C-48

# Periphyton Growth and Zinc Sequestration

M. Kalin and W.N. Wheeler
Boojum Research Limited
Toronto, ON M5A 1T7

## ABSTRACT

Periphyton populations composed mostly of green, filamentous algae have been found in mining waste water, together with large quantities of metal precipitates. These populations, because of the close interaction between precipitate and periphyton, have been termed periphyton-precipitate complexes (PPCs). These complexes can be over 80% precipitate, with zinc concentrations as high as 8%.

While growth of the periphyton and precipitate content of the complex are related to water chemistry, some commonalities have been found between PPCs growing in different waters from different mine sites. The elemental composition of precipitate is also similar to that of PPCs, suggesting that periphyton may play a role as sticky sieves. This paper discusses the relationship between zinc, precipitates, and periphyton growth, primarily from mining sites in northern Ontario and central Newfoundland.

## INTRODUCTION

Attached, or periphytic algae grow in mine effluent ponds and streams, characterized by extremes in pH and elevated metal and suspended solids concentrations (3, 5, 6, 10, 12, 13, 16). Reviews on metal/algae interactions (11, 12, 16) indicate that tolerance is achieved by several different means. Dissolved metals can be either bound to the cell walls, charged carbohydrates, or taken up into the cell and sequestered in specific organelles. All of these processes lead to high concentrations of metals in or on the algal biomass (3, 11).

Metal precipitates present as suspended solids can also attach to periphyton populations either to extracelluar carbohydrates or onto cell walls (4, 9, 15) resulting in Periphyton-Precipitate Complexes (PPCs) with high solids content and a small proportion of biomass. Precipitates, especially iron hydroxide, and iron hydroxide coprecipitated with zinc have been found on cell bacterial surfaces (1). This may also correlate with encrustation of iron and manganese found in a number of filamentous algae at sites contaminated by mine water (14). The periphyton populations inhabiting acidic effluents are multi-species complexes dominate by filamentous, benthic algae, which are associated with mosses and diatoms. Cyanophytes and charophytes dominate periphyton in ponds and ditches containing more alkaline waters.

Growth rates of PPCs have been quantified in the field at two sites by Kalin and Wheeler (7,8). It was found that in Newfoundland, in circumneutral pit water, PPCs "grew" at rates up to 4.6 gdw (sq. m of surface area)$^{-1}$ d$^{-1}$. In NW Ontario, PPCs in an acidic lake grew at rates of only 1.4 gdw (sq. m of surface area)$^{-1}$ d$^{-1}$. Quantifying growth of the periphyton portion of the PPC, without accompanying precipitates proved difficult.

This paper describes the relationship between metal precipitates and periphyton growing at two mine sites. Comparisons are made between the composition of PPCs and precipitates, as well as the growth of PPC and deposition rates of precipitates. A basic understanding of these relationships is essential to the development of biological polishing as a water cleansing process.

## METHODS AND MATERIALS

### Site and Algal Population Description

The first of two intensively-studied mine sites were located on Confederation Lake in northwest Ontario (Figures 1 and 2). The site included several water bodies which contained extensive periphyton populations. Large amounts of alkaliphillic cyanobacteria (*Oscillatoria*) occurred near the outflow of Decant Pond. Extensive populations of a *Ulothrix* spp.- dominated community were found on the beach end of Decant Pond and in an acidic lake (Boomerang Lake). Another population of *Ulothrix* spp. was found in Mill Pond (Table 1).

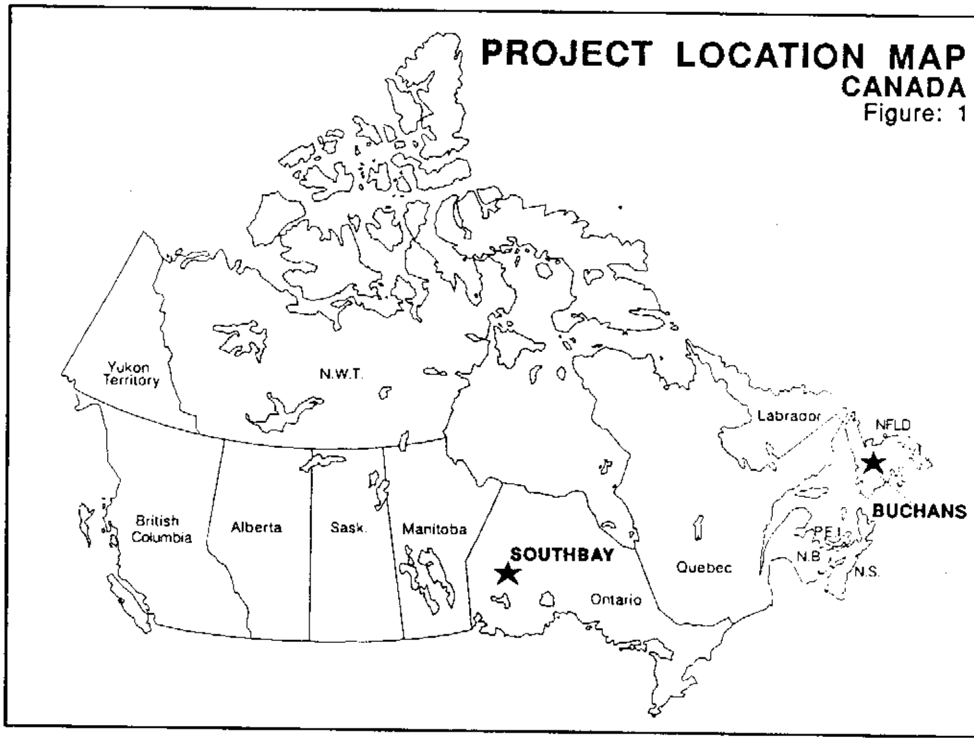


Figure 1: Location of study sites.

The second intensively studied site is the Buchans mine in central Newfoundland (Figures 1 and 3). In the Oriental East Pit and its effluent stream and polishing pond system, a *Microspora*-and moss-dominated community was proliferating (OEP). A *Ulothrix* community dominated the algal flora in the Oriental West Pit (OWP). Populations of a *Ulothrix/Microspora* -dominated community were growing in Second Meadow seepages (MDW), and in the Drainage Tunnel (DT) effluent water, another *Ulothrix/Microspora* -dominated population flourished (Table 1).

In the effluent of the Oriental East Pit, a series of six, serial experimental ponds were constructed in 1988. Each pond had a volume of approximately 40 cubic meters. Alder branches were placed in each pond to act as surface area (appx. 3.8 $m^2/m^3$) for the growth of PPCs. Flow through the ponds was controlled, to provide an overall residence time between 16 and 79 days.

## Field Sampling

PPCs were intensively studied over the summer of 1991 during site visits to the two mines. The habitat, i.e. pond, stream or lake shore was recorded, and pH and electrical conductivity were determined in the field. PPC and water samples were kept cool in plastic bags and bottles until processed in the laboratory.

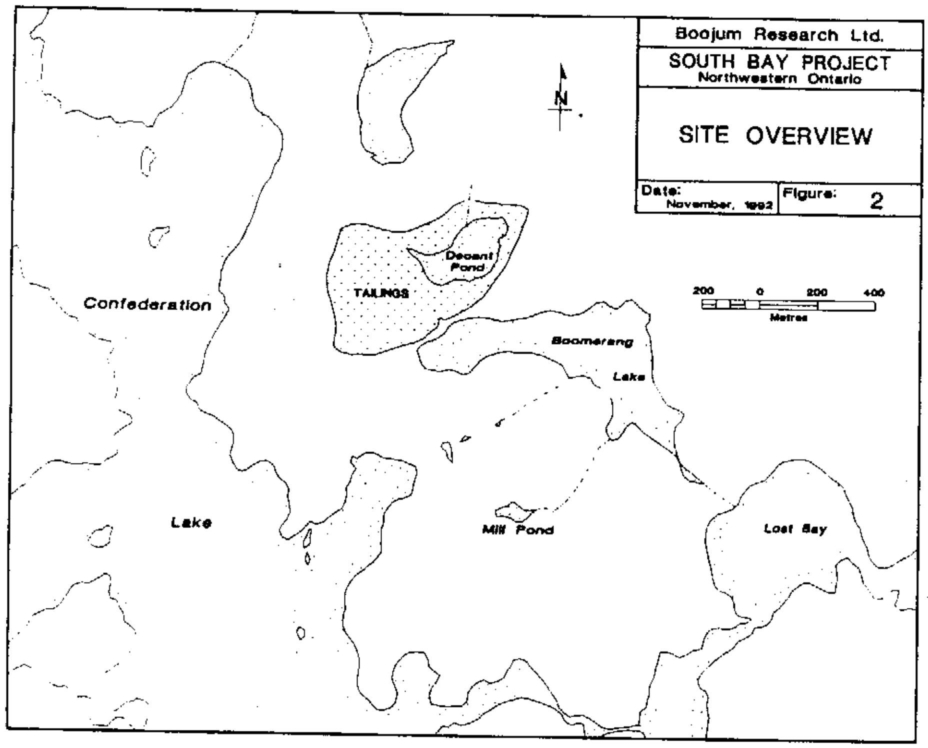


Figure 2: Location of study sites at South Bay.

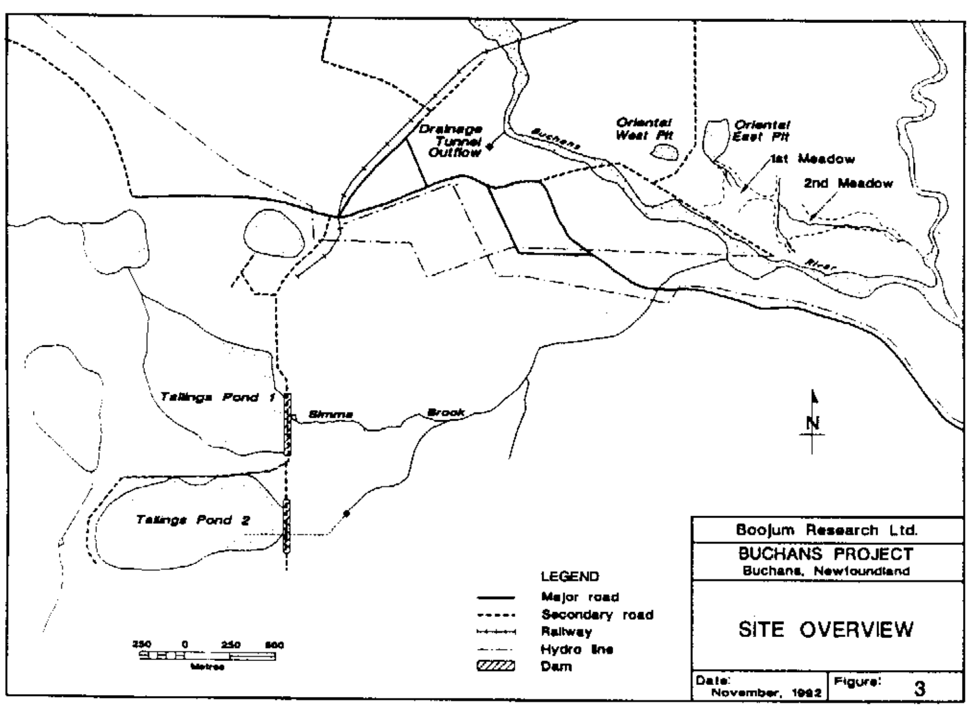


Figure 3: Location of study sites at Buchans.

TABLE 1: Description of Waste Water Sites .

| MINE SITE | HABITAT | pH | [Zn] mg/L | [Al] mg/L | [Fe] mg/L | [Cu] mg/L |
|---|---|---|---|---|---|---|
| NW Ontario | Boomerang Lake | 3.2-3.5 | 7-11 | 2.1 | 4.5 | 0.1 |
| NW Ontario | Decant Pond | 3.2-4.5 | 1-2 | 0.1 | 0.6 | 0.1 |
| NW Ontario | Decant Pond | 5-7 | 2-6 | 4 | 1* | 0.04 |
| NW Ontario | Mill Pond | 3.2-3.5 | 150-450 | 16 | 32 | 15.8 |
| Newfoundland | Oriental East Pit | 6.1-7.3 | 20-25 | 10 | 1* | 1* |
| Newfoundland | Meadow seepage | 6.5 | 7 | 4 | 1* | 0.1* |
| Newfoundland | Drainage Tunnel | 6.4-6.7 | 16-25 | 1* | 1* | 0.1* |
| Newfoundland | Polishing Ponds | 6.5-7.5 | 4-18 | 4-8 | 2 | 1* |
| Newfoundland | Oriental West Pit | 3.3-3.8 | 26-36 | 4-6 | 1 | 1* |

* indicates sample at or below detection limit

PPC growth rates were quantified at both locations using a combination of constructed, artificial substrates and natural, alder or black spruce branches. The artificial structures, called "peritraps" measured both growth and sloughing rates. The traps consisted of an artificial netting structure which housed alder or spruce branches. A plastic bag below the netting collected any PPC material falling from the netting or substrates. PPC growth rates could therefore be determined on both netting and branches. PPC mass was cleaned off the nets and branches, dried and weighed. Total growth was determined by adding PPC weights on nets and branches to that which had fallen into the bag. The cleaned traps were replaced for regrowth three times during the growing season. Peritraps were installed in Boomerang Lake and Decant Pond at South Bay, and in the OWP, OEP, and the polishing pond system at Buchans.

Precipitation rates were quantified at the South Bay site, in Boomerang Lake at 4 locations, including the outfall (2 m depth), the inflow area from Mill Pond (2 m), and in two deeper locations in the lake (4 and 5 m; Figure 2). Precipitation rates were also measured near the outfall in Decant Pond in 2 meters of water. At Buchans, precipitation rates were quantified at 4 locations in the Oriental East Pit, two near the outfall in 2.7 m of water and 2 in the centre of the pit in 20 m of water. The traps were a collection of 5 vertically-mounted tubes, 5 cm diam. and 50 cm in length (2). They were held vertically by a plate and harness, and lowered into the water to specific depths. The traps were hauled to the surface, and allowed to sit for 24 h. The material in the bottom of the tubes was collected, dried, and weighed. Precipitation rates per square meter of lake or pit bottom were calculated from the dry mass collected from the traps over several time intervals.

Specific comparisons between PPC composition and surrounding water can only be made using PPC populations of a similar biotic composition. Thus, *Ulothrix*-PPCs and surrounding water (Decant Pond, Mill Pond, and Boomerang Lake) were compared without precipitate correction with respect to their elemental composition (Figure 4a). For these comparisons, individual PPC samples were compared.

The distribution of elements and their concentrations in the waste water were similar to those found in PPCs, with the exception of iron, copper, and zinc. This was expected as the origin of the metals was either tailings or mill site. Sulphur, calcium, and zinc (Mill Pond only) were present in concentrations >100 mg $L^{-1}$. Those elements with concentrations greater than 10 mg $L^{-1}$ were magnesium, aluminum, silicon, iron (except Boomerang Lake), and copper (Mill Pond only).

In PPCs growing in the waters, the most accumulated element was iron (> 100,000 $\mu$g $gdw^{-1}$), but in the next highest concentration range (>10,000 $\mu$g $gdw^{-1}$) sulphur, manganese (Decant Pond only), and zinc (Decant Pond only) were present. The next highest concentration range (>1,000 $\mu$g $gdw^{-1}$) contained the elements calcium, aluminum, copper (Decant Pond only) and phosphorus. Potassium and sodium, as essential plant nutrients, can be expected to be present in high concentrations.

At Buchans, there were also 3 sites which contained similar periphyton populations composed of *Ulothrix* and *Microspora* (Table 2). Elemental distributions are shown for specific samples of populations in the Drainage Tunnel, the Oriental West Pit, and the Meadow seepages.

Figure 4b compares the concentrations of major elements in Buchans water with those found in corresponding PPCs. Only calcium and sulphur in the OWP and Meadow sites were found in concentrations greater than 100 mg $L^{-1}$. Of the elements, present in concentrations greater than 10 mg $L^{-1}$, only zinc was of concern. Iron concentrations at these sites were low, < 10 mg $L^{-1}$.

The PPCs found growing at these locations had somewhat different elemental distributions. Those elements found in concentrations greater than 10,000 $\mu$g $gdw^{-1}$, were calcium, sulphur, iron, and zinc (only for the Meadow Seep). For the Drainage Tunnel and OWP, only aluminum and iron greater than 10,000 $\mu$g $gdw^{-1}$. Zinc concentrations in the Drainage Tunnel and OWP PPCs were 2000 to 5000 $\mu$g $gdw^{-1}$.

## Precipitation Rates

The calculated average precipitate deposition rate in Boomerang Lake was around 2.2 gdw $m^{-2}$ $d^{-1}$ over the summer 1991 (Figure 5a). In Decant Pond, the average metal precipitation rate was 0.6 gdw $m^{-2}$ $d^{-1}$. In the Oriental East Pit at Buchans, one of the shallow traps caught 2.0 gdw $m^{-2}$ $d^{-1}$ over the winter, and 1.9 gdw $m^{-2}$ $d^{-1}$ over the following summer (Figure 5b). The deeper traps caught just over twice that amount, averaging about 5.0 g $m^{-2}$ $d^{-1}$ over the winter, and 5.3 gdw $m^{-2}$ $d^{-1}$ over the summer. Since the deepest traps in Boomerang Lake were in 4-5 m of water, precipitation rates were probably more comparable to the shallow traps in Buchans (2.7 m).

Table 2: PPC Composition

| LOCATION | TAXA | DW/FW | Algae | Fe | Fe(OH)3 | S | (SO4) | Zn | Zn(OH)2 | Mn | Al | Ca | CaCO3 | Cu | Other |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | % | % | | % | | % | | % | % | % | | % | % |
| Decant Pond | Oscillatoria | 0.28 | 29.5 | 17.8 | 34.9 | 2.5 | 7.5 | 3.3 | 5.1 | 1.1 | 3.9 | 1.4 | 3.6 | 1.1 | 13.3 |
| Decant Pond | Ulothrix | 0.309 | 93.0 | 3.2 | 6.3 | 1.0 | 3 | 1.8 | 2.8 | 2.1 | 0.3 | 1.0 | 2.6 | 0.1 | -10.1 |
| Mill Pond | Ulothrix | 0.264 | 14.0 | 28.9 | 56.6 | 2.6 | 7.8 | 0.2 | 0.3 | - | 0.6 | 0.6 | 1.5 | 0.2 | 18.9 |
| Boom. Lake | Ulothrix | n.d. | 35.4 | 20.1 | 39.4 | 1.8 | 5.4 | 0.3 | 0.5 | - | 0.4 | 0.5 | 1.3 | - | 17.7 |
| Drainage Tunnel | Ulothrix/Microspora | 0.376 | 34.0 | 3.1 | 6.1 | 0.6 | 1.8 | 0.7 | 1.1 | - | 2.1 | 0.5 | 1.3 | 0.2 | 53.5 |
| Meadow | Ulothrix/Microspora | 0.282 | 70.9 | 4.4 | 8.6 | 1.4 | 4.2 | 2.2 | 3.4 | 0.2 | 0.6 | 2.1 | 5.4 | - | 6.7 |
| Oriental West Pit | Ulothrix/Microspora | n.d. | 20.5 | 5.6 | 11.0 | 0.8 | 2.4 | 0.3 | 0.5 | - | 1.2 | 1.7 | 4.3 | - | 60.1 |
| Polishing Ponds | Microspora | n.d. | 18.7 | 10.9 | 21.4 | 0.5 | 1.5 | 8.2 | 12.7 | 1.4 | 1.2 | 1.6 | 4.1 | - | 39.0 |
| Oriental East Pit | Microspora | 0.206 | 35.3 | 25.8 | 50.6 | 0.4 | 1.2 | 3.6 | 5.6 | 0.2 | 0.5 | 1.3 | 3.3 | - | 3.3 |

- denotes percentage smaller than 0.1

n.d. - not determined

Specific comparisons between PPC composition and surrounding water can only be made using PPC populations of a similar biotic composition. Thus, *Ulothrix*-PPCs and surrounding water (Decant Pond, Mill Pond, and Boomerang Lake) were compared without precipitate correction with respect to their elemental composition (Figure 4a). For these comparisons, individual PPC samples were compared.

The distribution of elements and their concentrations in the waste water were similar to those found in PPCs, with the exception of iron, copper, and zinc. This was expected as the origin of the metals was either tailings or mill site. Sulphur, calcium, and zinc (Mill Pond only) were present in concentrations >100 mg $L^{-1}$. Those elements with concentrations greater than 10 mg $L^{-1}$ were magnesium, aluminum, silicon, iron (except Boomerang Lake), and copper (Mill Pond only).

In PPCs growing in the waters, the most accumulated element was iron (> 100,000 $\mu$g $gdw^{-1}$), but in the next highest concentration range (>10,000 $\mu$g $gdw^{-1}$) sulphur, manganese (Decant Pond only), and zinc (Decant Pond only) were present. The next highest concentration range (>1,000 $\mu$g $gdw^{-1}$) contained the elements calcium, aluminum, copper (Decant Pond only) and phosphorus. Potassium and sodium, as essential plant nutrients, can be expected to be present in high concentrations.

At Buchans, there were also 3 sites which contained similar periphyton populations composed of *Ulothrix* and *Microspora* (Table 2). Elemental distributions are shown for specific samples of populations in the Drainage Tunnel, the Oriental West Pit, and the Meadow seepages.

Figure 4b compares the concentrations of major elements in Buchans water with those found in corresponding PPCs. Only calcium and sulphur in the OWP and Meadow sites were found in concentrations greater than 100 mg $L^{-1}$. Of the elements, present in concentrations greater than 10 mg $L^{-1}$, only zinc was of concern. Iron concentrations at these sites were low, < 10 mg $L^{-1}$.

The PPCs found growing at these locations had somewhat different elemental distributions. Those elements found in concentrations greater than 10,000 $\mu$g $gdw^{-1}$, were calcium, sulphur, iron, and zinc (only for the Meadow Seep). For the Drainage Tunnel and OWP, only aluminum and iron greater than 10,000 $\mu$g $gdw^{-1}$. Zinc concentrations in the Drainage Tunnel and OWP PPCs were 2000 to 5000 $\mu$g $gdw^{-1}$.

## Precipitation Rates

The calculated average precipitate deposition rate in Boomerang Lake was around 2.2 gdw $m^{-2}$ $d^{-1}$ over the summer 1991 (Figure 5a). In Decant Pond, the average metal precipitation rate was 0.6 gdw $m^{-2}$ $d^{-1}$. In the Oriental East Pit at Buchans, one of the shallow traps caught 2.0 gdw $m^{-2}$ $d^{-1}$ over the winter, and 1.9 gdw $m^{-2}$ $d^{-1}$ over the following summer (Figure 5b). The deeper traps caught just over twice that amount, averaging about 5.0 g $m^{-2}$ $d^{-1}$ over the winter, and 5.3 gdw $m^{-2}$ $d^{-1}$ over the summer. Since the deepest traps in Boomerang lake were in 4-5 m of water, precipitation rates were probably more comparable to the shallow traps in Buchans (2.7 m).

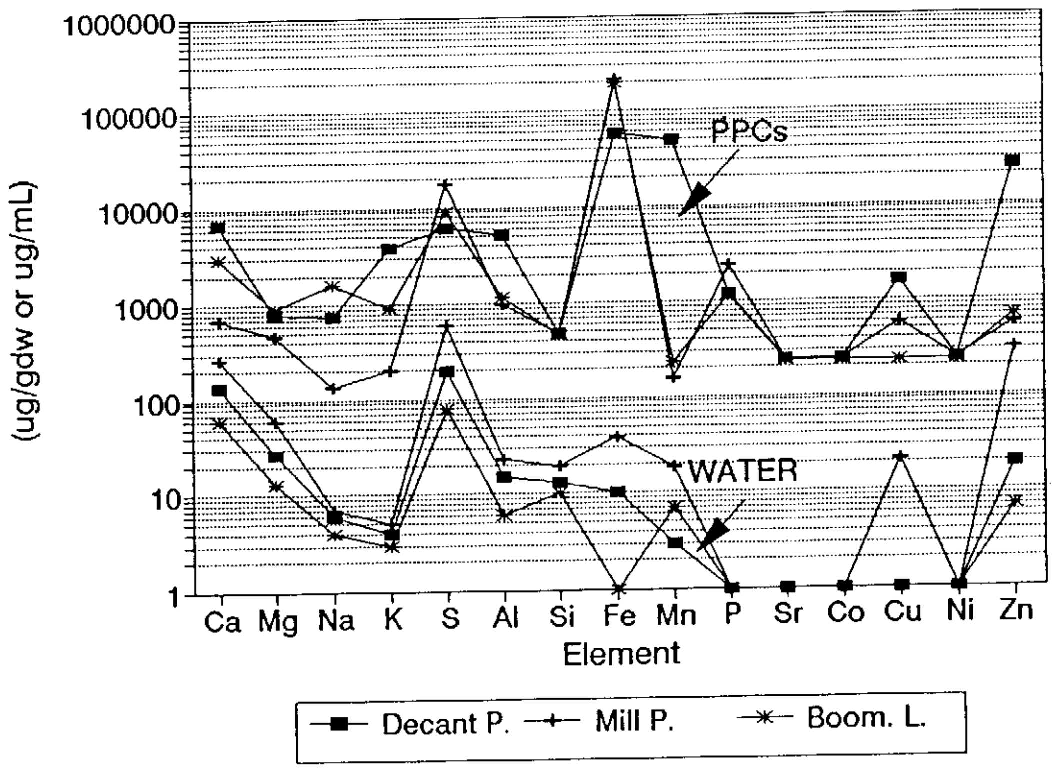


Figure 4a: Elemental scans of *Ulothrix* PPCs and surrounding water.

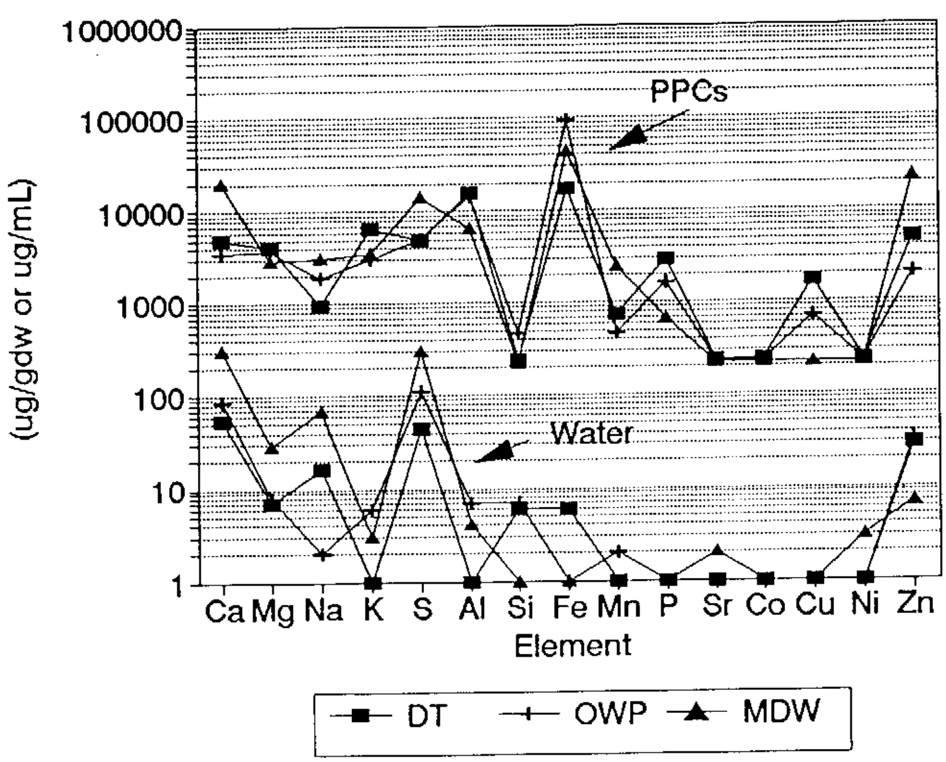


Figure 4b: Elemental scans of Ulothrix/Microspora PPCs and surrounding water.

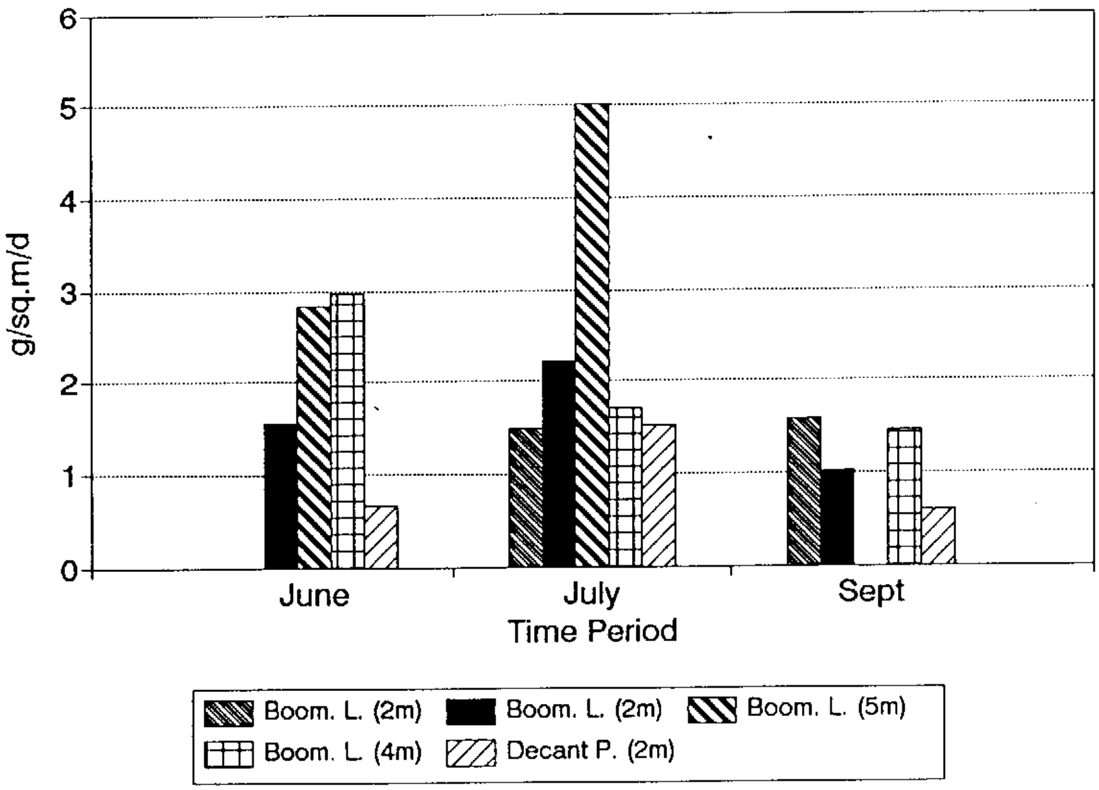


Figure 5a: Precipitation rates in Boomerang Lake and Decant Pond, 1991.

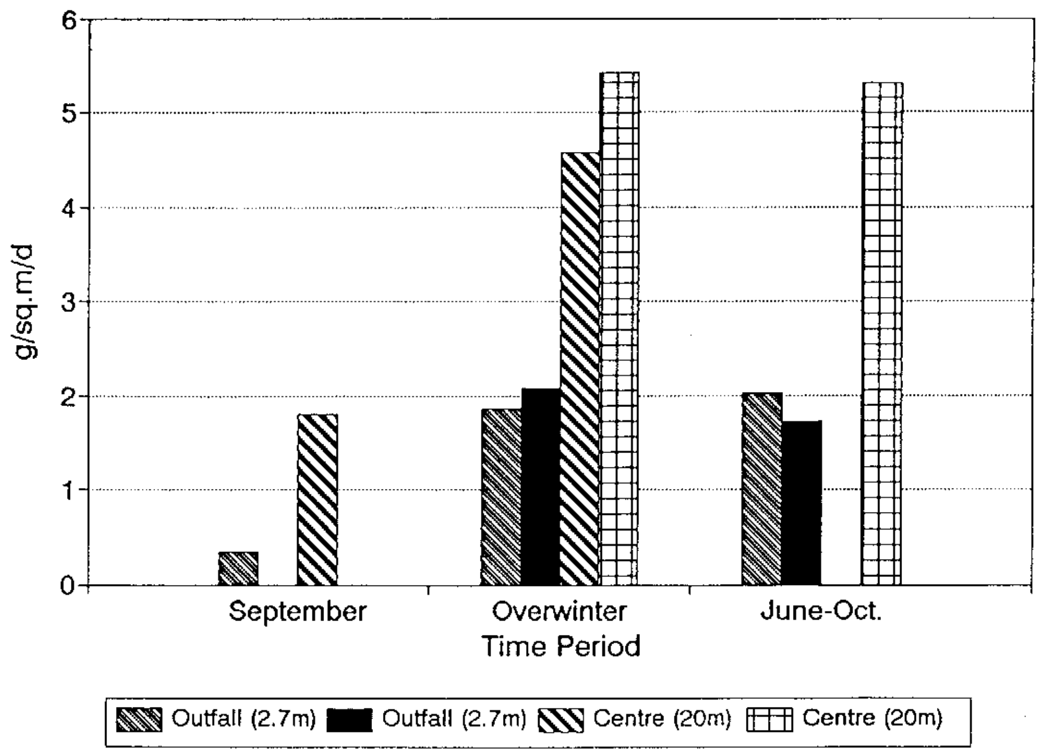


Figure 5b: Precipitation rates in the Oriental East Pit, 1990/1991.

PPC Growth Rates

At the South Bay site, 13 peritraps were set up at each of two locations in Boomerang Lake in early May. At Buchans, 5 peritraps were placed in each polishing pond at the end of May, with 6 in each of the pits. PPC growth rates over 3 periods in the summer were compared within mine sites (Boomerang Lake, and Decant Pond; OWP, OEP, PP1, and PP6) and between sites (Buchans, South Bay).

Growth rates of PPCs at South Bay and Buchans were calculated based on linear growth, i.e. the mass collected after a given submergence time per unit surface area. Growth rates of PPCs varied depending on site and time of year. In Newfoundland, in the OEP, for example, PPCs grew at rates up to 4.6 gdw (sq. m of surface area)$^{-1}$ d$^{-1}$. In NW Ontario, PPCs in Boomerang Lake grew at rates of only 1.4 gdw (sq m of surface area)$^{-1}$ d$^{-1}$. However, in order to compare algal growth at different sites, the precipitate content of the PPCs at each site had to be taken into account. Using the LOI correction for biomass described in the methods, the mean LOI-corrected periphyton growth rates for the summer of 1991 are shown in Figure 6. Due to the large precipitate component, the maximum periphyton growth rates are only about 0.75 gdw (sq. m of surface area)$^{-1}$ d$^{-1}$. With the exception of periphyton growth in the Oriental West Pit, and June samples from the polishing ponds, most of the growth rates are similar. The peritraps in Decant Pond were set up near the outflow and experienced water with a relatively high pH. Thus, the water bodies with high pHs (Decant Pond, OEP, PP1, and PP6) showed relatively high growth rates, with the exception of June and July data for PP1, and June data for PP6. However, among the acidic sites (all had pHs around 3.5), Boomerang Lake samples showed consistently higher growth rates than those in OWP.

PPC Growth and Precipitate Deposition

The growth of PPCs in South Bay and Buchans were compared to precipitate "growth" rates as measured by precipitate deposition rates. Comparisons were made for those periods when precipitate rates and PPC growth rates overlapped, and where precipitation traps were in close proximity to peritraps. The peritrap data were analyzed as if they were precipitate traps, i.e. growth rates were calculated based on the area of lake (or pit) bottom, rather than on a surface area basis. Data are presented in Table 3.

During peak growing periods, PPC mass "outgrew" precipitate deposition rates (Boomerang Lake July - 2.75 gdw m$^{-2}$ d-1 PPC growth vs. 1.65 gdw m$^{-2}$ d$^{-1}$ precipitate deposition rates), but at other times, rates were generally equal. The percentage precipitate was calculated to indicate which process dominated the "growth rate" of the PPC. Thus, in Boomerang Lake in June, 105% of the "growth" rate could be accounted for by precipitate deposition.

Another way of analyzing the contribution of the periphyton to the precipitate deposition process was to compare the composition of precipitate collected in precipitate traps and the composition of the PPCs collected in peritraps. These data are shown in Table 4. At South Bay, the LOI of PPCs was nearly identical with those of the precipitates (45.8 vs. 42.5%, respectively). The composition of the PPCs in Boomerang Lake were also nearly identical to the composition of the precipitate, for at least iron, aluminum, and

TABLE 3: Comparison of PPC "Growth" Rates With Precipitate Deposition Rate

| Treatment | June | July | Aug-Sep |
|---|---|---|---|
| | g/sq. m/day | | |
| NW Ontario - Boomerang Lake | | | |
| Precipitate | 1.60 | 1.65 | 1.60 |
| PPC | 1.52 | 2.75 | 1.45 |
| % Ppte | 105 | 60 | 110 |
| Newfoundland - Oriental East Pit | | | |
| Precipitate* | 1.9 | 1.9 | 1.9 |
| PPC | 1.77 | 3.33 | 3.3 |
| % Ppte | 107 | 57 | 58 |

* used over summer data (May-October)

TABLE 4: Elemental Composition of Precipitate and Periphyton Precipitate Complexes

| | LOI | Al | Fe | Zn | (n) |
|---|---|---|---|---|---|
| | % | ppm | ppm | ppm | |
| Newfoundland - Oriental East Pit | | | | | |
| Precipitate | 26.3 | 7699 | 299127 | 56267 | 4 |
| PPC | 36.6 | 5433 | 277716 | 40945 | 11 |
| NW Ontario - Boomerang Lake | | | | | |
| Precipitate | 42.5 | 3406 | 174771 | 618 | 2 |
| PPC | 45.8 | 2808 | 182490 | 619 | 13 |

zinc. At Buchans, however, Oriental East Pit precipitates appeared to be significantly enriched with metals over PPCs.

As an example of biological polishing capacity, zinc removal rates in the Buchans polishing ponds were calculated for selected days during the summer of 1991. Data on PPC mass, zinc concentrations in water and PPCs, and flow rates on Aug 25, 1991 were analyzed (Figure 7). Under conditions of: 1) 16 day residence time; 2) 15.5 mg $L^{-1}$ Zn in the inflow stream, and 3) 6.75 pH, 88% of the dissolved zinc was removed from the incoming water stream.

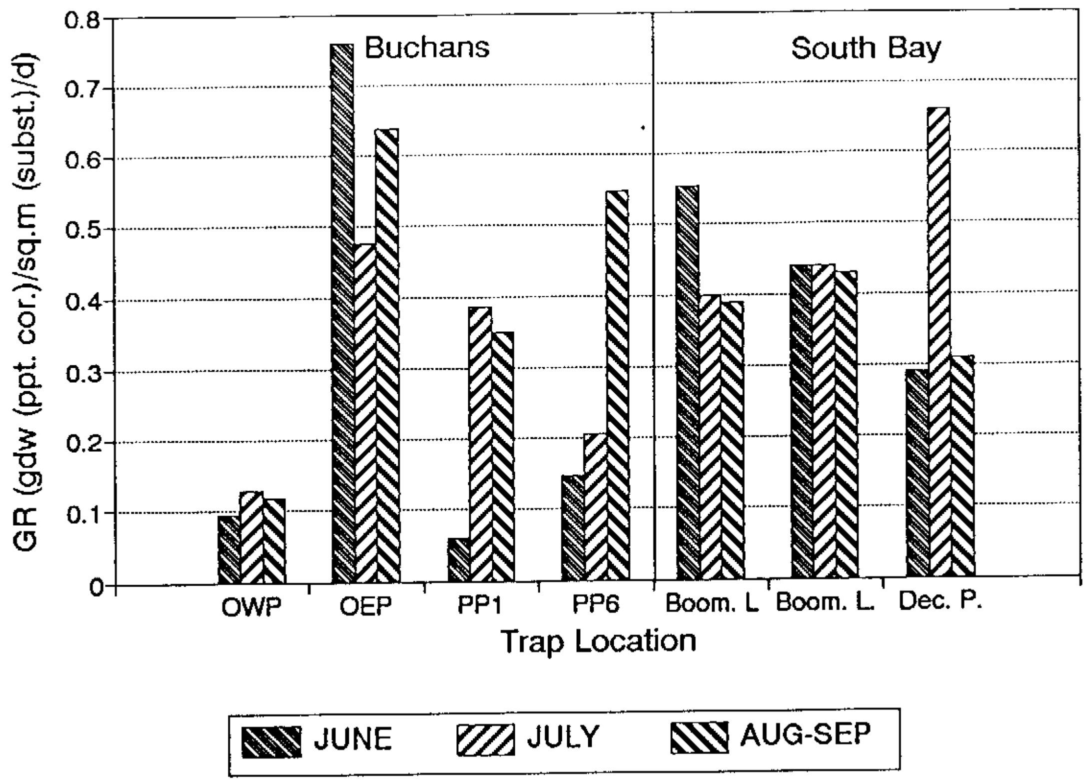


Figure 6: Periphyton growth rates on Peritraps in South Bay and Buchans waste waters.

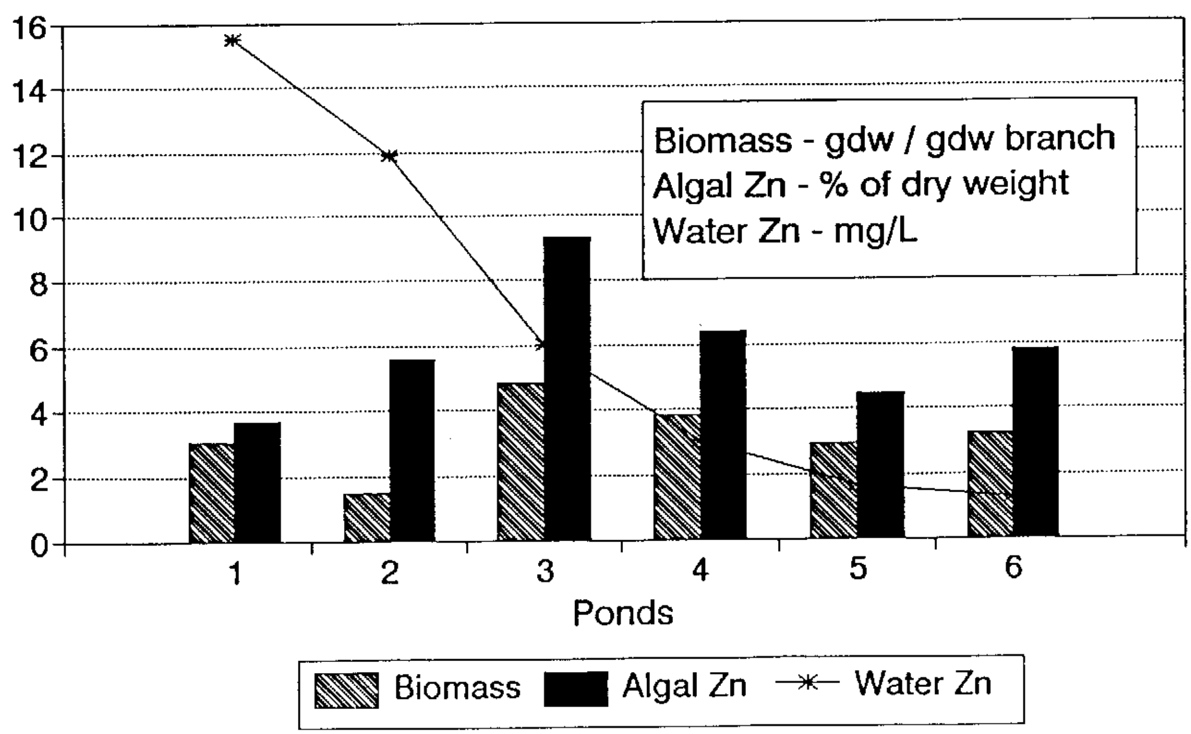


Figure 7: Biological polishing of zinc in Buchans polishing ponds, August 25, 1990.

## DISCUSSION

Our data suggest that algae can grow well in mine seepages, ponds, and lakes over a range of pHs, and metal concentrations. Metal precipitates, and dissolved metals can be found in association with algae at these sites. During peak growing periods, PPC mass can "outgrow" precipitate deposition rates, but at other times, rates are generally equal. This suggests that precipitate deposition rates dominate the PPC growth rates, and that already formed precipitates are simply being sieved from the water. This is further confirmed by analyzing the composition of the PPCs and precipitates.

Newman et al. (9) correlated the composition of *aufwuchs* with the composition of precipitate. Their finding, that negative correlations between cell density and elemental concentrations indicated that the role of metal hydroxides was more important than periphyton accumulation in explaining the metal concentrations found in *aufwuchs.*

Periphyton surfaces and associated polysaccharides are providing a "sticky" surface which appears to "sieve" precipitates from the water. Where precipitates are not in high concentrations, periphyton appear to be providing surface area for the direct binding of zinc, and other metals onto algal surfaces. Thus, at high concentrations of precipitate, the composition of the precipitate and PPC are nearly identical, and the PPC is a "sticky sieve". At low concentrations of precipitate or with only dissolved metals present, the periphyton are providing a surface on which metals can be bound.

Regardless of the role that periphyton plays, a significant fraction of the zinc, and other metal loadings can be removed from waste streams, especially at higher pHs.

## ACKNOWLEDGEMENTS

We wish to acknowledge partial support for this work by the joint venture group at Buchans, Newfoundland - ASARCO Inc. and Abitibi-Price Inc. Particular thanks are given to the General Manager/Consultant for ASARCO at Buchans, Mr. G. Neary. BP Resources, Development Corp. of Cape Breton, and CANMET also provided financial support, through CANMET contracts, DSS File #034SQ.23440-1-9009, DSS File # 034SQ.23440-1-9011, and DSS File #034SQ.23440-1-9010.

## LITERATURE


1. T.J. Beveridge and R.G.E. Murray, "Uptake and retention of metals by cell walls of *Bacillus subtilis*", J. Bacteriol. 127, 1976, 1502-1518.

2. J. Bloesch J. and N.M. Burns, "A critical review of sedimentation trap technique", Schweiz. Z. Hydrol., 42, 1980, 15-55.

3. P. Foster, "Species associations and metal contents of algae from rivers polluted by heavy metals", Freshwater Biology, 12, 1982, 17-39.

4. N.L. Gale and B.G. Wixson, "Removal of heavy metals from industrial effluents by algae", Dev. Ind. Microbiol., 20, 1979, 259-273.

5. J.W. Hargreaves, E.J.H. Lloyd, and B.A. Whitton, "Chemistry and vegetation of highly acidic streams", Freshwater Biology, 5, 1975, 563-576.

6. M. Kalin, M. Olaveson, and B. McIntyre, "Phytoplankton and periphyton in a shield lake receiving acid mine drainage in NW Ontario", Proceedings of the Fifteenth Annual Aquatic Toxicity Workshop, R. Van Coillie, A. Niimi, A. Champoux, and G. Joubert, Eds., Can. Tech. Rep. Fish. Aquat. Sci., 1714, 1989, 166-187.

7. M. Kalin, and W.N. Wheeler, "A study of algae-precipitate interactions", Final Report to CANMET, DSS File # 034sQ.23440-1-9011, 1992, 58 p.

8. M. Kalin, and W.N. Wheeler, "Algal biopolishing of zinc", Final Report to CANMET, DSS File #034SQ.23440-1-9009, 1992, 57 p.

9. M.C. Newman, J.J. Alberts, and V.A. Greenhut, "Geochemical factors complicating the use of *Aufwuchs* to monitor bioaccumulation of arsenic, cadmium, chromium, copper and zinc", Water Res., 19, 1985, 1157-1165.

10. M.M. Olaveson and P.M. Stokes (1989). "Responses of the acidophyllic alga *Euglena mutabilis* (Euglenophyceae) to carbon enrichment at pH 3", J. Phycol., 25, 1989, 529-539.

11. L.C. Rai, J.P. Gaur, and H.D. Kumar, "Phycology and heavy metal pollution", Biol. Rev., 56, 1981, 99-151.

12. R.H. Reed and G.M. Gadd, "Metal tolerance in eukaryotic and prokaryotic algae", Heavy metal tolerance in plants: evolutionary aspects, A.J. Shaw, Ed., CRC Press, Boca Raton, Fl., 1990, 105-118.

13. P.J. Say and B.A. Whitton, "Changes in flora down a stream showing a zinc gradient", Hydrobiol., 76, 1980, 255.

14. S.E. Stevens Jr., K. Dionis, and L.R. Stark, "Manganese and iron encrustation on green algae living in acid mine drainage", Constructed Wetlands for Wastewater Treatment, D.A. Hammer, Ed., Lewis Publishers, Chelsea MI, 1990, 765-773.

15. J.R.P. Strong, J.C. Madgwick, and B.J. Ralph, "Metal binding polysaccharide from the alga *Klebshormidium fluitans*", Biotechnol. Lett., 4, 1982, 239-242

16. B.A. Whitton, "Toxicity of zinc, copper and lead to Chlorophyta from flowing waters", Arch. Microbiol., 72, 1970, 353-360.