A STUDY OF ALGAE - PRECIPITATE INTERACTIONS

FINAL REPORT

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SUMMARY

The development of biological polishing, a waste water treatment system utilizing periphytic attached algae to remove metals, requires some understanding of the interaction between these periphyton communities and the metals which are removed from the water.

South Bay, an inactive Cu and Zn mine has waste water of three different pHs and dissolved metal concentrations. Boomerang Lake (pH 3.5, [Zn] 10 mg/L), Decant Pond (pH 7, [Zn] 2 mg/L), and Mill Pond (pH 3.5, [Zn] 150-250 mg/L). All water bodies contain extensive periphyton complexes.

Periphyton complexes were characterized, both chemically by ICP analysis and physically using SEM and EDS electron microscopy. Decant Pond complexes contained 17.2% iron and 2.5% sulphur on a dry weight basis. Mill Pond complexes contained 29% iron and 2.6% sulphur, while Boomerang Lake complexes contained 20% iron and 1.8% sulphur. Zinc, manganese, aluminum and copper were accumulated primarily by complexes in Decant Pond, where average zinc concentrations were 3.3% of dry weight with a maximum of 7% of dry weight. Zinc in the acidic Boomerang Lake and Mill Pond averaged 0.3% and 0.2% of dry weight, respectively. These elemental distributions suggest that zinc removal processes are different in acidic and alkaline waters.

The living algal fraction of the complex was determined using L.O.I. (500° C). This fraction ranged between 93% and 29.5% in Decant Pond. Mill Pond complexes averaged 14% periphyton, while Boomerang Lake complexes contained 35% periphyton.

In the first year of submergence about 0.3 grams dry weight (gdw) of complex were produced per gdw branch. In the second and third year the amount increased to 0.9 gdw gdw^{-1} and 1.2 gdw gdw^{-1}, respectively. As expected, the highest growth rates were observed in July in the neutral water of Decant Pond (0.7 gdw m^{-2} of substrate d^{-1}), followed by growth rates in Boomerang Lake (0.4 gdw m^{-2} substrate d^{-1}). These ranges are very similar to those reported for algal growth in neutral and acidic waste water on other mine sites.

Electron microscopy of a sample from Decant Pond showed two different precipitates near periphyton filaments and diatom frustules, a crystalline form and a plaque directly on cell walls. Semi-quantitatively, a large, low-density precipitate, composed mostly of iron, copper, zinc, and sulphur, could be differentiated from a high-density precipitate containing up to 60% indium and 22 % silver.

RÉSUMÉ

Le développement de l'épuration biologique, un système de traitement des eaux usées utilisant des algues périphytiques fixées pour éliminer des métaux, nécessite une certaine compréhension de l'interaction entre ces communautés de périphyton et les métaux qui sont éliminés de l'eau.

South Bay, une mine de Cu et de Zn désaffectée possède des eaux résiduaires de trois pHs et concentrations de métaux différents. Boomerang Lake (pH 3,5, [Zn] 10 mg/l), Decant Pond (pH 7, [Zn] 2 mg/l), et Mill Pond (pH 3,5 [Zn] 150-250 mg/l). Toutes les pièces d'eau contiennent des complexes de périphyton extensifs.

Les complexes de périphyton sont caractérisés, chimiquement par une analyse de PCI (spectroscopie du plasma couplé par induction) comme physiquement en utilisant une MEB (microscopie électronique par balayage) et une microscopie électronique SED (spectroscopie de densité d'électrons). Les complexes de Decant Pond contenaient 17,2% de fer et 2,5% de soufre, sur une base de poids sec. Les complexes de Mill Pond contenaient 29% de fer et 2,6% de soufre, alors que les complexes de Boomerang Lake contenaient 20% de fer et 1,8% de soufre. Le zinc, le manganèse, l'aluminium et le cuivre ont été accumulés principalement par les complexes dans Decant Pond, où les concentrations moyennes de zinc étaient de 3,3% de poids sec, avec un maximum de 7% de poids sec. Le zinc dans Boomerang Lake et Mill Pond, tous deux acides, se montait en moyenne, respectivement, à 0,3% et 0,2% de poids sec. Ces distributions élémentaires suggèrent que les procédés d'élimination du zinc sont différents dans des eaux acides et alcalines.

La fraction d'algue vivante du complexe a été déterminée en utilisant la PPC (500°C). Cette fraction allait de 93% à 29,5% dans Decant Pond. Les complexes de Mill Pond se montaient en moyenne à 14% de périphyton, alors que les complexes de Boomerang Lake contenaient 35% de périphyton.

Au cours de la première année de submersion, 0,3 gramme de poids sec (gps) environ de complexe était produit par gps de branche. Au cours de la seconde et de la troisième année, la quantité a augmenté respectivement jusqu'à 0,9 gps gps^{-1} et 1,2 gps gps^{-1}. Tel que prévu, les taux de croissance les plus élevés ont été observés en juillet dans les eaux neutres de Decant Pond (0,7 gps m^{-2} de substrat $jour^{-1}$), suivis par les taux de croissance dans Boomerang Lake (0,4 gps m^{-2} de substrat $jour^{-1}$). Ces intervalles sont très similaires à ceux rapportés pour la croissance de l'algue dans des eaux résiduaires neutres et acides dans d'autres sites miniers.

Une microscopie électronique d'un échantillon provenant de Decant Pond a mis en évidence deux précipités différents près des filaments du périphyton et des frustules de diatomées, une forme cristalline et une plaque directement sur les parois cellulaires. Semi-

quantitativement, un important précipité de basse densité, composé principalement de fer, de cuivre, de zinc et de soufre, pouvait être différencié d'un précipité de forte densité contenant jusqu'à 60% d'indium et 22% d'argent.

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1.0 INTRODUCTION

Attached, or periphytic algae grow in mine effluent ponds and streams, characterized by extremes in pH (2.2 to 8.5), elevated metal and suspended solids concentrations (Bennett 1969; Whitton 1970; Hargreaves et al. 1975; Say and Whitton 1980; Foster 1982; Kepler 1988; Kalin et al. 1989, Olaveson and Stokes 1989. Reviews on metal tolerance (Whitton 1970; Rai et al. 1981; Stokes 1983; Reed and Gadd 1990) indicate that metal tolerance is achieved by several different means, particularly in extreme aquatic environments, such as mine waste waters. Dissolved metals can be either excluded from cells by producing external, charged carbohydrates, or taken up into the cell and sequestered in specific organelles. Either of these methods leads to increased concentration of metals in or on the algal biomass (Rai et al. 1981; Foster 1982) resulting in decreased concentration in the water.

Periphyton populations were found growing profusely in a number of seeps, ponds, and ditches at mining sites across Canada. These periphyton populations contained a number of species, primarily benthic algae from the order Ulothricales. Diatoms, mosses, and aquatic macrophytes were also present in smaller numbers. Cyanophytes and charophytes dominated the periphyton in ponds and ditches containing more alkaline waters. Mixed periphyton populations were found growing well in mine effluent which had pHs as low as 2.5, zinc concentrations as high as 450 mg L^{-1}, and aluminum concentrations as high as 95 mg L^{-1}.

Metal precipitates present as suspended solids can attach to periphyton populations either, to extracellular carbohydrates or onto cell walls (Gale and Wixson 1979, Strong et al. 1982), resulting in bio-precipitate complexes with high solids content and a small proportion of biomass.

These Periphyton Precipitate Complexes (PPCs) have been analyzed from a number of mining sites across Canada in mine water effluent. The data set also includes the surrounding water chemistry, so that the ability of PPCs to concentrate dissolved metals can be calculated.

The primary goal of the 1991 research was to quantify the interaction between periphyton and precipitates, by measuring periphyton growth, precipitate sedimentation rates, and quantitatively describing the physical interaction between algae and precipitate.

This report is organized along the lines of the task list set out in the proposal. Thus, the physical description of these PPCs is described in section 2 for a number of mining sites, and South Bay in particular. In Section 3 the interaction between precipitation processes and periphyton growth rates is described for 3 key environments in South Bay. Extracellular carbon production rates are discussed in Section 4.

2.0 PHYSICAL DESCRIPTION OF PERIPHYTON-PRECIPITATE COMPLEXES

2.1 PPC Database Methods

Periphyton Precipitate Complexes (PPCs) were collected over a period of 3 years during site visits to many different mine sites. The habitat, i.e. pond, stream or lake shore was recorded and field measurements of pH and electrical conductivity were carried out. Samples were kept cool in plastic bags until processed in the laboratory. Water was collected at the same location and filtered within 12h through 0.45 μm filters and acidified with concentrated nitric acid to pH less then 1. Samples were subsequently analyzed for metal content by Inductively Coupled Plasma Spectroscopy (ICP) at a certified laboratory.

In the laboratory, reference samples for identification of the dominant groups in the algal complexes were taken and fixed in Lugol's fixative. In some cases, material was used to establish cultures in the laboratory to confirm the dominance of the algal group in the PPC.

The dominant periphyton groups in the sample collection, the habitat, the geographical location and the pH range of the sampling location are given in TABLE 1 along with Zn, Al and Fe concentrations in the water. Periphyton populations were found in water ranging from pH from 3.2 to 8.5. Zinc concentrations in these waters range from below detection limit of <0.001 to 450 mg L^{-1}. Aluminum concentrations ranged from the below detection limit of 0.01 to 95 mg L^{-1}.

Sticks and other debris were removed and the sample was dried in an oven at 40-60 °C for 2 to 3 days. The dry material was powdered in a hand mortar, and stored in sealed glass vials. Metal concentrations were determined by ICP at a certified laboratory. Loss on Ignition (LOI) values were derived from a subsample which was combusted for 30 min in a muffle furnace at 500 °C.

Most plants contain minerals and inorganic materials that give them an LOI of near 95%. Algae, on the other hand, can contain much higher inorganic content (Larcher 1975). Diatoms for example have silicious frustules, and some of the Characeae precipitate calcium carbonate on the cell wall (see Hutchinson 1975). Many algae, aquatic plants and bacteria in waters with high dissolved metal content can become armoured, i.e. metals such as iron and manganese will form a plaque on cell walls (Macfie and Crowder 1987; Ferris et al. 1989; Stevens et al. 1990). Periphyton can also trap metal precipitates in mucilages and external polysaccharides.

To separate periphyton from precipitate, the metal content of "clean periphyton" and "pure precipitates" were determined by LOI. After subtracting the LOI of "clean periphyton", the percentage precipitate was calculated based on a straight line interpolation to 100% at the LOI of the "pure precipitate". The conversion is graphically shown in FIGURE 1.

Once the algal percentage (or precipitate percentage) was calculated, a second conversion factor was used to approximate the fresh weight of the periphyton based on measured dry to fresh weight ratios from known periphyton populations. The general conversion factor was 0.25 grams dry weight per gram fresh weight (gdw gfw^{-1}), based on dry to fresh weights of field-collected *Microspora* from Newfoundland. Dry to fresh weight ratios vary from population to population, but are generally between 20-25% (depending on the amount of metal precipitate present; unpublished data).

Metal accumulations were calculated using the LOI conversion and fresh weight (fw) conversion. The resultant metal concentration in (μg gfw^{-1}) was plotted against water metal concentration (mg L^{-1}) in log-log format.

2.2 Periphyton Concentration Factors

Periphyton zinc accumulations are plotted in FIGURE 2. It can be seen that, for the most part, all periphyton samples were able to maintain a 500-1000 fold zinc concentration difference over ambient water concentrations. This accumulation was relatively stable even though water zinc concentrations ranged from 0.001 to about 10 mg L^{-1}. Above about 10 mg L^{-1}, the concentration difference decreased. It appears as if periphyton populations growing in water containing > 100 mg L^{-1} zinc, do not accumulate, but exclude zinc. One population of *Ulothrix sp.* was found growing in zinc concentrations in excess of 400 mg L^{-1} (Mill Pond, South Bay, ON).

A similar relationship can be shown for aluminum (FIGURE 3). For the entire range of water aluminum concentrations from 0.01 to 5 mg L^{-1}, periphyton were able, with the exception of one population, to maintain at least a 30 fold concentration difference over ambient. Populations also showed a maximum concentration of around 43 mg gfw^{-1}. Several populations over a wide range of water concentrations showed this maximum.

Both Coleman et al. (1971) and Foster (1982) found similar zinc accumulations in other algae. Accumulations increased with increasing water metal concentrations. Both passive and active mechanisms have been proposed for the ability of periphyton to accumulate zinc. Active uptake mechanisms, however, have generally been noted at water concentrations below 0.5 mg L^{-1}. Passive accumulation, primarily through cation exchange on cell walls and external carbohydrates is proportional to dissolved ion concentrations, probably giving rise to the relationships seen in FIGURES 2,3. Such sequestration of copper has been demonstrated by Mangi and Schumacher (1979), Fisher and Fabris (1982) and Sorrentino (1985). Because accumulation increased proportionally with rising water concentrations up to about 10 mg L^{-1}, surface adsorption was suspected. Water which contained more than 10 mg L^{-1} zinc also contained other cations in high concentrations. Competition for the number of available surface (-) sites would result in a marked reduction in zinc accumulation. The fact that there were no populations which exhibited significant accumulation abilities above 10 mg L^{-1} zinc, indicated that, at these high concentrations, competition with other metal ions may have restricted zinc binding.

Many populations of periphyton studied at mine sites live in contact with large amounts of precipitates. PPCs composed of more than 90% precipitate were not uncommon. Adsorption of these precipitates to periphyton communities has been observed by Gale and Wixson (1979), and Stevens et al. (1990). Further, Gale and Wixson (1979) indicated that changing redox and pH conditions near algal cell walls can redissolve precipitates, or conversely, precipitate dissolved metals. Precipitates, especially iron hydroxide, and iron hydroxide with zinc have been found on cell surfaces (Beveridge and Murray 1976). This may also correlate with encrustàtion of iron and manganese found in a number of filamentous algae at sites contaminated by mine water (Stevens et al. 1990).

The PPCs were composed of more than zinc, aluminum and iron. In SECTION 2.3 and 2.4 other elements which may be co-precipitated are discussed. This process is governed by pH, redox, and the waste material (tailings, waste rock or ore) through which water travels. In mine effluents a major factor contributing to the precipitation process is the oxidation and hydrolysis of reduced iron.

2.3 PPC Composition

The South Bay mine was an ideal site at which to analyze PPC composition, both from a chemical and physical point of view. Not only does it have extensive periphyton populations, but it has several different environments which could be used to analyze the effect of physical factors on periphyton and PPC growth. Large amounts of alkalophyllic cyanobacteria (*Oscillatoria*) occur in Decant Pond with pH 7, and [Zn] 2 mg L^{-1}. Extensive populations of *Ulothrix* spp. are found in a mildly acidic lake (Boomerang Lake; pH 3.5, [Zn] 7-11 mg L^{-1}). Another population of *Ulothrix* spp. is found in Mill Pond (pH 3.2-3.5; [Zn] 150-450 mg L^{-1}). MAP 1 describes the location of these water bodies.

2.3.1 Metal Scans

Analysis of ICP data for a number of these PPCs indicate that zinc and aluminum are not the only metals concentrated by periphyton. Three pairs of samples, *Ulothrix*-PPCs and surrounding water (Decant Pond (DPOND), Mill Pond (MPOND), and Boomerang Lake (BLAKE)) were compared without precipitate correction with respect to their elemental composition by ICP (FIGURE 4).

A first glance, the overall pattern of element concentrations in the waste water was similar, with the exception of concentrations of iron, copper, and zinc. This can be expected as the origin of the metals is either tailings or waste rock. Sulphur, calcium, and zinc (MPOND 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 BLAKE), and copper (MPOND only). The presence of elevated calcium levels in MPOND and DPOND was probably the result of earlier lime applications. Both ponds, prior to 1985, received regular lime treatment.

If the corresponding PPCs are analyzed, the most accumulated element was iron (> 100,000 μg gdw^{-1}), but in the next tier (>10,000 μg gdw^{-1}) were sulphur, manganese (DPOND only), and zinc (DPOND only). The next level (>1,000 μg gdw^{-1}) contained the elements calcium, aluminum, copper (DPOND only) and phosphorus. Potassium and sodium as essential plant nutrients can be expected to present in high concentrations.

Clearly, the fact that both Mill Pond (MPOND) and Boomerang Lake (BLAKE) are acidic, and Decant Pond (DPOND) is neutral affects the composition of of PPCs. Metals in PPCs are plotted by location in FIGURES 5 and 6 (see also MAPS 2,3,4). D = Decant Pond; M = Mill Pond; BL = Boomerang Lake station B8; BM = Boomerang Lake station B11, BO = Boomerang Lake station B2. FIGURE 5a-d shows iron, sulphur, zinc and manganese. FIGURE 6a-d shows aluminum, phosphorus, calcium and copper.

Three patterns of element occurrence are evident in FIGURES 5 and 6. The first pattern is represented by iron and sulphur, where concentrations in the PPC are independent of location, and average 1.9% for sulphur and 19.8% for iron. There seemed to be indiscriminate accumulation of these elements. The second pattern is represented by zinc, manganese, aluminum, copper, and calcium. These elements were accumulated by PPCs probably as a result of precipitation or adsorption due to the pH of the bulk solution, or were created in the complex. For example, aluminum precipitates at pHs in excess of 3.5 as a colloid, which can attract other metals such as copper and zinc. Copper precipitates at pH 4.5, zinc above pH 6.5, and manganese above pH 7. Since the pH of Decant Pond ranges from 6.5 and 7, most of the zinc is precipitated, while only some of the manganese precipitates.

The third pattern is exemplified by phosphorus. Phosphorus, for the most part, is found in low concentrations in South Bay mine waste water, usually less than 1 mg L^{-1} (detection limit). Those PPCs with elevated concentrations (above 5000 μg gdw^{-1}) have been influenced by fertilizer applications.

The distribution pattern of the elements Zn, Cu, Al, Mn, and Ca appears to be related to pH, where metals precipitate as hydroxides outside, on the periphyton. Some of the elemental concentrations could be due to the entrapment of precipitates on surfaces of the periphyton. A significant relationship between precipitate quantity (as LOI) and iron, sulphur, aluminum and potassium was noted ($p < 0.05$). FIGURE 7 shows the correlation between iron concentration and % precipitate (of PPC). The regression lines are shown for the acid sites (Boomerang Lake and Mill Pond) and the neutral site, Decant Pond. Both regression lines indicate that clean periphyton contained between 15 and 45 mg gdw^{-1} iron. "Pure" precipitates contained between 250 and 350 mg gdw^{-1} iron. Thus, 25-35% of the precipitate, on a weight basis, was iron. These data suggest that biological polishing is governed by pH, which in turn, facilitates the precipitation of metals.

Within Boomerang Lake, at two locations, the zinc concentration (dissolved zinc; pH 3.5) was replotted as a function of percentage precipitate in FIGURE 8. PPCs for three sites, B2, B11, and B8 are plotted (see MAP 2). B2 PPCs contained the most precipitate, while the B11 PPCs generally contained less precipitate. There is little to no relationship between LOI and zinc, hence no co-precipitation.

2.4 PPC - Microscopic Examination

PPCs from Decant Pond are found as large mats which cover most of the free surface area in the pond to a depth of 2-3 cm. Free surface area in the pond also includes a large amount of artificial surfaces (inert material from building dismantling) placed there in 1987 and 1988.

PPCs from Decant Pond contain cyanophyte filaments (primarily *Oscillatoria*) and the diatom, *Navicula*. The filaments are very narrow, usually less than 10 μm in diameter, most less than 1 μm (PLATE 1). *Navicula* spp. were about 20 μm in length and 3-4 μm in diameter.

PPCs were collected from Decant Pond, preserved in Lugol's fixative, and transported to the laboratory. Material was critical-point dried, and attached to aluminum stubs. The resulting surfaces were analyzed with SEM (Scanning Electron Microscopy) and EDS (Energy

Dispersive X-ray Spectroscopy). From the photographs and scans shown below, several interesting facts emerged.

Cyanophyte filaments shown in PLATE 1 were "encrusted" with a metallic coating consisting mostly of iron, with small amounts of copper and zinc. Analysis of the coating on the algal filaments using EDS shows that weight ratios of metals in the crust were (14:1:2, respectively; FIGURE 9). Diatom frustules (PLATE 2) were also coated with iron, copper and zinc. The crust contained these metals in ratios of (14:1:2; FIGURE 10).

Precipitates in the PPC could be divided up into electron dense and electron "light" particles (PLATE 3). The electron "light" particles were composed mostly of iron, copper, zinc, and sulphur (12.5:1:1.7:1.1; FIGURE 11). A similar particle contained precipitates with the ratios (19.6:1:2.6) without sulphur. These ratios are slightly different from those based on filaments and frustules.

The electron "dense" particles contained iron, copper and zinc, but in significantly different ratios (5.8:1:3.7) with large percentages of Ag and In (22 and 28%, respectively; PLATE 3, FIGURE 12). The precipitates also contained Ga, As, and Se. Another, similar particle contained Fe:Cu:Zn ratios of 6.2:1:2. with 3% and 60% Ag and In, respectively.

Water sampled from the area contained these elements in the ratio of (25:1:116). PPCs from Decant Pond contained Fe:Cu:Zn in a ratio of 19:1:7. Since the ratios found in precipitate alone, and in the water were considerably different from the precipitates found complexed with periphyton, biological components of the complex may have modified the precipitation process.

3.0 METAL PRECIPITATION AND PERIPHYTON GROWTH RATES

3.1 Precipitate Formation

In May 1991, 5 sediment "traps" were placed at a number of locations in Boomerang Lake and Decant Pond (see MAPS 2 and 3). A sediment trap is a collection of 5 vertically-mounted tubes, 5 cm diam. and 50 cm in length (SCHEMATIC 1). The tubes are held vertically by a plate and harness, and lowered into the water to specific depths. The traps capture precipitate that is formed above the trapsand sediments in the water column. Metal precipitation rates per square meter of bottom, or per cubic meter of water can then be calculated.

Traps were located at the outfall of Boomerang Lake in 2 meters of water, at B11 in 2.5 meters of water, at B4 in 10 meters of water, at B5 in 10 m of water (MAP 2). A trap was also located along the boom in Decant Pond in 2 m of water (MAP 3).

After 39 days in June, 31 days in July and 61 days in August and September, the traps were hauled to the surface, and allowed to sit for 24 h. The water was then slowly decanted.

Precipitate on the bottom and walls of the tubes was collected, dried, and weighed. The weights were divided by the total surface area of the tubes (98.17 cm^2), multiplied by 10,000 (per m^2), and divided by the depth of the water above the traps. This compensates for the fact that less precipitate can form in shallow water than in deeper water. The calculated average precipitate deposition rate was 0.6 g m^{-3} d^{-1} in June, 0.6 g m^{-3} d^{-1} in July, and 0.2 g m^{-3} d^{-1} in August and September (FIGURE 13). At this rate, (with a lake volume of 1.04 x 10^6 m^3), 210 tonnes of sediment and precipitate are collected each year in the lake. The mass of material in the traps was not only precipitate and sediment, as algae and invertebrates were found living in the tubes. Nevertheless, the amount of material collected was considerable.

In Decant Pond, the average metal precipitation rate was 0.6 gdw m^{-3} d^{-1}. Since the volume of the pond is approximately 37,700 m^3, the calculated annual precipitate formed in Decant Pond is 8 tonnes per year.

3.2 Periphyton Growth - Boomerang Lake

The metal content of Periphyton Precipitate Complexes has been presented in the previous section. To determine the rates at which these complexes grow, spruce branches, suspended for 0, 3, and 4 years, were sampled 4 times and plastic netting was sampled 3 times over the growing season.

3.2.1 Brush PPC accumulations

In 1987, brush from the area around the South Bay mine site, primarily black spruce, was used as a substrate for periphyton growth in the seepage areas (contained by a log boom) of Boomerang Lake.

Spruce needles and bark contain polyphenolic compounds and resins that are reported to act as herbicides. Therefore, submergence time (leaching time) was tested as a factor affecting the establishment and growth of PPCs. Spruce brush was put into Boomerang Lake in 1987, 1988, and 1991. In 1987, 2600 separate brush cuttings were put into the lake in 4 locations. The two areas which received the most brush were the outfall area (B2) and the area where Mill Pond runoff enters the lake (Map 2; B11). In 1988, additional brush was tagged before adding to the outflow of Boomerang Lake (B2) thus distinguishing it from the previous years' additions. In 1991, 30 truck loads of brush cuttings were placed behind the log boom at B8. With brush/trees from three separate years in the lake in different locations, it was possible to set up a sampling program to assess growth with time of submergence and season.

FIGURE 14 shows the weight of PPCs found on spruce branches with different submergence times. These data show that there is no clear relationship between long-term submergence time and PPC growth. In fact, the July PPC samplings showed the reverse trend; the most recently submerged trees had the highest PPC weights (FIGURE 14).

PPC samplings have been made one to four times a year since brush was first put into the lake in 1987. During that initial year, PPC growth was slow, weights did not exceed 0.3 gdw gdw^{-1} of branch (FIGURE 15). In the second year, weights were highest on the first sampling of spring (0.58 and 0.93 gdw gdw^{-1}), although samples taken in August were also high. In the third year of submergence samples were taken only in May and August. Again, August samples displayed the greatest weight (0.65 gdw gdw^{-1}). Only one sample was taken in 1990, the fourth year. The same trend was noticed in the fifth year, where growth of the PPCs clearly displayed the seasonality of periphyton blooms, producing highest weights in spring and fall. Growth after 5 years was similar to that after 2-3 years.

3.2.2 Peritraps

To determine the effects of fertilization on growth, toquantify the mass of sloughed material, and to obtain standardized seasonal growth rates for PPCs, periphyton "traps" were constructed. "Peritraps" (*in situ* periphyton growth/detritus system) are a combination of artificial and natural substrates, with a bag to catch detritus. The traps consist of a polypropylene net over a wooden frame (net) and a "spruce branch" within the net. The net structure is tied to a frame which supports a bag. The whole unit is then submerged in the waste water (SCHEMATIC 2; PLATE 4).

Traps were placed in Boomerang Lake at B2, B11, and in Decant Pond (MAPS 2,3). A modified version was also used in Mill Pond. Some of the traps contained cleaned spruce branches from 4-yr submerged, 3-yr submerged, 0-yr submerged spruce trees. Others contained 4-yr spruce branches and slow-release calcium phosphate, or 19:6:12 N:P:K fertilizer in the bag. Others, were kept empty of substrates and used as controls. One set was placed around station B2, and the other around station B11.

Growth should not be affected by location in Boomerang Lake unless metal precipitation processes are significantly different in the two locations. FIGURE 16 plots trap net PPC growth for identical treatments at both B2 and B11. Although there is some scatter, identical treatments gave similar weights.

The second hypothesis was that short-term effects of submergence time might be more noticeable than long-term effects. Data for the 3 growth periods measured in the summer (June, July, and August-September) indicated that different "natural" treatments, i.e. substrate submersion time (4 yr, 3 yr, 0 yr), did not significantly influence production on adjacent nets (FIGURE 17a). This was consistent with data from branch PPCs which indicated that submersion time was not consistently correlated with growth (FIGURE 14).

Nutrients in Boomerang Lake are low (NO_3 - < 0.02 mg L^{-1} with Hach test; < 0.1 mg L^{-1} PO_4 Hach test), therefore growth of PPCs in Boomerang Lake should have been limited by nutrients. However, since PPC "blooms" occur in May-June at the time of lake turnover, little difference was expected between fertilized and unfertilized traps during this period, and no significant difference in growth rates between fertilized and unfertilized traps was found. In July, growth rates were improved with added phosphate, but not NPK fertilizer (FIG 17b). In August-September, PPC growth with NPK fertilizer was considerably higher than growth without fertilizer, and with phosphate alone (FIG 17b).

Given the rather consistent weights of PPCs on branches of 0.4 to 0.6 g $branch^{-1}$ d^{-1} a significant proportion must have sloughed from the branches. The extent of sloughing was quantified using peritraps. The bags were emptied on each sampling date, providing an estimate of amount of mass contributed by sloughed PPCs (FIGURE 18). Sloughed PPC mass in Boomerang Lake was low in June, contributing only about 17% to the total PPC weight produced. In July, the percentage increased to about 53% of the total. The percentage contribution fell back to about 34% in August-September. For traps which were left submerged for the whole summer, sloughing contributed about 50% of the total biomass, and also the greatest weight of material. In Decant Pond sloughing rates were much lower, being 0% in June, 2.7% in July, and 2.1% of PPC mass in August-September. Lower sloughing rates in Decant Pond are consistent with growth habit of the cyanophyte populations in the pond, as contrasted with the chlorophyte populations in Boomerang Lake. Thus, sloughed biomass in Boomerang Lake was significant, contributing as much as 50% to the biological polishing capacity estimated, based on branches or netting, while it was insignificant in Decant Pond.

To determine differences in biological polishing capacity in different mine waters, PPC growth rates were compared between Boomerang Lake (B2 and B11) and Decant Pond and two gloryholes in Newfoundland (OWP; pH 3.5, Zn 31 mg L^{-1} and OEP; pH 6.5, Zn 25 mg L^{-1}). Traps were also put in pools with flow control (PP1; pH 7, Zn 5-15 mg L^{-1}). In this case, precipitate corrections to the PPC mass change were necessary to compensate for the different amounts and kinds of precipitates found complexed to different populations. These corrected data are presented in FIGURE 19. Comparing the different locations seasonally suggests that seasonal effects are much sharper in Buchans than in South Bay. Secondly, growth in the acid OWP water was considerably lower than growth in acidic Boomerang Lake, indicating that acidities may be playing a role (OWP 165 mg L^{-1} $CaCO_3$ vs. 87 mg L^{-1} $CaCO_3$, respectively).

3.3 Periphyton Growth - Mill Pond

Periphyton populations (as PPCs) grow well in Mill Pond. A dense population of *Ulothrix* grows well in the outflow area of the pond, forminmg floating mats. Filamentous strands of algae rise from the pond bottom in a number of places forming a surface canopy. The bottom of the pond at the outflow is covered in sawdust and straw, with a coating of metal precipitate. Zinc concentrations vary between 150 and 450 mg L^{-1} (FIGURE 4), copper concentrations vary between 10 and 20 mg L^{-1}.

Several methods were used to quantify PPC growth in Mill Pond. Because the pond was so shallow, especially at the outflow (<0.5 m), peritraps, as used in Boomerang Lake and Decant Pond could not be used. Several other methods using modified trap designs were tried.

The first method used was the quadrat. This consisted of marking out a square on the pond bottom with 4 permanent stakes the size of a peritrap netting frame, setting up four permanent areas of natural pond bottom. The quadrats allowed free movement of water and PPCs into and out of the area (See MAP 3).

The second method that was tried was the limnocorral. This was simply the peritrap bag frame and bag with the bottom cut out. The limnocorral enclosed a volume of pond water which was not exchanged with adjacent areas. The top of the limnocorral was open, allowing light penetration. Six limnocorrals were set up in the outfall end of the pond (See MAP 3).

The third method was the periplate. This consisted of a square plexiglas plate (23x23 cm^2), on which rough waterproof sandpaper (#60) had been glued. The six plates sat above the bottom slightly, providing a roughened surface which was open to outside colonization and transport.

Osmocote, slow-release 19:6:12 fertilizer, and Sierra custom blend PO4 slow-release fertilizer were added to the pond downstream from non-fertilized periplates and quadrats. Periplates and quadrats in these areas quantified the effects of the fertilizer applications (See MAP 3).

Quadrats and periplates produced the highest PPC masses. Both of these methods allowed transport of PPCs from adjacent areas, and may thus have imported more PPCs than actually grew there. Higher growth rates in quadrats could also be due to the high relief bottom surface in the pond which is covered with sawdust and straw. Because of the high metal precipitates in the area, growth estimates were based on only the periphyton component of the PPC. Corrections, notwithstanding, periphyton growth rate estimates were probably high. Rates as high as 3.7 gdw (precipitate corr.) m^{-2} day^{-1} were calculated. These were 4-5 times higher than recorded at other sites (FIGURE 20).

Limnocorrals gave the lowest growth estimates. This was due to the configuration of these system. Just as quadrats and plates allowed transport of PPCs from adjacent areas, limnocorrals sealed in a fixed water volume, allowing only PPCs which were actually present to grow. Since limnocorrals were cleaned on each trip, very little biomass remained on which to base growth, producing a low growth estimate. Rates measured by this technique varied between 0.01 and 1.5 gdw (precipitate corr.) m^{-2} d^{-1}. With the exception of the first growth measurement (1.5 gdw (precipitate corr.) m^{-2} d^{-1}) all were 0.01-0.02 gdw (precipitate corr.) m^{-2} d^{-1}. These rates are 10-100 x lower than those rates recorded at other sites (FIGURE 20).

PPC growth estimates in Mill Pond were 3-4 x higher than either Decant Pond or Boomerang Lake. Possible explanations include warm temperatures in the shallow water, high bottom relief, spread of downstream nutrients, and the data bias for growth methods which allow import of standing biomass.

PPCs at the downstream end of the outfall area (MAP 3) responded well to slow-release fertilizers. Both phosphate and NPK fertilizers increased PPC growth on periplates near the weir. PPCs in fertilized areas were a dark, grass green, more characteristic of clean algae than PPCs. Those in unfertilized areas were a golden brown, suggesting a larger precipitate component, and a more stressed condition. Assay results indicate that periphyton from fertilized areas had demonstrably more phosphorus (4,500 vs 400 $\mu g\ gdw^{-1}$), and in one case more zinc.

3.4 Periphyton Growth - Decant Pond

The periphyton in Decant Pond are of two distinct communities. A *Ulothrix* community dominates the western shore, where acid, leaching from the tailings, produces lower pH. Toward the middle of the pond, the pHs are circum-neutral, and a cyanobacterial community dominates, forming very dense mats. The mats contain only about 25-30% algae, the rest being solids, primarily precipitates (See Section 2.4).

Several methods were used to quanitify periphyton mat production in Decant Pond. The first was peritraps. Decant Pond is deep enough to allow their use. Four traps were used, two for fertilizer, one for natural recruitment, and one control (without substrate, MAP 4).

The second method involved scraping a known area off the surface of wooden 6x6s (in) on each of two rafts still floating in the pond. One raft was located near the centre of the pond in an area with a pH of 5.7. The other raft was near the outflow and log boom (MAP 4).

Periplates were installed on both rafts, one at the centre and one at the boom, 6 plates in total, 3 at each site. The three at the centre raft disappeared in summer storms between July and September visits.

Rope lines were also hung from the rafts. Earlier quantifications indicated that rope was a good growth substrate. Both cotton and polypropylene rope were used. Short (2m) segments were hung off the rafts and weighted at the lower end.

The results using the different substrates to quantify PPC growth are shown in FIGURE 21. Scrapings and suspended peritraps produced similar and high results, with periplates giving the lowest growth estimates. It was noted that PPC growth on periplates was initially quite slow, whereas rope was colonized almost immediately, with cotton rope giving even better results than polypropylene rope.

Rope lines were a surface which was readily colonized, but because the rafts were not anchored, they kept fouling with other lines, boards, etc. In several cases, periphyton seemed to have been stripped off whole sides of the rope. These data are thus underestimates.

Results indicate that growth in June was better than in either May or Aug-Sep. This may be explained by the observation of a massive cyanobacterial bloom occurred during the month of May, reducing the spring nutrient level in Decant Pond. Phosphorus levels at the pond outflow were below detection limit (< 0.1 mg L^{-1}) in April and May, climbing to 0.2 mg L^{-1} by the end of June. This bloom would necessarily have competed for nutrients with periphyton on traps, rafts and plates.

The highest growth rates recorded for each time period were made by periphyton growing in the presence of slow-release fertilizer (see F Net data in FIGURE 21). Not only were growth rates high, but P levels in the periphyton were also significantly higher (2,671 vs. 728 μg gdw^{-1}; FIGURE 22). In this case, peritrap 40 contained slow-release phosphate, peritrap 41 contained slow-release 19:6:12 fertilizer, and peritrap 42 contained no added fertilizer.

4.0 EXTRACELLULAR CARBON PRODUCTION

Algae release a large number of organic compounds into the water. These soluble compounds are mainly carbohydrates, fatty acids, amino acids, vitamins and enzymes (Fogg, 1971). Of those extracellular products, the organic carbon is the largest fraction. Extracellular organic carbon can be divided into two groups. The first group are the soluble carbohydrates (DOC), which are low molecular weight, and which are related directly to photosynthesis. The second group are surface-bound polysaccharides, which form sheaths, or jelly masses around algal cells and filaments. Their origin is not as easily determined.

Extracellular carbohydrates have been shown to be able to bind metals through cation exchange. For example, Strong, et al. (1982) found that carbohydrates can bind 0.26 mg Zn and 0.24 mg Cu per mg of carbohydrate. These carbohydrates can be produced in copious quantities as shown by Van den Berg et al. (1979) who reported that three species of algae produced 6.73, 2.86 and 0.66 umol L^{-1} of complexing ligands. The average rate of DOC production in freshwater is less than 20% of photosynthetic production (Wetzel 1983).

In general, production of extracellular products is proportional to algal biomass production. The higher the algal biomass, the more numerous the cellular carbohydrate production sites and the more extracellular materials produced.

The objectives of this research were to determine natural levels of external carbohydrate production. The research was carried out in both field and laboratory studies. The initial method used for measuring carbohydrate utilized the phenol-sulphuric acid test described by Kochert (1978).

To get an initial idea of the quantities of DOC produced by algae in waste water at South Bay, a field colorimetric test was modified from the method of Kochert (1978). The field modifications required making up a set of fresh glucose standards each day of the field trip. Color comparisons were made by eye, through interpolation between fixed standards.

Field measurements of dissolved carbohydrates varied considerably among the different locations (TABLE 2). In Mill Pond, for example, carbohydrates were measured in three different areas. In the middle of the pond, concentrations were low, < 0.01 g L^{-1}. In the outflow area of Mill Pond, among the PPCs, the concentration was higher, appx. 0.03 g L^{-1}. Water also passes through a layer of sawdust on the bottom of the pond and leaks beneath the weir. This water contained the highest concentration of dissolved carbohydrates, >0.08 g L^{-1}. Concentrations of dissolved carbohydrates in Boomerang Lake were likewise low, <0.01 g L-1.

PPC samples from Mill Pond of about 1 gfw were incubated in 20 mL vials for 5 hours, in situ (TABLE 2). Dissolved carbohydrate production by fertilized PPCs during that time amounted to >0.08 g L^{-1}, while carbohydrate production by "yellow" unfertilized algae was approx. 0.07 g L^{-1}. The conclusion is that extracellular dissolved carbohydrates are present in waste water at the South Bay mine site. These "glucose-equivalent" carbohydrates are due to the presence of periphyton in these systems. Thus, the concentration is extremely low in areas with out periphyton, such as the main part of Mill Pond and Boomerang Lake. As periphyton population density increases, the concentrations become greater.

5.0 SUMMARY AND CONCLUSIONS

The primary goal of this project was to quantify interactions between periphyton and precipitates, measuring growth, precipitate sedimentation rates, and quantitatively describing the physical interaction between periphyton and precipitates. Periphyton and metal precipitates have been found occurring together at many mine sites in Canada. These Periphyton Precipitate Complexes or PPCs grow under a range of physical and chemical conditions, in mining waste waters.

South Bay has three major waste water bodies with significant amounts of periphyton. Boomerang Lake contains extensive populations of *Ulothrix*-PPCs. Decant Pond contains thick mats of *Oscillatoria*-PPCs. Mill Pond harbors a large population of *Ulothrix*-PPCs. Major conclusions about PPCs and their environment are summarized below.

- PPCs can be fractionated into periphyton and precipitate using Loss On Ignition data. Some PPCs contain over 90% precipitate, others are nearly 100% periphyton.

- Using LOI as a means of correcting PPCs for the amount of precipitate present, it was calculated that clean periphyton would contain between 15 and 45 mg gdw^{-1} iron. "Pure" precipitate could contain between 250 and 350 mg gdw^{-1} iron. Thus, 25-35% of the precipitate, on a weight basis, would be iron.

- Zinc accumulations are as high as 7% in some Decant Pond PPCs, but less than 0.1% in Boomerang Lake and Mill Pond. In this case, zinc concentration in PPCs appears to be related to the pH of the waste water.

- Scanning Electron Microscopy (SEM) and Electron Dispersive Spectroscopy (EDS) observations on Decant Pond PPCs indicate that some of the metal accumulations are directly on periphyton surfaces, as a plaque. Major elements in the plaque are iron, copper and zinc. SEM and EDS analyses of precipitate particles also suggest two types of precipitates, low density and high density. The low density particles are composed mainly of iron, copper, zinc and sulphur. The dense particles contain high concentrations of indium, silver, and gallium.

- Precipitation rates (sedimentation rates) in Boomerang Lake were calculated based on sediment trap data. Rates averaged about 0.4 gdw per cubic meter (or 1.6 gdw m^{-2} d^{-1}) of lake per day. Since the average growth of PPCs in Boomerang Lake was 2.0 gdw m^{-2} d^{-1}, approximately 80% of the PPC growth was attributed to "sieving" sedimented precipitate from the lake water. Based on the LOI correction, Boomerang Lake periphyton were, on average, 35% of the PPC. In Decant Pond sedimentation rates were, on average, 0.5 g m^{-2} d^{-1}. PPC growth rates over the same time period averaged 1.4 gdw m-2 d^{-1}. This indicates that periphyton should be about 33% of the PPC. Precipitate-correction of Decant Pond PPCs suggests that this percentage should be about 30%. Thus, alarge fraction of the precipitates complexed with PPCs is simply "biosieved". Part of the interaction between PPCs and waste water is thus directed by geochemical conditions which allow precipitation to take place.

- Metal precipitates, together with natural periphyton communities, form PPCs in Boomerang Lake which grow on brush, grass, and any "surface" in the water. Growth is dependent on the submergence time for some substrates. After 1 year of submergence spruce branches produced about 0.3 gdw PPCs gdw $branch^{-1}$. After 2 years weights climbed to 0.9 gdw PPCs gdw^{-1}. After 3 years, peaks of 1.2 gdw gdw $branch^{-1}$ were recorded. However, at any given time of year, growth of PPCs from different-aged substrates were quite variable, and did not correspond to substrate age. Sloughing of PPCs on brush and peritraps in Boomerang Lake was substantial, ranging between 17% and 50% of the PPCs formed. Sloughing in Decant Pond, however, was minimal, suggesting that the growth form of cyanophyte-based PPCs is quite different from *Ulothirix*-based PPCs.

- PPCs from Mill Pond produce extracellular carbohydrates in large concentrations. Extracellular carbohydrates, such as polysaccharides, are responsible in other algae for sequestration of metals and protection from high metal concentrations.

6.0 LITERATURE

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FIG. 1: PPTE vs LOI
Theoretical

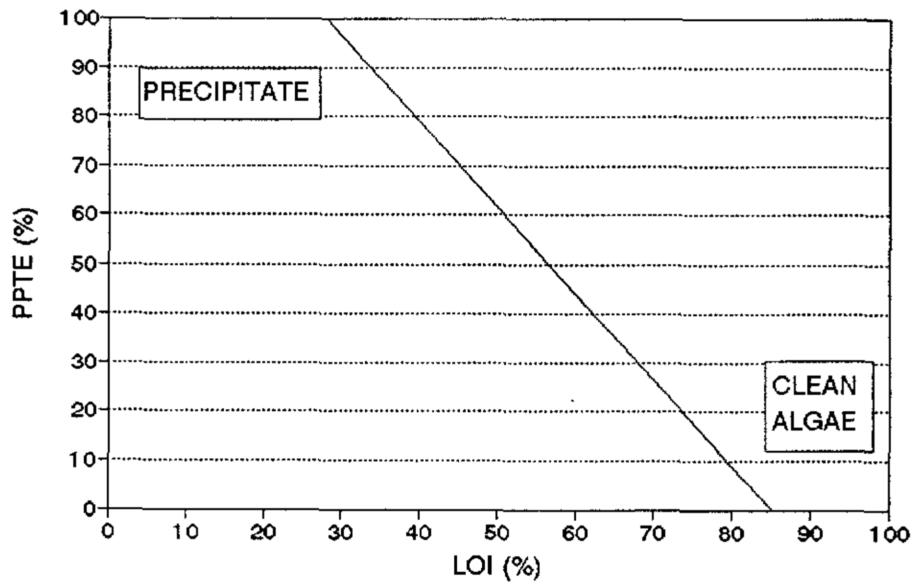


FIG. 2: Periphyton Zn Concentration

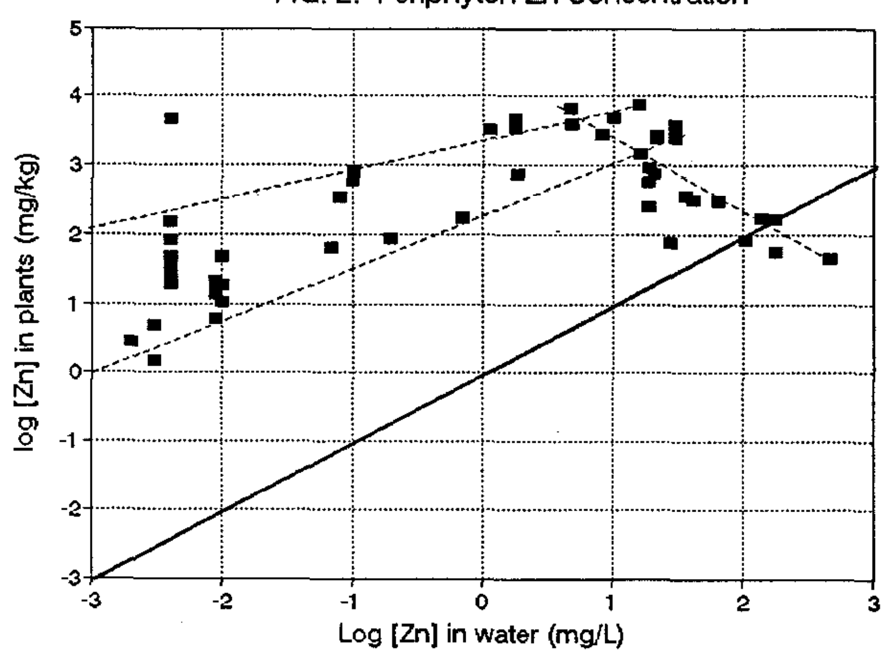


FIG. 3: Periphyton Al Concentration

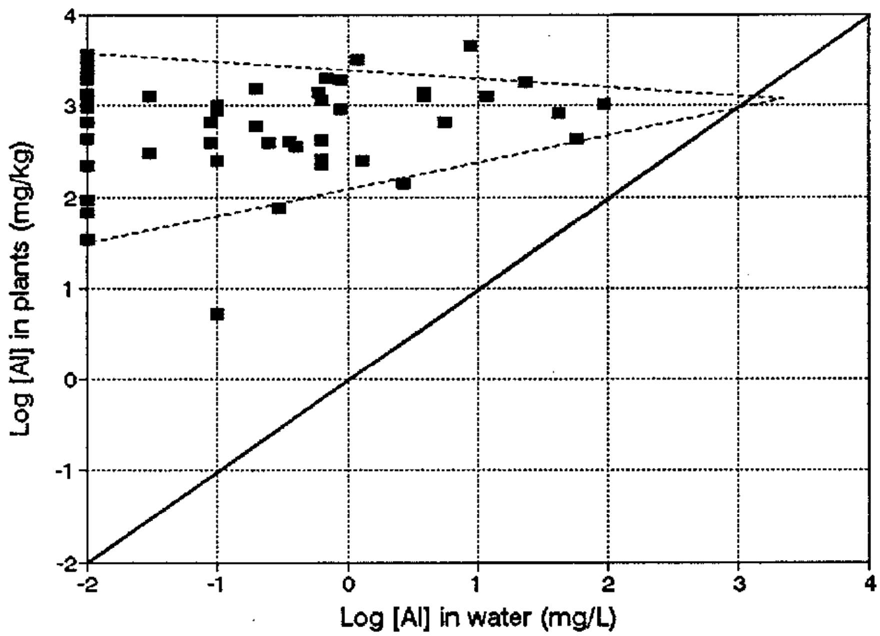


FIG. 4: Ulothrix-PPC and Water Elemental Scans

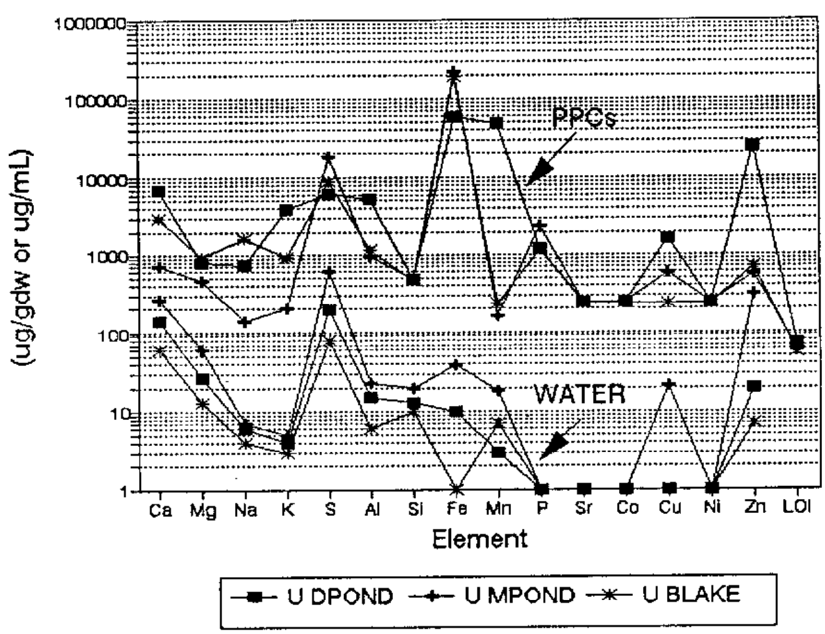
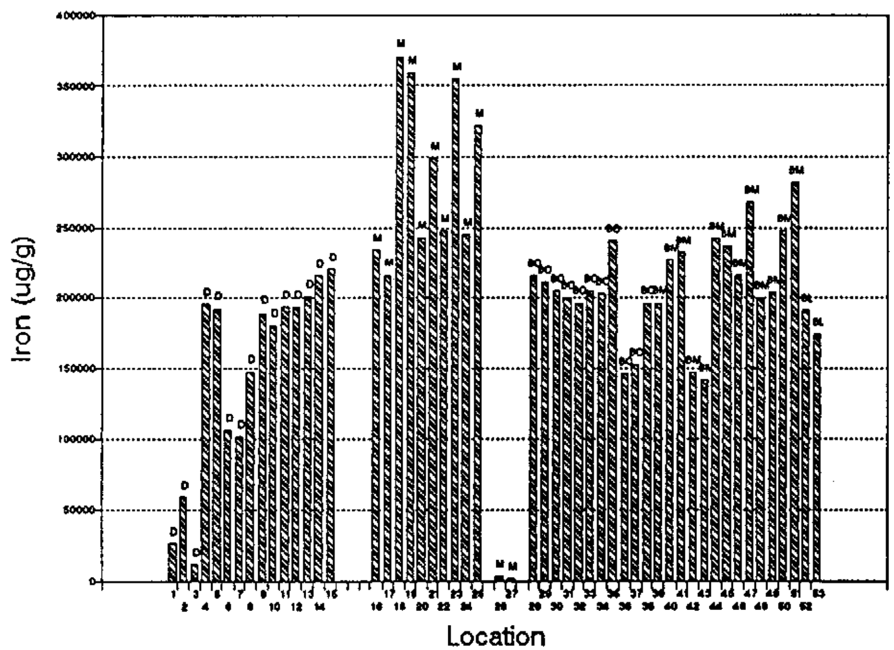
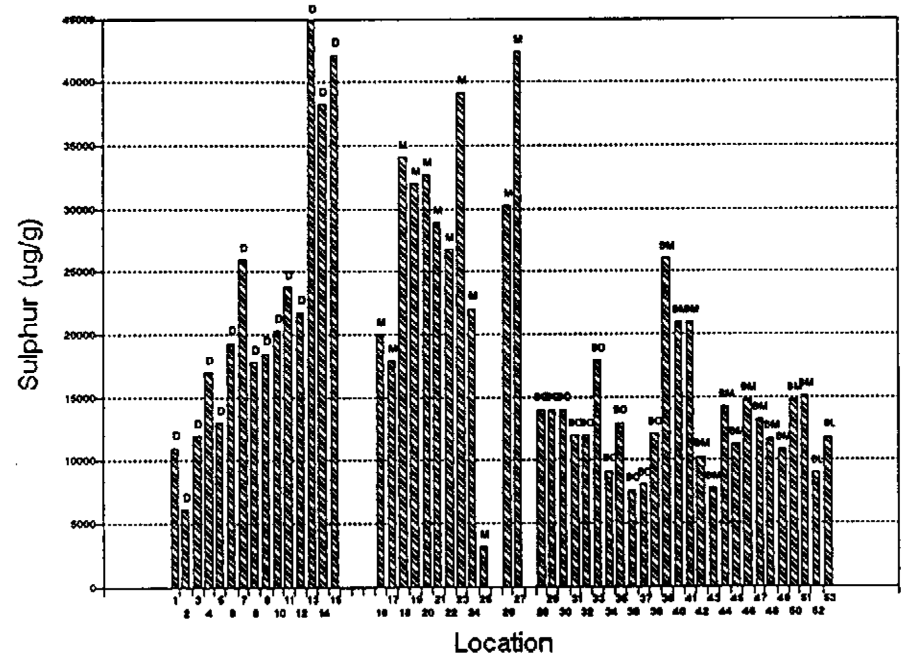


FIGURE 5

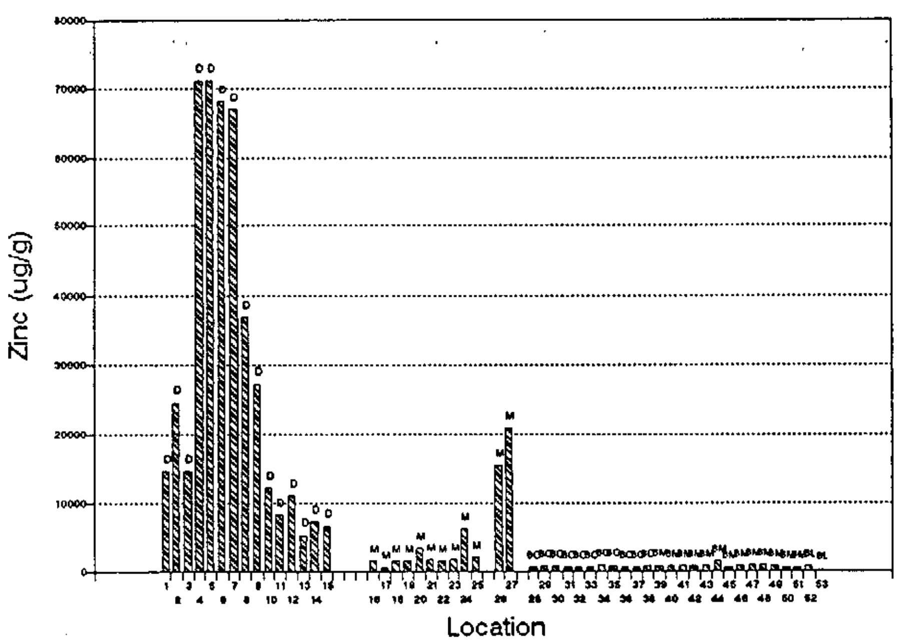
Metal Scan - Iron
South Bay



Metal Scan - Sulphur
South Bay



Metal Scan - Zinc
South Bay



Metal Scan - Manganese
South Bay

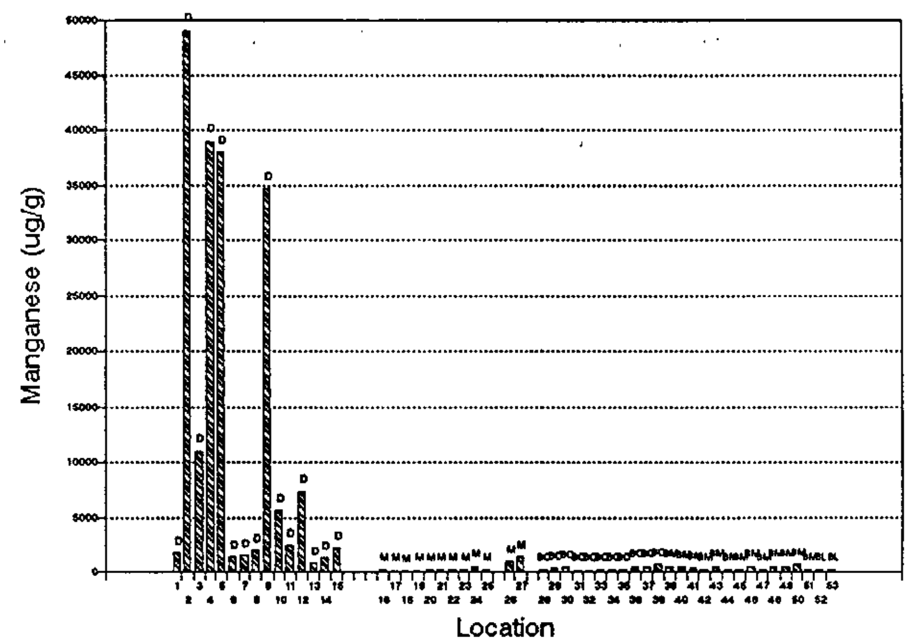


FIGURE 6

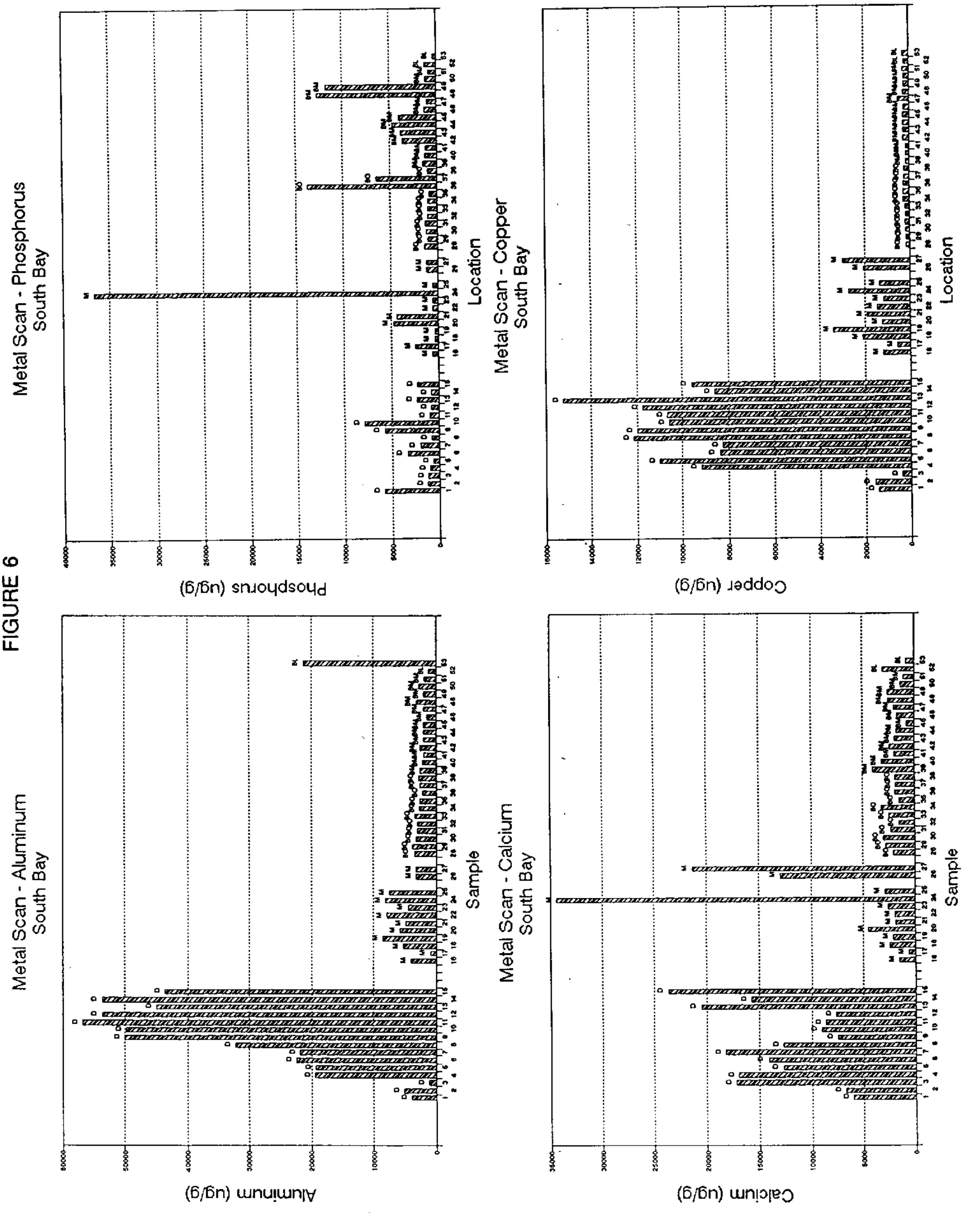

Metal Scan - Aluminum
South Bay
Aluminum (ug/g)
Sample
Metal Scan - Phosphorus
South Bay
Phosphorus (ug/g)
Location
Metal Scan - Calcium
South Bay
Calcium (ug/g)
Sample
Metal Scan - Copper
South Bay
Copper (ug/g)
Location

FIG 7: Precipitate vs. Iron
S. Bay

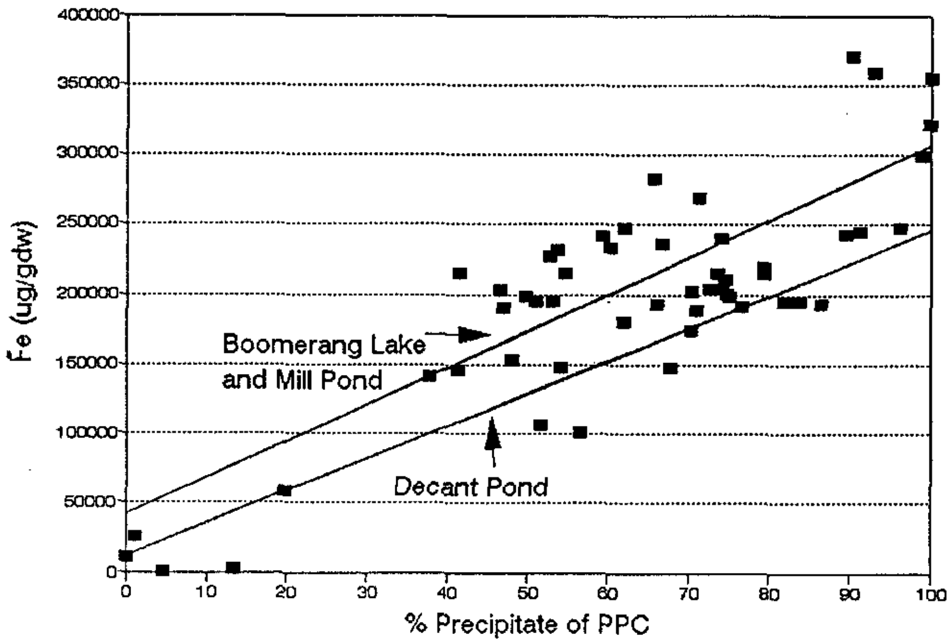
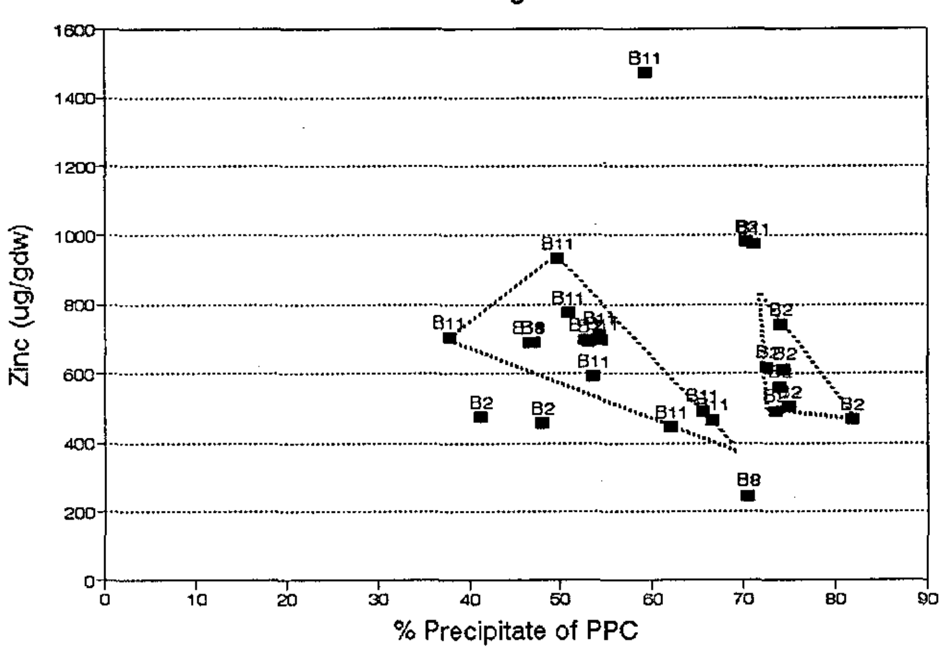
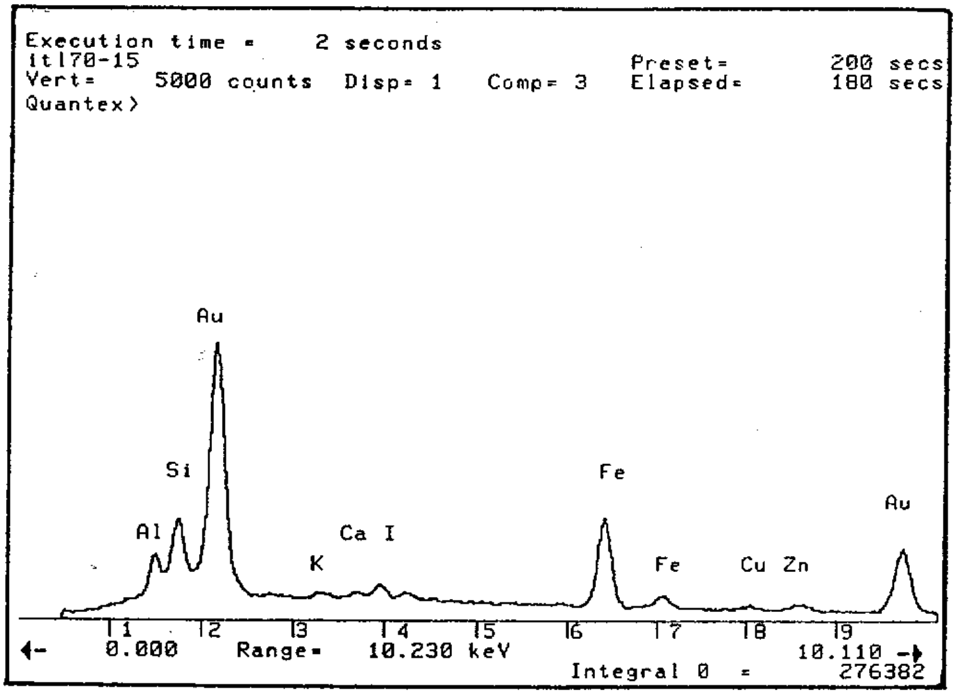


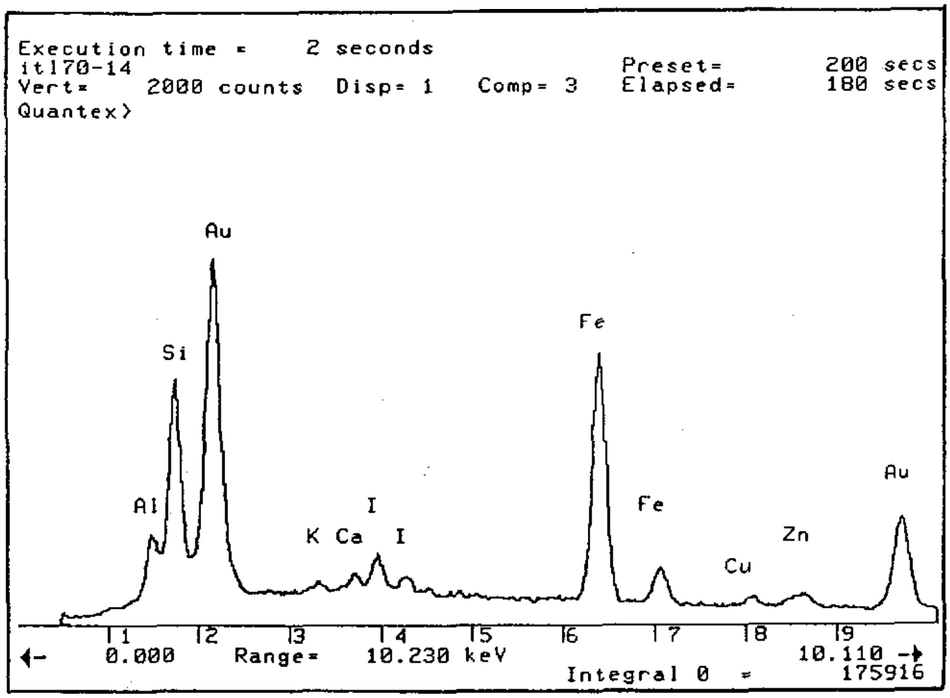
FIG. 8: Precipitate vs. Zinc
Boomerang Lake





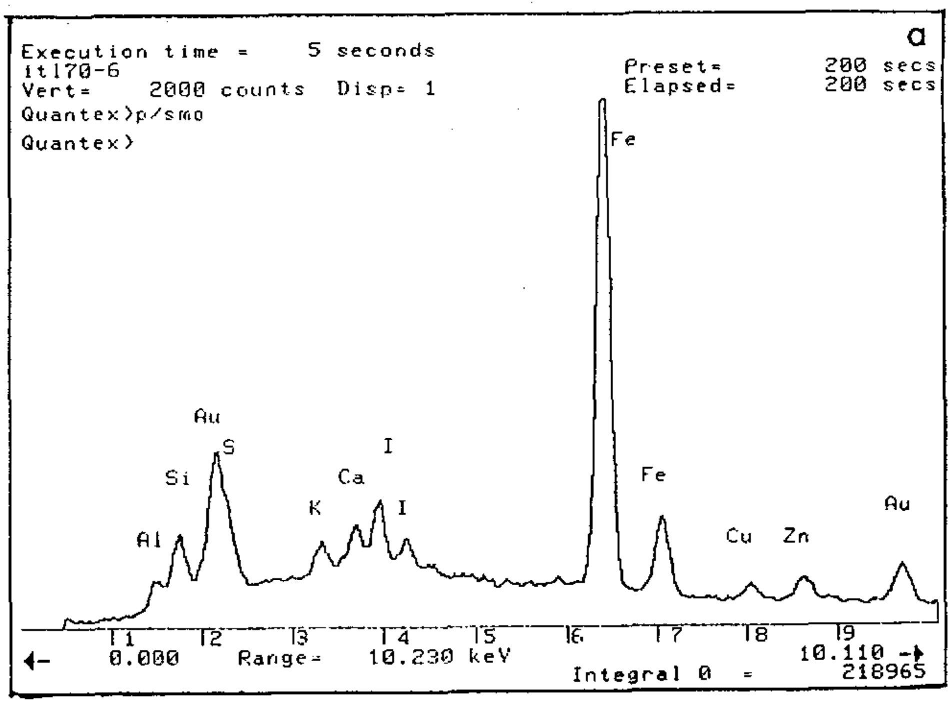
ELEMENT & LINE	WEIGHT PERCENT	ATOMIC PERCENT*
Si KA	28.08	43.79
K KA	1.39	1.56
Ca KA	2.38	2.60
Fe KA	55.29	43.35
Cu KA	4.52	3.11
Zn KA	8.34	5.59

FIG. 9: Energy Dispersive X-Ray analysis of an *Oscillatoria* filament in the Decant Pond PPC shown in Plate 1.



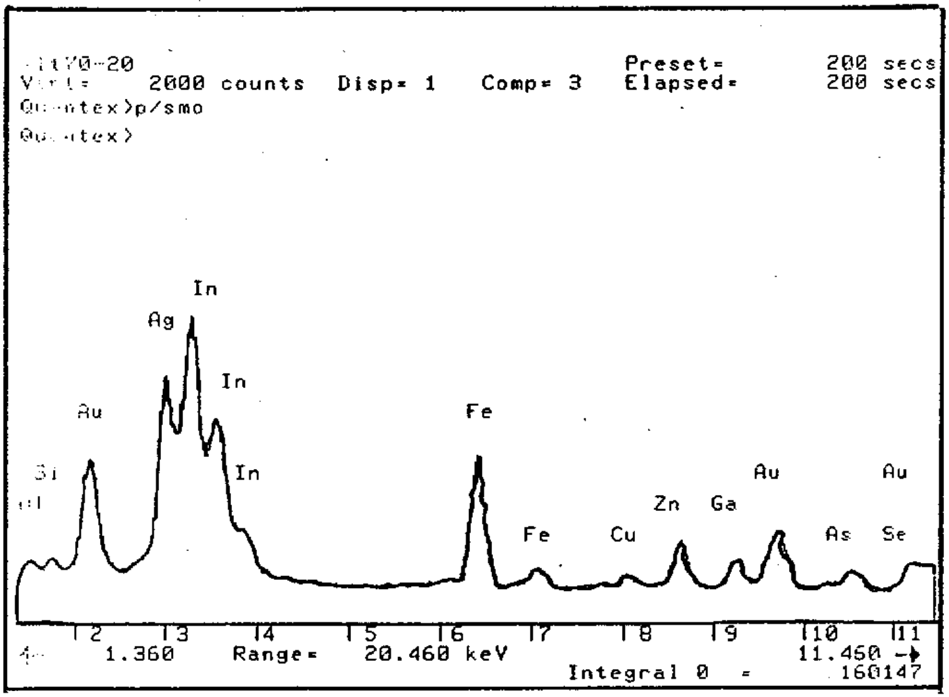
ELEMENT & LINE	WEIGHT PERCENT	ATOMIC PERCENT*
Si KA	30.22	46.47
K KA	0.85	0.93
Ca KA	1.88	2.02
Fe KA	55.14	42.63
Cu KA	3.97	2.69
Zn KA	7.95	5.25

FIG. 10: Energy Dispersive X-Ray analysis of a *Navicula* frustule in the Decant Pond PPC shown in Plate 2.



ELEMENT & LINE	WEIGHT PERCENT	ATOMIC PERCENT*
Si KA	2.86	5.41
S KA	3.52	5.83
K KA	1.83	2.48
Ca KA	3.04	4.04
Fe KA	71.10	67.71
Cu KA	6.45	5.40
Zn KA	11.21	9.12

FIG. 11: Energy Dispersive X-Ray analysis of low-density precipitate in Decant Pond PPC.



ELEMENT & LINE	WEIGHT PERCENT	ATOMIC PERCENT*
Al KA	6.40	16.98
Si KA	3.23	8.24
Fe KA	9.54	12.23
Cu KA	1.65	1.86
Zn KA	6.18	6.78
Ga KA	5.32	5.47
As KA	6.51	6.22
Se KA	11.21	10.17
Ag LA	21.76	14.45
In LA	28.21	17.60

FIG. 12: Energy Dispersive X-Ray analysis of a high-density precipitate in Decant Pond PPC.

FIG. 13: Sedimentation Rates
Boomerang Lake, Decant Pond

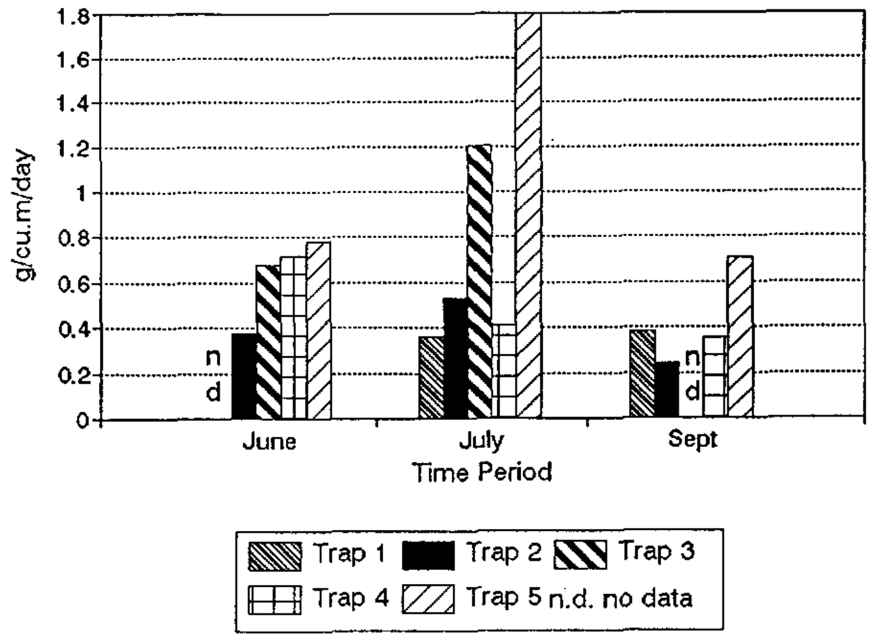


FIG. 14: PPC Densities on Brush
Boomerang Lake, 1991

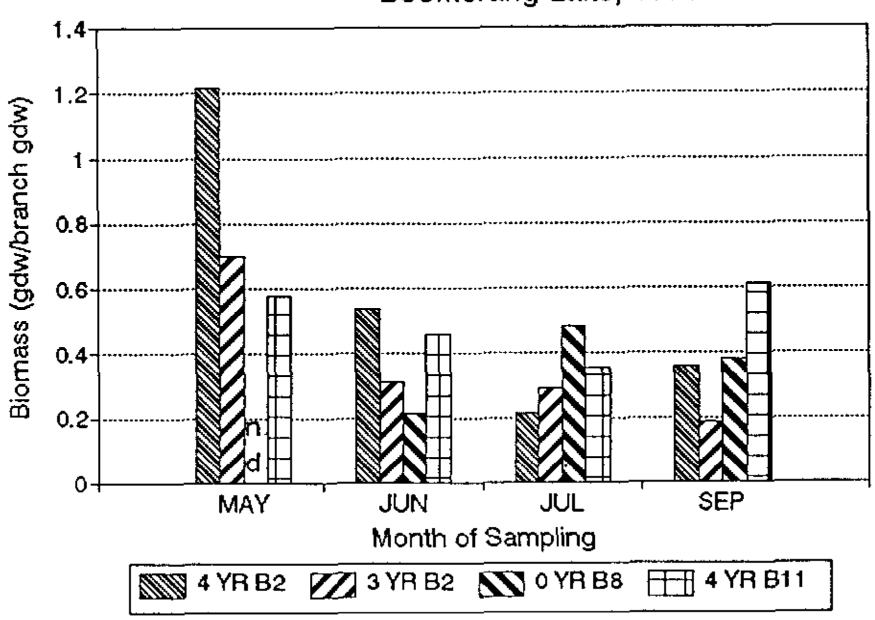


FIG. 15: PPC Densities on Brush
Boomerang Lake, Historical

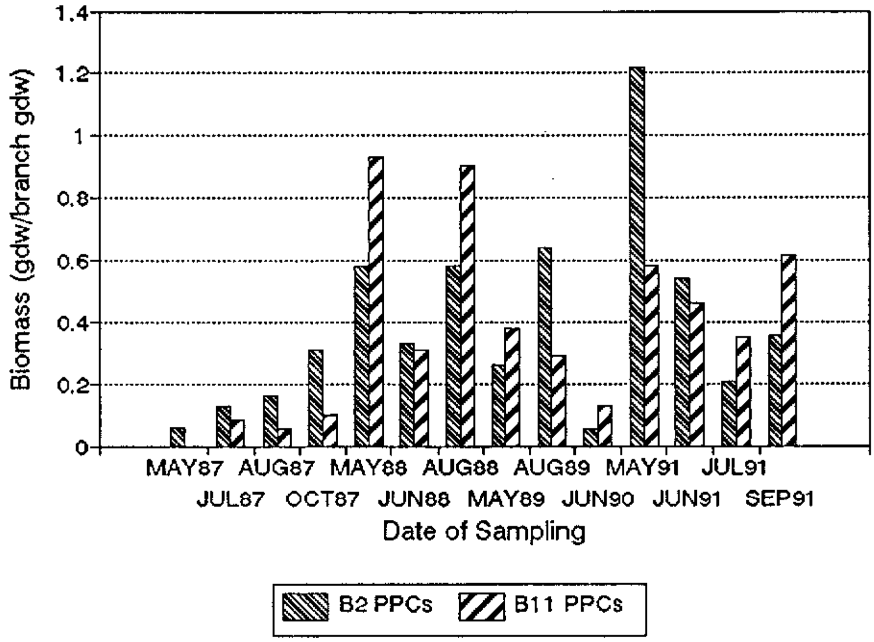


FIG 16: Netting PPC Growth
Stn B2 vs. B11, 1991

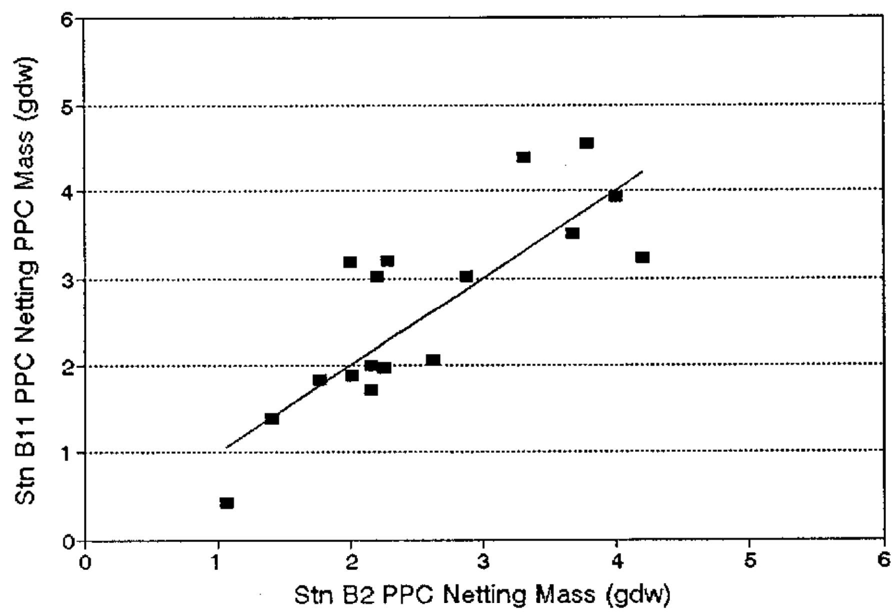


FIG 17a: Submergence Time vs. Growth
Boomerang Lake

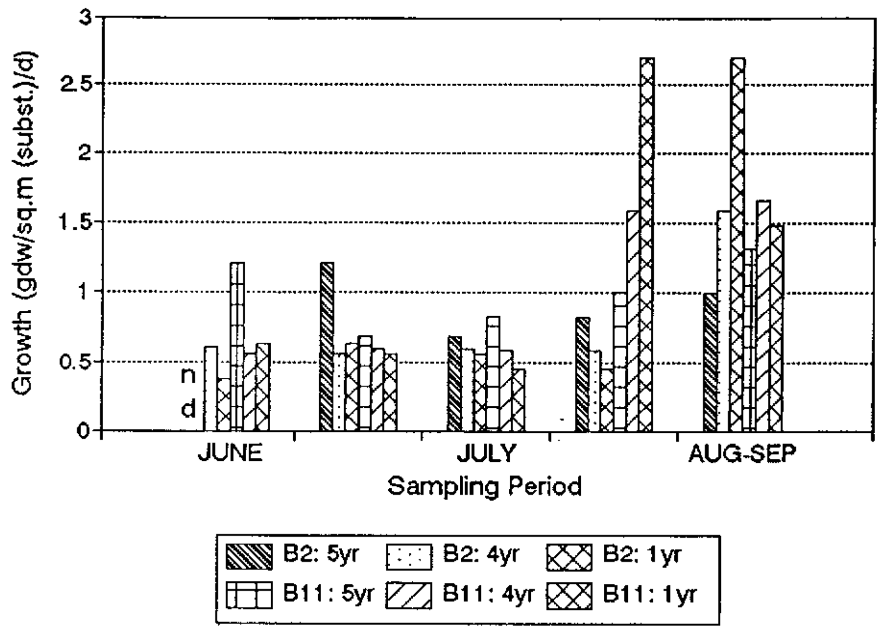


FIG 17b: Fertilizer Effects on Traps
Boomerang Lake

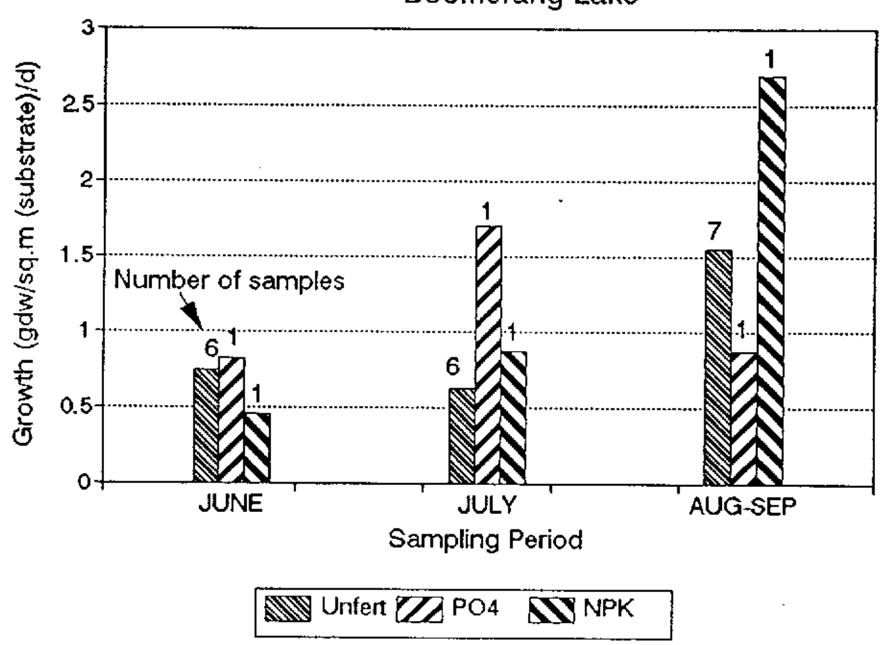


FIG 18: PPC Trap vs. Bag Weights
Boomerang Lake, 1991

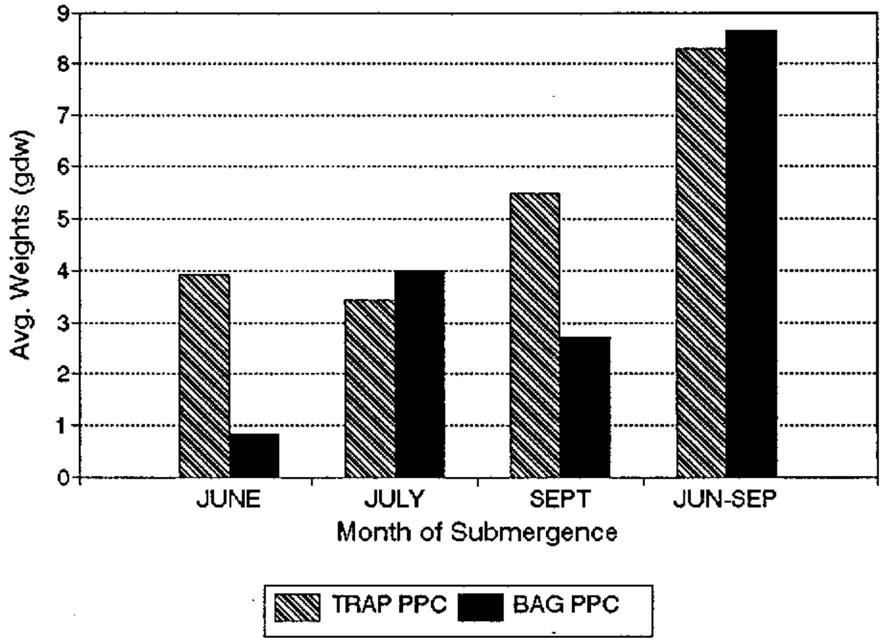


FIG. 19: Netting Periphyton Growth
Inter-Mine Site Comparisons

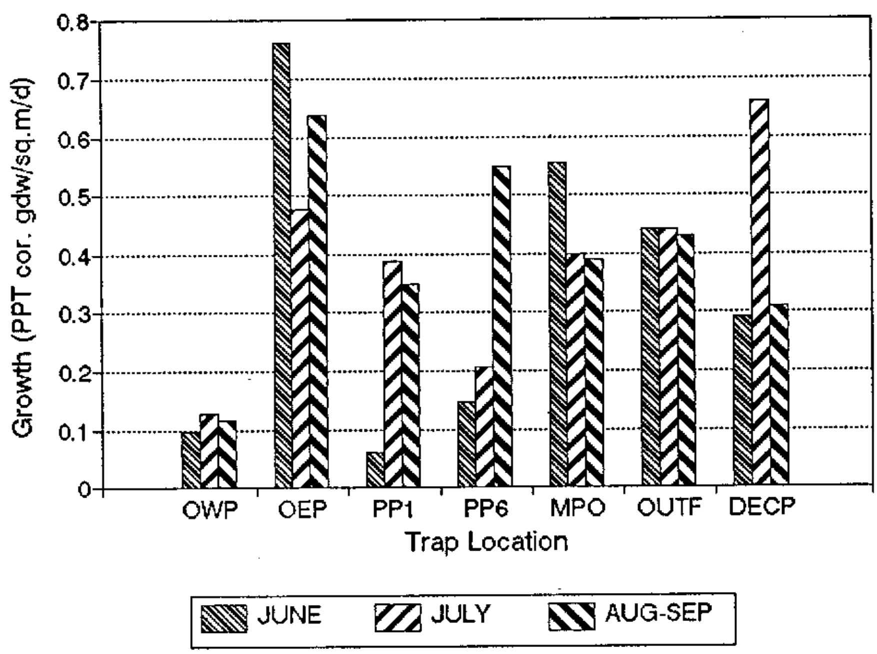


FIG. 20: Mill Pond Periphyton Growth Treatments, 1991

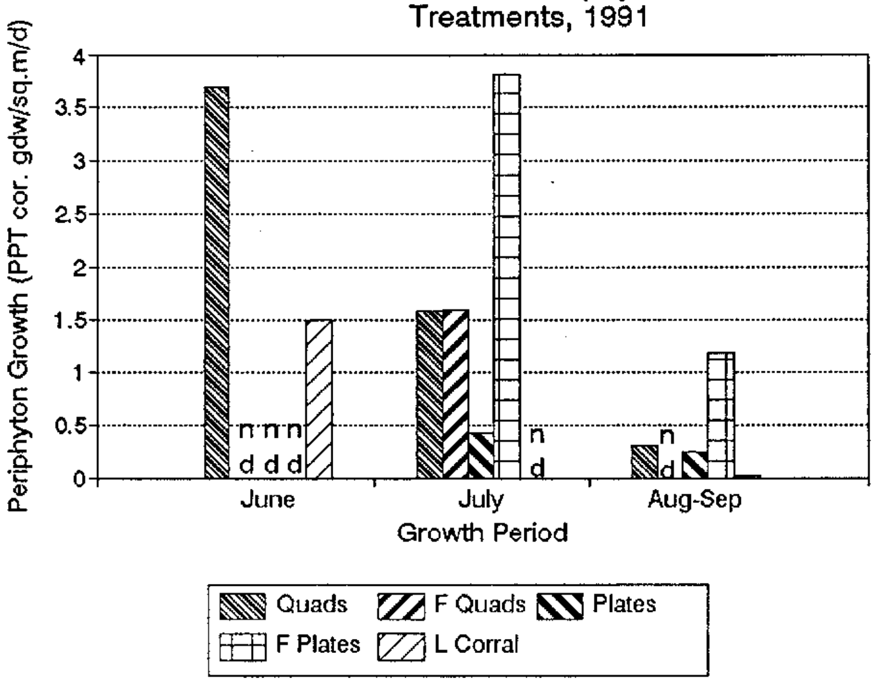


FIG. 21: Decant Pond Periphyton Growth Treatments, 1991

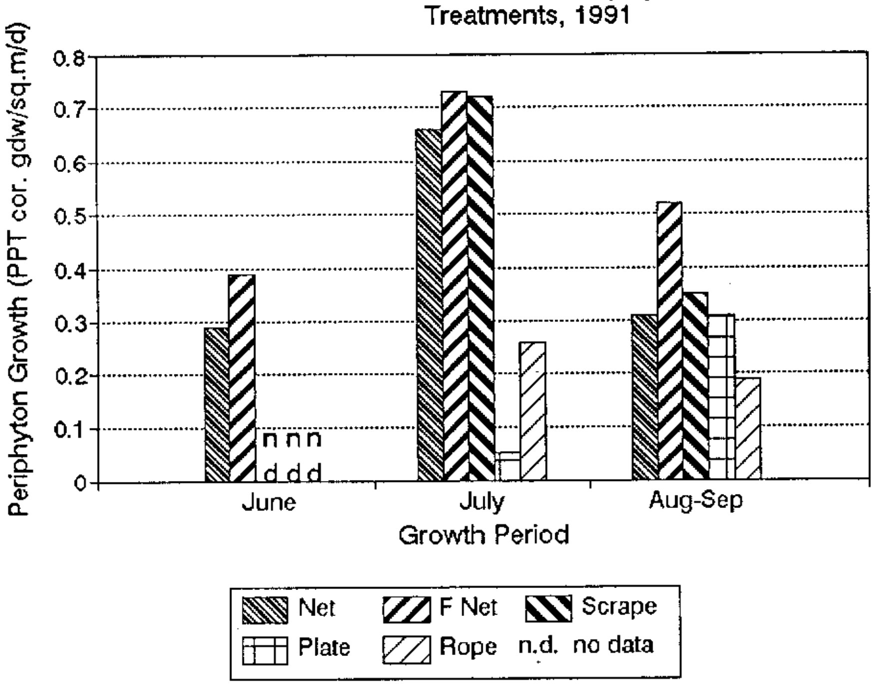


FIG. 22: Decant Pond PPCs and Water
Elemental Scans

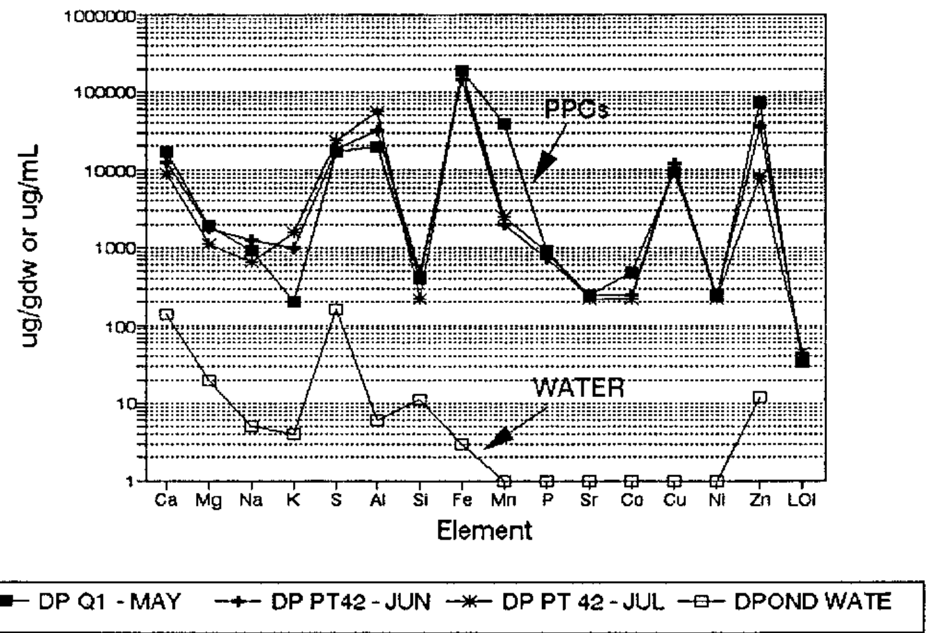


TABLE 1: Description of Waste Water Sites with Periphyton

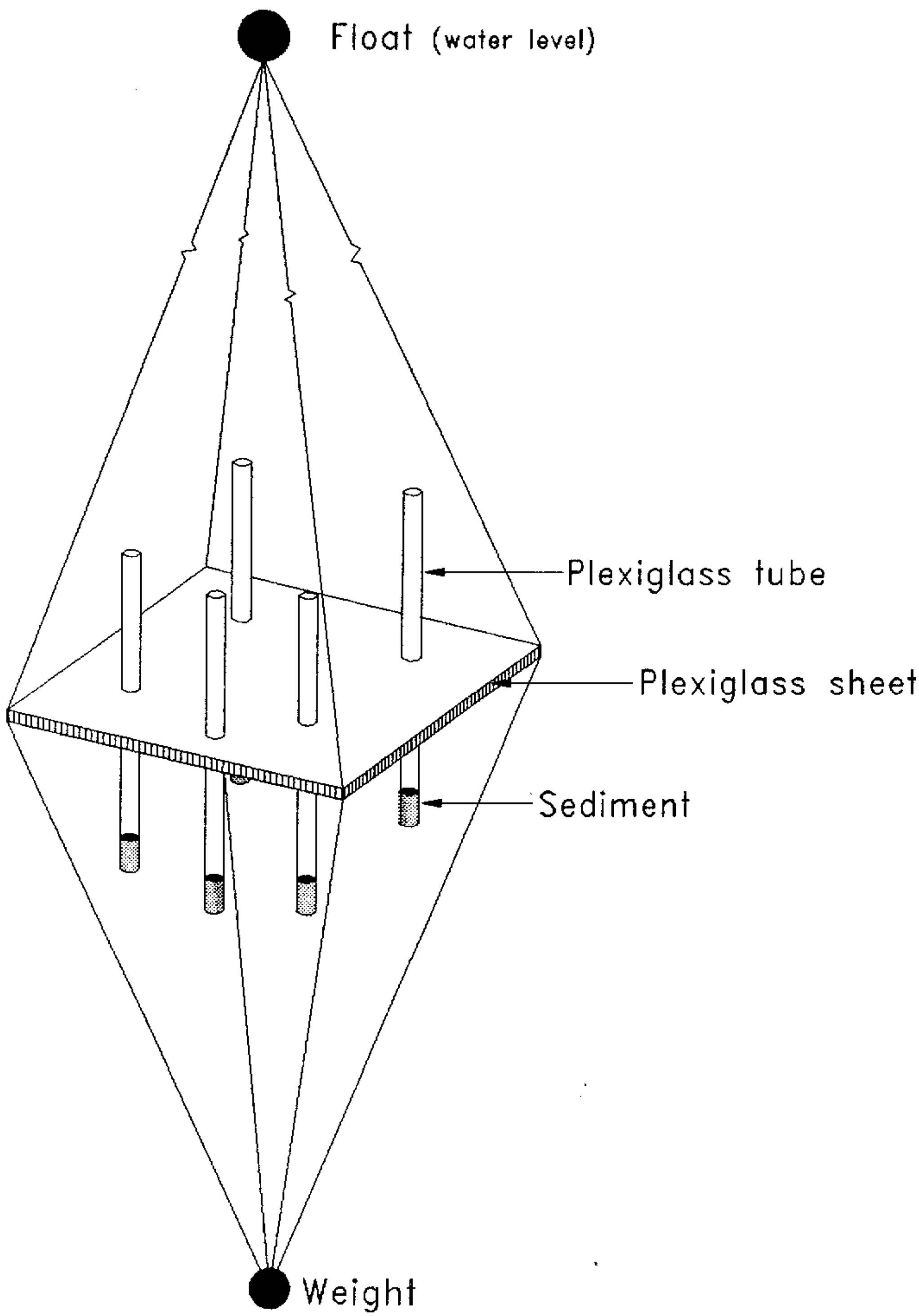
MINE SITE	HABITAT	MAIN TAXA	pH	[Zn]	[Al]	[Fe]
				mg/L	mg/L	mg/L
South Bay, Ontario	Lake	Ulothrix sp.	3.2-3.5	7-11	2.1	4.5
South Bay, On	Pond	Microspora sp.	3.2-4.5	1-2	0.1	0.6
South Bay, Ontario	Pond	Oscillatoria spp.	6.5-7	2-6	4	1*
South Bay, Ontario	Pond	Ulothrix sp.	3.2-3.5	150-450	16	32
Buchans, Nfld.	Pit	Stigeoclonium sp.	6.1-7.3	20-25	10	1*
Buchans, Nfld.	Stream	Microspora sp.	6.1-7.3	20-25	10	1*
Buchans, Nfld.	Seep	Ulothrix sp.	6.4-6.7	16-25	1*	1*
Buchans, Nfld.	Pond	Microspora sp.	6.5-7.5	4-18	4-8	2
Buchans, Nfld.	Pit	Ulothrix sp.	3.3-3.8	26-36	4-6	1
Faro, Yukon Terr.	Seep	Stigeoclonium spp.	7.1	.01*	.01*	6.5
Selbaie, Quebec	Seep	Ulothrix spp.	3.6	42	1.2	34
Selbaie, Quebec	Stream	Chara vulgaris	7.6	0.01*	0.01*	0.01*
Selminco, Nova Scotia	Seep	Ulothrix sp.	4	0.1-0.3	24-95	100-150
Victoria Junction, N.S.	Bog	Ulothrix sp.	3.5	.01*	1.6	8.6
Kidd Creek, Ontario	Pond	Chara vulgaris	7	.01*	0.09	0.2
Cameco, Sask.	Lake	Nitella flexilis	8.5	n.d.	n.d.	0.5
Cameco, Sask.	Lake	Aphanozememnon sp.	8.5	n.d.	n.d.	0.5
Cameco, Sask.	Pool	Oscillatoria spp.	7.1	n.d.	n.d.	0.1

* indicates sample at or below detection limit

TABLE 2: EXTRACELLULAR CARBOHYDRATE PRODUCTION

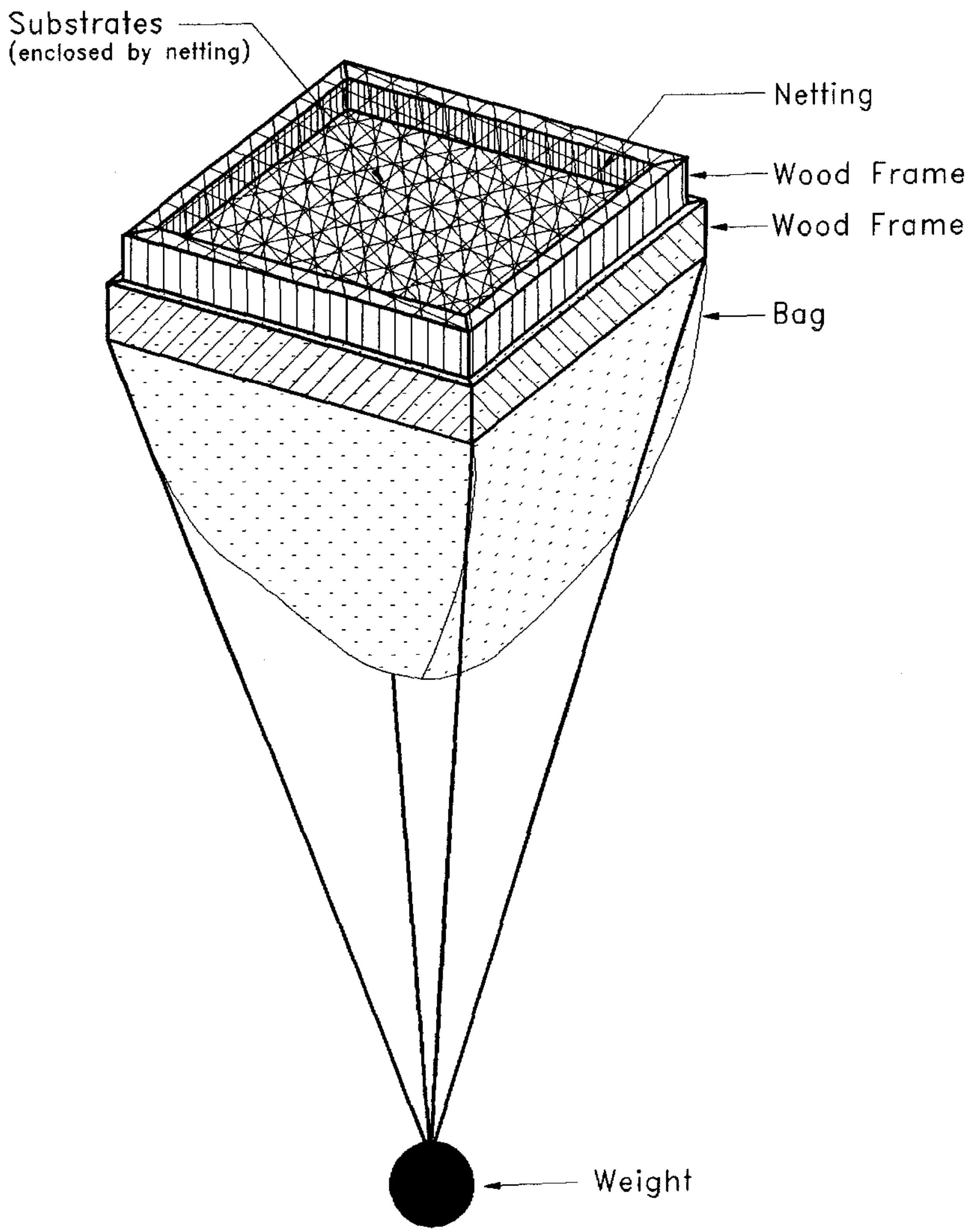
SITE	Glucose Equivalents (mg/L)
Mill Pond Centre	< 0.01
Outflow	0.03
Under dam	> 0.08
Boomerang Lake B8	< 0.01
INCUBATIONS	
Mill Pond "fert PPC"	> 0.40 mg/gfw/h
Mill Pond "unfert PPC"	0.35 mg/gfw/h

SCHEMATIC OF SEDIMENT TRAP

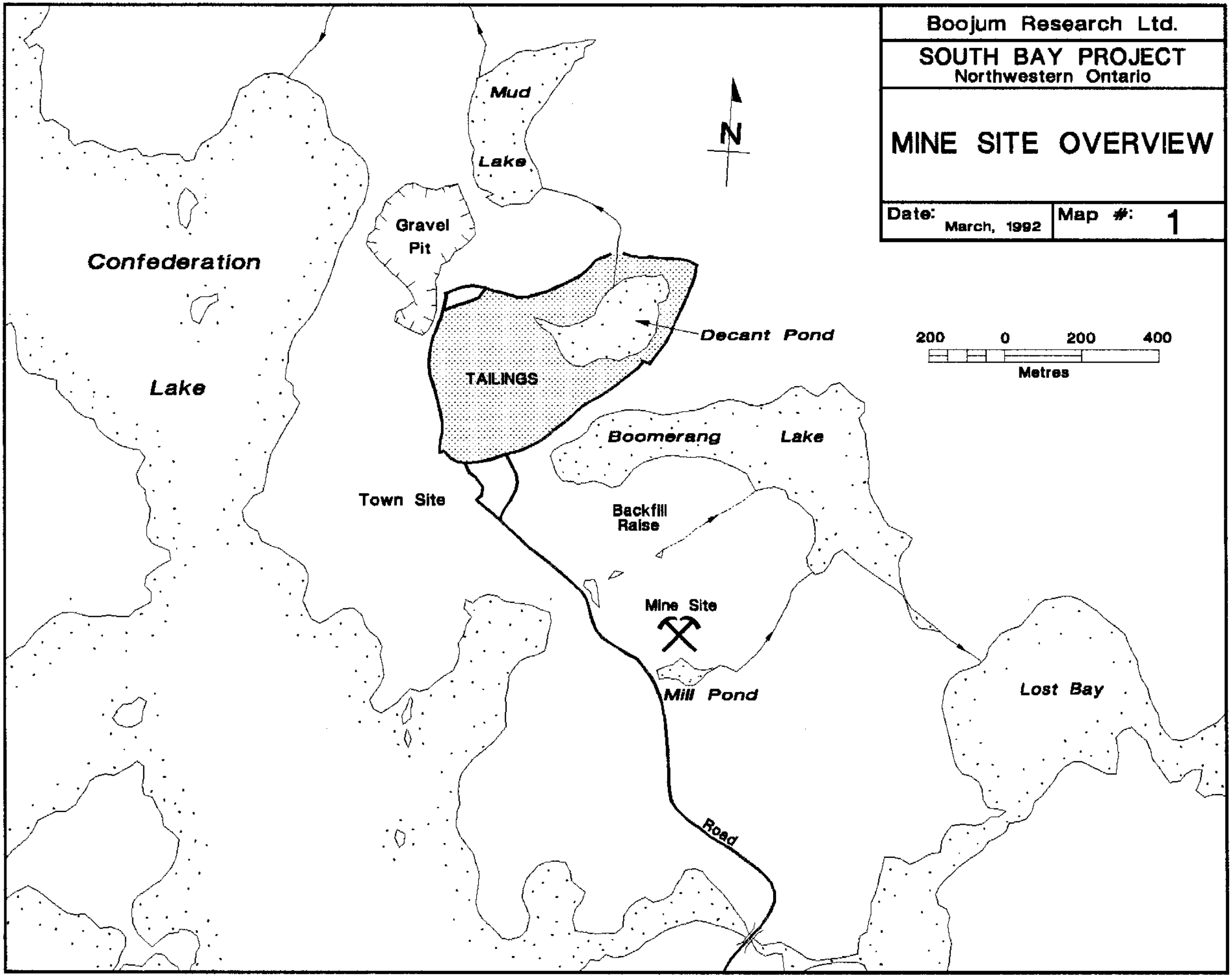


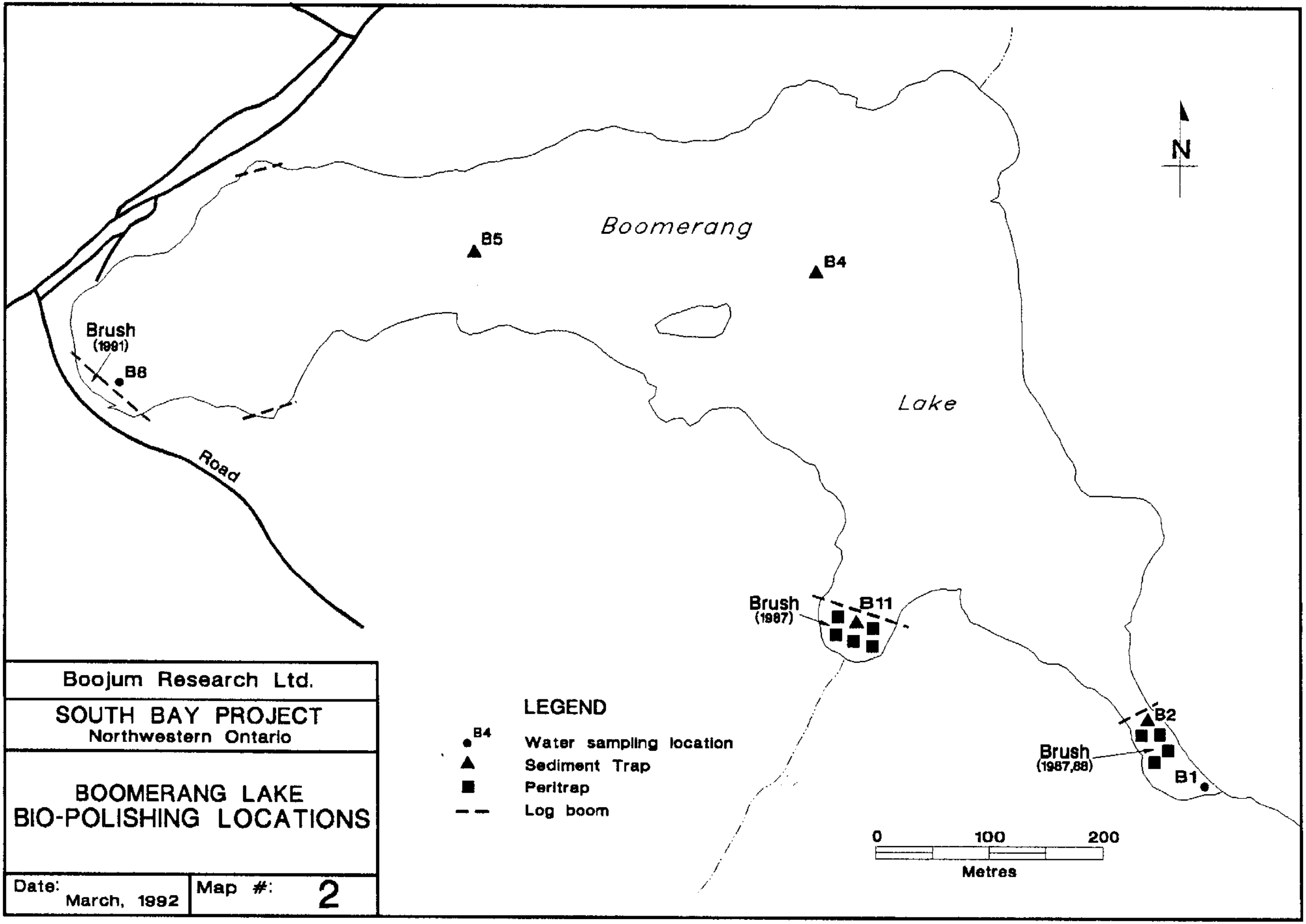
SCHEMATIC: 1

SCHEMATIC OF PERITRAP

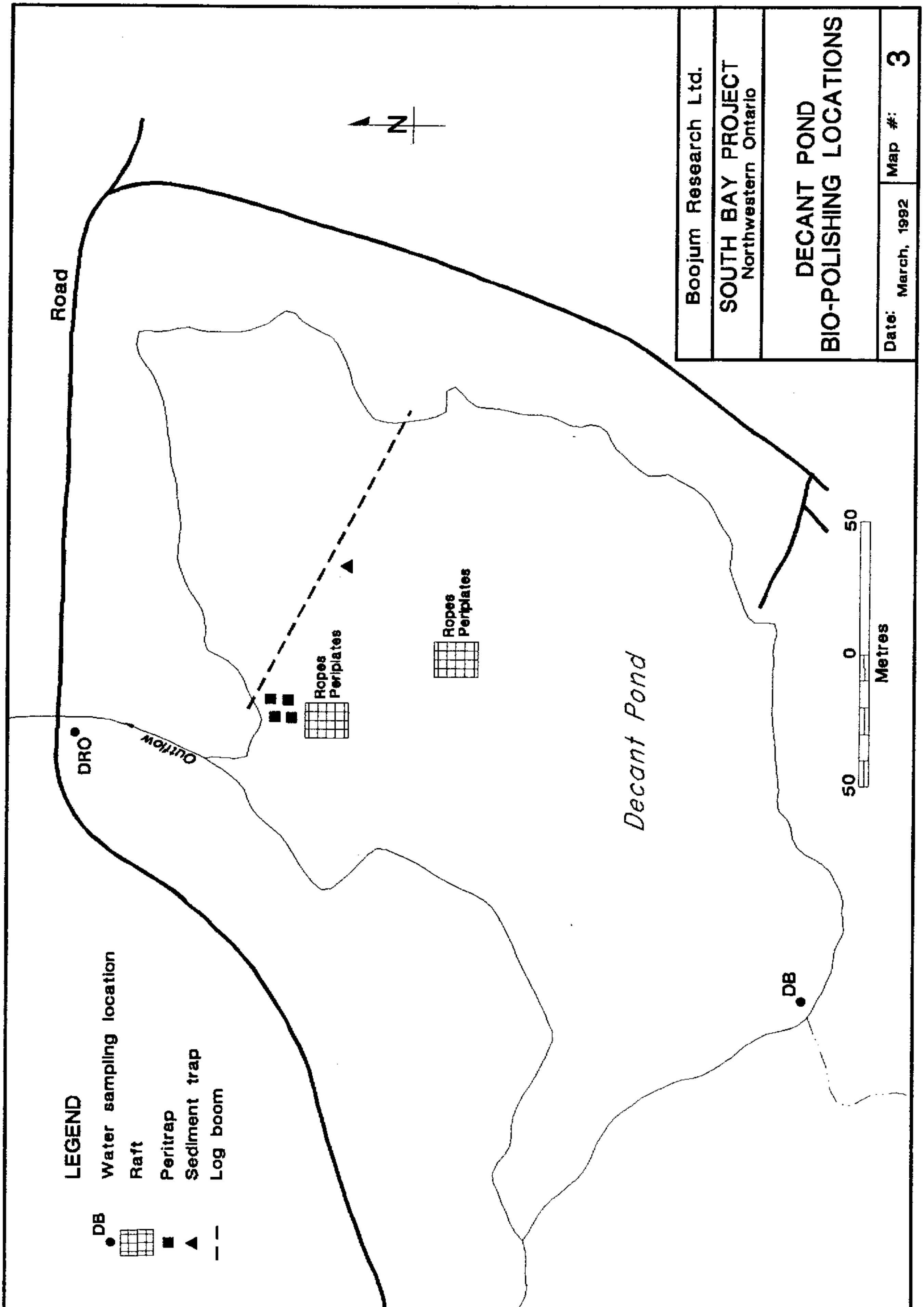


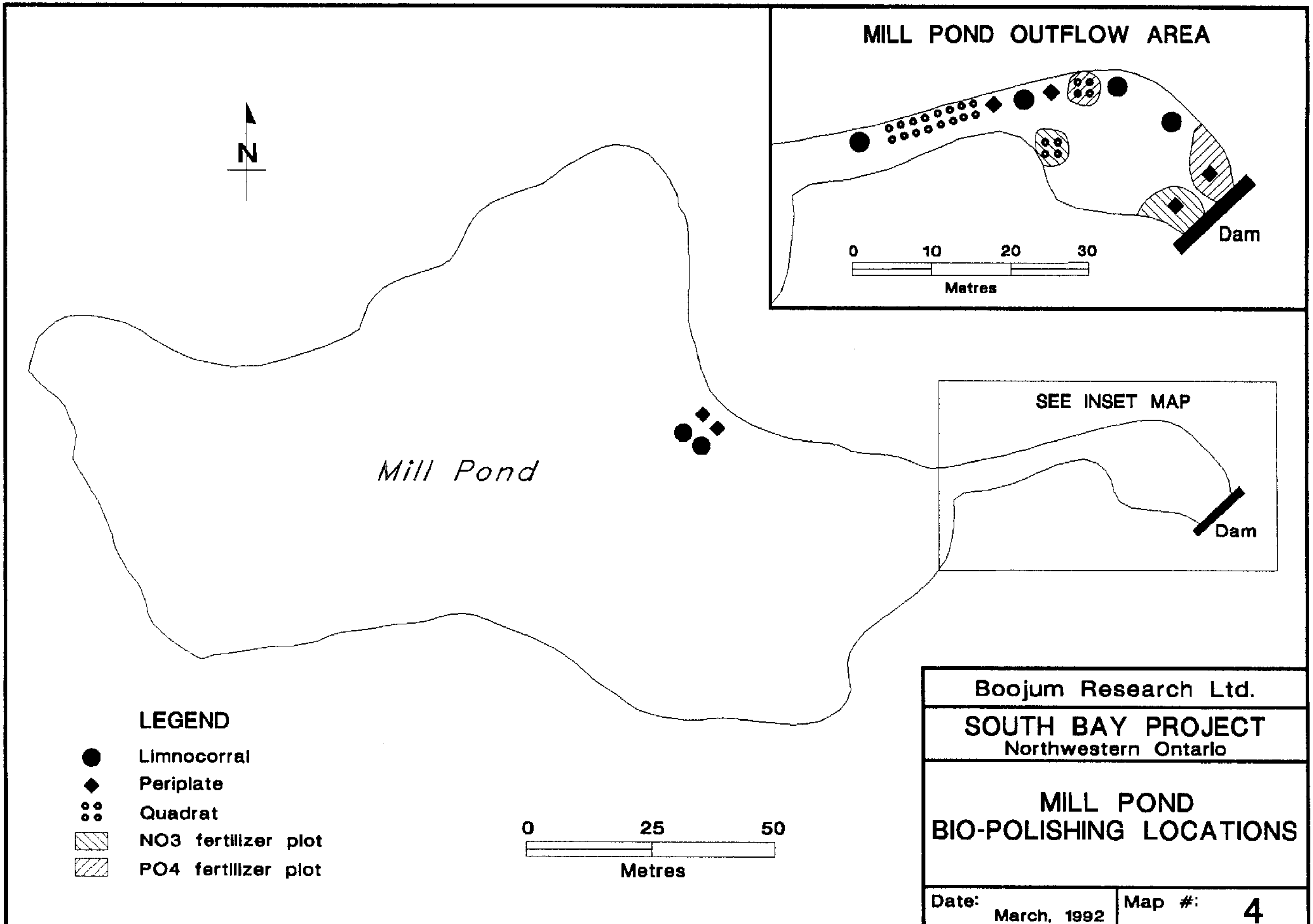
SCHEMATIC: 2

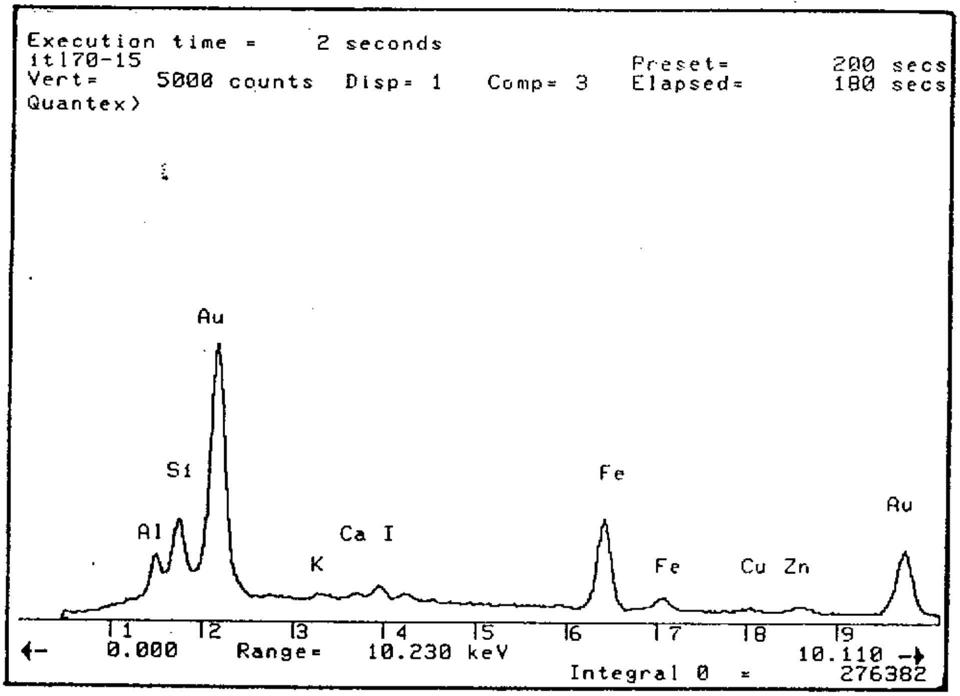

Boojum Research Ltd.
SOUTH BAY PROJECT
Northwestern Ontario
MINE SITE OVERVIEW
Date: March, 1992
Map #: 1
N
200 0 200 400
Metres
Mud Lake
Gravel Pit
Confederation Lake
TAILINGS
Decant Pond
Boomerang Lake
Town Site
Backfill Raise
Mine Site
Mill Pond
Lost Bay
Road



Boomerang
Lake
B5
B4
B6
Brush
(1991)
Road
Brush
(1987)
B11
B2
Brush
(1987,88)
B1
N
LEGEND
B4
Water sampling location
Sediment Trap
Peritrap
Log boom
0
100
200
Metres
Boojum Research Ltd.
SOUTH BAY PROJECT
Northwestern Ontario
BOOMERANG LAKE
BIO-POLISHING LOCATIONS
Date:
March, 1992
Map #:
2

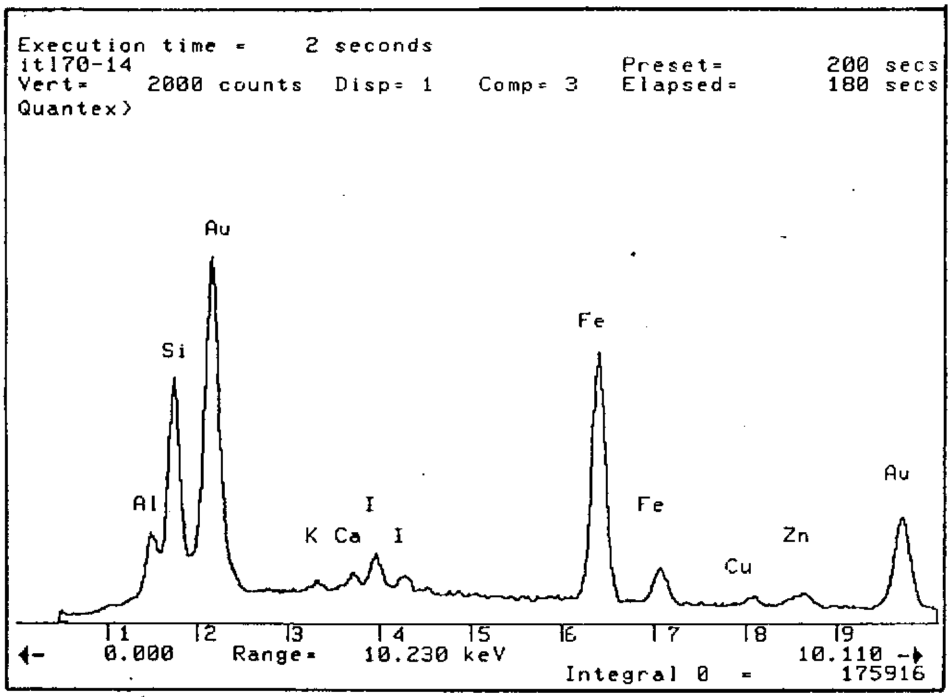

Boojum Research Ltd.
SOUTH BAY PROJECT
Northwestern Ontario
DECANT POND
BIO-POLISHING LOCATIONS
Date: March, 1992
Map #: 3
N
Road
Ropes
Periplates
Ropes
Periplates
Decant Pond
Outflow
DRO
DB
50
0
50
Metres
LEGEND
DB
Water sampling location
Raft
Peritrap
Sediment trap
Log boom


MILL POND OUTFLOW AREA
Dam
0 10 20 30
Metres
SEE INSET MAP
Dam
N
Mill Pond
LEGEND
Limnocorral
Periplate
Quadrat
NO3 fertilizer plot
PO4 fertilizer plot
0 25 50
Metres
Boojum Research Ltd.
SOUTH BAY PROJECT
Northwestern Ontario
MILL POND
BIO-POLISHING LOCATIONS
Date: March, 1992
Map #: 4



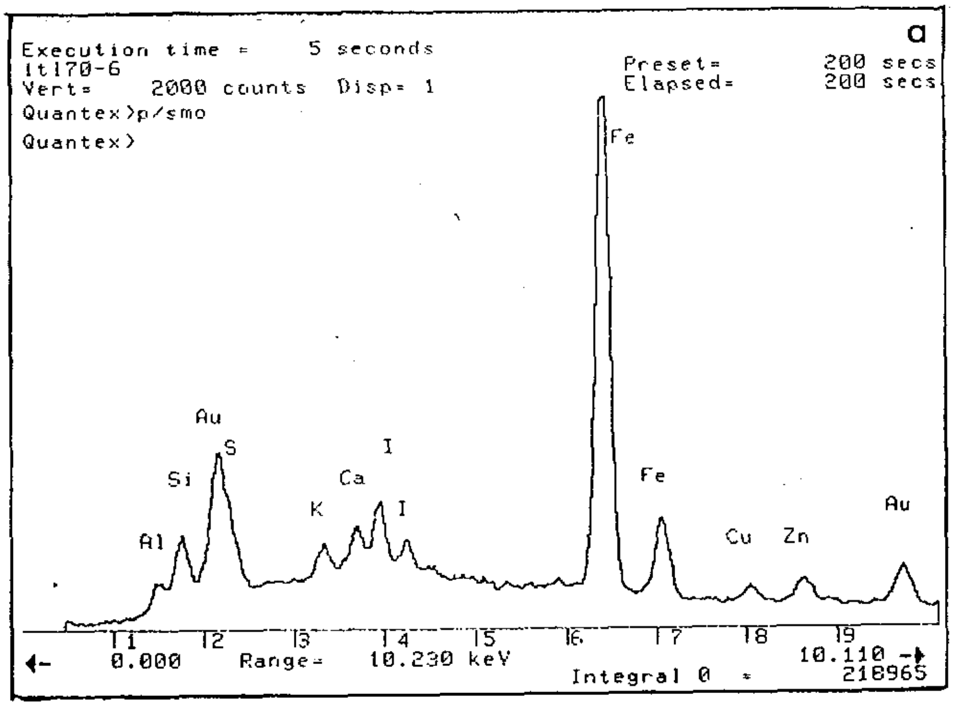
ELEMENT & LINE	WEIGHT PERCENT	ATOMIC PERCENT*
Si KA	28.08	43.79
K KA	1.39	1.56
Ca KA	2.38	2.60
Fe KA	55.29	43.35
Cu KA	4.52	3.11
Zn KA	8.34	5.59

FIG. 9: Energy Dispersive X-Ray analysis of an *Oscillatoria* filament in the Decant Pond PPC shown in Plate 1.



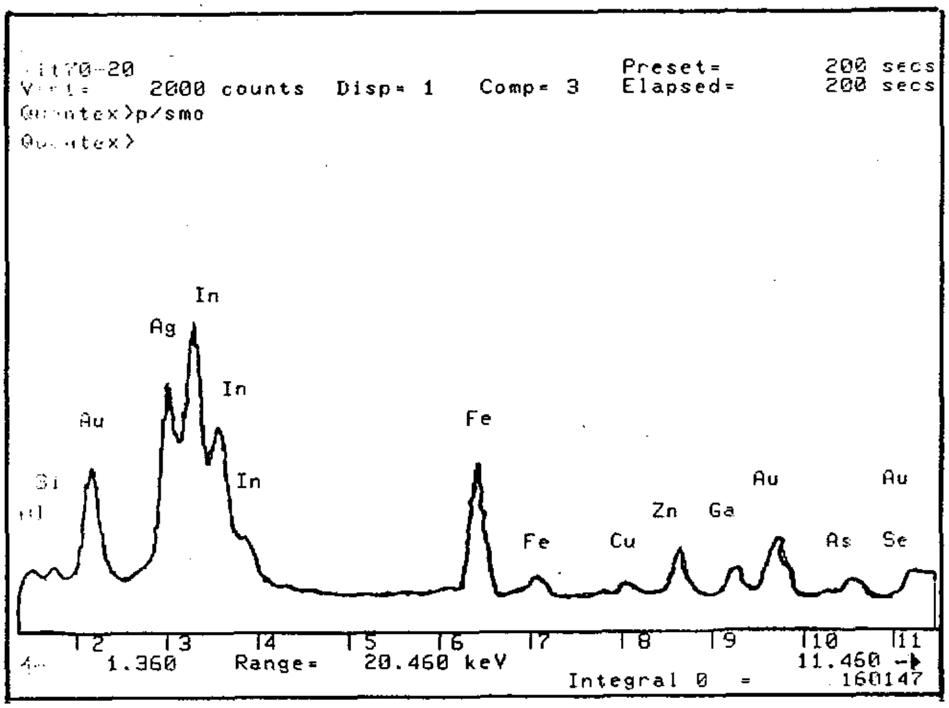
ELEMENT & LINE	WEIGHT PERCENT	ATOMIC PERCENT*
Si KA	30.22	46.47
K KA	0.85	0.93
Ca KA	1.88	2.02
Fe KA	55.14	42.63
Cu KA	3.97	2.69
Zn KA	7.95	5.25

FIG. 10: Energy Dispersive X-Ray analysis of a *Navicula* frustule in the Decant Pond PPC shown in Plate 2.



ELEMENT & LINE	WEIGHT PERCENT	ATOMIC PERCENT*
Si KA	2.86	5.41
S KA	3.52	5.83
K KA	1.83	2.48
Ca KA	3.04	4.04
Fe KA	71.10	67.71
Cu KA	6.45	5.40
Zn KA	11.21	9.12

FIG. 11: Energy Dispersive X-Ray analysis of low-density precipitate in Decant Pond PPC.



ELEMENT & LINE	WEIGHT PERCENT	ATOMIC PERCENT*
Al KA	6.40	16.98
Si KA	3.23	8.24
Fe KA	9.54	1[illegible].23
Cu KA	1.65	1.86
Zn KA	6.18	6.78
Ga KA	[illegible].32	5.47
As KA	5.51	6.22
Se KA	11.21	10.17
Ag LA	21.76	14.45
In LA	28.21	17.60

FIG. 12: Energy Dispersive X-Ray analysis of a high-density precipitate in Decant Pond PPC.

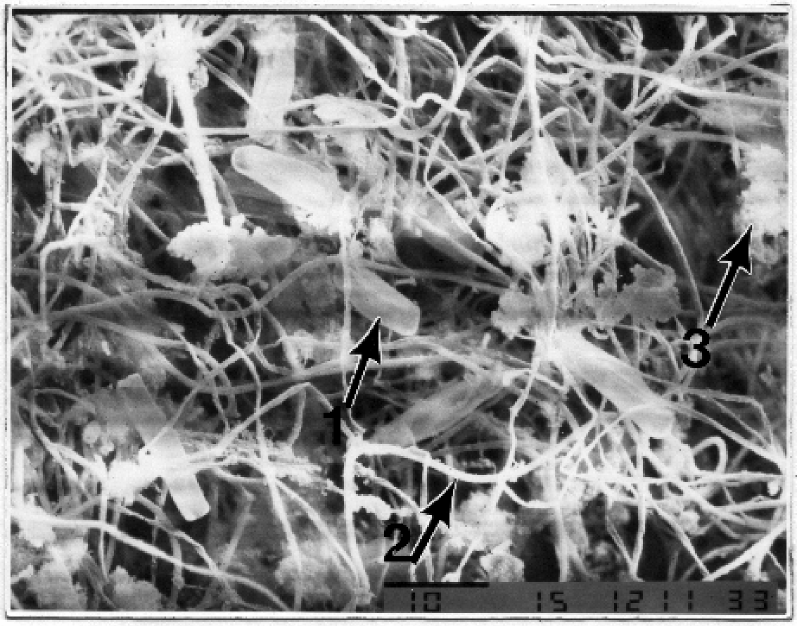


PLATE 1: Scanning Electron Micrograph of a PPC from Decant Pond. a) *Navicula* sp., b) *Oscillatoria* sp., and c) precipitate.

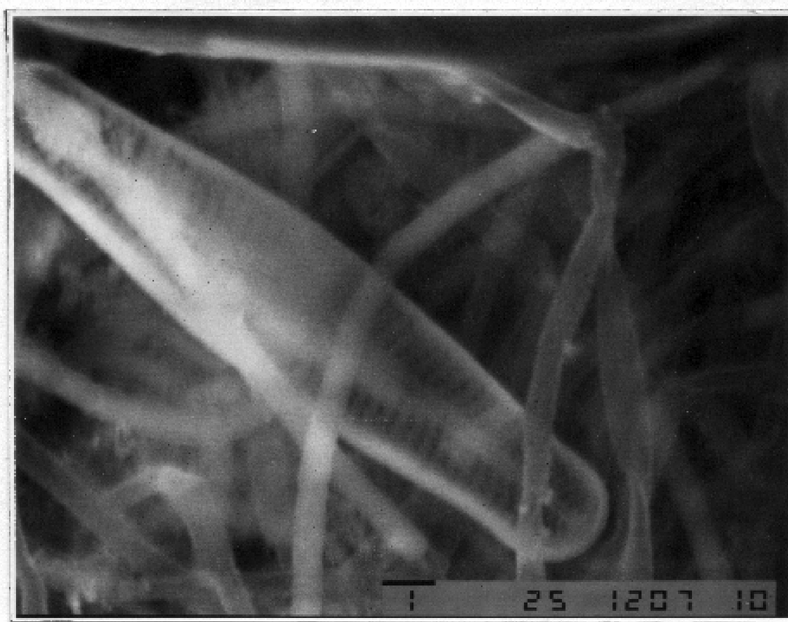


PLATE 2: Scanning Electron Micrograph of a *Navicula* sp. in a Decant Pond PPC.

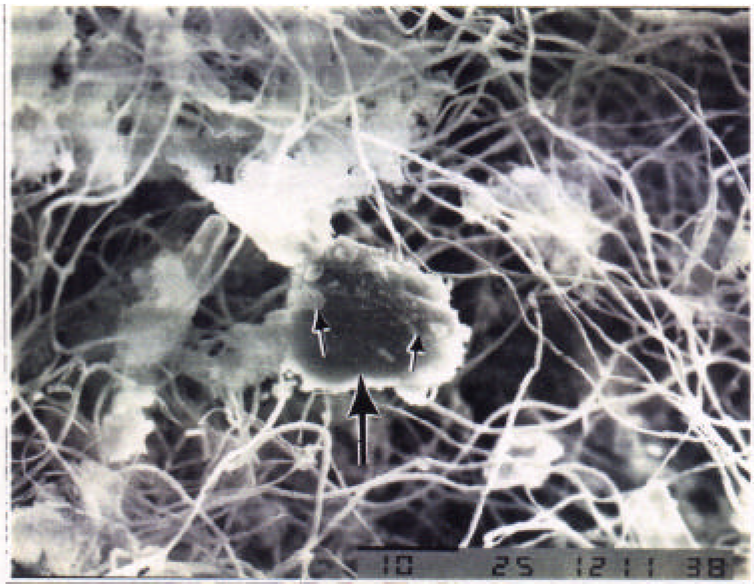


PLATE 3: Scanning Electron Micrograph of precipitates in a Decant Pond PPC. Both high density (gray) and low density (white) precipitates are shown.

PLATE 4: Peritraps in Boomerang Lake. Spruce trees for PPC substrate are also shown.