MORPHOLOGICAL/ANATOMICAL INVESTIGATION OF CATTAIL TRANSPLANTS AND BOG VEGETATION

FINAL REPORT

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SOMMAIRE

Bogs and coal acid mine drainage are closely linked at the Victoria Junction Coal processing plant of DEVCO in Sydney, Cape Breton, Nova Scotia. This geographical setting facilitates the investigation of these bogs with respect to the effects of AMD on the ecosystem and determine their use in ameliorating the acidic conditions. The species assemblage is typical of that encountered in dwarf-shrub bogs of the Northeastern regions of the United States and Canada. These bogs are dominated by _Chamaedaphne calyculata_.

This report describes the status of the vegetation in both bogs which have received AMD for varying times. It summarizes both on-site and laboratory investigations. Through the use of morphological-anatomical techniques, the death or growth of plant parts is determined.

The old bog, exhibits significant acid stress, although it had only received aerial deposition of coal and a diffuse flow of AMD. A second bog, the new bog, located immediately above the stressed bog was healthy and unaffected by acid mine drainage until AMD seepage was diverted into the bog at the end of summer 1988. In the new bog the vegetation damage is much more severe than in the old bog and prognosis for continued survival is not good for many of the species that form the natural species assemblage of the bogs. The

majority of shoot tips and lateral buds were found to be dead, suggesting little hope for recovery of the plants in subsequent years. Although damage was also observed in the same species of the old bog, the symptoms were not as severe as those seen in the new bog. Roots and rhizomes tended to show some damage but this was much reduced in comparison to that observed in the new bog.

The most important species being able to survive is Typha latifolia. Other grasses, sedges, and rushes may however compete in the colonizing of the dying bogs. Thus, a change in species composition of the bogs can be expected. To promote the growth of cattails in AMD conditions foliar fertilizers were tested. Treatment with noticeable beneficial effects was the application of 4-18-16 at a dilution of 10:1.

Morphological investigations of cattail roots indicated large accumulation of metals on the epidermis and the hypodermal layers. In dead lateral roots, metal concentrations are highest and have penetrated the entire root cross section. Analysis of X-ray spectra of the metals by SEM of root cross sections, indicated that high concentrations of Fe are associated with high levels of S and greatly reduced concentrations of Ca. Crystal formation was noted in the iron-sulphate plaque accumulation in the roots. X-ray scans of cattail leaves growing in AMD conditions indicate the presence of glandular cell regions, called hyropoten with Fe levels three times that of the adjacent epidermal regions. These findings suggest, that the cattail rhizosphere may be active in ameliorating AMD and that adaptations to high iron concentrations through activation of particular cell regions of the leaves may occur.

SOMMAIRE

Les tourbières et le drainage des houillères acides sont étroitement liés à l'usine de traitement de la houille de Victoria Junction, de DEVCO, à Sydney, au Cape Breton et en Nouvelle-Écosse. Cet emplacement géographique facilite l'étude de ces tourbières, en ce qui concerne les effets d'AMD (Drainages acides des mines) sur **l'écosystème,** et determine leur utilisation pour l'amélioration des conditions acidifiantes. L'assemblage d'espèces est typique de celui rencontré dans les tourbieres d'arbustes nains des regions nord-ouest des États-Unis et du Canada. Ces tourbieres sont dominées par les Chamaedaphne calyculata.

Ce rapport décrit l'état de la vegetation dans les deux tourbières ayant fait l'objet d'AMD pendant des périodes différentes. I1 resume les études sur place et en laboratoire. Par l'utilisation de techniques morphologiques et anatomiques, la mort ou la croissance des parties d'une plante sont déterminées.

L'ancienne tourbière **révèle** un stress acide significatif, bien qu'elle n'ait reçu qu'une deposition aérienne de houille et un écoulement diffus d'AMD. Une seconde tourbière, la nouvelle tourbière, située immédiatement au-dessus de la tourbière sous stress, était en bonne condition et n'était pas affectée par le drainage acide de la mine jusqu'à ce que des suintements d'AMD soient détournés dans la tourbière, à la fin de **l'été** 1988.

Dans la nouvelle tourbière, les **dégâts** causés **à** la végétation sont bien plus graves que dans l'ancienne tourbière, et les conjectures sur une survie continue ne sont pas positives pour plusieurs des espèces formant l'assemblage naturel des especes des tourbières. La plupart des apex de tige et des boutons latéraux étaient morts, ce qui suggère qu'il existe peu d'espoir de guérison pour les plantes, pour les années **à** venir. Bien que des dégâts furent également observes pour les mêmes espèces dans l'ancienne tourbière, les symptômes n'étaient pas aussi graves que ceux observes dans la nouvelle tourbière. Les racines et les rhizomes avaient tendance à laisser apparaître quelques dégâts, mais ils étaient bien moins grands que ceux observes dans la nouvelle tourbière.

L'espèce la plus importante capable de survivre est le typha latifolia. Cependant, les autres herbes, carex et joncs pourront se partager la colonisation des tourbières mourantes. Par conséquent, on peut s'attendre à un changement pour la composition des espèces des tourbières. Pour encourager la croissance des massettes* dans des conditons d'AMD, des fertilisants foliaires furent utilisés. Le traitement, qui eut des effets bénéfiques visibles, consistait en l'application de 4-18-16 à une dilution de 10:1.

Les etudes morphologiques des racines de massettes ont indiqué une grande accumulation de métaux sur l'épiderme et les couches hypodermiennes. Dans les racines latérales mortes, les concentrations de métaux sont plus élévées et ont pénétré toute la partie transversale de la racine. L'analyse du spectre de rayons X des métaux par MEB des parties transversales de la racine a indiqué que de fortes concentrations de Fe sont associées à des niveaux élevés de S, et réduisent considérablement les concentrations de Ca. La formation de cristal a été observée dans l'accumulation de plaques de sulfates de fer dans les racines. Des échogrammes aux rayons X des feuilles des massettes poussant dans des conditions d'AMD indiquent la présence de zones de cellules glandulaires, appelées «hyropoten» avec des niveaux de Fe représentant trois fois ceux des zones épidermiques adjacentes. Ces résultats suggèrent que la rhizosphère de massette peut être active pour améliorer l'AMD, et que les adaptations à des concentrations élévées de fer, par le fonctionnement de régions cellulaires des feuilles, peuvent avoir lieu.

* TRANSLATOR'S NOTE : «CATTAILS» ARE ALSO CALLED «TYPHA» IN FRENCH.

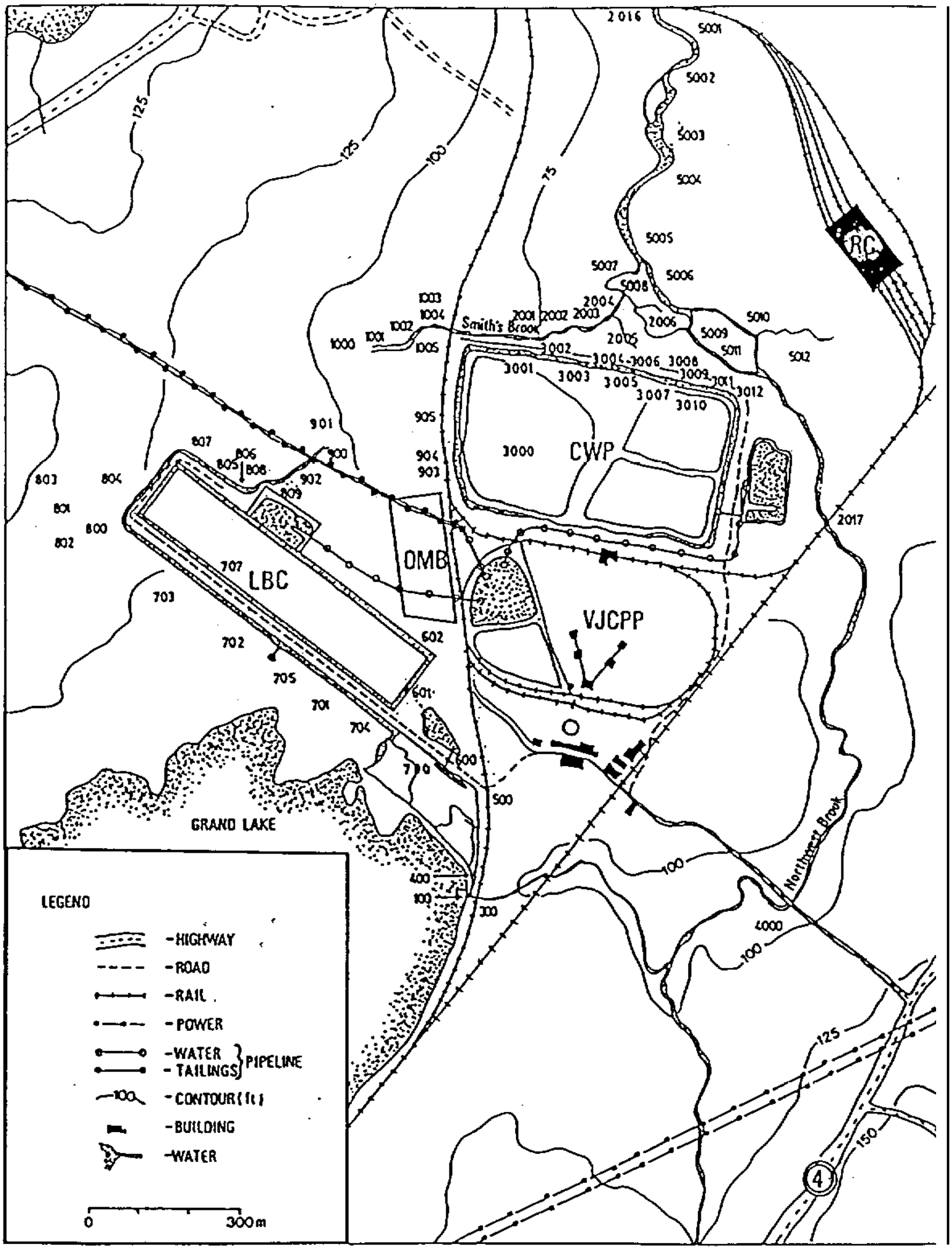
1.0 INTRODUCTION

The Victoria Junction Coal Processing Plant ("VJCPP") of DEVCO in Sydney, Cape Breton, Nova Scotia, is surrounded by bogs. Bogs are often acidic ecosystems which are expected to be tolerant to Acid Mine Drainage (AMD). Furthermore, bogs or wetlands are alleged to ameliorate these types of waste water by removal of their acidity.

Acid mine drainage from the Lifting and Banking Center ("LBC") (Map 1) and from settling ponds (Map 1, Location 600), drain along a ditch into a bog (Station 500) and leave the site (Station 300) to Northwest Brook. The drainage from the Old Met Bank ("OMB") and the Coarse Waste Rock Pile (CWP) are also received by bogs joining Smith Brook and, ultimately, Northwest Brook. Bogs and coal acid mine drainage are therefore closely linked in this site. This geographical setting facilitates the investigation of these bogs with respect to the effects of AMD on the ecosystem and determine their use in ameliorating the acidic conditions.

From an ecological point of view, the bog located between Stations 400 and 500 (Map 1), referred to as the Old bog, exhibits significant acid stress although it had only received aerial deposition of coal and a diffuse flow of AMD from the LBC ditch

MAP 1: OVERVIEW OF THE VICTORIA JUNCTION COAL PROCESSING PLANT



and the settling ponds. A second bog, referred to as the New bog located immediately above the stressed bog in the vicinity of Station 700 (Map 1) was healthy until August 1989 when it received AMD. The vegetation type of both bogsis similar and therefore a comparative investigation was possible. AMD was diverted into both bogs, the Old bog which had already deteriorated as well as into the healthy New bog, thus allowing for an assessment of the ecological responses of stressed and healthy vegetation to AMD from coal.

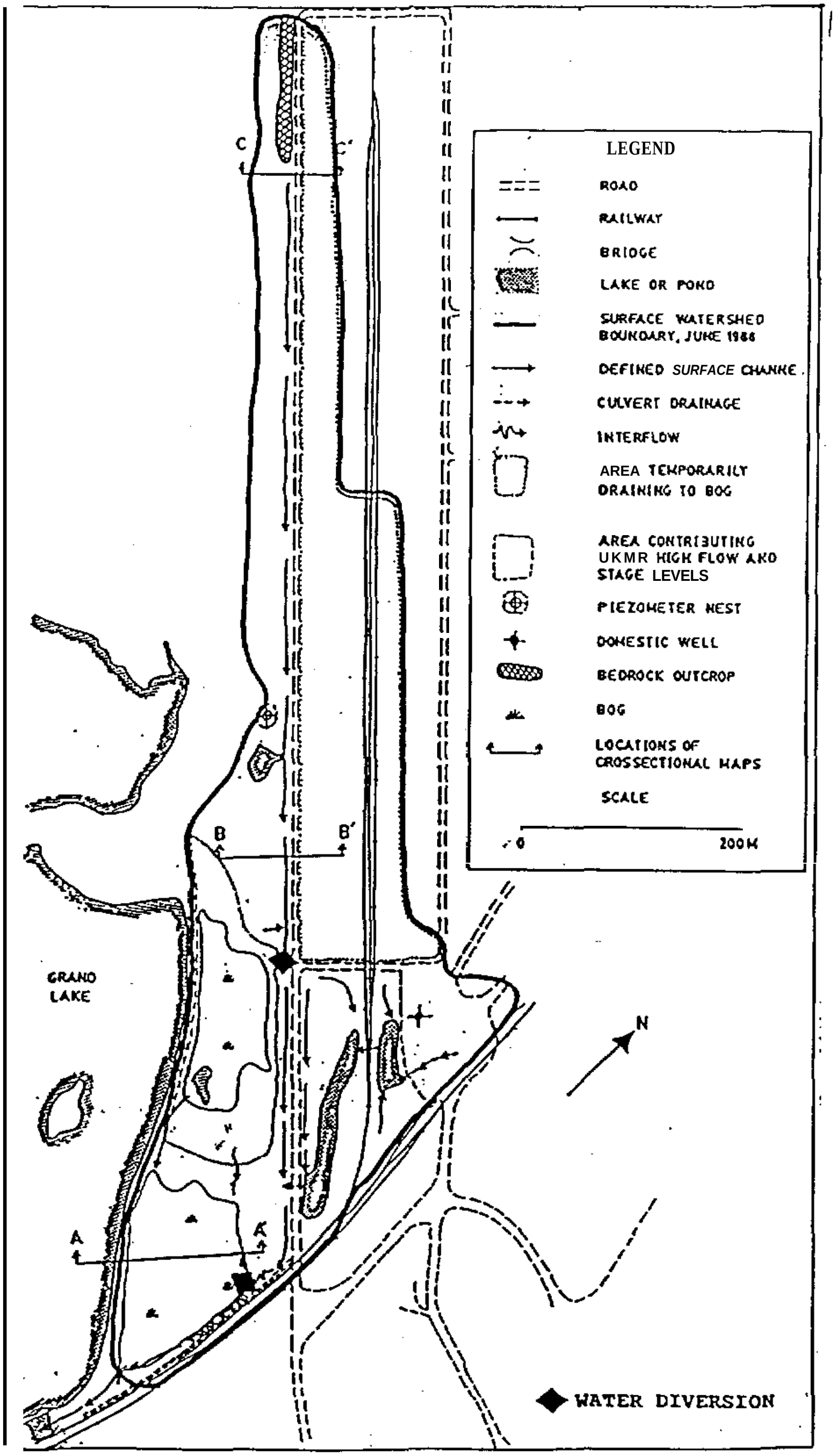
The study further encompasses the evaluation of the status of the growth and development of one and two year old cattail transplants and cattails in natural stands. These transplanted cattails are located at various points along the spill area of a tailings site in Elliot Lake. Cattail development was contrasted among sites among sites of increasing environmental severity in relation to pH. An assessment of the relative merits of several different fertilizer treatments in enhancing the growth and survivorship of cattail transplants was carried out.

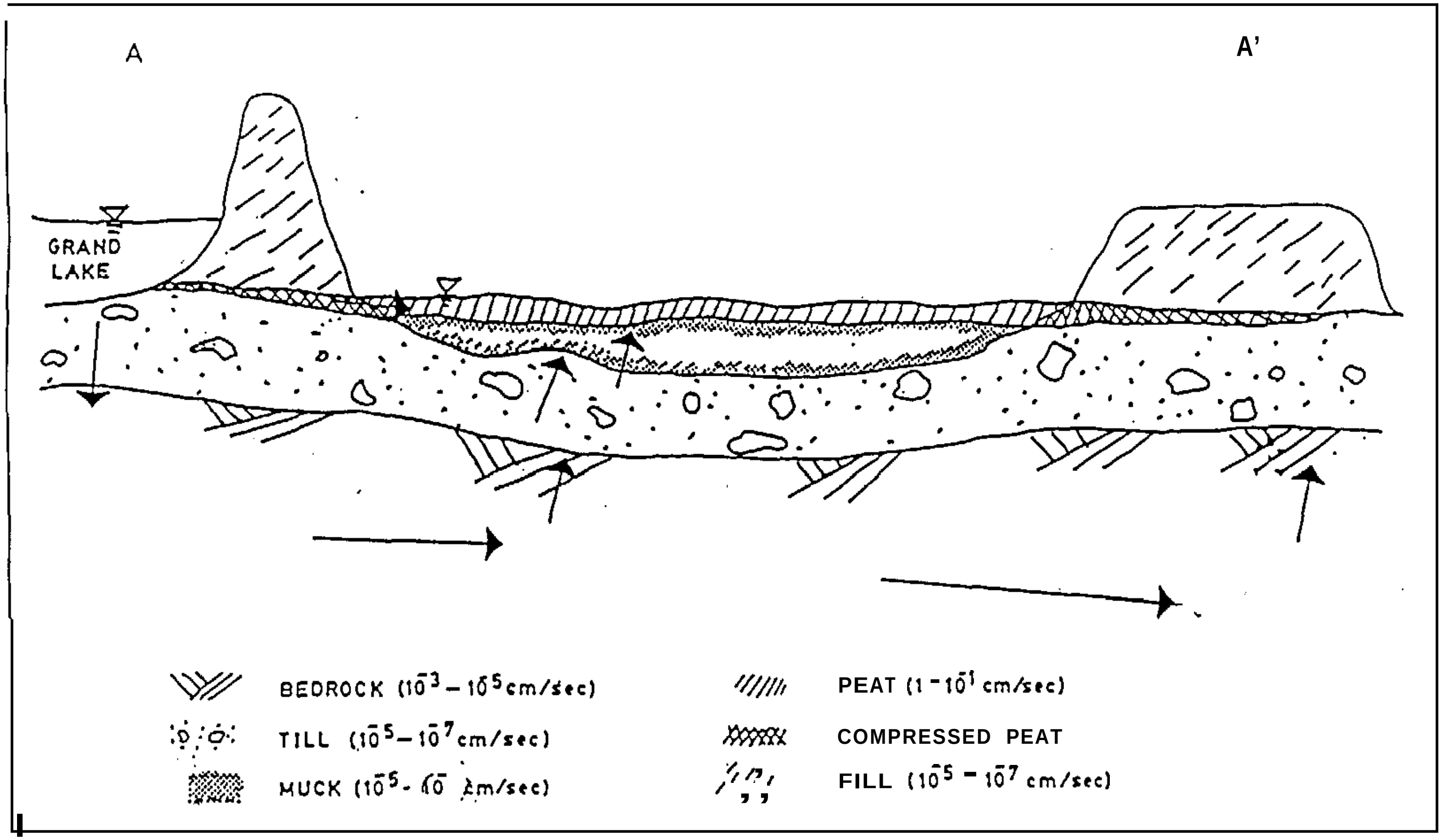
2.0 MATERIALS AND METHODS

2.1 Site Description: Hydrological conditions of the bogs

In Map 2, the drainage basin of the LBC is given schematically. The arrows indicate the general flow direction prior to the diversions of the AMD into the bogs. The location where the water was diverted is indicated by the diamomnds. Both diversions have been installed with clay berms and work effectively. The new bog is more extensively affected by Grand Lake water levels and high flow than the old bog. The New bog is a floating bog in contrast to the Old bog which is lodged to the ground. The cross section (Schematic 1) indicates the general ground and surface water hydrology in the bogs. The vegetation cover studied is growing on a layer of peat, which is underlaid by a layer of muck, lying above till.

Map 2: DRAINAGE BASIN OF THE LIFTING AND BANKING CENTER





SCHEMATIC 1: BOG GEOMORPHOLOGY AND PERMEABILITY

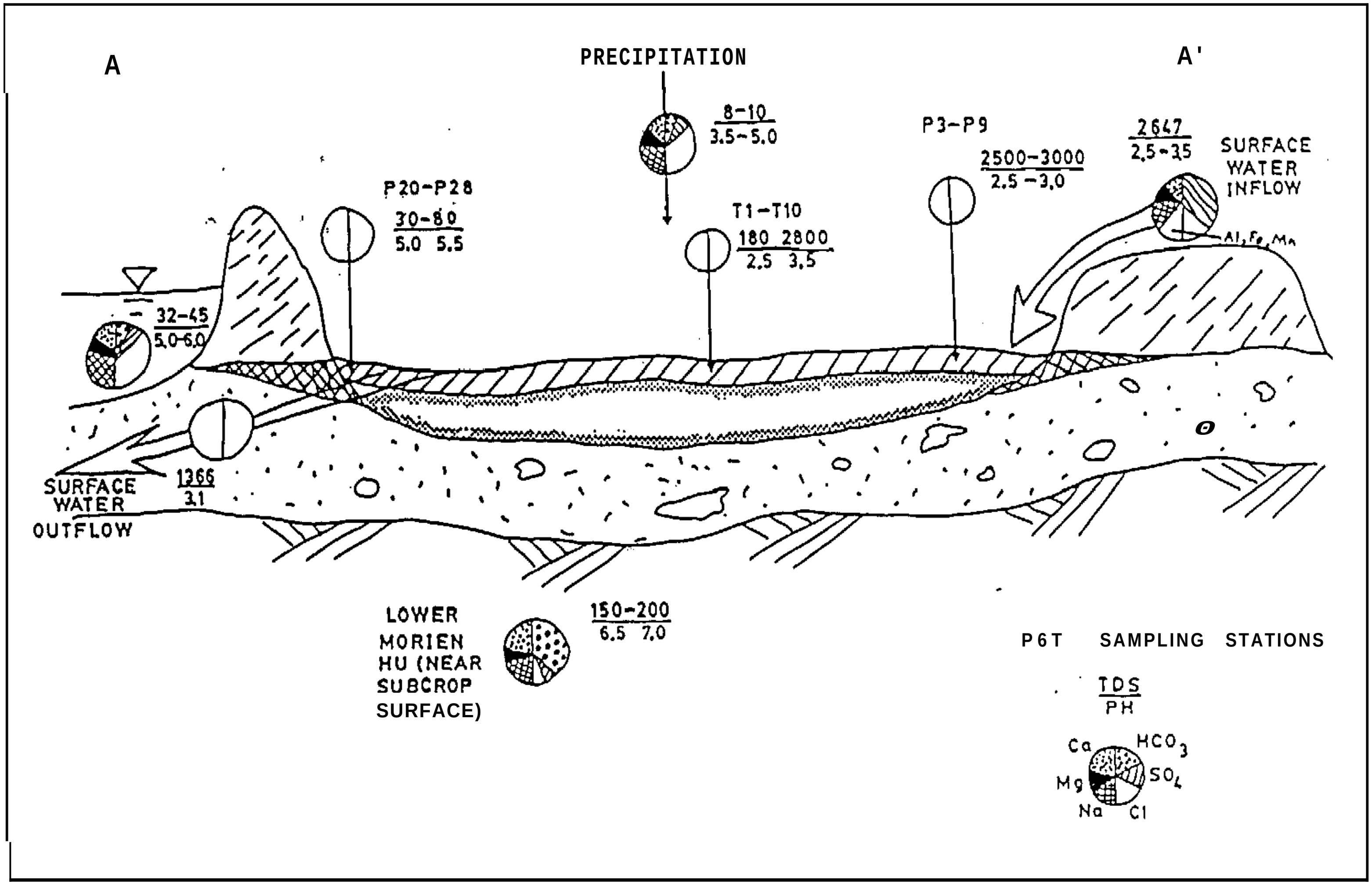
Thus ground water will enter the bog, together with water from Grand Lake, in addition to the acid mine drainage. The geomorphological and hydrological characteristics are summarized in Schemnatic 2 for conditions found in these bogs.

The relative composition of the groundwater is given for Ca, Mg, Na, Cl, SO, and HCO_3, which can be compared with the surface water entering from Grand Lake. The experimental layout, the treatments and the further details are given for the transplanted cattails used for comparitive investigation, in Kalin, Scribaillo, (1988).

2.2 Vegetation Assessment

The status of the overall growth of vegetation and of particular species was initially assessed in July 1989 by visual inspection. To allow a more continuous and quantitative assessment to be made,

SCHEMATIC 2: GROUNDWATER AND SURFACE WATER CHARACTERISTICS



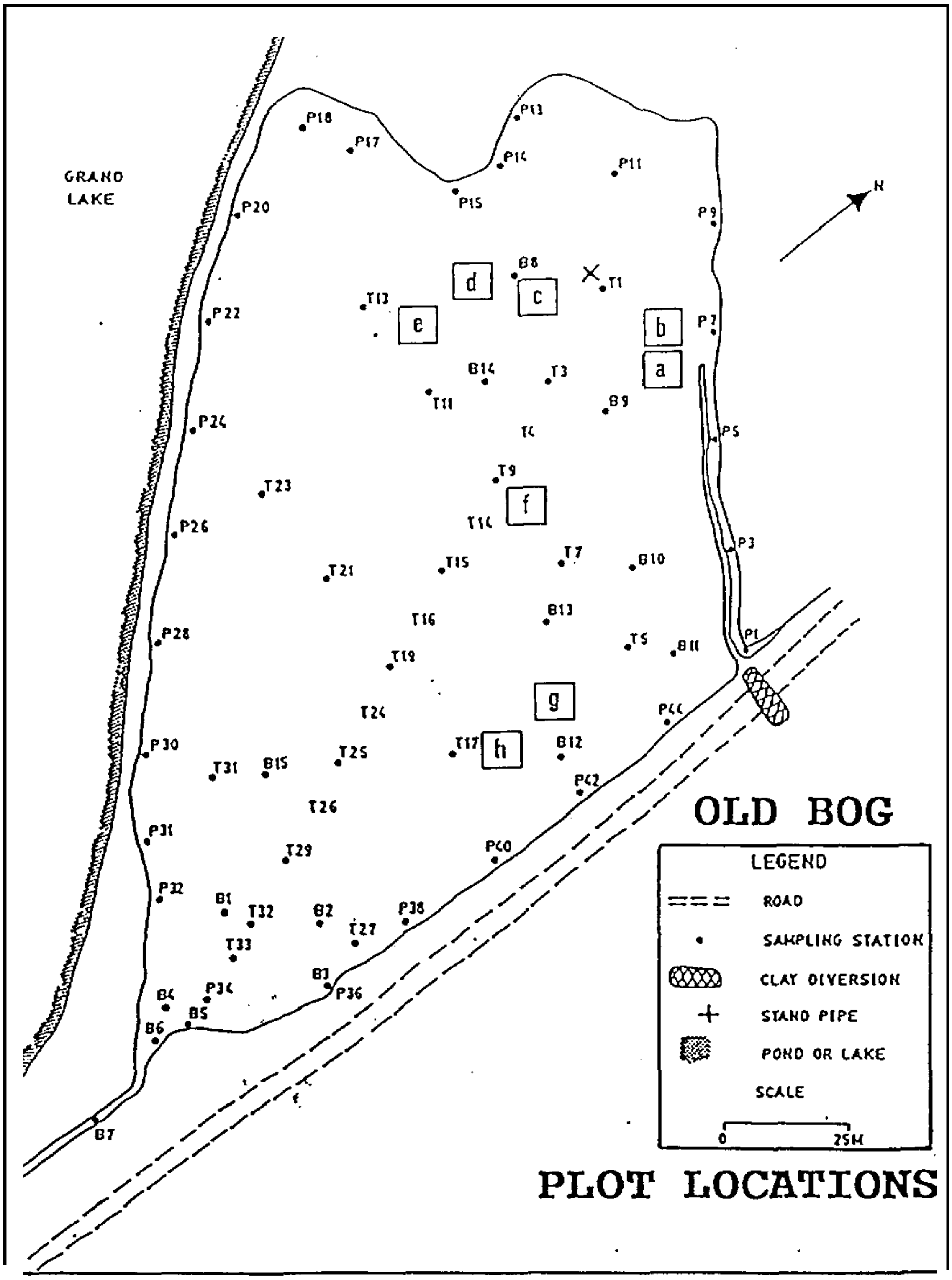
ten permanent quadrats were established.

Eight one meter squared quadrats were set up in the old bog (Map 3) and two one meter squared quadrats were set up in the new bog (Map 4). Quadrats were chosen to represent the major microhabitat sites present in the bogs. These were identified from an initial overall assessment of the bogs during a walk-through survey and collection of species during July, 1989.

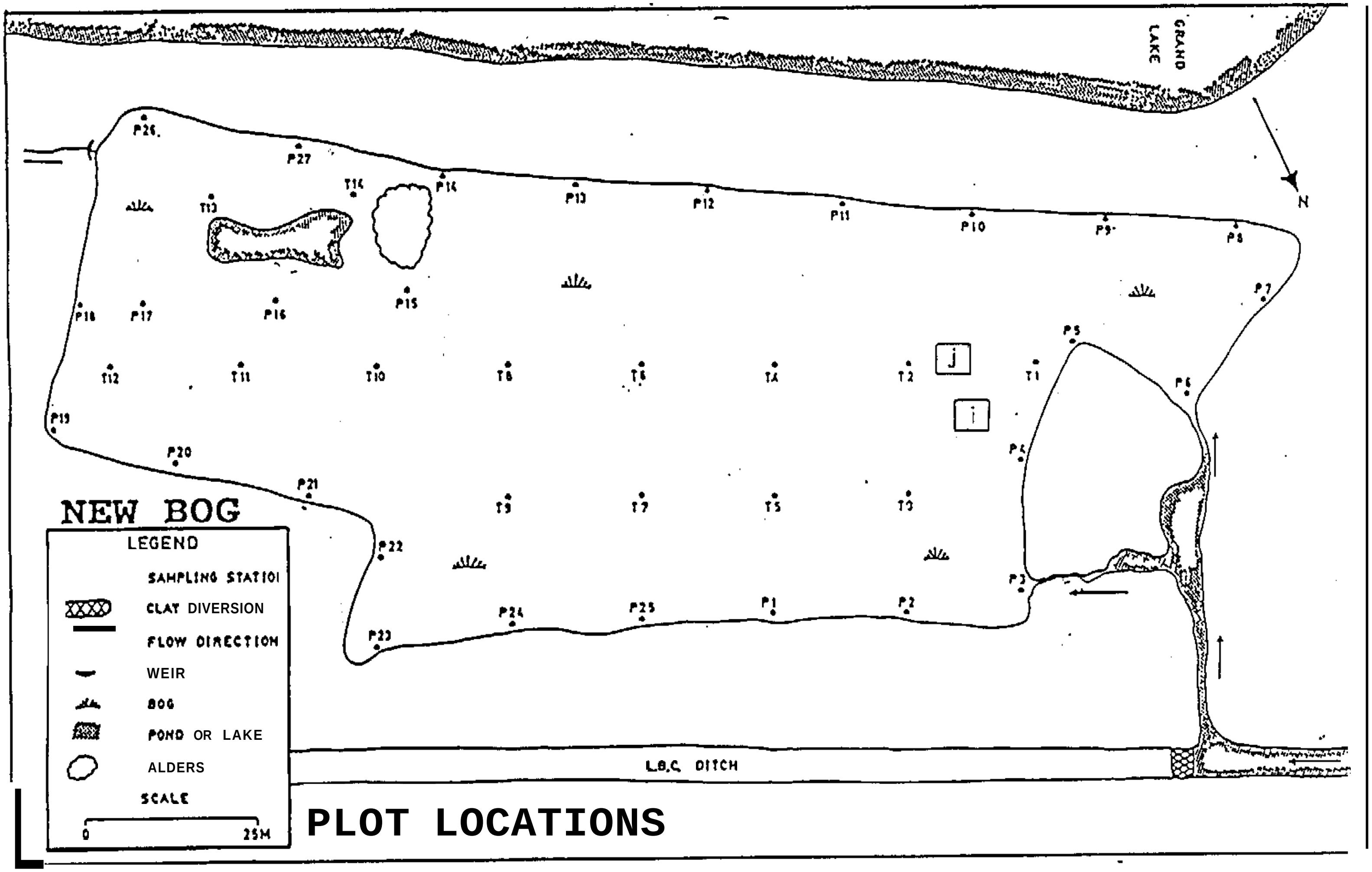
2.3 Microscopic Methods

Plants from both bogs and a control location, i.e. a bog not exposed to Acid Mine Drainage within the vicinity of the coal processing plant, were carefully excavated from the substratum and placed in sample bags for transport back to the lab. At the lab, plants were refrigerated for preservation until microscope examination. Selected plant organs were fixed in FAA (formalin: alcohol: acetic acid). Tissue was either hand-sectioned or sectioned on a Reichert sliding microtome. After mounting on slides, sections were either stained with TBO (toluidine blue O) to elucidate general structural features of the plant tissue (Feder and O'Brien, 1968) or with iodine: potassium iodide to determine locations of starch storage.

MAP 3: LOCATIONS OF PERMANENT QUADRATS IN OLD BOG



MAP 4: LOCATIONS OF PERMANENT QUADRATS IN NEW BOG



2.4 Sampling Methods

Representative cattails were excavated from the soil on-site and transported back to the lab in coolers. Individual cattails transplanted in 1987 at the 1st and 2nd openings which were amended with straw or straw and lime were excavated to evaluate their growth status compared to controls. Control cattails were collected from a site approximately 6 miles from the Elliot Lake tailings pond in a natural marsh/bog area next to the highway. Natural stand cattails and those transplanted in 1988, both having been fertilized with 20:1 or 10:1 4-18-16 (N P K) or 14-4-6 during summer 1989, were also excavated for comparisons with unfertilized controls.

2.5 Morphological Studies

Cattails were photographed to give a permanent record of their condition at time of sampling. General observations were made on the external growth characteristics of the cattails. Particular attention was paid to the extent of initiation of new roots and the level of damage to older roots and the production of new shoots and their status. After completion of the morphological assessment selected organs were fixed in FAA (formalin: alcohol: acetic acid).

2.6 Light Microscopy Methods

After being transferred from FAA through a graded alcohol series into water, control and tailing plant tissue was hand-sectioned prior to staining. Sections were stained with TBO (toluidine blue O) to elucidate general structural features (Feder and O'Brien, 1968).

For localization of metals unmordanted haematoxylin was used staining of iron, zinc, copper and manganese (Pizzolato and Lillie, 1967) and the Dithizone and Zincon methods for zinc and copper (McNary, 1960). Sections were stained for 30 minutes, rinsed and mounted in water. Sections were photographed with a Leitz Orthoflux Microscope using Kodak T-Max 100 ASA black and white or Kodacolor Gold 100 ASA color film.

2.7 Scanning Electron Microscopy (SEM) Energy Dispersive X-Ray Microanalysis

Tissue samples for x-ray microanalysis were prepared in the following manner. Samples were transferred from FAA, rinsed thoroughly in 70 percent ethyl alcohol, and moved into 100 percent. They were then critical point dried in a Tousimi Samri PVT-3 critical point drier using carbon dioxide as the intermediate fluid, mounted on metal stubs with double-sided tape and coated with carbon prior to viewing. Samples were examined at 20 kilovolts using an Amray model SEM equipped with an energy dispersive x-ray microanalysis probe. The probe was adjusted to a window size that allowed an average spectral plot to be taken of cells of a particular tissue type. Counts were taken for a minimum of 90 seconds to ensure accuracy in the spectral readout. The probe was adjusted to only give readouts of elements of higher atomic number since these were the only elements of importance.

3.0 RESULTS AND DISCUSSION

3.1 The Vegetation of the Bog

The major physical features of the overall site of the twin bogs is illustrated in Map 2 and Schematics 1 and 2. The new bog is a stray floating bog (Plate 1). The water flow through the bog is extensive and ubiquitous. Outflow occurs from the southern corner of the New bog into the old bog with a peripheral zone of cattails.

Plate 1: General view of the New stray bog

Vegetation types were mapped in each quadrat with particular attention being paid to the location and growth status of all cattails present in the quadrats (Schematics 3 and 4). Each quadrat was photographed to provide a visual record of the status of the vegetation (Plates 2 and 3). Future photographs will provide evidence of changes in the vegetation over time.

Throughout the Old bog (Plate 4), lower zones occur at three distinct troughs which almost run the length of the bog from the northwest to the southeast (Plate 5). At the southwest corner of the bog where water inflows from Grand Lake, there is a small strip of open water, dominated along its margin by cattails. The old bog is distinct from the New bog as extensive hummock formation has occurred with localized high (hummock) and low spots (hollow) (Plate 6). The majority of low spots contained, in July, either very slowly flowing or standing water.

Table 1 contains a list of all plant species identified at the two bog sites. The species assemblage is typical of that encountered in dwarf-shrub bogs of the Northeastern regions of the United States and Canada (Cowardin et al., 1979) (Plate 7). These bogs are

SCHEMATIC 3: Mapping of Plot A

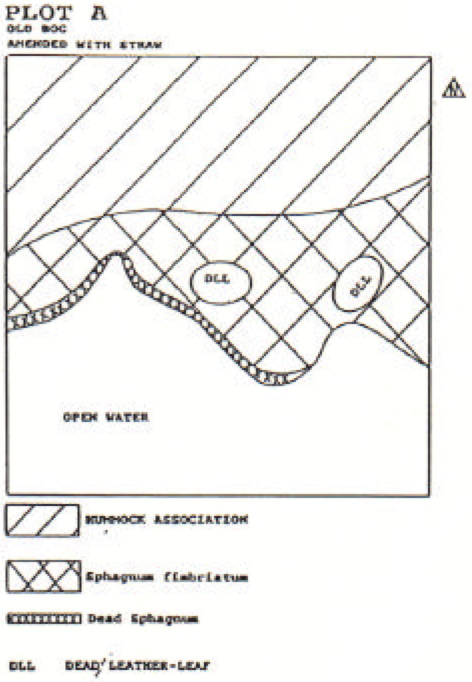


Plate 2: Photograph of Plot A

SCHEMATIC 4: Mapping of Plot G

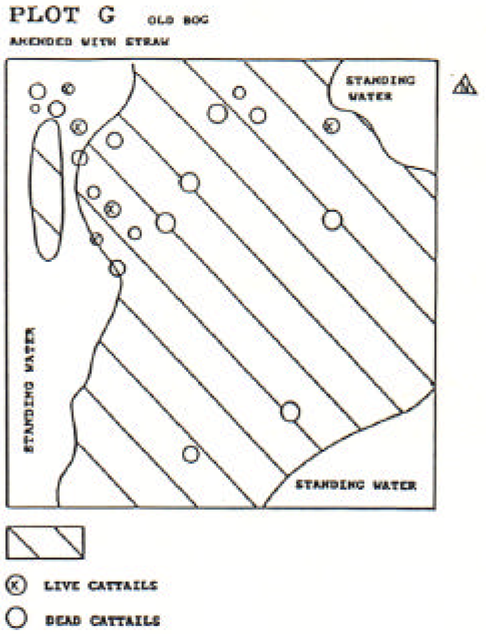


Plate 3: Photograph of Plot G

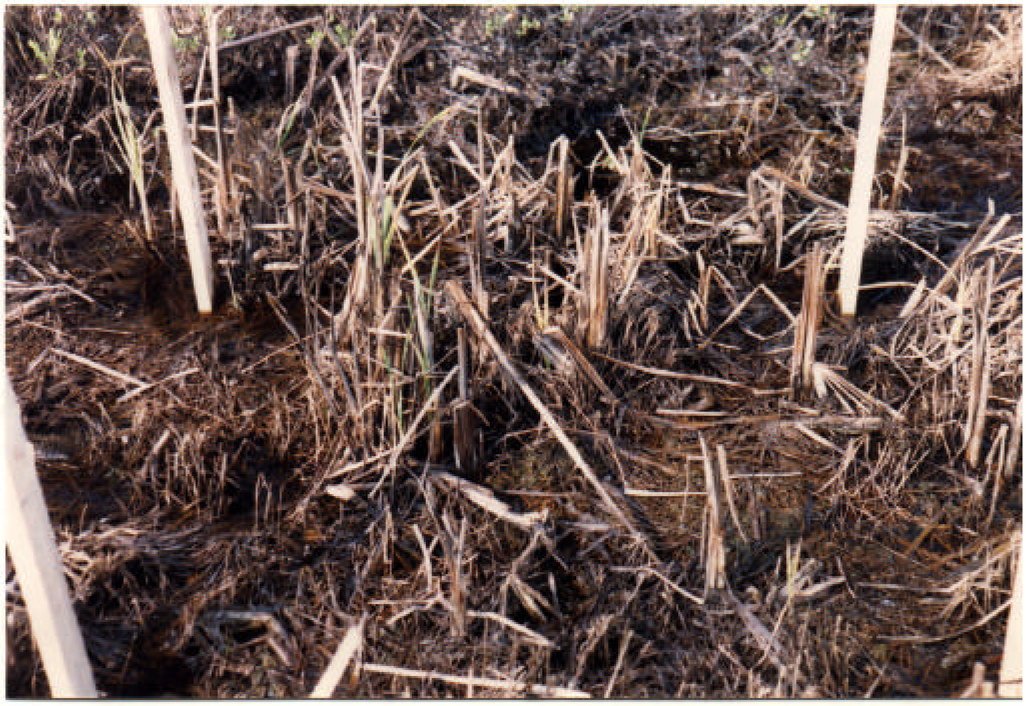


Plate 4: View of the Old bog in foreground

Plate 5: Lower zones in Old bog

TABLE 1: Plant Speicies Composition of DEVCO Bogs

Family	Genera and Species	Common Name
Typhaceae	Typha latifolia	Common Cattail
Liliaceae	Smilacina trifolia	Trifoliate Solomon's Seal
Gramineae	Calamagrostis canadensis	Bluejoint Reedgrass
Cyperaceae	Scirpus cespitosus	Bullrush
Junacaceae	Juncus inflexus Juncus canadensis	Rush Canadian Rush
Pinaceae	Larix laricina Picea mariana	Tamarack Black Spruce
Polygonaceae	Rumex domesticus	Dock
Droseraceae	Drosera anglica	Sundew
Ericaceae	Vaccinium oxycoccus Vaccinium macrocarpon Andromeda glaucophylla Chamaedaphne calyculata Kalmia angustifolia Kalmia polifolia Ledum glandilosum Ledum groenlandicum	Small Cranberry Large Cranberry Bog-Rosemary Leather-Leaf Sheep-Laurel Swamp-Laurel Labrador-Tea Labrador-Tea
Myricaceae	Myrica gale	Sweet Gale
Betulaceae	Betula pumila	Swamp-Birch
Fagaceae	Alnus rugosa	Speckled Alder
Musci*	Sphagnum fimbriatum	Mosses
	Polytrichum formosum	Mosses
Hepaticae*	Hepatic: cf. Cephaloziella	Liverwort
	Hepatic; cf. Cephalozia/Cephaloziella	

* Class

Plate 6: Hummock and hollow formation in Old bog

dominated by Chamaedaphne calyculata. The new bog can also be referred to as a moat-bog, with a partially developed moat separating the bog proper from the uplands. Bogs of this type are usually quaking and floating at their margins, although they may be grounded and raised towards the centre. Typha species are not uncommon in lower ground areas of these bogs (Damman and French,

Plate 7: A typical hummock association

1987). The presence of Typha latifolia, Juncus canadensis, Juncus inflexus and Scripus cespitosus indicates seasonal flooding of the bog and the presence of a high water table, particularly in the Spring.

3.2 Vegetation Assessment Comparisons Between the Bogs

3.2.1 Affects of hydrological characteristics on the distribution of acid damage.

A general survey of the status of the vegetation at the DEVCO site indicated both similarities and differences between the two bogs. The sudden inflow of acid waters into the New bog in Spring 1989, undoubtedly accounts for at least part of the reason why damage of the vegetation is much more severe in this bog than in the Old bog. Acid effects on the new bog may also be more uniform because the floating nature of the bog allows for a uniform movement of acid waters throughout the area. Unlike the Old bog, there is also little localized differentiation of the bog into hummocks and hollows.

In the old bog, acid water movement is diffuse, and it is uncertain how much lateral water movement is possible. In grounded bogs, in general, there is only limited movement of water beneath the surface of the bog, although seasonal flooding of the bog can cause extensive standing water areas. In the old bog, this is particularly the case in lower ground portions in the aforementioned troughs zones. In the immediately adjacent raised hummock areas, there is probably little water movement, thus allowing for less drainage of the vegetation.

3.2.2 Affects of acid conditions on the growth of natural bog vegetation

In the new bog, the prognosis for continued survival is not good for many of the species that form the natural species assemblage type common in the bogs of the area. This is particularly the case for Alnus rugosa and the woody dwarf shrubs Chamadaphne calyculata, Myrica *gale*, Kalmia angustfolia and Ledum species (Plate 7). Examination of plant parts of these species indicated similar trends. In all cases, assessments were made on the basis of comparisons with control plant material. Below ground damage (roots, rhizomes, etc.) was most extensive with many of the plants showing only scattered lateral and adventitious root hair development. Many rhizomes also showed extensive signs of internal damage and death from the cortical tissue inward. Above ground parts of the plants had few leaves and much of the woody older tissue was found to be dead. The majority of shoot tips and lateral buds were also found to be dead, suggesting little hope for recovery of the plants in subsequent years.

Affects on the aforementioned species tended to show little variation on a local scale, since little hummock formation was observed. Across the area of the New bog, damage was more extensive in the main channels of overland flow where plants were exposed more directly to acid water. At the edges of the New bog, and towards the southern limit of the bog, plants were generally healthier.

Although damage was also observed in the same species of the Old bog, the symptoms were not as severe as those seen in the New bog. Roots and rhizomes tended to show some damage but this was much reduced in comparison to that observed in the new bog. Many shoot tips were also healthy with prominent lateral buds. In the old bog, extensive variability was seen in the state of the plants between plants growing in hummocks versus hollows. Plants in the hollows showed much greater signs of acid associated damage. The extent of damage observed in plants found in the hollows of the Old bog was very similar to that observed in the New bog.

In the hollows, the prominent species present was Juncus canadensis but all biomass seen was dead plants heavily encrusted with ochre. The moss Sphagnum fibriatum also showed extensive signs of acid stress (Plate 2). It was noted that the dieback of Sphagnum fibriatum occurs only at the edges of the mat as it extends out into the water and becomes submerged.

On hummocks, a much greater diversity of species was observed in comparison to the hollows with the majority of natural species listed in Table 1 occurring (Plate 7). The mosses on these sites appeared healthy and showed signs of extensive growth. Of interest was the fact that a species replacement appeared to be occurring on the hummocks. The two species that were observed to be flourishing were Juncus inflexus and Calamagrostis canadensis. These weedy species replace the woody dwarf shrub community intolerant to the acid conditions depicted in Plate 7.

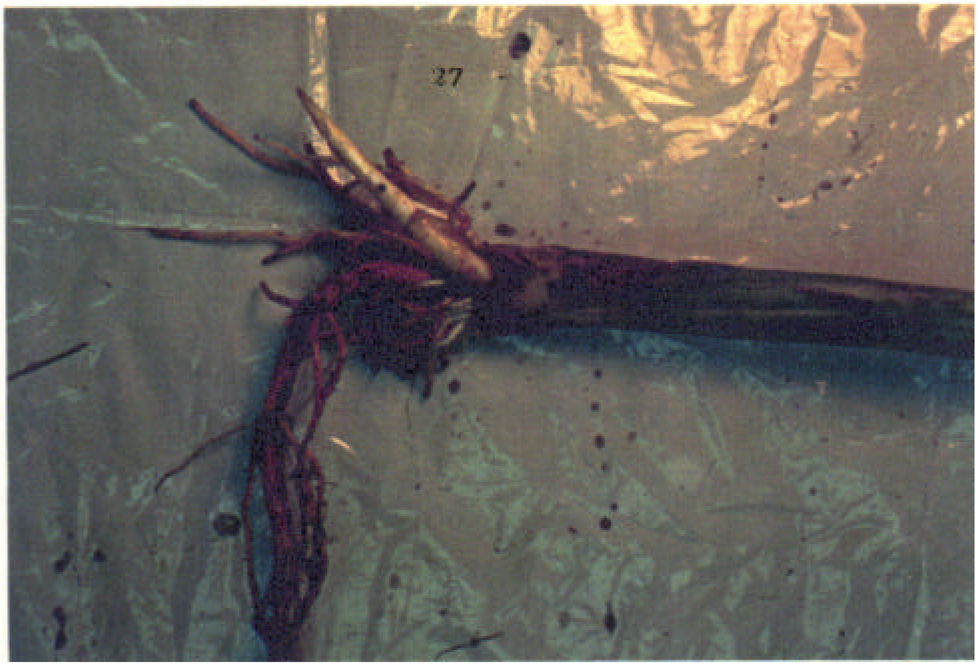
3.2.3 Effects of AMD on growth of cattail plants in the bogs

Examining the morphology and anatomy of the cattails indicated the same trends as the typical bog vegetation described above. Cattails were in better condition in the Old bog than in the New bog. The conditions described refer to the old bog. Symptoms were similar in the New bog but were much more severe.

In the Old bog, the rhizomes of the cattails showed a greatly reduced production of both lateral and adventitious roots. Few newly initiated roots were present on the rhizomes compared to controls (compare Plates 8 and 9). Many of these roots appeared to have been initiated but did not emerge from the rhizome. In anatomical characteristics, both types of roots often failed to show the typical aerenchyma type of development which is characteristic of healthy roots.

Plate 8: Cattail roots from the control bog

Plate 9: Cattail roots from the old bog



Recent studies have indicated that a continuous aeration channel connecting the previous year's stalks with new expanding lateral shoots is essential for the maintenance of healthy growth in the roots (Seago and Marsh, 1989). First signs of senescence in roots occur when their apical regions fail to undergo typical lysigenous development of aerenchyma and instead, differentiate with a solid cortex. This type of development was associated with a proliferation of adventitious roots near the tips of lateral roots shortly before growth ceased. In comparison with control plants, this phenomenon occurred very early in the elongation of lateral roots on plants from the new and old bogs.

The capacity of <u>Typha latifolia</u> rhizomes to grow buried deep in anoxic sediments is probably a function of their ability to both transport surface oxygen to growing roots and the fact that ground tissue starch reserves can be metabolized to provide the building blocks for structural tissues at least until new shoots can reach the surface, expand leaf laminas, and begin photosynthesis. This ability "to do without" has likely been an asset which has allowed <u>Typha</u> to become such a successful competitor in marsh habitats (Crawford et al., 1989).

Examination of above-ground biomass of cattail plants in both bog sites showed signs of considerable stress, demonstrating an incapacity to maintain healthy leaves. In most cases observed, the first six to eight leaves produced have died, and only the last two to four leaves were still green at the time of collection. This is in contrast to the situation at the control site and in the amended plots where all leaves produced remained photosynthetic (compare Plates 7 and 8). This observation suggests that conditions have possibly improved over the course of the season and it is only towards the latter part of the season, possibly associated with drawdown conditions, that the cattails could expand and maintain their photosynthetic tissue. Death of earlier leaves may indicate an earlier senescence in plants in the DEVCO bogs versus those seen at the control site. Cattails in acid tailings were also found to senescence somewhat earlier than the control sites (Kalin, 1984). This suggests that acid stress shortens the growing season.

Despite the detrimental effect of the acid conditions on the roots and rhizomes of the cattails observed, most plants had produced a lateral bud in the axil of each leaf initiated. Although approximately thirty percent of these were dead, the remaining buds were healthy and had the potential for expansion at some future date. All healthy buds had extensive starch reserves present at their base.

Examination of starch reserves present in rhizomes indicated that extensive starch was still present in the ground tissue, despite the fact that most plants had only expanded three or four leaves. Although this indicates that plants may be able to survive and produce further shoots for another year or two, unless a substantial photosynthetic input can be achieved, stored reserves could eventually become exhausted.

In anticipation of growth limitations due to AMD stress, foliar fertilizer was applied during the summer of 1989. The observations of the root development suggest that among fertilizer treatments, the most noticeable beneficial result of foliar fertilizer applications occurred with 10:1 4-18-16 in comparisons involving both natural stand and transplanted cattails. Cattails treated with this fertilizer showed pronounced increases in the initiation and growth of adventitious and lateral roots and root hairs. These roots were obvious by their contrasting white color with little buildup of an oxidation layer. Since root growth is the aspect of cattail growth most deleteriously affected by conditions on-site it seems feasible that the best fertilizer strategy to pursue is one maximizing root growth. Observations do indicate that application of the fertilizer at least allows cattails to increase the number of functional roots instead of just keeping pace with or experience a gradual decline in root number as the season progresses.

Nominal increases in the extent of shoot development were noted in those plants fertilized with high nitrogen fertilizer (14-4-6). Whether these are real differences will require observation in future years. Nevertheless the possibility of a gain from such an application suggest that the overall best strategy for fertilizer application may be a multipurpose 20-20-20 type of fertilizer.

3.3 Morphological Observations

Asessment of growth of all transplanted caatails is made through comparisons with "Control plants" (i.e. non-tailings, non-transplanted). Growth of these plants is discussed prior to that of tailing cattails. Typical control plants were characterized by the presence of, abundant rhizomatous shoot development and an extensive network of narrow but highly elongated adventitious roots (Plate 10, Fig. 1, 5). These roots were covered with lateral roots originating along their entire lengths. Lateral roots also exhibited an extensive development of root hairs. Roots showed no signs of abnormal growth and possessed little or fine layerings of iron hydroxides on their surfaces.

Despite the presence of considerable damage, discussed below, most cattails exhibited extensive new shoot development (Fig. 2,3). Most of this damage was associated with root death although rhizomes also showed characteristic signs of damage. Figure 4 shows the underside of the rhizome shown in Figure 3 at the point of the arrow. Characteristically the rhizome would split forming a parallel series of short cracks which then become heavily impregnated with iron.

Cattails sampled from the 1st and 2nd openings showed two types of damage syndromes. In the first, roots simply turned black and died without noticeable signs of iron plaque buildup. In the second, roots showed rapid buildups of iron plaque (Fig. 2) with death of older lateral roots (arrows, Fig. 3, 4) so that typically only a small number of stunted heavily encrusted lateral roots were present at the root apex (Fig. 6). An unusual proliferation of lateral roots was noted close to the tip on many adventitious roots (Fig. 6).

The first type of root damage discussed, most commonly occurred on cattails growing in running waters. The death of these roots may be caused simply by acid damage. The presence of flowing waters may inhibit the formation of an iron plaque layer that could act as a buffer zone against the acid conditions. The presence of iron

PLATE 10:

Fig. 1 - 10. Fig. 1. Root development at the control site. Fig. 2. Root and new shoot development of a transplant cattail from 1987. Note the two new shoots with damage to roots. Also note the heavy iron plaque buildup on the adventitious roots on the right. Fig. 3. Same as last figure. Again note the damage to roots indicated by the arrow. Fig. 4. Magnified view of rhizome at point of arrow from Fig. 3. Note parallel cracks heavily encrusted with iron plaque and stunted adventitious roots. Fig. 5. Adventitious and lateral root development from the control site. Note whitish coloration of adventitious root and extensive development of fine filamentous lateral roots. Fig. 6. Roots from transplanted cattails, Note the buildup of iron plaque, stunted and thickened appearance of lateral roots and their abnormal proliferation towards the adventitious root tip. Fig. 7 and 8. Plaque and crystal buildup on the surface of adventitious roots. Note the scars of lateral roots indicated by black arrows and the presence of crystals on the root surface. Fig. 9. Closeup of the root surface from Fig. 8. Note lateral root scar and cystals to the left. Fig. 10. SEM micrograph of a single silica crystal overlain with iron plaque.

Plate 10

1

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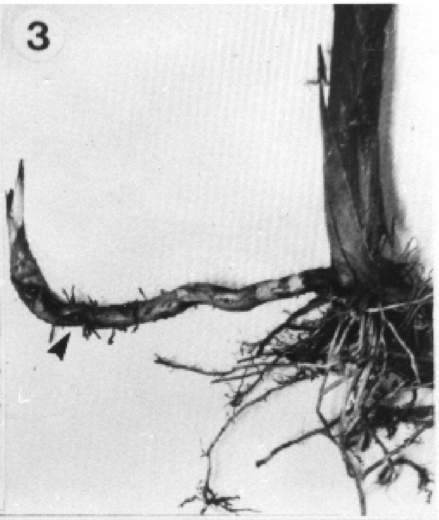

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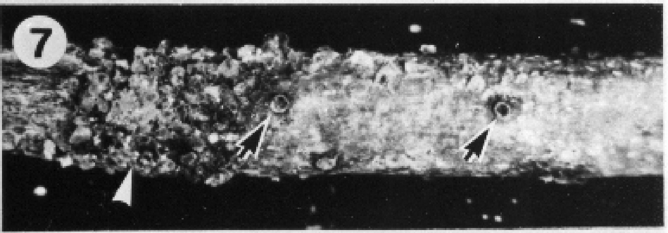

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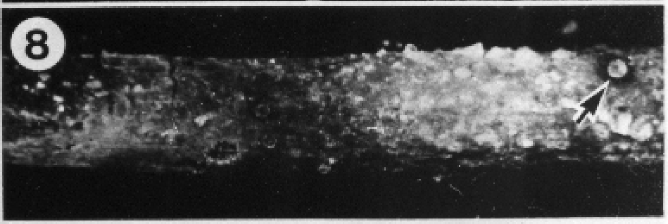

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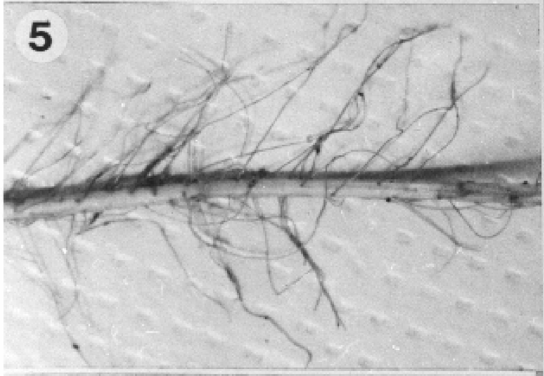

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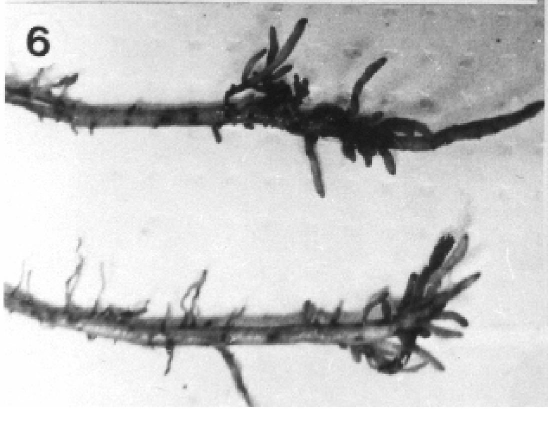

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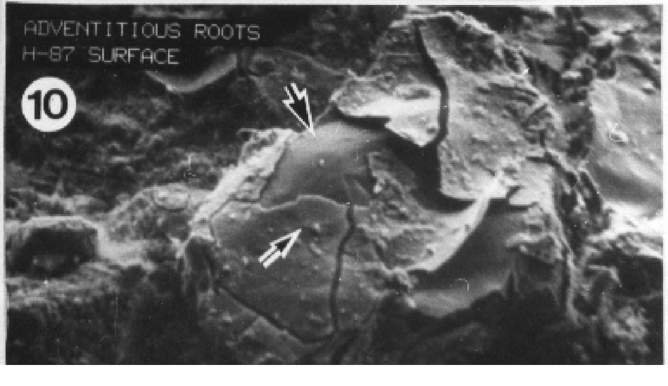


plaque layers has been postulated to slow the uptake of harmful metals in the substrate (Taylor, 1983).

Buildup of iron plaque was observed to be a cumulative process culminating in the adnation to or possible de novo formation of crystals on the root surface. The progression of buildup can be seen in Fig. 7. On the extreme left of the micrograph the plaque has been peeled away to expose the bare epidermis underneath. This area can be contrasted to the heavy plaque and crystal development adjacent to it (white arrow, Fig. 7). An intermediate level of plaque development is shown on the right of this root and to the left of the root in Fig. 8. Note the scars of dead lateral roots that are indicated in Fig. 7 and 8. In figure 9, a higher magnification view of part of the root in Fig. 7 shows some of the crystals present. The SEM micrograph in Fig. 10 indicates a common observation with various types of crystals (in this case silica, large arrow) overlain with a fine layer of iron plaque (small arrow).

The extent of crystal and iron plaque buildup appears to be most strongly correlated with the nature of the substrate immediately adjacent to the cattail roots. Height of the water table also appears to be an important factor with crystal and plaque formation increasing as the former decreases. The maintenance of a higher water table in the spring may provide more suitable conditions for cattail proliferation.

3.4 Anatomical Observations

Sectioning and staining of all types of rhizomes and roots for general anatomical features, for starch localization and for metals indicated the following trends, depicted in Plate 11, Figures 11 - 17. Control rhizomes exhibited extensive development of aerenchyma (air space tissue) in the cortex (area between HYP in Fig. 12 and EN in Fig. 11) and had a high level of starch buildup in the ground tissue (GT) (Fig. 11). Little to no plaque buildup was seen on control rhizomes.

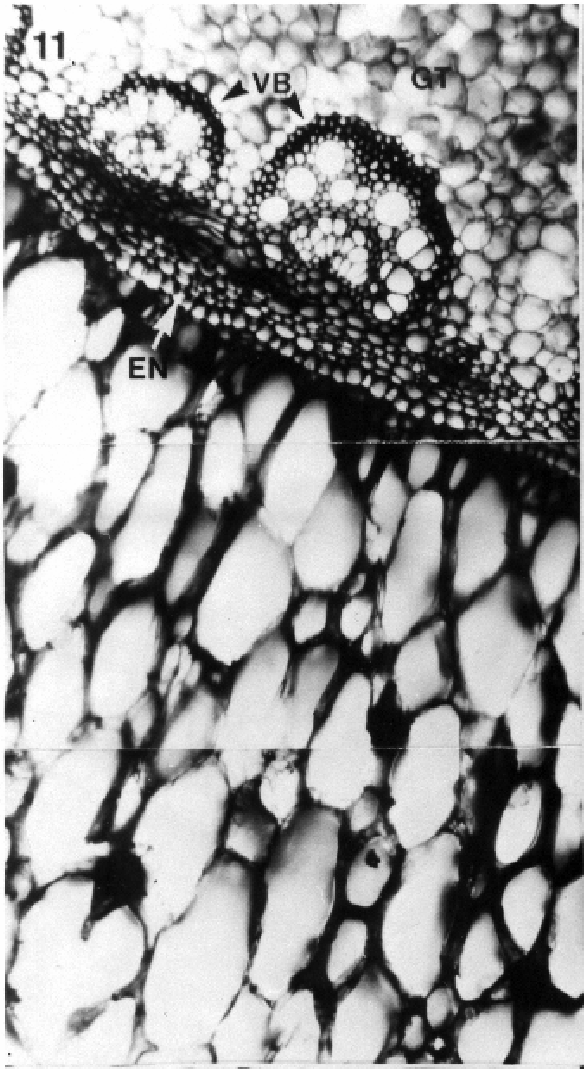
Control roots were characterized by the possession of a thin hypodermal layer (approx. 3 cell layers) relative to transplanted cattails (4-7 cell layers). Development of air spaces was also much more extensive in control cattails. The differences probably reflect a need for more structural supportive tissue to be present in roots growing on the compacted substrate on-site in comparison with the porous *Sphagnum* layer found at the control site. In an incidental fashion the consequent presence of a thicker hypodermal layer in the on-site roots appears to afford additional protection from acid damage by providing a buffer zone which can sustain considerable damage before the root is critically injured. Staining for metals on control roots indicated only peripheral

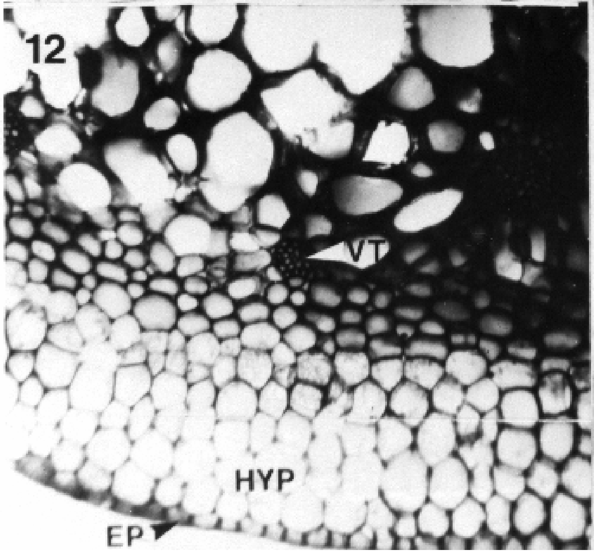
PLATE 11

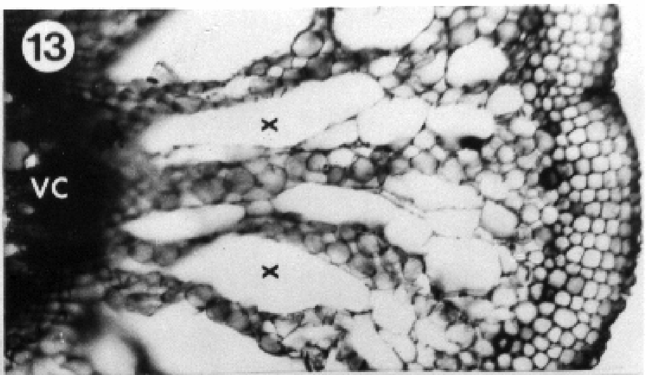
Fig. 11 - 17. Fig. 11 and 12. Cross section of a rhizome from the control site stained for general structural features. This rhizome section is continued in Fig. 12 although approximately one third of the cortex is not shown. Starting at the base of Fig. 12 note the epidermis (EP), thickened hypodermal layer (HYP) consisting of approximately 10 cell layers, central cortex tissue containing an extensive aerenchyma (air space) network, vascular tissue (VT) in the cortex, endodermis (EN) acting as a barrier to movement between the cortex and ground tissue (GT, inside endodermis). vascular bundles (VB) consisting of smaller tracheids and larger vessel elements and finally the ground tissue which is extremely important for storage of starch for new shoot development in the Spring. Fig. 13. Cross section of an adventitious root. Structural features are similar to those for rhizomes but note the radial expanded aerenchyma and solid vascular cylinder (VC) without ground tissue. Fig. 14. Cross section of root from transplanted cattails stained for metals with zincon. Note intense staining of the outer epidermal and hypodermal layers and sloughing off of partof this layer (large arrow). Fig. 15. Surface view of a young developing leaf approximately 4 cm in length. Note the presence of distinctive black hydropoten areas developing towards the base of the leaf. Fig. 16. Magnified view of several hydropoten from Fig.

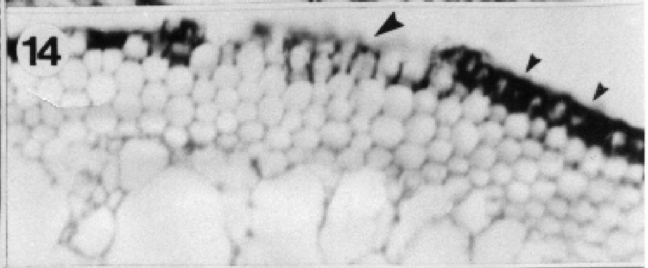
15. Note blakening of some of the cells indicating extensive metal uptake. Fig. 17. Hydropoten areas from a mature leaf. Note the intense coloration and size of hydropoten in comparison with those in Fig. 17 which are at the same magnification.

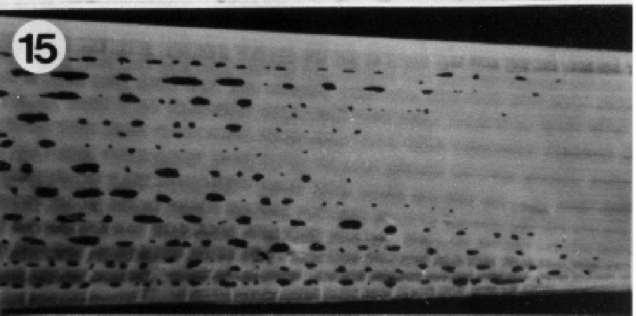
Plate 11

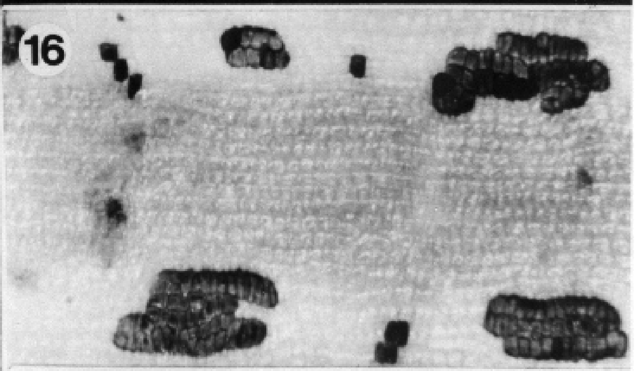


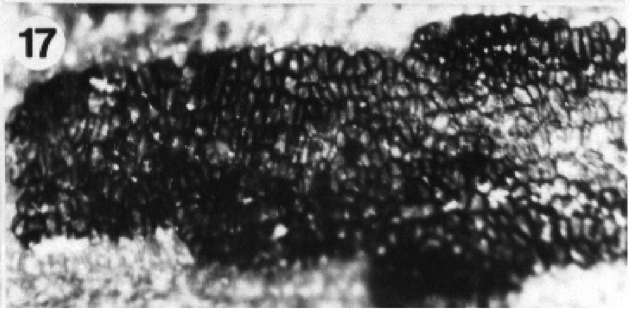












staining of the epidermis and outer one or two cell layers of the hypodermis.

Metal staining of cattail roots from on-site indicated large buildups on the epidermis and in the hypodermal layers. In many cases where buildup was extensive the epidermis and outer 3 or 4 cell layers were observed to slough off (large arrow, Fig. 14). In dead lateral roots metals concentrations were high right through the entire cross-section of the root. In rhizomes only slight buildup was observed on the epidermal and hypodermal layers although vascular bundles in the outer periphery of the cortex also showed considerable staining intensity.

Observations of young developing and mature leaves of new shoots indicated the presence of specialized glandular areas on the upper surface of the leaves identifiable by their prominent black coloration (without staining) against the white background of the leaf epidermal cells (Fig. 15, 16). Although little is known about the function of these glandular areas, referred to as hydropoten, they are commonly found on the leaves of submerged aquatic plants, and are thought to function in ion uptake (Sculthorpe, 1967). In young leaves, as in the one shown in Fig. 15 (approx. 4 cm long) the hydropoten have just begun to differentiate towards the base of the leaf. At this point the areas are very small and may consist of two to sixty modified cells (Fig. 16). Although some cells have taken up high levels of metals and are black the majority are of intermediate coloration. Figure 16 can be contrasted with figure 17 (both at the same magnification) showing the size of the these glands in the mature leaf and their intense staining indicating enormous levels of metal uptake.

Comparisons of these glandular areas to those seen in control plants indicated that although similar glandular regions were present in controls that they were much reduced in the latter case. The observations suggest proliferation of the hydropoten in response to the high metal conditions on-site and indicate a mechanism for new developing shoots to cope with the severity of the conditions in the immediate vicinity.

3.5 SEM Energy Dispersive X-Ray Microanalysis Observations

Figure 18 A - E show elemental plots for scans of specific tissue types across the lateral roots for the control site, and scans from a cattail from a transplant with intensive plaque buildup. Figure 18 G - K shows only an epidermal scan for the transplant since negligible difference was seen between the epidermal and hypodermal layers for the control site.

Figure 18 a, b, and c

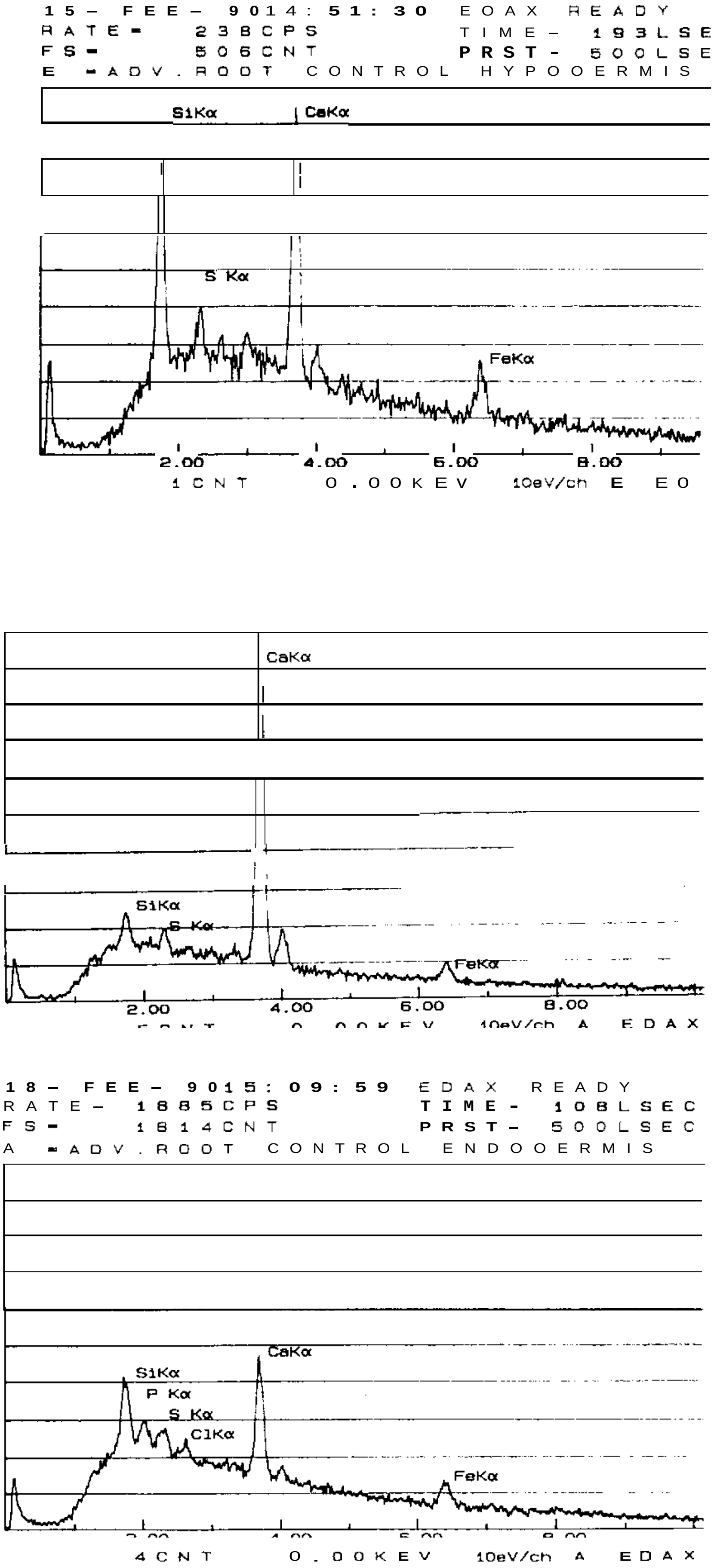
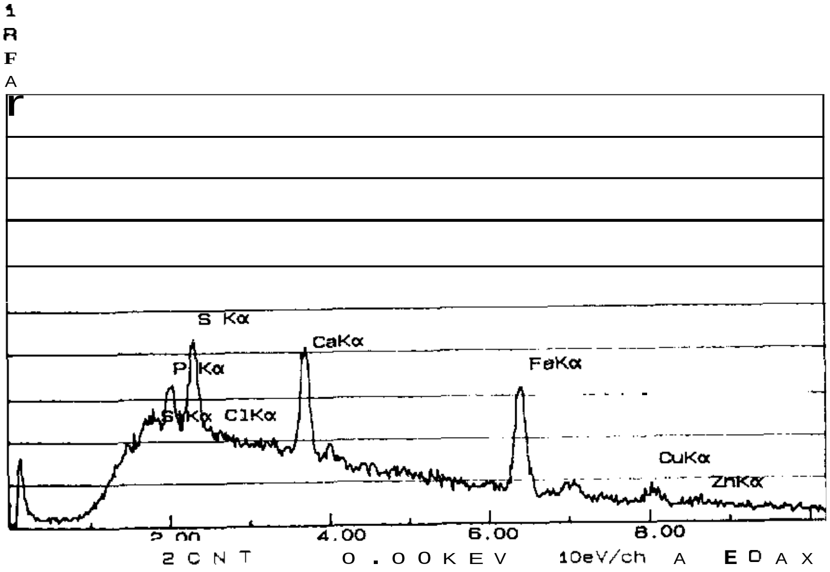


Figure **18** *g*, **h and i**



```
IS- FEW- 9015:59:40 EOAX READY
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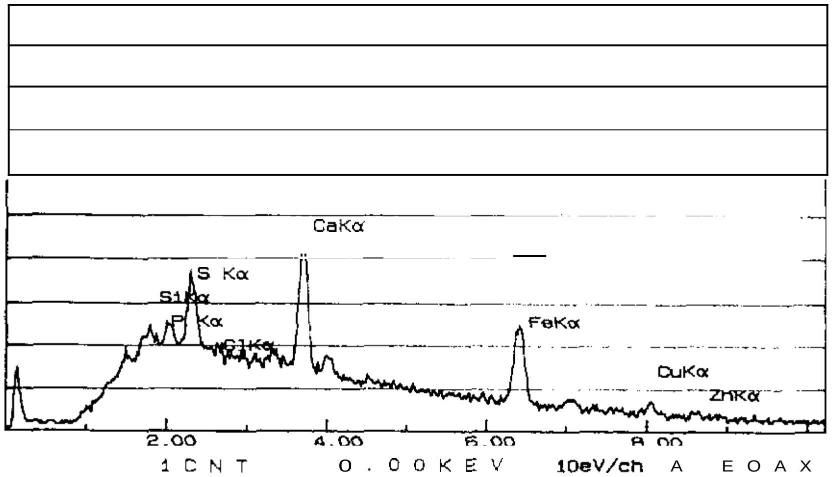
FS=    1888CNT         PRST= 500LSEC
A =ADV.ROOT U-87 CORTEX
```



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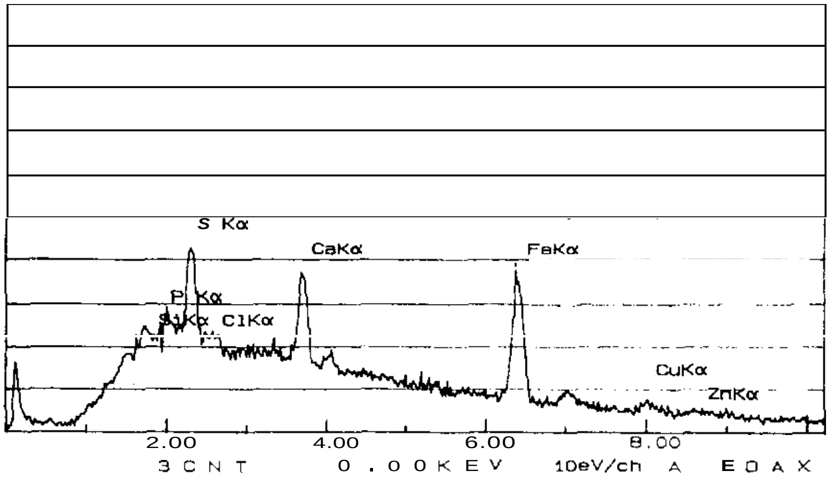
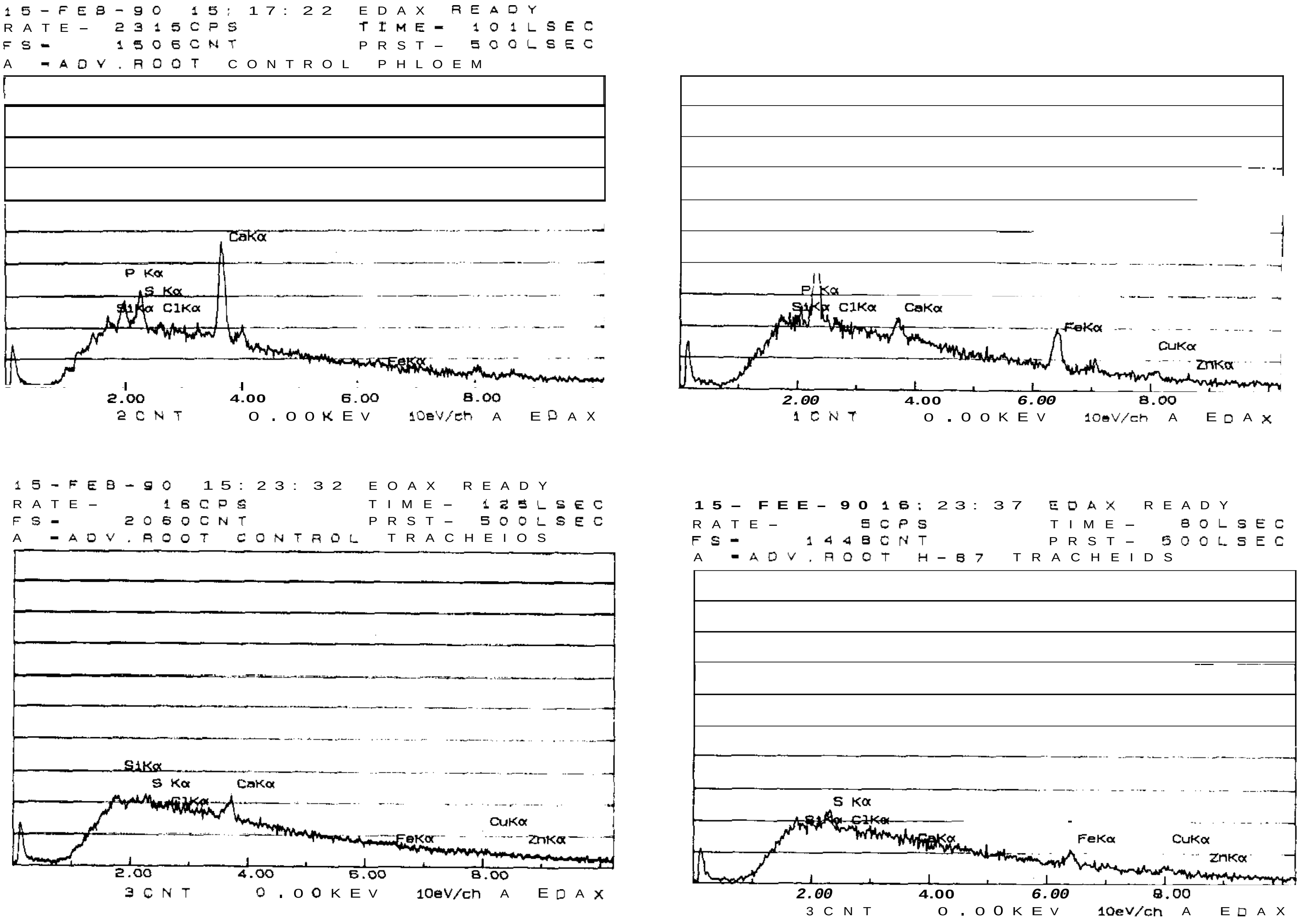
FS=    1476CNT         PRST- 500LSEC
A =ADV.ROOT H-87 HYPODERMIS
```





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RATE- 2315CPS           TIME- 101LSEC
FS- 1506CNT             PRST- 500LSEC
A =ADV.ROOT CONTROL PHLOEM
2CNT    0.00KEV   10eV/ch A   EDAX
```

```
1CNT    0.00KEV   10eV/ch A   EDAX
```

```
15-FEB-90 15:23:32 EDAX READY
RATE- 16CPS             TIME- 125LSEC
FS- 2060CNT             PRST- 500LSEC
A =ADV.ROOT CONTROL TRACHEIOS
3CNT    0.00KEV   10eV/ch A   EDAX
```

```
15- FEE- 9016:23:37 EDAX READY
RATE- 5CPS              TIME- 80LSEC
FS- 1448CNT             PRST- 500LSEC
A =ADV.ROOT H-67 TRACHEIDS
3CNT    0.00KEV   10eV/ch A   EDAX
```

Figure 18 d, e, j and k

Within the control site the most prominent peaks in the hypodermal layer are for calcium and silica with a small iron peak. The calcium peak reflects high levels of structural cell components. The high silica component is somewhat harder to explain. The low levels of iron and sulfur suggest a small amount of iron plaque buildup. All elements drop off in the cortex with only a high calcium peak, once again indicating a structural component. In the endodermis (EN, Fig. 11), which represents the barrier between the cortex and central vascular cylinder (VC, Fig. 13). In the endodermis and through the vessel elements (Figure 18 D) and tracheids (Figure 18 E) of the vascular tissue calcium continues to drop off as do all other elements.

Elemental scans from transplanted cattails indicate very high levels of iron in the epidermis associated with plaque and crystal development. Very small amounts of copper and zinc are also present. In the hypodermis high levels of iron are still present but have dropped substantially from the epidermis. Associated with the iron peaks are sulfur peaks and small calcium peaks. In the cortex and endodermis iron levels drop but still stay well above background. Iron levels also drop in the vascular tissue particularly in the tracheids. The results indicate that although iron adsorbs to the surface of the roots that some uptake through vascular tissue is occurring.

Comparisons of scans for the control versus transplanted roots indicate several important differences. The major difference is in the levels of iron present, the transplanted tissues having double to three times the levels of those in the control site. Of particular interest also was the presence of substantially smaller amounts of calcium in tissues of transplanted roots. The differences may reflect the presence of sulfate which could form $CaSO_4$ in the rhizosphere thus reducing the availbale Ca for uptake. Thus gypsum precipitation may adversely affect cattail growth. If this is the case a strategy for fertilizing may require supplements of calcium in addition to nitrogen,
phosphorus and potassium.

Figure 19 gives three scans for the surface epidermis of a control root and two from a transplanted cattail (Figure 20 - 21). The former indicates an array of small peaks associated with structural tissue and a small iron peak. Figure 20 shows the scan for the crystal depicted in Fig. 10 indicating that it is silica (quartz) and the overlying layer is iron plaques associated primarily with sulfur (Figure 21).

Figure 22 and 23 give overlay plots for scans taken of hydropoten areas (dark line) and adjacent epidermal areas (light line) on the leaves of shoots from transplanted cattails. Note the very high levels of iron on hydropoten versus epidermal cells particularly in Figure 22. All other peaks on the scans distinguish typical leaf cell structural tissues.

Figure 19: X-ray Microanalysis scan for control adventitious root

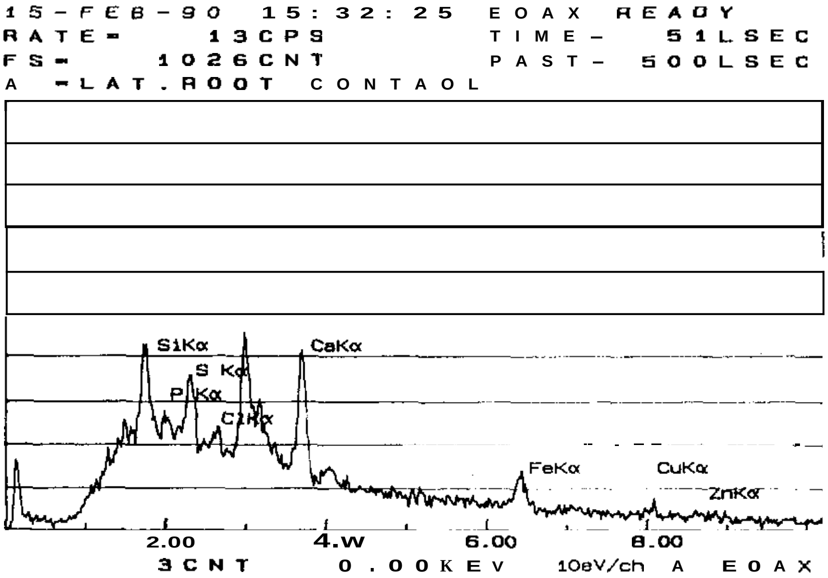


Figure 20: X-ray Microanalysis scan for a transplanted adventitious root

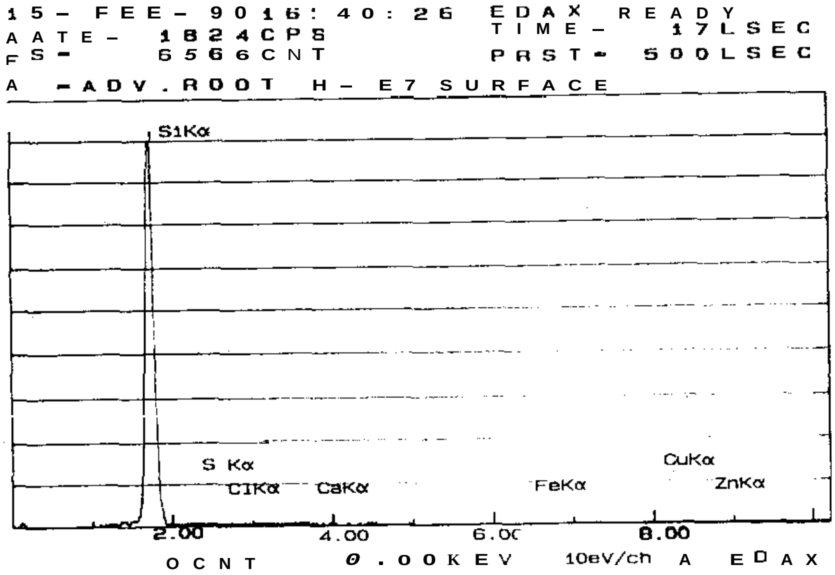


Figure 21: X-ray Microanalysis scan for an adventitious root

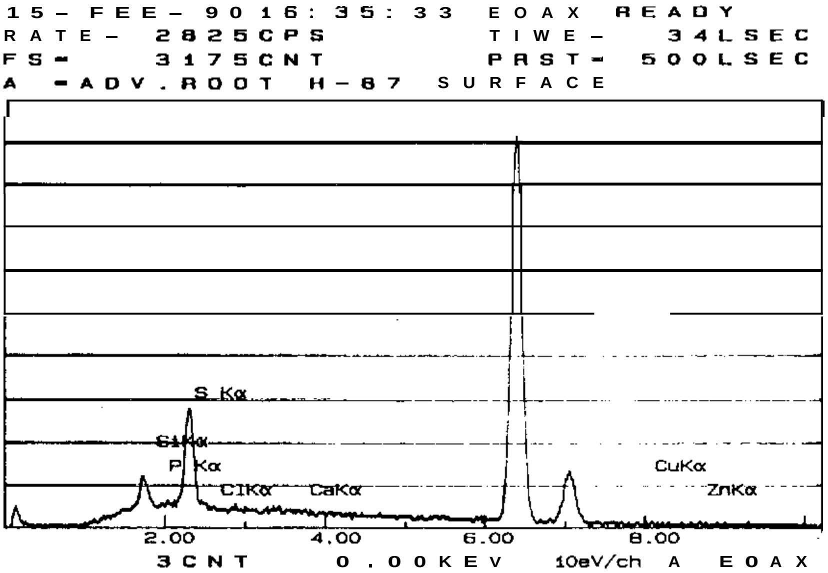


Figure 22: X-ray Microanalysis scan of a leaf hydropoten (dark line) overlayed with a plot for an adjacent epidermal cell area

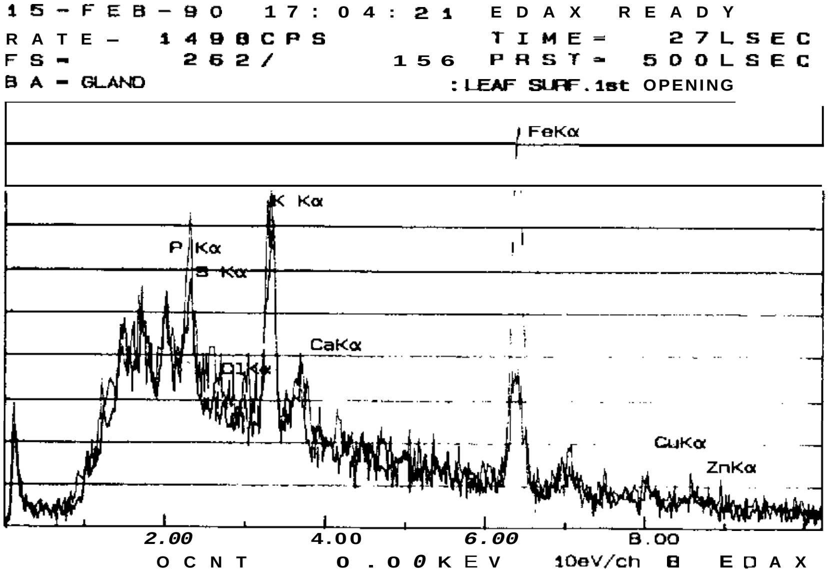
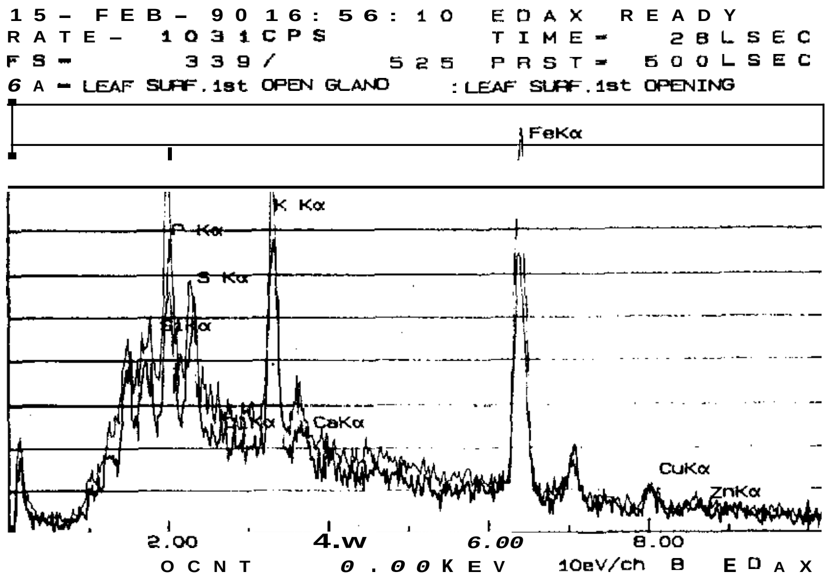


Figure 23: X-ray Microanalysis scan of a leaf hydropoten (dark line) overlayed with a plot for an adjacent epidermal cell area



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RATE-  1031CPS          TIME=   28LSEC
FS=      339/      525  PRST=  500LSEC
6 A = LEAF SURF.1st OPEN GLAND    :LEAF SURF.1st OPENING
```

```
0CNT     0.00KEV    10eV/ch  B    EDAX
```

4.0 CONCLUSIONS

An overall assessment of conditions at the bogs suggests that most of the natural species in the bogs will not survive as a result of the transition of chemical changes brought about by AMD. Ultimately, these species, because of their slow growth and low turnover rate, contribute considerably less decomposing matter which is required for the amelioration of AMD. The most important species in this regard is Typha latifolia. Other grasses, sedges, and rushes may however compete in colonizing of the dying bogs. Thus, a change in species composition of the bogs can be expected.

It is likely that the twin bogs evolved from open marshy bog to areas dominant with cattails. As acidity increased in the marsh, floating mats of Typha were formed in between the surviving hummock communities. Mallik (1989) has recently shownthatvigour of Typha glauca is strongly correlated with mat thickness, distance above the water table and pH decrease. The fact that the new bog is a floating bog probably indicates that it is of newer origin and has a higher water table than the old bog.

The observations on cattails in the acid stressed bogs indicate that the single most important detrimental factor is probably the acidity, since even cattails growing in the open water zones showed no signs of improved vigour. An alternative possibility is that cattails growing within the bog proper are suffering from lack of water, particularly as the season progresses and natural drawdown occurs. An important point to keep in mind is that bogs are basically xeric habitats. Vegetation types found on bogs (particularly Ericaceous shrubs) are adapted to require little water, primarily because most of the water is held tightly by the peat moss itself.

An alternative suggestion may be to burn the bogs in either late Fall after shoot senescence or in early Spring before shoot emergence to release nutrients into the bogs. A recent study has indicated that this strategy appears to substantially increase the vigour and number of new shoots produced (Krusi and Wein, 1988).

The other associated problem for a species such as Typha which exhibits rapid growth is that, as thickness of the Typha mat increases, so does the organic matter which is slowly decomposing in the mats. This results in "nutrient lock-up", an unsuitable condition for cattail growth.

Foliar applications of fertilizer may offer a possible solution to this problem. However, reshlts are too preliminary at this point to determine whether this strategy will increase survivorship and clonal growth in the Typha plants. Preliminary evidence on the affects of foliar fertilizer application at the Denison Mine project (Stanrock) indicates that high phosphorus and potassium fertilizer greatly increases root growth.

Several important findings from this study indicate possible methods to improve the growth and development of transplant cattails on-site. In particular, the study of fertilizer affects on growth suggest most pronounced benefits with high phosphorus potassium fertilizer primarily because of enhanced root production. Alternatively, high nitrogen fertilizer appears to slightly increase above ground leaf development. Therefore the best strategy would appear to involve usage of a 20 20 20 type fertilizer.

SEM x-ray microanalysis studies suggest a possible calcium deficiency in plant tissues probably because of it's high binding affinities for sulfur which is present in large quantities on-site. Growth of cattails may therefore be enhanced by addition of calcium to the fertilizer treatment.

One additonal point worthy of note concerns observations on the nature of metal damage to roots. These observations suggest that as the season progresses and water levels drop on-site that considerable localized tissue damage may occur because of direct contact. To lessen the magnititude of this affect it may be worthwhile to consider retaining water in the transplant area by damming late in July.

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