MORPHOLOGICAL/ANATOMICAL INVESTIGATION OF CATTAIL TRANSPLANTS AND BOG VEGETATION

FINAL REPORT

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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 élevées 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.

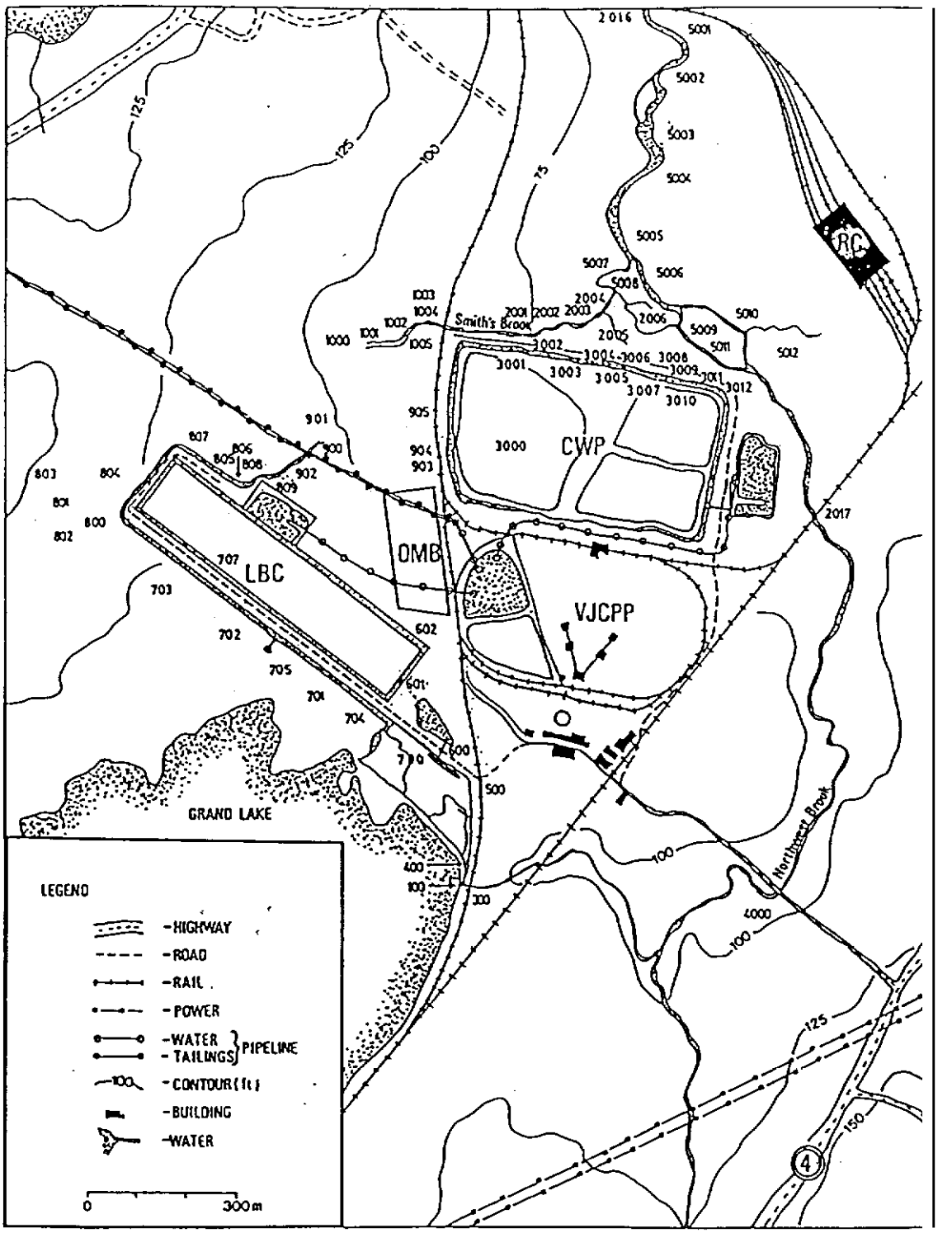
1.0 INTRODUCTION

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.

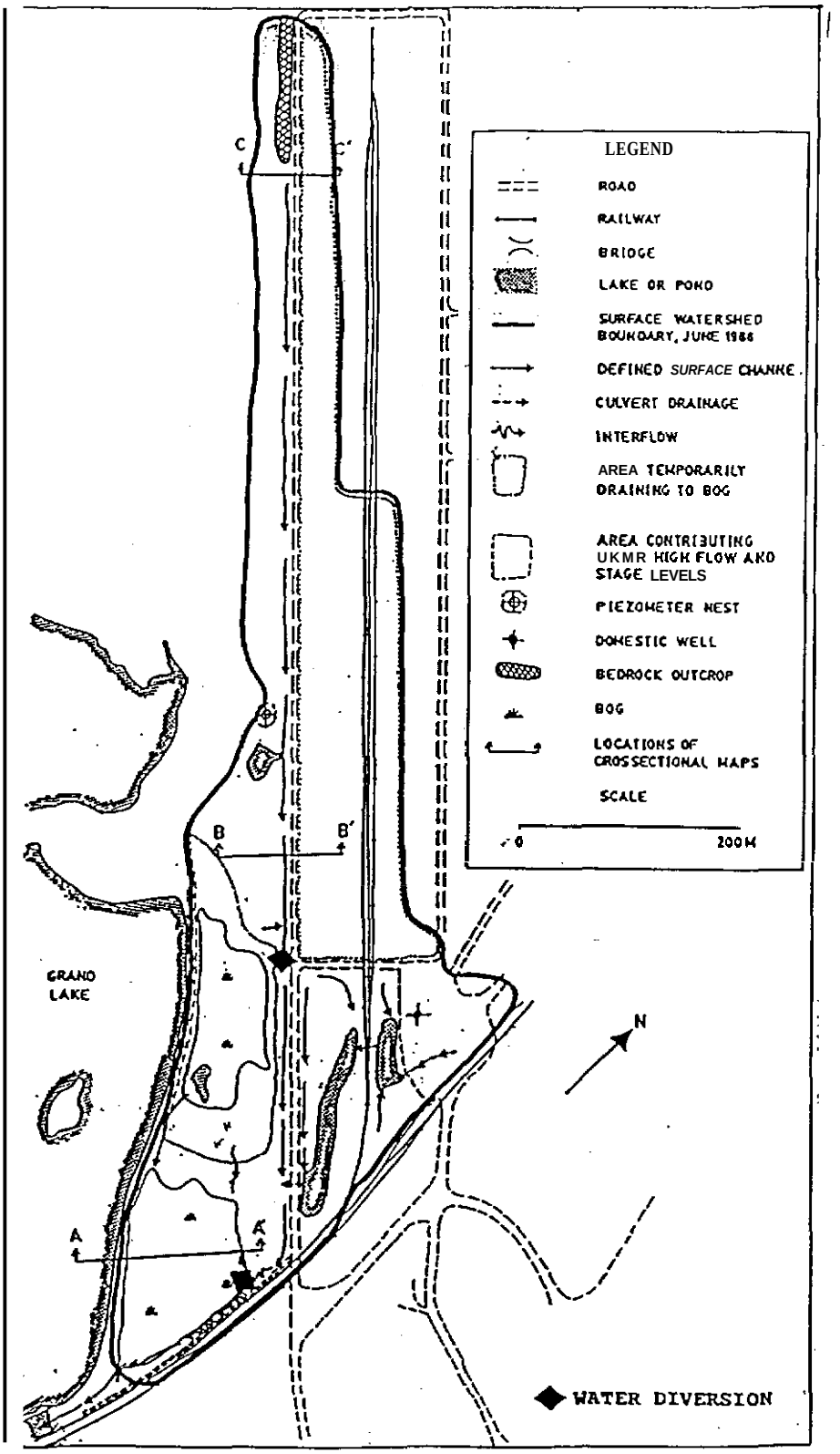
The study further encompasses the evaluation of the status of the growth and development of one and two year old cattail transplants and cattails in natural stands. These transplanted cattails are located at various points along the spill area of a tailings site in Elliot Lake. Cattail development was 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

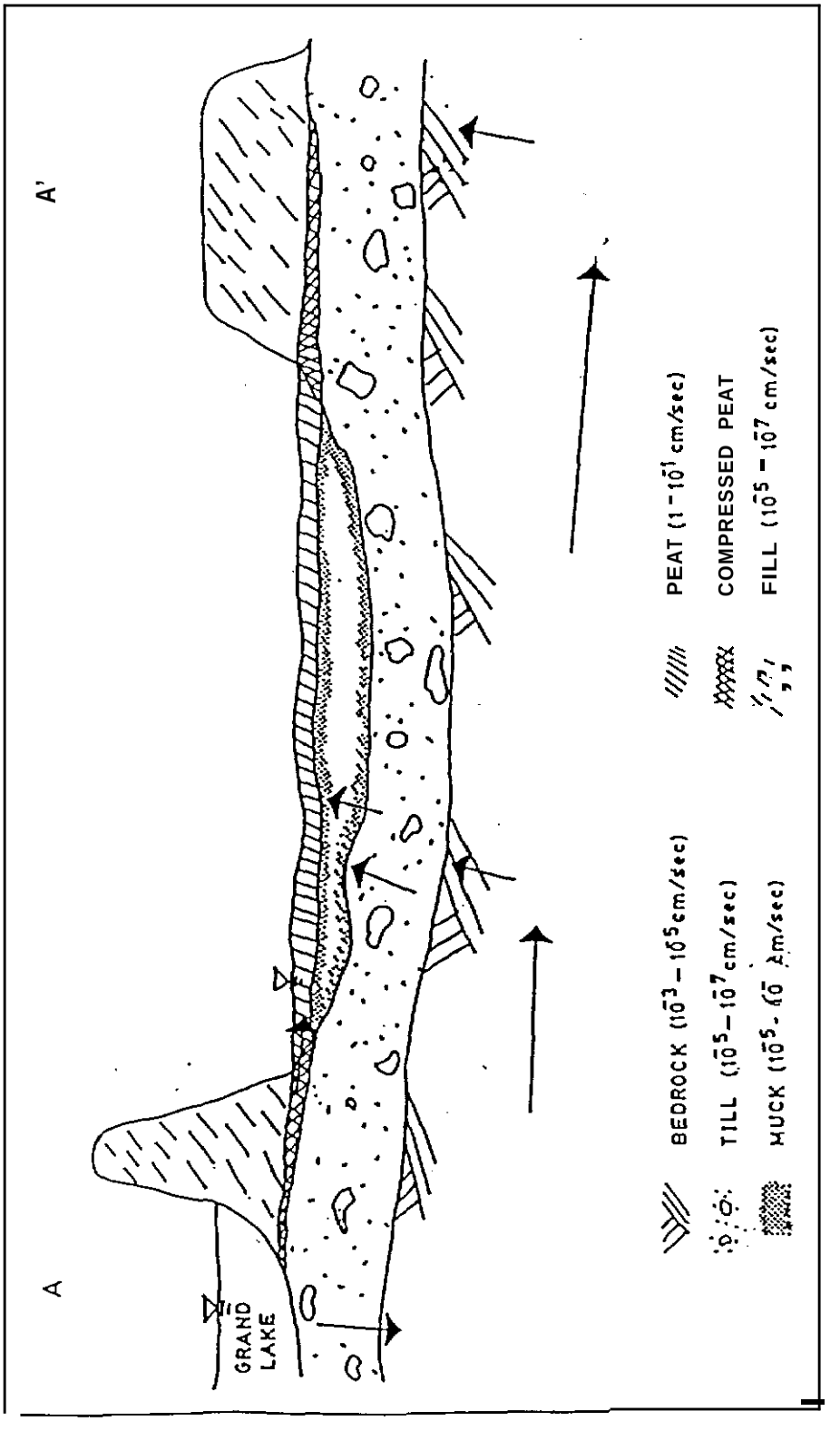
2.1 Site Description: Hydrological conditions of the bogs

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



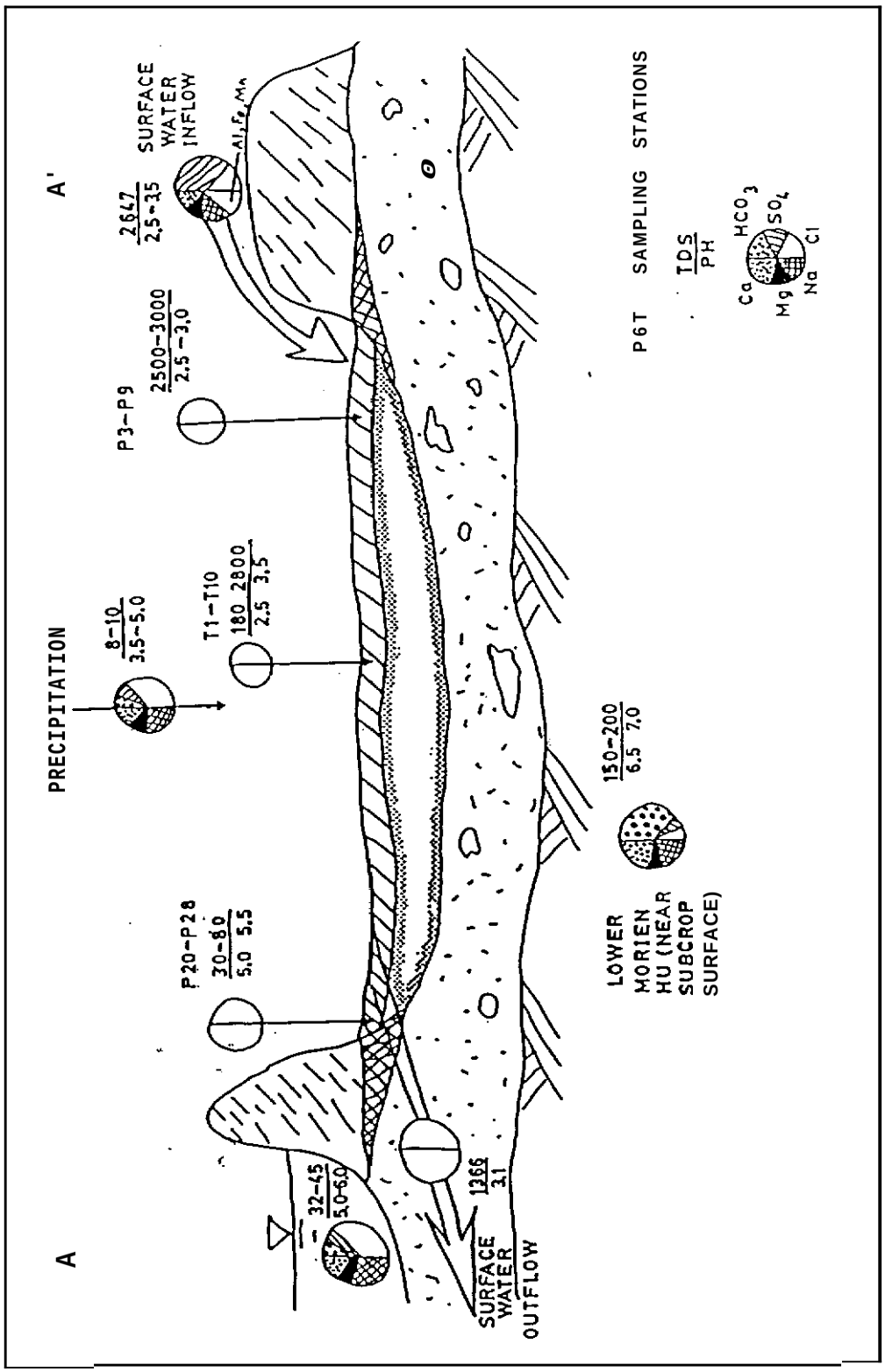
Thus ground water will enter the bog, together with water from Grand Lake, in addition to the acid mine drainage. The geomorphological and hydrological characteristics are summarized in Schemnatic 2 for conditions found in these bogs.

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



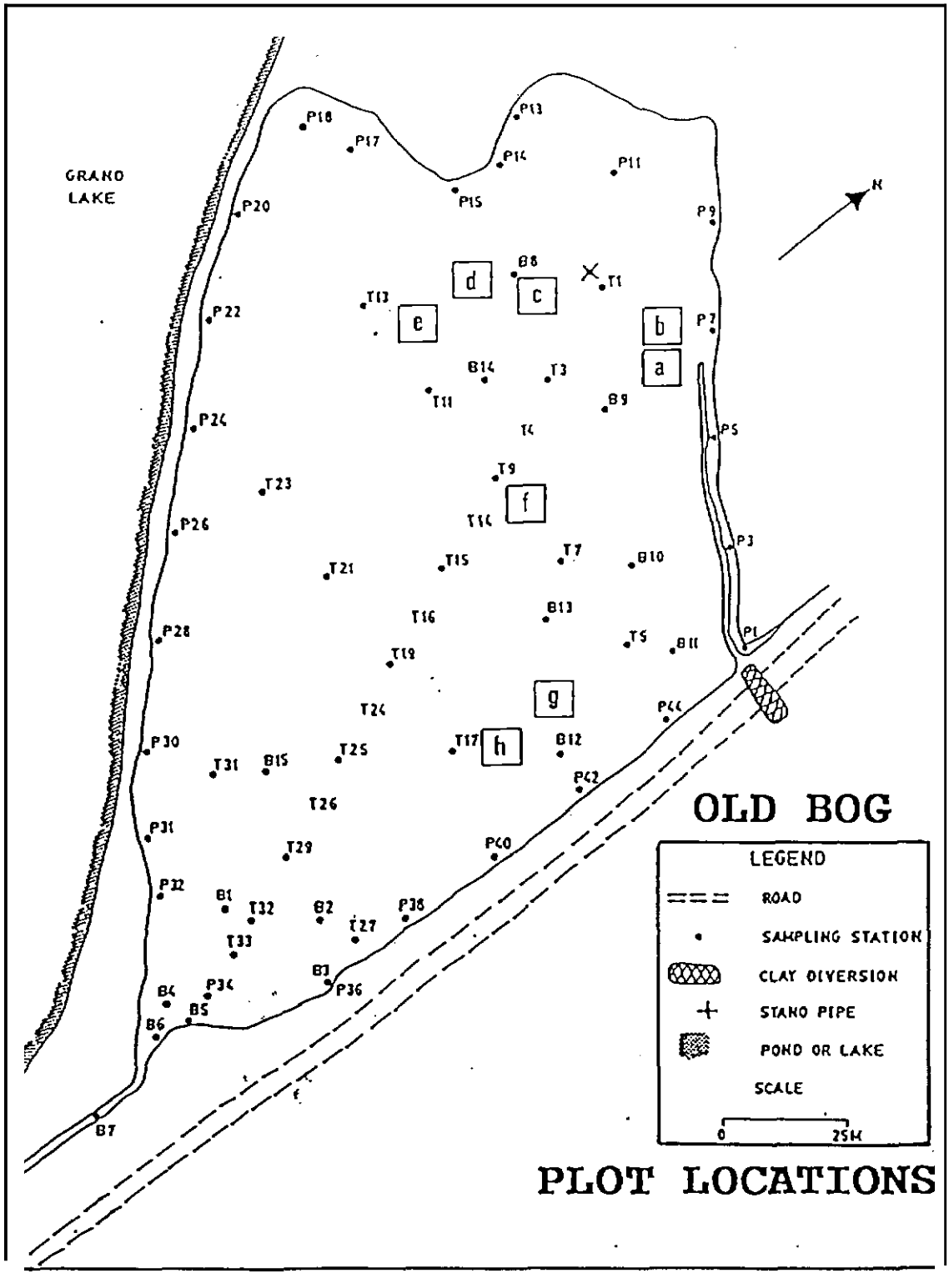
ten permanent quadrats were established.

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.

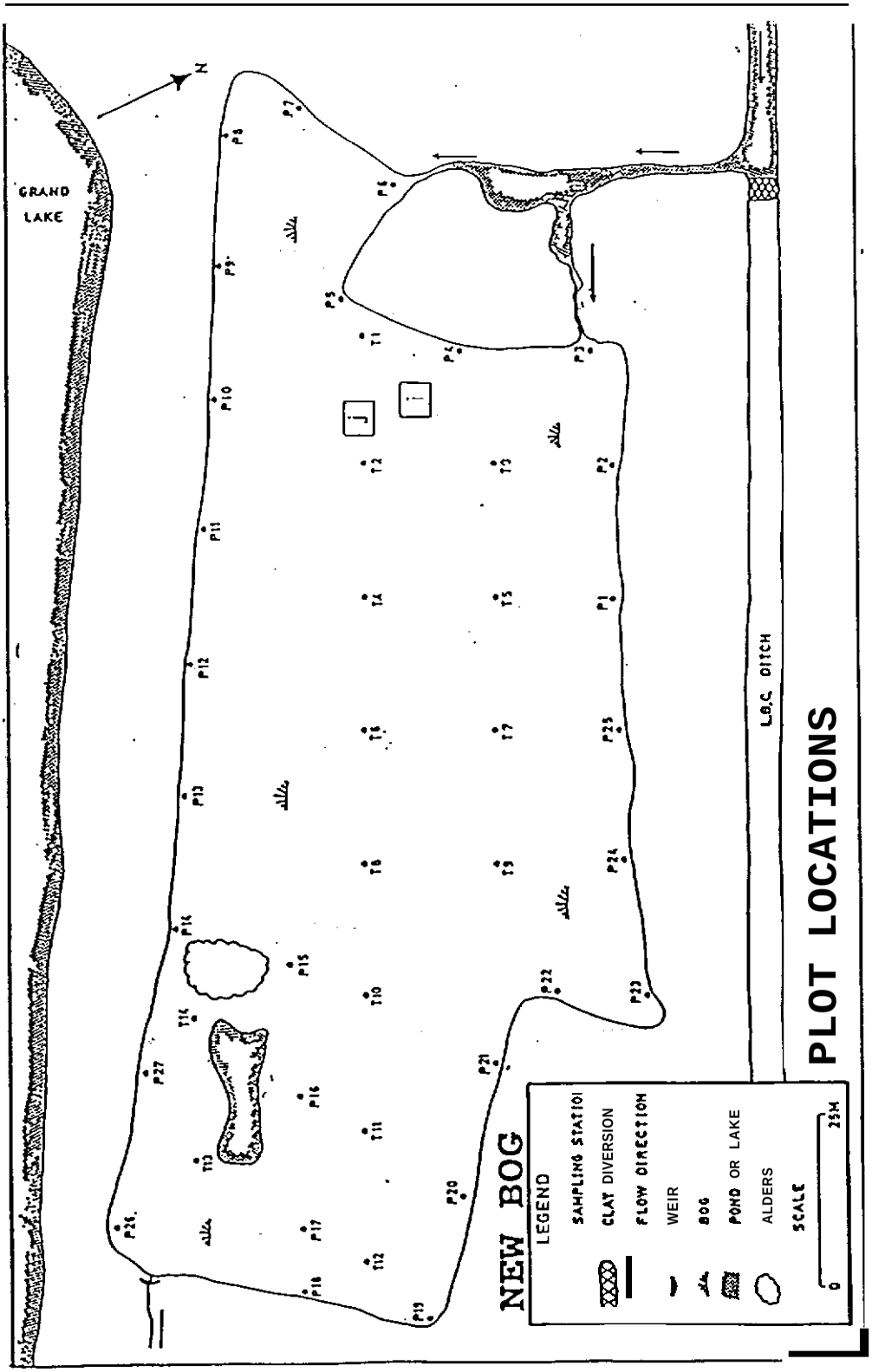
2.3 Microscopic Methods

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

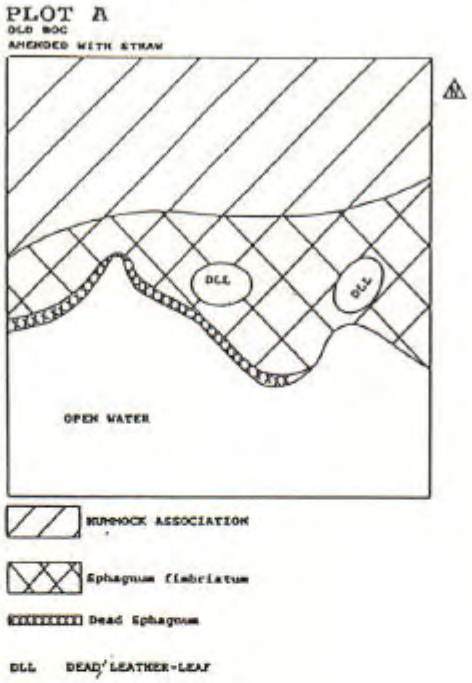


Plate 2: Photograph of Plot A

SCHEMATIC 4: Mapping of Plot G

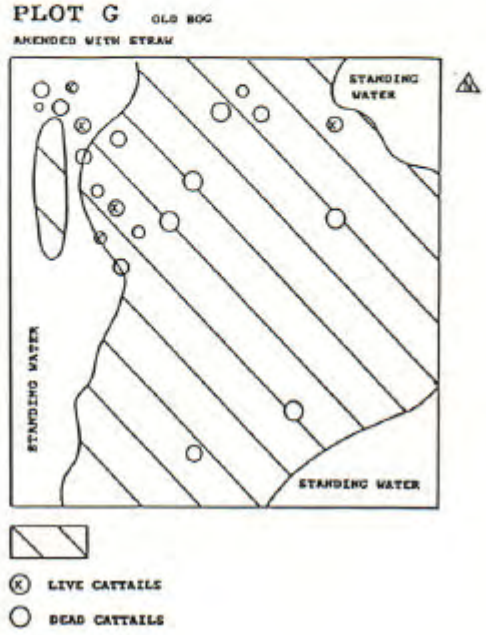


Plate 3: Photograph of Plot G

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 afore-mentioned 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 Typha latifolia 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 Typha 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.

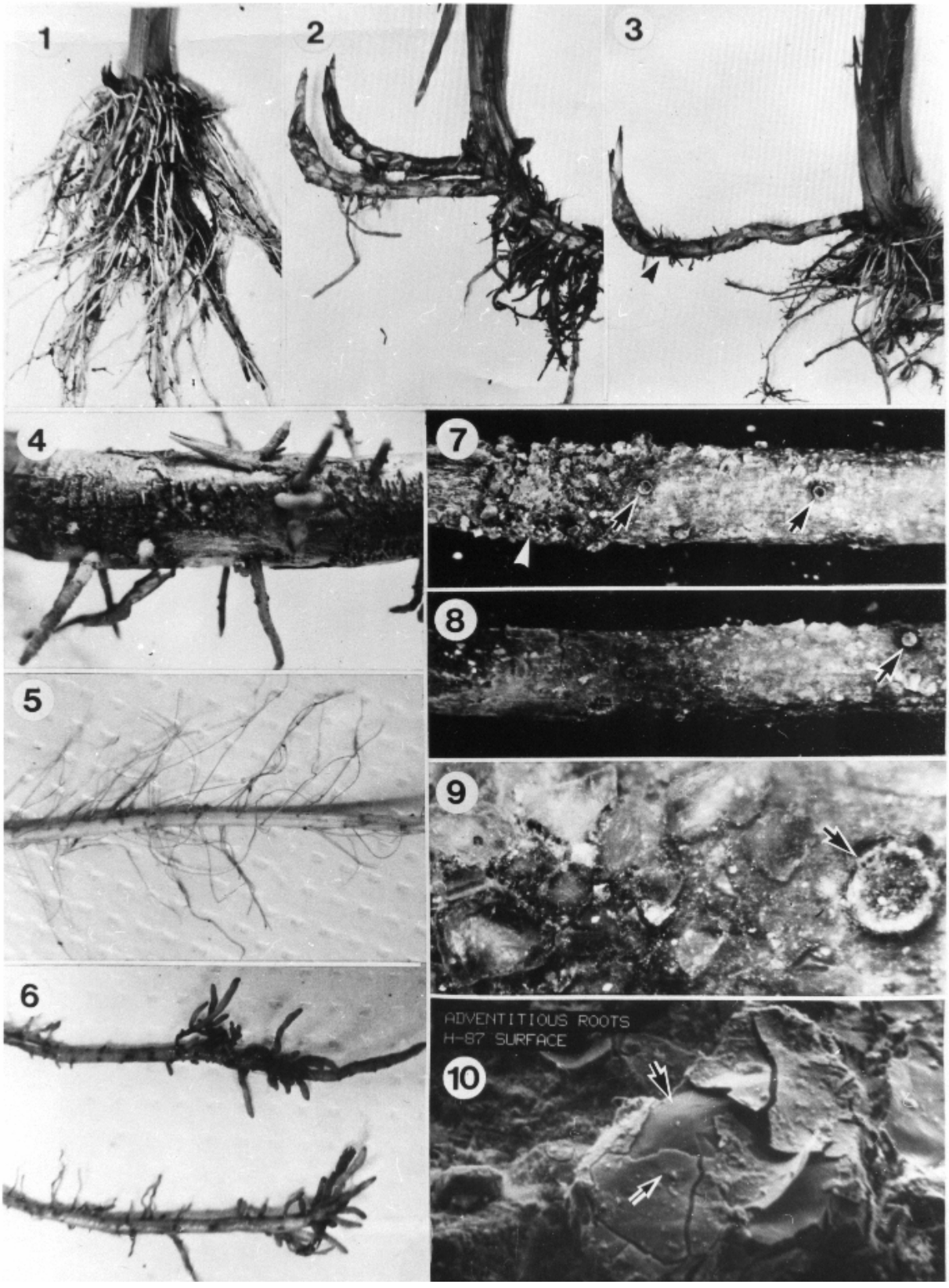
Cattails sampled from the 1st and 2nd openings showed two types of damage syndromes. In the first, roots simply turned black and died without noticeable signs of iron plaque buildup. In the second, roots showed rapid buildups of iron plaque (Fig. 2) with death of older lateral roots (arrows, Fig. 3, 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



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.

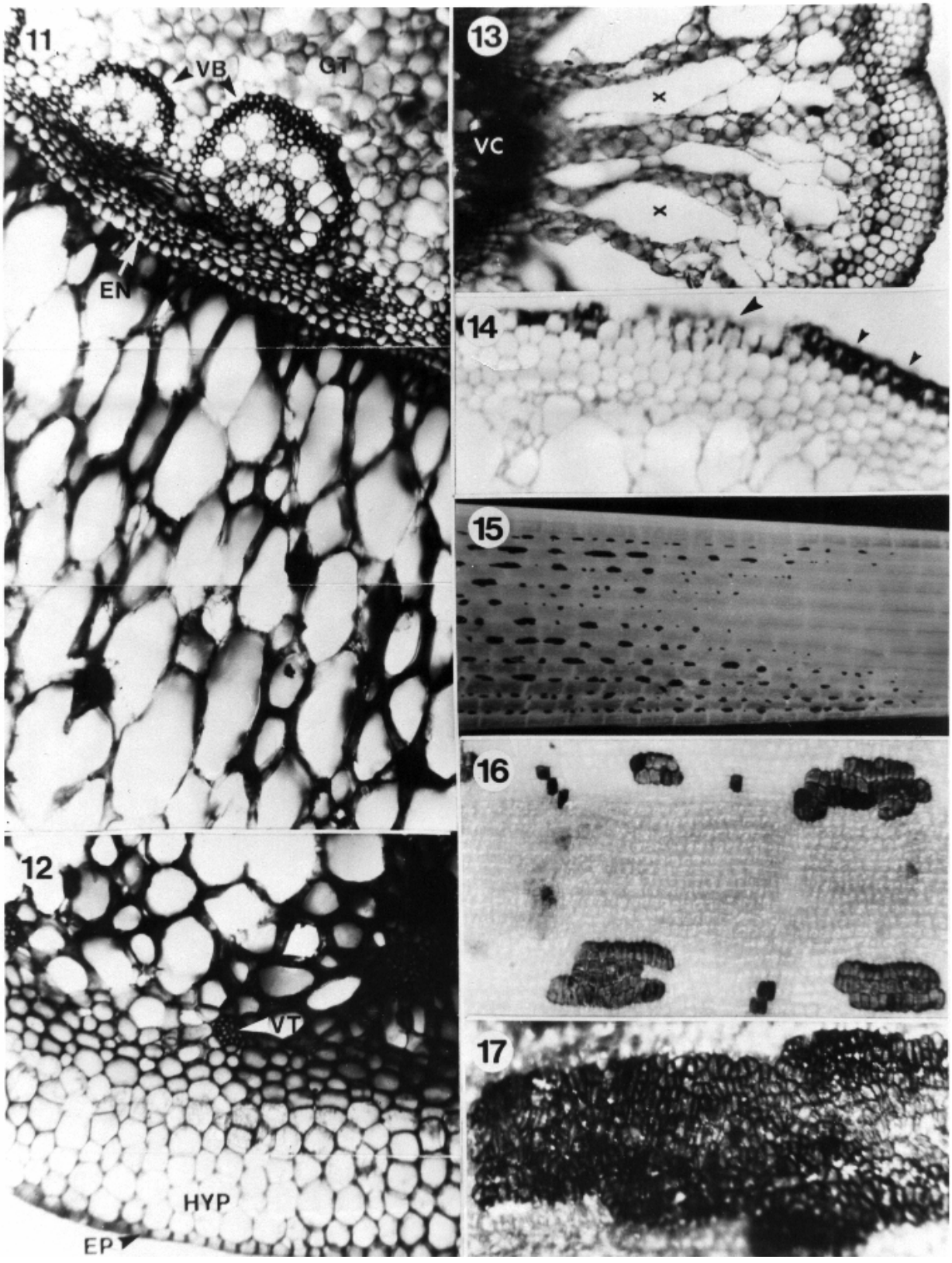
Control roots were characterized by the possession of a thin hypodermal layer (approx. 3 cell layers) relative to transplanted cattails (4-7 cell layers). Development of air spaces was also much more extensive in control cattails. The differences probably reflect a need for more structural supportive 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 !!



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

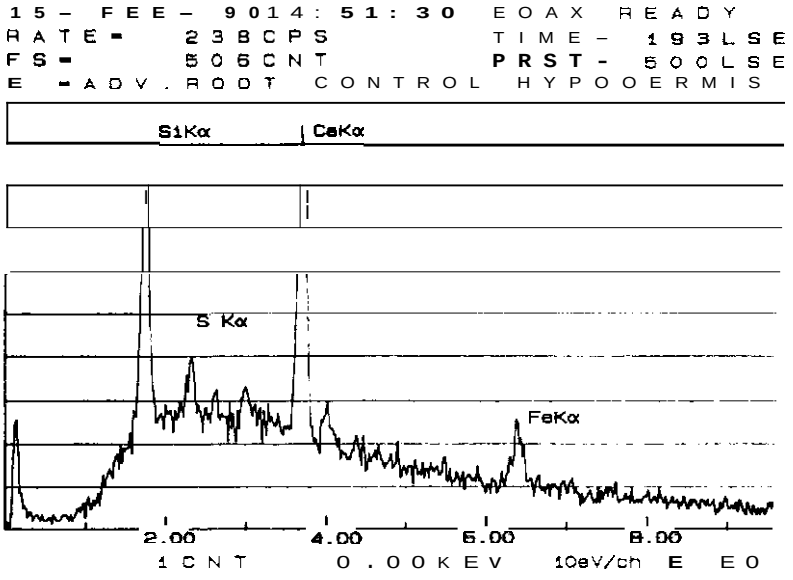
consist of two to sixty modified cells (Fig. 16). Although some cells have taken up high levels of metals and are black the majority are of intermediate coloration. Figure 16 can be contrasted with figure 17 (both at the same magnification) showing the size of the these glands in the mature leaf and their intense staining indicating enormous levels of metal uptake.

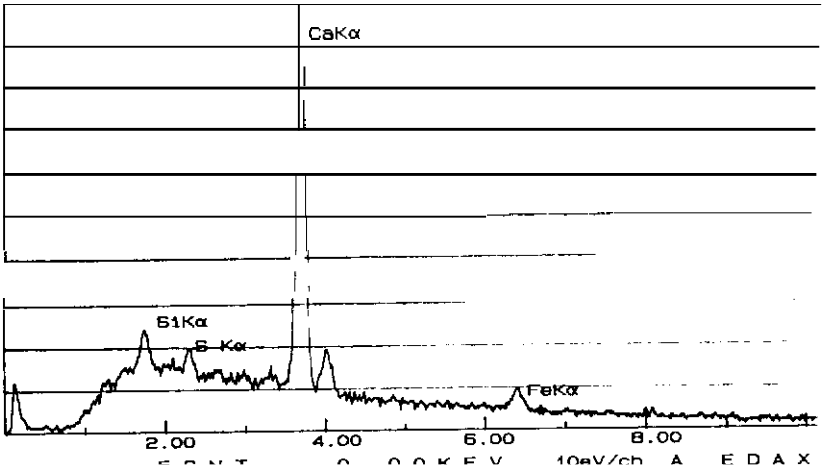
Comparisons of these glandular areas to those seen in control plants indicated that although similar glandular regions were present in controls that they were much reduced in the latter case. The observations suggest proliferation of the hydropoten in response to the high metal conditions on-site and 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





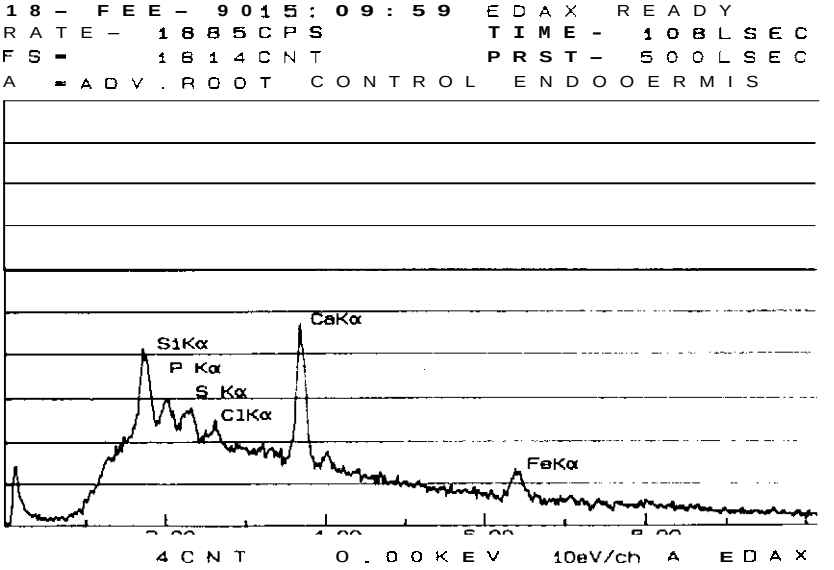
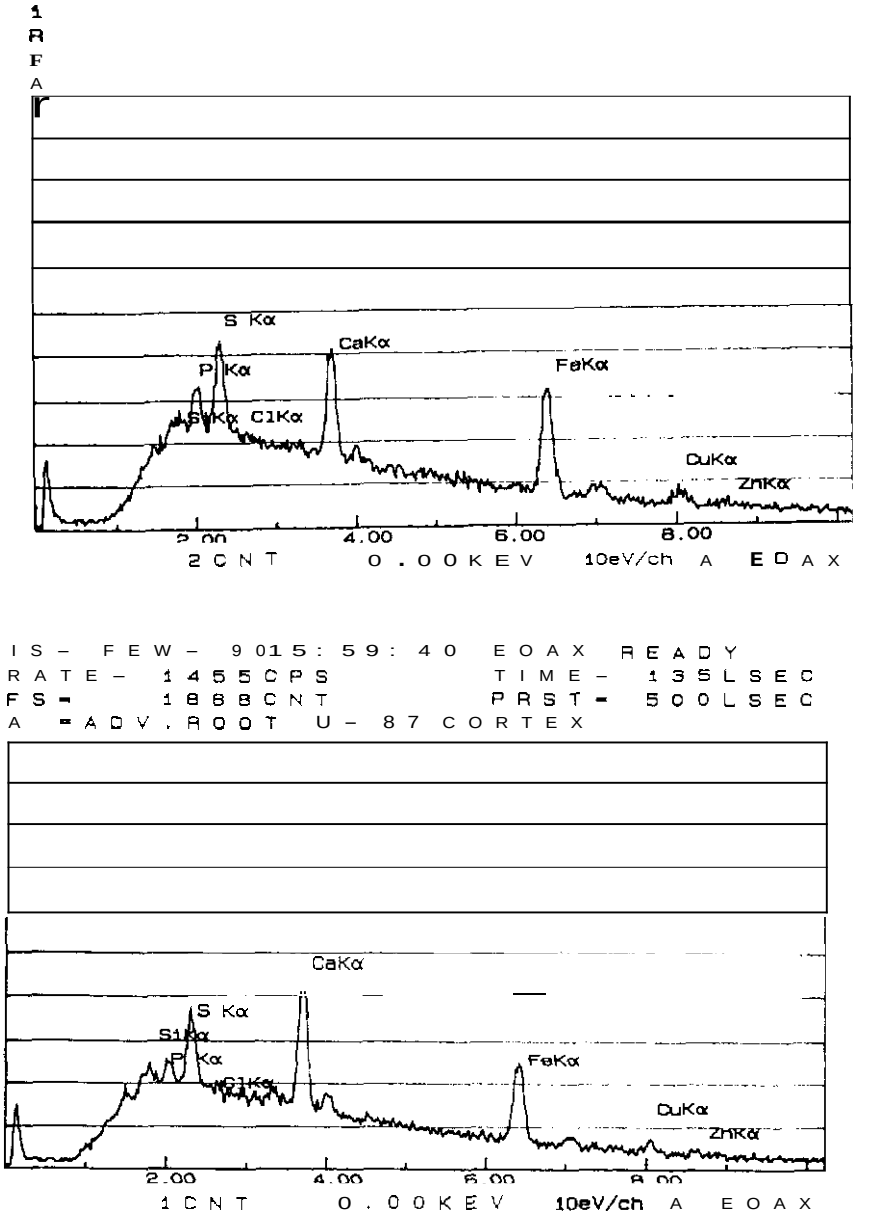
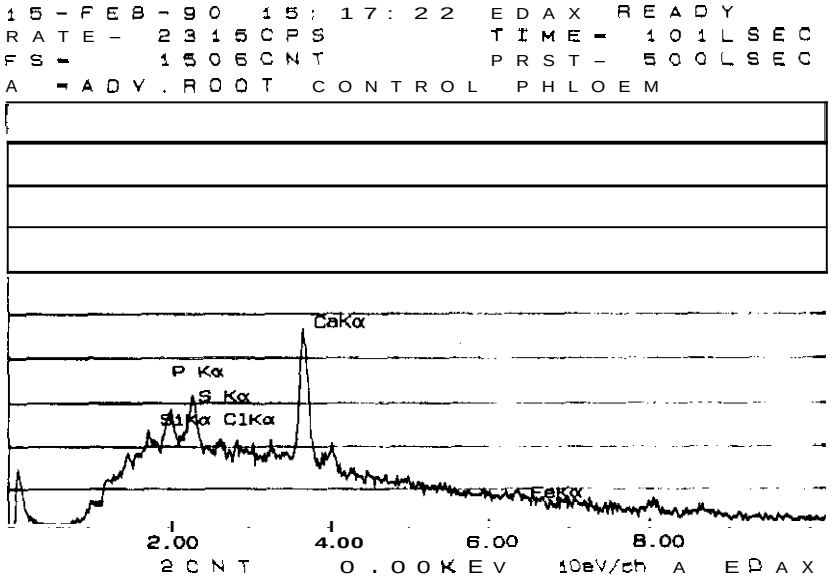
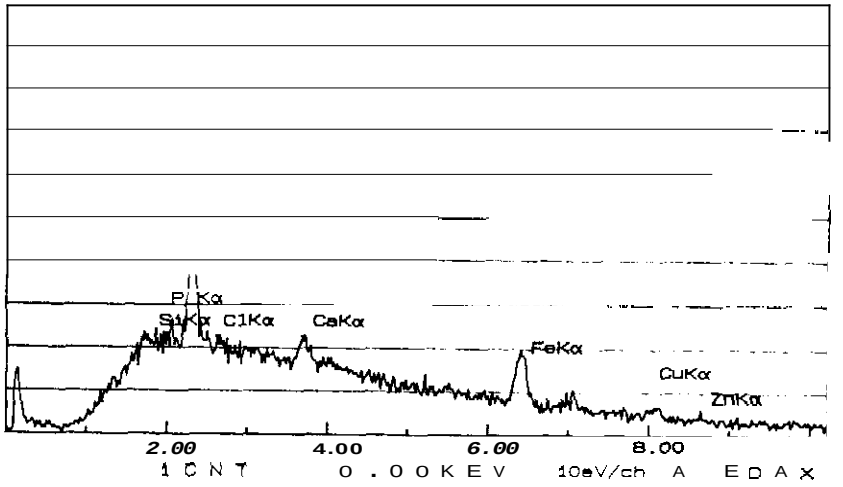
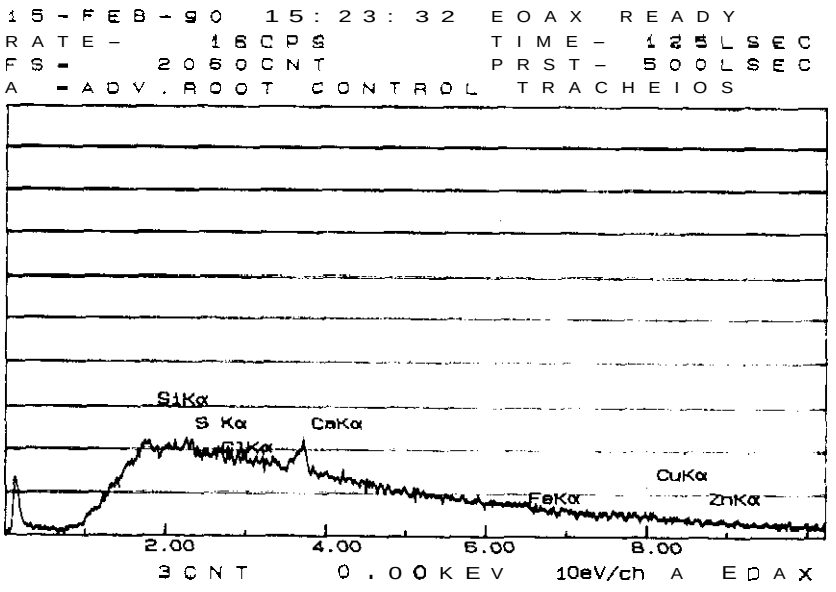


Figure **18** *g*, **h and i**









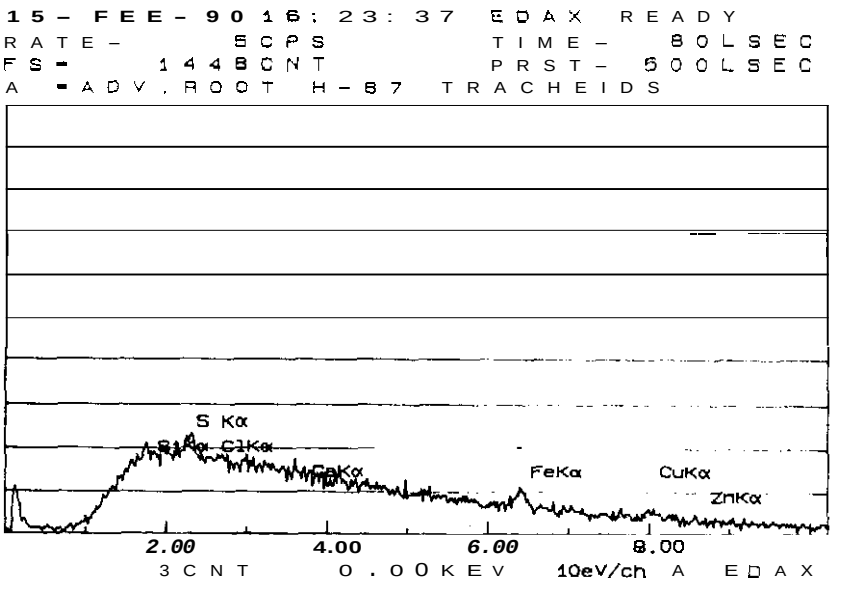


Figure 18 d, e, j and k

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**

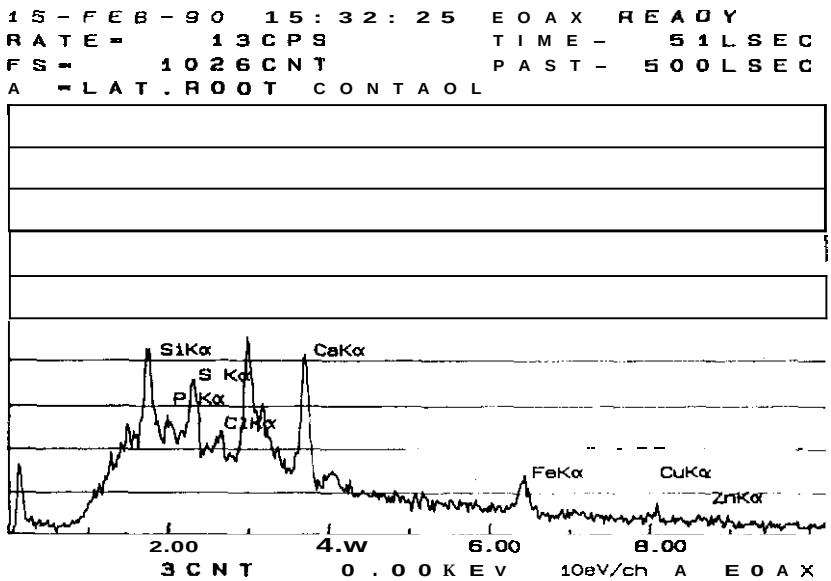


Figure 20: **X-ray Microanalysis scan for a transplanted adventitious root**

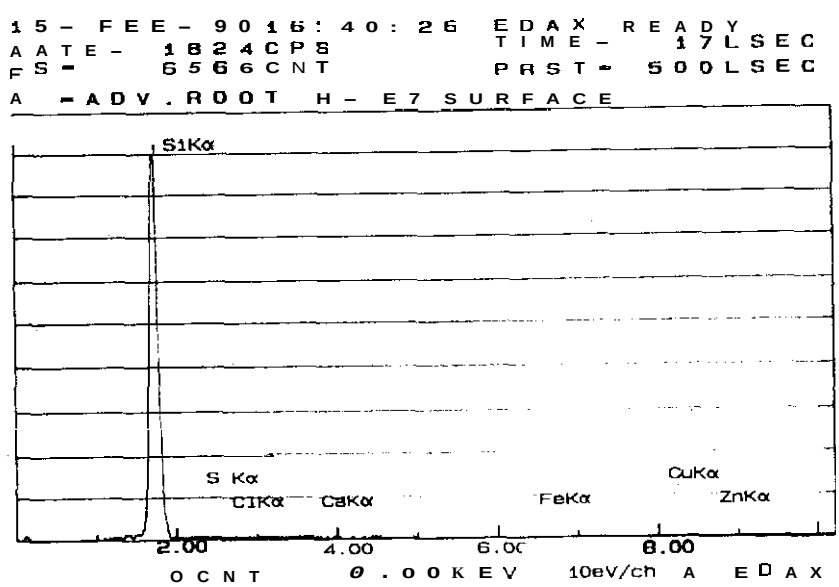


Figure 21: **X-ray Microanalysis scan for an adventitious root**

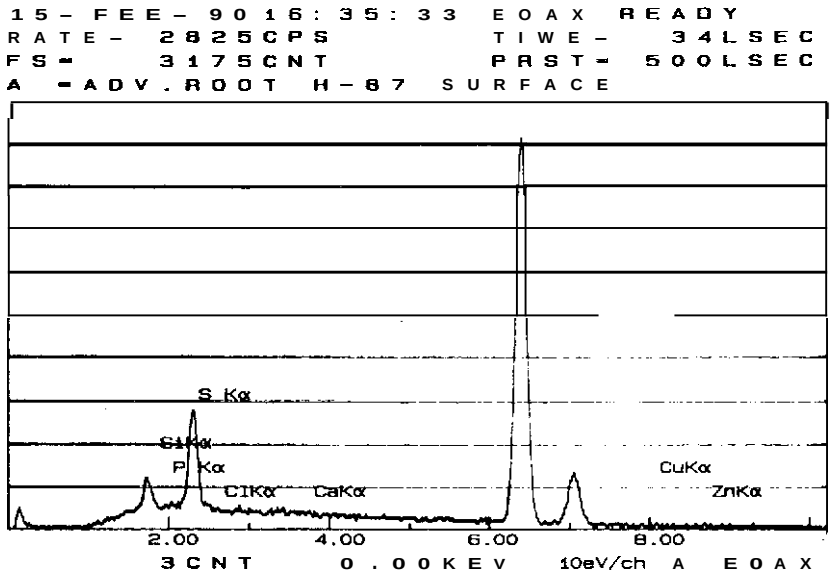


Figure 22: **X-ray Microanalysis scan of a leaf hydropoten (dark line) overlayed with a plot for an adjacent epidermal cell area**

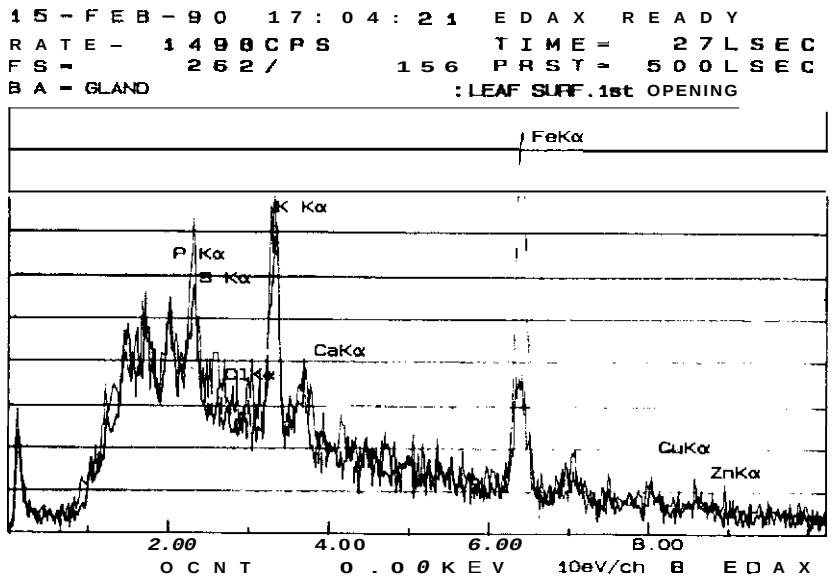
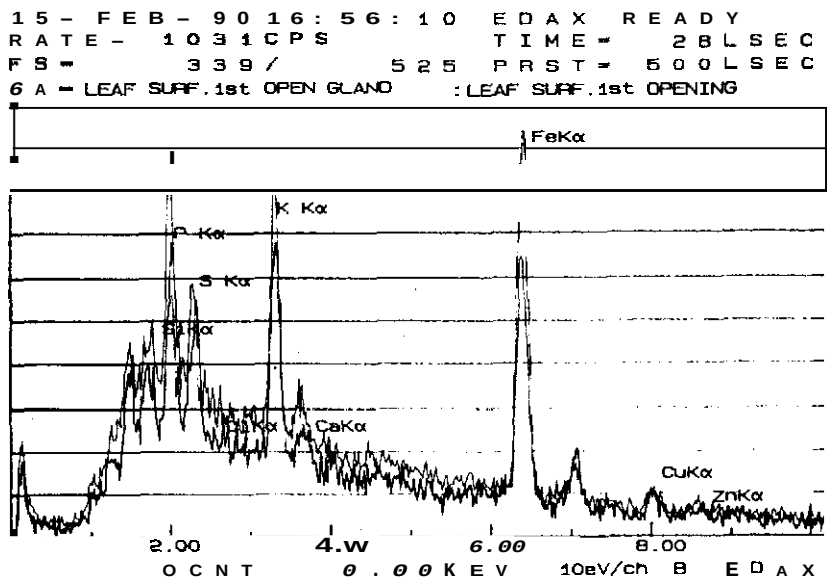


Figure 23: **X-ray Microanalysis scan of a leaf hydropoten (dark line) overlayed with a plot for an adjacent epidermal cell area**



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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SERIAL NO. 23440-8-9051/01SQ

MORPHOLOGICAL ASPECTS OF CATTAIL TRANSPLANTS and MICROBIOLOGICAL ASPECTS OF ORGANIC MATTER IN EXTREME ACIDIC CONDITIONS ON BASE METAL TAILINGS (UP-B7-020)

Morphological Assessment of Cattail Transplants in Acidic Tailings

FINAL REPORT

by

JIM CAIRNS, M. KALIN and R. SCRIBAILO

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

DSS FILE NO. 07SQ.23440-8-9051

DSS CONTRACT SERIAL NO. 23440-8-9051/01SQ

CANMET PROJECT NO.

CANMET SCIENTIFIC AUTHORITY Dr. R.G.L. McCready

DATE: June 30, 1988

TABLE OF CONTENTS

ABSTRACT

Two sets of experiments were brought together under this study - the establishment of cattail populations on acidic tailings and an investigation of the ameliorating effects of organic amendments on acid mine drainage water. Both these elements are essential components in the development of a self-sustaining biological treatment process for acid mine drainage.

Cattail transplant experiments yielded varying degrees of success. The root/rhizome system was investigated from a morphological point of view to obtain evidence on the processes which lead to either death or survival of the plants. It was found that root damage during transplanting is likely to be the main factor influencing survival and, accordingly, transplanting should take place prior to root development.

Several organic amendments were added to acidic water on a pyrrhotite-covered tailings area. By 1987, and more frequently in 1988, pH increases from 2.5 to 5 were noted in isolated pockets. A diverse microbiological fauna was identified in samples of the organic material. Based on a set of laboratory tests, the microbiological processes which are most likely to be responsible for the noted changes in pH, were defined in this study.

Samples obtained in the test cells in January 1988 contained significant numbers of sulfate reducing bacteria and ammonifying bacteria. The parameters for the microbiological neutralization process have been identified. The organic matter is decomposing to provide cellulose which, in turn, will be further broken down by fungi and bacteria to protein and sugars. These are the essential components required for the ammonifiers and iron and sulfate reducers.

Abstract

SOMMAIRE

On a rapproché deux séries d'expériences dans le cadre de cette étude : l'implantation de populations de quenouilles sur des étendues de résidus acides et une enquête ayant trait aux effets bénéfiques des modifications organiques sur les eaux d'écoulement acides des mines. Ces deux éléments sont essentiels pour mettre au point un procédé autonome de traitement biologique des écoulements de nature acide dans les mines.

Les expériences portant sur la transplantation de quenouilles ont apporté des résultats mitigés. Le systbme racines/rhizomes a été étudié d'un point de vue morphologique pour determiner quels sont les processus qui mènent soit à la mort, soit à la croissance des plantes. On s'est rendu compte que les chances de survie des plantes étaient vraisemblablement accrues si les racines n'étaient pas endommagées au moment de la transplantation et, par conséquent, celle-ci devrait être effectuée avant que les racines ne se développent.

On a ajouté des modificateurs organiques à l'eau acide d'un bassin de résidus couvert de pyrrhotine. *Dbs* 1987, et plus souvent encore en 1988, on remarquait que le pH passait de 2,5 à 5 dans des poches isolées. On a trouvé dans des échantillons de matière organique une faune microbiologique variée. D'après une série de tests en laboratoire, les processus microbiologiques qui sont le plus vraisemblablement responsables des changements de pH remarqués ont été définis dans cette Qtude.

Abstract

Les échantillons obtenus en janvier 1988 dans les cellules témoins contenaient une quantité importante de bactéries ammonifiantes et de bactéries faisant baisser le taux de sulfate. On a établi les paramètres du processus de neutralisation microbiologique. La matière organique se décompose pour donner de la cellulose, laquelle à son tour sera séparée en protéines et en glucides par des champignons et des bactéries. I1 s'agit là des éléments essentiels requis pour les agents ammonifiants et les réducteurs de fer et de sulfate.

ACKNOWLEDGEMENT

This study was made possible through the assistance of Inco, Sudbury Operations and Denison Mines Ltd.

Special thanks are due to Darl Bolton and Paul Davies for their help in the field.

1.0 INTRODUCTION

In many cases, acid generating waste sites produce seepages which require treatment. At present, in order to improve the seepage water, water is collected and subjected to neutralization. For the past years, Boojum Research has addressed various aspects of seepages on an experimental basis in an effort to develop a conceptual framework for a self-sustaining treatment system. This is part of the ongoing research of Ecological Engineering which aims to produce close-out conditions which are both environmentally acceptable and self-sustaining, no longer requiring the expensive treatment currently necessary.

The results from two series of experiments led to an impasse, as transplanted cattails gave inconsistent growth results following overwintering. In a second series of experiments, organic amendments which were intended to ameliorate the extreme acidic conditions produced only sporadic pH increases. This work, therefore, addressed the results of these experiments on a different level. Cattail root/rhizome morphology was used to determine the state of transplanted individuals and the organic amendments were tested for microbial activity in the laboratory.

The backgrounds and objectives of both experiments are given below by way of introduction to Sections 1 and 2 of this report.

It had been noted at various acidic tailings areas where cattail transplant experiments were underway, that growth was impaired or death occurred at various times after transplanting. Several variables could be contributing to the unsuccessful establishment of cattails in acidic tailings, some of which were indicated by the ongoing experiments, i.e. time of transplant and/or type of root/rhizome amendment.

The obvious reason for a continued search for those factors responsible for this failure is the existence of small but persistent stands of naturally colonized cattails on many highly acidic tailings sites (Kalin, 1984). It was clear therefore, that the pragmatic approach we were taking of continuing our transplant experiments would prove futile if we could not specifically delineate those factors responsible for the success or failure of the individual establishments.

Section 1 of this report has as its objective the investigation of cattail root/rhizome systems from previous transplant experiments at the morphological level to determine (a) the causes of death; and (b) the reason for successful growth.

The background for section two consists of an experimental test section of a pyrrhotite-covered waste management area which was

established in 1986, where various organic amendments were added to acidic water contained in series behind the tailings dam. In isolated pockets, pH increases from 2.5 to 5 were noted by 1987 and more frequently in 1988, A diverse microbiological fauna was identified in association with samples taken from the pools with amendments. Given the presence of this fauna, a more detailed assessment of the microbiological processes was carried out.

The objective of Section 2 has been to address the microbiological aspects of the test cells with organic amendment, specifically, to identify the sulphate reducing processes which are suspected to occur in test cells with AMD surface water.

By combining the results of section 1 and 2 of the investigation, it will be possible to define the parameters, which will hopefully lead to a self-sustaining microbiological treatment process of seepage water from acid generating waste material,

SECTION 1: Cattail Root/Rhizome Morphology

1.2 METHODS AND MATERIALS:

1.2.1 Cattail Collection Technique

Cattail rhizomes were collected from a number of locations along a spill area of an abandoned tailings site. The majority of these sites represented points of cattail transplants performed in August of 1987.

Several possible treatments had been done at each site at time of transplanting. These included: no amendment (N); straw (S); and, straw with lime (SL).

Transplant sites were designated as seepage, second opening, first opening and beach, corresponding to increasing distances from the tailings mass. Besides collection of previously transplanted cattails, cattails were also collected from a number of additional sites representing controls for comparisons with developmental status of transplanted individuals. These controls included cattails from the site which was used as a source for cattails for transplant (source); a natural stand of cattails present at the border of the second opening (Nat. Stand); individuals from Nat

Stand colonizing onto the tailings (Colonization),; an isolated clump of plants representing a seed germination and establishment event into the second opening from Nat. Stand (Seed Stand), from area Y1 which is a natural stand site existing for at least 15 years (Y1); and plants transplanted two years previously by Paul Davies onto tailings at an additional site (Paul).

Cattail plants were carefully excavated from the sites using a shovel, and were gently washed to remove excess soil. Plants were then stored in plastic bags in a cooler until the following day when they were examined and preserved for further study.

Observations on cattail growth were done at a number of levels which can be divided into stages. Stages correspond to those originally detailed in the unsolicited proposal preceding this report. Each stage is described below with particular reference to any techniques which were utilized during that stage.

1.2.2 Observation Methods

Stage 1: This involved an overall assessment of the developmental status of the plants, The following parameters were measured: number of new shoots/plants; length of rhizome between new shoots and the parent plant; and height of all new shoots. The plants were also photographed at this stage.

Stage 2: After examination of the plants from Stage 1, selected rhizomes, roots and shoots of plants from the different sites were fixed in 70% formalin; acetic acid; 95% ethyl alcohol (FAA) in a ratio of 1:1:18. Fixed plant material was hand sectioned for anatomical evidence of deleterious affects of tailings metals on cattail growth. Thick hand sections were photographed using a Zeiss stereomicroscope SV-8 at variable magnifications. To demonstrate the localization of metals in the rhizome tissue, we utilized a modification of the method proposed by McNary (1960). Unmordanted haematoxylin in pH 7.0 phosphate buffer binds to metals, producing a dark red coloration after several minutes of staining. This stain was particularly useful in enhancing observations on the localization of metals in thick sections of rhizomes.

rhizomes.

Stage 3: Plants fixed from stage 3 were sectioned for use in scanning electron microscopy (SEM). To prepare tissue for SEM, plant material preserved in FAA was first washed in 70% ethanol, before being transferred through a graded ethanol series to absolute 100% ethanol. Tissue was then critical point dried in an Omar SPC-1500 critical point dryer, mounted on metal stubs and coated for five minutes with gold palladium in a Techron Hummer V sputter coater. Material was observed at 10 kv using a Hitachi 570 SEM.

1.3 RESULTS AND DISCUSSION:

Before proceeding with a discussion of results from this study, some introductory remarks are necessary to describe basic features of the anatomy, morphology and development of cattail plants. Figure 1.1 diagrammatically illustrates many of these features.

Individual cattail plants consist of a rosette of opposite leaves (about 6 per side) which reproduce vegetatively through the production of underground stems (rhizomes). These rhizomes initially develop as small vegetative buds in the axil (at the base) of each leaf. Thus each plant has the capacity to produce

vertically (S_L, Figure 1.2A), and becomes an upright shoot (new plant) (S_V, Figure 1.2A: arrows, Figure 1.2B). Rhizomes characteristically produce roots at regular intervals along their length (arrow, Figure 1.2A), although the majority of roots tend to be produced at the bases of upright shoots (Figures 1.2B, 1.2D).

Anatomically (in section), rhizomes consist of two distinct zones, an outer cortex (C, Figure 1.2G, Figures 1.3A, 1.3B), and an inner pith (Pi, Figure 1.2G, Figure 1.3B). The pith is predominantly composed of tightly packed storage cells containing large quantities of starch granules (large arrow indicating starch granules in pith cells, Figure 1.3A). The cortex is largely composed of stellate (star-shaped cells) with large amounts of air space between cells (Figure 1.3A). The pith is bordered on its periphery by an endodermis (EN, Figure 1.3A), which acts as a barrier to lateral movement of substances from the cortex into the pith. The cortex is bordered on the outside by the exodermis which forms the outer 'skin' of the rhizome and acts as a barrier to transport of materials from the external environment into the rhizome (EX, Figure 1.2G, Figure 1.3A). Vascular bundles are found scattered throughout the cortex and pith in particular (small arrows, Figure 1.2G; VB, Figure 1.3A; small arrows, Figure 1.3B). The primary function of vascular bundles is to transport water from the roots to the shoots and to transport sugars produced during

photosynthesis from the leaves to the rhizome for storage (as starch in the pith).

The anatomy of roots is very similar to that of rhizomes, except that there is no pith region and this is replaced by a solid cylinder of vascular tissue (rather than many individual vascular bundles) termed a stele (large arrow, Figure 1.3C; Figure 1.3D). Rhizomes produce roots laterally from the pericycle, which is a thin layer of tissue found just beneath the endodermis. Root initiation is shown clearly in Figure 1.2I. Roots may also produce lateral roots from their pericycle (large arrow, Figure 1.3D).

Stage 1: Examinations of gross morphology of cattails indicated substantial information on the affects of tailing metals and the amendment treatments on growth. Quantitative data comparing parameters of growth indicate that transplanted cattails performed better the greater the distance from the tailing source (Figure 1.4). Furthermore, and of interest to the transplanting program, the amendments had significant positive affects on cattail development. This is particularly true in the case of the straw and lime amendments which had the greatest number of new shoots per plant at both the first and second openings (Figure 1.4). For length of rhizome and new shoot height, straw and straw and lime amendments both showed similar development, both performing better

than no amendment cattails (Figures 1.5B, 1.5C).

At the second opening site, transplanted cattails with straw and straw and lime amendments grew better in all respects (Figures 1.5A-C), than cattails in the natural stand and natural colonization. They also performed as well as or better than control plants from the Source population and from Y1, both of the latter representing natural stands.

Of particular interest is the fact that plants from the Seed Stand far outperformed any other plants in this investigation. Implications of this are referred to more fully in the discussion,

Root development was assessed by examining the level of new root initiation from the bases of new cattail shoots. These roots could be identified by their whitish colour and intact root tips (Figures 1.2A, 1.2B)). In contrast, older roots were invariably brown in colour and heavily mineralized. This latter point will be discussed in more detail in the results presentation for Stage 2 work.

Amendment type had a dramatic affect on root development. The straw and lime amendment in particular, greatly enhanced both the extent of new root initiation as well as the growth of these new

roots. In unamended sediments, few new roots were initiated and those present were only a maximum of several cms. in length, The straw treatment showed similar, but slightly better development. With the straw and lime amendment, 20 - 30 new roots tended to be present on larger new shoots and these often had grown as much as 10 cms. in length (Figure 1.2B). At the first opening, development on the latter plants was comparable to or better than that of plants from the natural stand or that of plants from Y1 (Compare Figures 1.2A and 1.2B). The dramatic effect of straw and lime amendments on the enhancement of root and rhizome growth is shown when one compares Figure 1.2C (seepage, no amendment) with 2D (seepage, straw/lime). Note the almost complete lack of roots and rhizome growth in Figure 1.2C, in contrast with substantial root growth in Figure 1.2D.

Overall growth, besides varying significantly between amendments versus controls, also showed a large decrease as one progressed from the beach towards the tailings seepage. This decline was evidenced by almost total loss of new shoot initiation and new root development, Deleterious affects of metals also became much more obvious towards the tailing source. This is discussed in detail under results for Stage 2 analyses of development.

Stages 2 and 3: Results from Stage 2 and Stage 3 studies of cattail development confirmed findings from Stage 1 concerning the value of the amendment treatments for promoting growth. In addition though, these results also provided considerable information about progressive stages in cattail death which are useful in indicating the best possible transplant method for ensuring future success.

Microscopic studies of roots, rhizomes and shoot, using both sectioned and whole material, indicated the following findings:

- Damage to roots and rhizomes from transplanting (i.e. loss of the growing tip) caused rapid uptake of metals through cut surfaces leading to the rapid mineralization of roots
- In the case of new roots, the following steps leading to their death were observed: Root tips would begin to accumulate metal oxides on their tips and external surfaces (arrow, Figure 1.3F versus Control, Figure 1.3E). These metals would then begin to encroach through the exodermis of the root causing death of the exodermal layers and underlying cortex (Figures 1.3G and 1.3H). As this accumulation increased and root tips began to blacken, a profusion of secondary roots would often be initiated and grow out just back from the tip (Figure 1.2K).

These roots often exhibited abnormal growth with multiple branching occurring on the same root. Shortly after this time, the primary roots would die, becoming heavily mineralized. Generally, roots showed earlier onset of this dying syndrome at sites closer or on the tailings mass. On the tailings mass itself, roots died rapidly almost at initiation, leaving black scars on the rhizome or shoot base where they had previously been initiated. Within the different amendment types, this process always occurred slowest with the straw and lime amendment.

Root death was closely tied in with death of rhizomes. Sectioned rhizomes showed first signs of mineral damage at points of entry of lateral roots into the cortex. This can be seen clearly in Figure 1.2G, where darkening of the cortex tissue indicates localized death of cells. After staining of this section (Figure 1.2H), the zone of damage can be seen to be much more widespread. An interesting fact to note is that this damage does not extend into the pith area, which remains white. Close examination of rhizomes with the naked eye indicated that the location of these internal zones of death could be seen externally as darkened spots appearing beneath the rhizome surface.

Anatomically areas of metal uptake in the cortex, as further elucidated by haematoxylin staining, would grow in size until most

of the cortex was blackened. Only in the final stages of rhizome death was this zone seen to encroach into the pith area (Figure 1.2I). Therefore, rhizomes were often seen which had a completely black cortex but a pith which remained white. The method of slow rhizome death just described was more typically seen in cattails with less extreme conditions, such as those sites further away from the tailings mass, or in amended conditions. Staining of young shoots with haematoxylin identified young leaves as containing large amounts of metals, although the young developing leaves appeared to be healthy.

In sites closer to the seepage or in places where direct contact occurred between rhizomes and tailing sediments, death of rhizomes was more dramatic. At these locations external damage to the rhizome surface was often visible. Metals, because of their corrosive nature, appeared to eat their way right through the rhizome surface leading to rapid spread of metals through the cortex, with subsequent death of the rhizome following shortly thereafter (arrow, Figure 1.2J).

1.4 *SUMMARY* AND CONCLUSIONS:

Results from the morphological/anatomical study have yielded valuable information which can be used towards planning a sound cattail transplant program for the year of 1988.

This study has clearly shown that amendments at time of transplanting can be very beneficial towards ensuring cattail growth and reproduction. This is particularly the case with the straw and lime amendment, indicating that it is the best treatment to continue investigation with.

Nevertheless, overall findings from the study suggest that direct contact between roots and rhizomes with tailing sediments result in rapid death of the organs involved. This suggests that difficulties in the promotion of further growth will be reached when lateral spread of cattail organs extends beyond the immediate area of the initial amendment.

Evidence from studies of iron plaque formation on cattail roots indicate that it is highly pH specific. At low pH, iron is predominantly in ferrous soluble form and as pH increases, iron is precipitated out into the substrate (MacFie and Crowder, 1987). This in part explains the tendency for much greater root mineral

damage (from plaque) to occur as one gets closer to the seepage. Furthermore, at a given site, the addition of lime to the tailings causes a rapid rise in pH, probably causing precipitation of harmful metals. This in turn would allow root growth to proliferate without metal damage.

Another crucial factor which appears to hamper cattail transplant success, is the damage done to roots at the time of excavation prior to subsequent transplant. Evidence presented here indicates that cut faces of roots may lead to rapid mineralization and death of these roots. The process of this mineralization also leads to metal accumulation in the rhizome cortex and in the shoot system. In both cases, vigour of the plants is undoubtedly reduced, making them more susceptible to death, Nevertheless, evidence indicates that although localized death may occur in the cortex of the rhizomes via this method, death of rhizomes would only occur after a protracted period of exposure. A crucial factor here is whether metals actually can penetrate the pith area, causing loss of storage materials essential for renewed growth, particularly in the Spring months. This does not appear to be an immediate result of metal uptake by roots, since observations here indicate pith damage only occurs under extreme conditions, as, for example, at the seepage.

1.5 RECOMMENDATIONS:

The observations discussed with respect to cattail establishment strongly suggest that it is imperative to transplant cattails with minimal damage. The best possible method to ensure this would be to transplant cattails in the Spring prior to initiation of new roots.

An alternative approach to overcome the damage problem would be to use plants grown from seed. Such a strategy might allow the additional problem of contact death described above to be overcome in the following way. If seedlings are hardened during development by growth in diluted tailings sediments, it may be possible to get seedlings to acclimatise to sediments with high metal concentrations. Seedlings treated in this way may suffer less of a shock, and show more resistance upon exposure to tailings conditions.

In the process of producing plants from seed, it is possible that certain plants may turn out to be more resistant to metals than others. Ideally, if plants showing that characteristic are found, they could be cloned for future considerations for mass transplanting. Certainly evidence from the natural stands at the 2nd opening, Y1 and Source, indicate variability for resistance to

metal damage. This point is best exemplified by evidence of the successful establishment and prolific growth of the clone Seed Stand from the 2nd opening.

SECTION 2: Microbiology of Organic Amendments

2.2 METHODS AND MATERIALS

2.2.1 Sample Collection and Handling

Samples of water and amendment material were collected in the different test cells through holes drilled with an ice auger on January 27, 1988. The samples were transported in a cooler and stored in a refrigerator at Dearborn. After 24 h, all samples were subjected to an ATP test, microscopic observations and tested for SRB (sulphate reducing bacteria). Five days later, pH was determined with a probe specifically designed to obtain the measurement in the samples with minimal introduction of oxygen. After 19 to 27 days, the microcosms were set up, addressing several conditions. At the same time, a second set of observations and determinations was obtained, consisting of pH, ATP, microscopic observations, SRB and ammonifiers determinations.

The microcosms were set up inside an anaerobic hood in 40 ml Wheaton vials representing layers in the pre-bog acid creek (water, amendment and amendments and tailings). These microcosms were incubated at an ambient temperature of 22°C. The conditions tested were anaerobic plus nutrient (0.1% peptone / 0.25% lactate),

anaerobic no nutrients added (sealed vials), aerobic/anaerobic (cotton plug), and Pentachlorophenol (3,000 ppm) treated microcosms, representing an abiotic control. Pentachlorophenol (PCP) is a strong toxic substance, killing most living organisms. After 12 to 19 days, the tests previously described were repeated for all treatments.

Figures 2.1 to 2.3 are photographs of the appearance of the field samples. The arrows indicate the regions of the sample bottles from which aliquots were removed for analysis. It was decided to analyze these sub-samples rather than homogenize the samples, in order to minimize possible trauma to the microorganisms caused by changes in pH and redox conditions during mixing.

It was noted during testing on January 28, that both the amendment 3 test cell samples had a sulphide odour. Odour was not detected in the other samples.

Figures 2.4 to 2.8 are representative photographs of the sample microcosm vials.

2.2.2 ATP (Adenosine Triphosphate) Assays

ATP was measured by the firefly luciferase method using a Turner Designs Model 20 e Photometer,

ATP was extracted from the microorganisms in the samples by a technique developed by Dearborn and found to be successful for AMD samples. Cell walls were lysed with ethanol; the ethanol was removed by filtration through a glass fibre filter; the cells were further lysed by passing acetone through the filter; finally, ATP from the organisms in the filter were leached with trisodium phosphate buffer. The extracts were further diluted in trisodium phosphate buffer containing Mg-EDTA in order to prevent inhibition of firefly luciferase due to metal ions,

2.2.3 Test for SRB's (Sulphate reducing bacteria)

In order to estimate the order of magnitude number of viable SRB's, decimal serial dilutions of sample were performed in media capable of supporting the growth of these organisms, All media contained lactate as a carbon source and iron to indicate growth which resulted in blackening of the media when the organisms produced H_2S.

Initially, API medium RP38 was used for this purpose. Each vial contained a nail to poise the redox potential.

In samples where sulphide odour was detected, subcultures of positive cultures were performed in order to ensure that blackening of the medium was not due to sulphide already formed in the sample.

In subsequent analyses, tests were performed in deep agar tubes of Postgate E and F media in an attempt to obtain more quantitative precise results. Postgate E medium was selected to provide a count of sulphate reducing bacteria while Postgate F medium was used to detect additional sources of microbially produced sulphide such as sulphide from sulphite reduction and decomposition of organo-sulphur proteins. Since counts obtained using Postgate F medium never exceeded those with Postgate E, only Postgate E medium was used in the microcosm analyses.

Although it was possible to obtain actual black colony counts for many samples, counts for many other samples were obscured by extensive blackening. Therefore, to simplify reporting, results are only shown as order of magnitude levels.

2.2.4 Estimation of Ammonifying Microorganisms

Order of Magnitude estimates of ammonifying microorganisms were obtained by preparing decimal serial dilutions of sample in Caslin medium (Methods for Microbiological Analysis of Waters, Wastewaters, and Sediments, Canada Centre for Inland Waters, 1978).*

2.3 RESULTS AND DISCUSSION

In Table 2.1, the pH measurements for the samples and their microcosms are presented. The three amendment types are coded as previously described in Figures 2.1 to 2.3. The values taken on January 27 were determined on-site, where sample temperatures were -1° C. The samples were placed in an insulated container and transported to Dearborn where they were immediately stored at 4° C. All pH readings taken during January and February therefore occur when the microbial populations in the sample are in a cold environment. Consequently, it is important to observe that in two of the samples (1.3 and 3.7), an increase in pH of more than one pH unit occurred during this cold storage period.

The "Microcosm" experiment, initiated between February 16 and 24, demonstrates the pH changes in the samples which are subjected to various conditions at room temperature (approximately 22° C)

The "anaerobic" condition essentially represents shifting a portion of the sample from refrigerated to room temperatures. It can be seen that this results in a further pH increase in the 1.3 and 3.8, 3.10 and 1.1 samples. The highest pH observed for the anaerobic test condition was a value of 5.7. Both the 3.7 sample and the 1.3 sample attained this pH.

The trends for pH increases in the microcosms supplemented with nutrients (peptone and lactate) are slightly more difficult to interpret for many of the samples because nutrient addition itself immediately caused a pH rise in the samples to pH 4.2. However, it is evident that the nutrients stimulated pH increases in the 1.1, 1.3 and 3.7 and 3.8 samples.

No pH rise was observed in any of the aerobic microcosms. On the contrary, aerobic conditions resulted in a pH depression. It is speculated that the drop in pH was caused by the growth of Thiobacillus ferroxidans.

With the exception of the 1.1 sample, no pH rise was observed in any of the microcosms poisoned with PCP (abiotic control). This indicates that acid neutralization process is biological. By examining Figure 2.7, it can be seen that sample 1.1 had a very high proportion of sediment. The presence of this sediment probably restricted access of the PCP to the microbial populations in the vial. Therefore, some limited biological activity may still have been occurring.

Tables 2.2 to 2.10 summarize the results of the microbiological tests conducted on the samples and their microcosms. In addition, some of this data has been extracted from these tables to prepare Tables 2.11 and 2.12.

By overviewing Tables 2.2 to 2.10, it can be seen that a diverse group of microorganisms were observed in the site samples. Representatives of bacterial, fungal and algal species were observed. Only bacteria were detected in the control sample.

Samples from the test cells all had significantly more viable biomass levels than the test cell samples as indicated by their ATP (Adenosine Triphosphate) content. Although ATP levels were indicative of the biovolumes observed during the microscopic examinations, no correlation with the neutralization process was

evident.

In contrast, as summarized in Table 2.11, there appears to be a direct correlation with the neutralization process and SRB12 (sulphate reducing bacteria) SRB'S and ammonifiers.

Four of the five samples in the anaerobic microcosms displayed pH increases of at least one pH unit after February 2. However none of these organisms grew in the samples which displayed no pH increase or an increase less than one pH unit after February 2.

The only exception in the above relationship appears to be sample 3.10. Nevertheless, SRB's and ammonifiers did grow in this sample when the nutrients peptone and lactate had been added. Furthermore, when peptone and lactate were added to the other microcosms, SRB's and ammonifiers grew only those which displayed a pH increase of at least one unit.

Table 2.12 summarizes the effects that these nutrients had on the samples which displayed one unit pH increase. In every case, a pH increase was observed and in the majority of cases, increases in the SRB counts and ammonifiers counts occurred.

Thus, the results in Table 2.11 and Table 2.12 demonstrate the probable involvement of SRB and ammonifiers with the neutralization process. The possibility of the involvement of iron reducers cannot be excluded based on the present data and those could also contribute to the neutralization process.

It is surprising that in most cases, the SRB and ammonifier counts are relatively low. Perhaps, the counts are underestimates. This could occur if the cultural conditions of the microbial tests were insufficient to support the growth of the major strains of these organisms. In addition, it is probable that many of the cells of these types of organisms are associated in clumps and could attach to particulates. In microbial enumeration techniques based on cultural methods, a clump of microorganisms or group of microorganisms attached to a particle is counted as a single cell. It is also possible that additional types of microorganisms could be responsible for neutralization of seepage acidity. Iron reducing bacteria are such a group.

Further examination of Table 2.12 also suggests that there is a relationship in occurrence of SRB's and ammonifiers. In each sample where SRB's were detected, ammonifiers were also found. Two ecological relationships of these organisms are probable. First, ammonia, which is produced by the ammonifiers is a nitrogen source

for SRB's. Second, the production of ammonia may raise the pH of the samples to pH 4. Growth of SRB's below pH 4 has not been documented.

It is of interest to note that sometime after February 2, 1988, during storage in the refrigerator, the 1.1, 1.3 and 2.4 samples became frozen. In the microcosm experiment, growth of both SRB's and ammonifiers occurred in all of these samples. Therefore, the members of the ecosystem leading to the process may be considered safe from irreversible damage caused by winter temperatures.

The interaction of these organisms is one of many interactions which probably occur in this ecosystem. Figure 2.9 summarizes the activities which are likely to be involved.

2.4 SUMMARY AND CONCLUSIONS

A. The experiments have demonstrated that microbial neutralizationof acid seepage is possible in samples obtained under winter conditions and AMD originating from pyrrhotite.

B. The microbial process was active at 4°C and recovered following one freeze/thaw cycle.

C. Ammonifiers and sulphate reducing bacteria appear to play key roles in the process. Compared to the control, the samples from the experimental sites had greater microbial population diversity (according to microscopic analyses) and higher biomass levels. The correlation of ATP levels with the bio-volume quantities (estimated microscopically) indicates that the majority of the organisms were viable.

D. Some of the amendments provided sufficient nutrients for the neutralization process. However, the effect of the nutrient additions in the microcosm studies indicate that there is a potential to improve the process.

E. It was demonstrated that the microbial acid neutralization process requires anaerobic conditions. Aerobic incubation of the samples resulted in generation of acidity rather than alkalinity.

2.5 RECOMMENDATIONS

A literature review was carried out to define the approach which could be taken to proceed with refinement of the process and determine parameters which could be utilized to develop an effective seepage treatment system. In Table 2.13, sulphate reduction rates are summarized fromthe literature. It is evident, that reduction rates can be increased in a bio-film lab reactor by several orders of magnitude. The approach to be taken for the development of a self-sustaining neutralization process however, is not immediately apparent. Clearly the microbiology itself and the interactions of decomposition and aerobic and anaerobic degradation are complex. One must assess the microbial interactions as outlined in Figure 2.9 in detail.

The identification of all aspects which are likely to contribute to the neutralization of the acidic water, particularly taking account of the rate limiting aspects of the process would require an extensive financial commitment without guarantee of success. It is, therefore, recommended that a more pragmatic approach be taken, utilizing the existing literature as a guide (list of references given in the Appendix).

APPENDIX

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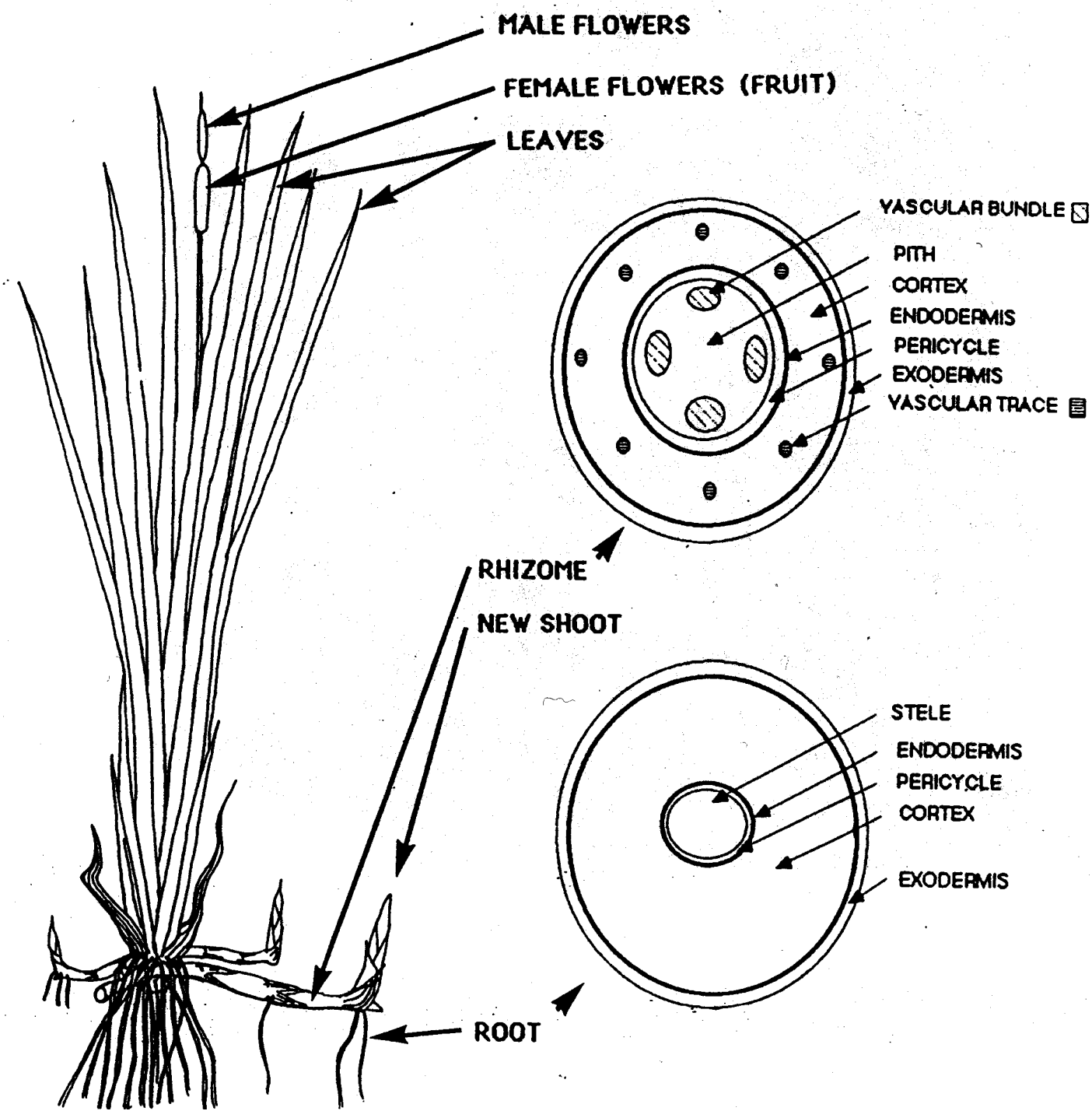


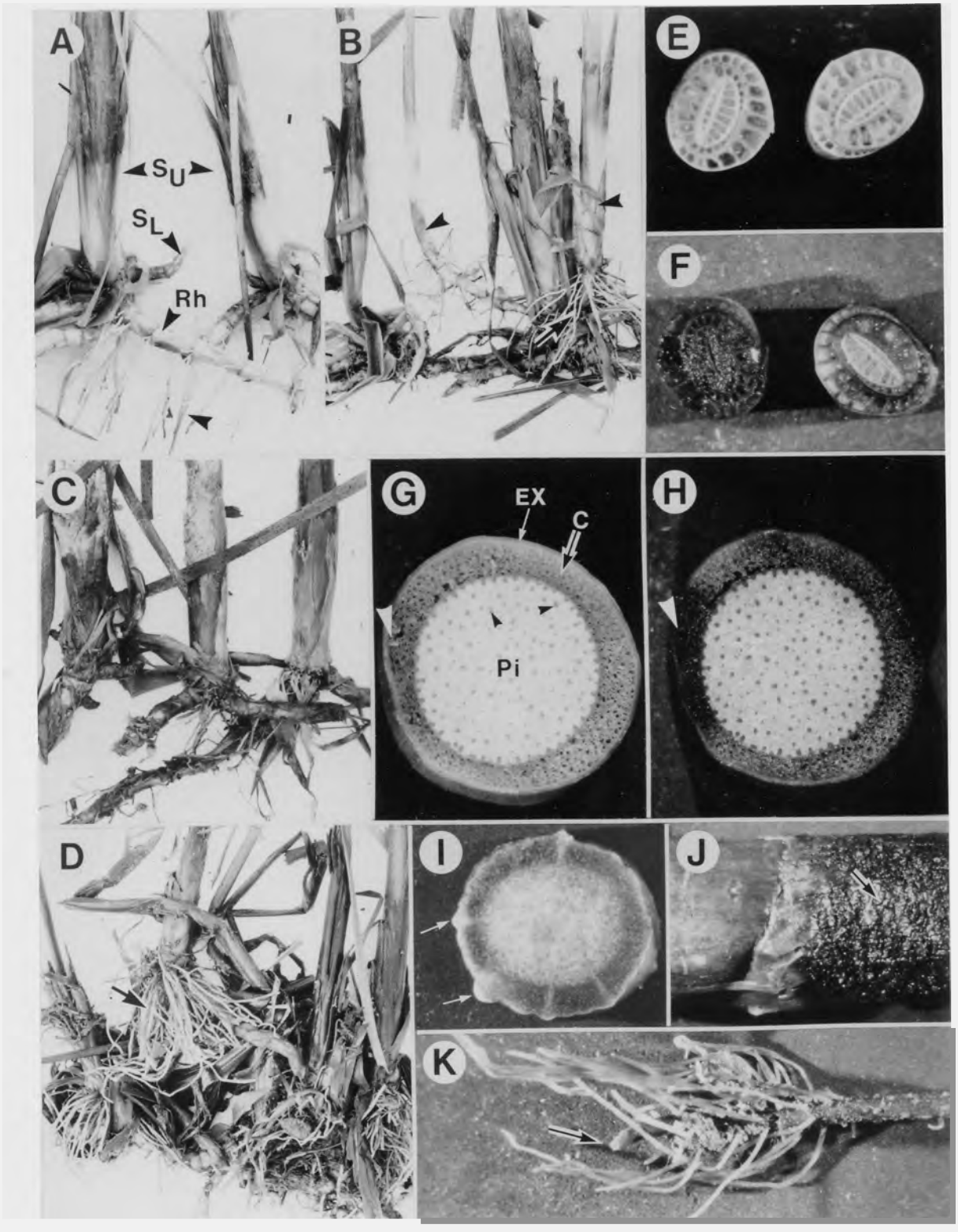


Fig. 1. 2

FIGURE 1.2

GROSS MORPHOLOGICAL FEATURES OF CATTAIL GROWTH

A. Cattail from site Y1, upright shoots (S_u), lateral shoot (S_L), rhizome (Rh), roots (arrow).

B. Cattail from the 2nd opening treated with straw and lime. Arrows indicate upright shoots.

* Note the root proliferation on the upright shoot to the right.

C. Cattail from the seepage with no amendment.

* Note the lack of new growth.

D. Cattail from the seepage amended with straw and lime. Extensive new root growth has occurred (arrow).

E & F. Sectioned shoot showing young leaves. F has been stained with haematoxylin to show the tremendous accumulation of metals in the shoot system. X 4

G & H. Section of a rhizome unstained (g) and stained (H). Metal damage from uptake is indicated by the large white arrow. Note that this damage has not spread to the pith (Pi). Exodermis (EX), cortex (C), small arrows indicate vascular bundles. X 4

I. Damage from metal uptake which has spread into the pith. Arrows indicate lateral roots.

J. External metal damage on a rhizome (arrow) due to direct contact with tailing sediments. X 4

K. Lateral root proliferation on a root after damage to the root tip. X 8

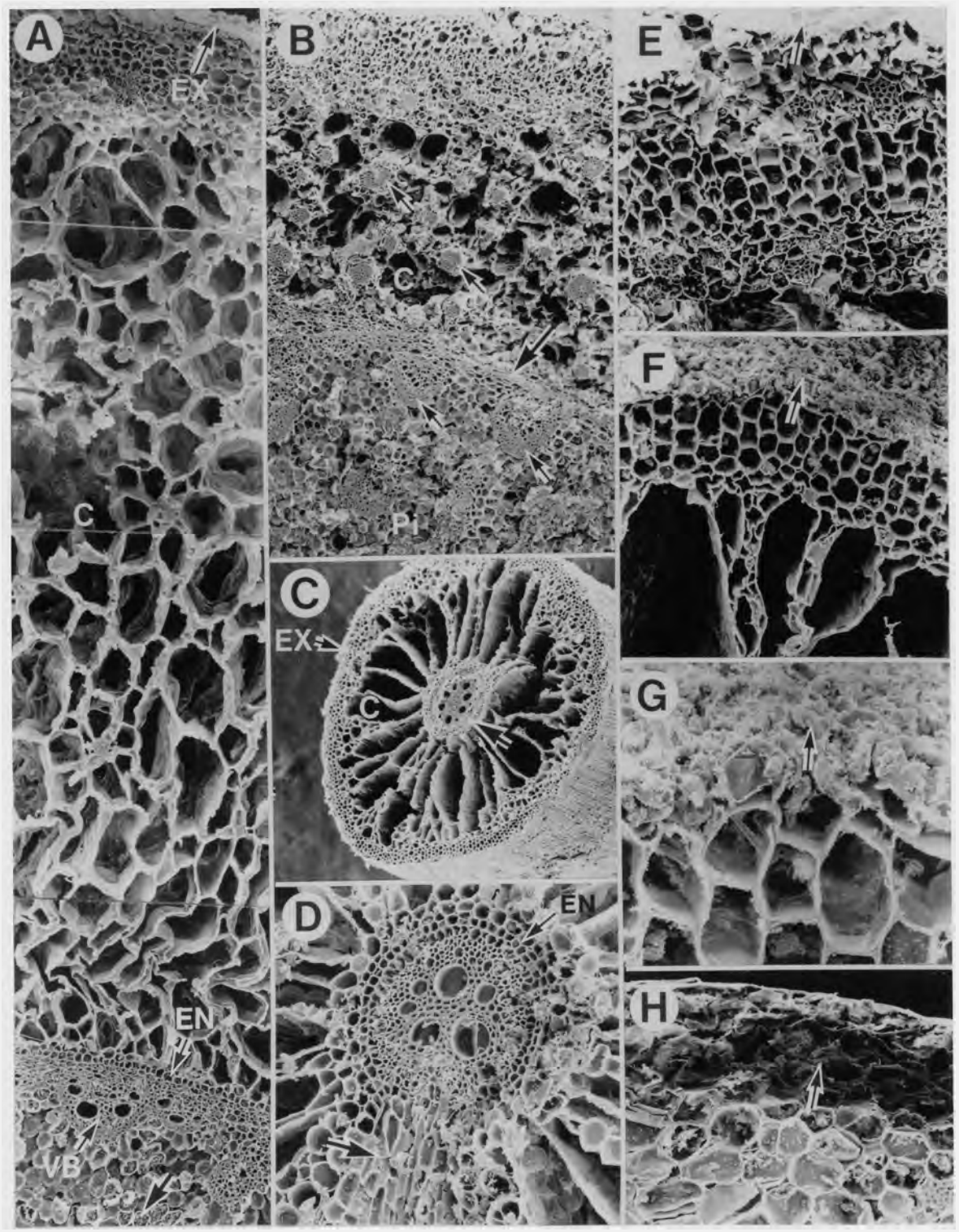


Fig. 1. 3

FIGURE 1.3

SEM SECTIONS OF CATTAIL RHIZOMES

A & B. Sections of rhizomes

A. Note the overall anatomy of the rhizome. Cortex (C), Endodermis (EN), Vascular bundle (VB), large arrow indicating pith cells containing starch granules, exodermis (small arrow). X 100

B. A younger, more compact rhizome than that shown in Figure A, Endodermis (large arrow), vascular bundles (small arrows), X 100

C. Section of a root. Stele (large arrow). X 50

D. Close-up of a stele from a root similar to that shown in Figure C. large arrow indicates a site of lateral root initiation, X 250

E. Close-up of the exodermis of a healthy root. Note that there is no metal accumulation on the outer surface of the exodermis. X 250

F. Root showing signs of metal accumulation. The exodermis surface is coated with metal particles and these metals are beginning to encroach into the cortex layers. X 250

G. Close-up view of metals in the outer layers of the cortex and beginning to accumulate in cortical cells. X 1000

H. Later stage of root death due to metal accumulation. A band of dead cells is present within the cortex. X 250

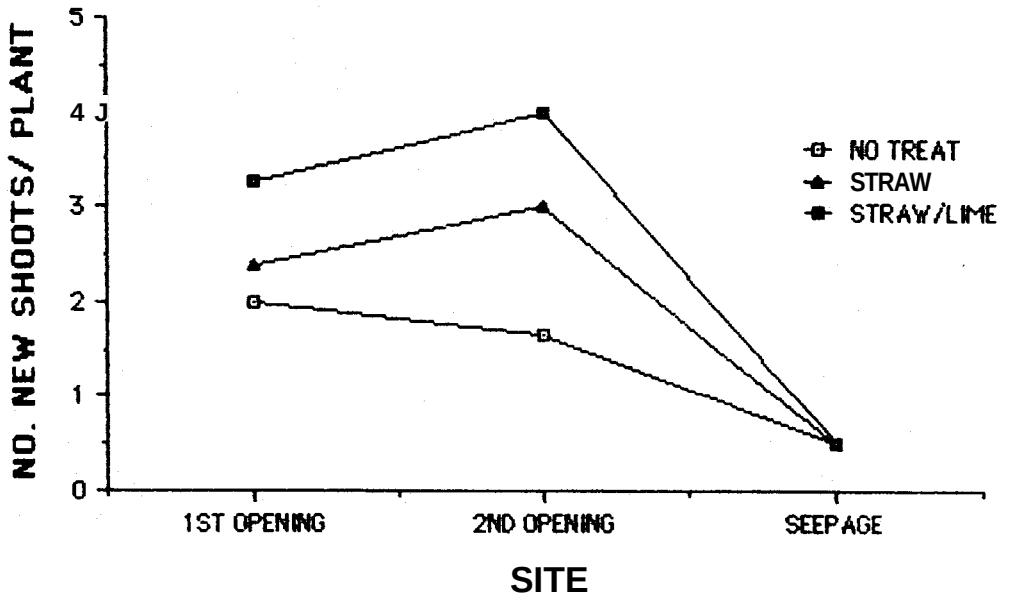
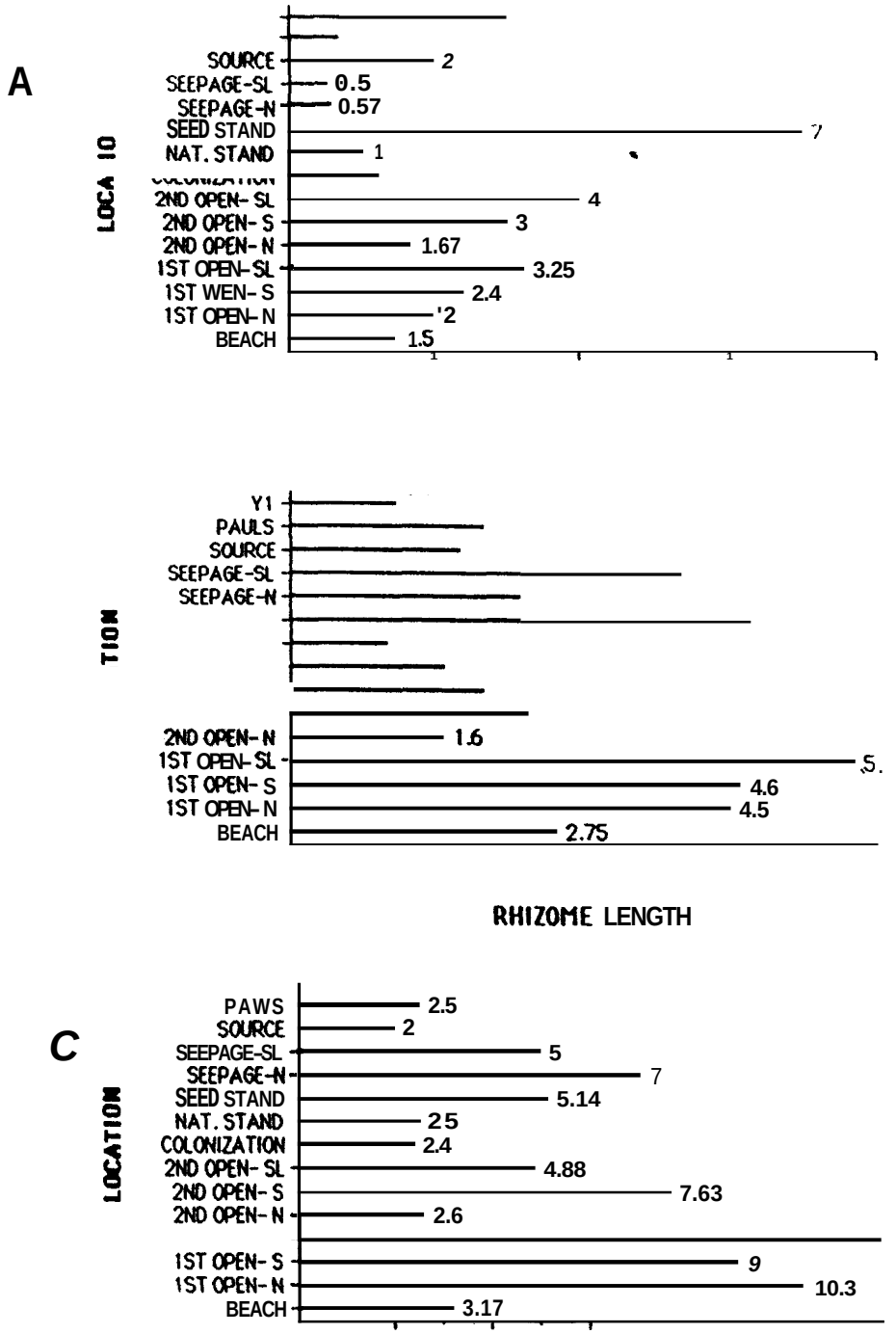


FIGURE 1.4: Affect of amendment type on number of new shoots produced per plant, expressed as a function of transplant location.

Note: Most adverse conditions (pH 1.5) encountered at seepage, and best conditions (pH 3.5) at first opening.



FIGURES 1.5A,B and C: Growth characteristics expressed as new shoots per plant, length of rhizome and height *of* new shoots

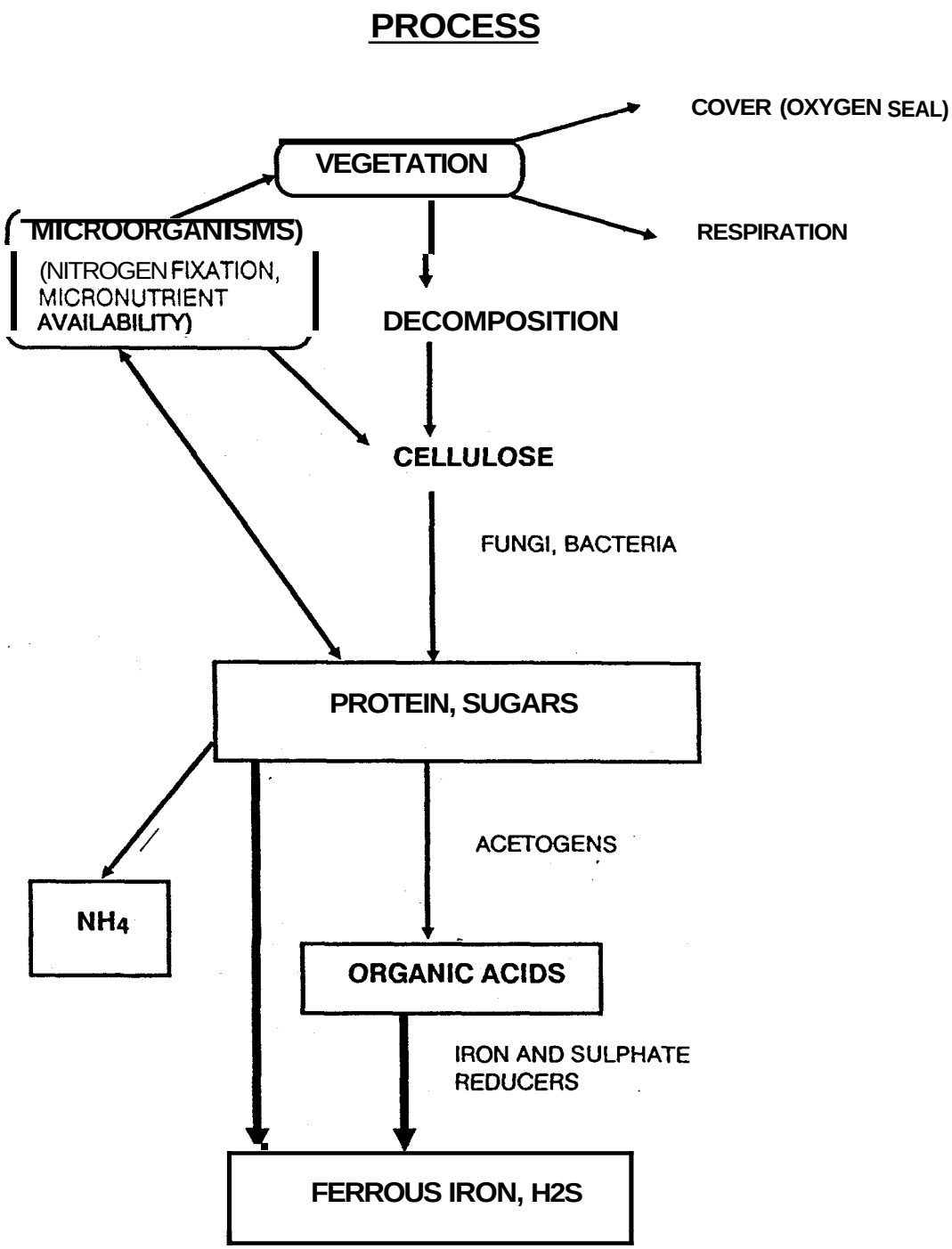


Figure 2.9: Interactions Proposed in Neutralization Process

Table 2.1: pH Values of Samples and Their Microcosms
April 5, 1988

1988 Date	0ω	2.4	2 5	1.2	3.10	1.1	1.3	3 ד	3.8
Jan. 2ד	3.6	--	--	--	3 ד	--	--	3·ב	ס 2
Feb. 2	3.5	3.1	3.3	3.3	3.5	3.4	4.1	4.4	3.6
Feb.16-24	3.2	2.6	2.9	2.9	3.0	2.6	5.3	4.7	3.9
Microcosm Experiment: March 7, 1988									
Anaerobic & Nutrient	ס 5	5 ד	4 2	4.2	4.8	5.2	6.3	5.9	6.0
Anaerobic	2 5	2.8	2.4	2 3	ס.6	4ל	ד 7	5 7	5 1
Aerobic	2 2	2 5	2.0	2ב	2 5	2 5	3.0	2ל	2.4
Abiotic Control PCP	2 ד	3.0	2 5	2 5	2 7	4.1	ס 2	3 ד	3 ה

Note: pH Values could not be obtained due to frozen pH probe

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Summary of Microbiological Tests
January to March 1988

TABLE 2.2: Sample 0.0 - Control. No Amendment

Date	Test Zone	pH	ATP (ng/mL)	Sulfate Reducing Bacteria (number/mL)	Ammonifiers (number/mL)	Microscopic Direct Count (counts/mL) Bacteria	Fungi	Algae
Jan.28/88	Water Bottom	3.6	0.083	NG (API)	--	10 E5	<10 E4	<10 E4
Feb.5/88	Water-Top	3.5	--	--	--	--	--	--
Feb.16/88	Shaken Sample	3.2	0.17	NG (E) NG (F)	NG	10 E5	<10 E4	<10 E4
Mar.7/88	Microcosms:							
	PCP	2.7	0.18	NG (E)	NG	--	--	--
	Aerobic	2.2	0.41	NG (E)	NG	--	--	--
	Anaerobic	2.5	0.44	NG (E)	NG	--	--	--
	Lactate + Peptone	4.5	0.55	NG (E)	NG	--	--	--

NOTES: A blank (-) indicates that a test was not done
NG = no growth from an innoculum of 1.0 mL
API = American Petroleum Institute Medium RP 38
(E) = Postgate Medium E
(F) = Postgate Medium F
* = Sample had frozen in refrigerator and was allowed to stand for 4 to 7 days before initiation of microcosm experiment

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Summary of Microbiological Tests
January to March 1988

TABLE 2.3: Amendment type 2.4

Date	Test Zone	pH	ATP (ng/mL)	Sulfate Reducing Bacteria (number/mL)	Ammonifiers (number/mL)	Microscopic Direct Count (counts/mL) Bacteria	Fungi	Algae
Jan.28/88	Water (law)	--	1.10	10 (API)	--	10 E6	<10 El	<10 E4
	Upper Sediment	--	0.69	1 (MI)	--	10 E5	<10 E4	<10 E4
	Lower Sediment	--	2.00	lo (MI)	--	10 E6	10 E6	10 E4
Feb.5/88	Water	3.1	--	lo (API)	--	--	--	--
Feb.24/88*	Water (law)	2.6	1.10	NG (F)	NG	10 E7	<10 E4	<10 E4
	Upper Sediment	2.4	0.96	NG (F)	NG	10 E7	<10 E4	<10 E4
	Lower Sediment	--	2.60	NG (E)	10	10 E7	10 E6	10 E4
Mar.8/88	Microscoms:							
	PCP	3.0	1.70	NG (E)	NG	--	--	--
	Aerobic	2.5	4.40	NG (E)	NG	--	--	--
	Anaerobic	2.8	10.00	1 (E)	NG	--	--	--
	Lactate + Peptone	5.7	78.00	10 E3 (E)	10	--	--	--

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Summary of Microbiological Tests
January to March 1988

TABLE 2.4: No Treatment, between amendment type 2 and 1

Date	Test Zone	pH	ATP (ng/mL)	Sulfate Reducing Bacteria (number/mL)	Ammonifiers (number/mL)	Microscopic Direct Count (counts/mL) Bacteria	Fungi	Algae
Jan.28/88	Water	--	0.42	NG (AA)	--	10 E5	<10 E4	<10 E4
	Sediment	--	27.00	10 (API)	--	10 E5	<10 E4	10 E4
Feb.5/88	Water	3.3	--	--	--	--	--	--
Feb.17/88	Shaken Sample	2.9	0.92	1 (E)	NG	10 E6	<10 E4	<10 E4
Mar.7/88	Microcosms:							
	PCP	2.5	0.22	NG (E)	NG	--	--	--
	Aerobic	2.0	0.36	NG (E)	NG	--	--	--
	Anaerobic	2.4	0.34	NG (E)	NG	--	--	--
	Lactate + Peptone	4.2	0.62	NG (E)	NG	--	--	--

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PRE-BOG ACID CREEK

Summary of Microbiological Tests
January to March 1988

TABLE 2.5: Amendment type 1.1

Date	Test Zone	pH	ATP (ng/mL)	Sulfate Reducing Bacteria (number/mL)	Ammonifiers (number/mL)	Microscopic Direct Count (counts/mL) Bacteria	Fungi	Algae
Jan.28/88	Water	--	0.85	1 (API)	--	10 E6	10 E4	<10 E4
	Sediment	--	470.00	NG	--	10 E5	10 E6	10 E5
Feb.5/88	Water	3.3	--	--	--	--	--	--
Feb.19/88	Shaken Sample	2.9	4.30	NG (E)	1	10 E7	10 E4	10 E4
Mar.7/88	Microcosms:							
	PCP	2.5	0.31	NG (E)	NG	--	--	--
	Aerobic	2.0	2.40	NG (E)	NG	--	--	--
	Anaerobic	2.3	2.90	NG (E)	NG	--	--	--
	Lactate + Peptone	4.2	0.72	NG (E)	NG	--	--	--

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Summary of Microbiological Tests
January to March 1988

TABLE 2.6: Amendment Type 1.2

Date	Test Zone	pH	ATP (ng/mL)	Sulfate Reducing Bacteria (number/mL)	Ammonifiers (number/mL)	Microscopic Direct Count (counts/mL) Bacteria	Fungi	Algae
Jan.28/88	Water	--	4.60	10 (API)	--	10 E6	<10 E4	<10 E4
	Upper Sediment	--	21.00	10 (API)	--	10 E6	<10 E4	<10 E4
	Lower Sediment	--	41.00	10 (API)	--	10 E5	<10 E4	<10 E4
Feb.5/88	Water	3.4	--	--	--	--	--	--
Feb.24/88*	Water	2.6	2.80	NG (E) 1 (F)	1	10 E8	<10 E4	10 E6
	Upper Sediment	2.6	23.00	10 (E) 1 (F)	10	10 E6	<10 E4	<10 E4
	Lower Sediment	--	42.00	10 (E) 1 (F)	10 E2	10 E5	<10 E4	<10 E4
Mar.7/88	Microcosms:							
	PCP	4.7	0.31	NG (E)	NG	--	--	--
	Aerobic	2.5	41.00	10 (E)	10	--	--	--
	Anaerobic	4.6	12.00	10 (E)	10 E3	--	--	--
	Lactate + Peptone	5.2	11.00	10 E5 (E)	10 E3	--	--	--

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Summary of Microbiological Tests
January to March 1988

TABLE 2.7: After amendment type 1

Date	Test Zone	pH	ATP (ng/mL)	Sulfate Reducing Bacteria (number/mL)	Ammonifiers (number/mL)	Microscopic Direct Count (counts/mL) Bacteria	Fungi	Algae
Jan.28/88	Water (top)		3.00	1 (MI)	--	10 E6	10 E4	<10 E4
	Water (bottom)		4.40	10 (AA)	--	10 E6	10 E5	10 E5
	Sediment		100.00	lo (MI)	--	10 E6	10 E6	10 E6
Feb.5/88	Water	4.1	--	--	--	--	--	--
Feb.18/88*	Water (top)	5.3	1.80	10 E2 (E)	10	10 E8	10 E6	<10 E4
	Water (bottom)			1 (F)				
	Sediment	--	6.70	10 (E)	10 E3	10 E9	<10 E4	10 E4
				10 (F)				
Mar.7/88	Microcosms:							
	PCP	4.2	0.30	NG (E)	NG	--	--	--
	Aerobic	3.0	4.10	10 (E)	10	--	--	--
	Anaerobic	5.7	8.10	10 (E)	10	--	--	--
	Lactate + Peptone	6.3	0.23	10 (E)	10	--	--	--

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Summary of Microbiological Tests
January to March 1988

TABLE 2.8: Amendment type 3. first test call

Date	Test Zone	pH	ATP (ng/mL)	Sulfate Reducing Bacteria (number/mL)	Ammonifiers (number/mL)	Microscopic Direct Count (counts/mL) Bacteria	Fungi	Algae
Jan.28/88	Water (top)	3.8	7.90	1 (API)	--	10 E6	10 E5	10 E4
	Water (bottom)	3.8	4.10	1 (API)	--	10 E5	10 E5	<10 E4
	Sediment	--	16.00	10 (API)	--	10 E6	10 E5	<10 E4
Feb.5/88	Water	4.4	--	--	--	--	--	--
Feb.19/88	Water (top)	4.7	3.50	10 E2 (E) 10 (E)	10 E2 10 (F)	10 E7	<10 E4	<10 E4
	Water (bottom)	4.7	4.50	10 E2 (E) 10 (F)	10 E2 (E) 10 (F)	10 E8	10 E6	<10 E4
	Sediment	--	5.60	10 (E) 10 (F)	10 (E) 10 (F)	10 E2	10 E7	10 E4
Mar.7/88	Microcosms:							
	PCP	3.7	0.17	NG	NG (E)	--	--	--
	Aerobic	2.6	6.10	10 (E)	10	--	--	--
	Anaerobic	5.7	5.20	10 (E)	10 E3	--	--	--
	Lactate + Peptone	5.9	0.75	10 E3 (E)	10 E3	--	--	--

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Summary of Microbiological Tests
January to March 1988

TABLE 2.9: Amendment type 3. second test cell

Date	Test Zone	pH	ATP (ng/mL)	Sulfate Reducing Bacteria (number/mL)	Ammonifiers (number/mL)	Microscopic Direct Count (counts/mL) Bacteria	Fungi	Algae
Jan.28/88	Water (top)	4.2	2.90	10 (API)	--	--	--	--
	Water (bottom)	4.2	3.80	1 (API)	--	10 E6	10 E4	<10 E4
	Sediment	--	14.00	--	10 E6	10 E5	<10 E4	
Feb.5/88	Water	3.6	--	--	--	--	--	--
Feb.19/88	Water (top)	3.9	2.80	10 E2 (E) 10 (F)	10 E3	10 E7	10 E7	<10 E4
	Water (bottom)	3.9	2.20	10 E2 (E) 10 (F)	10 E3	10 E7	10 E6	<10 E4
	Sediment	--	16.00	10 E4 (E)	10 E3	10 E8	10 E7	<10 E4
Mar.7/88	Microcosms:							
	PCP	3.5	0.72	NG (E)	NG	--	--	--
	Aerobic	2.4	21.00	10 (E)	10 E3	--	--	--
	Anaerobic	5.1	7.60	10 (E)	10 E3	--	--	--
	Lactate + Peptone	6.0	3.20	10 E5 (E)	10 E5	--	--	--

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PRE-BOG ACID CREEK

Summary of Microbiological Tests
January to March 1988

TABLE 2.10: After Amendment type 3, No Treataent

Date	Test Zone	pH	ATP (ng/mL)	Sulfate Reducing Bacteria (number/mL)	Ammonifiers (number/mL)	Microscopic Direct Count (counts/mL) Bacteria	Fungi	Algae
Jan.28/88	Water (bottom)	3.5	0.29	NG (API)	--	10 E5	<10 E4	<10 E4
Feb.5/88	Water	3.5	--	--	--	--	--	--
Feb.17/88	Shaken Sample	3.0	0.80	1 (E) 1 (F)	1	10 E6	<10 E4	>10 E4
Mar.7/88	Microcosms:							
	PCP	2.7	2.30	NG (E)	NG	--	--	--
	Aerobic	2.5	1.80	NG (E)	NG	--	--	--
	Anaerobic	4.6	1.60	NG (E)	10	--	--	--
	Lactate + Peptone	4.8	1.30	1 (E)	10	--	--	--

Levack - Pre-Bog Acid Creek

Table 2.11: Relation of SRB and Ammonifier Counts
To Acid Neutralization in Anaerobic Microcosms

Samples which displayed pH increases of at least one pH unit after Feb.2	SRB's (Numbers per mL)	Ammonifiers (Numbers per mL)
No Treatment after type 3	NG	NG
Amendment type 1	10	10 E3
After Amendment type 1	10	10
Type 3 cell - #1	10	10 E3
Type 3 cell - #2	10	10 E3
Samples which displayed no pH increase or an increase less than one pH unit after Peb. 2		
Control, no amendment	NG	NG
type 2	NG	NG
No treatment, after type 3	NG	NG
Water layer. type 1	NG	NG

NG = no growth from an inoculum of 1 mL

Note: SRB's and ammonifiers grew in the Sample 3.10 microcosm in which the nutrients peptone and lactate had been added. When peptone and lactate were added to the other microcosms, SRB's and ammonifiers grew only those which displayed a pH increase of at least one unit.

Effect of Lactate/Peptone Supplement

TABLE 2.12

	final pH without addition	final pH with addition	increase in SRB count	increase in ammonifiers count
No Treatment After Type 3	4.6	4.8	+	+
Amendment Type 1	4.6	5.2	+	+
Type 3 cell #1	5.7	6.3	0	0
Type 3 cell #2	5.7	5.9	+	0
	5.1	5.9	+	+

\+ = increase was observed.

0 = no change was observed.

Examples of Sulphate Reduction Rates

TABLE 2.13

ENVIRONMENT	SULPHATE REDUCTION RATE mg SO_4 L^{-1} day^{-1}	COMMENTS	REFERENCE
Acidic peat	**17**	Highest seasonal rate observed.	Spratt et al **(1987)**
Fresh water sediments receiving acid mine drainage.	**864**	These rates are higher than any other values reported previously for coastal marine or lake sediments. Results are average of triplicate samples (CV<50%)	Herliky & Mills **(1985)**
Lab reactor	**1920**	Lactic acid as carbon source (Starkey's medium).	Cork & Cusanovich **(1978)**
Lab reactor	**2300**	Molasses as carbon source (assume lactic acid produced).	Maree et al **(1987)**
High rate sulfate reduction annular biofilm lab reaction.	**214,000**	Lactic acid as carbon source. Temperature = 20°C.	Neilsen **(1987)**

MORPHOLOGICAL/ANATOMICAL INVESTIGATION OF CATTAIL TRANSPLANTS AND BOG VEGETATION

Milestone 1

By: R.W. Scribailo and M. Kalin

DSS File No.: 28SQ.23440-9-9140

DSS Contract Serial No.: 23440-9-9140

CANMET Scientific Authority: Dr. R.G.L. McCready

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MORPHOLOGICAL/ANATOMICAL INVESTIGATION OF CATTAIL TRANSPLANTS AND BOG VEGETATION

Milestone 1

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1.0 INTRODUCTION

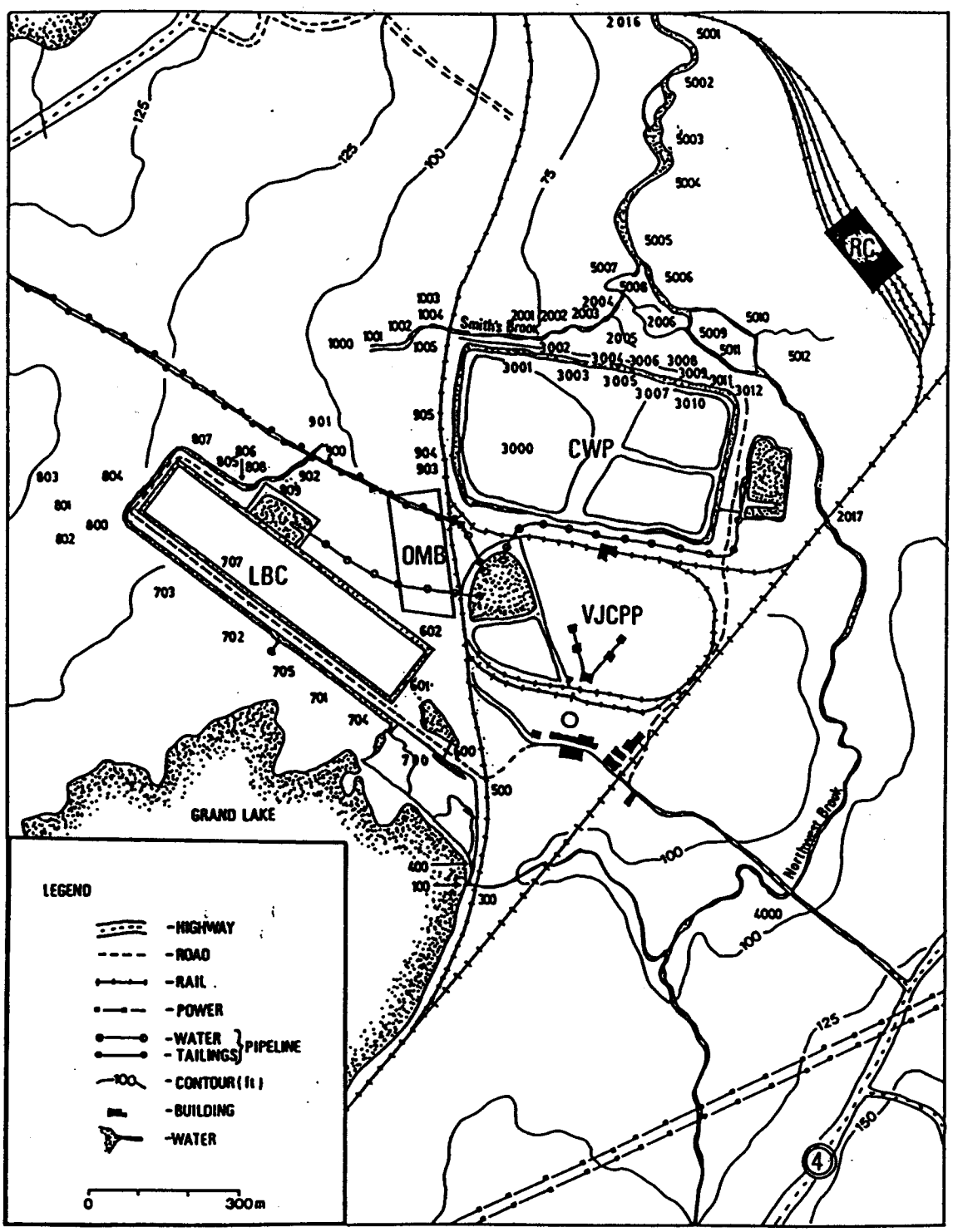
The Victoria Junction Coal Processing Plant ("VJCPP") of DEVCO in Sydney, Cape Breton, Nova Scotia, is surrounded by bogs. Bogs are often acidic ecosystems which are expected to be tolerant to Acid Mine Drainage (AMD). Furthermore, bogs or wetlands are alleged to ameliorate these types of waste water 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 and the settling ponds. A second bog, located immediately above the stressed bog in the vicinity of Station 700 (Map 1) was healthy and unaffected by acid mine drainage. The vegetation type of both bogs

MAP 1: OVERVIEW OF THE VICTORIA JUNCTION COAL PROCESSING PLANT

VICTORIA JUNCTION COAL PROCESSING PLANT.



is very similar and therefore a comparative investigation was possible. Acid mine drainage was diverted into both bogs, the old bog which had already deteriorated as well as into the healthy new bog.

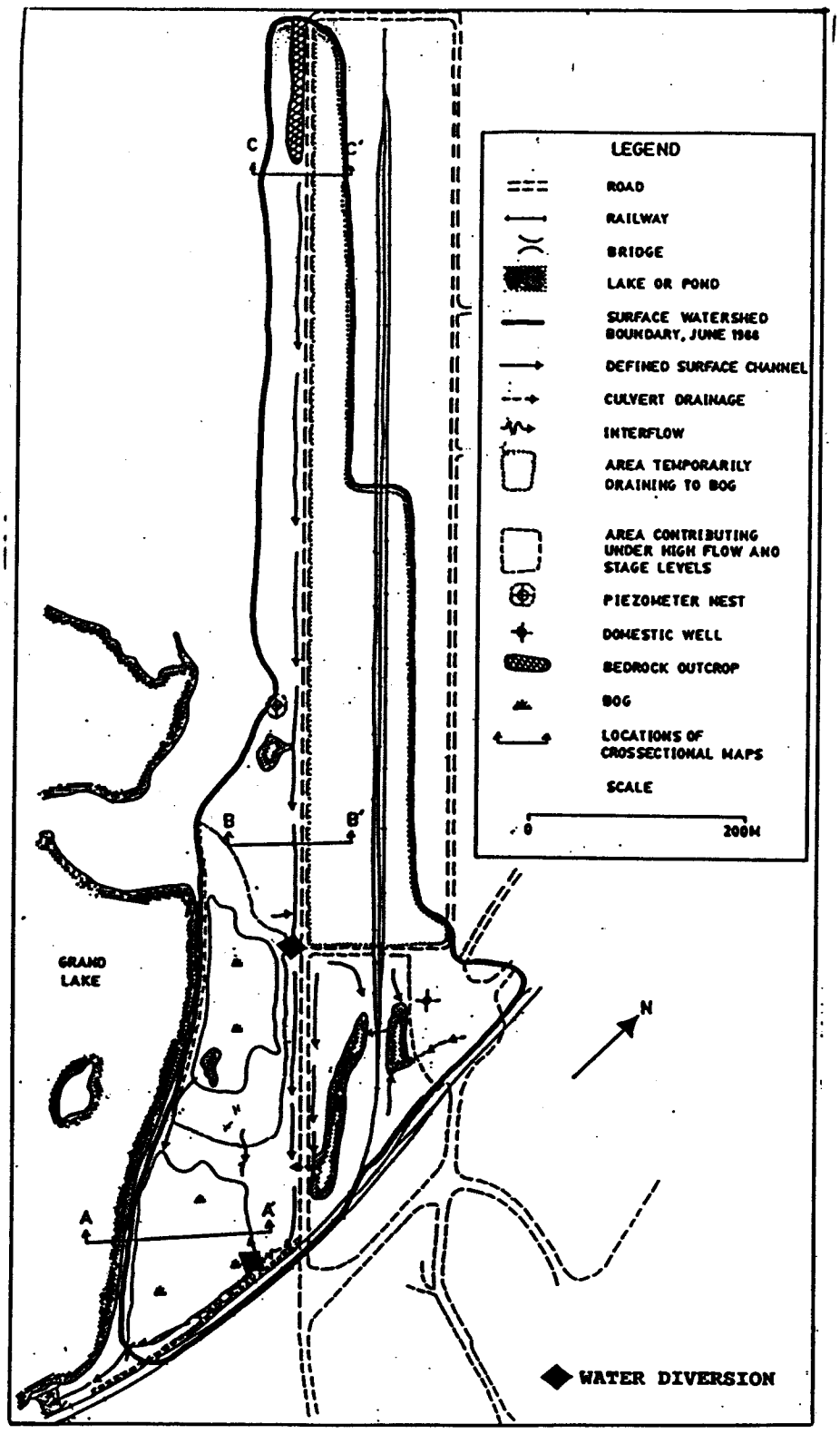
This report describes the status of the vegetation in both bogs after one growing season. It summarizes both on-site and laboratory investigations. Through the use of morphological-anatomical techniques, the death or growth of plant parts can be determined.

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 of the general flow direction indicate the conditions prior to the diversions of the surface water flows which are indicated by the diamonds. 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. In effect, 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 flows in the bogs. The vegetation cover studied is growing on a layer

Map 2: DRAINAGE BASIN OF THE LIFTING AND BANKING CENTER



of peat, which is underlaid by a layer of muck, lying above till. Thus ground water will enter the bog, together with water from Grand Lake, and in addition to the acid mine drainage.

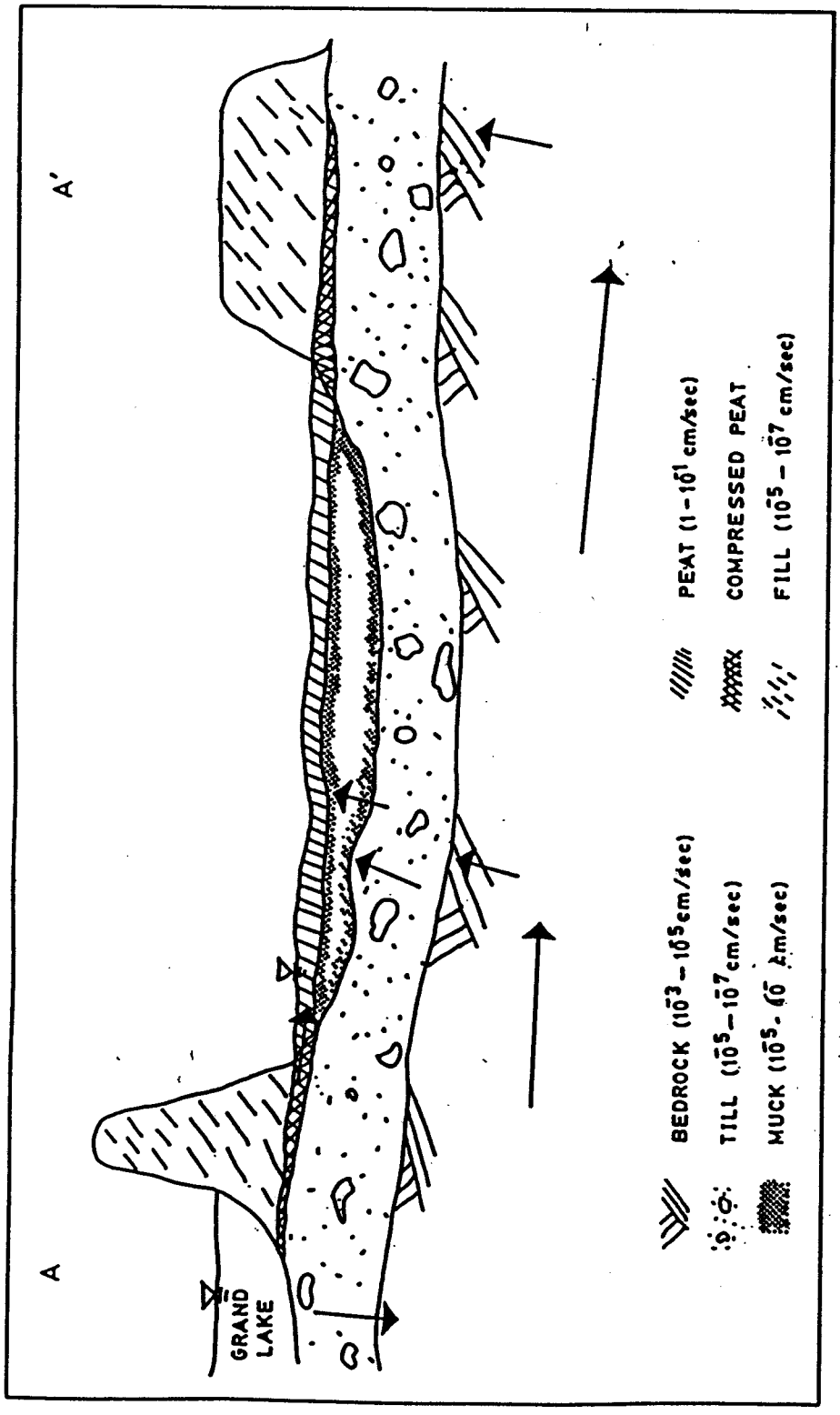
These differences are evident, and are summarized in Schematic 2 for conditions found in the old bog during the 1989 summer. Along the side of Grand Lake, the pH is high and the electrical conductivities are low, whereas acid conditions prevail in the remainder of the old bog.

The relative composition of the groundwater is given for Ca, Mg, Na, Cl SO_4 and HCO_3, which can be compared with the surface water entering from Grand Lake and the Acid mine drainage. The vegetation cover clearly reflects the water regime. The vegetation along the Grand Lake inflow is not stressed by the acid conditions and is healthy.

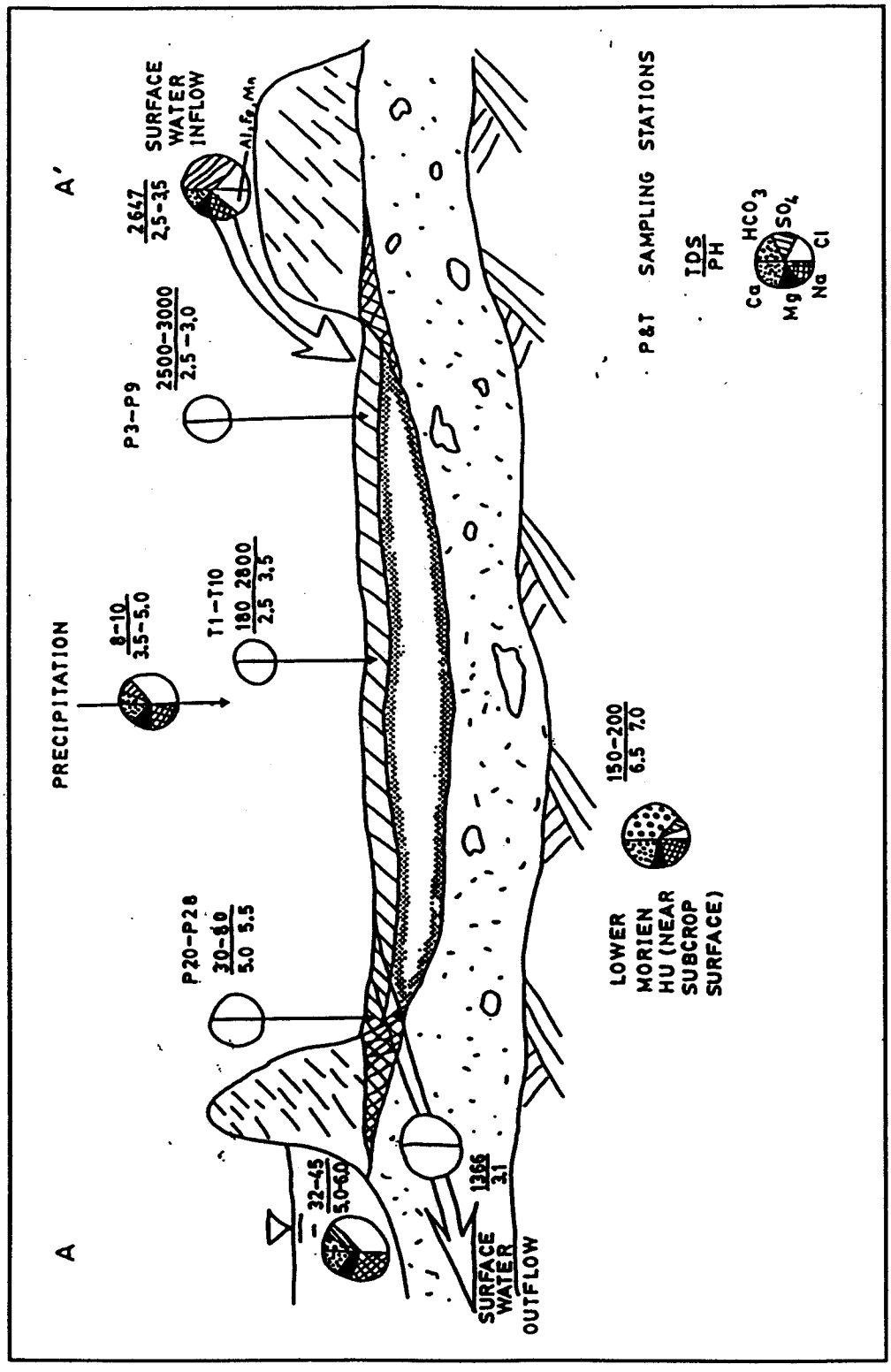
2.2 Vegetation Assessment

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 1: BOG GEOMORPHOLOGY AND PERMEABILITY



SCHEMATIC 2: GROUNDWATER AND SURFACE WATER CHARACTERISTICS



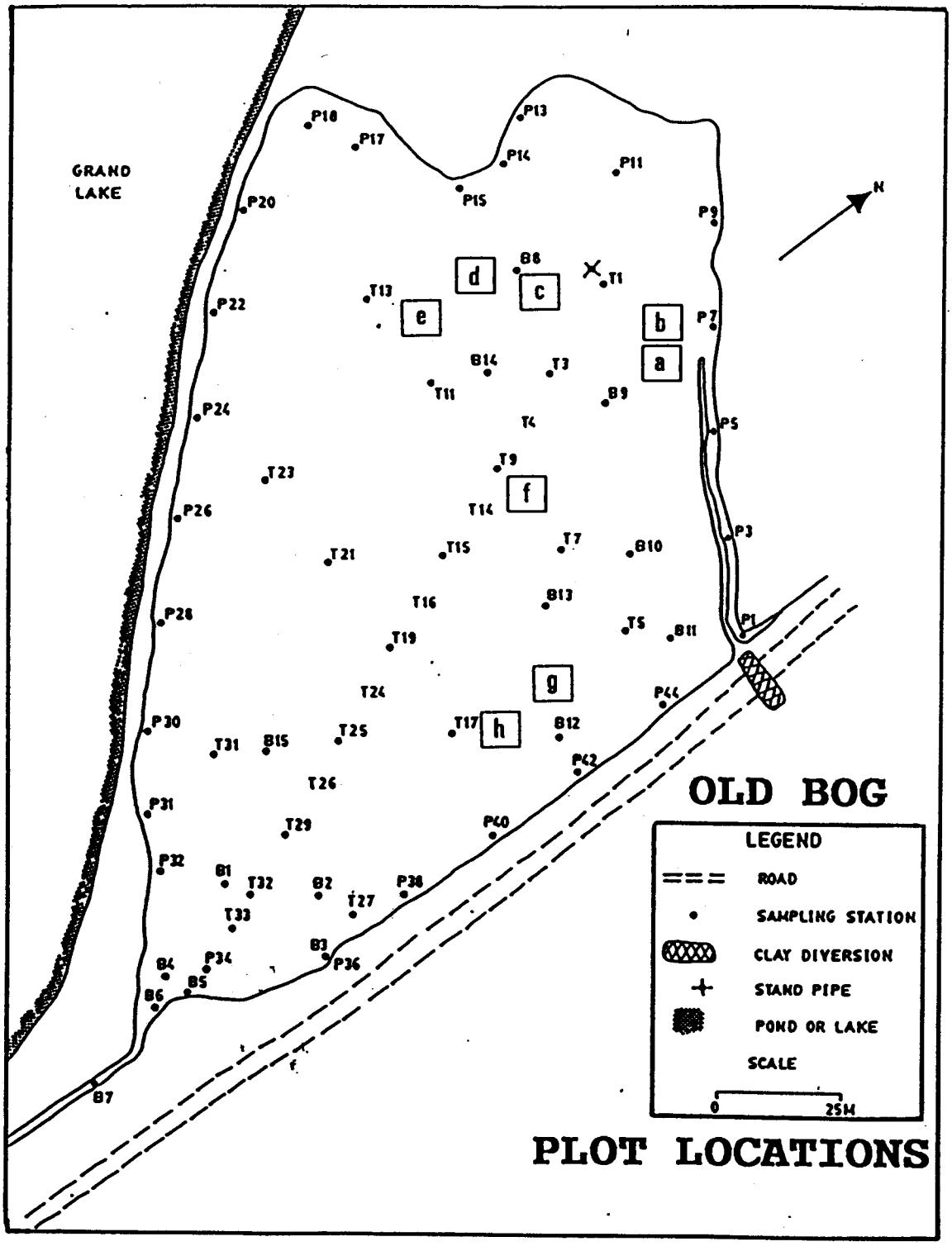
ten permanent quadrats were established.

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.

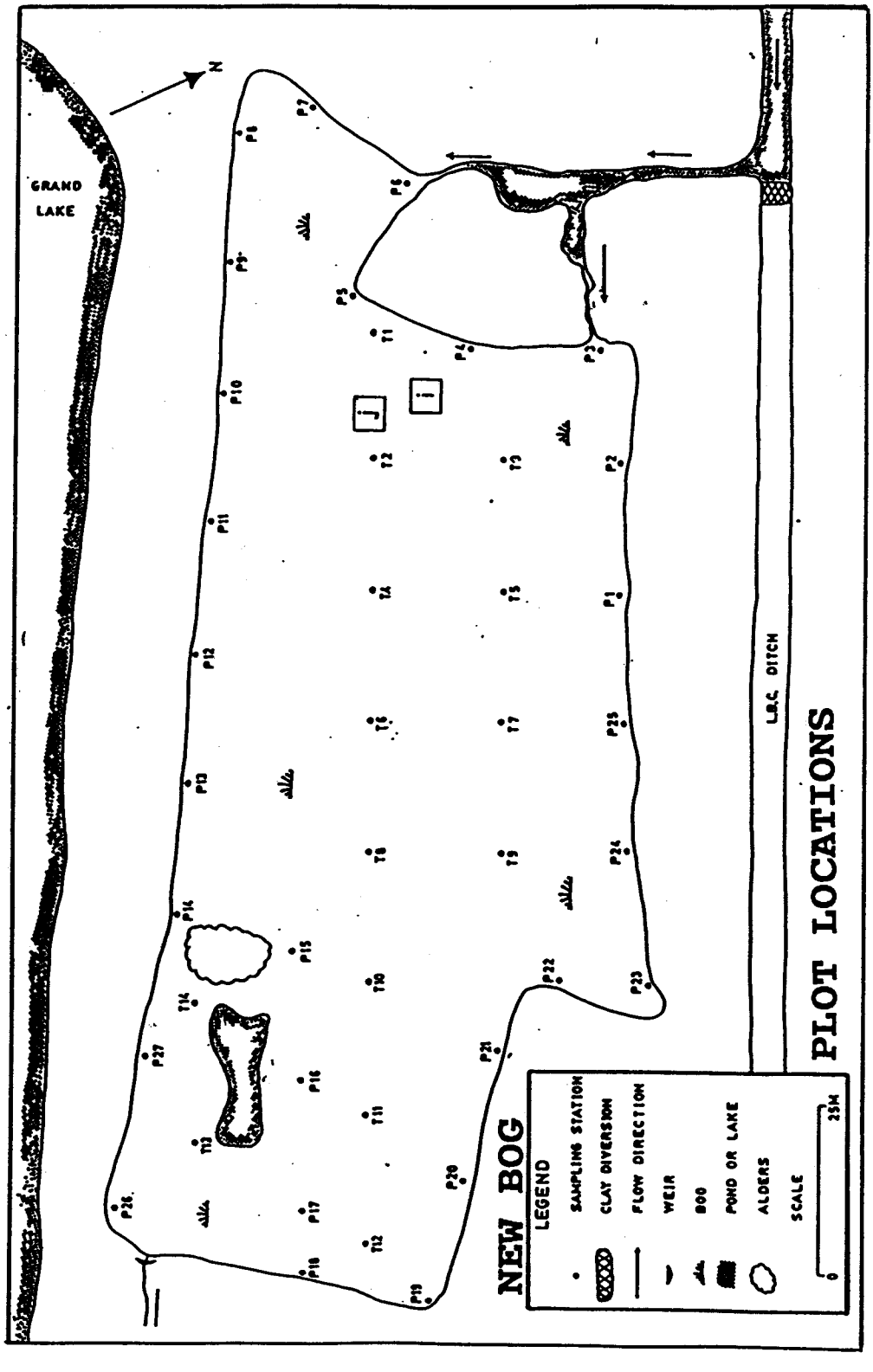
2.3 Microscopic Methods

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 bogs 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

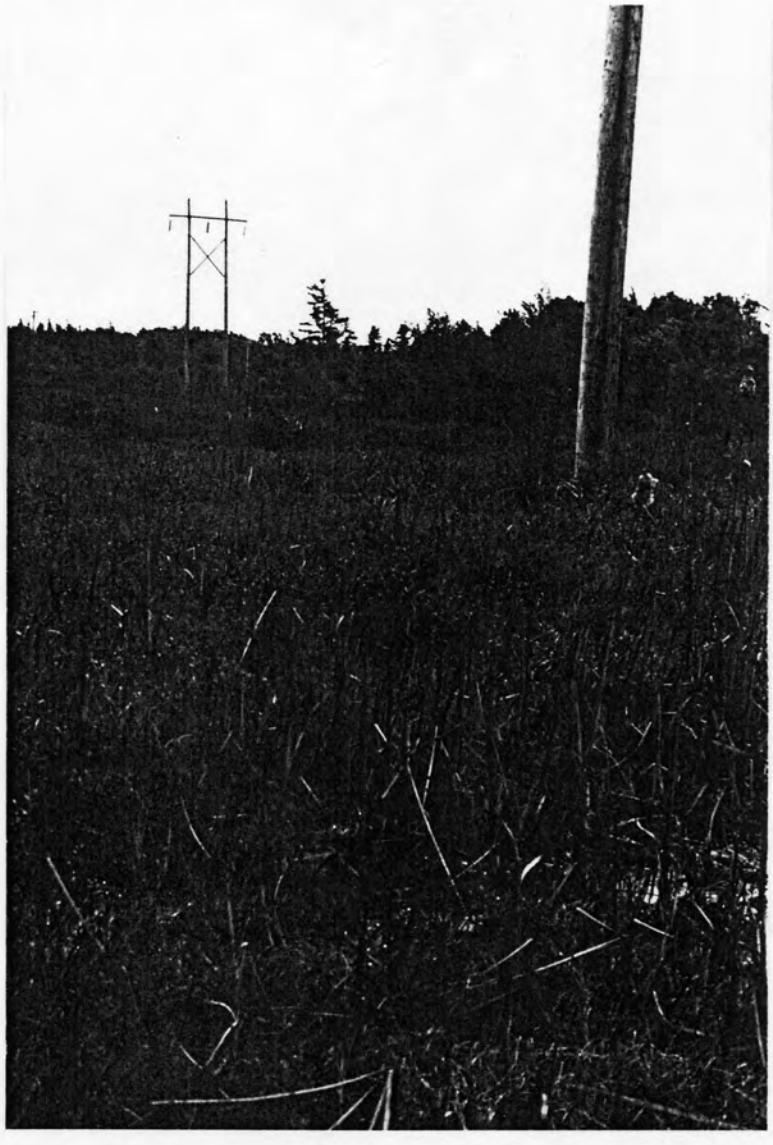


3.0 RESULTS AND DISCUSSION

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 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 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

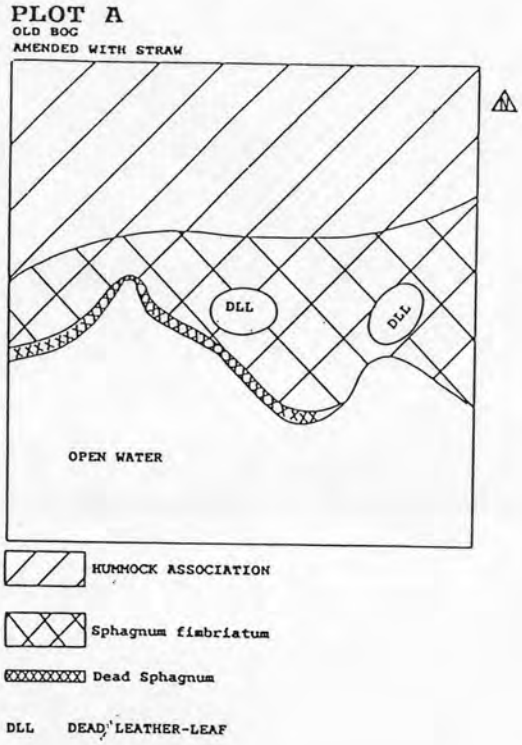


Plate 2: Photograph of Plot A

SCHEMATIC 4: Mapping of Plot G

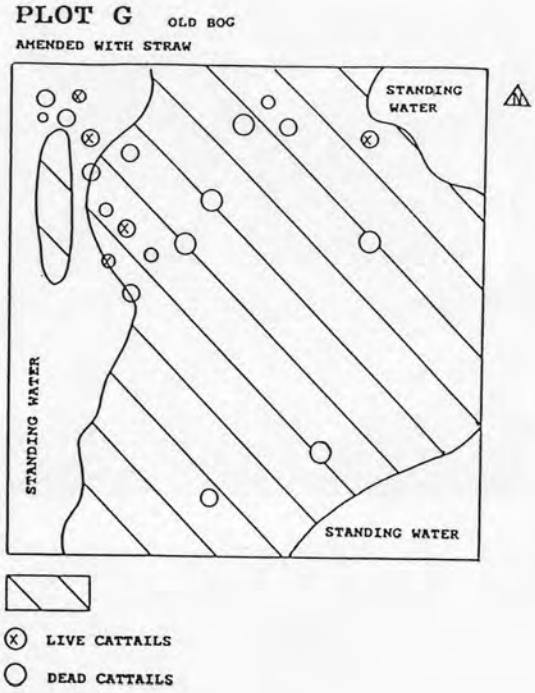


Plate 3: Photograph of Plot G

Plate 4: View of the old bog in foreground

Plate 5: Lower zones in old bog

TABLE 1: PLANT SPECIES 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 afore-mentioned 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 acid conditions on growth of cattail plants

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 Typha latifolia 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 Typha 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.

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.

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.

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 the 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 shown that vigour 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, results 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.

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AN EVALUATION OF METHODS REQUIRED TO DETERMINE OVERWINTERING PLANT HEALTH IN CATTAILS GROWING ON ACIDIC MINE TAILINGS

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ABSTRACT

As part of an overall Ecological Engineering strategy to ameliorate acid mine drainage - a water pollution problem associated with the mining industry - by enhancing natural water treatment processes, this report has evaluated (and discussed data obtained from) methods and analyses used to determine below-ground cattail starch quantities and distribution, metal uptake, and general morphology. With the vision of increasing plant biomass and biological polishing capacity through fertilization, results from this report will be used to formulate a sampling and analytical strategy for determining the effects of foliar fertilization on below-ground biomass of cattails growing on acidic tailings at a uranium mine in Elliot Lake, Ont. Preliminary cattail samples were collected locally and subjected to the procedures deemed appropriate for determining whether applications of foliar fertilizer on tailings-grown cattails would have an effect on plant health. The methods used during the preliminary trial were appropriate for providing data on a number of growth-related parameters and required only minor sample-specific adjustment to obtain accurate results. Data obtained while evaluating the methods, along with findings from other studies, indicated that pith size may be an important below-ground morphological indicator of plant health.

CONCLUSIONS

Cattails can be extracted in the winter with a spade, provided there is minimal ice build-up over the substrate. Plant damage due to extraction was kept to a minimum by digging approximately 0.5m away from the chosen senescent shoot in a circular fashion and periodically checking for submerged new shoots in the substrate.

Minimal disfiguring of rhizome hand sections occurred when samples, before sectioning, were placed back in the freezer for one-half hour after laboratory washing.

Storing hand sections in 95% denatured ethyl alcohol did not change the distribution of starch in the rhizome. Changes in actual starch quantity as a result of alcohol storage were not confirmed.

Removing roots at the exodermis was not microscopically precise. It could not be determined whether any remaining root tissue affected rhizome weight measurements.

Iodine/potassium iodide staining for starch distribution was an effective method for elucidating the presence and location of starch within a rhizome section. Colouration of tissue resulting from stain application was consistent throughout the samples.

Hematoxylin staining for metal uptake in tissue was very time-specific, with the 2-4 hr. retention interval producing the best colouration. Colours produced by stock hematoxylin application were too intense for effective examination; those produced from fresh solutions were adequate.

Photographing rhizome sections produced the best results when the camera shutter speed was slowed down from the suggested setting. Measuring sections was made effective and easy using a movable, graduated (mm^2 grid) cover slip.

Blending fresh cattail tissue produced considerable variability in final quantitative starch determinations. Because the tissue could not be blended into a consistent slurry, accuracy of the starch data was questionable.

Twenty-seven of 29 rhizome sections had piths whose diamaters were greater than 50% of their respective rhizome's overall diameter. Ninety percent of the rhizomes had piths that became proportionately larger toward the new shoot. Pith diameter may be an important parameter in determining plant health.

RECOMMENDATIONS

Once extracted from the substrate, cattails should be kept frozen until analyses are undertaken. Ice-filled coolers should be used to transport samples from the field to the laboratory.

Below-ground biomass claculations should be performed in the summer months when the conditions are suitable to do so.

Random sampling of cattails may be done by pacing out the experimental plots and using a table of random numbers to locate samples.

Total soluble carbohydrate tests should be carried out to determine what percentage leaves the plant cells and passes into solution.

Fresh hematoxylin solution should be prepared daily and allowed to develop colour for at least two hours, but no longer than four.

To ensure proper exposure when taking pictures of rhizome sections, small samples should be photographed at a ¼s slower exposure setting than suggested by the camera's light meter. Exposure times for large samples need only be moved to the maximum setting on the fine adjustment control while keeping the macro-adjustment as suggested.

A micro-ruler with 0.1mm graduations should be useful and more accurate for rhizome dimensioning under the microscope.

To decrease data variability between replicates of a single sample, tissue for quantitative starch analysis should be dried at 110°C to constant weight and ground into a powder.

Graduated 5ml pipettes should be used to improve accuracy and consistency when taking

aliquots.

Attention should be paid to measuring pith and rhizome diameters of the Denison cattails since a relationship between proportional pith size and plant health has been suggested.

1. INTRODUCTION

1.1 Problem Areas

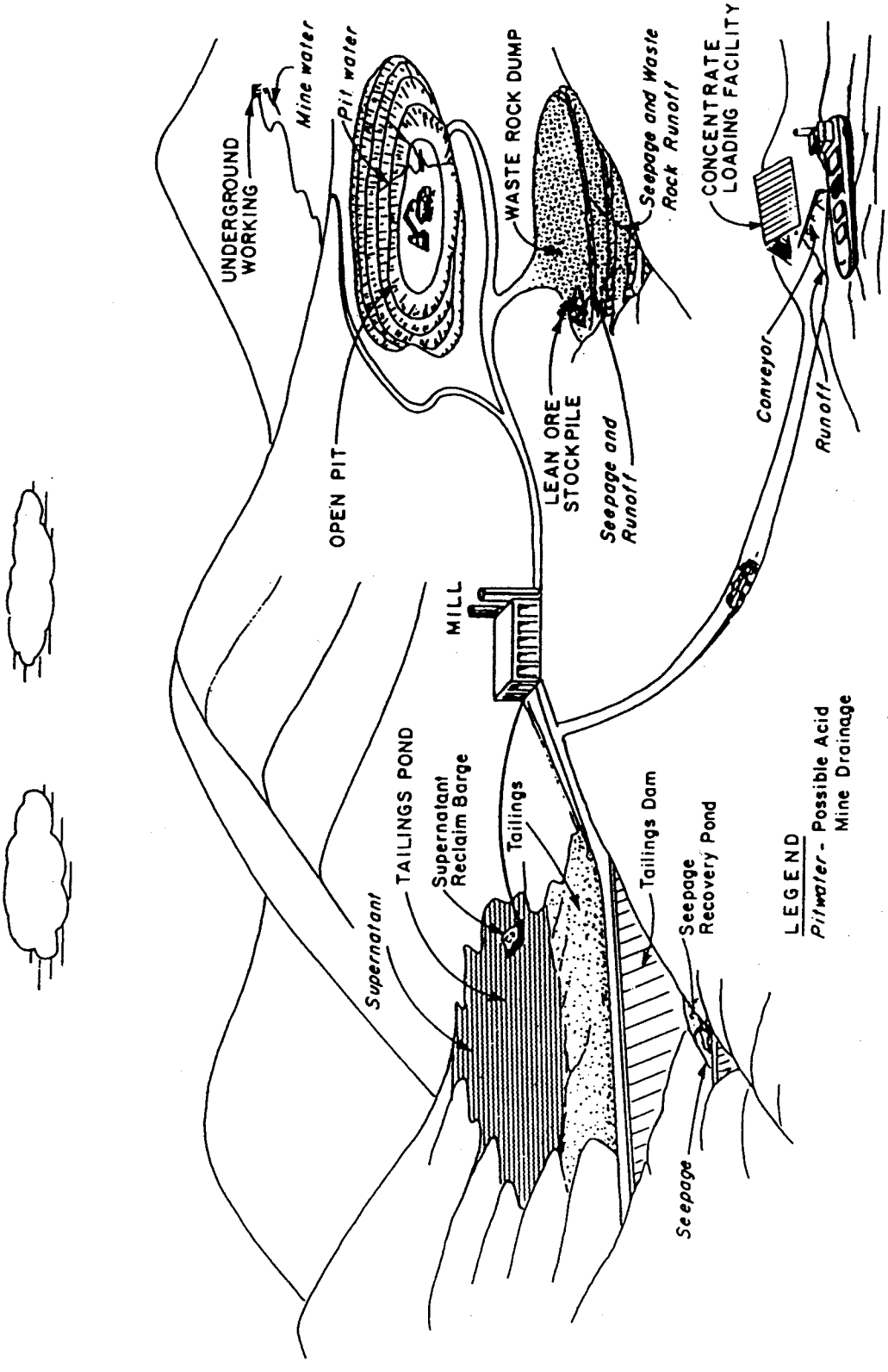
Conventional approaches to wastewater treatment at acid-generating waste sites focus on collecting and neutralizing contaminated water, typically with the use of costly treatment plants and liming programs. Unfortunately for the operators, these costs are perpetual as wastes continue to produce acid long after revenue operations have ceased. To ensure environmental integrity, government abatement regulations are now mandating operators of base metal and precious metal mines, for example, to set aside money for environmental initiatives like wastewater treatment. Because, from a business standpoint, the environment represents a long-term liability, novel and cost-effective treatment methods need to be developed so that honest and effective efforts can be made to protect the natural environment.

Ecological Engineering addresses these concerns in its development of a conceptual framework for a self-sustaining treatment system. Cattail studies are only part of a broader scheme whose aim is to produce close-out conditions which are environmentally acceptable, self-sustaining, and cost-effective; other areas of investigation include microbiological sulphate-reducing processes, biological filtration systems, and solid waste research.

In recent years it has been acknowledged that cattail wetlands possess the ability to treat wastewater (Lakshman, 1987; Kadlec, 1987; Watson et al, 1987), and attempts have been made to use this quality for ameliorating acid mine drainage (Kalin, 1986). Recognizing that small but persistent stands of naturally-colonized cattails do grow on acidic tailings sites, ecological studies have been carried out on the growth of these emergent macrophytes (Kalin, 1984).

Resulting from the oxidation and hydrolysis of sulphides in waste rock and tailings, acid mine drainage (AMD) is a water quality problem commonly associated with metal mining activities (Tucker at al, 1987) - see Schematic 1. Acid is produced, in the presence

Schematic 1. Major Components of a Metal Mine and Sources of Acid Mine Drainage



of water and oxygen, when Fe and other metallic sulphides oxidize to form soluble hydrous Fe sulphates:

$$(1)\quad 2FeS_2 + 7O_2 + 2H_2O \leftrightarrow 2Fe^{+2} + 4SO_4^{-2} + 4H^+$$

In contact with water, ferrous Fe oxidizes to the ferric state where it further complexes with ferrous and ferric oxyhydroxides to form a precipitate and more acid:

$$(2)\quad Fe^{+2} + 1/4O_2 + H^+ \leftrightarrow Fe^{+3} + 1/2H_2O$$
$$(3)\quad Fe^{+3} + 3H_2O \leftrightarrow Fe(OH)_3 + 3H^+$$

In addition, *Thiobacillus ferrooxidans*, an Fe bacteria which occurs in aqueous environments with a pH range of 2.8 to 3.2, can bring about reactions 2 & 3 and produce more H^+ ions, thereby further increasing the acidity of the system (Tucker et al, 1987).

Cattails play a role in reducing acid generated in the above processes by providing an organic source for sulphate reducing bacteria (SRBs); these microorganisms reduce acid in wastewater back to hydrogen sulphide:

$$(4)\quad 2CH_2O + SO_4^{-2} + 2H^+ \rightarrow 2CO_2 + H_2S + 2H_2O$$

With this reduction of acidity comes the precipitation of ferric hydroxide and other metals. In addition, cattails require oxygen in the rhizosphere for respiration; under circumneutral conditions, CO_2 released by respiration around cattail rhizomes remains in solution thus making surrounding water more resistant to changes in pH. This buffering capacity, however, is greatly reduced in acidic conditions where CO_2 does not remain in solution and quickly bubbles away as gas (Wetzel, 1983).

Cattails also help ameliorate AMD by providing, over waterlogged areas, an

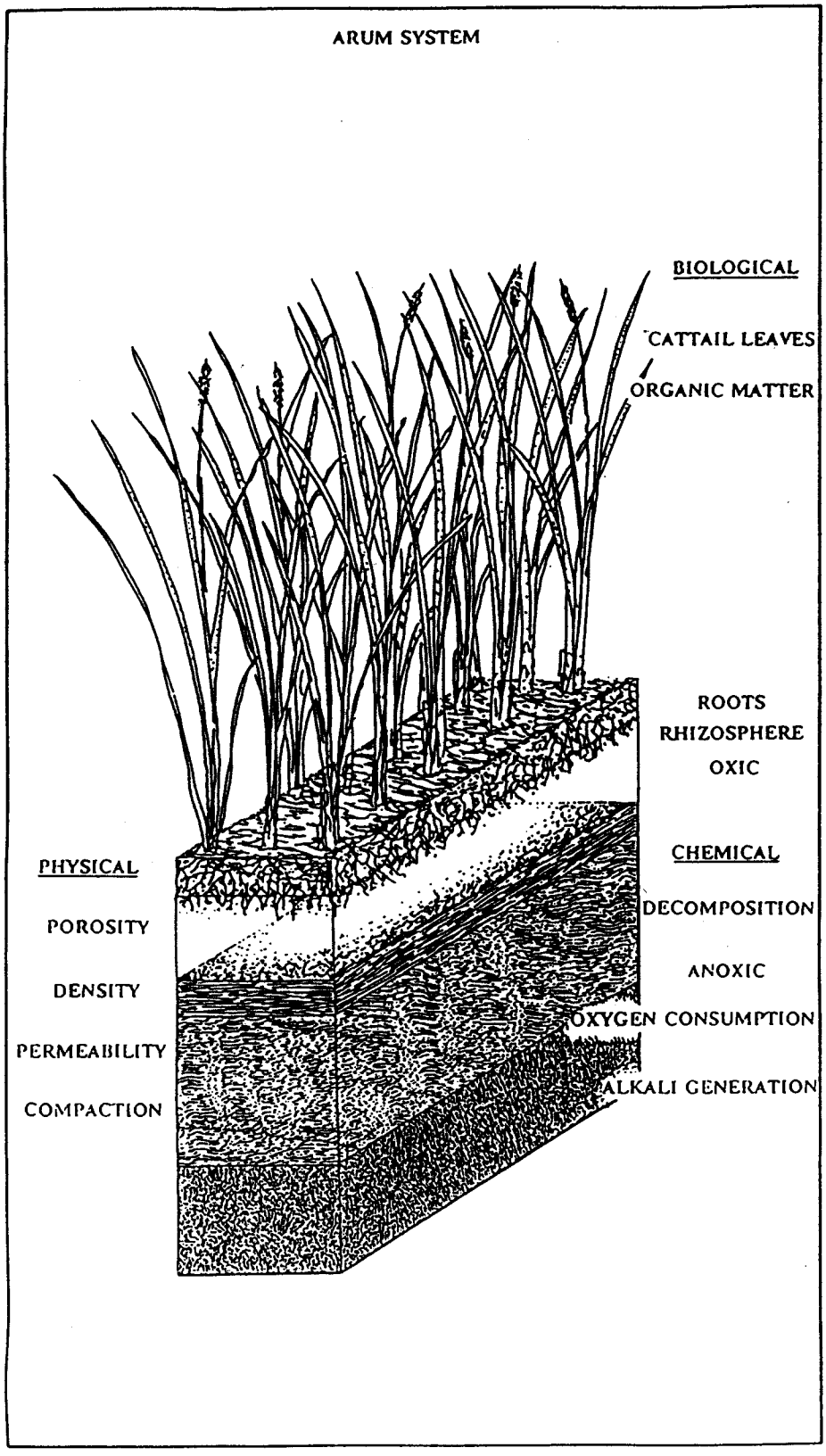
intercepting cover that prevents the mixing of oxygen (an essential component in the acid-generating process) with water - see oxygen consumption and alkalinity generation in the anoxic layer in Schematic 2. Furthermore, emergent wetland biota like the cattail prevent the accumulation of water in these areas by intercepting and transpiring much of the precipitation incident on a wetland (Larcher, 1987).

In light of these phenomena it becomes clear that cattails play an important role in AMD treatment. In response, then, to their significance in natural wastewater treatment and industry's growing need for cost-effective solutions to its waste problems, studies commenced in 1988 to determine the growth and development patterns of cattails in acidic waters. Investigations focused on comparing cattail development among sites of increasing environmental severity in relation to pH and associated increasing levels of metal toxicity (Kalin & Scribailo, 1989; Kalin, 1990). In addition, and of particular relevance to this study, several different fertilizer treatments were applied to cattail plots at Denison Mines (Elliot Lake, Ont.) to determine their effectiveness in enhancing growth and survivorship.

Results of experiments on acidic tailings at a uranium mine in Elliot Lake, Ontario indicated that roots of cattails showed large accumulations of metals on their exodermal and hypodermal layers (for a biological description of cattails see Section 2.1). Scanning Electron Microscopy (SEM) analyses, employing X-ray spectral determinations of metal concentrations, showed that high levels of iron were associated with high concentrations of sulphur and greatly reduced levels of calcium; crystal formation was also noted in the iron-sulphate plaque accumulation on the roots. These findings suggested that the cattail rhizosphere may have been active in ameliorating AMD (Kalin & Scribailo, 1989).

The latter part of this study attempted to determine the morphological and growth effects of foliar fertilizers on cattails with the rationale that if these macrophytes have an ameliorative effect on AMD, then by increasing plant biomass through fertilization in areas where growing conditions are harsh, their treating effect should also be increased (i.e. the more biomass, the greater the treatment capacity). Foliar fertilizers, as the name implies,

Schematic 2. The ARUM (Acid Reduction Using Microbiology) System

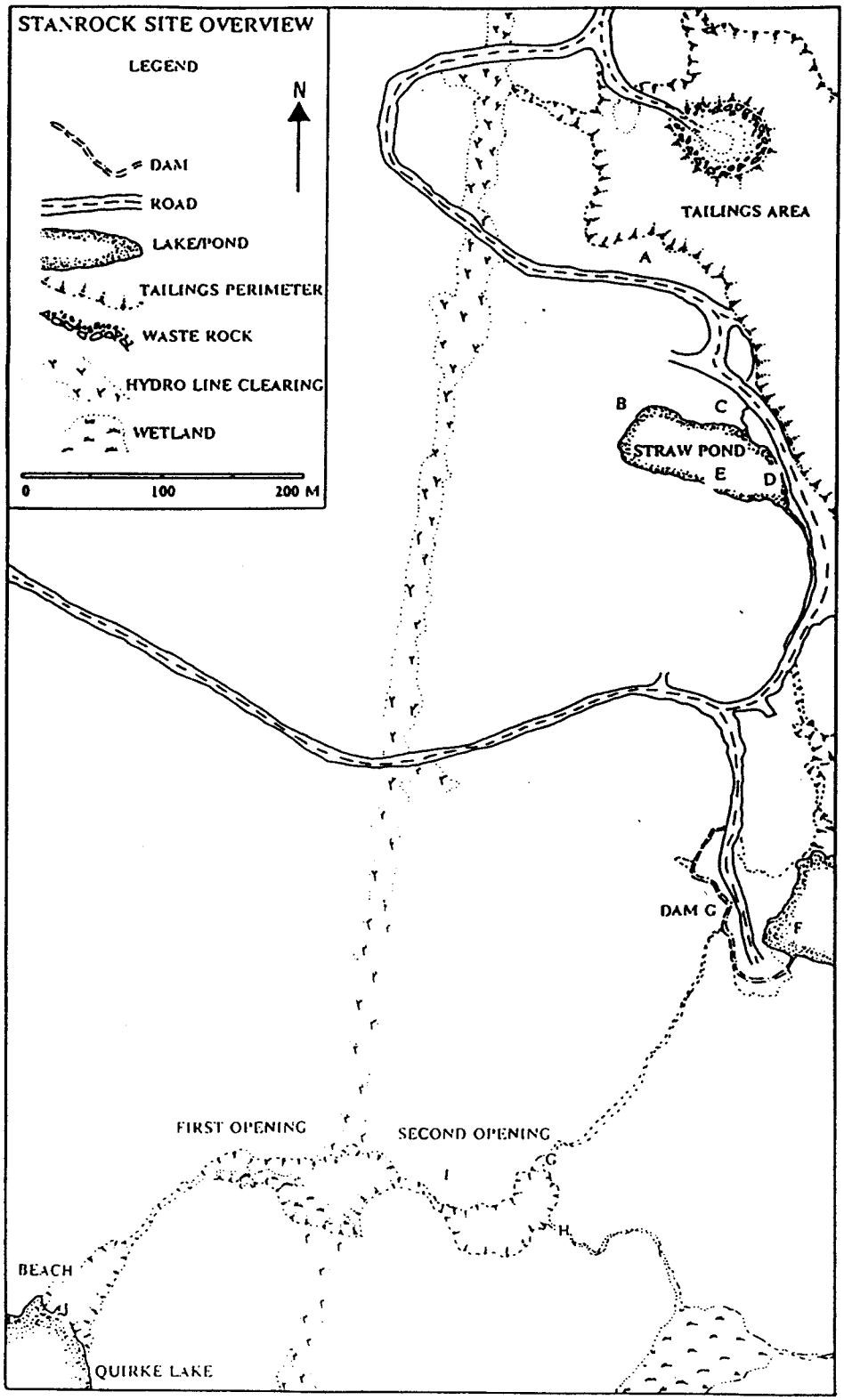


are applied to the above-ground portion - the shoots and leaves - of plants; this method is deemed more effective, in terms of nutrient uptake, than ground-applied fertilizers in areas where the potential of moving surface water and resultant transportation of nutrients away from the plant exists (as is the case at Denison). From a chemical perspective, foliar fertilizers are appropriate because, under acidic conditions (in this case, acid is abundant in the tailings where the cattails have taken root), the plant availability of nitrogen and phosphorus is greatly reduced due to a lower rate of organic matter mineralization. As acidity increases the solubility of P in Fe phosphates decreases, as does the activity of N-fixing bacteria (Tucker et al, 1987); the implication here is that ground-applied fertilizers will result in minimal N and P uptake because of the acidic conditions.

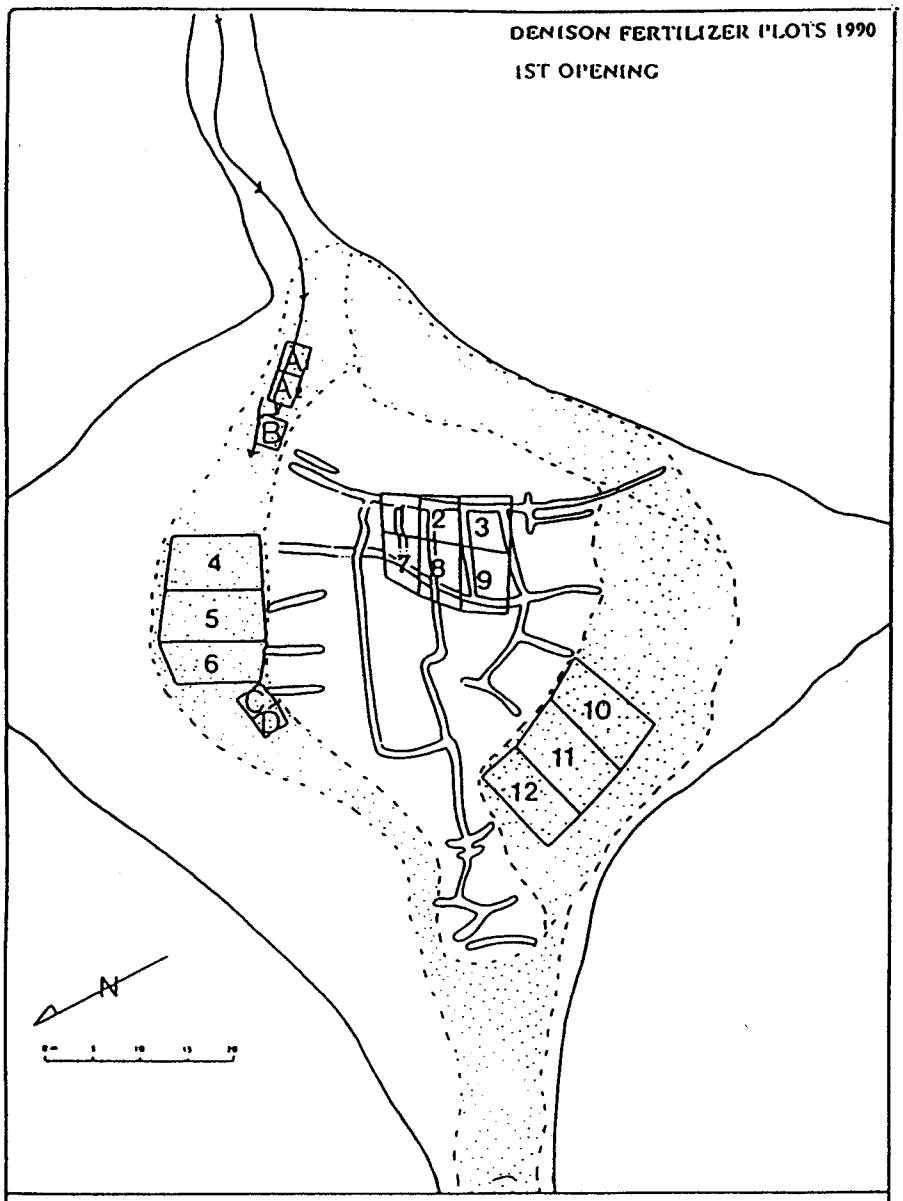
To maximize fertilizer-induced nutrient uptake in cattails, preliminary foliar applications began in 1989 at Denison in the First Opening of the Stanrock Dam G site (see Map 1.) to determine concentrations of fertilizer that would not produce leaf burn and, at the same time of course, enhance growth. Separate applications of 14-4-6 and 4-18-6 NPK (Nitrogen-Phosphorus-Potassium) fertilizers in 1/10 and 1/20 dilutions were applied in the summer of 1989, and results indicated some leaf burn using all four combinations. Greatly increased root growth was observed with high phosphorus and potassium fertilizer relative to unfertilized control plots, while high nitrogen fertilizer increased above-ground leaf development (Kalin, 1990).

Based on these observations, the 1990 fertilizer campaign saw the use of 14-4-6 NPK applications at a dilution of 1/10 with the addition of 1/50 diluted calcium fertilizer - see Map 2. As indicated in Kalin (1990), results to this point are too preliminary to determine whether foliar fertilizer applications will increase survivorship and clonal growth in cattails. Comparative studies need to be undertaken to find out what the effects of applying foliar fertilizer are on root and rhizome development relative to unfertilized controls in order to determine if, in fact, nutrient addition of this nature will help to maximize polishing capacity in cattail stands.

Map 1. Stanrock Site Overview



Map 2. Denison Fertilizer Plots in the First Opening



PLOT #	DESCRIPTION	PLOT #	DESCRIPTION
1	NPK LEAVES	11	CONTROL
2	NPK LEAVES	12	NPK LEAVES
3	NPK LEAVES	A1	Ca LEAVES
4	NPK LEAVES	A2	NPK LEAVES, Ca GROUND
5	CONTROL	B	NPK LEAVES, Ca LEAVES
6	NPK LEAVES	C	NPK GROUND
7	NPK LEAVES	D	Ca GROUND
8	NPK LEAVES		
9	NPK LEAVES		
10	NPK LEAVES		

1.2 Purpose and Objectives of Study

This study is, in a sense, a "stepping stone" to achieving the ultimate goal of determining whether foliar fertilizer applications on tailings-grown cattails is a viable method for increasing biomass production and, hence, biological polishing capacity. By comparing root and rhizome development between fertilized and non-fertilized control plots, it should be feasible to determine the morphological and biochemical (i.e. starch reserves) effects of NPK and Ca-fertilized cattails.

An investigation of below-ground tissues is appropriate because they act as regions of material storage for future growth and development. In particular, the pith region of the rhizome is relevant to the study because it is there that sugars produced during photosynthesis are stored as starch. And when there is an unlimited availability of nutrients the pith sees a reduction in sugar and starch accumulations (Lakshman, 1987). Based on this point, all parameters otherwise being equal, cattails in control plots should see greater levels of starch in their piths than those receiving additional fertilizer-induced nutrients.

During winter months, cattails store sugars and starch at relatively stable levels; carbohydrate use during this period is minimal with only small amounts utilized for suppressed respiration. Winter sampling and experimentation, then, is useful under this "steady state" condition because it eliminates sampling problems arising from daily and growing-season fluctuations in starch levels. Starch content in rhizomes of fertilized cattails relative to that in the unfertilized ones should indicate to some degree the relative availability of fertilizer-induced nutrients to the plants. Analyses will focus on pith diameter relative to overall rhizome diameter, starch distribution, and starch concentration. Since Kalin and Scribailo (1989) observed tissue damage from metal uptake in cattail rhizomes and roots, metal distribution will also be looked at.

Before commencing field work at Denison Stanrock, however, samples for preliminary testing will be collected locally to first determine if it is at all possible to extract cattails

during winter, and then to work out appropriate techniques for starch analysis. In the latter case, it will need to be determined how to sample cattail populations representatively, while doing the same for individual plants before morphological and starch investigations. This report, then, presents findings from a preliminary study on overwintering, below-ground cattail morphology, starch storage, and metal uptake using locally-collected samples. An evaluation of the methods used to examine these parameters is provided along with a discussion of actual data obtained. Conclusions drawn, and recommendations made from this preliminary study will be used to develop an appropriate strategy for extracting and analysing the Denison cattails.

2. BACKGROUND INFORMATION

2.1 Cattail Anatomy, Morphology, and Development

The following description is based on that found in Kalin & Scribailo (1989).

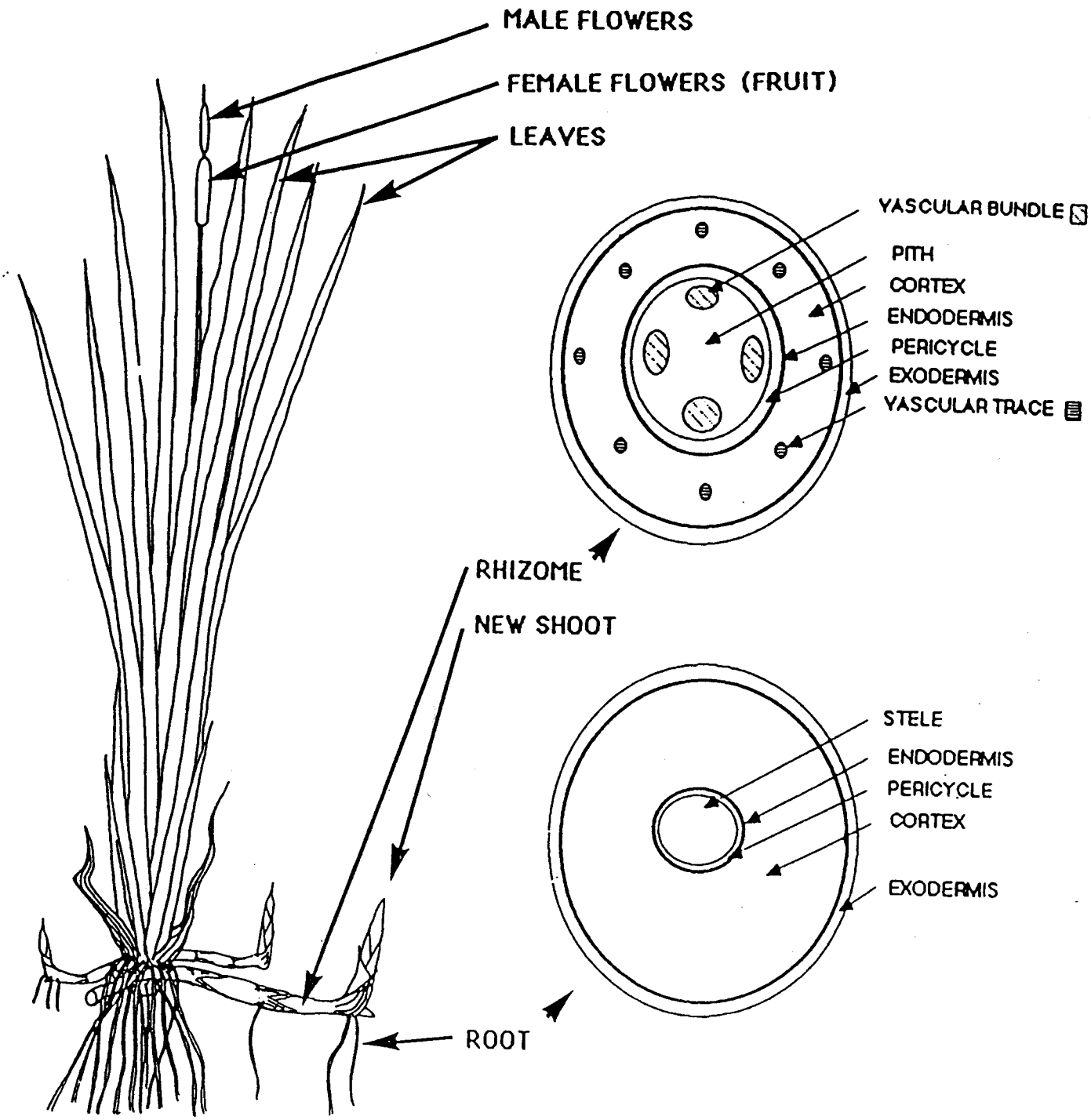
Cattails reproduce vegetatively, with the production of rhizomes (underground stems), to produce individual plants consisting of a rosette of opposite leaves, typically six per side - see Schematic 3. By developing small vegetative buds at the base of each leaf, referred to as the axil, these emergent macrophytes possess the ability to develop vertical, upright shoots. Rhizomes characteristically produce roots at regular intervals along their length, although the majority of roots tend to develop at the bases of upright shoots.

In cross section, rhizomes consist of two distinct zones - an outer cortex and an inner pith. The pith is mainly composed of tightly-packed storage cells used for holding starch for future growth and development. To inhibit lateral movement of substances from the cortex into the inner zone, an endodermis borders the pith's periphery.

The cortex is largely composed of star-shaped cells, collectively referred to as stellate, with significant quantities of air between them. This outer region of the cattail rhizome is bordered on the outside by an exodermis which acts as a barrier to the transportation of materials from the external environment into the rhizome. Vascular bundles are scattered throughout the cortex, but are more concentrated in the pith. Their primary function is to transport water from the roots to the shoots, and to transport sugars produced during photosynthesis from the leaves to the rhizome for storage as starch in the pith.

Root anatomy is similar to that of the rhizome with the exception of the pith region. In cattail roots, rather than many individual bundles, the pith is replaced by a solid cylinder of vascular tissue called a stele. Roots are produced laterally from a thin layer of tissue just beneath the rhizome's endodermis called the pericycle; these roots may also produce lateral roots from their pericycle.

Schematic 3. Cattail Anatomy and Morphology



2.2 The Cattail Rhizosphere

The significance of cattails, and particularly the rhizosphere, in ameliorating AMD has been suggested by Kalin and Scribailo (1989) and is one reason why this study focuses on the below-ground tissue of these emergent macrophytes. There are other reasons, as well, for concentrating on the rhizome, yet before effective data interpretation can take place these reasons need to be elucidated in light of an understanding of the phenology and development of the cattail rhizome.

Cattail plants are connected together by an underground network of stems, or rhizomes, growing parallel to the surface of the bottom (Linde et al, 1976). By reproducing vegetatively with aerial shoots arising from rhizomes, cattails produce colonies that migrate outward to invade new areas. Because each shoot originates from a common ancestor, all are genetically identical - thousands of aerial shoots, composed of leaves and occasional fruiting stalks, can be produced by a single parent.

Rhizomes have a number of functions which are important to the survival and propagation of the cattail. During the growing season, reserves of carbohydrates are built up in the pith region of the rhizome and stored through winter to provide food for the pre-photosynthetic (i.e. submerged) development of the new aerial shoot in the spring. In the summer months, migration of the cattail beyond its periphery is brought about by the growth of rhizomes, thereby maintaining established populations while also expanding them into new, suitable habitats. During the winter, rhizomes also serve to carry the plant through this dormant period by being able to obtain oxygen for survivability. Even though the aerial shoot is dead at this time, aerenchyma cells in the rhizome, which are connected to those in the shoot, receive conducted oxygen from the shoot and ensure the cattail's survival during winter-induced anaerobic conditions.

Growth and development studies of cattails in a shallow marsh in Wisconsin, U.S.A. receiving heavy nutrient loading were undertaken by Linde et al (1976) during a three-year

period to gain an understanding of how to control and manage the plant for associated wildlife habitat. Growth and development of leaves, fruiting heads, and rhizomes were measured weekly throughout each growing season. Of particular relevance to this study was the weekly collection and analysis of total nonstructural carbohydrates (TNC); this was seen as a means for determining stored energy levels on which the plant depended for initial growth in the spring (Linde et al, 1976).

Findings from that study showed that old rhizomes - those produced during a previous growing season - were the principal storage organ with maximum levels of TNC occurring during the early winter period (Linde et al, 1976). Levels of TNC gradually declined with the onset of growth in the spring to a minimum in mid-summer during fruiting head development. Beyond this point, carbohydrates were produced in excess of the plant's needs so they were translocated to the rhizomes for storage. Conclusions from the study indicated that the stored carbohydrates were available to help the plant recover from severe injuries and to develop new shoots the following spring before it is capable of photosynthesizing sufficient carbohydrates for its needs (Linde et al, 1976).

Other findings on cattail rhizomes indicated that adventitious buds at the base of growing aerial shoots developed into new rhizomes in late spring, and maximum rhizome growth of one inch per day. Sprouts were observed forming on the new rhizomes during mid summer and were later found to have produced the following summer's crop of aerial shoots; the sprouts went into dormancy in late fall and resumed growth in early spring (Linde et al, 1976).

2.3 Overwintering Starch Distribution in Cattail Rhizomes

Although the literature is scant in dealing with the exact subject, one paper was found which might be of significance to this examination. To determine the amount of starch storage during the overwintering period in different tissues of the cattail, a study was

conducted in 1977-78 at a single clonal cattail community near the southeast shore of Lake Ontario in New York State (Kausch et al, 1981).

In keeping with previous studies of the same nature, results from analyses of starch distribution in cattail leaves, stems, and rhizomes showed the rhizome as the major storage organ for starch. The central core region of the rhizome - the pith - showed extensive accumulations of globose starch grains during late fall and early winter with significant reductions from late winter through to early spring (Kausch et al, 1981). Small amounts of starch were also noted in the rhizome endodermis and adjacent cortical parenchyma in late fall and winter, with almost none in spring. Beneath the outer cortex, large accumulations of starch were found in scattered parenchyma cells; by spring, this layer lost most of its starch.

The outer cortex of intact roots contained starch in late fall, while the middle cortex remained starch-free all year. Starch was also noted in a number of cells in the inner cortex, but very little was seen in the endodermis. Overall, cattail roots saw a gradual decrease in starch levels in midwinter followed by a sharp drop in late winter with almost zero deposits by spring.

Coupled with findings from investigations of cattail buds and young leaves it was concluded that a decrease in starch in storage tissues of rhizomes and roots by late winter and early spring coincided with increasing bud growth and starch accumulation in or near zones of rapid development (Kausch et al, 1981). This strongly supported the idea that most of the stored starch was transferred from the storage organs to tissues of the developing shoot until the new shoots emerged from the water and became photosynthetic. It was apparent from the study that cattails are extremely productive plants with as much as 45.03% starch, dry weight, accumulating in rhizomes in early winter and 22.80% in roots; interestingly, not all stored starch was used for productivity as rhizomes retained a significant amount (27.40% dry weight) into the start of their second year (Kausch et al, 1981).

3. METHODS

3.1 Field Sampling and Commentary

On January 29, 1991 cattails for preliminary testing were extracted from a seepage/seasonal surface flow area at the base of the Scarborough Bluffs in Metropolitan Toronto. Because the daytime temperature was below 0°C, and had been that way for two days prior, most of the cattail population was "locked-up" in frozen water. This state made it impossible to successfully extract any plants with a spade; efforts were made to chip ice away from around the cattail, but after considerable time and effort without substantive results the strategy was deemed ineffective under conditions present at that time.

After testing various locations for ice build-up, a small ditch was discovered adjacent to the parking lot where a number of cattails existed. Upon testing the ice cover with the spade it was found that the ice was softer here than the areas previously tried. The ice chipped away easily to reveal a soft, unfrozen substrate layer that accepted the spade to the depth of the rhizome layer without any problem.

Due to time limitations, only a small area with approximately five plants was excavated. Any upright shoot with intact rhizomes was kept for testing along with detached sections of rhizomes found after dredging the water-filled, excavated hole with the spade. As much substrate as possible was then removed from around the cattail rhizomes by hand in the field; some blackening of rhizome and root tissue was noticed at this time. To keep the cattails in a frozen state, samples were transferred to an ice-packed cooler for transportation to the laboratory freezer.

Because of the conditions previously described, sampling was restricted to one small area of the population. Techniques, therefore, for representatively sampling a population were not used. The same applied to extracting individual plants - the combination of ice and spade made it difficult to accurately cut and excavate a prescribed plot around each cattail.

During the second week of February, 1991 a warm spell presided over the Toronto area so a second trip was made to the Scarborough Bluffs to excavate additional cattails. Much of the seepage area that was frozen solid during the previous sampling trial was free of ice and, as such, made extraction easy. A location within the overall stand was found where a number of cattails were rooted under free-flowing water. Digging commenced with great success - the substrate in which the cattails were rooted was soft, thereby making extraction simple and fast.

After a number of plants were excavated by locating emergent, senescent shoots and digging around them to obtain their intact rhizomes, it was found that this approach often damaged the year-old underground stems. Because the year-old rhizomes were underground there was no way of determining how far out from the parent shoot one needed to dig in order to keep the rhizome intact. As a result, many of the initial samples had broken rhizomes where the spade had cut through the tissue when digging.

In order to alleviate the problem, an attempt was made to locate emergent shoots and work "backwards" by digging approximately 0.5m away from the parent in a circular fashion around the plant. This method proved highly successful, and within an hour ten plants with intact rhizomes were extracted. The samples underwent an initial field washing in adjacent Lake Ontario and were placed in an ice-filled cooler for transportation to the laboratory. Once at the lab, the samples were placed in a freezer to await examinations.

3.2 Sample Preparation

To prepare the cattails for morphological and chemical investigations, individual plants were taken from the freezer and quickly washed under hot tap water to remove any excess substrate remaining after field washing. Year-old rhizomes with yet-to-emerge new shoots were then located on each plant, measured for their length, and described as to their appearance. The central 12cm portion of each rhizome was kept for further investigations,

as per Kausch et al (1981). Because sectioning problems arose from thawing plant material during this initial preparation period, an additional step was later incorporated into the protocol; to ensure that the rhizome was frozen to such an extent that hand sectioning had no damaging or mishaping effects on the tissue, individual plants were placed back in the freezer for half an hour immediately after laboratory washing.

All intact root material on each 12cm length was removed with a razor blade at the outer surface of the rhizome exodermis and placed in individually-labelled sample jars containing 95% denatured ethyl alcohol. Two immediately-adjacent hand sections - one for IKI staining for starch and the other for hematoxylin staining of metal uptake -were made at both ends and in the middle of the rhizome sample, for a total of six. The two sections at each location were placed together in a scintillation vial containing the 95% alcohol and labelled according to rhizome number and position within the 12cm portion. Sections at the parent-shoot end of the rhizome were labelled "A", those in the middle "B", and the sections closest to the new shoot "C." The remaining tissue was placed in appropriately-labelled sample jars (containing alcohol) for quantitative starch analysis.

3.3 Iodine/Potassium Iodide (IKI) Staining for Starch

Before staining took place, the alcohol inside the cells of the rhizome sections had to be washed out. To accomplish this, one hand section from each grouping of two was removed from its respective scintillation vial and immersed in each of a series of four beakers containing distilled water. After the final immersion, the section was placed on a microscope slide ready to accept the iodine/potassium iodide (IKI) solution. Two to three drops of pre-prepared IKI were placed on the section and allowed to develop into the stain's characteristic bluish-purple colour when in contact with zones of starch accumulation.

Once sufficient colour had developed, excess stain was washed off and the section was put in a petri dish modified with a movable glass cover slip. The dish was filled with distilled

water and the section was placed under the cover slip to prevent it from moving around while under microscopic examination. Sections were viewed under a Ziess stereomicroscope, model SV 8, fitted with a MC 63 M 35 photomicrographic camera. Visual observations were recorded and, where appropriate, slides were taken using Kodak Ektachrome 160T professional slide film.

3.4 Measuring Rhizome Section Dimensions

After recording observations and photographing the IKI-stained rhizome tissue, each section was removed from the viewing dish and placed in another petri dish fitted with a mm^2 grid superimposed on a movable, clear acetate cover. Each section was placed under the acetate cover, making sure the cover was in contact with the entire surface of the section, and centred about a prescribed grid intersection point. Pith diameters as well as overall rhizome diameters were recorded in both the X and Y directions by counting the number of millimetre squares each feature took up. Sections were then discarded.

3.5 Hematoxylin Staining for Metals Uptake

In order to elucidate the presence and distribution of metals in cattail rhizomes, sections were stained with 1: 10 000 dilution of unoxidized hematoxylin in 0.01M phosphate buffer, as per Pizzolato and Lillie (1967). Hematoxylin staining for metals in tissue produces the following colours: Al, Cr, Ga, Hf, In, Fe, and Zr - dark blue granules; Be, Dy, Ho, Ir, Pb, Mn, Mo, Nd, Ni, Pt, Rh, Tb, U, Yb, and Zn - lighter blue granules; Bi - purple; Ta - brown-red; Nb and Ti - brown; Cu - greenish blue; Sn and Th - purplish red; and, Os -blue- or green-brown (Pizzolato & Lillie, 1967).

A stock solution was prepared by dissolving 1g hematoxylin in 100ml posphate buffer, 0.01M and pH 7.0. Fresh solutions were made by diluting 2ml stock solution with 200ml

phosphate buffer. The remaining rhizome section from each scintillation vial was prepared, viewed, and photographed for hematoxylin staining in the same manner as that for IKI. Colour development was compared between stock and fresh solutions and between different stain retention times. Linear measurements were not taken since those obtained from immediately adjacent sections during IKI-starch viewing were deemed applicable. Again, sections were discarded after examination.

3.6 Quantitative Determination of Starch

A modified version of the Nielson method (1943) was used. Leftover material from each 12cm rhizome portion was taken out of the storage alcohol, wrapped in absorbant towel to remove excess liquid, and weighed to two decimal places of a gram on a Sauter electronic balance. The weighed material was placed in a Waring Blendor and ten times that weight of water was added to make a 10% tissue slurry. After the blendor was allowed to run at the high-speed position for 4-5 minutes, 2ml of the resultant slurry was measured into a graduated cylinder and then placed in a 50ml beaker.

Exactly 2.7ml of 72% perchloric acid was added to the aliquot while stirring thoroughly to ensure no momentary high concentrations of acid in any portion of the sample. The mixture was allowed to stand with occasional stirring for approximately ten minutes. A 1ml aliquot of the solution was then pipetted into another 50 ml beaker and 6ml of distilled water was added. The solution was brought to a pH of 8.3 with a few drops of 6N sodium hydroxide and then to a pH of 4.5 with 2N acetic acid.

Two and a half millilitres of 2N acetic acid was then added in excess, followed by 0.5ml 10% potassium iodide, and 5ml of 0.01N potassium iodate. The solution was allowed to stand for 5 minutes so that effective colour development could take place. Using a graduated cylinder, the coloured solution was made up to 50ml with distilled water and transferred to a suitable cuvette for colour estimation in a photoelectric colourimeter.

Colourimetric readings were made on the final solutions using a Coleman Junior II Spectrophotometer set at 680 nm with a red filter. The spectrophotometer was calibrated to zero absorption prior to each reading using a reference blank containing all reagents except for actual starch material. To provide a control against turbidity in the final solutions, a blank was also made up with the slurried rhizome material and all reagents except for the potassium iodide and iodate, and checked against distilled water.

3.6.1 Standardization

Starch content in cattail rhizome and root tissue was calculated on a fresh weight basis (mg starch/g of tissue) from a standard curve prepared from the colourimetric readings of a known range of starch concentrations. Various amounts of corn starch were accurately weighed to the nearest 10μg on a Sartorius analytical balance and made to final 50ml solutions in the same manner as the cattail tissue. The amount of starch in the final solutions was calculated, and colourimetric readings were taken on the spectrophotometer. A curve was developed by plotting starch in 50ml solution against spectrophotometer absorbance.

Absorbance readings of cattail tissue solutions were then read from the curve to obtain starch concentrations in 50ml. Starch content in fresh weight of cattail material was then calculated by relating the observed concentration to the amount of tissue in the 50ml solution (while incorporating the turbidity blank result). Leftover material from the blending operation was air dried to constant weight for dry weight biomass calculations.

4. DATA RESULTS

4.1 Staining

Results from the staining of cattail rhizome sections are presented in Tables 1 and 2. Iodine/potassium iodide staining for starch yielded consistent results with the pith showing extensive bluish-purple colouration (indicative of starch) as well as a thin band beneath the epidermal layer. Starch was detected at the base of roots in some sections but not in others. A number of sections experienced damaged piths in the form of lost material.

Hematoxylin staining of the entire sample run for metal uptake produced no apparent colouration with a >18 hr. stain retention time. A test of fresh hematoxylin on a rhizome section showed excellent colouration after only three hours of retention with a pale orangy-brown pith and a dark blue epidermis fading to a lighter blue through the cortex.

4.2 Rhizome Dimensioning

Results of cattail rhizome measurements are presented in Table 3 and graphically in Figures 1 and 2. Figure 1 is a general overview of results obtained from rhizome dimensioning showing a relatively steady ratio, from location to location within each rhizome, between pith diameter and overall rhizome diameter. On closer inspection, the data reveal that in 90% of the rhizome samples the pith becomes proportionately larger toward the new shoot, as revealed in Figure 2.

Table 1. VISUAL OBSERVATIONS OF IKI STAINING FOR STARCH

Section #	Description
1A	- starch concentrated in pith, purple-blue colouration (colour consistent for all remaining observati - starch also located in a thin band beneath epidermal layer - some starch concentrated around roots in cortex
1B	- cortex retained blue colouration, but no starch granules present - starch distribution the same as above (no roots present) - some white areas in pith (vascular bundles)
1C	- same as 1A (no roots)
2A	- same as 1B
2B	- expected starch distribution - pith damaged from sectioning
2C	- same occurence as 2b
3A	- characteristic starch pattern - stray starch granules from unstable pith present in cortex
3B	- expected starch distribution - concentration of starch around roots in cortex
3C	- pith destroyed after stain rinsing - material left showed expecected pattern
4A	- expected starch distribution, but decreased amounts relative to the above sections - no starch around root
4B	- characteristic starch pattern
4C	- reduced amount of starch in pith - concentration of starch between two roots and damaged epidermis - overall, expected distribution present
5A	- loose pith with subsequent scattering of its tissue - basic starch pattern
5B	- normal distribution
5C	- normal distribution
6A	- characteristic starch pattern - vascular bundles in pith very definite
6B	- normal starch distribution - good accummulation of starch around root
6C	- expected starch pattern
7A	- section damaged - characteristic pattern in intact material
7B	- section damaged - little starch under epidermis - starch accummulatio around root
7C	- blue tinge, but no starch granules, in cortex - otherwise, normal starch pattern
8A	- darker colouration to one side of pith in cortex - expected distribution otherwise
8B	- badly damaged section - material left shows expected starch pattern
8C	- irregularly-shaped rhizome, jagged on one side - pith butts up against jagged edge - pith filled with starch - more starch under jagged epidermis than under smooth portion
9A	- expected distribution - pericycle pronounced with blue colouration
9B	- expected distribution
9C	- characteristic pattern
10A	- expected distribution
10B	- normal starch pattern
10C	- expected distribution

Table 2. VISUAL OBSERVATIONS OF HEMATOXYLIN STAINING FOR METALS UPTAKE

Section #	Description
Test Stock Sol. (<15 min.)	- bright red pith - dark blue pockets in cortex
Test Stock Sol. (>3 hr.)	- purple & pink cortex - strong pink/red in pith - epidermis black
Test Final Sol. (<15 min.)	- no apparent colouration
Test Final Sol. (>3 hr.)	- dark blue epidermis fading to lighter blue towards pith - pith pale orange/brown
After 18 hrs.:	**Description**
1B	- some of pith washed out - pale purple beneath epidermis - pale purple in pith with scattered red spots (vascular bundles)
1C	- same as 1B - pale orange at root base
2A	- pith washed out - pale purple cortex
2B	- same pattern as above - darker blue towards one side of rhizome
2C	- stain concentrated around vascular traces in cortex
3A	- concentrated stain around root
3B	- expected pattern (as in 1B)
3C	- no cortical colouration
4A	- pale pink pith - no blue tinge in cortex
4B	- same as 4A
4C	- same as 4A
5A	- dark blue where pre-stain blackened conditions existed
5B	- same as 5A
5C	- more blue colouration in one side of cortex
6A	- no blue tinge in cortex - pale pink pith
6B	- no cortical colouration - pale pink pith
6C	- same as 6A&B
7A	- light brown cortex - some blue colouration under epidermis
7B	- slight blue tinge in cortex
7C	- even colouration throughout rhizome
8A - 10C	- evident that hematoxylin stain lost its colouring effects from 1A onward

Table 3.

CATTAIL RHIZOME MEASUREMENTS

Sample	Rhizome Length (cm)	Axis	Pith Width (mm)	Average	Cortex & Epidermal Layer (mm)	Axis	Rhizome Width (mm)	Average	Ratio Pith: Rhizome Diameter
1A	14.0	x	7.0	7.40	5.60	x	12.50	13.00	1: 1.69
		y	7.8			y	13.50		
1B		x	4.7	4.10	3.95	x	9.00	8.05	1: 2.20
		y	3.5			y	7.10		
1C		x	4.3	4.10	4.05	x	8.60	8.15	1: 2.10
		y	3.9			y	7.70		
2A	25.0	x	8.2	8.25	6.20	x	14.10	14.45	1: 1.71
		y	8.3			y	14.80		
2B		x	7.9	7.75	4.95	x	11.90	12.70	1: 1.54
		y	7.6			y	13.50		
2C		x	7.5	7.15	4.55	x	11.50	11.70	1: 1.61
		y	6.8			y	11.90		
3A	25.0	x	5.8	5.85	4.30	x	10.60	10.15	1: 1.81
		y	5.9			y	9.70		
3B		x	6.7	6.35	4.05	x	12.10	10.40	1: 1.91
		y	6.0			y	8.70		
3C		x	5.4	6.50	4.30	x	9.50	10.80	1: 1.46
		y	7.6			y	12.10		
4A	13.0	x	5.9	5.90	3.60	x	9.80	9.50	1: 1.66
		y	5.9			y	9.20		
4B		x	7.3	7.10	3.80	x	10.10	10.90	1: 1.42
		y	6.9			y	11.70		
4C		x	8.2	7.75	3.25	x	11.40	11.00	1: 1.47
		y	7.3			y	10.60		
5A	21.5	x	6.8	5.45	3.75	x	11.10	9.20	1: 2.04
		y	4.1			y	7.30		
5B		x	5.7	4.90	3.95	x	9.80	8.85	1: 2.00
		y	4.1			y	7.90		
5C		x	5.9	6.05	3.50	x	9.60	9.55	1: 1.59
		y	6.2			y	9.50		
6A	14.0	x	6.6	5.95	3.70	x	10.90	9.65	1: 1.83
		y	5.3			y	8.40		
6B		x	5.7	6.25	3.20	x	9.50	9.45	1: 1.52
		y	6.8			y	9.40		
6C		x	6.1	6.30	3.85	x	9.90	10.15	1: 1.57
		y	6.5			y	10.40		
7A	9.5	x	6.9	5.75	3.20	x	9.10	8.95	1: 1.58
		y	4.6			y	8.80		
7B		x	7.1	6.55	3.65	x	11.70	10.20	1: 1.79
		y	6.0			y	8.70		
7C		x	10.3	10.10	3.80	x	14.20	13.90	1: 1.41
		y	9.9			y	13.60		
8A	16.5	x	7.4	7.00	3.55	x	11.30	10.55	1: 1.61
		y	6.6			y	9.80		
8B	Section Too Damaged for Measuring								
8C		x	7.9	7.05	2.05	x	9.50	9.10	1: 1.35
		y	6.2			y	8.70		
9A	24.0	x	5.3	5.70	6.50	x	11.60	12.20	1: 2.04
		y	6.1			y	12.80		
9B		x	7.1	7.10	7.90	x	13.10	15.00	1: 1.85
		y	7.1			y	16.90		
9C		x	4.4	4.85	3.80	x	8.50	8.65	1: 1.75
		y	5.3			y	8.80		
10A	15.5	x	8.5	8.15	2.65	x	13.00	10.80	1: 1.60
		y	7.8			y	8.60		
10B		x	8.8	9.15	4.30	x	13.70	13.45	1: 1.50
		y	9.5			y	13.20		
10C		x	10.1	9.85	2.90	x	13.20	12.75	1: 1.34
		y	9.6			y	12.30		

Figure 1.

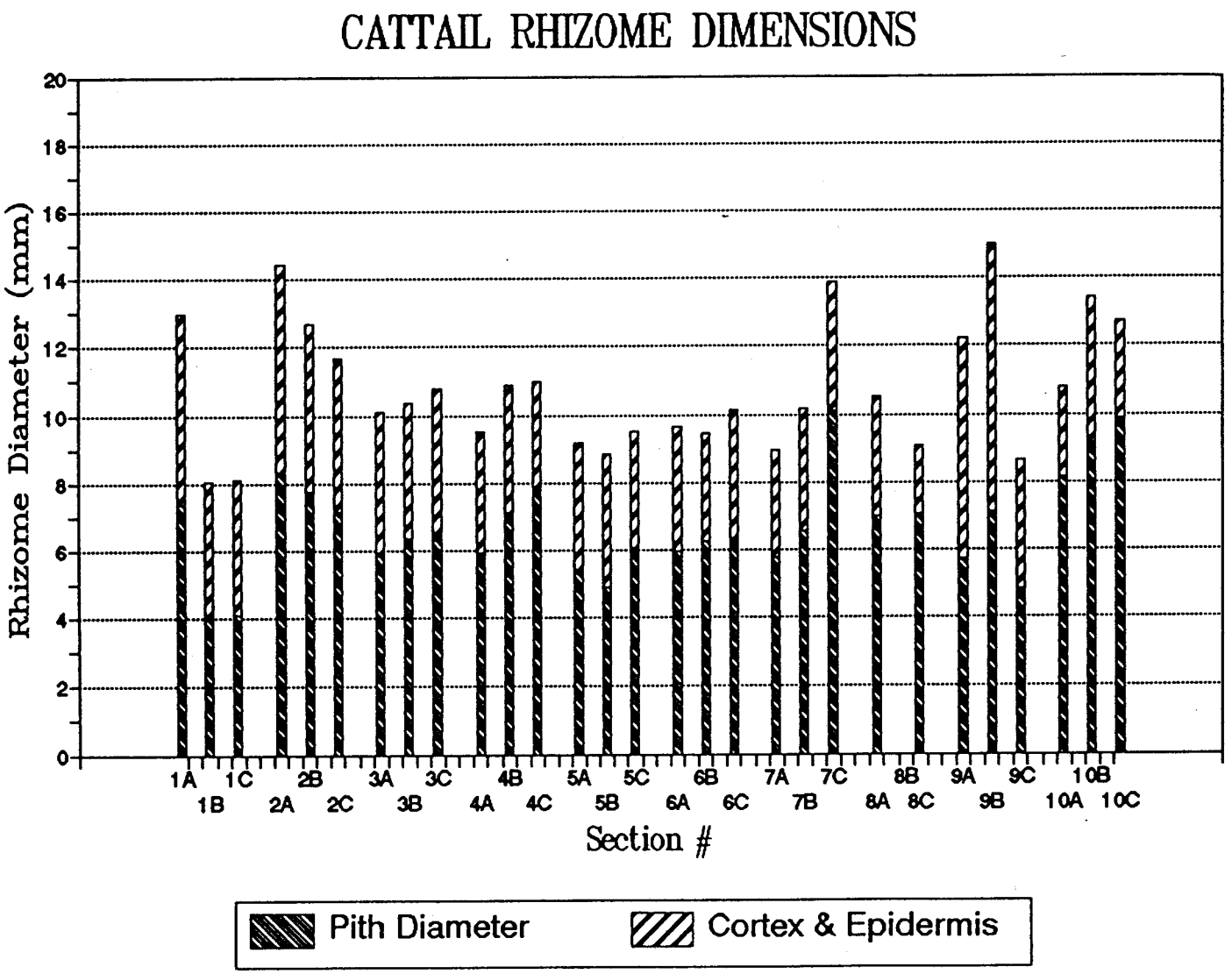
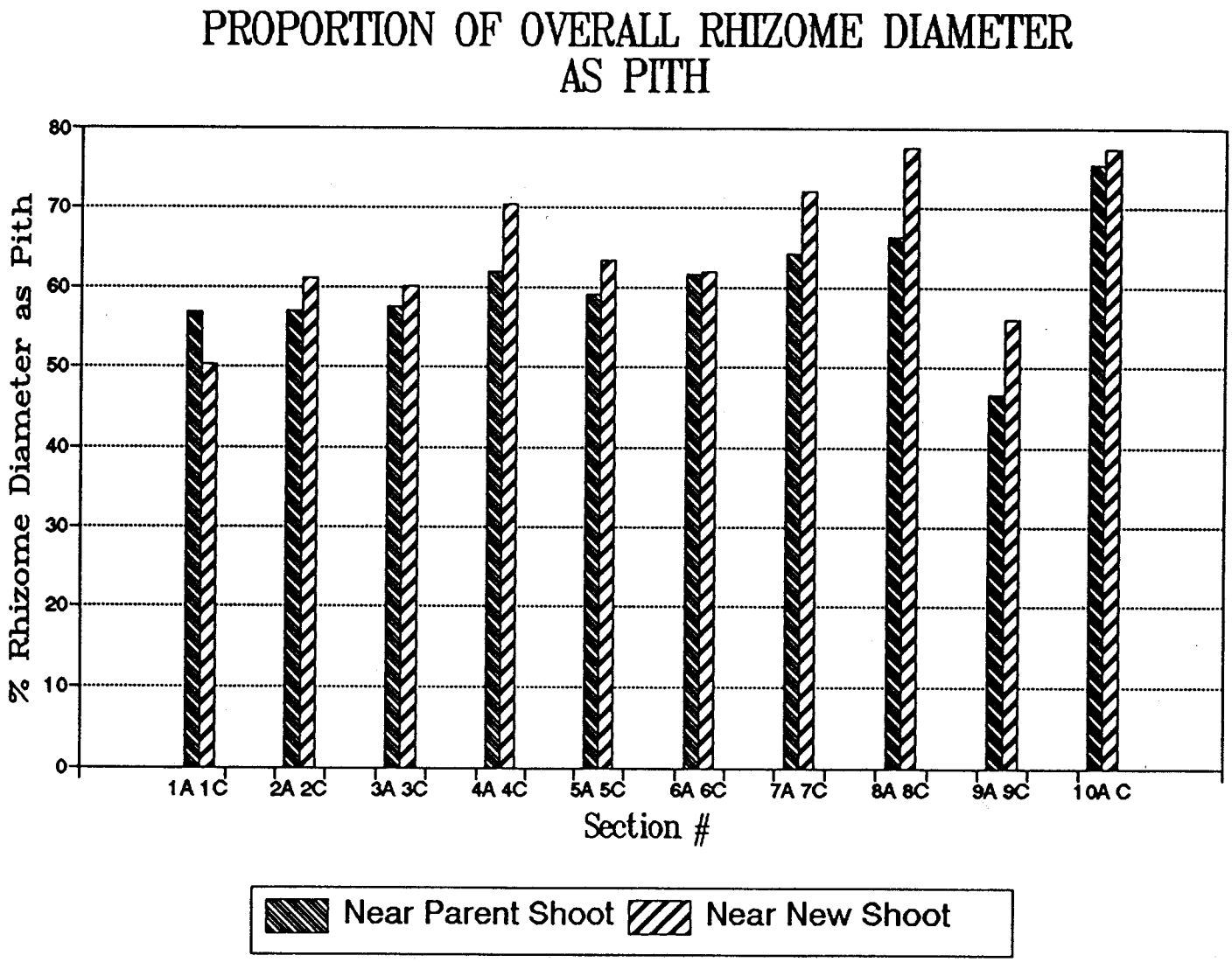

CATTAIL RHIZOME DIMENSIONS
Rhizome Diameter (mm)
0
2
4
6
8
10
12
14
16
18
20
1A 1B 1C 2A 2B 2C 3A 3B 3C 4A 4B 4C 5A 5B 5C 6A 6B 6C 7A 7B 7C 8A 8B 8C 9A 9B 9C 10A 10B 10C
Section #
Pith Diameter
Cortex & Epidermis

Figure 2.


PROPORTION OF OVERALL RHIZOME DIAMETER AS PITH
% Rhizome Diameter as Pith
0
10
20
30
40
50
60
70
80
1A 1C
2A 2C
3A 3C
4A 4C
5A 5C
6A 6C
7A 7C
8A 8C
9A 9C
10A C
Section #
Near Parent Shoot
Near New Shoot

4.3 Quantitative Determination of Starch

A standard curve is presented in Figure 3 with supporting data in Table 4 showing the absorption of light in a spectrophotometer by accurately known amounts of starch. With an R Squared of 0.950 in Figure 3, the amount of starch in solution almost perfectly predicts absorption readings on the spectrophotometer.

Table 5 shows the results of cattail rhizome and root tissue determinations. Absorption readings were obtained from the spectrophotometer, read from the standard curve to determine starch quantity in 50ml solution, and a ratio of starch weight to tissue weight calculated. For calculations on a dry weight basis, rhizome and root dry weight/fresh weight ratios of 17.66% and 14.35% respectively were obtained.

Both pooled samples of rhizomes and roots showed significant variability in final starch determinations with coefficients of variation (standard deviation expressed as a percentage of the mean) of 23% and 41% respectively, as indicated in Figures 4 and 5.

Figure 3. Standard Curve for Starch Concentrations

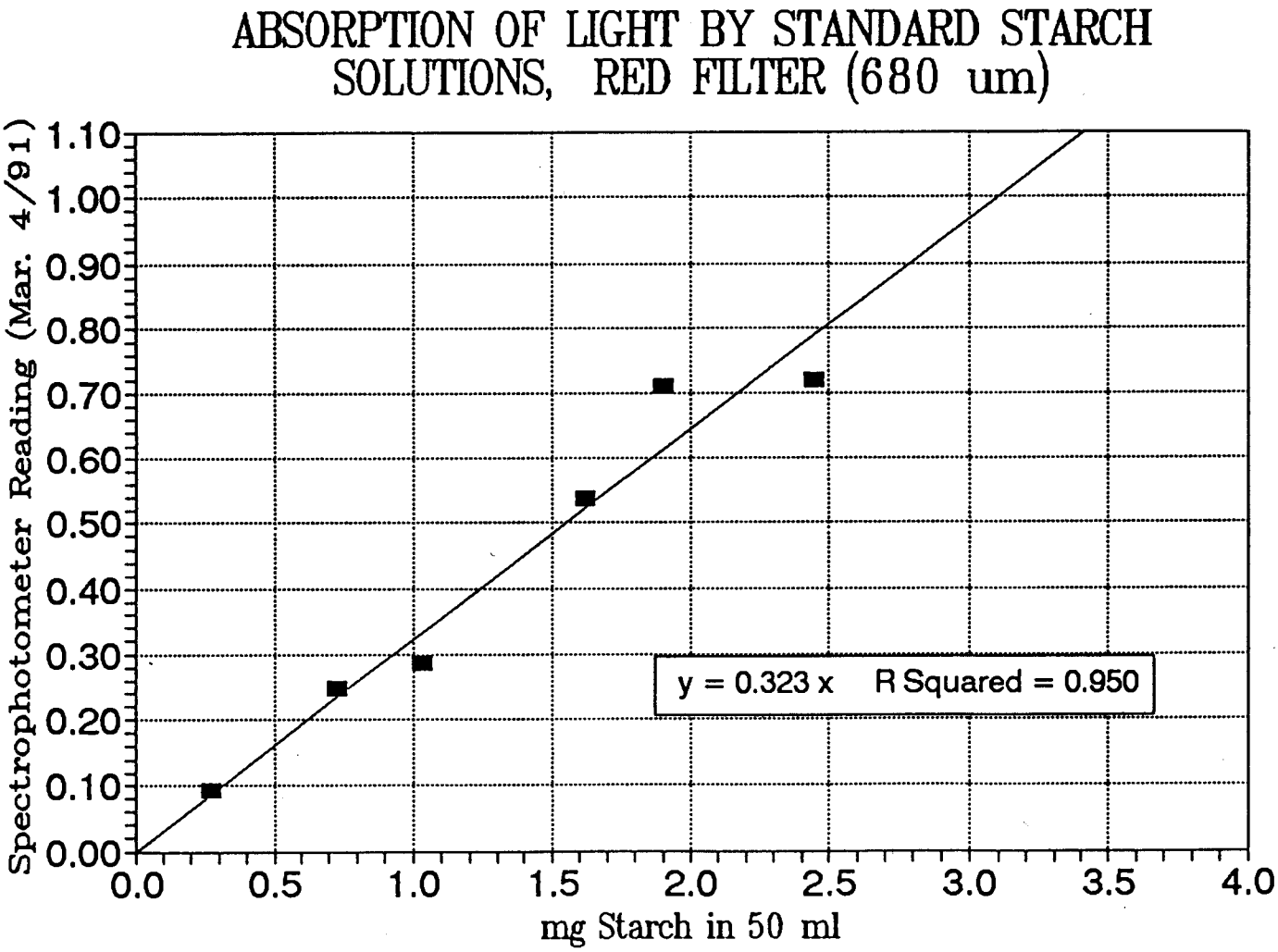


Table 4.

RESULTS OF STANDARD STARCH SOLUTION PREPARATIONS - MAR. 4/91

Standard #	Weighing Paper Weight (mg)	Weight of Paper + Starch (mg)	Starch Weight (mg)	Calc. Starch in 50 ml (mg)	Spectrophotometer Absorbance	% Transmittance
1	403.37	404.65	1.28	0.27	0.094	80.5
2	397.02	400.46	3.44	0.73	0.248	56.4
3	416.90	421.73	4.83	1.03	0.286	51.9
4	391.11	398.74	7.63	1.62	0.538	29.0
5	397.53	406.46	8.93	1.90	0.710	19.3
6	397.88	409.38	11.50	2.45	0.720	19.0

Table 5.

STARCH CONTENT IN FRESH TISSUE OF CATTAIL RHIZOMES & ROOTS

Sample		Spectrophotometer % Transmittance	Absorbance	mg Starch in 50 ml	Fresh Weight (g)	Starch Content (mg/g)
Turbidity Blank		93.0	0.030	-	-	-
Rhizome #	(i)	37.0	0.430	1.37	4.57	10.06
	(ii)	32.5	0.485	1.55		11.35
Rhizome #	(i)	12.3	0.900	2.87	11.6	8.30
	(ii)	15.8	0.800	2.55		7.38
Rhizome #	(i)	9.1	1.000	3.19	7.12	15.02
	(ii)	8.1	1.000	3.19		15.02
Rhizome #4		not analysed			6.89	
Rhizome #	(i)	20.3	0.690	2.20	5.31	13.90
	(ii)	32.0	0.490	1.56		9.87
Pooled Sample Rhizome #	(i)	47.3	0.325	1.04	49.2	3.53
	(ii)	58.3	0.233	0.74		2.53
	(iii)	65.8	0.181	0.58		1.97
	(iv)	60.9	0.216	0.69		2.35
	(v)	59.6	0.224	0.72		2.43
Pooled Sample Roots #1-1	(i)	73.0	0.160	0.26	5.32	16.08
	(ii)	64.0	0.194	0.31		19.50
	(iii)	78.0	0.107	0.17		10.76
	(iv)	88.5	0.053	0.08		5.33
	(v)	67.8	0.170	0.27		17.09

Variability:

Pooled Rhizomes #6-10		Pooled Roots #1-10	
Mean	2.563	Mean	13.751
Stand. Dev.	0.583	Stand. Dev.	5.691
Coefficient of Variance (%)	22.747	Coefficient of Variance (%)	41.386

Figure 4.

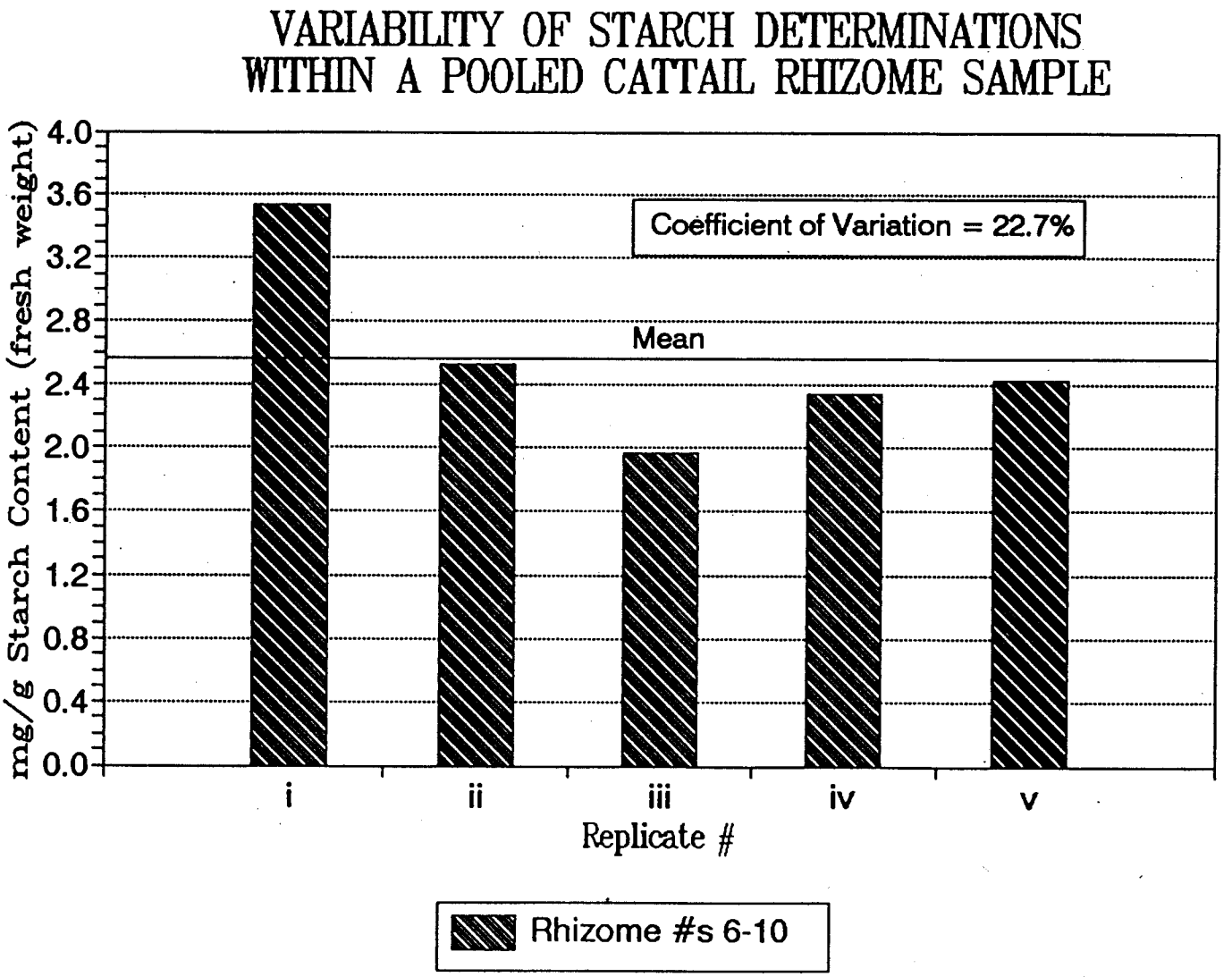
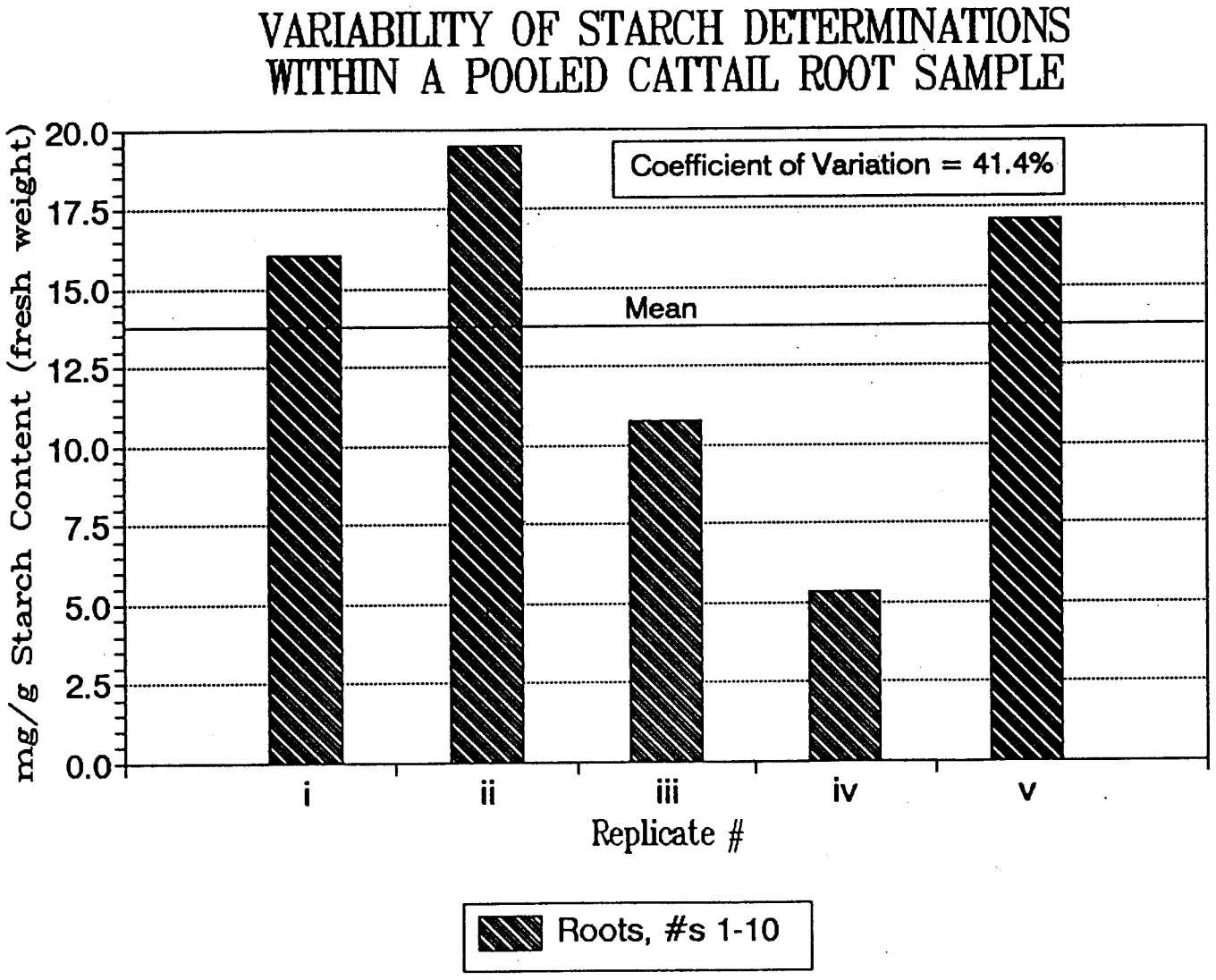

VARIABILITY OF STARCH DETERMINATIONS
WITHIN A POOLED CATTAIL RHIZOME SAMPLE
mg/g Starch Content (fresh weight)
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
Coefficient of Variation = 22.7%
Mean
i
ii
iii
iv
v
Replicate #
Rhizome #s 6-10

Figure 5.


VARIABILITY OF STARCH DETERMINATIONS
WITHIN A POOLED CATTAIL ROOT SAMPLE
mg/g Starch Content (fresh weight)
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
Coefficient of Variation = 41.4%
Mean
i
ii
iii
iv
v
Replicate #
Roots, #s 1-10

5. DISCUSSION

5.1 Cattail Extraction

Evidence from both sampling trials at the Scarborough Bluffs indicates that, provided there is no solid ice cover nor frozen substrate, cattails can be extracted quite easily with a spade. The winter season, at least in the Toronto area, sees great fluctuations in daily and weekly temperatures so attention needs to be payed to weekly forecasts for the coming of winter thaws. This is extremely applicable to the Scarborough Bluffs cattail stand because there is not enough flow through the area to keep water moving and, hence, unfrozen during cold spells. By extracting two to three days into a warm spell, sufficient time is allowed for accumulated ice to melt thereby making cattail sampling quick and easy.

Elliot Lake, however, is a different matter. Because it is farther north, daytime temperatures are not as warm as they are in Toronto, and winter thaws are less frequent. Fortunately, the cattails of concern at the Denison Stanrock site are located in a flow area so it is hoped that there will not be a buildup of ice in the fertilizer plots. Because the 1st Opening site is not easily accessed in the winter it is not likely that on-site staff will be able to report on conditions at the stand. Should ice buildup be a problem, though, when Boojum staff arrive, the use of an ice pick to extract the cattails may be a necessity.

The most effective way of extracting cattails with intact rhizomes, with a spade at least, is to locate a senescent, emergent shoot and dig at least 0.5m away from it in an encompassing fashion. Periodic checks should be made while digging to locate any year-old rhizomes with new shoots so as not to damage them. To save transportation weight it is advisable to wash as much substrate as possible from the extracted cattails in the field. As well, plants should be kept as frozen as possible in the field, and during transportation, so that starch levels do not start changing as a result of tissue thawing.

If below-ground biomass results are required, individual cattails should be stored in separate plastic bags so that loose material associated with a particular plant does not get

mixed up with that of others; otherwise, several plants can be transported and stored in a single bag. This, however, should not be an issue during the winter season since snow and ice conditions make it extremely difficult to cut and extract a prescribed plot of substrate around an individual cattail. On that point, below-ground biomass estimations should be performed in the summer months when conditions are more suitable to do so.

Random sampling of cattails could not be carried out at the Scarborough Bluffs because ice conditions dictated exactly where it was feasible to extract samples. Even during the second trial only small pockets of spade-acceptable cattails were found. If conditions allow for effective random sampling at Denison it is suggested that the width and depth of the cattail plots be paced out and a table of random digits used to locate plants. Starting at the bottom right hand corner of each plot, the first number in the table will dictate how many paces left, and the second how many forward. Any number encountered in the table greater than either the width or depth should be discarded and the following digit used. Once the destination is reached, the nearest cattail will be the one to extract.

5.2 Sample Preparation

Again, it is critical that the sample stay frozen until absolutely needed - tissue starch levels may change if the plant is allowed to thaw. It became evident while making hand sections through the rhizomes that the tissue was thawing and, as a result, softening up. This created problems because pressure had to be applied to the razor blade to make it cut, thereby mishaping the rhizome section and often squeezing out the contents of the pith. Fortunately, the samples were relatively homogenous in terms of starch distribution so lost material was not critical. However, the case may be extremely different in the Denison samples where the difference between fertilized and non-fertilized plants could be great. For this reason it is important to do as little damage to the tissue as possible.

By slightly altering the preparation protocol order, the problem was solved. After

washing the samples in the laboratory, instead of immediately commencing with hand sectioning, the cattails were placed back in the freezer for at least a half hour to allow them to harden again. With this change, no compression of the rhizome, or loss of material was encountered when sectioning.

To see if storing hand sections in 95% denatured ethyl alcohol had any effects on starch distribution, two test sections were cut from an unused portion of a rhizome. One was immediately stained with IKI and examined under the microscope while the other was stored in alcohol overnight, washed, stained, and examined in the same manner. There was no appreciable difference between the two in terms of distribution, but in terms of actual quantity nothing was confirmed. During the Denison work it may be useful to do a total soluble carbohydrate test on the storage alcohol to determine the amount of carbohydrates that leave the tissue cells and pass into solution. The Dubois et al (1956) method also makes use of colourimetric estimations to obtain results and may, therefore, be appropriate.

Removing roots at the surface of a rhizome is a lengthy and tedious process. Determining the percentage of rhizome weight (including roots) as roots must be looked at with the realization that the precision with which roots are removed accurately at the exodermis surface is questionable. Macroscopically speaking, it may appear that all roots have been accurately removed at the exodermis, but under the microscope the case is different. Microscopic examinations show that no matter how much time is spent removing root material, there will always be some amount still attached to the rhizome. Though it cannot be concluded from this report that those "leftovers" are neglible, it is worth highlighting the point before future data interpretation takes place.

5.3 Iodine/Potassium Iodide Staining

IKI staining for starch distribution proved highly successful with the production of a dark, bluish-purple colour in areas of starch accumulation in plant tissue. Results were

consistent throughout the samples with the pith showing strong colouration as well as a thin band underneath the epidermal layer. Some samples showed an uncharacteristic blue tinge in the cortical layers, indicative of starch, but on closer inspection no granules were seen. The blue colouration in the cortex may be a result of ineffective post-stain rinsing, or the movement/smearing of residual stain-retaining alcohol from the pith across the cortex. Evidence from the microphotographs depicting some smearing, and the fact that post-stain rinsing was thorough in all cases suggests that the latter reason may be the cause.

5.4 Hematoxylin Staining

Hematoxylin staining of cattail rhizome sections for metals uptake proved to be a very exacting task. Initial staining with fresh hematoxylin showed little tissue colouration immediately following application (unlike that for starch which had fast results) so it was thought that the fresh mixture was too dilute. As a means of comparison, a few drops of stock hematoxylin were added to another section - colour development was immediate with a bright red pith and dark blue pockets in the cortex. After three hours the colours were more intense with much of the exodermis and cortex black. The section stained with the fresh solution at that time had developed good colouration with a dark blue exodermis fading to lighter blue in the cortex, and a pale orangy-brown pith.

Some of the metals known to produce the above colouration, and which may be of significance at Denison, include: iron and aluminum - dark blue; nickel, manganese, and zinc - light blue: and, copper - greenish blue (Pizzolato and Lillie, 1967).

The test indicated that the stock solution was too concentrated and that, given time to develop, the final solution was appropriate. Too much time, however, meant a loss of stain effectiveness as evidenced by the lack of any real colouration in the entire run of samples, from #1B-10C, left sitting in the hematoxylin overnight. Though the fresh hematoxylin solution is effective in elucidating metal uptake in plant tissue, it is very time-

specific. As reported in Pizzolato and Lillie (1967), the 2-4 hour interval for stain retention appears to be the best.

Furthermore, the fresh solution does not last more than one day. After attempting to stain a section with day-old 'fresh' hematoxylin, and with no appreciable colour development after three hours it was decided that fresh solutions needed to be prepared daily (not clear from the Pizzolato and Lillie paper). To further strengthen this argument, a sample of the day-old solution was placed in a vial alongside one containing fresh hematoxylin; that in the first vial was much paler than that in the second. Because of this rapid change (i.e. within 24 hours) in solution colour, it is recommended that the 2-4 hour stain retention time reported in Pizzolato and Lillie be strictly adhered to.

5.5 Microphotography and Rhizome Dimensioning

Photographing rhizome sections under the stereomicroscope has the obvious benefit of providing an everlasting record of tissue conditions; notes made at the time of examination can be cross-checked with results from permanent pictures for accuracy. A number of observations with respect to the process of photographing sections need to be highlighted to ensure the success of future attempts in recording material for permanency.

First of all, cameras are sensitive to subject movement, especially at slower shutter speeds. It is therefore essential to keep, in this case, the tissue section as still as possible when taking a photograph so that no blurring of the image occurs. To prevent glare from the material (the Ziess scope used in this study shines light onto the sample from above) caused by reflected light, samples must be immersed in a sufficient depth of water (approx. 1cm). Because a tissue sample in water has a tendency to move, it must be secured in a manner that does not alter the overall effect of the image. By installing a lifting clear glass cover plate in a petri dish it is possible to do just so; a sample can be secured in water by placing it underneath such a lifting cover (anchored to the dish, by silicone, with a flexible

plastic arm) without harming the photographic effect. As a further check against sample movement, the area around the scope should be clear of people moving back and forth.

The MC 63 M 35 camera on the Ziess scope is equipped with an automatic light meter that reads the amount of light coming into the camera from the image surface and tells the photographer what exposure setting to use. It was found during the study, however, that a number of rhizome sections which did not fill the entire image plane were underexposed; those sections that did were just slightly underexposed. This occurs because the light meter obtains its reading from the entire image plane, so when there is a lot of white background the meter suggests a fast exposure - the resultant image has a very nice white background but a dark subject. It was found that by setting the exposure control ¼ of a second slower than the suggested setting, small subjects could be properly exposed. Subjects that took up the entire image plane gave more accurate exposure readings, but it was found that adjusting the fine exposure control to the maximum level (rather than the suggested mid-point) for any particular macro-setting gave the best results.

Other things that need to be watched for in order to obtain good photographs are air bubbles and floating particles in the petri dish. Air bubbles that get trapped in the tissue or between the material and the cover plate can cause incoming light to reflect unevenly producing unwanted glare; one way to get rid of air bubbles is to vaccuum them out of the water with a syringe. The best way to keep solid particles out of the water is to thouroughly rinse the petri dish between samples.

Dimensioning rhizome sections was made quick and easy by replacing the clear glass cover slip previously mentioned with a grid superimposed on a clear acetate cover. It is important to note that once magnified, grids that appear precise to the naked eye are quite rough; for the purposes of this preliminary study, though, the grid used was sufficient. To increase accuracy in the future, a micro-ruler with one-tenth of a millimeter graduations may be useful.

5.6 Quantitative Determination of Starch

The Nielson method (1943) proved to be an effective and rapid way of quantifying the amount of starch stored in the rhizome and root of a cattail. Results from replicate determinations of the same sample, however, do indicate some variability in the process; this variability is either due to the sampling technique, the instrumention, or the random turbidity of the tissue slurry (or a combination thereof).

Variability due to instrumention (i.e. the spectrophotometer) was shown to be negligible since the prepared reference blank continually gave consistent readings, with only minor fine adjustment, before each tissue sample was estimated. An important point to note here is that the spectrophotometer sample cuvettes must be thoroughly rinsed between samples and wiped clean of any solid particles if accurate data are required. Because this method is a colourimetric determination (i.e. the amount of light a given sample absorbs), it is paramount that particles foreign to the actual sample be removed so as not to interfere with light penetration and give misleading results.

Sampling variability, in the case of starch standard determinations, was also neglible as indicated by the standard curve prepared using very precise amounts of starch. With an R Squared value of 0.950, the graph shows that the predictability of spectrophotometer readings from the amount of starch in solution is close to perfect. In this case, 95% of the variance in spectrophotometer readings is accounted for. Not only does this strengthen the argument that the spectrophotometer is consistent, but it also suggests that, given the accuracy of the sampling during this stage, variability due to sampling was negligible as well. The key to minimizing sampling variability here was the use of 1ml and 3ml syringes to very consistently obtain, from standard to standard, the appropriate aliquots at the various stages in the process. Furthermore, the absence of interfering material (like the strands of tissue in the rhizome/root samples) of different shapes and sizes moving through the final solution in a random fashion makes for very consistent light transmission in the spectrophotometer.

The latter point, in fact, appears to be the primary reason why there is variability in the starch concentrations between replicates of the same sample, especially the roots. Because rhizomes and roots contain large amounts of cellulose, blending the tough material results in an unsatisfactory haphazard mixture of liquid, strands of tissue, and other chopped up particles. Much of the material does not get ground up to anything near the fineness required for homogeniety, and as such the resultant random slurry offers little consistency from one replicate sample to another. Because a substantial amount of material remains intact throughout the blending operation it cannot even be assured that the results obtained are indicative of conditions in the plant tissue; because the blended slurry is not homogenous there is no way of knowing whether or not an aliquot taken from it has the correct percentage of starch weight to tissue weight.

This variability in the slurry contents and, hence, turbidity causes problems for sampling and spectrophotometer operation. Because of the large particles in the slurry, the only way to take a 2ml aliquot was to measure that amount into a graduated cylinder - pipettes and syringes were tried but were instantly blocked up with large particles thereby rendering them useless. Trying to actually detect the miniscus of such a rough mixture in a graduated cylinder was anything but consistent, and further added to the variability of the process. Though there is no data to support the claim, it is likely that the apparent randomness of the slurry also created inconsistent turbidity results from sample to sample thereby making comparative spectrophotometer estimations inaccurate. Nevertheless, a single turbidity blank was produced and its results incorporated into the calculation of starch content in all tissue samples.

It is clear from the above that attempting to blend belowground cattail tissue into a homogenous, workable solution does not work and leads to variability and inaccuracy in the final data. To solve the problem it is suggested that, in the future, samples for starch determination be oven dried to constant weight at 110°C, weighed, and ground into a powder with a mill using a 60- to 80- mesh screen [as recommended by McCready et al (1950)]. This method should allow for a much more workable and consistent solution. Another

improvement to minimize sampling variability is the use of precision graduated 5ml pipettes (in 0.1ml graduations) rather than syringes or minimum capacity pipettes for taking aliquots - attempting to measure 2.7ml of perchloric acid with only a 1ml graduated pipette meant drawing up liquid an extra two times for one sample and increasing the possibility for error by three.

5.7 Rhizome Pith Width and Starch Content

Though this report focusses on evaluating the methods for collecting cattails, describing their morphology, and examining tissue for starch content and metal uptake, the data do point to some interesting ideas. These ideas will undoubtedly guide the investigation of future work at Denison Mines to determine the effects of foliar fertilizer on cattail below-ground biomass (growing on tailings).

In this study, roots showed significant amounts of starch to be present in the tissue. Though the variability between five replicates from the same sample was high, starch had definitely accumulated. A study done at a community near Lake Ontario on overwintering starch distribution in cattails showed that roots contained as much as half the starch that rhizomes did. It appears, then, that roots play a significant role in starch storage during the winter month so it is suggested that sections be made to examine starch distribution. The argument for making hand sections of cattail roots is further strengthened by Kalin and Scribailo's (1988) observations of metal damage to transplanted cattail rhizome and roots at the Denison site. Provision, then, should be made for hematoxylin staining of root sections.

The most interesting data to come of this preliminary study are the diameters of the pith in the rhizome. Of the 29 sections measured, only two had piths whose diameter was less than 50% of the overall rhizome diameter. In addition, nine out of ten times the pith got proportionately larger toward the new shoot-end of the rhizome (it is suspected, but not

proved, that the one time it did not was a result of unknowingly reversing the rhizome, thereby doing the same to the measurements). If rhizome biomass, then, is related to productivity, as implicated by Fiala (1971), and the pith is the major overwintering storage organ of the cattail (Kausch et al, 1981), then the percentage of the rhizome diameter as pith may be an important morphological indicator of plant health.

If this is the case then particular attention must be paid to measuring pith and rhizome diameters so that comparisons can be made between fertilized and non-fertilized tailings-grown cattails, natural ones, and even those on floating mats. Should pith size be an overwintering indicator of plant health and future growth (i.e. the coming growing season) capability, those plants who are healthy should see larger piths than those that are not. To reinforce this statement, quantitative starch measurements should be made to determine the actual amount of overwintering growth capacity cattails have.

The relationship between pith size and starch content can be determined by considering the dimensioning results of this study. If the pith becomes proportionately larger toward the new shoot it would make sense to do separate starch determinations at each end of the rhizome and relate those results to corresponding pith diameters. The sample preparation method used in the preliminary study could be altered to test the pith size/starch content hypothesis. Rhizomes from future cattail samples could be measured for length, and a constant central percentage of that length used for morphological and chemical investigations. Portions sufficient enough for quantitative starch determination can be cut at each end of the rhizome sample (i.e. parent shoot and new shoot ends) and bracketed by two hand sections for dimensioning. This should provide the means for relating pith size to starch content.

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THE EFFECTS OF FOLIAR FERTILIZATION ON PLANT PRODUCTIVITY AND BIOMASS IN CATTAILS GROWING ON ACIDIC MINE TAILINGS

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September 1991

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ABSTRACT

Acid mine drainage (AMD) is a water pollution problem associated with the mining industry. When pyritic material in mine tailings and waste rock weathers it produces high acidity in seepage and runoff water. A natural treatment alternative exists that utilizes cattail populations for the microbial generation of alkaline conditions to combat that acidity.

Because cattails can grow in acidic conditions, and can have an ameliorative effect on AMD, experiments are presently being carried out on acidic tailings in the Elliot Lake area of Ontario to determine if their productivity and hence biological polishing capacity can be increased through foliar fertilization. Findings from this study indicate that fertilization had no significant effects on proportional pith size, starch content, or starch distribution in overwintering rhizomes (parameters indicative of cattail productivity). Fertilization significantly reduced the weight of roots on the rhizome sections examined.

This study further strengthens the idea that cattails are tolerent of harsh environmental conditions. Additional studies will help optimize their role in ameliorating AMD and other water pollution scenarios.

CONCLUSIONS

Foliar fertilization had no significant effect (probability ≤ 0.05) on proportional pith diameter or rhizome starch content.

Root weight on rhizome sections from fertilized plots was significantly less (probability ≤ 0.05) than on those from unfertilized plots.

In terms of starch content and proportional pith size, foliar fertilization had no significant effect on cattail productivity/biomass.

Based on qualitative observations, foliar fertilization had no effect on starch distribution in the rhizome.

Cattails are tolerant of harsh conditions, and can grow effectively on acidic mine tailings.

RECOMMENDATION

A total biomass count should be performed in the Denison fertilizer plots to determine if foliar fertilization has any effect on cattail biomass.

1. INTRODUCTION

1.1 Problem Areas

Conventional approaches to wastewater treatment at acid-generating waste sites focus on collecting and neutralizing contaminated water, typically with the use of costly treatment plants and liming programs. Unfortunately for the operators, these costs are perpetual as wastes continue to produce acid long after revenue operations have ceased. To ensure environmental integrity, government abatement regulations are now mandating operators of base metal and precious metal mines, for example, to set aside money for environmental initiatives like wastewater treatment. Because, from a business standpoint, the environment represents a long-term liability, novel and cost-effective treatment methods need to be developed so that honest and effective efforts can be made to protect the natural environment.

Ecological Engineering addresses these concerns in its development of a conceptual framework for a self-sustaining treatment system. Cattail studies are only part of a broader scheme whose aim is to produce close-out conditions which are environmentally acceptable, self-sustaining, and cost-effective; other areas of investigation include microbiological sulphate-reducing processes, biological filtration systems, and solid waste research.

In recent years it has been acknowledged that cattail wetlands possess the ability to treat wastewater (Lakshman, 1987; Kadlec, 1987; Watson et al, 1987), and attempts have

been made to use this quality for ameliorating acid mine drainage (Kalin, 1986). Recognizing that small but persistent stands of naturally-colonized cattails do grow on acidic tailings sites, ecological studies have been carried out on the growth of these emergent macrophytes (Kalin, 1984).

Resulting from the oxidation and hydrolysis of sulphides in waste rock and tailings, acid mine drainage (AMD) is a water quality problem commonly associated with metal mining activities (Tucker at al, 1987) - see Schematic 1. Acid is produced, in the presence of water and oxygen, when Fe and other metallic sulphides oxidize to form soluble hydrous Fe sulphates:

$$(1)\ 2FeS_2 + 7O_2 + 2H_2O \leftrightarrow 2Fe^{+2} + 4SO_4^{-2} + 4H^{+}$$

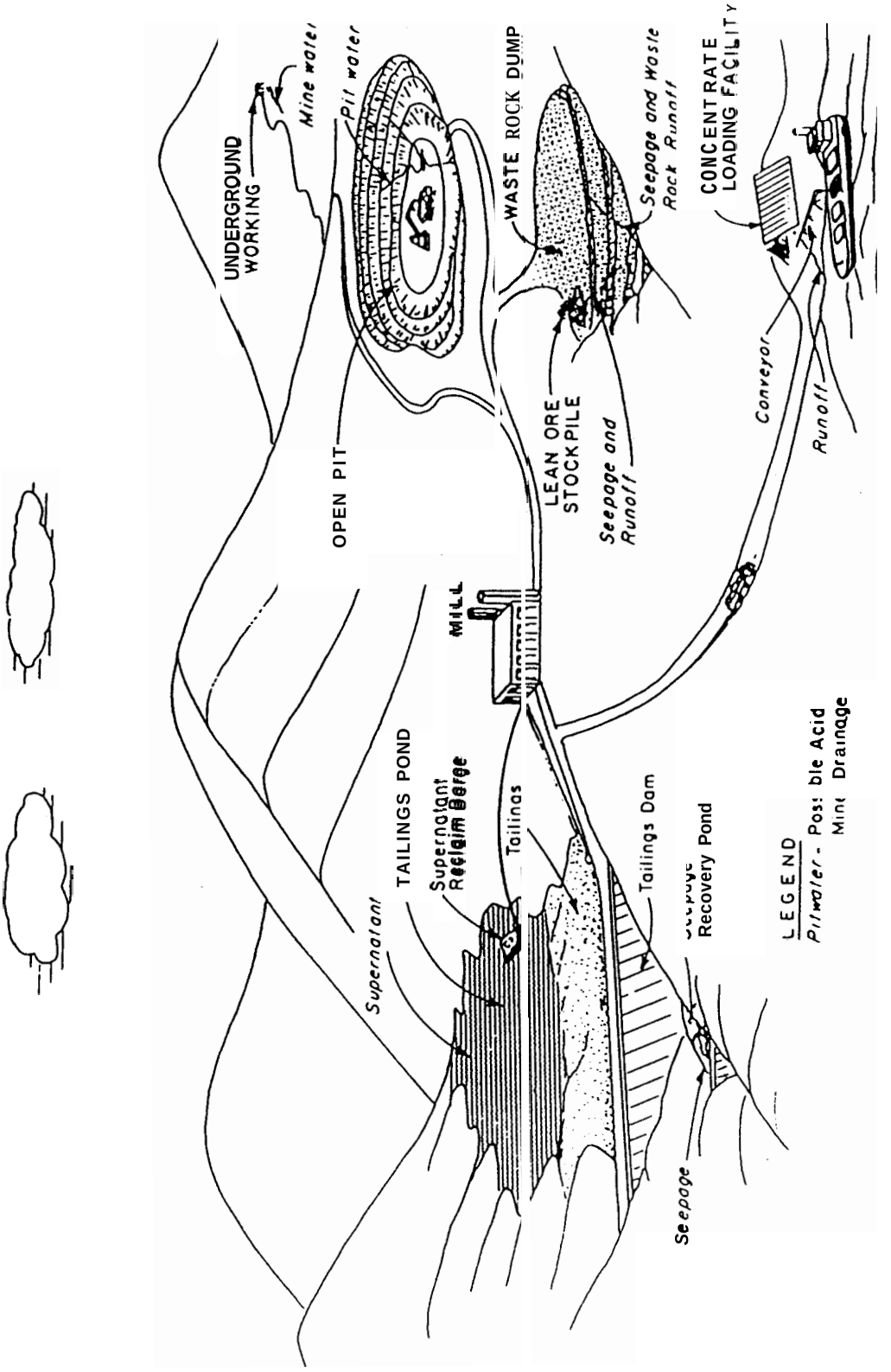
In contact with water, ferrous Fe oxidizes to the ferric state where it further complexes with ferrous and ferric oxyhydroxides to form a precipitate and more acid:

$$(2)\ Fe^{+2} + 1/4O_2 + H^{+} \leftrightarrow Fe^{+3} + 1/2H_2O$$

$$(3)\ Fe^{+3} + 3H_2O \leftrightarrow Fe(OH)_3 + 3H^{+}$$

In addition, *Thiobacillus ferrooxidans*, an Fe bacteria which occurs in aqueous environments with a pH range of 2.8 to 3.2, can bring about reactions 2 & 3 and produce more H^{+} ions,

Schematic 1. Major Components of a Metal Mine and Sources of Acid Mine Drainage



thereby further increasing the acidity of the system (Tucker et al, 1987).

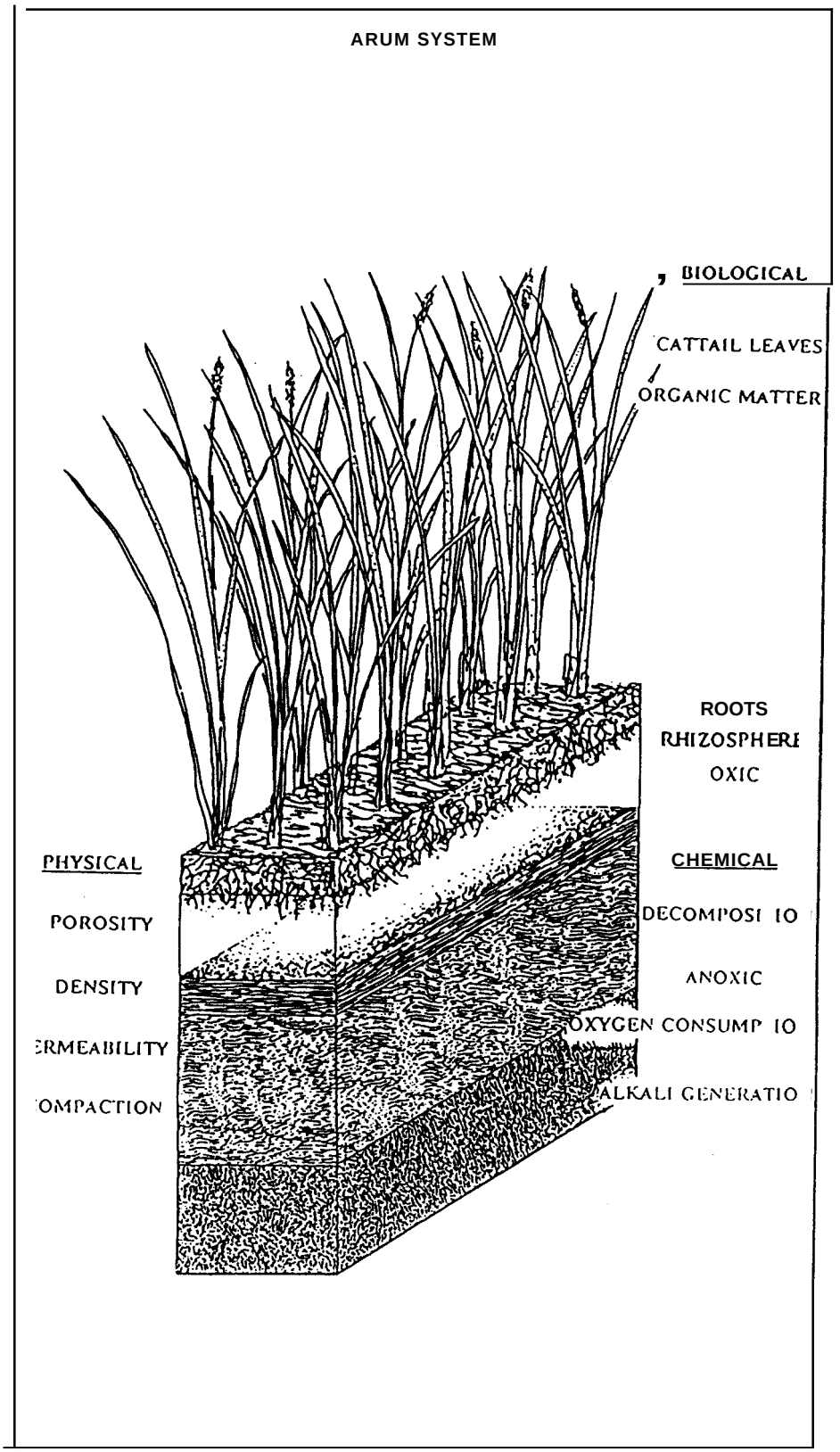
Cattails play a role in reducing acid generated in the above processes by providing an organic source for sulphate reducing bacteria (SRBs); these microorganisms reduce acid in wastewater back to hydrogen sulphide:

$$(4)\quad 2CH_2O + SO_4^{-2} + 2H^+ \rightarrow 2CO_2 + H_2S + 2H_2O$$

With this reduction of acidity comes the precipitation of ferric hydroxide and other metals. In addition, cattails require oxygen in the rhizosphere for respiration; under circumneutral conditions, CO_2 released by respiration around cattail rhizomes remains in solution thus making surrounding water more resistant to changes in pH. This buffering capacity, however, is greatly reduced in acidic conditions where CO_2 does not remain in solution and quickly bubbles away as gas (Wetzel, 1983).

Cattails also help ameliorate **AMD** by providing, over waterlogged areas, an intercepting cover that prevents the mixing of oxygen (an essential component in the acid-generating process) with water - see oxygen consumption and alkalinity generation in the anoxic layer in Schematic 2. Furthermore, emergent wetland biota like the cattail prevent the accumulation of water in these areas by intercepting and transpiring much of the precipitation incident on a wetland (Larcher, 1987).

Schematic 2. The ARUM **(Acid Reduction Using** Microbiology) **System**



In light of these phenomena it becomes clear that cattails play an important role in AMD treatment. In response, then, to their significance in natural wastewater treatment and industry's growing need for cost-effective solutions to its waste problems, studies commenced in 1988 to determine the growth and development patterns of cattails in acidic waters. Investigations focused on comparing cattail development among sites of increasing environmental severity in relation to pH and associated increasing levels of metal toxicity (Kalin & Scribailo, 1989; Kalin, 1990). In addition, and of particular relevance to this study, several different fertilizer treatments were applied to cattail plots at Denison Mines (Elliot Lake, Ont.) to determine their effectiveness in enhancing growth and survivorship.

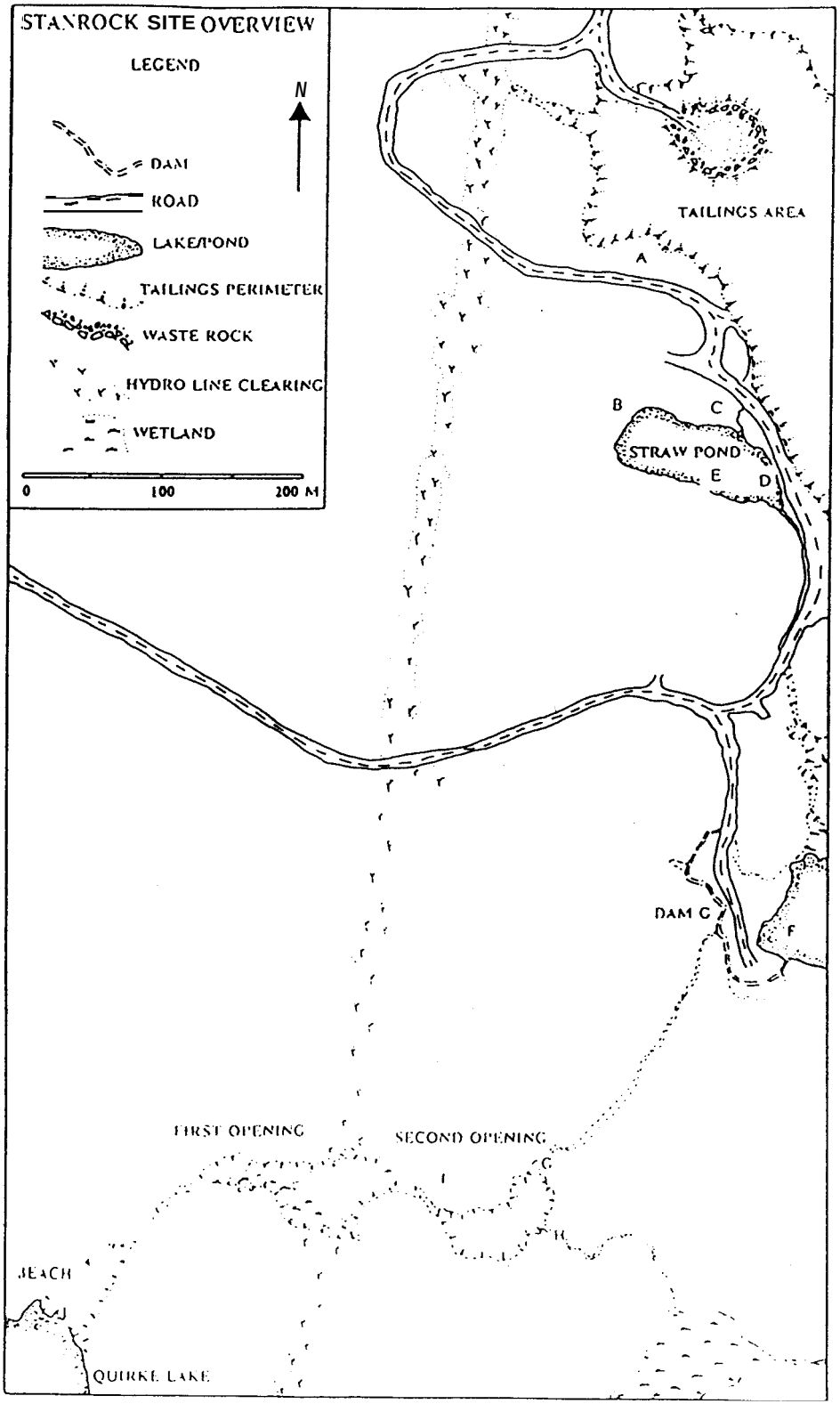
Results of experiments on tailings at a uranium mine in Elliot Lake, Ontario indicated that roots of cattails showed large accumulations of metals on their exodermal and hypodermal layers (for a biological description of cattails see Section 3.1). Scanning Electron Microscopy (SEM) analyses, employing X-ray spectral determinations of metal concentrations, showed that high levels of iron were associated with high concentrations of sulphur and greatly reduced levels of calcium; crystal formation was also noted in the iron-sulphate plaque accumulation on the roots. These findings suggested that the cattail rhizosphere may have been active in ameliorating AMD (Kalin & Scribailo, 1989).

The latter part of this study attempted to determine the morphological and growth effects of foliar fertilizers on cattails with the rationale that if these macrophytes have an ameliorative effect on AMD, then by increasing plant biomass through fertilization in areas

where growing conditions are harsh, their treating effect should also be increased (i.e. the more biomass, the greater the treatment capacity). Foliar fertilizers, as the name implies, are applied to the above-ground portion - the shoots and leaves - of plants; this method is deemed more effective, in terms of nutrient uptake, than ground-applied fertilizers in areas where the potential of moving surface water and resultant transportation of nutrients away from the plant exists (as is the case at Stanrock). From a chemical perspective, foliar fertilizers are appropriate because, under acidic conditions (in this case, acid is abundant in the tailings where the cattails have taken root), the plant availability of nitrogen and phosphorus is greatly reduced due to a lower rate of organic matter mineralization. As acidity increases the solubility of P in Fe phosphates decreases, as does the activity of N-fixing bacteria (Tucker et al, 1987); the implication here is that ground-applied fertilizers will result in minimal N and P uptake because of the acidic conditions.

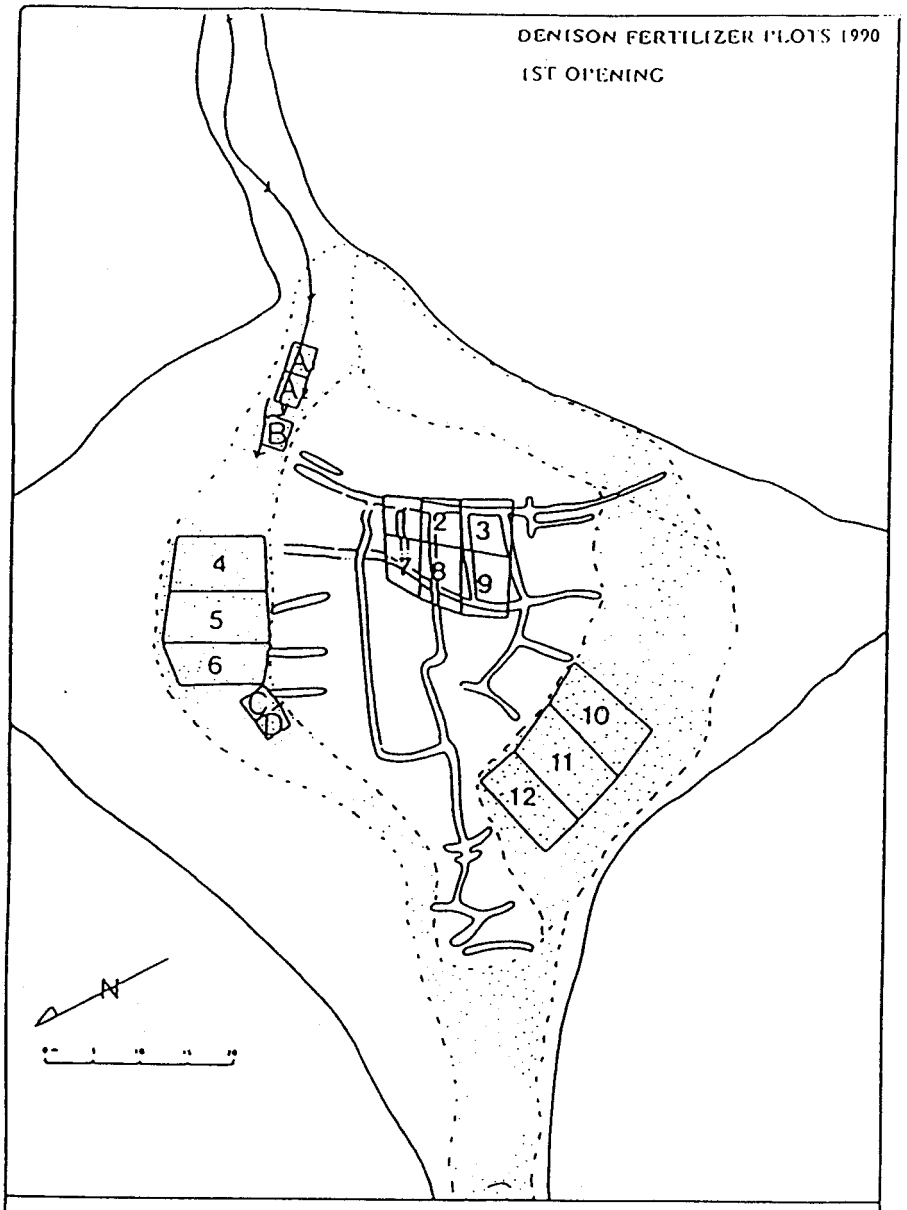
To maximize fertilizer-induced nutrient uptake in cattails, preliminary foliar applications began in 1989 at the First Opening of the Stanrock Dam G site (see Map 1.) to determine concentrations of fertilizer that would not produce leaf burn and, at the same time of course, enhance growth. Separate applications of 14-4-6 and 4-18-6 NPK fertilizers in 1/10 and 1/20 dilutions were applied in the summer of 1989, and results indicated some leaf burn using all four combinations. Greatly increased root growth was observed with high phosphorus and potassium fertilizer relative to unfertilized control plots, while high nitrogen fertilizer increased above-ground leaf development (Kalin, 1990).

Map 1. Stanrock Site Overview



Based on these observations, the 1990 fertilizer campaign saw the use of 14-4-6 NPK applications at a dilution of 1/10 with the addition of 1/50 diluted calcium fertilizer - see Figure 4. As indicated in Kalin (1990) results to this point are too preliminary to determine whether foliar fertilizer applications will increase survivorship and clonal growth in cattails. Comparative studies need to be undertaken to find out what the effects of applying foliar fertilizer are on root and rhizome development relative to unfertilized controls in order to determine if, in fact, nutrient addition of this nature will help to maximize polishing capacity in cattail stands.

Map 2. Denison Fertilizer Plots in the First Opening



PLOT #	DESCRIPTION	PLOT #	DESCRIPTION
1	NPK LEAVES	11	CONTROL
2	NPK LEAVES	12	NPK LEAVES
3	NPK LEAVES	A1	Ca LEAVES
4	NPK LEAVES	A2	NPK LEAVES, Ca GROUND
5	CONTROL	B	NPK LEAVES, Ca LEAVES
6	NPK LEAVES	C	NPK GROUND
7	NPK LEAVES	D	Ca GROUND
8	NPK LEAVES		
9	NPK LEAVES		
10	NPK LEAVES		

12 Purpose and Objectives of Study

This study is, in a sense, a "stepping stone" to achieving the ultimate goal of determining whether foliar fertilizer applications on tailings-grown cattails is a viable method for increasing biomass production and, hence, biological polishing capacity. By comparing root and rhizome development between fertilized and non-fertilized control plots, it should be feasible to determine the morphological and biochemical (i.e. starch reserves) effects of NPK and Ca-fertilized cattails.

An investigation of below-ground tissues is appropriate because they act as regions of material storage for future growth and development. In particular, the pith region of the rhizome is relevant to the study because it is there that sugars produced during photosynthesis are stored as starch. And when there is an unlimited availability of nutrients the pith sees a reduction in sugar and starch accumulations (Lakshman, 1987). Based on this point, all parameters otherwise being equal, cattails in control plots should see greater levels of starch in their piths than those receiving additional fertilizer-induced nutrients.

During winter months, cattails store sugars and starch at relatively stable levels; carbohydrate use during this period is minimal with only small amounts utilized for suppressed respiration. Winter sampling and experimentation, then, is useful under this "steady state" condition because it eliminates sampling problems arising from daily and growing-season fluctuations in starch levels. Starch content in rhizomes of fertilized cattails

relative to that in the unfertilized ones should indicate to some degree the relative availability of fertilizer-induced nutrients to the plants.

This report, then, presents findings from a study undertaken during March and April of 1991 to determine the effects of foliar fertilization on overwintering cattail rhizome morphology and starch reserves. Experiments were carried out on cattails growing on tailings and in a natural bog to determine their below-ground starch distribution, starch weight/plant weight percentages, rhizome dimensions, and root weight/rhizome weight percentages. At Denison's Stanrock Dam G site, cattails were extracted from two different foliar fertilizer plots (NPK and NPK+Ca) and a control plot on the tailings. As a means of off-site comparison, cattails growing in a natural wetland near the mine site were also used in the study.

Findings from this investigation, as well as future total biomass studies, should indicate whether or not foliar fertilization is a viable strategy to increase clonal growth in cattail populations, and with it biological polishing capacity.

2. BACKGROUND INFORMATION

2.1 Cattail Anatomy, Morphology, and Development

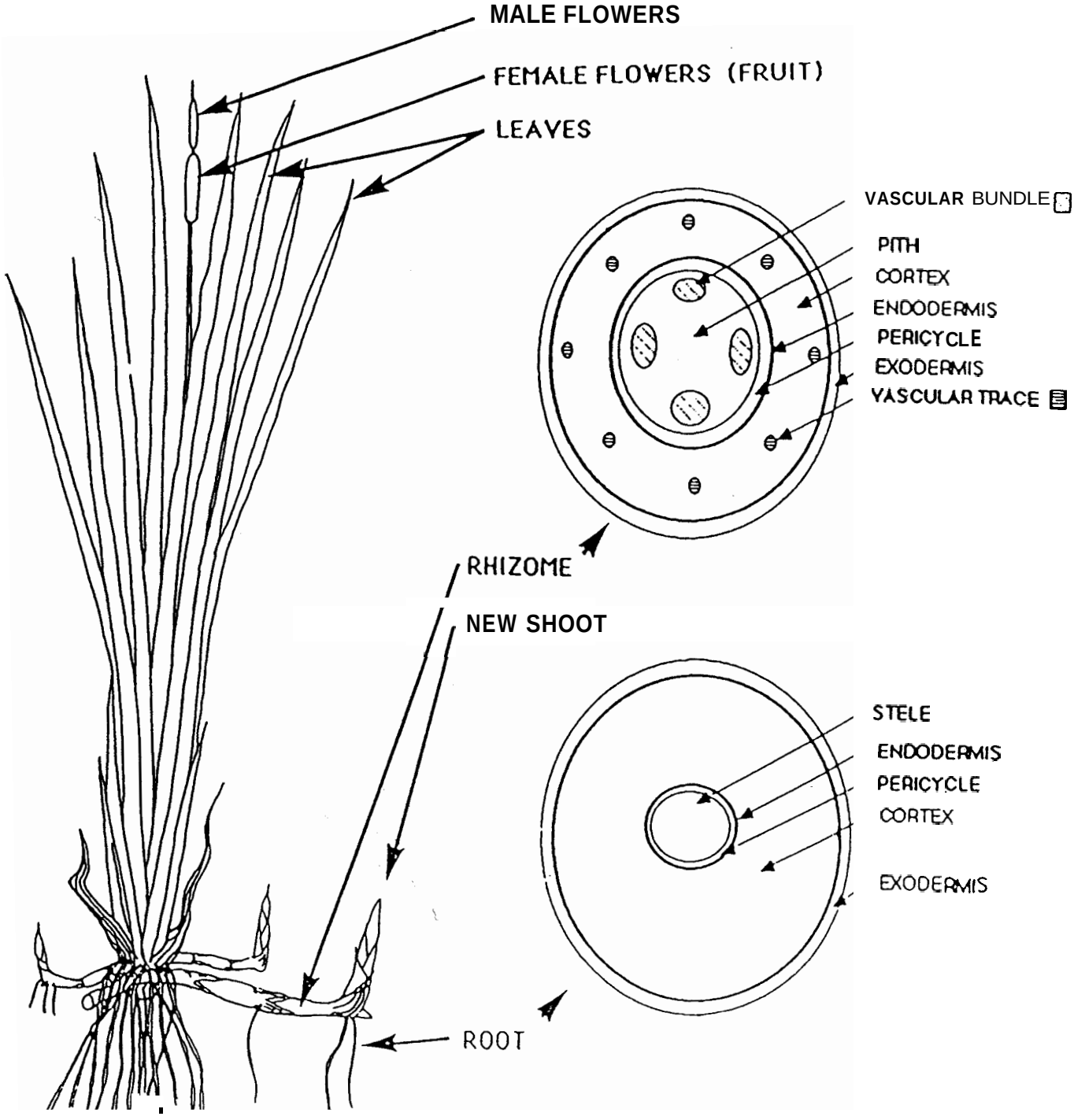
The following description is based on that found in Kalin & Scribailo (1989).

Cattails reproduce vegetatively, with the production of rhizomes (underground stems), to produce individual plants consisting of a rosette of opposite leaves, typically six per side - see Schematic 3. By developing small vegetative buds at the base of each leaf, referred to as the axil, these emergent macrophytes possess the ability to develop vertical, upright shoots. Rhizomes characteristically produce roots at regular intervals along their length, although the majority of roots tend to develop at the bases of upright shoots.

In cross section, rhizomes consist of two distinct zones - an outer cortex and an inner pith. The pith is mainly composed of tightly-packed storage cells used for holding starch for future growth and development. To inhibit lateral movement of substances from the cortex into the inner zone, an endodermis borders the pith's periphery.

The cortex is largely composed of star-shaped cells, collectively referred to as stellate, with significant quantities of air between them. This outer region of the cattail rhizome is bordered on the outside by an exodermis which acts as a barrier to the transportation of materials from the external environment into the rhizome. Vascular bundles are scattered throughout the cortex, but are more concentrated in the pith. Their primary function is to

Schematic 3. Cattail Anatomy and Morphology



transport water from the roots to the shoots, and to transport sugars produced during photosynthesis from the leaves to the rhizome for storage as starch in the pith.

Root anatomy is similar to that of the rhizome with the exception of the pith region. In cattail roots, rather than many individual bundles, the pith is replaced by a solid cylinder of vascular tissue called a stele. Roots are produced laterally from a thin layer of tissue just beneath the rhizome's endodermis called the pericycle; these roots may also produce lateral roots from their pericycle.

2.2 The Cattail Rhizosphere

The significance of cattails, and particularly the rhizosphere, in ameliorating AMD has been suggested by Kalin and Scribailo (1989) and is one reason why this study focuses on the below-ground tissue of these emergent macrophytes. There are other reasons, as well, for concentrating on the rhizome, yet before effective data interpretation can take place these reasons need to be elucidated in light of an understanding of the phenology and development of the cattail rhizome.

Cattail plants are connected together by an underground network of stems, or rhizomes, growing parallel to the surface of the bottom (Linde et al, 1976). By reproducing vegetatively with aerial shoots arising from rhizomes, cattails produce colonies that migrate

outward to invade new areas. Because each shoot originates from a common ancestor, all are genetically identical - thousands of aerial shoots, composed of leaves and occasional fruiting stalks, can be produced by a single parent.

Rhizomes have a number of functions which are important to the survival and propagation of the cattail. During the growing season, reserves of carbohydrates are built up in the pith region of the rhizome and stored through winter to provide food for the pre-photosynthetic (i.e. submerged) development of the new aerial shoot in the spring. In the summer months, migration of the cattail beyond its periphery is brought about by the growth of rhizomes, thereby maintaining established populations while also expanding them into new, suitable habitats. During the winter, rhizomes also serve to carry the plant through this dormant period by being able to obtain oxygen for survivability. Even though the aerial shoot is dead at this time, aerenchyma cells in the rhizome, which are connected to those in the shoot, receive conducted oxygen from the shoot and ensure the cattail's survival during winter-induced anaerobic conditions.

Growth and development studies of cattails in a shallow marsh in Wisconsin, U.S.A. receiving heavy nutrient loading were undertaken by Linde et al (1976) during a three-year period to gain an understanding of how to control and manage the plant for associated wildlife habitat. Growth and development of leaves, fruiting heads, and rhizomes were measured weekly throughout each growing season. Of particular relevance to this study was the weekly collection and analysis of total nonstructural carbohydrates (TNC); this was seen

as a means for determining stored energy levels on which the plant depended for initial growth in the spring (Linde et al, 1976).

Findings from that study showed that old rhizomes - those produced during a previous growing season - were the principal storage organ with maximum levels of TNC occurring during the early winter period (Linde et al, 1976). Levels of TNC gradually declined with the onset of growth in the spring to a minimum in mid-summer during fruiting head development. Beyond this point, carbohydrates were produced in excess of the plant's needs so they were translocated to the rhizomes for storage, Conclusions from the study indicated that the stored carbohydrates were available to help the plant recover from severe injuries and to develop new shoots the following spring before it is capable of photosynthesizing sufficient carbohydrates for its needs (Linde et al, 1976).

Other findings on cattail rhizomes indicated that adventitious buds at the base of growing aerial shoots developed into new rhizomes in late spring, and maximum rhizome growth of one inch per day. Sprouts were observed forming on the new rhizomes during mid summer and were later found to have produced the following summer's crop of aerial shoots; the sprouts went into dormancy in late fall and resumed growth in early spring (Linde et al, 1976).

2.3 Overwintering Starch Distribution in Cattail Rhizomes

Although the literature is scant in dealing with the exact subject, one paper was found which might be of significance to this examination. To determine the amount of starch storage during the overwintering period in different tissues of the cattail, a study was conducted in 1977-78 at a single clonal cattail community near the southeast shore of Lake Ontario in New York State (Kausch et al, 1981).

In keeping with previous studies of the same nature, results from analyses of starch distribution in cattail leaves, stems, and rhizomes showed the rhizome as the major storage organ for starch. The central core region of the rhizome - the pith - showed extensive accumulations of globose starch grains during late fall and early winter with significant reductions from late winter through to early spring (Kausch et al, 1981). Small amounts of starch were also noted in the rhizome endodermis and adjacent cortical parenchyma in late fall and winter, with almost none in spring. Beneath the outer cortex, large accumulations of starch were found in scattered parenchyma cells; by spring, this layer lost most of its starch.

The outer cortex of intact roots contained starch in late fall, while the middle cortex remained starch-free all year. Starch was also noted in a number of cells in the inner cortex, but very little was seen in the endodermis. Overall, cattail roots saw a gradual decrease in starch levels in midwinter followed by a sharp drop in late winter with almost zero deposits

by spring.

Coupled with findings from investigations of cattail buds and young leaves it was concluded that a decrease in starch in storage tissues of rhizomes and roots by late winter and early spring coincided with increasing bud growth and starch accumulation in or near zones of rapid development (Kausch et al, **1981).** This strongly supported the idea that most of the stored starch was transferred from the storage organs to tissues of the developing shoot until the new shoots emerged from the water and became photosynthetic. It was apparent from the study that cattails are extremely productive plants with as much as **45.03%** starch, dry weight, accumulating in rhizomes in early winter and **22.80%** in roots; interestingly, not all stored starch was used for productivity as rhizomes retained a significant amount **(27.40%** dry weight) into the start of their second year (Kausch et al, **1981).**

3. METHODS

3.1 Field Sampling

Cattails for experimentation were extracted from the First Opening of Denison's Stanrock Dam G site on March 21, 1991. Five plants from each of three test plots, for a total of fifteen, were excavated from holes (approximately 1m wide) dug within each plot. The first plot (#4 - see Map 2) was foliar fertilized with 14-4-6 NPK in the summers of 1989 and 1990, the second (#B) with NPK and Calcium, and the third (#5) left as an unfertilized control. To act as an off-site control, five cattails were also extracted from a natural waterlogged area adjacent to the Stanrock access road.

In order to extract cattails with intact year-old rhizomes (i.e. with yet-to-emerge new shoots), snow had to be cleared away from the extraction holes. Any ice at the substrate surface was chipped away, then substrate removed to the depth of the rhizomes (approx. 15-30cm). Samples were removed by hand, washed, labelled, photographed, and placed in ice-packed coolers for transportation back to laboratory freezers.

3.2 Sample Preparation

To prepare the cattails for morphological and chemical investigations, individual plants were taken from the freezer and quickly washed under hot tap water to remove any excess substrate remaining after field washing. Samples were then placed back into the freezer so that tissue was firm enough for hand-sectioning. After one hour, individual plants were removed from the freezer and their year-old rhizomes identified and measured for length. The central 50% of each rhizome was cut from its overall length, making sure to note the "parent-shoot" and "new shoot" ends; a previous study by the author found that cattails' overwintering starch storage organs (i.e their piths) became proportionately larger toward the new shoot-end of the rhizome.

One hand section from each end of the cut length of rhizome was made and stored in 95% denatured ethyl alcohol for starch distribution experiments; those hand sections at the parent shoot end of the segment were labelled "p", and those near the new shoot, "n". Three-centimeter-long segments were then cut from each end, and their roots removed at the exodermis. Each 3cm segment and its corresponding root material was appropriately labelled and oven dried for dry weight measurements and quantitative starch determinations.

3 3 Iodine/Potassium Iodide (IKI) Staining for Starch

Before staining took place, the alcohol inside the cells of the rhizome sections had to be washed out. To accomplish this, one hand section from each grouping of two was removed from its respective scintillation vial and immersed in each of a series of four beakers containing distilled water. After the final immersion, the section was placed on a microscope slide ready to accept the iodine/potassium iodide (IKI) solution. Two to three drops of pre-prepared IKI were placed on the section and allowed to develop into the stain's characteristic bluish-purple colour when in contact with zones of starch accumulation.

Once sufficient colour had developed, excess stain was washed off and the section was put in a petri dish modified with a movable glass cover slip. The dish was filled with distilled water and the section was placed under the cover slip to prevent it from moving around while under microscopic examination. Sections were viewed under a Ziess stereomicroscope, model SV 8, fitted with a MC 63 M 35 photomicrographic camera. Visual observations were recorded and, where appropriate, slides were taken using Kodak Ektachrome 160T professional slide film.

3.4 Measuring Rhizome Section Dimensions

After recording observations and photographing the IKI-stained rhizome tissue, each section was removed from the viewing dish and placed in another petri dish fitted with a mm^2 grid superimposed on a movable, clear acetate cover. Each section was placed under the acetate cover, making sure the cover was in contact with the entire surface of the section, and centred about a prescribed grid intersection point. Pith diameters as well as overall rhizome diameters were recorded in both the X and Y directions by counting the number of millimetre squares each feature took up. Sections were then discarded.

3.5 Dry Weight Measurements

A complete set of rhizome segments and roots was oven-dried at 110°C to constant weight, cooled in a dessicator, and weighed on a Sartorious balance to the nearest 10μg. Root weight/rhizome weight percentages were calculated for each segment. The dried plant material was then set aside for quantitative starch determinations.

3.6 Quantitative Determination of Starch

A modified version of the Nielson method (1943) was used. Each 3cm rhizome portion was ground in a Wiley mill using a 40 mesh screen and re-weighed on the Sartorius balance. Because there was significantly less root material, all roots from the new shoot segments in each plot were pooled together, and milled/weighed in the same manner as the rhizomes; the same was done for the parent shoot-end roots. For each sample, the milled tissue was placed in a 50mL beaker and 150 times its weight of distilled water was added to make a 0.67% tissue slurry. The plant material was then suspended in the water using a magnetic stirrer. Five 2mL aliquots were taken from the suspension and placed in separate beakers using a fast delivery pipette.

Exactly 2.7ml of 72% perchloric acid was added to each aliquot while stirring thoroughly to ensure no momentary high concentrations of acid in any portion of the sample. For each aliquot, the mixture was allowed to stand with occasional stirring for approximately ten minutes. A lml aliquot of the solution was then pipetted into another 50 ml beaker and 6ml of distilled water was added. The solution was brought to a pH of 8.3 with a few drops of 6N sodium hydroxide and then to a pH of 4.5 with 2N acetic acid.

Two and a half millilitres of 2N acetic acid was then added in excess, followed by 0.5ml 10% potassium iodide, and 5ml of 0.01N potassium iodate. The solution was allowed to stand for 5 minutes so that effective colour development could take place. Using a

graduated cylinder, the coloured solution was made up to 50ml with distilled water and transferred to a suitable cuvette for colour estimation in a photoelectric colourimeter.

Colourimetric readings were made on the final solutions using a Coleman Junior II Spectrophotometer set at 680 nm with a red filter. The spectrophotometer was calibrated to zero absorption prior to each reading using a reference blank containing all reagents except for actual starch material. To provide a control against turbidity in the final solutions, a blank was also made up with the slurried rhizome material and all reagents except for the potassium iodide and iodate, and checked against distilled water.

3.6.1 Standardization

Starch content in cattail rhizome and root tissue was calculated on a dry weight basis (mg starch/mg of tissue) from a standard curve prepared from the colourimetric readings of a known range of starch concentrations. Various amounts of potato starch were accurately weighed to the nearest 10μg on a Sartorius analytical balance and made to final 50ml solutions in the same manner as the cattail tissue. The amount of starch in the final solutions was calculated, and colourimetric readings were taken on the spectrophotometer. A curve was developed by plotting starch in 50mL solution against spectrophotometer absorbance.

Absorbance readings of cattail tissue solutions were then read from the curve to

obtain starch concentrations in 50ml. Starch content in fresh weight of cattail material was then calculated by relating the observed concentration to the amount of tissue in the 50ml solution (while incorporating the turbidity blank result). Leftover material from the blending operation was air dried to constant weight for dry weight biomass calculations.

3.7 Statistical Analyses

In order to determine whether differences in morphological and biochemical parameters between treatments were significant, a number of statistical calculations were performed. Sample means and standard deviations were calculated for each treatment. To allow for more meaningful comparisons of the variability of the results, standard deviations were reported as Coefficients of Variation (i.e. standard deviation expressed as a percent of the mean). To test the significance of differences between the experimental plots (i.e. probability statements), the Student's *t* test (Parker, 1973) was performed on the treatments' mean values.

4. RESULTS

4.1 IKI Staining for Starch

Iodine/potassium iodide staining for starch yielded consistent results throughout the samples with the pith showing extensive bluish-purple colouration (indicative of starch) as well as a thin band beneath the exodermal layer. Starch was detected at the base of roots in some sections but not in others. A number of sections experienced damaged piths in the form of lost material.

4.2 Rhizome Dimensioning

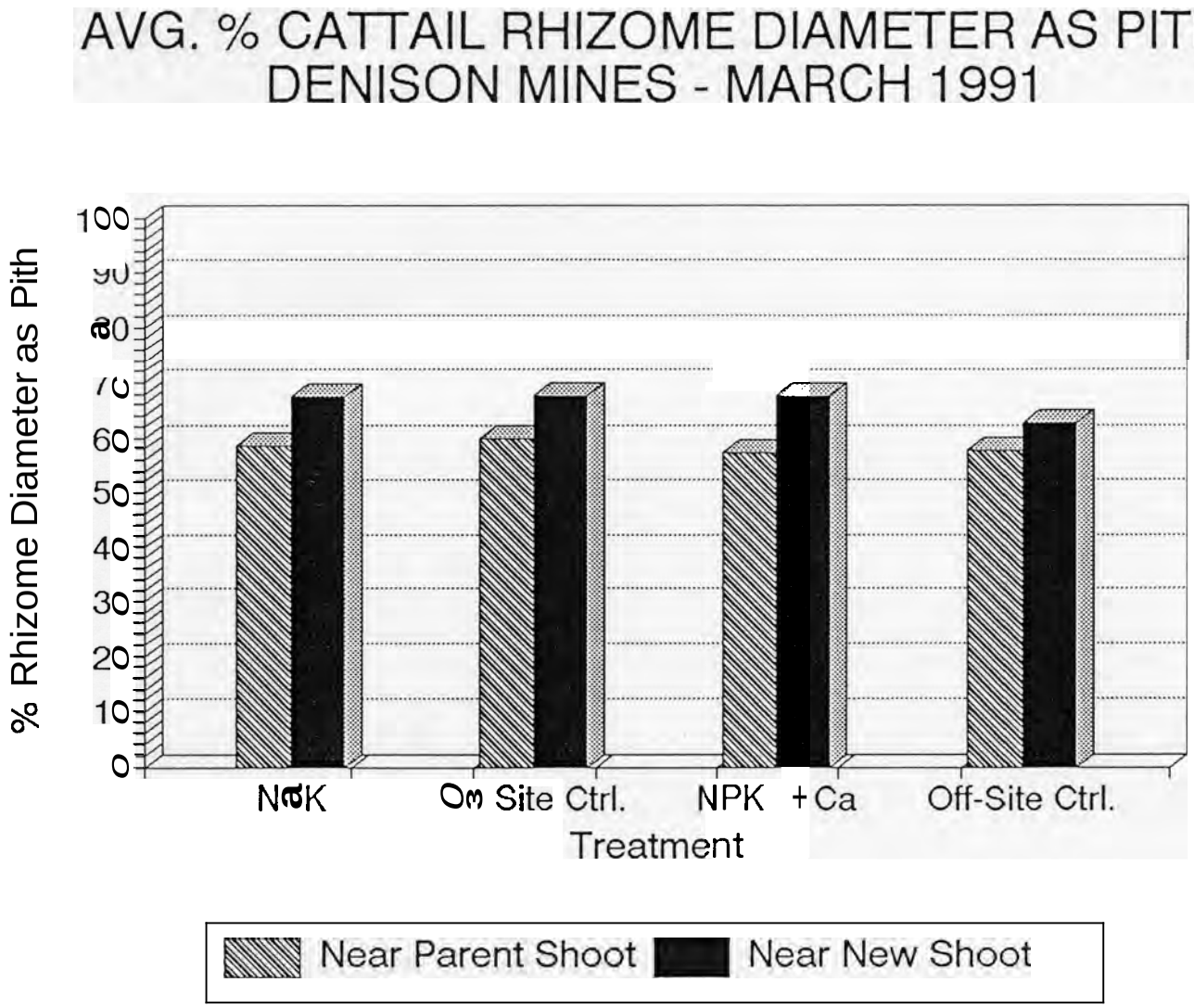
Results of cattail rhizome dimension measurements are presented in Table 1. Measurements were taken in the X and Y directions for the pith diameter and overall rhizome diameter, then averaged. Percent rhizome diameter as pith was then calculated using the two averages. Figure 1 reveals that, under all four treatments, the pith occupies more cross-sectional space in the cattail rhizome toward the new shoot. Actual differences in the data between treatments are not significant (probability ≤ 0.05). Low Variation Coefficients in Table 1 strengthen the validity of these findings.

Table 1.

DIMENSIONS OF DENISON CATTAIL RHIZOME SAMPLES - MARCH 1991

Plot- Cattail #	Rhizome Length(cm)	Section #	Pith Diam. (mm) X Axis	Pith Diam. (mm) Y Axis	Average Diam.(mm)	Rhiz. Diam.(mm) X Axis	Rhiz. Diam.(mm) Y Axis	Average Diam.(mm)	% Rhi. Diam. as Pith	Plot Statistics (for % rhi. diam. as pith)	
4-1	18.0	p	5.8	6.0	5.9	8.5	10.6	9.6	61.8	Parent Shoot:	
4-2	19.5	p	8.2	9.0	8.6	11.5	14.3	12.9	66.7	Mean =	58.5
4-3	21.0	p	8.7	7.6	8.2	14.3	12.7	13.5	60.4	Variation Coef. =	10.8 %
4-4	31.3	p	5.9	6.3	6.1	12.5	12.9	12.7	48.0		
4-5	48.5	p	7.5	7.2	7.4	13.8	12.6	13.2	55.7		
4-1		n	8.1	8.4	8.3	11.5	12.0	11.8	70.2	New Shoot:	
4-2		n	11.5	12.0	11.8	16.1	17.1	16.6	70.8	Mean =	67.4
4-3		n	9.7	9.7	9.7	12.2	14.5	13.4	72.7	Variation Coef. =	8.0 %
4-4		n	6.3	6.6	6.5	10.4	12.0	11.2	57.6		
4-5		n	10.3	10.7	10.5	15.7	16.3	16.0	65.6		
5-1	29.0	p	7.1	7.9	7.5	13.8	11.0	12.4	60.5	Parent Shoot:	
5-2	41.5	p	6.8	6.8	6.8	12.3	14.0	13.2	51.7	Mean =	59.9
5-3	25.0	p	8.0	7.5	7.8	12.5	12.0	12.3	63.3	Variation Coef. =	7.1 %
5-4	19.0	p	4.6	4.3	4.5	7.1	7.0	7.1	63.1		
5-5	15.5	p	6.0	6.3	6.2	10.7	9.5	10.1	60.9		
5-1		n	9.9	9.2	9.6	14.4	13.0	13.7	69.7	New Shoot:	
5-2		n	9.5	9.0	9.3	14.7	14.1	14.4	64.2	Mean =	67.6
5-3		n	10.4	10.7	10.6	14.3	14.3	14.3	73.8	Variation Coef. =	7.1 %
5-4		n	5.6	5.2	5.4	8.3	7.1	7.7	70.1		
5-5		n	6.0	6.2	6.1	9.8	10.5	10.2	60.1		
B-1	22.5	p	7.4	1.0	7.2	13.2	13.0	13.1	55.0	Parent Shoot:	
B-2	24.5	p	6.9	7.0	7.0	12.3	13.3	12.8	54.3	Mean =	57.4
B-3	10.0	p	6.8	6.6	6.7	10.9	11.8	11.4	59.0	Variation Coef. =	5.9 %
B-4	11.5	p	5.1	4.9	5.0	9.3	8.7	9.0	55.6		
B-5	12.0	p	4.7	5.0	4.9	7.4	7.9	7.1	63.4		
B-1		n	8.6	8.3	8.5	14.3	12.0	13.2	64.3	New Shoot:	
B-2		n	9.0	9.4	9.2	13.2	14.5	13.9	66.4	Mean =	67.6
B-3		n	8.6	7.5	8.1	12.1	11.8	12.0	67.4	Variation Coef. =	3.9 %
B-4		n	7.9	7.7	7.8	11.5	10.1	10.8	72.2		
B-5		n	7.3	7.5	7.4	10.1	11.7	10.9	67.9		
Ctrl.-1	38.1	p	6.8	6.1	6.5	13.9	11.0	12.5	51.8	Parent Shoot:	
Ctrl.-2	36.0	p	10.8	9.3	10.1	17.8	12.6	15.2	66.1	Mean =	57.9
Ctrl.-3	30.0	p	5.6	5.7	5.7	9.3	11.7	10.5	53.8	Variation Coef. =	8.9 %
Ctrl.-4	28.5	p	8.0	6.9	1.5	13.8	12.4	13.1	56.9		
Ctrl.-5	11.5	p	7.9	7.5	7.7	13.5	11.8	12.7	60.9		
Ctrl.-1		n	8.6	8.9	8.8	13.0	14.0	13.5	64.8	New Shoot:	
Ctrl.-2		n	10.5	8.5	9.5	18.7	11.5	15.1	62.9	Mean =	62.8
Ctrl.-3		n	Section too damaged for measuring							Variation Coef. =	2.6 %
Ctrl.-4		n	8.2	8.1	8.2	13.4	13.4	13.4	60.8		
Ctrl.-5		n	Section too damaged for measuring								

Figure 1.


AVG. % CATTAIL RHIZOME DIAMETER AS PITH
DENISON MINES - MARCH 1991
% Rhizome Diameter as Pith
100
90
80
70
60
50
40
30
20
10
0
NPK
Off Site Ctrl.
NPK +Ca
Off-Site Ctrl.
Treatment
Near Parent Shoot
Near New Shoot

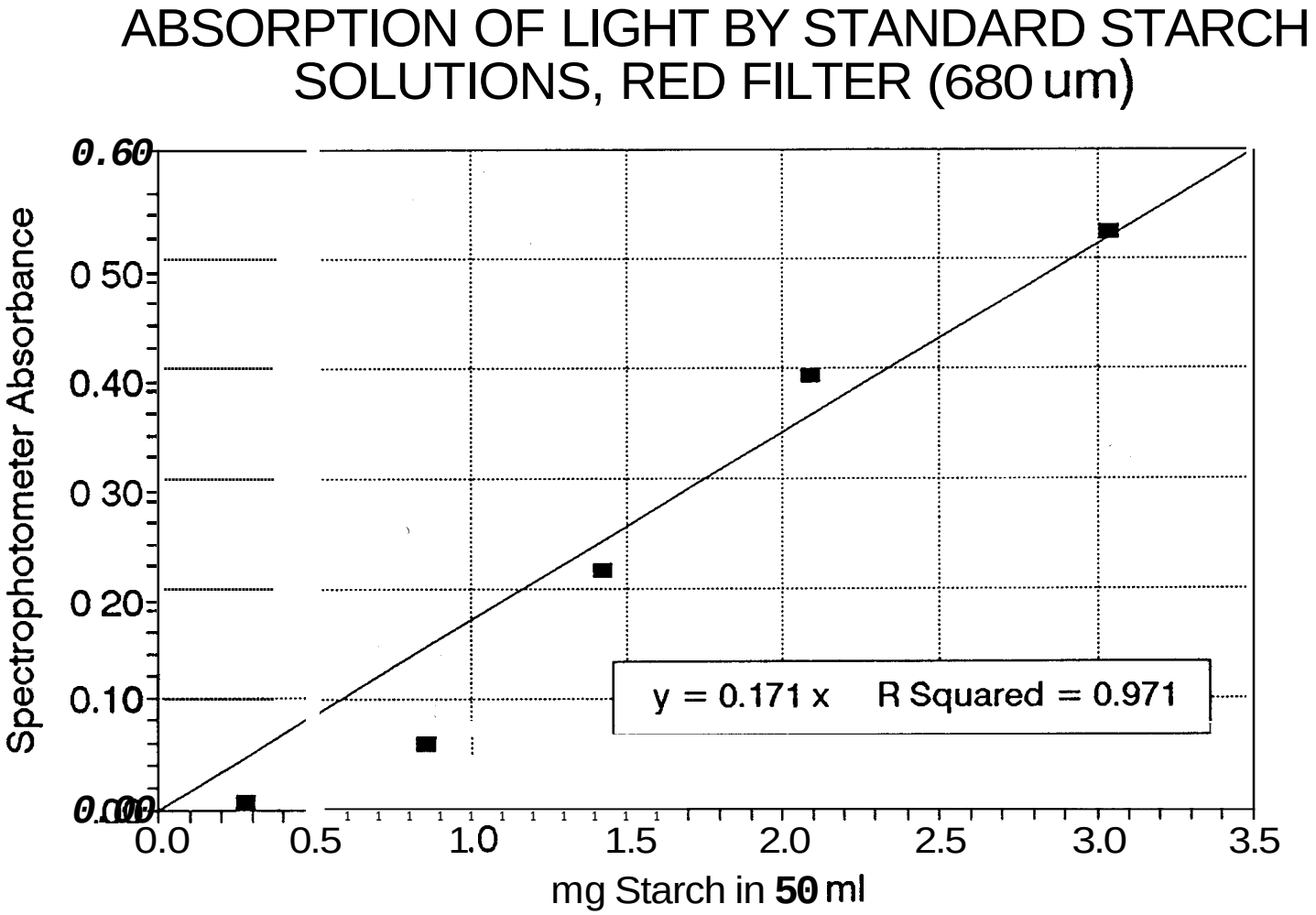
4 3 Quantitative Determination of Starch

A standard curve is presented in Figure 2 showing the absorption of light in a spectrophotometer by accurately known amounts of starch. With an R Squared of 0.971 in Figure 2, the amount of starch in solution almost perfectly predicts absorption readings on the spectrophotometer.

Table 2 shows the results of cattail rhizome and root tissue determinations. Five replicates were used for each sample. Absorption readings were obtained from the spectrophotometer, read from the standard curve to determine starch quantity in 50ml solution, and a ratio of starch weight to tissue weight calculated.

Figure 3 reveals that differences in starch content from the parent shoot-end of the cattail rhizome to the new shoot-end are only slight. Differences in rhizome starch levels between treatments are not significant (probability $\leq$ 0.05). Variation Coefficient numbers in Table 2 indicate some variability in the data.

Figure 2. Standard Curve for Starch Concentrations



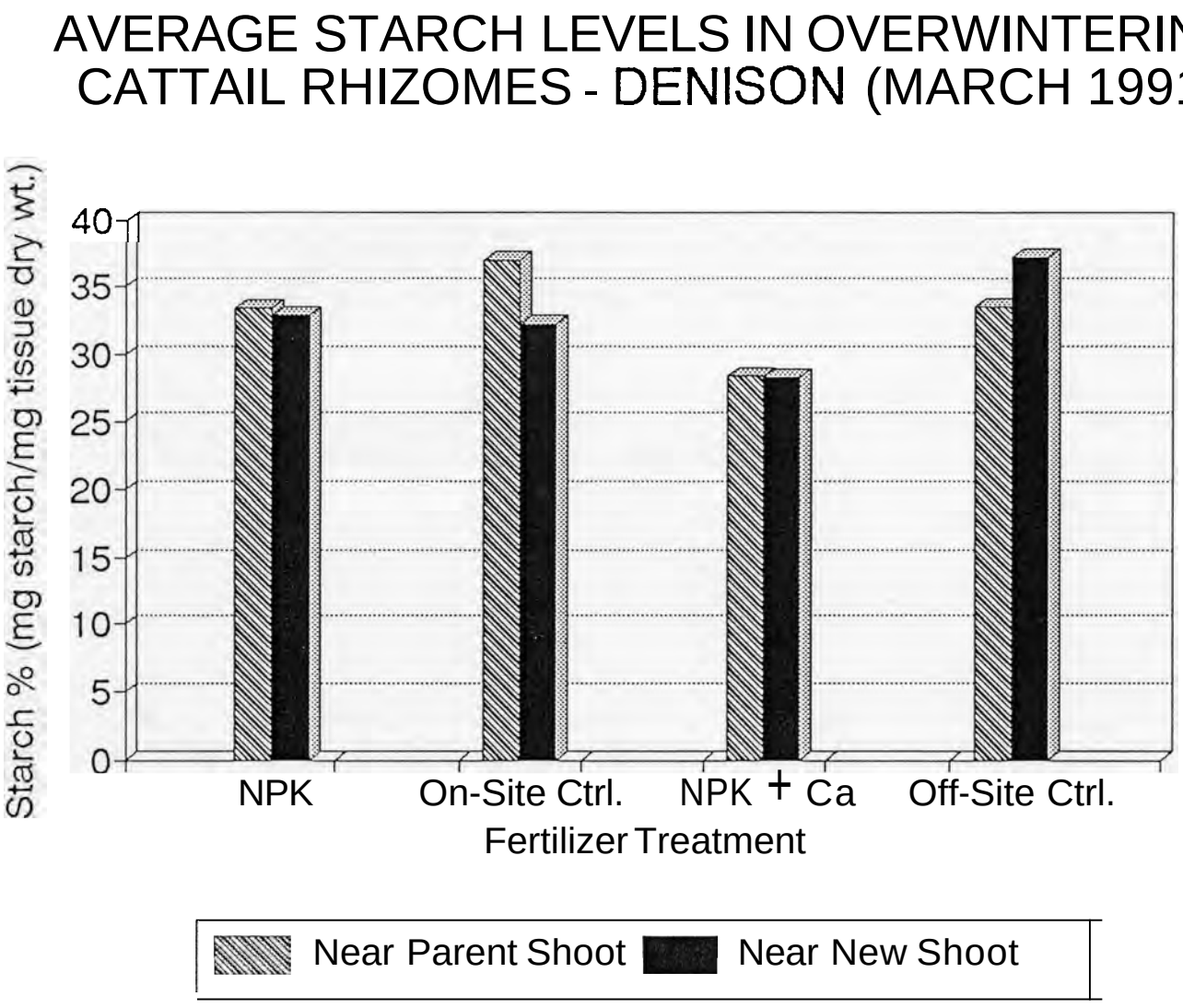
STARCH LEVELS IN DENISON CATTAIL RHIZOME AND ROOT SAMPLES - MARCH 1991

Table 2.

RHIZOMES

Plot	Sample	Spectrophotometer Absorbance					mg Starch in 50 ml					Starch % (mg starch/mg plant material dry weight)					Sample Mean	Plot Statistics			
4	1p	0.362	0.389	0.308	0.306	0.287	212	227	1.80	1.79	1.68	3117	35.64	28.22	28.04	2630	30.28	mean	3146		
	2p	0.383	0.428	0.460	a425	0.429	224	250	269	2.48	251	35.09	39.22	4215	38.94	39.31	38.94	mu	4298		
	3P	0.391	a408	0.469	0.420	0.338	229	239	2.74	2.46	1.98	35.83	37.39	42.98	38.49	30.97	37.13	min	26.30		
	4P	0.352	0.326	0.310	0.305	a335	206	1.91	1.81	1.78	1.96	3225	29.87	28.41	27.95	30.70	29.64	st. dev.	4.73		
	5p	0.380	0.340	0.340	0.308	0.331	222	1.99	1.99	1.80	1.94	34.62	31.15	31.15	a 2 2	30.33	31.14	Variation Coef.	14.13	96	
4	1n	0.300	0.310	0.294	0.300	0.256	1.75	1.81	1.72	1.75	1.50	27.49	28.41	2694	27.49	23.46	2676	mean	32.95	Plot 4 mean	33.21
	2n	0.361	0.405	0.400	0.418	0.420	211	237	234	2.44	246	33.00	37.11	36.65	38.30	38.49	3673	m u	U.90		
	3n	0.371	0.349	0.382	a307	0.329	217	204	223	1.79	1.92	34.00	31.98	35.00	28.13	30.15	31.05	min	23.46		
	4n	0.313	0.332	0.335	a287	a294	1.83	1.94	1.96	1.68	1.72	28.68	30.42	30.70	26.30	26.94	28.61	st. dev.	5.58		
	5n	0.428	0.420	a490	0.439	0.449	250	246	286	257	262	39.22	38.49	44.90	40.23	41.14	40.79	Variation Coef.	1692	%	
5	1p	0.461	0.379	0.378	0.365	0.327	2.70	222	221	213	1.91	4224	34.73	34.64	3145	29.96	35.00	mean	37.06		
	2p	0.481	a390	0.520	0.445	a445	281	2.28	3.04	260	260	44.07	35.74	47.65	40.78	40.78	41.60	m u	51.31		
	3P	0.490	0.520	0.543	0.484	0.560	2.86	3.04	117	263	3.27	44.90	47.65	49.76	44.35	51.31	47.59	min	25.47		
	4P	0.442	0.316	0.298	0.278	0.317	258	1.85	1.74	1.63	1.05	40.50	28.96	27.31	25.47	29.05	30.26	st. dev.	7.70		
	5p	0.333	0.360	0.358	0.303	0.318	1.95	210	209	1.77	1.86	3a51	3299	3280	27.76	29.14	M64	Variation Coef.	20.77	%	
5	1n	0.491	a440	0.380	a436	0.502	287	2.57	222	2.55	293	44.99	40.32	34.82	39.95	46.00	41.22	mean	3233	Plot 5 mean	34.69
	2n	0.390	0.494	0.340	a362	0.387	228	289	1.99	212	226	35.74	45.27	31.15	33.17	35.46	3616	m u	46.00		
	3n	0.495	0.415	0.373	0.337	a472	289	243	218	197	216	45.36	38.03	34.18	30.88	43.25	38.34	min	13.93		
	4n	0.338	0.379	0.338	0.331	0.2%	1.98	222	1.98	1.94	1.73	30.97	34.73	M97	30.33	21.12	30.82	at. dev.	10.04		
	5n	0.175	0.156	0.171	0.170	a152	1.02	0.91	1.00	0.99	0.89	16.04	14.29	15.67	15.58	13.93	15.10	Variation Coef.	31.06	%	
B	1p	0.208	0.250	0.233	a239	a273	1.22	1.46	1.36	1.40	1.60	19.06	2291	21.35	21.90	25.02	2205	wan	28.36		
	2p	0.177	0.252	0.231	a220	a237	1.03	1.47	1.35	1.29	1.39	16.22	23.09	21.17	20.16	21.72	20.47	mu	50.95		
	3P	0.386	0.330	0.450	0.361	0.358	226	1.93	263	211	209	35.37	30.24	41.23	33.00	3260	34.55	min	16.22		
	4P	0.216	0.338	0.225	0.279	0.322	1.26	1.98	1.32	1.63	1.88	19.79	30.97	20.62	25.57	29.51	25.29	st. dev.	8.55		
	5p	0.480	0.556	0.381	0.387	0.348	281	325	223	2.26	2.03	43.98	50.95	34.91	35.46	31.89	39.U	Variation Coef.	30.14	%	
B	1n	0.212	0.159	0.226	0.214	0.238	1.24	0.93	1.32	1.25	1.39	19.43	14.57	20.71	19.61	21.81	19.22	mean	28.29	Plot B mean	28.32
	2n	0.198	0.238	0.250	0.308	0.264	1.16	1.39	1.46	1.80	154	18.14	21.81	2291	28.22	24.19	23.05	max	43.53		
	3n	0.446	0.360	0.475	0.419	0.348	261	210	2.78	245	203	40.87	3299	43.53	38.39	31.89	37.53	min	14.57		
	4n	0.310	0.312	0.280	0.300	0.327	1.81	1.82	1.64	1.75	1.91	28.41	28.59	25.66	27.49	29.96	28.02	st. dev.	7.35		
	5n	0.341	0.400	0.397	0.324	0.372	1.99	234	232	1.89	217	31.25	36.65	3638	29.69	34.09	33.61	Variation cod.	25.98	96	
Ctrl.	1p	0.384	0.376	0.371	0.294	0.320	224	220	217	1.72	1.87	35.19	34.45	34.00	26.94	29.32	31.98	wan	33.54		
	2p	0.525	a573	0.513	a597	0.488	3.07	3.35	3.00	3.49	285	4all	5251	47.01	54.70	44.72	49.41	max	54.70		
	3P	0.287	0.330	0.339	0.340	a292	1.68	1.93	1.98	1.99	1.71	2630	30.24	31.06	31.15	26.76	29.10	min	20.16		
	4P	0.342	a379	0.365	0.404	a407	2.00	222	213	236	238	31.34	34.73	3145	37.02	37.29	34.77	st. dev.	9.31		
	5p	0.239	0.220	0.254	0.264	0.248	1.40	1.29	1.48	154	1.45	21.90	20.16	23.27	24.19	22.72	2245	Variation Coef.	27.77	%	
Ctrl.	1n	0.441	a475	0.494	0.491	0.420	258	2.78	289	287	246	40.41	43.53	45.27	U.99	38.49	4254	mean	37.21	Ctrl. mean	35.38
	2n	0.533	0.560	0.540	0.540	0.568	3.12	127	116	3.16	3.32	48.84	51.31	49.48	49.48	5205	MU	max	5205		
	3n	0.392	0.367	0.400	0.337	0.351	229	215	234	1.97	205	35.92	33.63	36.65	30.88	3216	3105	min	14.66		
	4n	0.436	0.495	0.493	0.501	a490	255	289	288	293	2.86	39.95	45.36	45.17	45.91	U.90	44.26	st. dev.	1235		
	5n	0.160	0.169	0.164	0.165	aim	0.94	0.99	0.96	0.96	0.99	14.66	15.49	15.03	15.12	15.58	15.17	Variation Coef.	3119	%	
ROOTS																					
4P		nd	nd	nd	nd	nd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
4n		nd	nd	nd	nd	nd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
5p		nd	nd	nd	nd	nd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
5n		nd	nd	nd	nd	nd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
Bp		nd	nd	nd	nd	nd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
Bn		nd	nd	nd	nd	nd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
Ctrl.p		nd	nd	nd	nd	nd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
Ctrl.n		nd	nd	nd	nd	nd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				

Figure 3.


AVERAGE STARCH LEVELS IN OVERWINTERING CATTAIL RHIZOMES - DENISON (MARCH 1991)
Starch % (mg starch/mg tissue dry wt.)
40
35
30
25
20
15
10
5
0
NPK
On-Site Ctrl.
NPK + Ca
Off-Site Ctrl.
Fertilizer Treatment
Near Parent Shoot
Near New Shoot

4.4 Rhizome and Root Dry Weight Measurements

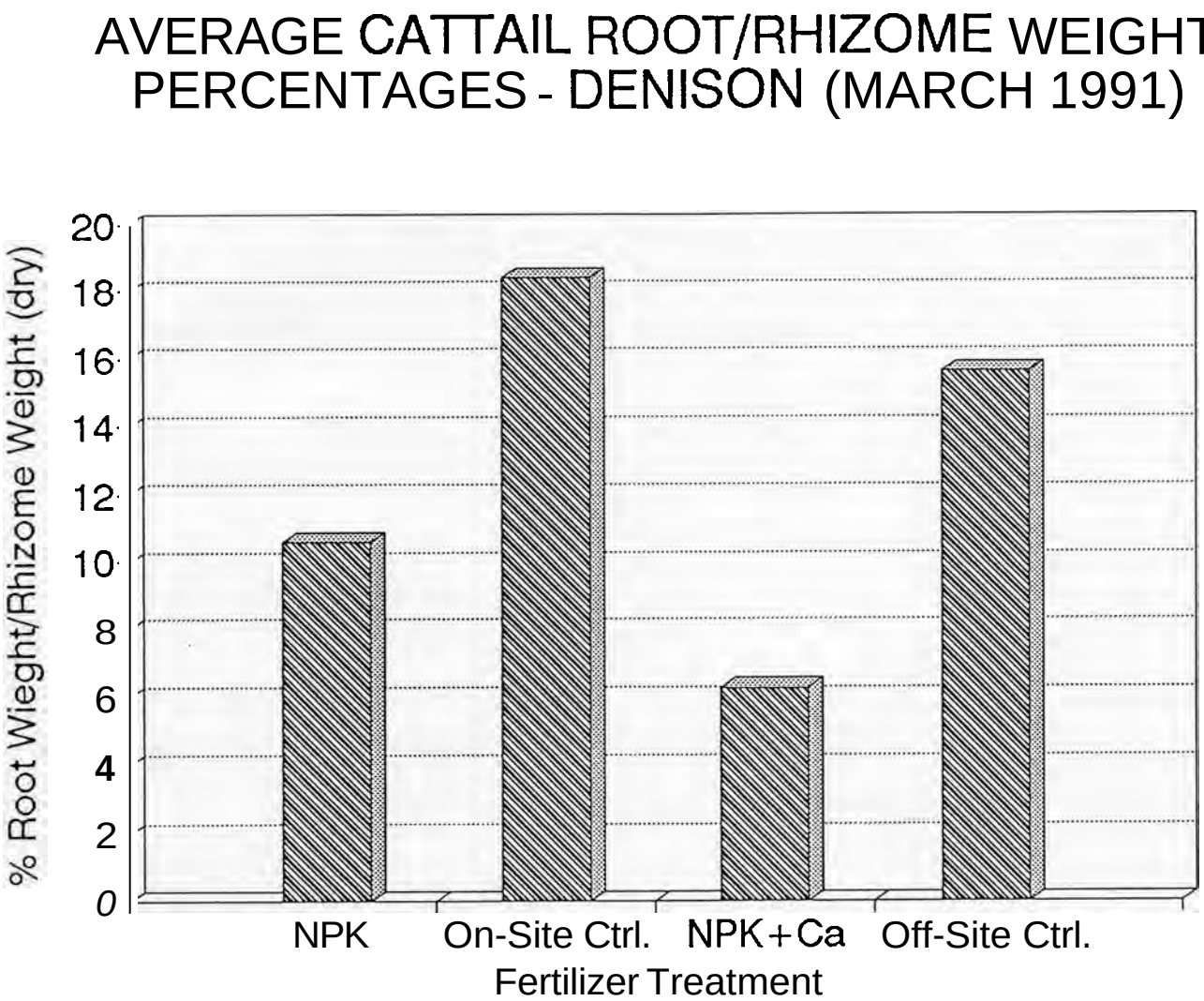
Results from the dry weight measurements of rhizome segments and associated root material are presented in Table 3. Weights are given for the weighing paper, the paper and tissue, and the tissue itself. The weights are recorded for each rhizome segment and its root material, and percentages of root weight to rhizome weight calculated. Figure 4 shows that fertilized cattails have significantly less root mass (probability ≤ 0.05) on their rhizomes than both the on and off-site controls. Root biomass differences between the treatments are significant despite the high variability reported in Table 3.

Table 3.

RHIZOME & ROOT DRY WEIGHTS (g) OF DENISON CATTAIL SAMPLES - MARCH 1991

			RHIZOMES			ROOTS					
Plot	Sample #		Wt.P	Wt.P+M	Wt.M	Wt.P	Wt.P+M	Wt.M	%Rt./Rhi.Wt	Plot Statistics	
4	1	p	0.39985	0.79207	0.39222	0.40011	0.44337	0.04326	11.03	Parent Shoot:	
		n		1.03983	0.63998		0.50148	0.10137	15.84	Mean =	4.72
	2	p		1.36216	0.96231		0.42399	0.02388	248	Variation Coef. =	*84.60* %
		n		1.57435	1.17450		0.89212	0.49201	41.89		
	3	P		1.16727	0.76742		0.41606	0.01595	2.08	New Shoot:	
		n		133582	0.93597		0.45213	0.05202	556	Mean =	16.44
	4	P		0.87232	0.47247		0.40165	0.00154	0.33	Variation Coef. =	80.07 %
		n		0.86880	0.46895		0.44935	0.04924	10.50		
	5	p		1.12967	0.72982		0.45603	0.05592	7.66	Plot 4 Mean =	10.58
		n		1.66351	1.26366		0.50625	0.10614	8.40		
5	1	p		1.09879	0.69894	0.40126	0.51885	0.11759	16.82	Parent Shoot:	
		n		1.39914	0.99929		0.51418	0.11292	11.30	Mean =	20.49
	2	p		1.06357	0.66372		0.57753	0.17627	2656	Variation Coef. =	59.96 %
		n		1.48025	1.08040		0.57492	0.17366	16.07		
	3	P		1,14136	0.74151		0.55157	0.15031	20.27	New Shoot:	
		n		1.61629	1.21644		0.51657	0.11531	9.48	Mean =	16.27
	4	P		0.65550	0.25565		0.49875	0.09749	38.13	Variation Coef. =	71.46 %
		n		0.71034	0.31049		052109	0.11983	38.59		
	5	p		0.89632	0.49647		0.40455	0.00329	0.66	Plot 5 Mean =	18.38
		n		0.79200	0.39215		0.42451	0.02325	5.93		
B	1	p		0.94050	0.54065	0.40155	0.41072	0.00917	1.70	Parent Shoot:	
		n		1.08444	0.68459		0.45241	0.05086	7.43	Mean =	4.39
	2	p		0.98167	0.58182		0.42838	0.02683	4.61	Variation Coef. =	63.51 %
		n		1.05928	0.65943		0.41270	0.01115	1.69		
	3	P		0.71352	0.31367		0.43156	0.03001	9.57	New Shoot:	
		n		0.77869	0.37884		0.41207	0.01052	278	Mean =	8.12
	4	P		0.68406	0.28421		0.41219	0.01064	3.74	Variation Coef. =	86.77 %
		n		0.80549	0.40564		0.43104	0.02949	7.27		
	5	p		0.82094	0.42109		0.41127	0.00972	231	Plot B Mean =	6.26
		n		0.90657	0.50672		0.51013	0.10858	21.43		
Ctrl.	1	p		0.79907	0.39922	0.39999	0.50385	0.10386	26.02	Parent Shoot:	
		n		1.18355	0.78370		0.87287	0.47288	60.34	Mean =	16.76
	2	p		1.15217	0.75232		0.47358	0.07359	9.78	Variation Coef. =	58.41 %
		n		1.57019	1.17034		0.72671	0.32672	27.92		
	3	P		0.76907	0.36922		0.47578	0.07579	20.53	New Shoot:	
		n		0.94599	0.54614		0.46007	0.06008	11.00	Mean =	14.45
	4	P		1.04097	0.64112		0.56820	0.16821	26.24	Variation Coef. =	141.21 %
		n		1.14965	0.74980		0.47320	0.07321	9.76		
	5	p		0.82984	0.42999		0.40535	0.00536	1.25	Ctrl. Mean =	15.61
		n		0.84432	0.44447		no mat.				

Figure **4.**


AVERAGE CATTAIL ROOT/RHIZOME WEIGHT PERCENTAGES - DENISON (MARCH 1991)
% Root Wieght/Rhizome Weight (dry)
20
18
16
14
12
10
8
6
4
2
0
NPK
On-Site Ctrl.
NPK+Ca
Off-Site Ctrl.
Fertilizer Treatment

5. DISCUSSION

This study has set out to determine whether foliar fertilization is a viable method for increasing plant biomass and biological polishing capacity in cattails growing on acidic mine tailings. Studies by Fiala (1971) and Linde et al (1976) indicated that rhizome size and biomass were good indicators of cattail productiuity. Kausch et al (1981) pointed to the fact that the pith was the major overwintering storage organ of the cattail. When combined, these studies suggest that pith size may be an important morphological indicator of cattail productivity. Furthermore, starch stored in the pith during the winter should be at a maximum and reflective of the previous growing season's productivity. These findings provided the framework for the sampling and analytical strategies utilized in this study.

Results of this investigation suggest that foliar fertilization has no significant effects on rhizome and pith size, or starch content and distribution. Comparisons of those parameters between "parent shoot" and "new shoot" portions of cattail rhizomes may, therefore, not be necessary. Despite high variability (perhaps due to very localized growing conditions or genetic inheritance), the differences in root biomass between fertilized and unfertilized plots is significant. This finding indicates that, perhaps, root growth is stimulated by stress, such as the nutrient-limited conditions of AMD (Fyson et al, 1991). As roots comprise less than 10% of total plant dry weight in cattails however, it is unlikely that fertilization has an effect on productivity (Hogg & Wein, 1987).

Despite the lack of effectsthat foliar fertilization has on cattail pith size and rhizome starch content, there exists another, yet more tedious, method for determining plant productivity. A total above-and-below-ground biomass count can be made in the various experimental sites by marking prescribed plots, removing all cattail material both above and below-ground, and obtaining dry-weight estimations for comparison. Based on the studies of Fiala (1971) and Linde et al (1976), this method may provide more detail on the effects of foliar fertilization.

In conclusion, this study does point to an important observation. It reinforces the idea that cattails are tolerant of harsh conditions and can grow effectively on acidic mine tailings. With findings from Kalin (1984, 1986) and others (Lakshman, 1987; Kadlec, 1987; Watson et al, 1987) that suggest these emergent macrophytes possess the ability to treat wastewater, a concerted effort must be made to further research this capability so that areas suffering from aqueous contamination can be restored to a natural state.

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RHIZOME DIMENSIONS

Plot/ Cattail #	Rhizome Length(cm)	Section #	Pith Diameter (mm) X Axis	Pith Diameter (mm) Y Axis	Average Diam.(mm)	Rhizome Diam.(mm) X Axis	Rhizome Diam.(mm) Y Axis	Average Diam.(m	% Rhi. Diam. as Pith	Average % for Plot
4-1	18.0	B	5.8	60	59	aS	10.6	9.6	61.8	
4-2	19.5	B	a2	9.0	8.6	11.5	14.3	12.9	66.7	
4-3	21.0	B	8.7	7.6	a2	14.3	127	13.5	6a4	
4-4	3L3	B	5.9	63	61	12.5	129	127	48.0	
4-5	48.5	B	7.5	7.2	7.4	13.8	126	13.2	55.7	58.5
4-1	18.0	C	8.1	a4	8.3	11.5	120	11.8	70.2	(nuParent Shoot)
4-2	19.5	C	11.5	12.0	11.8	16.1	17.1	166	70.8	
4-3	21.0	C	9.7	9.7	9.7	122	14.5	13.4	R 7	
4-4	31.3	C	6.3	66	6.5	10.4	120	11.2	57.6	
4-5	48.5	C	10.3	10.7	lo5	15.7	16.3	16.0	65.6	67.4
5-1	29.0	B	7.1	79	7.5	13.8	11.0	124	60.5	(near New Shoot)
5-2	4Ls	B	6.8	68	6.8	123	14.0	13.2	51.7	
5-3	25.0	B	8.0	7.5	7.8	125	120	123	63.3	
5-4	19.0	B	4.6	43	4.5	7.1	7.0	7.1	63.1	
5-5	15.5	B	60	6.3	6.2	10.7	9.5	10.1	60.9	59.9
5-1	29.0	C	99	9.2	9.6	14.4	13.0	13.7	69.7	(near Putat Shoot)
5-2	41.5	C	95	9.0	9.3	14.7	14.1	14.4	64.2	
5-3	25.0	C	10.4	10.7	10.6	14.3	14.3	14.3	73.8	
5-4	19.0	C	5.6	5.2	5.4	a3	7.1	7.7	70.1	
5-5	15.5	C	60	6.2	6.1	9.8	10.5	10.2	60.1	67.6
B-1	22.5	B	7.4	7.0	7.2	13.2	13.0	13.1	55.0	(near New Shoot)
B-2	24.5	B	6.9	7.0	7.0	123	13.3	128	54.3	
B-3	10.0	B	6.8	6.6	6.7	10.9	11.8	11.4	59.0	
B-4	11.5	B	5.1	4.9	5.0	9.3	8.7	9.0	55.6	
B-5	12.0	B	4.7	5.0	4.9	7.4	7.9	7.7	63.4	57.4
B-1	22.5	C	8.6	8.3	8.5	14.3	120	13.2	64.3	(nuPutat Shoot)
B-2	24.5	C	9.0	9.4	9.2	13.2	14.5	13.9	66.4	
B-3	10.0	C	8.6	75	8.1	12.1	11.8	120	67.4	
B-4	11.5	C	7.9	7.7	7.8	11.5	10.1	10.8	72.2	
B-5	120	C	7.3	7.5	7.4	10.1	11.7	10.9	67.9	67.6
Ctrl-1	38.1	B	6.8	6.1	6.5	139	11.0	125	51.8	(near New Shoot)
Ctrl-2	36.0	B	10.8	9.3	10.1	17.8	126	15.2	66.1	
Ctrl-3	30.0	B	S6	5.7	5.7	9.3	11.7	10.5	53.8	
Ctrl-4	28.5	B	8.0	6.9	7.5	13.8	124	13.1	S6.9	
Ctrl-5	11.5	B	79	7.5	7.7	13.5	11.8	127	609	57.9
Ctrl-1	38.1	C	h6	8.9	8.8	13.0	14.0	13.5	64.8	(near Parent Shoot)
Ctrl-2	36.0	C	10.5	8.5	95	18.7	11.5	15.1	62.9	
Ctrl-3	30.0	C	Section too damaged for measuring							
Ctrl-4	28.5	C	8.2	8.1	8.2	13.4	13.4	13.4	60.8	62.8
Ctrl-5	11.5	C	Section too damaged for measuring							(near New Shoot)

Rhizomes						Roots					
Plot			Wt.P	Wt.P+M	Wt.M	Wt.P	Wt.P+M	Wt.M	%Rt./Rhi	Avg. p %	Avg. n %
4	1	p	0.39985	0.79207	0.39222	0.40011	0.44337	0.04326	11.03	11.03	15.84
		n		1.03983	0.63998		0.50148	0.10137	15.84	2.48	41.8%
	2	p		1.36216	0.96231		0.42399	0.02388	2.48	2.08	5.56
		n		1.57435	1.17450		0.89212	0.49201	41.89	033	10.5
	3	P		1.16727	0.76742		0.41606	0.01595	2.08	7.66	8.4
		n		1.33582	0.93597		0.45213	0.05202	5.56		
	4	P		0.87232	0.47247		0.40165	0.00154	0.33	4.716	16.438
		n		0.86880	0.46895		0.44935	0.04924	10.50		
	5	p		1.12967	0.72982		0.45603	0.05592	7.66		
		n		1.66351	1.26366		0.50625	0.10614	8.40		
5	1	p		1.09879	0.69894	0.40126	0.51885	01 1759	16.82	16.82	11.3
		n		1.39914	0.99929		0.51418	0.11292	11.30	26.56	16.07
	2	p		1.06357	0.66372		0.57753	0.17627	26.56	20.27	9.48
		n		1.48025	1.08040		0.57492	0.17366	16.07	38.13	38.59
	3	P		1.14136	0.74151		0.55157	0.15031	20.27	0.66	5.93
		n		1.61629	1.21644		0.51657	0.11531	9.48		
	4	p		0.65550	0.25565		0.49875	0.09749	38.13	20.488	16.274
		n		0.71034	031049		0.52109	0.11983	38.59		
	5	p		0.89632	0.49647		0.40455	0.00329	0.66		
		n		0.79200	0.39215		0.42451	0.02325	5.93		
B	1	p		0.94050	0.54065	0.40155	0.41072	0.00917	1.70	1.7	7.43
		n		1.08444	0.68459		0.45241	0.05086	7.43	4.61	1.69
	2	p		0.98167	0.58182		0.42838	0.02683	4.61	9.57	2.78
		n		1.05928	0.65943		0.41270	0.01115	1.69	3.74	7.27
	3	P		0.71352	0.31367		0.43156	0.03001			
		n		0.77869	0.37884		0.41207	0.01052			
	4	P		0.68406	0.28421		0.41219	0.01064			
		n		0.80549	0.40564		0.43104	0.02949			
	5	p		0.82094	0.42109		0.41127	0.00972			
		n		0.90657	0.50672		0.51013	0.10858			
Ctrl.	1	p		0.79907	0.39922	0.39999	0.50385	0.10386			
		n		1.18355	0.78370		0.87287	0.47288			
	2	p		1.15217	0.75232		0.47358	0.07359			
		n		1.57019	1.17034		0.72671	0.32672			
	3	P		0.76907	0.36922		0.47578	0.07579	20.53	1.25	9.76
		n		0.94599	0.54614		0.46007	0.06008	11.00		
	4	P		1.04097	0.64112		0.56820	0.16821	26.24	16.764	14.45
		n		1.14965	0.74980		0.47320	0.07321	9.76		
	5	p		0.82984	0.42999		0.40535	0.00536	1.25		
		n		0.84432	0.44447		no mat.				