

**ECOLOGICAL ENGINEERING**  
**TESTS OF CONCEPTS AND ASSUMPTIONS ON LEVACK**  
**PHASE III: BIOLOGICAL SEEPAGE TREATMENTS**

**BY**  
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**IN FULFILLMENT OF INCO CONTRACT # A6073**  
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FINAL REPORT  
ECOLOGICAL ENGINEERING: PHASE III

BIOLOGICAL SEEPAGE TREATMENTS

1. OVERVIEW OF OBJECTIVES

On completion of Phase II, we had identified three tasks to be completed in the field on the Levack site as Phase III. These were:

1. More detailed investigation of pre-bog acid creek;
2. Reducing conditions in seepage test cells;
3. Evaluation of alkaline cattail transplant methods.

2. SUMMARY OF WORK COMPLETED

Due to cracks developing in the mine slimes which had accumulated behind the Boomerang dams, we evaluated the thickness of the slimes and carried out preliminary tests on wetland seed sources. We measured the depth of the mine slimes and concluded that at least 1 m of slime is required to prevent crack development to the bottom of the pyrrhotite. We tested two types of wetland sediments, one more peaty material and one from a cattail stand. The material from the cattail stand showed germinating plants which appeared to be grasses and cattails.

Until the results of Daryl's seeding have been evaluated in 1988 and further mine slime cover has been placed, no more work is warranted at this time.

### **Task 3: Alkaline cattail transplant**

Basically, all transplanted cattails overwintered and continued to grow in 1987. It appears that in all three experimental areas, the cattail populations at least doubled. In Table 1, the results of the number of cattails growing after one year, are presented for each area and a schematic outlines the experimental areas. The results indicate that successful methods have been developed for transplanting cattails into extreme alkaline areas.

### **Task 2: Reducing conditions in seepage creek**

We placed organic material into the test cells at the beginning of the summer. However, the summer was very dry and the test cells dried out. It is obvious that without water no reducing conditions will develop.

### **Task 1: Pre-bog acid creek conditions**

This was the most important part of the work, since the trends noted in 1986 suggested that we may indeed have the beginning of a biological acid mine drainage treatment system.

We amended the cells again in 1987 with additional organic material as outlined in Schematic 2. We monitored the water characteristics twice throughout the year (Table 2), and measured pH and conductivity as frequently as possible (Table 3). In general, it can be noted that the upper range of pH in 1987 was slightly higher than in the previous year, and increases in the electrical conductivities were also noted.

These data, total metal analysis, pH and conductivity ranges, do not appropriately reflect the processes which are occurring. Frequently, we measured pH as high as 5 and 6 in the organic material directly after insertion. Such a measurement can only be maintained for a short period of time, since the insertion of the pH probe and the disturbance by the investigator's hand produced mixing and the pH drops again to 2.5 or 3.

The metals analysis in total concentrations is also an inappropriate indicator for two reasons. If the metals are reduced and precipitated in the pre-bog acid creek test cells, the concentrations would not necessarily be lower, since no control is exercised on input from the sides and the origin of the creek. Secondly, if they are in a reduced form as metal sulphides, the total analysis would not indicate the difference. Although the sample (B4) collected after the straw treatment in April has generally lower concentrations of some elements (Fe, Mg, Mn, P, Ni and Al) compared to the previous year's range, this

can hardly be taken as a result of the treatment. That some water improvement has to occur can be taken from the solids analysis given in Table 4, as the percentages of iron, aluminium and sulphur are rather high. However, this would be passive adsorption and thus a finite treatment system. The organic material is to produce the food source for the microbiological populations which in turn are to produce reducing conditions in the acidic waters.

Reducing bacteria of the group Desulphovibrio are the key components of such a biological treatment system, in addition to an organic matter supply to the reducing layer in the form of cattail leaves. Therefore, efforts have been extended in two directions: (1) to determine the presence/absence of reducing bacteria, and (2) to establish cattails in the treatment cells.

These microbiological investigations carried out in samples of the solid material (overburden, straw, peat and sawdust) and mixed liquid/solid samples collected one month after addition of the 1987 amendments, produced very positive results. In essence, since the initiation of the experiments, where the material contained only one type of bacteria, after one year the number of bacteria have increased, as well as the diversity of species encountered. In comparison to samples taken from other locations, such as piezometers in pyrrhotite/pyrite tailings, the population density is very high. For further details, see report by CANMET enclosed in the Appendix.

Reducing bacteria continue to be present after the second year of organic matter placement. From the literature, it appears that Japanese workers (Wakao, et al, 1979) have proposed similar microbiological approaches to the treatment of acid mine drainage. The paper is enclosed in the Appendix. The group has been contacted by mail to enquire about their progress since publication of this work.

The transplanting of cattails into these acidic conditions had to be approached from a broader point of view. Success of the growth of the transplants is ephemeral. The transplant methods are clearly inappropriate, as cattail stands exist naturally having been established in pre-bog acid creek conditions, and their continued growth in very acidic conditions in tailings in the Elliot Lake area, has been studied and documented. A different approach was therefore taken, namely the comparative study of death and successful growth in acidic conditions. The observations on the root/rhizome system from a morphological viewpoint are summarized in the enclosed research plan.

In conclusion, the work on pre-bog acid creek, both in relation to producing conditions for reducing microbial populations and for organic matter production has progressed as far as is possible in the present framework. A joint research program has been developed with Denison Mines, Inco Sudbury operations, and CANMET. The outline of the overall approach and the details for Phase 1 are enclosed in the Appendix.

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2. Metal concentrations in pre-bog acid creek cells
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4. Analysis of solid material placed in treatment cells

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1. Cattail transplant areas
2. Pre-bog acid creek treatment cells

## APPENDIX

- A. Joint research program: **BOOJUM-CANMET-INCO-DENISON**
- B. CANMET report: Microbiological analysis of tailings samples provided by Boojum Research
- C. Copy of paper by Wakao, 1979: A treatment of Acid mine water using sulphate reducing bacteria. J. Ferment. Technol. Vol. 57 p 445-452

TABLE 1.  
LEVACK MINE SITE - 1987 RECOUNT OF 1986 CATTAIL TRANSPLANTS

| LOCATION          | JULY/86<br>COUNT | JULY/87<br>COUNT | %<br>INCREASE |
|-------------------|------------------|------------------|---------------|
| AREA 1 - stake 13 |                  |                  |               |
| A                 | 2                | 9                | 350           |
| B                 | 3                | 12               | 300           |
| C                 | 1                | 7                | 600           |
| D                 | 3                | 14               | 367           |
| E                 | 3                | 7                | 133           |
| stake 14          |                  |                  |               |
| A                 | 3                | 7                | 133           |
| B                 | 3                | 6                | 100           |
| stake 15          |                  |                  |               |
| A                 | 4                | A+B = 18         | 157           |
| B                 | 3                | -                | -             |
| C                 | 4                | 9                | 125           |
| D                 | 3                | 20               | 567           |
| E                 | 3                | 15               | 400           |
| F                 | 3                | 11               | 267           |
| AREA 2 - stake 10 |                  |                  |               |
| A                 | 5                | 9                | 80            |
| B                 | 4                | 10               | 150           |
| C                 | 4                | 13               | 225           |
| D                 | 3                | 22               | 633           |
| E                 | 2                | 7                | 250           |
| F                 | 3                | 8                | 167           |
| G                 | 2                | 8                | 300           |
| H                 | 2                | 10               | 400           |
| I                 | 2                | 5                | 150           |
| J                 | 2                | 3                | 50            |
| stake 11          |                  |                  |               |
| A                 | 4                | 7                | 75            |
| B                 | 4                | 9                | 125           |
| C                 | 4                | 13               | 225           |
| D                 | 3                | 10               | 233           |
| E                 | 4                | 5                | 25            |
| F                 | 2                | 5                | 150           |
| G                 | 3                | 9                | 200           |
| H                 | 2                | 4                | 100           |
| I                 | 2                | 6                | 200           |
| J                 | 2                | 4                | 100           |
| stake 12          |                  |                  |               |
| A                 | 4                | 7                | 75            |
| B                 | 3                | 5                | 67            |
| C                 | 5                | 10               | 10            |
| D                 | 2                | 10               | 400           |
| E                 | 3                | 5                | 67            |
| F                 | 2                | 3                | 50            |
| G                 | 2                | 3                | 50            |
| H                 | 2                | 10               | 400           |
| I                 | 3                | 6                | 100           |
| J                 | 2                | 5                | 150           |

TABLE 1 (cont'd).

LEVACK MINE SITE - 1987 RECOUNT OF 1986 CATTAIL TRANSPLANTS

| LOCATION | JULY/86<br>COUNT | JULY/87<br>COUNT | %<br>INCREASE |
|----------|------------------|------------------|---------------|
| AREA 3   |                  |                  |               |
| group 1  | 15               | 27               | 80            |
| group 2  |                  |                  |               |
| A        | 4                | 6                | 50            |
| B        | 3                | 4                | 33            |
| C        | 2                | 4                | 100           |
| D        | 2                | 5                | 150           |
| E        | 3                | 6                | 100           |
| group 3  |                  |                  |               |
| A        | 3                | 9                | 200           |
| B        | 1                | 2                | 100           |
| C        | 2                | 4                | 100           |
| D        | 2                | 4                | 100           |
| E        | 2                | 4                | 100           |
| group 4  |                  |                  |               |
| A        | 2                | 6                | 200           |
| B        | 3                | 3                | 0             |
| C        | 3                | 7                | 133           |
| D        | 2                | 3                | 50            |
| E        | 3                | 9                | 20            |
| group 5  |                  |                  |               |
| A        | 4                | 9                | 125           |
| B        | 2                | 7                | 250           |
| C        | 3                | 7                | 133           |
| D        | 3                | 9                | 200           |
| E        | 3                | 11               | 267           |
| group 6  |                  |                  |               |
| A        | 2                | na               | na            |
| B        | 3                | na               | na            |
| group 7  |                  |                  |               |
| A        | 2                | 13               | 550           |
| B        | 5                | 8                | 60            |
| C        | 1                | 11               | 1000          |
| D        | 3                | 16               | 433           |
| E        | 3                | 10               | 233           |
| group 8  |                  |                  |               |
| A        | 4                | 10               | 150           |
| B        | 3                | na               | na            |

Table 2.  
ELEMENTAL CONCENTRATIONS IN WATER OF PRE-BOG ACID CREEK AMENDMENT CELLS

| CELL | A0      |        |         |        |         |        |         |        |         |      | A2      |  |         |  |           |  |         |        |         |        | A3      |       |         |  |         |         |         |        |         |  |
|------|---------|--------|---------|--------|---------|--------|---------|--------|---------|------|---------|--|---------|--|-----------|--|---------|--------|---------|--------|---------|-------|---------|--|---------|---------|---------|--------|---------|--|
|      | 6/22/86 |        | 7/10/86 |        | 7/29/86 |        | 9/05/86 |        | 3/25/87 |      | 4/20/87 |  | 8/18/87 |  | 18/18/87* |  | 6/22/86 |        | 7/10/86 |        | 7/29/86 |       | 9/05/86 |  | 3/25/87 |         | 4/20/87 |        | 8/18/87 |  |
| Al   | 4.84    | 11.8   | 9.62    | 8.47   | 18.40   | 9.11   | 5.48    | 8.77   | 11.7    | 88.2 |         |  |         |  |           |  |         | 10.30  | 15.40   | 26.00  | 15.90   |       |         |  |         | 2.44    | 8.77    | 6      |         |  |
| Ca   | 461     | 393    | 408.00  | 426.00 | 142.00  | 366.00 | 36.2    | 250    | 404     |      |         |  |         |  |           |  |         | 341.00 | 496.00  | 158.00 | 302.00  |       |         |  |         | 120     | 250     | 330    |         |  |
| Cd   | 0.083   | <0.042 | 0.17    | 0.12   | 0.30    | 0.11   | 0.04    | <0.026 | <0.052  |      |         |  |         |  |           |  |         | 0.10   | 0.43    | 0.05   | 0.12    |       |         |  |         | 0.04    | <.0260  | <0.052 |         |  |
| Co   | 0.097   | 0.129  | 0.14    | 0.11   | 0.24    | 0.49   | 0.06    | 0.23   | <0.116  |      |         |  |         |  |           |  |         | 0.15   | 0.22    | 0.32   | 0.38    |       |         |  |         | <0.0580 | 0.23    | <0.116 |         |  |
| Cu   | 0.086   | 0.455  | 0.53    | 0.60   | 2.33    | 1.27   | 0.66    | 0.803  | 2.18    |      |         |  |         |  |           |  |         | 0.51   | 0.71    | 3.22   | 1.84    |       |         |  |         | 0.38    | 0.8     | 1.01   |         |  |
| Fe   | 1300    | 1659   | 1511    | 1801   | 915     | 1980   | 115     | 1665   | 1990    |      |         |  |         |  |           |  |         | 1194   | 1690    | 1040   | 1500    |       |         |  |         | 324     | 1665    | 1448   |         |  |
| Mg   | 85.5    | 147    | 152.00  | 182.00 | 47.30   | 195.00 | 8.13    | 97.2   | 215     |      |         |  |         |  |           |  |         | 108.00 | 162.00  | 51.40  | 122.00  |       |         |  |         | 24.7    | 97.2    | 103    |         |  |
| Mn   | 5.3     | 8.01   | 8.08    | 9.16   | 2.53    | 10.30  | 0.63    | 5.96   | 10.7    |      |         |  |         |  |           |  |         | 14.1   | 9.04    | 2.77   | 7.21    |       |         |  |         | 1.71    | 5.96    | 6.45   |         |  |
| Na   | 44.9    | 5.9    | 8.63    | 6.69   | 4.37    | 6.65   | 1.37    | 11.2   | 9.98    |      |         |  |         |  |           |  |         | 51.6   | 28.30   | 10.50  | 3.94    | 6.18  |         |  |         | 11.9    | 11.2    | 14.5   |         |  |
| Ni   | 9.79    | 19.2   | 23.30   | 18.40  | 13.50   | 41.50  | 3.62    | 27.7   | 6.01    |      |         |  |         |  |           |  |         | 7.6    | 19.10   | 24.00  | 17.50   | 27.40 |         |  |         | 4.26    | 27.7    | 9.17   |         |  |
| P    | 0.552   | <0.259 | <0.259  | <0.496 | 0.36    | 0.30   | <0.259  | <0.259 | 0.932   |      |         |  |         |  |           |  |         | 8.87   | <0.259  | <0.496 | 0.49    | 0.21  |         |  |         | <.259   | <.259   | <0.518 |         |  |
| Zn   | 0.308   | 0.571  | 0.94    | 0.62   | 1.06    | 1.90   | <0.0470 | 1.64   | 0.866   |      |         |  |         |  |           |  |         | 1.18   | 0.61    | 0.89   | 0.27    | 1.07  |         |  |         | <.0470  | 1.64    | 0.282  |         |  |

Table 2 (cont'd).  
ELEMENTAL CONCENTRATIONS IN WATER OF PRE-BOG ACID CREEK AMENDMENT CELLS

| CELL | B1      |         |         |         | B2      |         |         |         | B3*     |         |         |         |         |         |
|------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| mg/L | 6/22/86 | 7/10/86 | 7/29/86 | 9/05/86 | 3/25/87 | 4/20/87 | 8/18/87 | 6/22/86 | 7/10/86 | 7/29/86 | 9/05/86 | 4/20/87 | 8/18/87 | 8/18/87 |
| Al   | 18.50   | 16.80   | 118.0   | 16.10   | 19.5    | 12.6    | 8.52    | 18.70   | 16.90   | 118.00  | 17.70   | 12.1    | 13.9    | 13.9    |
| Ca   | 503.0   | 552.00  | 443.0   | 466.00  | 284     | 353     | 300     | 523.0   | 560.00  | 463.00  | 443.00  | 367     | 422     | 480     |
| Cd   | 0.07    | 1.53    | 0.05    | 0.12    | <0.026  | <0.026  | 0.26    | 0.10    | 0.35    | <0.026  | 0.13    | <0.026  | <0.052  | <0.052  |
| Co   | 0.20    | 0.15    | 0.88    | 0.21    | 0.12    | 0.19    | <0.116  | 0.20    | 0.16    | 0.94    | 0.25    | 0.182   | <0.116  | 0.178   |
| Cu   | 0.43    | 0.27    | 8.21    | 0.45    | 1.52    | 0.4     | 0.684   | 0.41    | 0.48    | 8.76    | 0.72    | 0.442   | 1.24    | 1.12    |
| Fe   | 1628    | 2018    | 2763    | 2210    | 1050    | 1847    | 1772    | 1797    | 1893    | 2820    | 2150    | 1685    | 2184    | 2254    |
| Mg   | 103.0   | 130.00  | 206.0   | 111.00  | 75.2    | 116     | 112     | 125.0   | 137.00  | 219.00  | 139.00  | 112     | 139     | 206     |
| Mn   | 6.17    | 7.78    | 11.10   | 7.45    | 5.01    | 7.09    | 7.79    | 7.43    | 8.05    | 12.00   | 8.63    | 7.09    | 9.1     | 10.2    |
| Na   | 46.70   | 51.20   | 27.90   | 45.20   | 31      | 38.3    | 47      | 47.20   | 49.00   | 26.50   | 36.00   | 47.7    | 63.4    | 64.6    |
| Ni   | 13.90   | 14.50   | 53.10   | 14.10   | 10.8    | 24      | 6.15    | 16.70   | 13.90   | 56.60   | 16.80   | 22.2    | 7.1     | 6.28    |
| P    | 0.30    | <0.496  | 2.80    | 0.28    | <0.259  | 0.3     | 0.55    | 0.68    | <0.496  | 2.67    | 0.30    | <0.259  | 0.838   | 0.81    |
| Zn   | 0.20    | 0.40    | 1.35    | 0.55    | 0.22    | 2.05    | 2.78    | 0.25    | 0.28    | 1.60    | 0.70    | 0.736   | 0.504   | 1.41    |

# ELEMENTAL CONCENTRATIONS IN WATER OF PRE-BOG ACID CREEK AMENDMENT' CELLS

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Table 3.  
 Prebog Acid Creek pH and Conductivity Ranges for 1986 and 1987

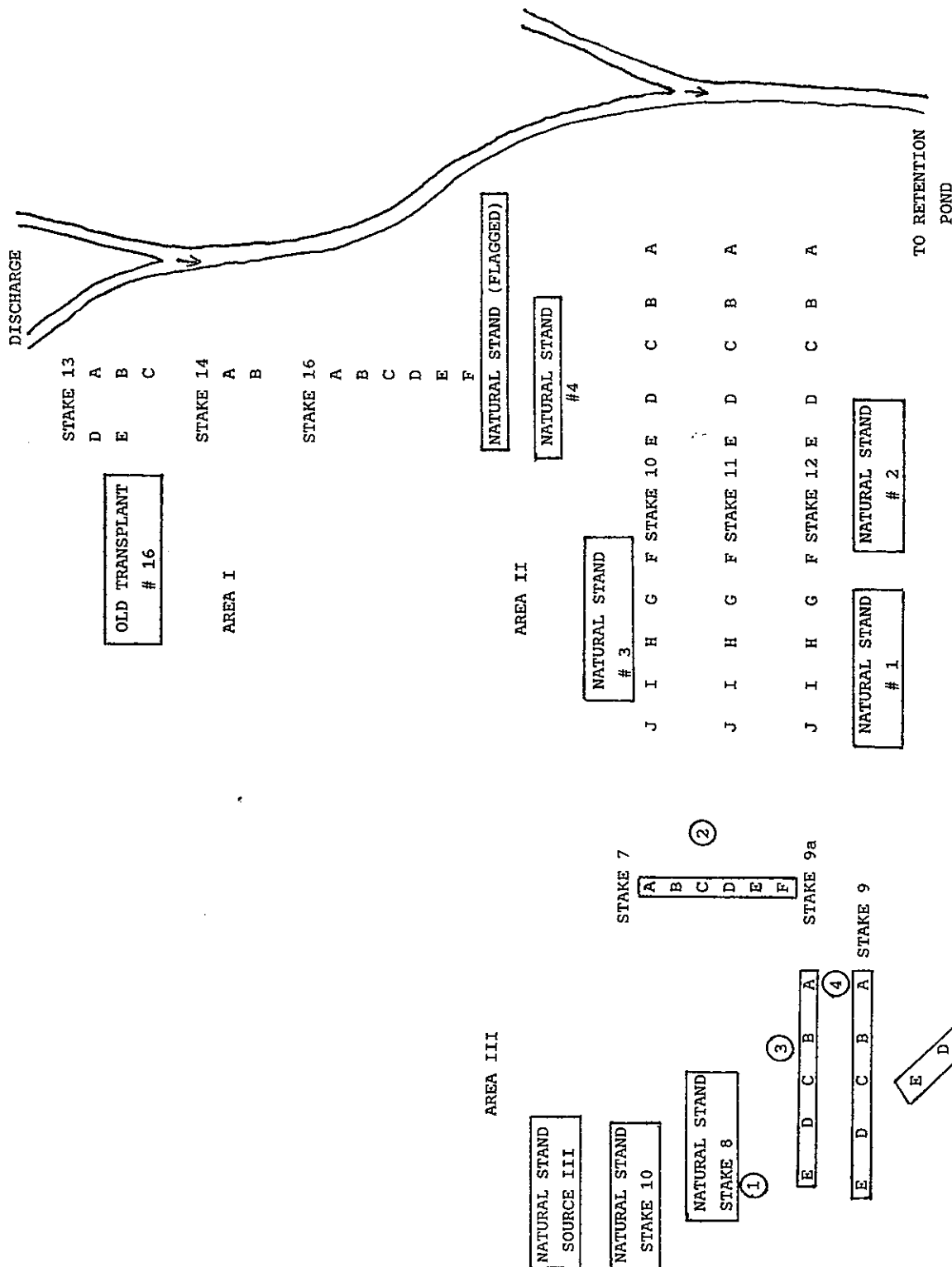
| Cell         | A0        | 1986      | 1987      | A3        | 1986      | 1987      | B1        | 1986      | 1987      | B2        | 1986      | 1987      | B4      | 1986    | 1987    | C1       | 1986 | 1987 | C4 | 1986 | 1987 |
|--------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|---------|---------|---------|----------|------|------|----|------|------|
| pH Range     | 2.0-2.9   | 2.5-3.7   | 2.4-3.0   | 2.5-3.01  | 2.6-3.2   | 2.5-3.57  | 2.6-3.2   | 2.5-3.57  | 2.6-3.2   | 2.02-5.01 | 2.5-2.8   | 2.2-2.6   | 2.5-2.7 | 2.5-3.8 | 2.2-2.7 | 2.56-2.7 |      |      |    |      |      |
| Conductivity |           |           |           |           |           |           |           |           |           |           |           |           |         |         |         |          |      |      |    |      |      |
| Range        | 2300-5600 | 4800-6200 | 2500-6000 | 6000-8600 | 3600-8000 | 4700-7000 | 4300-8000 | 3600-6000 | 4500-4900 | 4800-8050 | 4200-8900 | 3750-7500 | 4900    |         |         |          |      |      |    |      |      |

Table 4.

Elemental Concentrations in Solids of Pre-bog Acid Creek, May 20, 1987

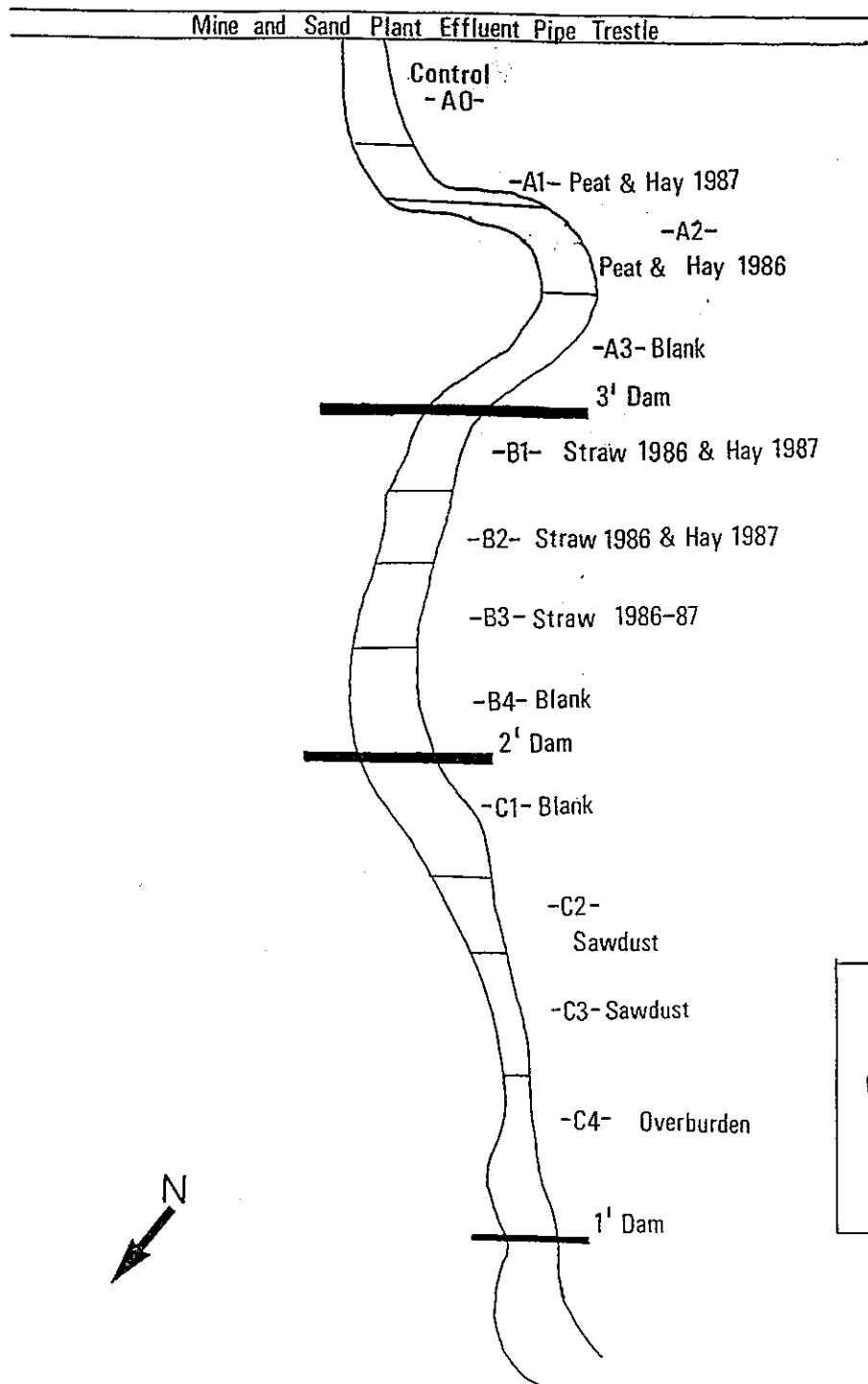
| Element (%) | Inco Peat | Inco Sawdust | Inco Straw |
|-------------|-----------|--------------|------------|
| Ag          | <.001     | <.001        | <.001      |
| As          | 0.01      | 0.01         | 0.01       |
| B           | 0.02      | 0.07         | 0.04       |
| Ba          | 0.006     | 0.01         | 0.005      |
| Be          | <.001     | <.001        | <.001      |
| Bi          | <.001     | <.001        | <.001      |
| Ce          | 0.001     | 0.002        | 0.003      |
| Cr          | 0.008     | 0.007        | 0.007      |
| Hg          | <.01      | <.01         | <.01       |
| La          | 0.002     | 0.003        | 0.002      |
| Mo          | 0.01      | <.001        | 0.01       |
| Nb          | 0.001     | 0.004        | 0.004      |
| Pb          | 0.01      | 0.01         | 0.01       |
| S           | 1.8       | 6.4          | 4.4        |
| Sb          | <.001     | 0.06         | 0.02       |
| Se          | <.001     | 0.05         | 0.02       |
| Sn          | 0.007     | <.001        | 0.01       |
| Sr          | 0.007     | 0.01         | 0.005      |
| Te          | 0.01      | 0.01         | 0.01       |
| Th          | 0.006     | 0.02         | 0.01       |
| U           | 0.02      | 0.05         | 0.03       |
| V           | 0.002     | 0.005        | 0.002      |
| W           | 0.005     | <.001        | <.001      |
| Y           | <.001     | <.001        | <.001      |
| Zr          | 0.01      | 0.04         | 0.01       |
| K2O         | 0.6       | 3.3          | 2.1        |
| TiO2        | 0.06      | 0.1          | 0.04       |

| Element (%) | Inco Peat | Inco Sawdust | Inco Straw |
|-------------|-----------|--------------|------------|
| Al2O3       | 1.2       | 2.7          | 0.7        |
| CaO         | 1.2       | 1.6          | 0.7        |
| Cd          | 0.001     | 0.002        | 0.001      |
| Co          | 0.001     | 0.006        | 0.004      |
| Cu          | 0.02      | 0.08         | 0.006      |
| Fe2O3       | 10.9      | 36           | 27         |
| MgO         | 0.6       | 0.8          | 0.4        |
| MnO         | 0.03      | 0.05         | 0.03       |
| Na2O        | 0.4       | 1.1          | 0.2        |
| Ni          | 0.03      | 0.1          | 0.01       |
| P2O5        | 0.1       | 0.1          | 0.2        |
| Zn          | 0.01      | 0.04         | 0.01       |



SCHEMATIC 1: CATTAIL TRANSPLANT AREAS ON THE LEVACK SITE. LETTERS REFER TO SPECIFIC BUNCHES OF CATTAILS. CIRCLED NUMBERS ARE GROUP NUMBERS LISTED IN TABLE FOR THIS AREA.

# PREBOG ACID CREEK



| LEGEND                      |                |
|-----------------------------|----------------|
| XO-                         | Cell           |
| Organic Material- Treatment |                |
| —                           | - Cell Curtain |
| —                           | - Manmade Dam  |

## APPENDIX A

**DEVELOPMENT OF METHODS TO ESTABLISH REDUCING CONDITIONS IN  
ACIDIC WATERS LEADING TO SELF-MAINTAINING TREATMENT METHODS FOR  
SEEPAGES AND ACIDIC WATER ON THE SURFACE OF WASTE MANAGEMENT  
AREAS**

**BACKGROUND**

The experiments carried out over the last 3 years by Boojum Research, with the support of Canmet (RATS), Inco, BP-Selco, Kidd Creek and Denison, have finally led to results which indicate that not only are the concepts of Ecological Engineering valid, but that the experimental foundation is at hand to proceed with a focused research program to develop the treatment process.

The program needs the joint effort of Canmet's expertise (Microbiology), and Boojum Research's experience and data acquired to date, and not least importantly, a commitment from Inco and Denison to provide their continued support with respect to maintaining the experimental sites. Only in this manner can we proceed with further work based on the results obtained in a stepwise program.

Step 1: Investigate Cattail roots as per proposal Appendix 1.

**Proposed supporter:** CANMET, Biotechnology

**Estimated costs:** \$20,000.00

Step 2: Set up swimming pool size experimental cells in the field to establish the same pre-bog acid creek conditions, but allow flow control, and water quality measurements (Levack)

**Proposed supporter:** Inco, Sudbury operations.

**Estimated costs:** \$35,000.00

Step 3: Implement acid cattail transplant experiments on Stanrock and in existing pre-bog acid creek (Levack).

**Proposed supporter:** Denison Mines

Estimated costs: \$25,000.00

Step 4: Microbiological processes in existing (running since 1985), pre-bog acid creek and Stanrock bog (established in 1987), and swimming pools (to be established in 1988).

Proposed supporter: CANMET, Denison and Inco

Estimated costs: \$120,000.00

### Proposed Schedule of project

| Task                               | Nov 1987 to March 1988 | April 88 to Oct 88 | Nov to Nov89 |
|------------------------------------|------------------------|--------------------|--------------|
| 1. Develop a detailed project plan |                        |                    |              |
| 2. Obtain necessary resources      |                        |                    |              |
| 3. Implement project plan          |                        |                    |              |
| 4. Monitor project progress        |                        |                    |              |
| 5. Report project status           |                        |                    |              |
| 6. Close project                   |                        |                    |              |

Step 1 XXXXXXXXXXXXXXXXXXXXXXXX

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Step 2                                XXXXXXXXXXXXXXXX
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Step 3 XXXXXXXXXXXXXXXXXXXXXXXXXXXX

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Step 4      XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
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After Step 4, we anticipate having sufficient information to design a pilot field testing program.

ORGANIZATION:

Boojum would require two new technicians, Position 1, in Microbiology, working in CANMET under Dr. Ron McCready, and one field technician to set up the experiments for Steps 2 and 3.

The total project volume to start in 1988 would be \$200,000.00, of which more than 60% would be for labour costs, including supervision by M. Kalin. In Year 2, it is estimated that only 1.5 technicians would be required, since the experiments would have been set up, and the estimated costs for this year would be \$120,000.00. In Year 3, the costs would again be lower, since we would be ready to start pilot testing, hopefully with NRC support.

STEP 1 PROPOSAL  
IN SUPPORT OF ONGOING EXPERIMENTS BY BOOJUM RESEARCH

CARRIED OUT AT DENISON MINES and INCO LTD.

Assistance requested from CANMET Biotechnology

September 24, 1987

Completion - March 31, 1988

INTRODUCTION

Organic matter produced from populations of macrophytes which grow virtually maintenance-free and are self-sustaining, has many ameliorating aspects on acid-generating waste sites. The main beneficial aspects will result from:

- (1) a layer of organic matter intercepting infiltrating precipitation before reaching the lower tailings layers;
- (2) reduction in the thickness of the active layer of acid generating tailings mass by the action of the rhizosphere; and
- (3) provision of organic matter as food sources for reducing bacteria.

It follows that the establishment of cattail populations over large areas of acid generating wastes would be desirable. Three aspects of cattail establishment and growth are presently being investigated, one of which is addressed here.

# 1. Cattail transplanting in acidic and alkaline conditions

Cattail transplanting into extremely alkaline mine wastes (pH 8-10) has been carried out successfully and growth continues after transplanting under the correct conditions. This was the result of three years of investigation and experimentation. Concurrently, transplanting has been carried out in extremely acid waters (pH 2.5), and marginal success has been obtained. Plants initiate new shoots and subsequently continue to grow. They succumb however, at some point, certainly after over-wintering. These results were gained mainly from hydroponic experiments. From the above, it was concluded that water conditions have to be improved prior to further transplanting experiments.

Cattails are known to survive, and in fact to thrive in acidic conditions as documented by Kalin (1982). The root region of these cattails is indeed 1 to 2 pH units higher than the surface pH, which could be due in part to the saturation of the tailings and reduced oxygen availability. Measurements of the same nature in the same location, without cattail roots, were more acidic. Accordingly, transplanting experiments in tailings areas in Elliot Lake, with pH 1.5 to 2.0 were initiated in 1987, for examination of the response of cattails to the hydroponic acidic conditions.

The responses of the transplants proved very interesting. Two types of death occurred: the leaves turning brown or turning black. Most importantly though, many transplants grew and made new shoots. These responses suggested that an investigation of the root-hair/root/rhizomes, post mortem, compared to those surviving and making new shoots, might indicate the reasons for growth. Some roots were collected, preserved and investigated from a morphological-developmental point of view.

Based on these preliminary investigations, we are now in a position to formulate a systematic approach to address the differences between surviving and dying populations.

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Cattail root systems from clumps which were transplanted at the same time in the same location (pH 1.5 to 2.5) were collected, representing black or brown death, and those making new shoots. These were compared to the root system of transplants on the Levack site, i.e. ameliorated hydroponic acidic condition.

#### OBSERVATIONS BLACK/BROWN DEATHS (Elliot Lake)

Black and brown death observations are combined, since morphological signs of mortality on the roots/rhizomes appear similar for the two types of death of the leaves. Black deaths appear to

represent those plants dying rapidly after transplant, while brown deaths required a longer period after transplant to die.

Rhizomes and roots of the observed brown and black death plants showed substantial levels of mineralization. In rhizomes, this mineralization was very heavy within the outer cortical layers, but was also noticeable in the pith to a lesser extent. A decreasing gradient of mineralization could be seen extending from the tips of the rhizomes to older tissue.

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If we assume that the new rhizomes initiated after transplanting, it can be said that for the brown death and the growing cattail, 1.3 and 1.6 rhizomes are present per plant, whereas for the black death, a ratio of 0.6 rhizomes per plant are noted.

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Unlike the *Typha* plants which died and are discussed above, the single transplant which survived showed vigorous rhizome growth and two new shoots developed. Rhizomes were firm and showed no signs of decay. Interestingly though, these rhizomes had black pith areas, although the cortex was white as in normal growth. The significance of this observation is unclear at the present time. There were no signs of internal mineralization in the three plants. No consistent differences in the extent or root or root hair development could be seen between these plants and the earlier transplants (black/brown deaths).

There were five new rhizomes initiated on the three plants examined. One of these plants however, was itself a product of

growth after transplant. Unlike the above plants, all new rhizomes showed substantial growth and were at least 5 cm. in length.

#### LEVACK HYDROPONIC TRANSPLANT

The Levack transplant showed a small degree of mineralization of rhizomes, although root development appeared to be normal. Cause of death is not immediately apparent, and will require more detailed investigation. Only one new rhizome had been initiated. The fact that these root systems, although they had been transplanted earlier than those at Elliot Lake, showed no mineralization and lived longer, is of particular significance to this root system investigation.

#### PRELIMINARY INTERPRETATION

Several possible hypotheses can be proposed to explain the observations on successful and unsuccessful Typha transplants. The first hypothesis is based on the premise that the type of transplant substrate is irrelevant to transplant success. According to this hypothesis, the likelihood of transplant survival is primarily governed by the timing of transplant. It is plausible to suggest that transplants later in the season are likely to have higher success rates, because these plants with lower metabolic

and growth rates are likely to suffer a smaller degree of transplant shock. Thus, death of early season transplants would be due to shock, with mineralization being a secondary result of this original effect.

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In contrast, in undisturbed sediments, the appropriate rhizosphere chemistry may have been more rapidly achieved, increasing the frequency of survival. Until more data is available, it seems worthwhile to continue to explore all possible factors which could contribute to transplant success.

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Investigation of the root system of the transplanted cattails from the following experiments:

1. Root systems from cattails transplanted by the same methods, the same way, at different times.

2. Root systems from cattails transplanted into straw, peat, lime, and tailings without amendment.
3. Root systems from cattails transplanted into pre-bog acid creek.
4. Root systems from cattails transplanted and growing in alkaline mine slimes on Levack.
5. Root systems from various source populations (Stanrock edges, first and second openings, South mine road, and mine slime cattails).

#### METHODS OF INVESTIGATION

Preserve plants in FAA (Formalin acidic acid) prior to freeze up in 1987. The plants will then be inspected visually for aberrations, and thick sections prepared for further description. Documentation will require some photography and quantification of root systems.

Morphological features to monitor would include the number of new shoots initiated, the extent of rhizome development (length, diameter) mineralization and extent thereof, root and root hair development, and the number of new leaves.

## EXPECTED RESULTS

In spring 1988, a sound basis for the transplant experiments, after assessment of overwintering of the existing populations, could be formulated and carried out.

## APPENDIX B

~~MICROBIOLOGICAL~~  
~~MINERALOGICAL~~ ANALYSIS OF TAILINGS  
SAMPLES PROVIDED BY  
BOOJUM RESEARCH LTD.

by

R.G.L. McCready and J. Salley  
Extractive Metallurgy Laboratory

August 1987

Project: 30.86.99  
Cost Recovery - Environmental Controls

Job No: 025510

MINERAL SCIENCES LABORATORIES  
DIVISION REPORT MSL 87-8 (CR)

CONFIDENTIAL

MICROBIOLOGICAL  
~~MINERALOGICAL~~ ANALYSIS OF TAILINGS SAMPLES  
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R.G.L. McCreedy and J. Salley  
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ABSTRACT

Samples of sulphide saturated steel wool were treated to recover the sulphide to obtain presumptive data indicating the presence of sulphate-reducing bacteria (S.R.B.). Two sets of organic amendments used in tailings treatment were analyzed for the presence of SRBs and the later set were analyzed for the presence of heterotrophic bacteria.

Keywords: sulphate-reducing bacteria, Desulphovibrio, organic degradation, acid mitigation

## 1. Estimation of $H_2S$ Production in Tailings Piezometers

Five samples of steelwool labelled 27, 27b, Bock Hill, Clara Pond and Dave Dun, were treated anaerobically with 1:1 HCl and the evolved gas was trapped as silver sulphide in a 5% silver nitrate trap (see Figure 1).

### Results:

The silver sulphide was recovered by filtration, dried, weighed and the amount of sulphide released was calculated using the following equation.

$$\text{mg of } S^{2-} = \frac{\text{wt of silver sulphide}}{248} \times 32$$

| Sample     | Mg $Ag_2S$ | Calculated mg $S^{2-}$ |
|------------|------------|------------------------|
| Dave Dun   | 123.0      | 15.9                   |
| Bock Hill  | 125.4      | 16.2                   |
| Clara Pond | 115.0      | 14.8                   |
| 27         | 170.0      | 21.9                   |
| 27b        | 112.6      | 14.5                   |

## 2. Analyses of Organic Treatments for the Presence of Sulphate-Reducing Bacteria (SRB)

Samples were received from Boojum Research Ltd. labelled saw-dust, overburden, straw and liquid. An aliquot of each sample was placed in a sterile 250 mL screw-cap jar which was subsequently filled with modified Starkey's Liquid Medium. These anaerobic cultures were incubated at 24°C and checked periodically for the presence of black colonies or the production of hydrogen sulphide.

## Results:

Sulphate-reducing organisms, presumably *Desulphovibrio*, were detected in aliquots of three of the four samples submitted. No sulphate-reducing organisms could be detected in the sample labelled "overburden".

The initial cultures obtained from these samples were subcultured into tubes of Starkey's Solid Medium to determine if the punctiform black colonies typical of *Desulphovibrio* would be observed. Such colonies were observed except from the subculture of the "overburden" sample. A higher cell population was observed from the "saw-dust" and "liquid" sample than from the "straw" sample.

---

 Starkey's Liquid Medium
 

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| Component  | g/L  |
|--|--|
| Peptone  | 5  |
| Beef Extract                                       | 3  |
| Yeast Extract                                      | 0.2  |
| MgSO <sub>4</sub> ·2H <sub>2</sub> O               | 1.5  |
| *Na <sub>2</sub> SO <sub>3</sub>                   | 0.25   |
| Na <sub>2</sub> SO <sub>4</sub>                    | 0.75   |
| *FeNH <sub>4</sub> (SO <sub>4</sub> ) <sub>2</sub> | 0.10   |
| Glucose  | 5.0 q.s. to 1L with H <sub>2</sub> O, pH 7.0 |

\* Added as filter sterilized solutions after cooling of the autoclaved medium.

Starkey's Solid Medium is as above with 20 g of Bacto Agar added per litre.

### 3. Analyses for Sulphate-Reducing Bacteria in Organic Residues from the Levack Site

Mid-straw, Cell #3a  
 Peat/Hay, Cell #2  
 Straw/Sediment, Cell #3  
 Straw/Hay, Cell #1  
 Peat, Cell #3, no 1987 addition  
 Control, No amendment

Aliquots of the first five samples were incubated in 250 mL sterile glass jars containing Starkey's Liquid Medium. All of the samples contained sulphate-reducing bacteria with the highest cell population and hydrogen sulphide generation occurring with the straw/sediment sample from Cell #3.

#### 4. Analyses of Heterotrophic Organisms in the Organic Amendments

Aliquots of the six samples were streaked onto triplicate plates of nutrient agar adjusted to four different pH values; 2.5, 3.0, 4.0 and pH 7.0. Heterotrophic growth occurred at all pH values from all the samples with the exception of the unamended control sample. Fungi and actinomycetes, both of which are capable of metabolizing cellulose and lignocellulose, were the predominant organisms. However, an aciduric, Klebsiella ozaenae was detected on the nutrient agar plates at pH 4.0 and 7.0

In contrast to previous analyses of this material in which a monoculture of a cellulase-producing *Bacillus* was isolated, the larger diversity of heterotrophs from these samples suggest that extensive degradation and metabolism of the organic amendments has occurred.

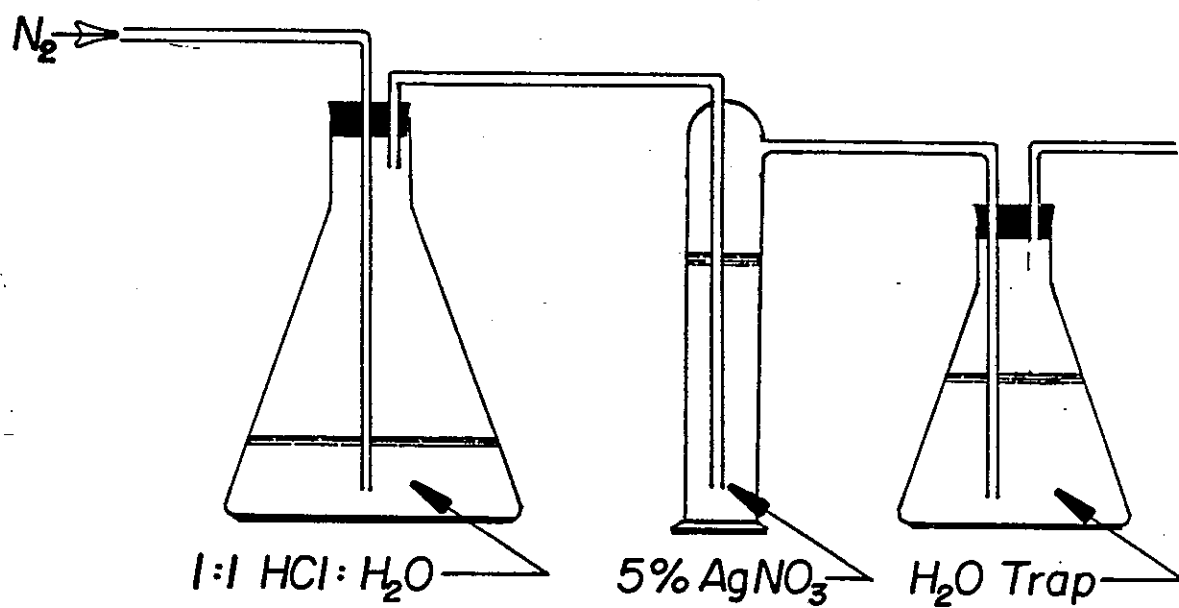
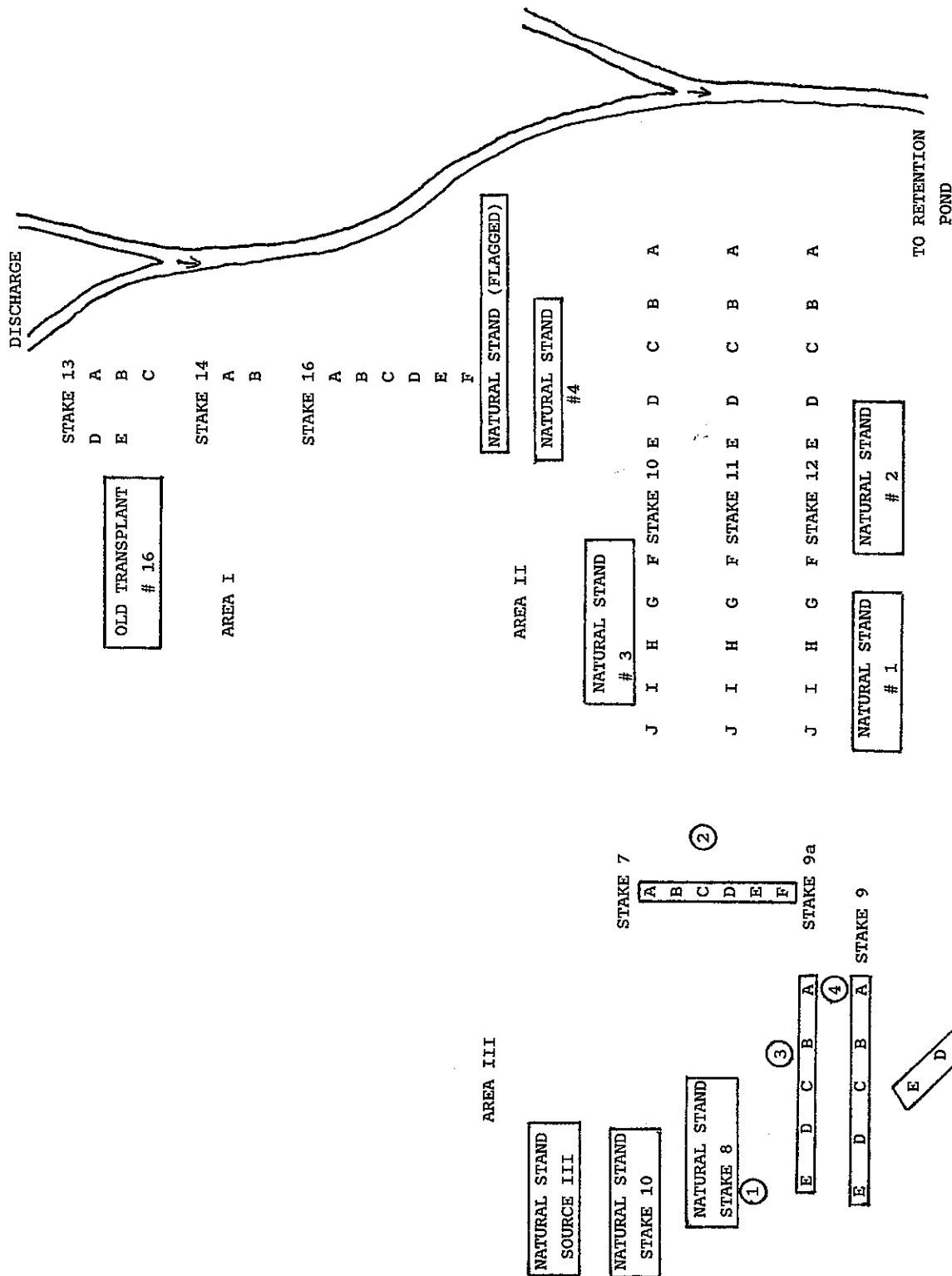


Fig. 1 - Sulphur recovery from steel wool

## APPENDIX C



SCHEMATIC 1: CATTAIL TRANSPLANT AREAS ON THE LEVACK SITE. LETTERS REFER TO SPECIFIC BUNCHES OF CATTAILS. CIRCLED NUMBERS ARE GROUP NUMBERS LISTED IN TABLE FOR THIS AREA.

**DEVELOPMENT OF METHODS TO ESTABLISH REDUCING CONDITIONS IN  
ACIDIC WATERS LEADING TO SELF-MAINTAINING TREATMENT METHODS FOR  
SEEPAGES AND ACIDIC WATER ON THE SURFACE OF WASTE MANAGEMENT  
AREAS**

**BACKGROUND**

The experiments carried out over the last 3 years by Boojum Research, with the support of Canmet (RATS), Inco, BP-Selco, Kidd Creek and Denison, have finally led to results which indicate that not only are the concepts of Ecological Engineering valid, but that the experimental foundation is at hand to proceed with a focused research program to develop the treatment process.

The program needs the joint effort of Canmet's expertise (Microbiology), and Boojum Research's experience and data acquired to date, and not least importantly, a commitment from Inco and Denison to provide their continued support with respect to maintaining the experimental sites. Only in this manner can we proceed with further work based on the results obtained in a stepwise program.

Step 1: Investigate Cattail roots as per proposal Appendix 1.

**Proposed supporter:** CANMET, Biotechnology

**Estimated costs:** \$20,000.00

Step 2: Set up swimming pool size experimental cells in the field to establish the same pre-bog acid creek conditions, but allow flow control, and water quality measurements (Levack)

**Proposed supporter:** Inco, Sudbury operations.

**Estimated costs:** \$35,000.00

Step 3: Implement acid cattail transplant experiments on Stanrock and in existing pre-bog acid creek (Levack).

**Proposed supporter:** Denison Mines

Estimated costs: \$25,000.00

Step 4: Microbiological processes in existing (running since 1985), pre-bog acid creek and Stanrock bog (established in 1987), and swimming pools (to be established in 1988).

**Proposed supporter:** CANMET, Denison and Inco

Estimated costs: \$120,000.00

### Proposed Schedule of project

| Task                               | Nov 1987 to March 1988 | April 88 to Oct 88 | Nov to Nov89 |
|------------------------------------|------------------------|--------------------|--------------|
| 1. Develop a detailed project plan |                        |                    |              |
| 2. Obtain necessary resources      |                        |                    |              |
| 3. Implement project plan          |                        |                    |              |
| 4. Monitor project progress        |                        |                    |              |
| 5. Report project status           |                        |                    |              |
| 6. Complete project                |                        |                    |              |

Step 1 XXXXXXXXXXXXXXXXXXXXXXXX

Step 2 XXXXXXXXXXXXXXXX

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Step 3                                XXXXXXXXXXXXXXXXXXXXXXXXXX
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Step 4 XX

After Step 4, we anticipate having sufficient information to design a pilot field testing program.

ORGANIZATION:

Boojum would require two new technicians, Position 1, in Microbiology, working in CANMET under Dr. Ron McCready, and one field technician to set up the experiments for Steps 2 and 3.

The total project volume to start in 1988 would be \$200,000.00, of which more than 60% would be for labour costs, including supervision by M. Kalin. In Year 2, it is estimated that only 1.5 technicians would be required, since the experiments would have been set up, and the estimated costs for this year would be \$120,000.00. In Year 3, the costs would again be lower, since we would be ready to start pilot testing, hopefully with NRC support.

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#### **EXPECTED RESULTS**

In spring 1988, a sound basis for the transplant experiments, after assessment of overwintering of the existing populations, could be formulated and carried out.