TREATMENT OF ACIDIC SEEPAGES USING WETLAND ECOLOGY AND MICROBIOLOGY:

OVERALL PROGRAM ASSESSMENT

MEND Project 3.11.1

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TREATMENT OF ACIDIC SEEPAGES

USING WETLAND ECOLOGY

AND MICROBIOLOGY:

OVERALL PROGRAM ASSESSMENT

FINAL REPORT

by: Margarete Kalin

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SUMMARY

The MEND Project 3.11.1: ***"Treatment of Acidic Seepages Employing Wetland Ecology and Microbiology"*** has reached the completion of its fourth year. The project has been financially supported by Inco, Denison, Environment Canada, CANMET and by the Centre de Recherche Minerales (CRM). The objectives of the project are to determine the conditions which will lead to the treatment and amelioration of acid mine drainage (AMD) through the use of ecological microbial processes. Those occur naturally in wetlands, lake and ocean sediments. The Makela Test Cell System was intended to provide flow control for a typical seepage from a base metal tailings dam. Under flow control, natural Fe^{3+} hydroxide precipitation and acidification rates were determined. Conditions which are required to promote microbial sulphate reduction and alkalinity-generation were to be identified. The microbially-mediated treatment of acid mine drainage is referred to as ARUM (Acid Reduction Using Microbiology).

The construction of retention cells at the perimeter of the phreatic line of the tailings dam was complex. This terrain is hydrologically unstable and dikes are prone to slumping. Permeable dikes were used to provide sheet flow, and impermeable dikes, providing flow control, were required to separate the retention cells. Frequent repairs on the cell system were needed from the beginning of construction in 1989 until summer 1991. By the end of the summer of 1991 flow control was achieved, and a prototype of a floating cattail cover, which allows the ARUM system to develop, was finally installed. Project activities are given below for each year.

Year 1 (1989/1990): In the first year, the Test Cell System was constructed and hydraulic adjustments were made to control flow. Test work in 200 L drums (ARUMators), containing organic amendment and equipped with sampling ports, showed that microbial alkalinity-generation in tailings seepages is possible. ARUMator 3, a 12 m^3 fibreglass tank with an 800 L inner sleeve containing the organic

amendment, was installed at the end of the Test Cell System. This would facilitate testing of the ARUM processes under completely controlled conditions.

Increases in pH in the ARUMators were reported from 2.5 to 5.7. Decreases in nickel concentrations from 91 mg/L in a sample from a surface port to 1.7 mg/L in a bottom sample of the same ARUMator were noted. These observations lead to the recognition that the process is sediment-bound and that a floating cattail cover was needed. Cattails rooted in the organic amendment would rapidly deplete the nutrients required for the ARUM ecosystem. A floating vegetation mat, however, would not only provide organic matter to the sediment below, but also enhance reducing conditions in the water column between the sediment and the floating cattail mat.

The research of the first year was reported in June 1990 in a report entitled:

> MEND Project 3.11 .1 "Treatment of Acidic Seepages Employing Wetland Ecology and Microbiology, Final Report", by M. Kalin, June 1990. DSS Contract Number 23440-8-9264.

A peer review was carried out on the report, and the project was found technically sound.

Year 2 (1990/1991): After the first winter, the Test Cell System required readjustment of the hydraulic conditions. It was established that the lowest controllable flows were 3 - 5 L/min. The maximum flow, which the system could sustain without structural failure, was determined to be 300 L/min. Baseline chemistry of the system was defined in the second year.

Ground water contributions, amounting to less than 1 L/min, were found to have no detectable effects on the water chemistry. The conditions under which precipitation of ferrous (Fe^{2+}) and ferric (Fe^{3+}) hydroxide takes place in the precipitation cell (Cell 1) were defined. A baffle system was installed in Cell 1 which facilitated settling of

the hydroxides. This cell discharged a clear, acidic solution with low iron concentrations to Cell 2.

Organic amendment was placed in Cells 3 and 4, between snowfencing curtains. Flax bales mixed with hay bales were used to provide the substrate on which the microbial ecosystem would grow and where alkalinity would be generated. Through the activity of the sulphate reducers, hydrogen sulphide is generated, which results in the precipitation of metal sulphides. An extensive microbiological investigation was carried out in the laboratory to define the growth requirements of the alkalinity-generating microbes.

A report on the work completed in the second year was submitted in March 1991.

> MEND Project 3.11 .1 "Treatment of Acidic Seepage Employing Wetland Ecology and Microbiology, Final Report", by M.Kalin, March 1991. DSS Contract Number 23440-o-9065.

Year 3 (199111992): In the first two years of the project, the ecological conditions required for microbial alkalinity-generation were defined. Floating cattail mats were installed on Cells 3 and 4 in 1991. The third year was, therefore, the first opportunity to demonstrate the ARUM process under defined flow conditions. The optimum configuration required for the establishment of the ARUM process had only been achieved by late July, due to problems encountered with bank stability in late May 1991. Slumping of the tailings dam blocked the bypass ditch, preventing regulation of the flow to the Test Cell System.

The ARUM process works from the sediment upwards, and thus, its effects would first be seen in the lower part of the water column in Cells 3 and 4. The flow was adjusted to 1 L/min by mid July. By mid September 1991, differences in metal concentrations of water on the surface and in the lower parts of the water column were large. In Cell 4, the nickel concentrations at the surface ranged from 43 mg/L

to 74 mg/L. The range in the lower part of the water column (50 - 60 cm) was 12 mg/L to 33 mg/L. In Cell 3, the first ARUM cell receiving the low pH AMD, the nickel concentrations ranged between 23 and 51 mg/L at the surface, while the lower part of the water column had concentrations between 15 and 24 mg/L. This represents approximately a 50 % reduction of the nickel concentrations. Copper was present in both cells at the surface in concentrations ranging from < 1 to 4 mg/L and reduced by the ARUM process in the lower part of the cells to 1 or < 1 mg/L.

At a flow rate of 1 L/min, the water in Cells 3 and 4 has a retention time of just over 4 months. The surface water, however, short-circuits and therefore, the pH of the discharged water had only slightly increased from 2.5 to 3.2. However, 27 kg of alkalinity has been generated in water leaving Test Cell 4 after passing over the actively ARUMating lower water column, where the pH is as high as 6.0. If water were to be discharged from the bottom of Cell 4, reduced metal concentrations with a high pH water would leave the system by the end of the third year.

The results of the third year indicated that, in the Test Cell System, alkalinity-generation had taken place. The work of the third year was reported in March 1992.

> MEND Project 3.11 .1 "Treatment of Acidic Seepages Employing Wetland Ecology and Microbiology, Final Report", by M. Kalin, March 1992. DSS Contract Number 23440-o-9065.

Year 4 (1992/1993): Due to dam stability problems during spring thaw and freezing of the dikes along with the control valve, the system was closed during the winter of 1992 to 1993.

As the cattail rafts were planted late in 1991 growing season, growth was restricted to a few plants. By the beginning of the 1992 growing season, adjustments were made in the root zone. The floating cover was functional by July 1992 and the system was ready to be monitored.

In 1989 and 1990, there was no flow control and flows were very variable. With short retention times (4.2 days in Cells 1 and 2 and 3.26 days in Cells 3 and 4 at 40 L/min) Fe^{3+} hydroxide precipitation occurred throughout the system. When flow control was established at 1 L/min, retention time could be increased to an estimated 168 days in Cells 1 and 2. In 1992, Fe^{3+} hydroxide precipitation facilitated the removal of at least 94 % of the iron load in Cell 1 and produced an acidity loading of 100 to 600 g/day in the water entering the ARUM cells (Cells 3 and 4).

The final configuration, established by the end of 1991, allowed for the establishment of ARUM in Cells 3 and 4. In 1992, with a retention time of 131 days, the ARUM system (Cells 3 and 4) removed 80 - 87 % of the nickel loading, 77 - 98 % of the copper loading, 10 - 20 % of the sulphur loading, and 47 - 73 % of the acidity loading from the seepage water.

This report presents the summary of those components of the microbial ecosystem which play major roles in the ARUM process. The relationships between wetland ecosystems and ARUM processes are given in Section 2. In Section 3, the Test Cell System is described, outlining the events which finally lead to flow control and floating cattail rafts in 1992. The water chemistry, the hydrology with and without microbial activity, as well as the iron hydroxide precipitation, are described in Section 4. In Section 5, the data obtained in the research program are used to define the operating parameters, such as nutrient supply and chemical conditions. The expected performance and the applications of the process are discussed in Sections 6 and 7. In Section 8, the limitations of the microbial approach are outlined. Some economic considerations are presented in Section 9. It is concluded in Section 10 that the project has provided the technical basis to define the conditions required to utilize microbial amelioration of AMD in decommissioning seepage collection ponds, open pits and polishing ponds.

RÉSUMÉ

Le projet NEDEM 3.11.1, intitulé «*Traitement de la percolation acide par l'emploi de l'écologie des marécages et de la microbiologie*», en est à la fin de sa quatrième et dernière année. Ce projet a été finance par Inco, Denison, Environnement Canada, CANMET et par le Centre de recherches minerales (CRM). Il visait à determiner les conditions qui permettront de traiter le drainage minier acide (DMA) au moyen de processus écologiques impliquant des microorganismes. Ces processus existent naturellement dans les mar&ages, dans les sediments des lacs et des oceans. Le systeme d'essai du site Makela visait à contrôler l'ecoulement d'eaux d'infiltration typiques provenant d'un barrage constitué de résidus miniers de métaux de base. L'écoulement étant contrôlé, on a determine les taux naturels de precipitation de l'hydroxyde de Fe^{3+} et d'acidification. On devait determiner les conditions nécessaires pour promouvoir la reduction microbienne des sulfates et la generation d'alcalinité. Le traitement microbien du drainage minier acide est appelé ARUM (Acid Reduction Using Microbiology).

La construction de cellules de retention sur le périmètre de la ligne phréatique du barrage de residus miniers s'est révélée complexe. À cet endroit, le terrain est instable au point de vue hydrologique et les digues menaçaient de s'affaisser. On a utilisé des digues perméables pour assurer un Bcoulement en nappe et des digues imperméables, contrôlant l'écoulement, pour séparer les cellules de retention. A partir du debut de la construction, en 1989, jusqu'à l'été de 1991, le systeme a nécessité des reparations fréquentes. À la fin de l'été 1991, on avait réussi à contrôler l'ecoulement et on a install6 un prototype d'une couverture flottante de quenouilles, permettant au systeme ARUM de se developper. Les activités effect&es dans le cadre de ce projet et correspondant à chaque année sont données ci-après.

Premiere année (1989/1990) : Au cours de la premiere annee, on a construit le systeme d'essai et on a effectué des ajustements hydrauliques pour contrôler

l'ecoulement. Les essais réalisés dans des barils de 200 I (« ARUMators ») contenant de la matière organique et munis d'orifices d'echantillonnage ont montré qu'il était possible de générer, sous l'action des microorganismes, de l'alcalinité dans les eaux d'infiltration provenant de résidus miniers. Pour faciliter la mise à l'essai du processus ARUM dans des conditions totalement contrôlées, on a installé à une extrémité du système d'essai un « ARUMator 3 », c'est-a-dire un reservoir en fibre de verre de 12 m^3 muni d'un manchon interieur de 800 I contenant la matière organique.

Dans les ARUMators, le pH est passe de 2,5 à 5,7. On a également note que la concentration de nickel, qui était de 91 mg/l dans un echantillon prélevé à travers un orifice situé près de la surface, tombait à 1,7 mg/l dans un echantillon prélevé dans le fond du même ARUMator. Ces observations ont permis de constater que le processus intervenait dans les sediments et qu'une couverture flottante de quenouilles était nécessaire. En s'enracinant dans la matière organique, les quenouilles auraient épuisé rapidement les elements nutritifs nécessaires à l'écosystème ARUM. Une couverture flottante de vegetation constituerait non seulement une source de matière organique pour les sediments sous-jacents, mais elle accentuerait aussi les conditions reductrices dans la colonne d'eau, entre les sediments et la couverture flottante de quenouilles.

Les travaux de recherche réalisés au cours de la premiere année ont été présentés en juin 1990 dans un rapport intitulé :

> Projet NEDEM 3.11 .1 « Treatment of Acidic Seepages Employing Wetland Ecology and Microbiology, Final Report », par M. Kalin, juin 1990, numéro de contrat du MAS 23440-8-9264.

Ce rapport a fait l'objet d'un examen par les pairs et le projet a été jugé techniquement correct.

Deuxième année (1990/1 991) : Après le premier hiver, les conditions hydrauliques du systeme d'essai ont dû être rajustées. On a établi que les plus faibles debits qui pouvaient être obtenus étaient de 3-5 l/min. Par ailleurs, on a determine que le debit maximal auquel la structure du systeme pouvait resister était de 300 l/min. La chimie de base du systeme a été définie au cours de la deuxieme année.

Les contributions des eaux souterraines, qui representaient moins de 1 l/min, n'avaient aucun effet décelable sur la chimie de l'eau. On a défini les conditions dans lesquelles l'hydroxyde ferreux (Fe^{2+}) et l'hydroxyde ferrique (Fe^{3+}) precipitaient dans la cellule de precipitation (cellule 1). On a installé dans la cellule 1 un systeme de déflecteurs pour faciliter le dépôt des hydroxydes. Cette cellule rejetait dans la cellule 2 un drainage acide clair 8 faible teneur en fer.

On a depose de la matière organique dans les cellules 3 et 4, entre des écrans pare-neige. On a utilisé des balles de lin mélangées avec des balles de foin comme substrat pour faire croitre l'écosystème microbien dans lequel l'alcalinité pourrait être générée. Sous l'action des bactéries sulfato-réductrices, du sulfure d'hydrogène est produit, ce qui fait precipiter les sulfures métalliques. Une etude microbiologique approfondie a été effectuée en laboratoire pour définir les conditions de croissance des microbes producteurs d'alcalinité.

Un rapport sur les travaux réalisés au cours de la deuxieme année a été présenté en mars 1991.

> Projet NEDEM 3.11.1 « Treatment of Acidic Seepages Employing Wetland Ecoloav and Microbioloav, Final Report », par M. Kalin, mars 1991, numéro de contrat du MAS 23440-8-9065.

Troisième année (1991/1992) : Au cours des deux premieres années du projet, on a défini les conditions Bcologiques necessaires à la generation microbienne d'alcalinité.

En 1991, des couvertures flottantes de quenouilles ont été installées dans les cellules 3 et 4. C'est donc pour la premiere fois au cours de la troisieme année que l'on a pu faire la demonstration du processus ARUM dans des conditions d'écoulement définies. La configuration optimale nécessaire à l'établissement du processus ARUM n'a été obtenue qu'à la fin juillet à cause de problèmes de stabilité des berges survenus à la fin de mai 1991. En s'affaissant, le barrage de résidus miniers a bloqué le fossé de derivation, ce qui a empêché le contrôle de l'écoulement jusqu'au système d'essai.

Le processus ARUM s'amorce dans les sediments et se poursuit vers le haut. Ses effets devraient donc se manifester d'abord dans la partie inferieure de la colonne d'eau des cellules 3 et 4. Le debit a été ajusté à 1 l/min à la mi-juillet. Des la mi-septembre 1991, on a note des differences importantes entre les concentrations de metal dans l'eau à la surface et dans les parties inférieures de la colonne d'eau. Dans la cellule 4, les concentrations de nickel à la surface variaient de 43 mg/l à 74 mg/l. L'intervalle dans la partie inferieure de la colonne d'eau (50-60 cm) était de 12 mg/l à 33 mg/l. Dans la cellule 3, premiere cellule ARUM à recevoir les eaux de DMA de faible pH, les concentrations variaient entre 23 et 51 mg/l 8 la surface, alors qu'elles se situaient entre 15 et 24 mg/l dans la partie inferieure de la colonne d'eau. Cet écart représente une reduction d'environ 50 % des concentrations de nickel. Le cuivre était present dans les deux cellules, à la surface, en concentrations variant de < 1 à 4 mg/l et, sous l'action du processus ARUM, ces concentrations tombaient à 1 ou à < 1 mg/l dans la partie inferieure des cellules.

A un debit de 1 l/min, l'eau sejournait un peu plus de 4 mois dans les cellules 3 et 4. L'eau superficielle a toutefois un effet neutralisant et le pH de l'eau rejeté n'avait donc que légèrement augmenté de 2,5 à 3,2. Toutefois, 27 kg d'alcalinité avaient été produits dans l'eau quittant la cellule d'essai 4 après être passée sur la partie inferieure de la colonne d'eau en « ARUM-ation » active, où le pH s'élevait jusqu'à 6,0. Si de l'eau était soutirée à partir du fond de la cellule 4, elle presenterait de faibles concentrations de metal et un pH élevé dès la fin de la troisieme année.

Les résultats obtenus au cours de la troisieme année ont indiqué qu'il y avait eu production d'alcalinité dans le systeme d'essai. Les travaux effectués au cours de la troisième année ont été présentés en mars 1992.

> Projet NEDEM 3.11 .1 « Treatment of Acidic Seepages Emplovina Wetland Ecology and Microbioloav, Final Report », par M. Kalin, mars 1992, numéro de contrat du MAS 23440-8-9065.

Quatrième année (1992/1993) : À cause de problèmes d'instabilite du barrage pendant la fonte printaniere et le gel des digues et de la vanne de regulation, le systeme a été fermé pendant l'hiver 1992-1993.

Les quenouilles ayant été plantées vers la fin de la saison de croissance de 1991, seules quelques plants ont poussé. Au debut de la saison de croissance de 1992, on a fait des ajustements dans la zone des racines. La couverture flottante est devenue fonctionnelle en juillet 1992 et le systeme était alors prêt à être contrôlé.

En 1989 et en 1990, il n'y a pas eu de contrôle de l'écoulement et celui-ci a été très variable. Dans le cas de courts temps de retention (4,2 jours dans les cellules 1 et 2 et 3,26 jours dans les cellules 3 et 4 8 40 l/min) l'hydroxyde de Fe^{3+} précipitait dans l'ensemble du systeme. Lorsque le debit a été limité à 1 l/min, on a estimé que le temps de retention passait à environ 168 jours dans les cellules 1 et 2. En 1992, la precipitation de l'hydroxyde de Fe^{3+} a facilité l'élimination d'au moins 94 % de la charge en fer de la cellule 1, ce qui s'est traduit par la production d'une charge en acidité de 100 à 600 g/jour dans l'eau qui entrait dans les cellules ARUM (cellules 3 et 4).

La configuration finale, obtenue à la fin de 1991, a permis l'établissement du procédé ARUM dans les cellules 3 et 4. En 1992, avec un temps de retention de 131 jours, le systeme ARUM (cellules 3 et 4) a permis d'éliminer 80-87 % de la charge en nickel,

77-98 % de la charge en cuivre, IO-20 % de la charge en soufre et 47-73 % de la charge en acidité des eaux d'infiltration.

Le present rapport decrit sommairement les constituants de l'écosystème microbien qui jouent un rôle important dans le processus ARUM. Les relations existant entre les écosystèmes des marécages et le processus ARUM sont donnés dans la section 2. Dans la section 3, on decrit le système d'essai en insistant sur les événements qui ont finalement permis d'obtenir le contrôle de l'écoulement et les couvertures flottantes de quenouilles en 1992. La chimie de l'eau, l'hydrologie avec et sans activité microbienne ainsi que la precipitation de l'hydroxyde de fer sont décrits dans la section 4. Dans la section 5, on utilise les données obtenues au cours du programme de recherche pour définir les paramètres de fonctionnement, notamment les conditions d'approvisionnement en elements nutritifs et les conditions chimiques. La performance prévue et les applications du traitement sont traitées dans les sections 6 et 7. Dans la section 8, on decrit les limites de l'approche microbienne. Certaines considerations Bconomiques sont présentées dans la section 9. On conclut dans la section 10 que le projet fournit une base technique permettant de définir les conditions nécessaires à l'utilisation du traitement microbien du DMA pour fermer des lagunes servant à recueillir des eaux d'infiltration, des mines à ciel ouvert et des lagunes tertiaires.

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1.0 INTRODUCTION

Mining any commodity which is associated with sulphide-bearing minerals can result in large quantities of acid-generating waste rock or tailings. During the operation of a mine, effluent treatment plants neutralize the acid waste streams and hydroxide sludges are collected in ponds for disposal. It can be expected, in some cases, that the volume of sludge production exceeds several times that of waste materials producing the acidic effluent. Oxidation rates are slow and quantities of oxidisable materials are large. Therefore effluent treatment is needed for a very long time. For the mineral sector decommissioning of mining operations represents a major technical challenge, which requires an environmentally-acceptable and economically-viable solution.

When mining activities cease, effluent volumes decline but remain substantial. The volumes are determined at the time of decommissioning by the size of the drainage basin in which the waste rock and tailings are placed, and both the hydrological and meteorological conditions of the area. Effluent characteristics, in particular the acidity, are generally determined by the oxidation rates which prevail in the waste material. The metal concentrations in the effluent are generally a function of both hydrology of the area and mineralogy of the wastes.

Acid generation is a natural process. Acid generation is mainly an oxidative process of pyrite, which in nature is balanced by reductive processes such as iron- and sulphate-reduction, which result in alkalinity generation. Therefore, providing the conditions which facilitate alkalinity-generating processes is the technical foundation to counter-balance the acid generation.

Treatment plants are required to neutralize the effluents which result in the accumulation of metal-laden sludge. Microbial sulphate reduction is used successfully in treating acid mine drainage in reactors where food sources (e.g. fatty acids, methanol, etc.) are added for the microbes.

The maintenance of a treatment plant to neutralize AMD, be it microbially- or chemically-mediated, does not represent an economically, sustainable solution for the long-term. In the past 10 years, wetlands have been suggested as a natural means of treating AMD.

The acid reduction processes in wetlands, however, are sediment-bound. Therefore, in order to affect AMD which flows over sediments, the reducing conditions have to be extended from the sediment into the water column. The concept of ARUM (Acid Reduction Using Microbiology), developed under this project, is intended to provide those conditions. A Test Cell System was designed, and constructed to test concept, and derive design parameters for a full-scale ARUM treatment system.

The design of a biological treatment system is fundamentally different from an engineered system in that the necessary microbial activity in the sediment, the engine driving the treatment process, is not directly measurable. Therefore, design criteria for a biological system consist of identifying those conditions which support the microbial activity. The measurements and observations summarized in this report reflect the development of the ARUM process over three years. The configuration of the system was stabilized in 1992, and the microbial ecosystems required for the treatment process were present only in the last year of the program.

To reduce the rate of oxidation and provide reducing conditions for the effluents, a self-sustaining or low maintenance scenario for a mining waste management area is required. In nature, reducing conditions occur in wetland and lake sediments. Ecosystems can be designed and constructed to promote the establishment of sediment-like conditions, i.e. where reducing conditions favourable for alkalinity-generating processes prevail. To counterbalance oxidation rates, the ecosystems must produce alkalinity at rates which are able to deal with annual contaminant loadings. Contaminants are generated by oxidation and transported by the hydrology within the waste management area.

To achieve a self-sustaining system or low maintenance solution to the decommissioning of mine waste management areas, it is necessary to balance the rates of oxidation and reduction and also the rates of dissolution and precipitation. The volume of effluent is determined by the hydrological conditions of the mine site. The containment of the effluents within holding ponds where the reductive processes are installed has to accommodate the seasonal hydrological cycle. In principle, the design and engineering of mining waste management areas aim to minimize drainage basins into which tailings or waste rock are placed. Retention ponds are designed to contain or collect spring and fall run-off for treatment.

Ecological Engineering technology is an approach to decommissioning mining waste management areas which exploits natural biological processes within the tailings and retention ponds to curtail oxidation rates and to treat annual contaminant generation. The rates by which the biological processes improve water quality have to balance those rates at which the contaminated water is generated.

Two biological processes have been identified as key components in the Ecological Engineering approach. One of the processes, biological polishing, utilizes attached algae to remove contaminants in the waste water. The algal biomass provides surface area onto which metals are concentrated by co-precipitation and/or cation exchange reactions. The second process is a microbial process referred to as ARUM (Acid Reduction Using Microbiology). ARUM generates alkalinity through microbial reduction of iron and sulphate. Engineering of appropriate retention structures, for example, berms with low permeability, within the waste management area is required to implement the operations of the Ecological Engineering systems.

Over the last 10 years, field tests at several different mine sites addressed the overall objective of developing this passive biological decommissioning technology. The key aspect which needed to be addressed was the quantification of the expected contaminant removal rates by these biological processes. Field tests were carried out

which, although costly, are the only means of arriving at realistic operating parameters, since simulation of environmental conditions in chemically-reactive effluents, such as AMD or ARD is not feasible.

The ARUM work carried out within the MEND program represents only a part of the overall R&D which was required to understand the process. This project provided a test system where a microbially-active sediment could be configured as conceptualized. Through the provision of flow control in the Makela Test Cell System, built at one of the seepage stations of the INCO Copper Cliff tailings area, it was possible to determine minimum retention time and flow rates for the treatment of a tailings seepage.

The construction of retention ponds with flow control at the foot of a seeping tailings dam was technically difficult, and hence costly. Several efforts to re-stabilize or fix the berms were needed. In the last year of the project, the final configuration was achieved with flow control of about 1 L/min entering the two ARUM cells.

The Test Cell System has facilitated the quantification of acidification rates, iron hydroxide precipitation rates, alkalinity-generation rates and sulphate-reduction rates in a tailings seepage. The ARUM process, although originally sediment-bound can operate in the water column if reducing conditions are present. To decrease water mixing and provide proper conditions a floating, living macrophyte cover was necessary. The conditions for the establishment of such a vegetation mat were developed during the MEND program. In addition, a floating cattail mat was grown over the ARUM cells in 1992. Contaminant removal was quantified under controlled flow conditions and in the required configuration for a period of about 5 months.

Although scientific and technical information suggested that the ARUM process should be viable, the Makela Test Cell System has provided the first purely microbially-treated acid mine drainage.

The scope of work for this report called for a broader framework to outline applications of the ARUM process, and to specifically address the MEND objectives. One of the main objectives of the joint industry and government program, MEND, is to find self-sustaining or low maintenance decommissioning solutions.

To date, three reports have been issued on the project and this fourth and final report provides an overview of the activities and the results obtained. The biological conditions which drive ARUM are summarized in Section 2. Section 3 describes the Test Cell System and Section 4 discusses the noted water quality changes brought about by the system. In Section 5, the technical basis for operating parameters of ARUM sediments are given. Section 6 discusses ARUM performance, while Section 7 draws together the data from various applications at different test sites. Section 8 summarises the limitations of the system. Although costs to establish the process are expected to vary with respect to site conditions and availability of material, some general economic considerations are presented in Section 9.

The conclusion in Section 10 represents a general discussion of ecological thought with respect to mining waste management. The work carried out to date provides evidence that if the fundamental requirements for ecosystem recovery are provided, mining waste management areas can develop into new ecosystems.

2.0 MICROBIOLOGICAL CONDITIONS FOR ARUM

Waste rock and tailings from mining operations weather when exposed to the atmosphere. This weathering is accelerated when the material has commenced acidification through biological oxidation. Oxidation proceeds when reduced iron liberated from the pyritic material is oxidised and more acid is released. The acid acts as a chemical leach solution. Tailings seepages contain a large fraction of Fe^{2+} which on exposure to air is oxidised, and following hydrolysis, is precipitated as Fe^{3+} hydroxide as long as pH exceeds 3.5. Fe^{2+} oxidation and Fe^{3+} hydroxide precipitation increases the acidity, and removes iron from the water via the reaction:

$$4\ Fe^{2+} + O_2 + 10H_2O = 4Fe(OH)_3 + 8H^+$$

Seepages emerging from a waste rock pile, in contrast to tailings seepages, are already oxidised, and, therefore, contain mainly Fe^{3+} which precipitates as $Fe(OH)_3$ following hydrolysis, via the following overall reaction:

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

Thus, for practical purposes, the difference between a tailings seepage and acid rock seepage, is the location at which the acid is generated; for waste rock it is in the pile, and for tailings seepages it is after emergence from the dam.

The resulting AMD effluents from both waste rock and tailings are acidic and contain high metal concentrations. The acid generation process is relatively slow, and therefore the effluents from tailings and waste rock will be contaminated for a long time.

The acid generation process occurs naturally, and can be counteracted by natural alkalinity-generating processes. Wetlands, both natural and constructed, have been used to clean up a variety of waste waters (Hammer, 1990). Wetlands, particularly those dominated by *Typha* survive the passage of AMD, and accumulate quantities of Fe^{3+} hydroxide sludge (yellow boy). Therefore, wetlands have been investigated as a means of treating AMD. Constructed wetlands for the treatment of AMD have met with mixed success (Brodie, 1990; Wildeman and Laudon, 1990).

The potential of wetlands to treat AMD anaerobically is suggested by thermodynamic consideration of aquatic ecosystems. The order of reducing reactions as predicted from thermodynamic considerations can be found in vertical profiles of aquatic sediments (Zehnder and Stumm, 1988). Mills et al. (1989) reported the presence of microbially-mediated anaerobic processes which generate alkalinity in sediments.

Bacteria have evolved to exploit electron acceptors other than oxygen under reducing conditions. In AMD, the principle electron acceptors are sulphate, which is always present, and iron, which is present most of the time. When oxygen is used up in the sediment by heterotrophic (decomposing) bacteria, in the presence of Fe^{3+}, reduction to Fe^{2+} will proceed prior to sulphate-reduction. Ferric iron-reducing bacteria have only recently been isolated from sediments (Lovley and Phillips, 1988), and hence, their importance in AMD treatment and wetlands is only now beginning to be appreciated (Vile and Weider, 1993). Ferric iron reduction generates the alkalinity, which was lost during oxidation, where the Fe^{3+} was precipitated as Fe^{3+} hydroxide at a pH above 3.5.

Sulphate reduction and fermentation of organic materials will proceed when Fe^{3+} is no longer available. After sulphate is used up, methanogens can utilise remaining carbon sources for respiration, without generating alkalinity. Sulphate reduction is much better understood than Fe^{3+} reduction. Most studies on the use of wetlands to ameliorate AMD have considered this process alone as a means of reducing acidity and removing metals (Hedin et al., 1990; Kleinmann et al., 1991).

For sulphate reduction, bacteria use SO_4^{2-} as an electron acceptor and a variety of organic substrates as donor. The SO_4^{2-} is reduced to H_2S at low pHs. At high pHs, SO_4^{2-} is reduced to S^{2-} and HS^-, and, in the presence of metal cations, metal sulphides are precipitated.

All the alkalinity-generating micro-organisms require organic carbon as a source of energy. For most organisms, short chain fatty acids, alcohols or sugars can provide

this requirement. Some iron reducers and sulphate reducers are reported to utilise H_2 as an energy source.

The breakdown products required for the reducing bacteria are formic, acetic, butyric and propionic acids (together with H_2). These are produced through the activity of heterotrophic bacteria which breakdown organic matter under aerobic conditions in the upper layer of the sediment, first to simple carbohydrates. Through acetogenesis, which occurs in the anaerobic sediment layer, the required organic acids are produced as the result of breakdown of simple carbohydrates. Therefore, the nature of the organic matter in the sediment and the rate at which the fermentation of organic matter takes place is a very important aspect in utilizing microbial processes in AMD treatment.

Wetlands can be considered as sediment treatment systems. At the surface, among the roots of aquatic plants, decomposition is favoured by the good oxygen supply and relatively high temperatures. Within the sediment, roots and rhizomes will supply oxygen locally but, oxygen will be readily used up. Much of the organic matter will be decomposed completely to CO, in the oxic zone, and would therefore not provide the required food sources for the sulphate and iron reducers.

Anaerobic decomposition of plant material occurs at a lower rate than aerobic decomposition (Colberg, 1988). The breakdown of lignin, which comprises 20-30 % dry weight of most woody plant-derived materials, under anaerobic conditions remains controversial, but is certainly slow, at best (Benner et al., 1984). Therefore mixtures of organic materials are likely required to initiate and promote the required decomposition. The recalcitrance (resistance to breakdown) of organic matter in wetlands is reflected by the accumulation of dead plant materials which results in peat formation. It is important to note that other factors such as nutrient availability and temperature are also potentially limiting to decomposition. In AMD, some of the

problems of decomposition of organic material may be overcome, as acid hydrolysis may increase the availability of food substrates for the micro-organisms.

Materials such as cellulose or starch are much more rapidly broken down than lignin and can be utilised in totally anaerobic conditions. For long-term maintenance of ARUM, it is essential that the microbial processes be fed. Readily available substrates such as potato waste will establish ARUM, but for long term maintenance, it will be necessary to occasionally add readily degradable carbon sources.

ARUM has been developed as a means of extending anaerobic microbial sediment processes throughout the water column. This is achieved through the establishment of a floating cattail mat installed over the organic sediment. Between the living organic root mat and the sediment, the AMD will be treated. By this means, the interception of AMD with reducing conditions may be optimised. The generation and maintenance of reducing conditions to treat the AMD are dependent on maintaining high rates of microbial activity which, in turn, depend on the presence of available substrates. Therefore, maintaining ARUM activity with a living vegetation mat is a logical avenue to pursue, as living plants would provide a continuous source of biodegradable organic matter.

2.1 Quantifying ARUM Activity

Thermodynamic considerations can accurately predict when a reaction will occur. If an energy yielding reaction is possible, there is generally an organism present that can carry that process out (Zehnder and Stumm, 1988). Much work has been devoted to enumeration of bacteria, based on their ability to grow on media selective for a particular taxonomic group, which is the case for sulphate-reducing bacteria.

It is becoming accepted that, in nature, bacteria do not grow in homogenous media but in biofilms, where chemical gradients determine the ability of bacteria to survive and function in specific niches. The interactions between micro-organisms and the reactions they carry out are of paramount importance. For example, the decomposition of organic material represents a complex series of microbial interactions, none of which can proceed without the previous or concurrent reactions taking place. In sulphate-reducing systems, H_2 utilising bacteria, which maintain H_2 concentrations sufficiently low to allow other processes to proceed at the appropriate E_h are essential (Zehnder and Stumm, 1988).

Local micro-niches such as an individual biofilm can provide conditions for processes to take place which would not be possible in the bulk media. The elegant studies with microelectrodes by Jorgensen's group (Kuhl and Jorgensen, 1992) have shown that sulphate reduction occurs in biofilms growing in aerobic (trickling filters) environments. In such biofilms, sulphur is tightly cycled (oxidation/reduction) within the biofilm and may have little effect on bulk medium changes. However, sulphate-reducing bacteria would be isolated in such circumstances. Sulphate reduction has also been found at significant rates in oxic sediments (Jorgensen and Bak, 1991). These findings indicate that microbial activity cannot be appropriately measured by isolating the organisms, and the measurements in the bulk solution do not reflect microbial activity, but are the result of the microbial ecosystem.

A further shortcoming of working with individual bacterial groups, as suggested by the traditional approach, is indicated by the recent findings that bacteria are very versatile in the reactions they carry out. For example, sulphate-reducing bacteria can oxidise sulphide as well as disproportionation (simultaneous oxidation and reduction) of thiosulphate (Jorgensen and Bak, 1991). Thiobacilli, which have been reported as the key culprits of AMD generation (through sulphide oxidation), may in some anaerobic circumstances reduce Fe^{3+} (Suzuki et al., 1990; Pronk et al., 1992). Sulphate-reducing

bacteria may be the same organisms which carry out Fe^{3+} reduction (Coleman et al., 1993).

A recent contribution to the field of microbial ecology is the concept that genetic information, in the form of plasmids, may fairly readily pass from one organism to another in nature and confer the ability to carry out particular processes to a wide variety of bacterial taxa (Trevors et al., 1986). All these observations point to the fact that enumeration of a particular 'class' of bacteria is a dubious practice and probably not worth the time and money. Unfortunately, a significant effort was extended during the third year of ARUM development to quantify and identify sulphate reducers, at the request of the reviewers.

In understanding and quantifying the microbial ecology of ARUM, we have therefore chosen the chemical approach. Analysis of the chemistry of an environment, particularly the E_h and pH, can indicate what microbial processes are expected. With this information at hand, it will be possible to formulate models for the design of ecosystems to treat particular AMDs and derive the carbon requirements for the system.

3.0 DESCRIPTION OF THE MAKELA SYSTEM

The Test Cell System was set up in the fall of 1989. It is about 100 m long by 20 m wide. The system has evolved throughout the project, to the present configuration (as documented in Table 1). The system is comprised of four cells, Cells 1 to 4, with two small holding ponds A and B (which were originally intended to supply nutrients if needed) located after Cell 2 and between Cells 3 and 4 (Schematic 1).

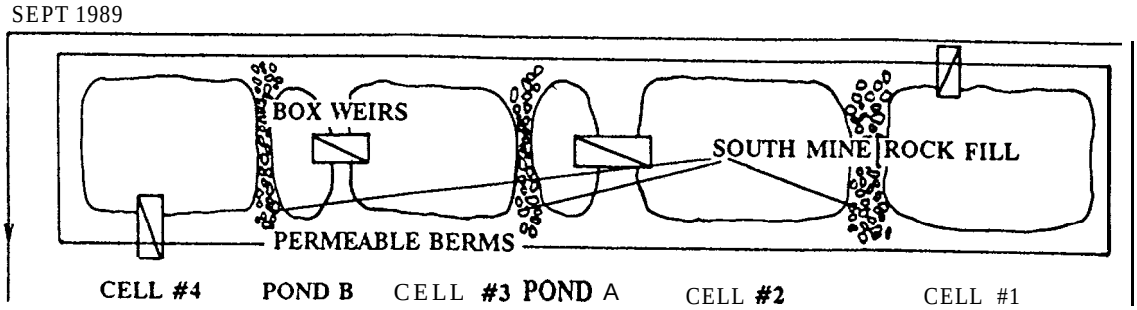
The dimensions of the system and estimated retention times (times to replace volume) are summarised in Table 2. Prior to the establishment of flow control, which was achieved through installation of a control valve between Cell 2 and Pond A (Stn 6) in May 1991, flows were quite variable. An estimated average flow of 40 L/min gave the system a retention time of only 8.53 days. The flow was too high, preventing complete oxidation of the seepage. Precipitation of Fe^{3+} hydroxide occurred throughout the system, which coated the organic material added for the microbial populations. With a flow rate of 1 L/min, the retention time is 168 days for Cells 1 and 2 and 131 days for Cells 3 and 4.

The actual retention time of water entering and leaving the system is less than the calculated time, as water passes through as surface sheet flow and some ground water is added throughout the system. Due to slumping of the main tailings dam, the diversion ditch, which controls the level in the cell system, required replacement. Thus flow control was lost briefly in 1991, affecting the flow and quality of water entering and leaving the system. The system has since settled down. Once the cattail rafts were in place (summer 1991), sheet flow was reduced.

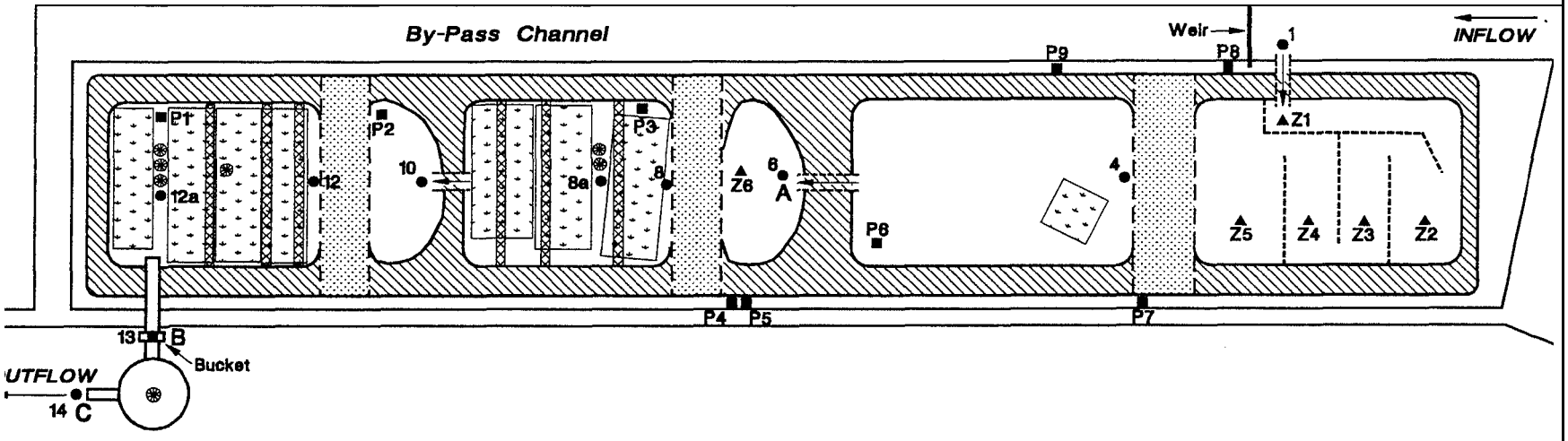
In 1992, the only changes to the system involved replanting the cattail rafts in Cell 3, adding alfalfa pellets to the Cell 3 cattail rafts, and adding potato waste to Cell 4 sediment.

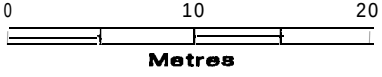
Table 1: Flows and modifications of the Test Cell System

The Test Cell System after construction



Date	Flow L/min	Modifications
August 1989	0-100	System constructed
October 1989	0-100	ARUMators installed
November 1989	0-100	Erosion blanket/gravel
May 1990	40	Weirs replaced with pipes Amendment curtains in Cell 4
July 1990	4-5	Control valve installed Amendment curtains in Cell 3 Baffles in Cell 1 to help Fe settling Decomposition bags in Cell 3
June 1991	0	Bypass ditch filled-system closed
July 1991	1	System reopened Prototype cattail raft installed in Pond B
August 1991	1	Cattail rafts in Cells 3 & 4
October 1991	0	System closed for winter
May 1992	1	System opened
June 1992	1	Alfalfa pellets added to Cell 3 rafts
July 1992	1	Sphagnum added to cattail rafts Cattail rafts replanted
August 1992	1	Potato waste added to Cell 4
October 1992	0.1	System closed for winter





LEGEND

- Water sampling location
- Piezometer
- A — Flow measurement location
- Precipitate bucket
- Aerator
- Amendment curtain
- Baffle
- Culvert
- Floating cattail raft
- Impermeable dam
- Permeable dam

Boojum Research Ltd.	
MEND PROJECT Makela, Sudbury. Ontario	
MAKELA TEST CELLS	
Date: March, 1993	Schematic: 1

Table 2: Makela Test Cell System
Volumes and retention times

Cell	Volume (m3)	Retention time (days) @1 L/min	@40 L/min
Cell 1	128	89	2.22
Cell 2	114	79	1.98
Pond A	36	25	0.63
Cell 3	86	60	1.49
Pond B	26	18	0.45
Cell 4	102	71	1.77
Overall	492	342	8.54

Flow rates at Stn 6 and Stn 13 are shown in Figure 1. Flow rates at Stn 13 were similar to those at Stn 6, indicating that little ground water entered the ARUM cells, certainly much less than the flow through the system. Any ground water entering the system would undoubtedly be seepage water and therefore add to the loading which to be treated. In Table 3, the concentrations of relevant elements in water sampled from piezometers I-3 are summarized. These piezometers are located in and above Cells 3 and 4, and water sampled from them would most likely represent ground water which enters the system. Thus, the putative ground water entering these cells has negative E_m values and acidities ranging from 190 to 620 mg/L equiv. of $CaCO_3$. The nickel concentrations ranged from 1 .0 to 5.5 mg/L. Sulphur concentrations varied from 413 to 966 mg/L.

Fig. 1: Stn 6 and Stn 13
Flow Rates, 05/91 - 10/92

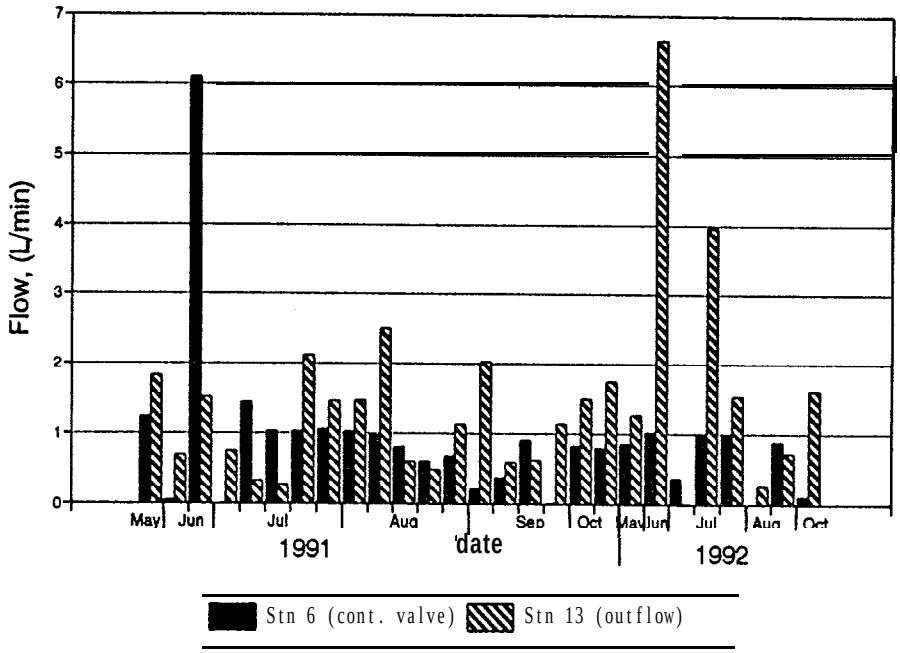


Table 3: Chemistry of piezometers around ARUM cells

Date		10-May-90			7-Jul-90			22-Nov-90			5-Jul-91		
Piezo		P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
Temp.	C	17	17	17	20	21	23	6	6	6	23.9	23	24
pH	units	7	6.78	7.04	6.54	8.37	6.34	6.05	5.73	5.77	6.61	6.42	6.4
Cond.	uS/cm	2680	2720	2970	2760	2805	3080	1900	1750	1700	2580	3500	1800
Em	mV				-10	130	107	-251	-224	-225	39	-48	14
Alkalinity	mg/L										80	190	110
Acidity	mg/L							620	320	650	370	260	190
Al	mg/L	<0.01	0.02	<0.01	0.12	0.2	0.4	<0.01	<0.01	<0.01	8	8	5
Ca	mg/L	592	771	746	592	757	840	442	513	452	320	357	213
cu	mg/L	0.08	<0.01	<0.01	0.01	0.01	0.03	<0.01	<0.01	<0.01	<1	<1	<1
Fe	mg/L	<0.01	<0.01	<0.01	1.6	0.1	2.1	1.6	0.5	0.9	cl	Cl	<1
K	mg/L	8.7	8.6	55	6.4	9.6	77	<0.01	3.1	48	13	29	27
Mg	mg/L	273	257	283	227	172	181	174	133	138	155	116	81
Mn	mg/L	2.2	44	28	1.5	41	29	0.6	34	17	cl	33	10
Na	mg/L	180	163	201	198	140	138	160	119	140	113	83	66
Ni	mg/L	1.9	5.5	3.7	0.3	2.1	0.8	0.3	1.8	0.4	<1	2	<1
S	mg/L	805	951	966	741	782	818	525	562	511	413	438	250

3.1 Sampling Stations for System Chemistry

The data presented here summarise the chemistry of Test Cell water at 6 locations in the system (Schematic 1, page 14). Stn 1 is the seepage entering the system. Stn 4 is at the entry to Cell 2. Stn 6 is in Pond A and water samples from there represent the chemical changes taking place in Cells 1 and 2. Stn 13 is the outflow point from Cell 4, and the point where water leaves the ARUM treatment system. Stn 8A and Stn 12A are located in Cell 3 and Cell 4, respectively. At these locations, vertical profiles of the ARUM water chemistry (which will develop from the bottom upwards) are recorded.

A total of 9 piezometers were installed around the system (Schematic 1, page 14). They were monitored to assess ground water level changes and to sample seepage entering the treatment cell. Piezometers 1 to 3 are relevant with respect to addition of contaminant loadings to Cell 3 and 4.

Comparing Stn 1 (the inflowing seepage) with Stn 6 (Pond A) chemistry indicates the overall effects of Cells 1 and 2 (precipitation and oxidation cells). Changes between Stn 6 and Stn 13 indicate the effects of Cells 3 and 4 (ARUM cells).

Field measurements of pH, conductivity, E_7 and acidity, which are the essential criteria for assessing changes in AMD chemistry are summarized for Stn 1, Stn 6 and Stn 13. The ICAP analyses of filtered, acidified water samples for the same stations are summarised as concentrations and loadings for aluminum, copper, iron, sulphur and nickel. The results are discussed in Section 4. Loadings show more clearly than concentrations, the overall effects of the Cell system on the quality of seepage water, and hence, they are used to assess the treatment capacity of the ARUM system. Loadings for each station were determined by multiplying the concentration of an element by the measured flow rate at the control valve (Stn 6) on the same day.

In utilizing loadings for assessment, it has to be recognized that, at a flow rate of 1 L/minute, the water sampled at Stn 6 would not leave Stn 13 on that day. More intensive monitoring of the system would have been required to trace one batch of water through the treatment process. Unfortunately, economic restraints prohibited the required sampling periods.

3.2 Field and Laboratory Methods

Standard methods were used for field chemistry. Redox potential, E_m, and pH were measured with portable meters such as Corning Model 103, with Fisher E_h electrodes. Conductivity was measured with a YSI Model 33 or Orion (WTW) 140. The pH meter was calibrated with buffers after every 5 to 10 measurements.

E,, the measured electrode potential 'Eh meter' is converted to an E_h value standardised for 25 °C from the following formula:

$$E_h\ (mV) = E_m\ (mV) + (241 - 0.66(T - 25))$$

where T is the measured temperature (°C). Redox potential is also affected by pH. E_h is corrected for pH by the following formula (Wetzel 1983):

$$E_7\ (mV) = E_h\ (mV) + 58(pH - 7)$$

Water samples were filtered through 0.45 µm cellulose acetate filters in the field. Acidity and alkalinity were determined by manual titrations in the field. The filtered, acidified sample was accompanied by a duplicate sample which was kept cool for determination of changes which might take place after collection. In the laboratory, for the last two years, titrations have been carried out with a Beckmann Auto-titrator Titrino and E,, pH and conductivity remeasured with the same instruments used in the field. The ARUM treated water had stable chemistry, i.e. no changes were found after several months of storage.

3.3 Internal quality control

Detailed field sampling methods and the storage and handling of samples are summarised in MEND summary report, February 1990. This document also describes in detail methods used for determinations of pH, conductivity, acidity and alkalinity. Microbiology and analytical chemistry methods used over the four year duration of the project are given in each of the annual reports referenced in the introduction.

3.4 External Quality Control (Quality Assurance)

Metal concentrations in solids and water were carried out by ICAP (Inductively Coupled Plasma Spectrophotometry), U.S. EPA Method No.200.7 at certified laboratories. Anions were determined by a number of methods, see Appendix. The QA/QCs of EPL and X-Ral are enclosed. To assure the validity of the results, blanks and standards were sent together with field samples. These samples were packaged and marked like field samples.

Standards with different concentrations of metals were sent (0.1, 1, 10, 100, 1000 mg/L of metals) every few months. U.S. National Bureau of Standards 1645 (River Sediment) and 1571 (Orchard Leaves) samples were sent as solid standards.

In 1991, 12 standards were sent to Chauncey laboratories, and 24 to X-Ral. In 1992, 4 Boojum standards were sent to X-Ral, and 18 standards were sent to EPL. The standard analyses were not consistently accurate for any of the three laboratories. The quality control data for the blank analysis is available on request.

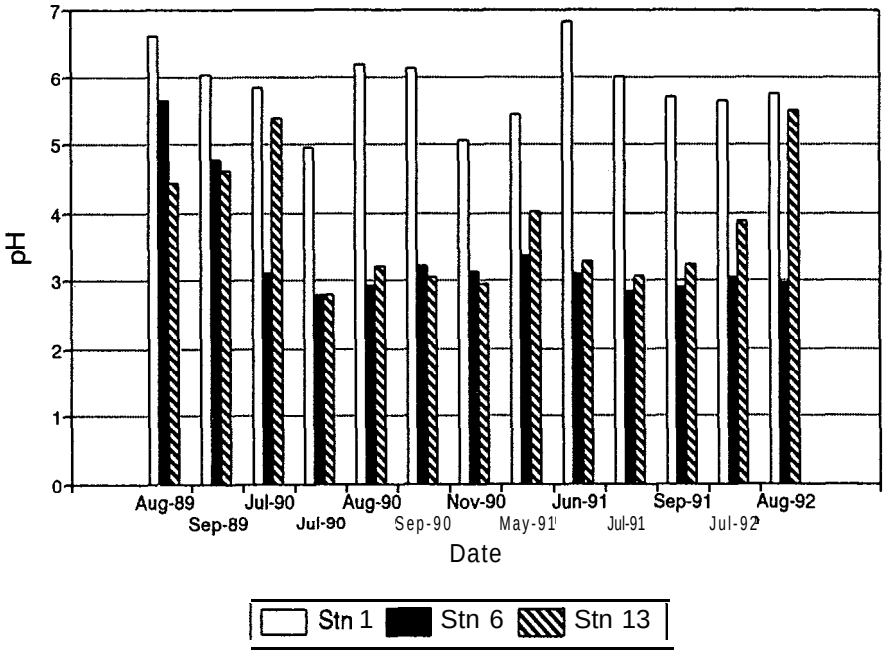
These analytical results obtained from the various laboratories were subjected to cation and anion balances to determined major errors in the results. Samples with obvious inaccuracies or anomalies were submitted for reanalysis to the respective laboratory to obtain the actual result.

4.0 WATER CHARACTERISTICS

4.1 Changes in Water Quality Through the Test Cell System

The pH of seepage water entering the Test Cell System (Stn 1) ranged from 4.76 to 6.83, but was usually between 5.5 and 6.0 (Figure 2).

Fig. 2: Stn 1, Stn 6 and Stn 13
pH, 08/91 - 08/92



There was a slight, but clear, decreasing pH trend from 1989 to 1992 in the incoming seepage water. Stn 6 remained consistent from 1990 onward, at a pH between 3 and 3.5. The 1989 pH values for Stn 13 were around 4.5 (Figure 2). During 1990, a decline to around pH 3.0 was observed at Stn 6 due to the precipitation of $Fe(OH)_3$. Such readings continued through 1991. Higher values were observed in 1992 with a peak of 5.52 on July 21.

Acidity fluctuated considerably without any clear trend, but, from 1991 onward, water with lower acidity left the system (Figure 3a). The mean value for all readings at Stn 1 was 581 mg/L equiv. of $CaCO_3$. With the decline in flow rate in 1991, there was a considerable reduction in acidity loading to the system. Loadings in 1992 ranged from 131 to 1212 g $CaCO_3$ equiv./day (Figure 3b). By August 1992, acidity was reduced by 80 % in Cells 3 and 4.

E_7 values around + 100 to + 250 mV were typically found in the seepage water at Stn 1, suggesting that oxidising conditions prevailed throughout the system (Figure 4). There was no clear trend in E_7 values at Stn 6 during the 1989-1992 period (Figure 4). Water leaving the system (Stn 13) showed some decline in E_7 attributable to ARUM.

Conductivity (indicative of total ion loading and dissolved materials) at Stn 1 fluctuated considerably between sampling dates (Figure 5). Substantial rain storms would result in reduced conductivities. Thus, this parameter provides a check on rain dilution. Values were usually in the range 1700 to 3200 μhmos/cm from 1989 until the fall of 1990. Lower values were found during the winter period, a period when little flow entered the system. At the end of the monitoring period, in the summer of 1992, conductivity rose to over 4000 μmhos/cm. There were no clear changes in conductivity through the system.

Aluminum concentrations were below detection limits (< 0.7 mg/L) for much of 1989 and 1990 (Figure 6a). In 1991 and 1992, there were increases in aluminum concentrations at Stn 1 and Stn 6. These increases were attributable to the dissolution of clay minerals in the berms, which were mobilized during construction and repair activities. In 1992, with the functioning ARUM system, the loadings at Stn 6 were almost entirely removed by the time water passed through Stn 13 (Figure 6b). The increase in pH resulted in the precipitation of aluminum. Adsorption of aluminum to surfaces in Cells 3 and 4 was also possible.

Fig. 3a: Stn 1, Stn 6 and Stn 13
Acidity, 08/90 - 08/92

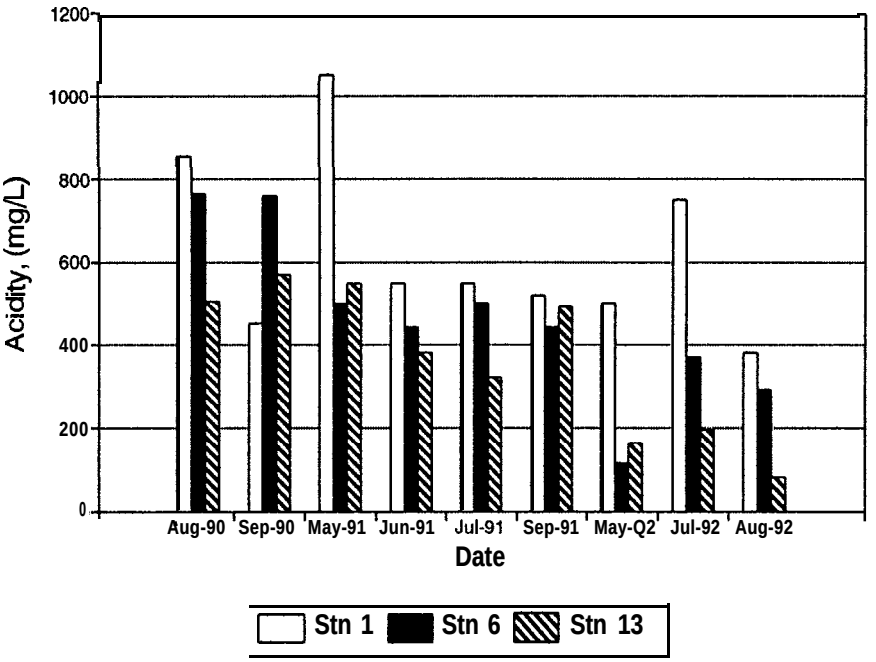


Fig 3b: Stn 1, Stn 6 and Stn 13
Acidity Loadings, 07/91 - 08/92

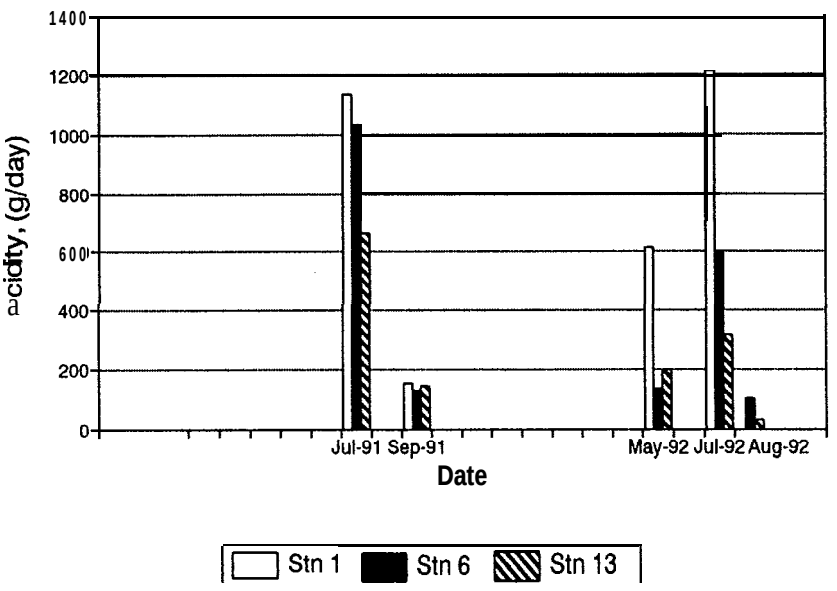


Fig. 4: Stn 1, Stn 6 and Stn 13
Redox Potential (E7), 07/90 - 08/92

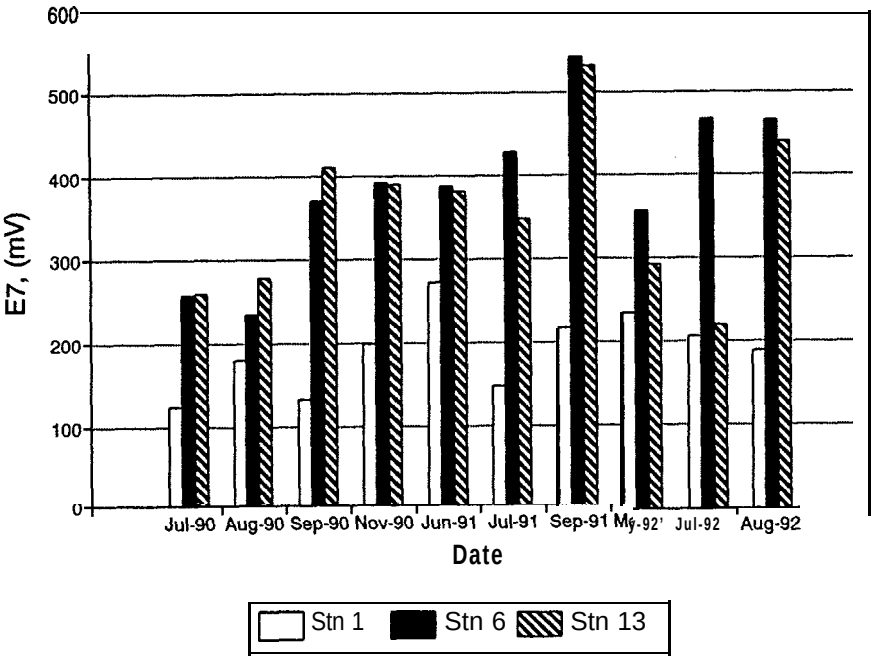


Fig. 5: Stn 1, Stn 6 and Stn 13
Conductivity, 08/89 - 08/92

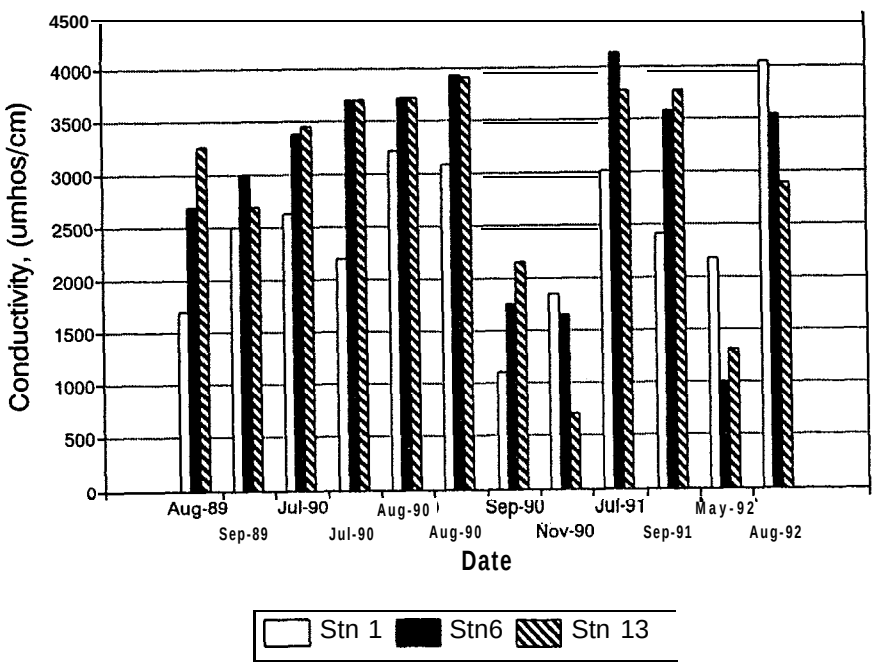


Fig. 6a: Stn 1, Stn 6 and Stn 13
Aluminum Concentration, 09/89 - 08/92

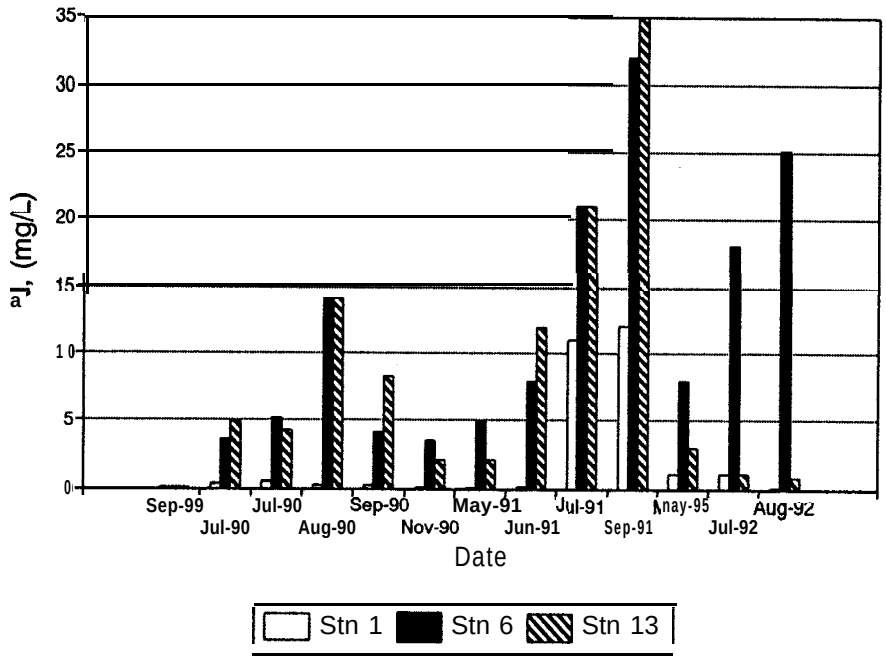
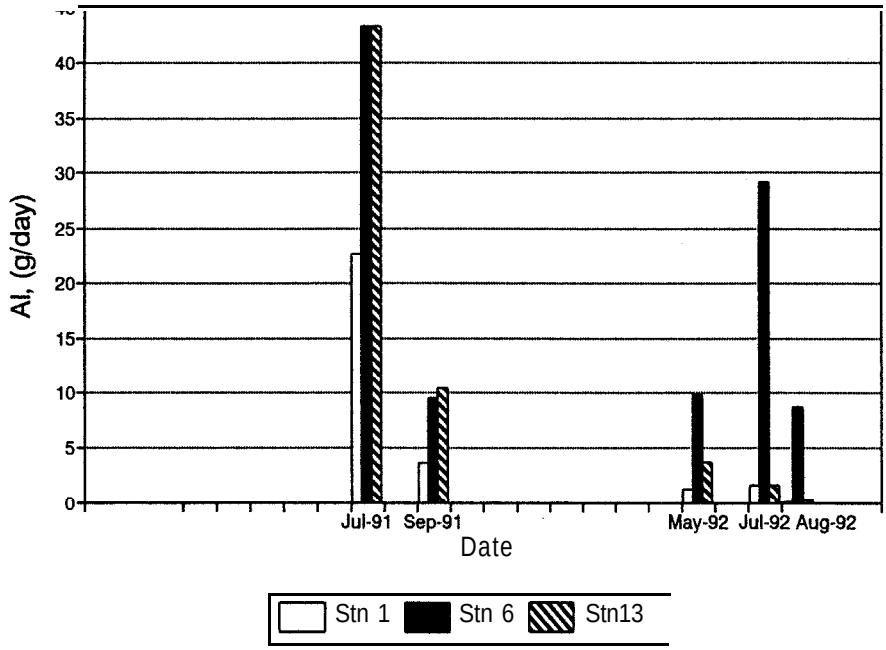


Fig. 6b: Stn 1, Stn 6 and Stn 13
Aluminum Loadings, 07/91 - 08/92



Copper concentrations were generally < 1 mg/L (detection limit for July 1991 to July 1992 was 1 mg/L) at Stn 1, except for a sharp peak in the spring of 1990 (Figure 7a, page 27). Copper concentrations at Stn 6 were always higher than at Stn 1, ranging from around 3 mg/L to as high as 5.5 mg/L in the summer of 1992. At Stn 13 the copper concentrations were again at or below the detection limit of 1 mg/L. The copper increases at Stn 6 were a result of the dissolution of evaporites which formed on the side of Cell 2. In Figure 7b (page 27) the loadings of copper at Stn 6 were as high as 7 g/day. There was a dramatic decline between Stn 6 and Stn 13 in 1992 indicating that the ARUM cells were removing copper.

Iron concentrations in the seepage showed a seasonal trend, with high concentrations in the summer around 250 to 300 mg/L, and lower concentrations in winter, ranging from 50 to 150 mg/L (Figure 8a, page 28). There was a dramatic decline in iron at Stn 6 (Figure 8a, page 28), which commenced in the summer of 1990 and continued until 1992 when concentrations were < 17 mg/L. With both high and low flow rates, most of the iron entering the system precipitated as $Fe(OH)_3$ in Cells 1 and 2. In 1989, iron concentrations increased from Stn 6 to Stn 13 (Figure 8a, page 28). Indeed, values exceeded those of the seepage water (Stn 1). This may be attributed to the dissolution of $Fe(OH)_3$ precipitates with the decline in pH. Iron loadings at Stn 1 were around 0.5 kg/day in 1991 and as low as 0.08 kg/day at the end of the 1992 summer season (Figure 8b, page 28).

Nickel concentrations of water entering the system ranged from 16 to 37 mg/L (Figure 9a, page 29). Ni loadings ranged from 4.4-60.3 g/day after the establishment of flow control in 1991 (Figure 9b, page 29). Nickel concentrations were always higher leaving the system, than entering, until ARUM became operational in the summer of 1992. At Stn 6, nickel concentrations were generally similar to those at Stn 1 until 1992, when a steady decline became apparent (Figure 9a, page 29). Before 1992, nickel concentrations were consistently greater in the cells than in the water entering the system. The same was the case for copper. Both these metals accumulated along the

sides of Cell 2 during the summer due to evaporation. At Stn 13, except for one exceptionally high reading in December 1989, nickel concentrations were similar to those at Stn 6 through 1991 (Figure 9a, page 29). In 1992, a dramatic decline was apparent at Stn 13. In July of that year, loadings declined from 70 g Ni/day at Stn 6 to 2 g/day at Stn 13. As for copper, it is likely that removal was through adsorption to precipitates and organic amendment surfaces.

Sulphur concentrations were generally in the range 600-1000 mg/L (Figure 10a, page 30), with loadings of 298-1742 g/day (Figure 10b, page 30). As with iron, sulphur concentrations exhibited a seasonal trend, with low concentrations in the winter and high concentrations in the summer. The overall patterns of sulphur concentration at Stn 6 and Stn 13 were very similar to that at Stn 1. In 1992, however, sulphur removal was apparent at both stations. Overall, the system removed 35 to 53 % of the sulphur in 1992. Sulphate reduction was occurring in Cells 3 and 4 and much of the removal of sulphur was attributable to precipitation of Fe^{2+} sulphides.

Fig. 7a: Stn 1, Stn 6 and Stn 13
Copper Concentration, 09/89 - 08/92

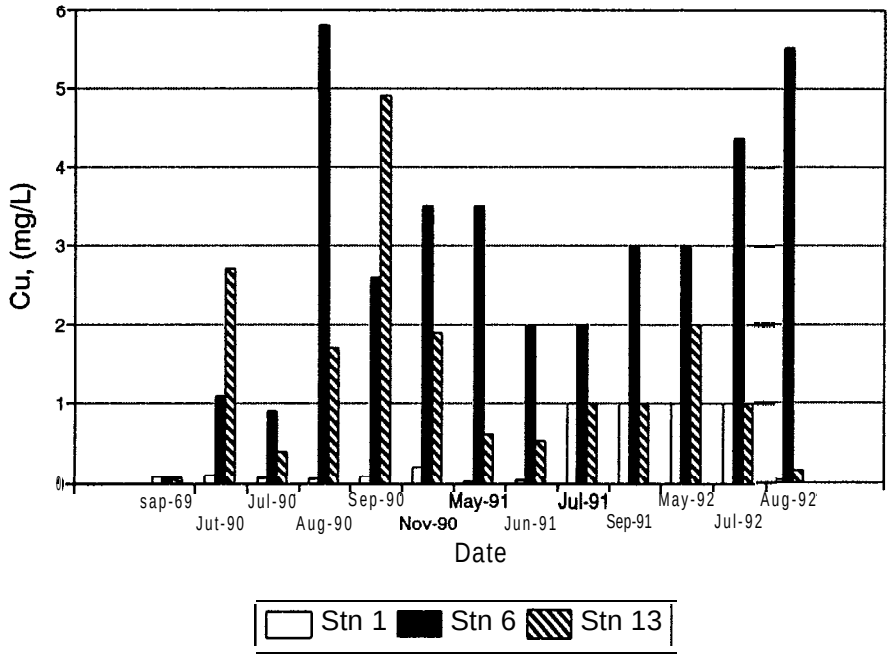


Fig. 7b: Stn 1, Stn 6 and Stn 13
Copper Loadings, 07/91 - 08/92

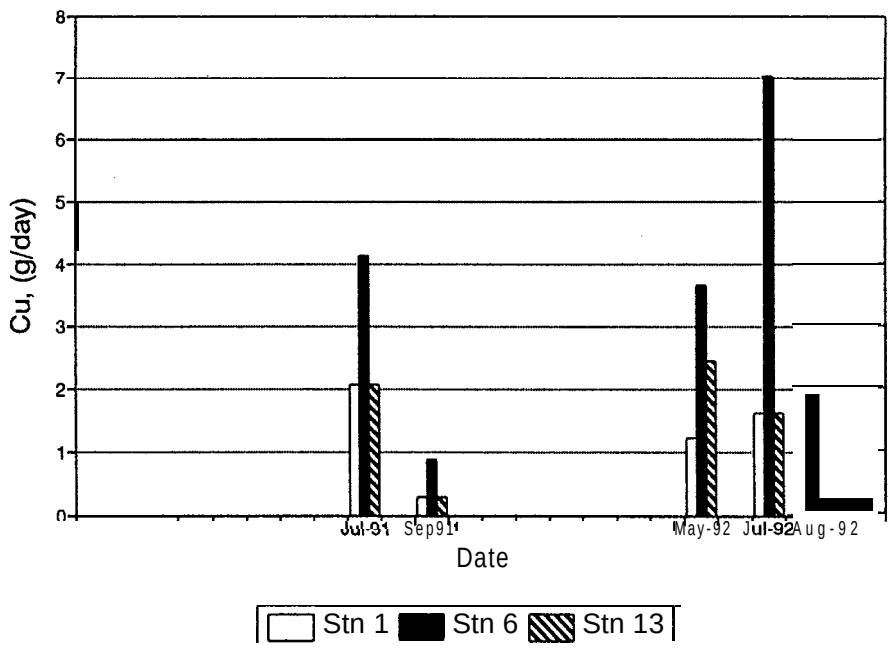


Fig. 8a: Stn 1, Stn 6 and Stn 13
Iron Concentration, 09/89 - 08/92

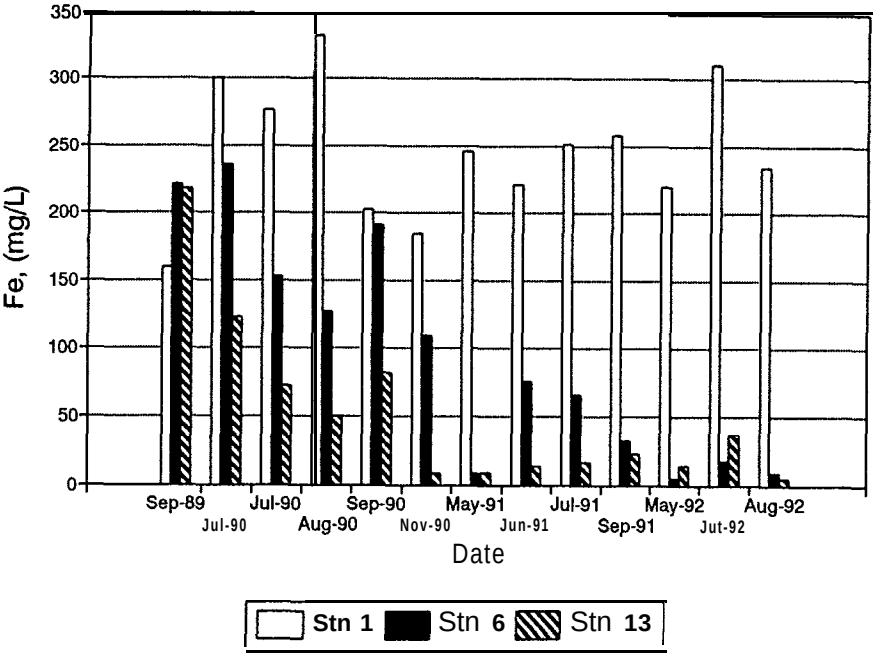


Fig. 8b: Stn 1, Stn 6 and Stn 13
Iron Loadings, 07/91 - 08/92

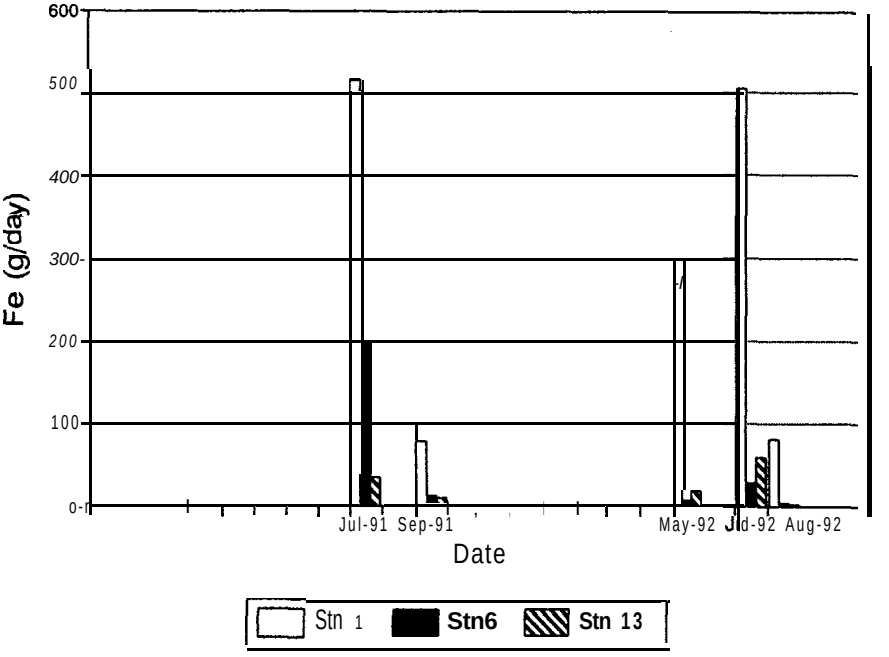


Fig. 9a: Stn 1, Stn 6 and Stn 13
Nickel Concentration, 09/89 - 08/92

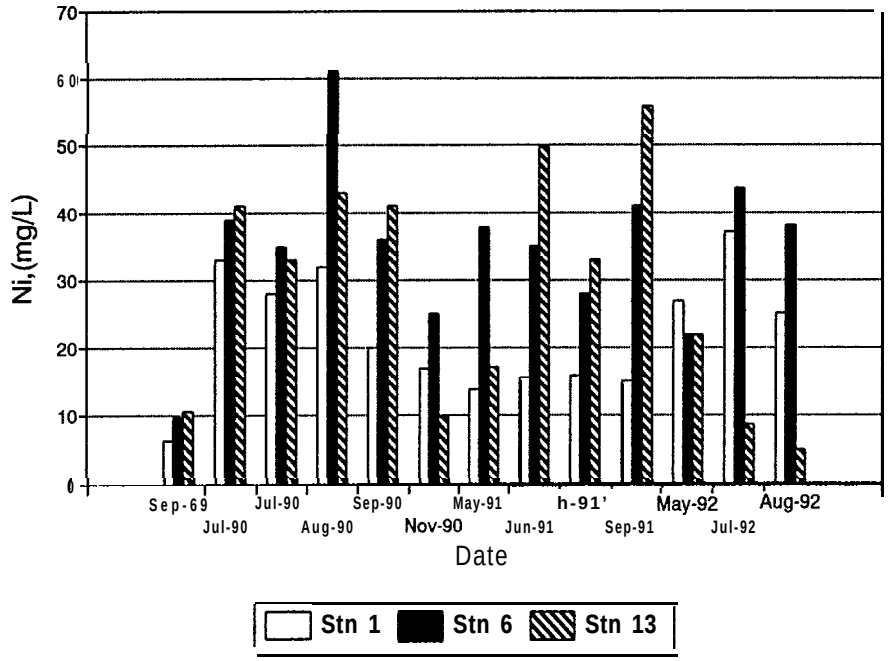


Fig. 9b: Stn 1, Stn 6 and Stn 13
Nickel Loadings, 07/91 - 08/92

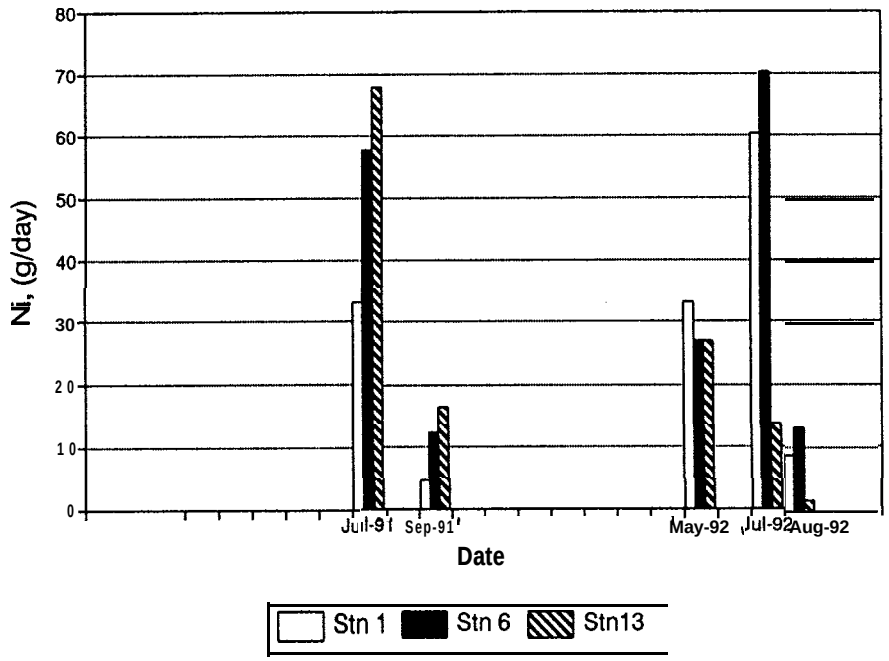


Fig. 10a: Stn 1, Stn 6 and Stn 13
Sulphur Concentration, 07/90 - 08/92

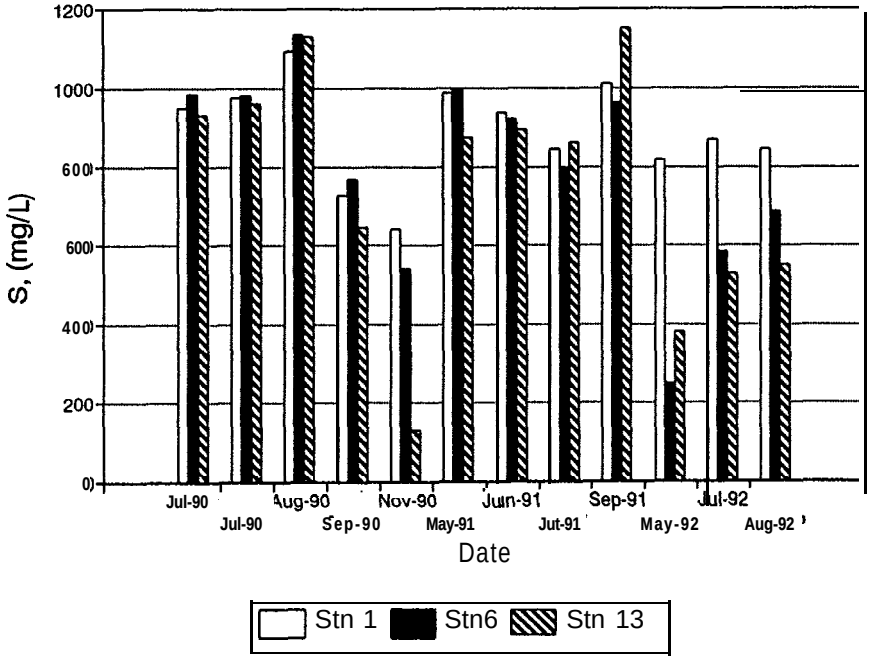
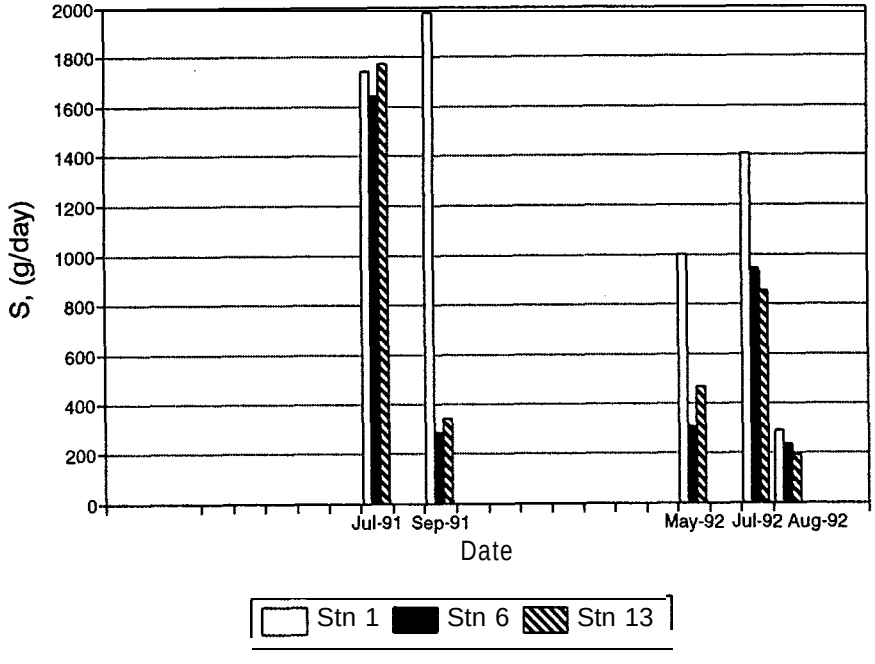


Fig. 10b: Stn 1, Stn 6 and Stn 13
Sulphur Loadings, 07/91 - 08/92



In February 1993, further analytical data for samples collected on October 22, 1992 became available through INCO analytical services. These data are presented in Table 4, together with earlier 1992 data. The sampling locations within the Test Cell System are given in Schematic 1 (page 14).

In 1992, with flow control and a cover of floating cattails on the ARUM cells (Cells 3 and 4), the Test Cell System effectively removed acidity and metals from the Makela seepage water. The percentage removal of acidity and metals by the system are summarised in Table 5.

Table 4: Test Cell System - 1992 Loadings Stn 1, Stn 6, Stn 13

	July 1992 Flow 1.125 L/min			August 1992 Flow 0.24 L/min			October 1992 Flow 0.1 L/min		
	Stn 1	Stn 6	Stn 13	Stn 1	Stn 6	Stn 13	Stn 1	Stn 6	Stn 13
Temp. (C)	17.2	15.5	21.5	15	20.9	19.3	8.4	8.2	8.1
pH	5.65	3.05	5.52	5.76	2.96	3.34	5.7	2.94	6.14
Em (mV)	38	440	62	12	444	408	37	428	-18
Acidity (g/day)	1215	599	315.9	131	100	27.7	84.9	35.9	23.4
Al (g/day)	<1.62	29.2	<1.62	0.01	7.02	0.24	0.32	2.76	0.14
Cu (g/day)	<1.62	7.06	<1.62	0.01	1.9	0.05	0.03	0.4	0.001
Fe (g/day)	505	27.4	59	81.2	3.12	1.71	32	2.52	3.18
Ni (g/day)	60.4	70.6	14	8.71	13.2	1.75	3.66	4.2	0.46
S (g/day)	1408	948	855	292	237	190	122	86.7	60.1

Table 5 Test Cell System - Changes in Element Loadings, 1992

	Stn1-Stn6			Stn6 - Stn13			Stn1 - Stn13		
	July Change (%)	August Change (%)	October Change (%)	July Change (%)	August Change (%)	October Change (%)	July Change (%)	August Change (%)	October Change (%)
Acidity	-51	-24	-58	-47	-72	-35	-74	-79	-72
Al	NA	NA	NA	-95	-96	-95	NA	NA	-56
Cu	NA	NA	NA	-77	-98	-100	NA	+315	-96
Fe	-95	-96	-92	+215	-45	+26	-88	-98	-90
Ni	+17	+51	+15	-80	-87	-89	-77	-80	-88
S	-33	-33	-29	-10	-10	-31	-39	-39	-51

NA = not applicable, + = increase, - = decrease

The seepage entering the system (Stn 1) has an E_m a little over 0 mV. The water is oxidised in the precipitation cells (Cells 1 and 2). By Pond A (Stn 6), E_m values were in the range 428 to 444 mV on the 3 sampling dates in 1992. Redox values declined again in the ARUM cells. In October, a negative E_m value (-18 mV) was obtained for the first time in water leaving Cell 4, suggesting that reducing conditions previously confined to the vicinity of the amendments and cattail rafts had become established throughout the water column. ARUM was therefore able to function effectively.

In July, the pH of water entering the system (5.65) was almost the same as that leaving Cell 4 (5.52). However, there was a substantial decrease in acidity, from 1215 g $CaCO_3$ equiv./day at Stn 1 to 599 g $CaCO_3$ equiv. at Stn 6 (Pond A) to 316 g $CaCO_3$ equiv./day at Stn 13 (Cell 4 effluent). Fifty one percent of the acidity was lost in Cells 1 and 2 and 47 % of the remainder in Cells 3 and 4. Similar trends were observed in August and October. Overall, the system removed 74 %, 79 % and 72 % of the acidity on the 3 sampling dates.

In July and August, substantial amounts of aluminum were released to the water from the clay walls and floor of the cells as the pH dropped with $Fe(OH)_3$ precipitation. Most of this was removed in the ARUM cells, probably as $Al(OH)_3$ precipitate which will form as the pH rises. In October, there was less aluminum in the water leaving the system (0.98 mg/L) than entering it (2.2 mg/L). Copper showed a similar trend to aluminum with increases between Stn 1 and Stn 6. Before flow control was established, copper co-precipitated with iron and nickel. This copper would be re-released when the iron precipitation dropped the pH to 3. In the ARUM cells, copper can be removed by precipitation as copper sulphide. By October, the overall system was removing 96 % of the copper entering the system.

Iron was effectively removed from the seepage water in the precipitation cells (Cells 1 and 2). On two of the three sampling dates, there was no further net removal in Cells 3 and 4. A large pool of iron from $Fe(OH)_3$ precipitates accumulated in the ARUM cells

prior to flow control. At the bottom of the ARUM cells, considerable concentrations of iron were in solution (211 mg/L at 8a in Cell 3 and 298 mg/L at 12a in Cell 4 in October 1992). This was undoubtedly ferrous iron, as the E_m was negative. Overall, in 1992, the Test Cell System removed 88 to 98 % of the iron entering the system.

Nickel, like copper and aluminum, increased in concentration between Stn 1 and Stn 6 due to the dissolution of previously co-precipitated sediment material and evaporites. However, the ARUM cells were very effective in removing nickel. On the three sample dates, 80-89 % of the nickel was removed from the water in the ARUM cells. The water leaving the system in October contained only 3.17 mg/L, compared to 25.4 mg/L entering the system.

Sulphur loadings were reduced, both in the precipitation cells, and the ARUM cells. The reduction in Cells 1 and 2 may be partially attributed to the precipitation of $CaSO_4$. For example in October, the total concentration of calcium declined by 1.9 mmol between Stn 1 and Stn 6. Sodium declined from 103 to 68.9 mg/L between Stns 1 and 6. Since sodium solubility is very high, its concentration is not affected by chemical and biological reactions. As such, it is used as a tracer for determining dilution effects. Since sodium concentrations declined by 33 % between stations 1 and 6, it is likely that dilution from rain could fully account for the reduction noted in sulphur concentration.

The Test Cell System was consistent in its performance in the summer and fall of 1992. The October numbers were the best yet, with most of the acidity and heavy metals (aluminum, copper, iron, nickel and sulphur) removed. As long as flow within the system was low (1 L/min or less) and the iron was precipitated as $Fe(OH)_3$ in Cell 1, the ARUM cells operated very effectively.

4.2 Iron Precipitation in Cells 1 and 2

In the previous section, it was indicated that the tailings seepage undergoes oxidation which is associated with acid generation and iron hydroxide precipitation. To prevent the precipitation of iron hydroxide on the organic materials in the ARUM cells, it was necessary to provide a precipitation/ oxidation pond. This is the function of Cells 1 and 2 of the Makela system.

Between seepage inflow (Stn 1) and Stn 6, the pH is expected to drop from an average value around 5 to about 3. This is due to hydrogen ions generated during the precipitation of iron as $Fe(OH)_3$. In Cells 1 and 2, oxidising conditions developed rapidly, and were maintained through the monitoring period with E_7 readings usually around + 400 mV at Stn 6 (Figure 4, page 23).

A dramatic reduction in iron at Stn 6 commenced in the summer of 1990 and continued for the remainder of the project (Figure 8a, page 28). With both high and low flow rates, most of the iron entering the system precipitated as $Fe(OH)_3$ in Cells 1 and 2.

The iron loadings (Figure 8b, page 28) indicate that in 1991 and 1992, most of the iron entering the system was removed before Stn 6, i.e. in Cells 1 and 2. Percentage iron removal was calculated for the differences at Stn 4 (at the entry to Cell 2) and Stn 1. More than 94 % of the iron was removed before Cell 2 (Figure 11).

Five buckets were placed in Cell 1 to collect iron hydroxide precipitates, and sampled since the summer of 1990 to quantify the precipitate settling rates. The amount of precipitate in these buckets should give a reasonable estimate of iron removal capacity of the Test Cell System. Dry weight loadings in kg/day are given in Figure 12. The highest precipitate loadings were found, as expected, in the summer, when higher iron concentrations entered Cell 1. Precipitation rates of 5 kg/day to 8 kg/day were observed in 1991 and 1992.

Fig. 11: Stn 1 - Stn13
Change in Iron, 09/89 - 08/92

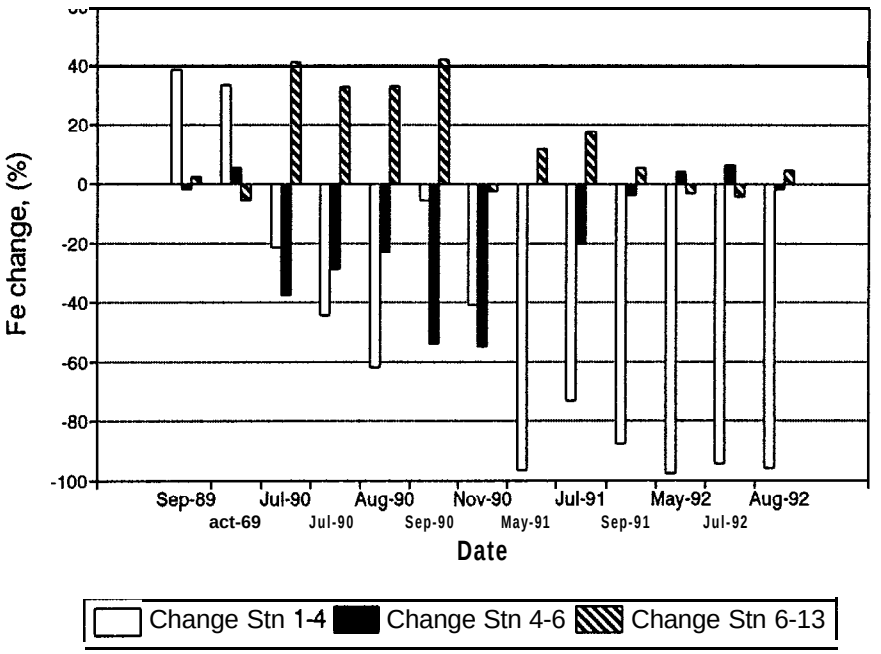
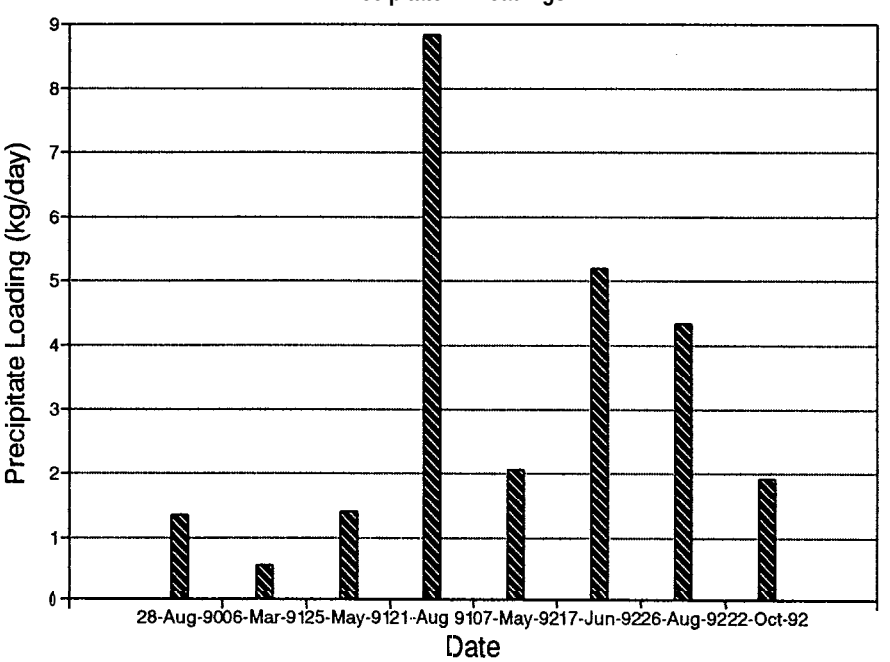


Fig. 12: Cell 1
Precipitate Loadings



During the winter months of 1991 and 1992, when the system was mostly shut down, precipitate in the buckets amounted to about 2 kg/day.

The volume/weight ratio of the precipitates has been determined. Bucket precipitates were placed in measuring cylinders and left to stand until a more or less constant volume had settled out (about 6 days). The final volume was divided by the dry weight. Details of the settling characteristics of the precipitate were presented in the June 1990 report.

A mean value of 10.1 mL of wet volume per gram dry precipitate was obtained for May 1992 and October 1992 samples. Using this value and the bucket surface area, it was calculated that about 1049 kg were accumulated in Cell 1 during the period between August 21, 1991 and August 26, 1992. This represents about 10.6 m^3 of precipitate accumulated over the year. At this rate, and with the same flow rates, it will take approximately 12 years to fill Cell 1 with precipitate.

The precipitate accumulation in Cell 1 should be reflected in the iron loadings. Based on changes in iron loadings, with an average flow of 1 L/min, about 462g/day should have precipitated. Since about a quarter or 25 % of the precipitate dry weight is iron, 2 kg of precipitate should have formed in Cell 1. However, in the buckets, the estimate ranged from 5 to 8 kg/day, which suggests, that a higher flow was entering Cell 1.

Although flow rates into Cell 1 were not easily measured, some attempts were made based on the tracking of an object through the inflow pipe. Assuming that all water passed through the pipe, the dimensions of the pipe were used to estimate a flow of 4-10 L/min. This was the flow estimate obtained when 1 L/min was passing through the control valve. The excess flow entering Cell 1 is seeping through the permeable dam alongside the cells, thus leaving Cells 1 and 2 through the bottom of the cell and the dam walls.

To effectively precipitate Fe^{3+} hydroxide from tailings seepages, retention times need to be long. With a summer average iron concentration (< 300 mg/L), and a flow ranging from 4 to 10 L/min, a retention time of 89 days (and the presence of baffles), effectively facilitated iron precipitation. With the higher flow rates and thus loadings, the calculated fill time for Cell 1 decreases to 5 to 10 years.

4.3 ARUMators in Cell 4

At the onset of the project, there was a considerable amount of concern about the AMD tolerance levels of the proposed natural, microbial processes. To simulate the stagnant flow conditions which would prevail in the deeper part of the sediment, ARUMators were set up. Details of the design and the contents have been reported in previous reports, along with results obtained. ARUMators are 200 L drums filled with flax and Cell 4 water and fitted with sampling ports so that changes in water chemistry from various locations in the drum could be monitored.

These ARUMators were batch systems which tested the ability of the amendments to treat seepage water. For the overall understanding of the ARUM process, a brief discussion of the results obtained in ARUMators 1 and 2 is given. Water samples taken from the surface, middle and bottom of ARUMators 1 and 2 are shown in Figures 13a to 13h and Figures 14a to 14h (pages 39-42), for the same parameters discussed for the Test Cell System. By June 1990, the pH in the ARUMators had risen throughout the drums to around 5. By July 1991, the pH increased to 7.5 (Figures 13a and 14a, page 39).

The acidity deceased from 600 to 100 mg/L equiv. of $CaCO_3$ over the same period in ARUMator 1 (Figure 13b, page 39), but in ARUMator 2 the decrease was more drastic: from 1,500 mg/L equiv. of $CaCO_3$ at the start to a reduction of 200 mg/L at the end of the measurement period (Figure 14b, page 39).

Reducing conditions (negative E_7 values) had been established in the ARUMators when first measurements were taken in the summer of 1990. In May 1991, the ARUMators received new seepage water from Cell 4 after being submerged during the high run-off period. Positive E_7 values were noted at that time in ARUMator 1, but redox conditions remained reducing in ARUMator 2 (Figure 14c, page 40). However, the new water additions did not change the pH or the acidity. Sufficient buffering capacity must have been available to accommodate the new seepage water.

Electrical conductivity decreased with time in both ARUMators slightly (Figures 13d and 14d, page 40). A massive increase in aluminum concentrations was noted with the entry of new water (Figures 13e and 14e, page 41). The new seepage must have redissolved the previously precipitated aluminum, since high concentrations were noted in the next measurement period in ARUMator 1. However, in ARUMator 2 increases were present immediately. Aluminum concentrations returned to the same low levels by the last measurement period.

Iron concentrations in both ARUMators dropped very quickly, and remained at very low levels throughout the observation period (Figures 13f and 14f, page 41). The same pattern noted for iron was observed for nickel (Figures 13g and 14g, page 42). For sulphur, the concentrations decreased slowly over the measurement period in both ARUMators (Figures 13h and 14h, page 42). In ARUMator 1, there were higher concentrations noted over the last measurement period, which could not be explained. Overall, the results from sampling the ARUMators proved conclusively that microbial treatment of AMD is possible.

Fig. 13a: Arumator 1
pH

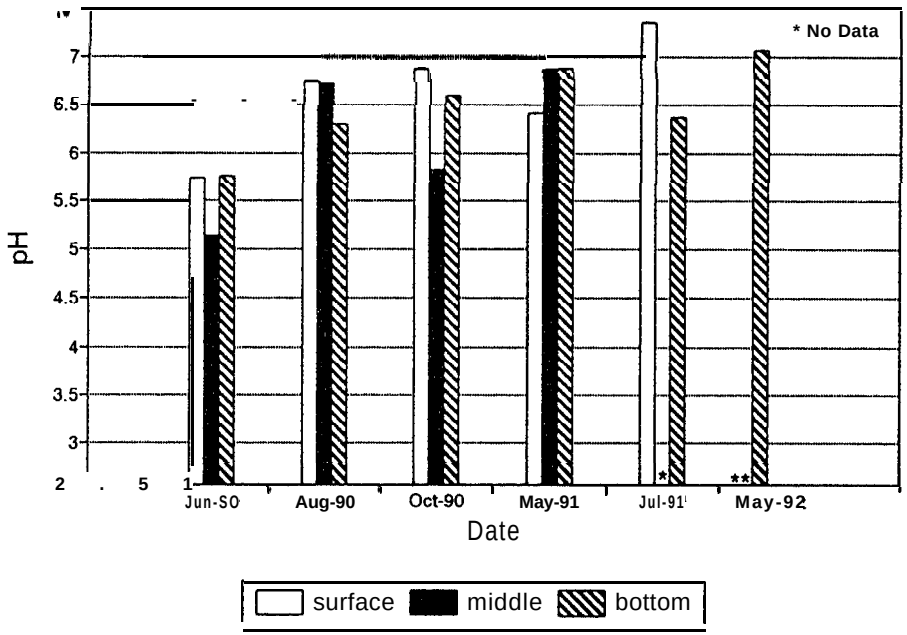


Fig. 14a: Arumator 2
pH

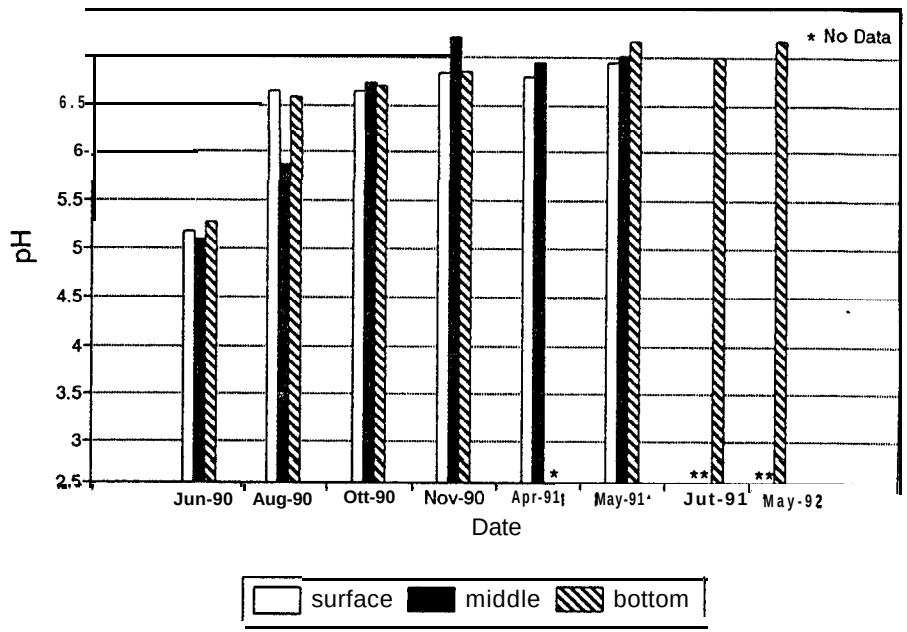


Fig. 13b: Arumator 1
Acidity

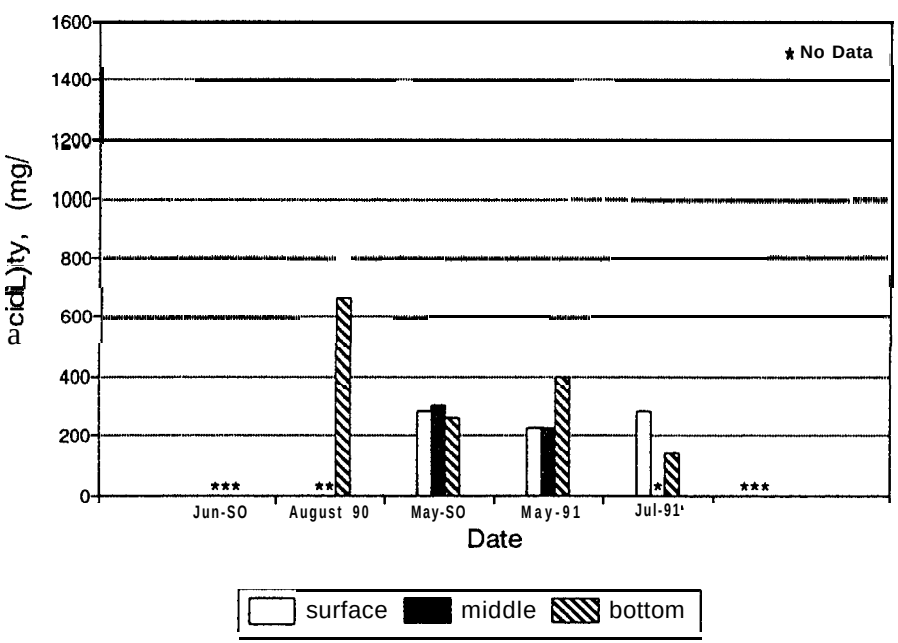


Fig. 14b: Arumator 2
Acidity

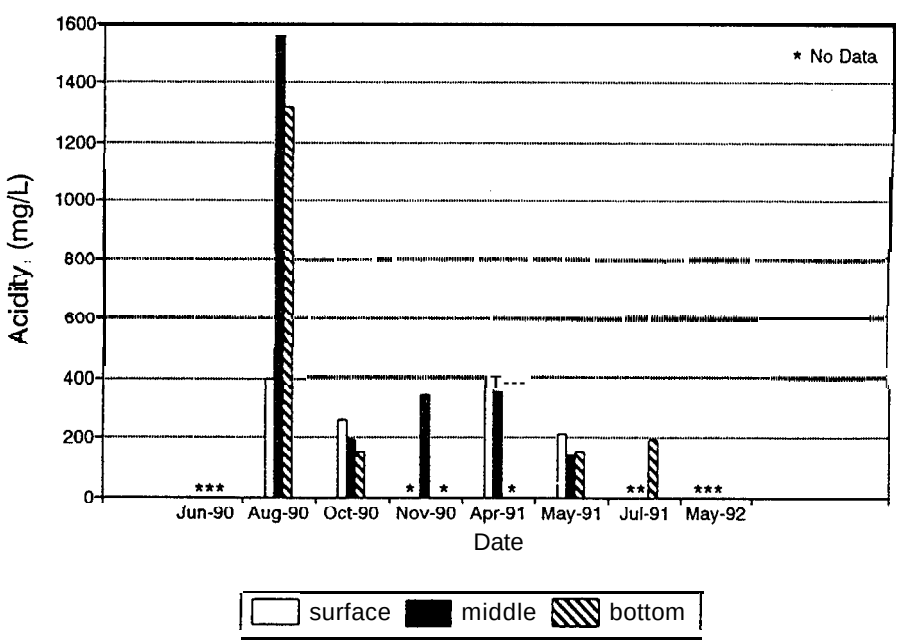


Fig. 13c: Arumator 1
Redox Potential (E7)

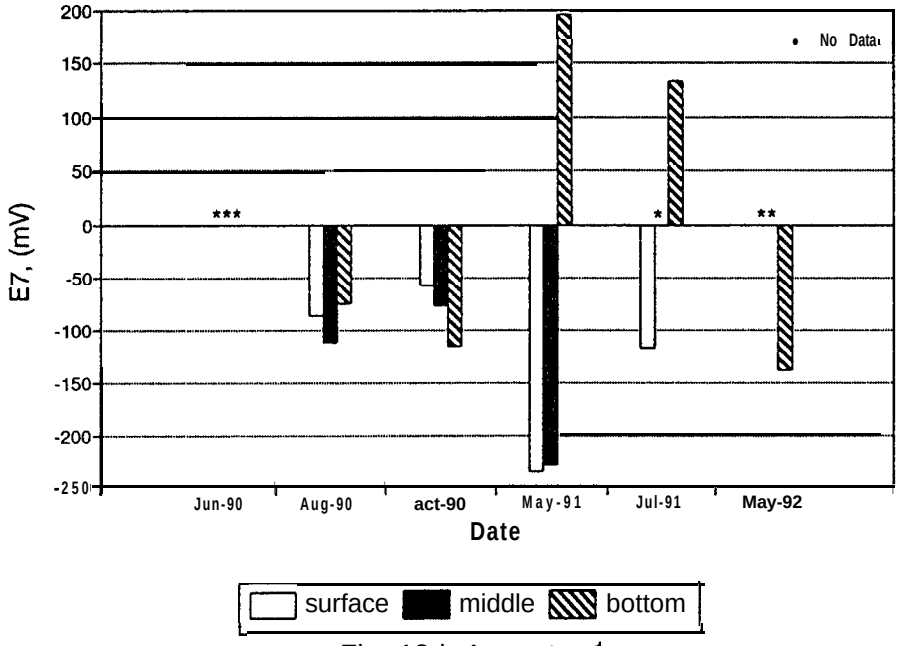


Fig. 14c: Arumator 2
Redox Potential (E7)

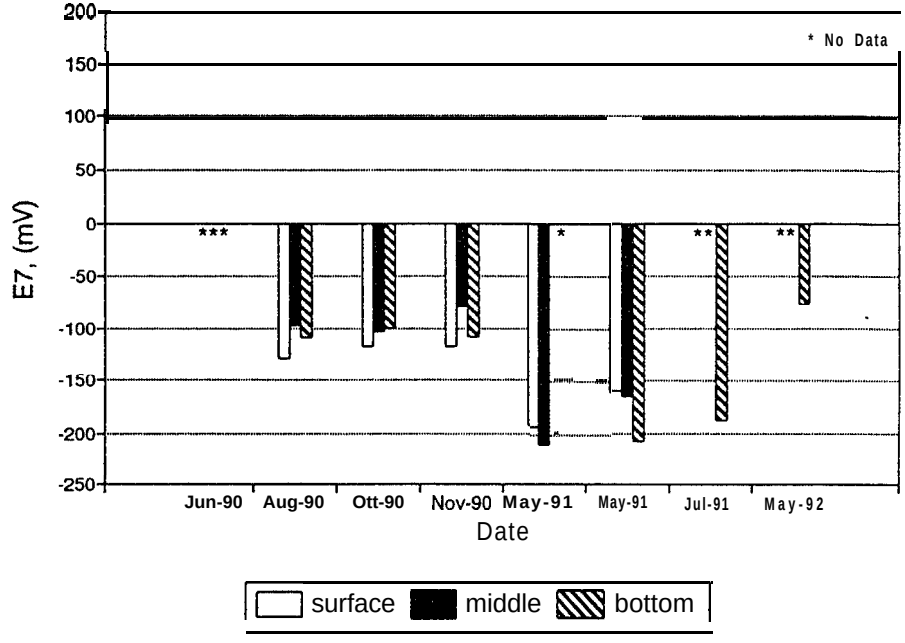


Fig. 13d: Arumator 1
Conductivity

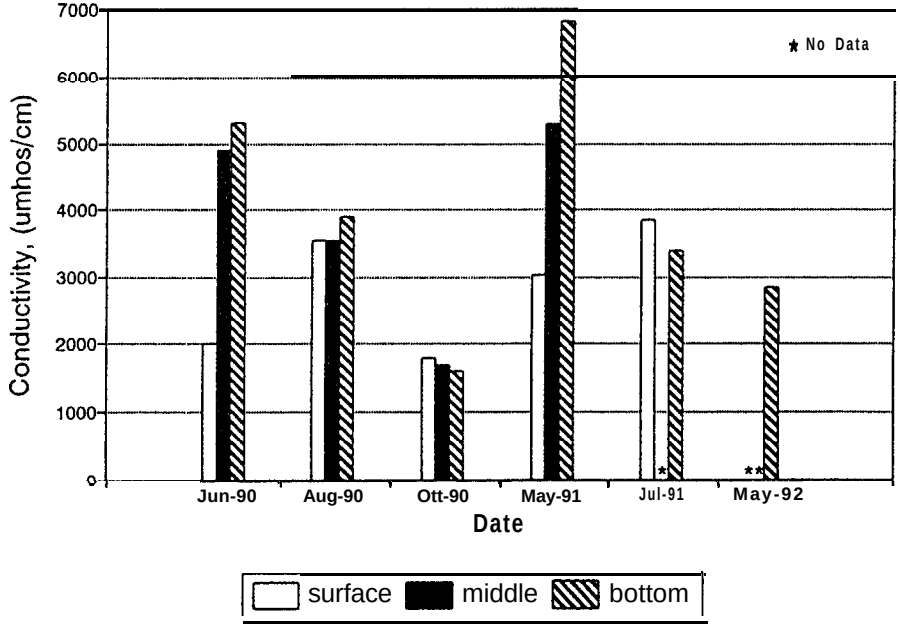


Fig. 14d: Arumator 2
Conductivity

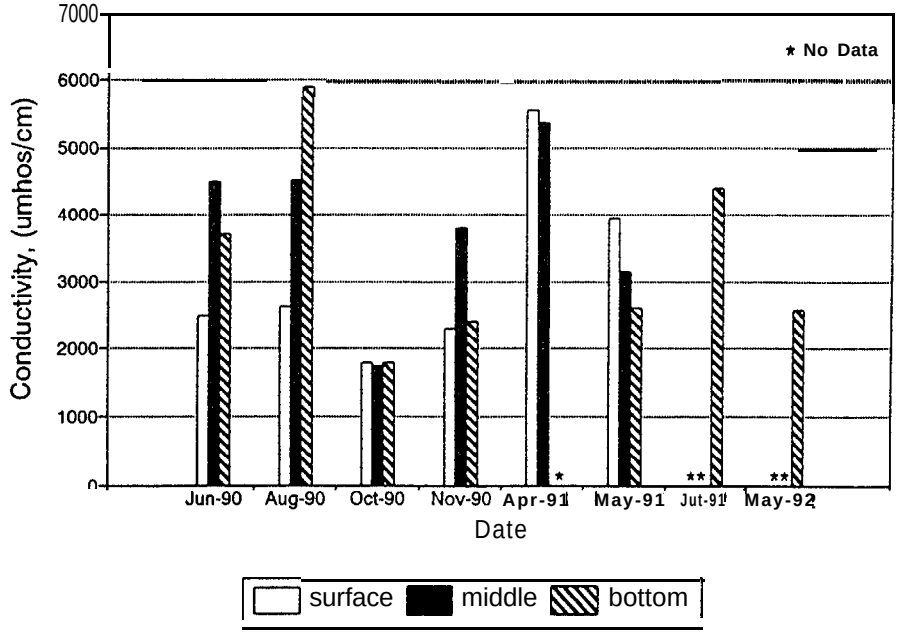


Fig. 13e: Arumator 1
Aluminum Concentration

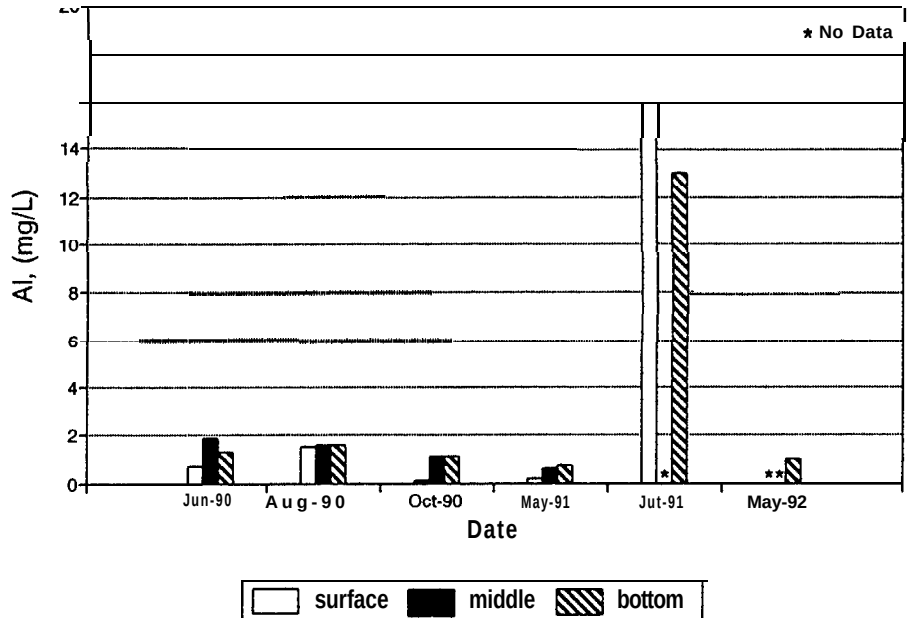


Fig 14e: Arumator 2
Aluminum Concentration

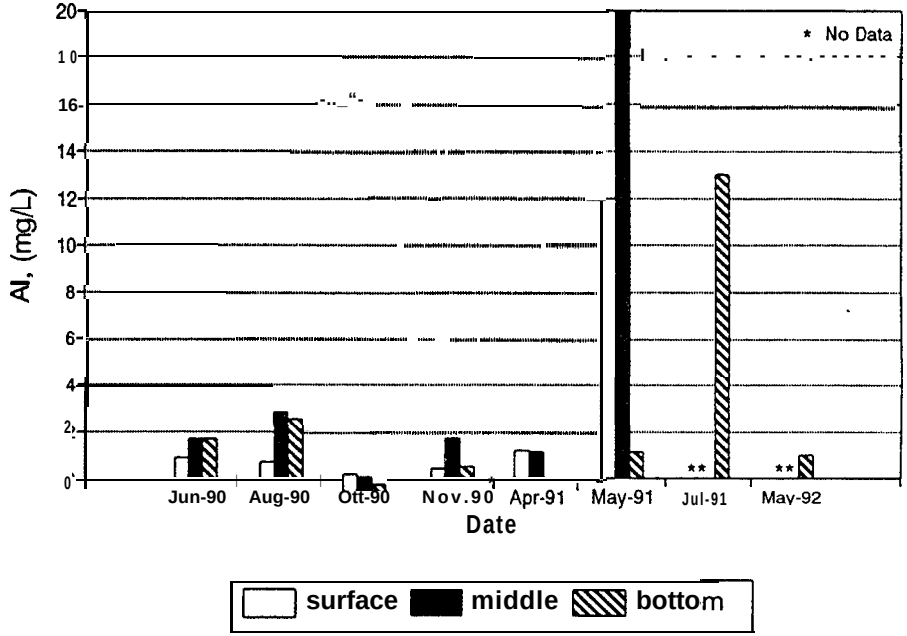


Fig. 13f: Arumator 1
Iron Concentration

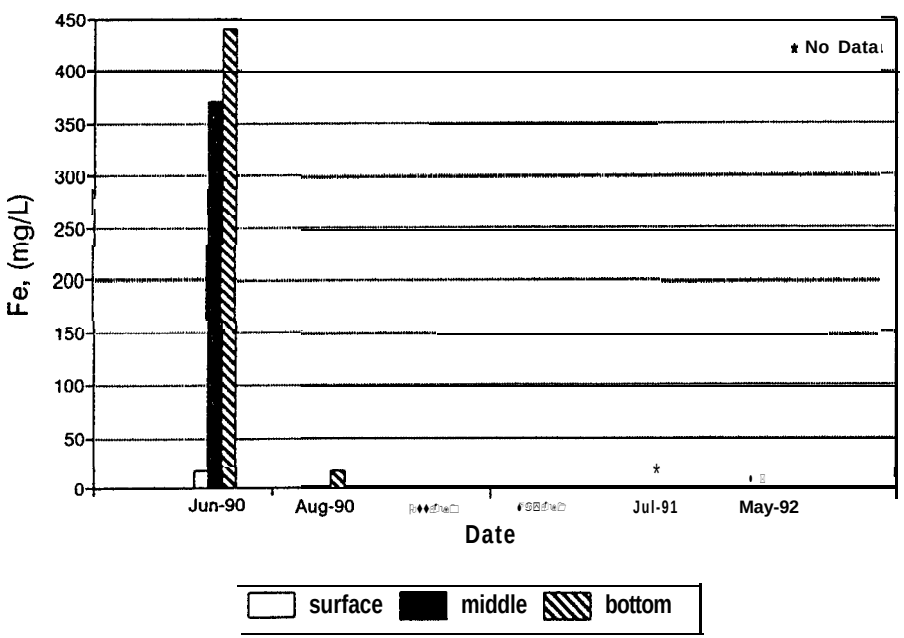


Fig. 14f: Arumator 2
Iron Concentration

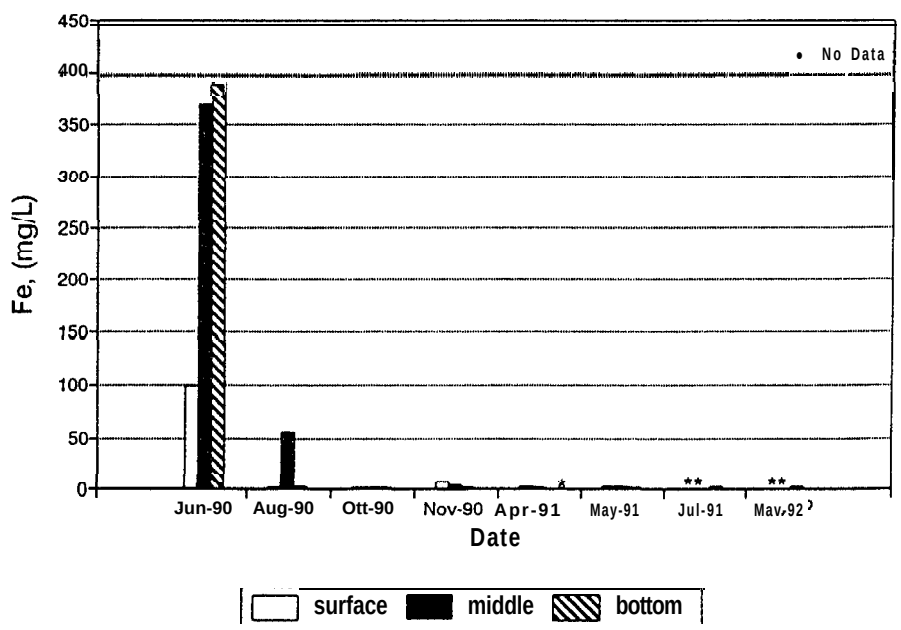


Fig. 13g: Arumator 1
Nickel Concentration

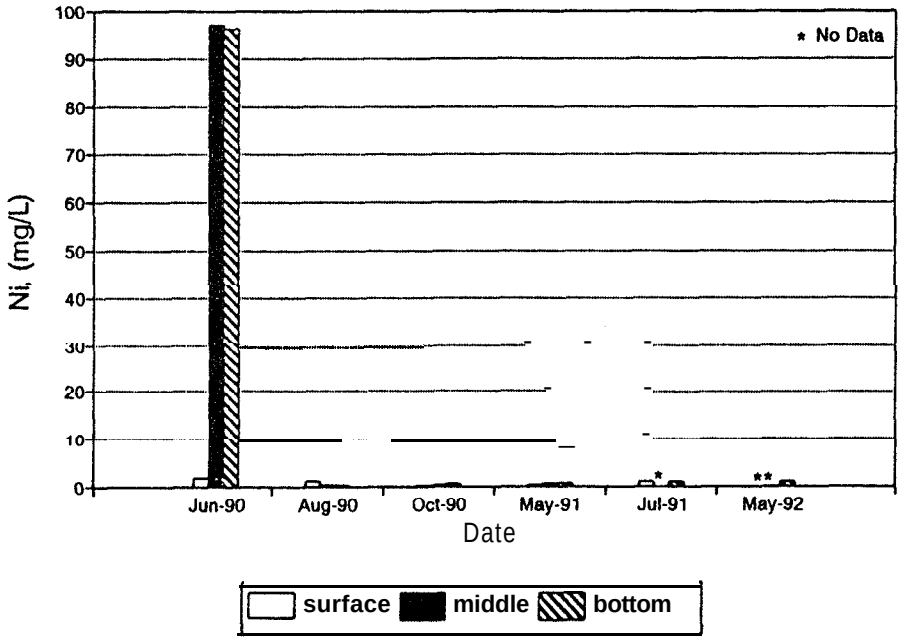


Fig 14g: Arumator 2
Nickel Concentration

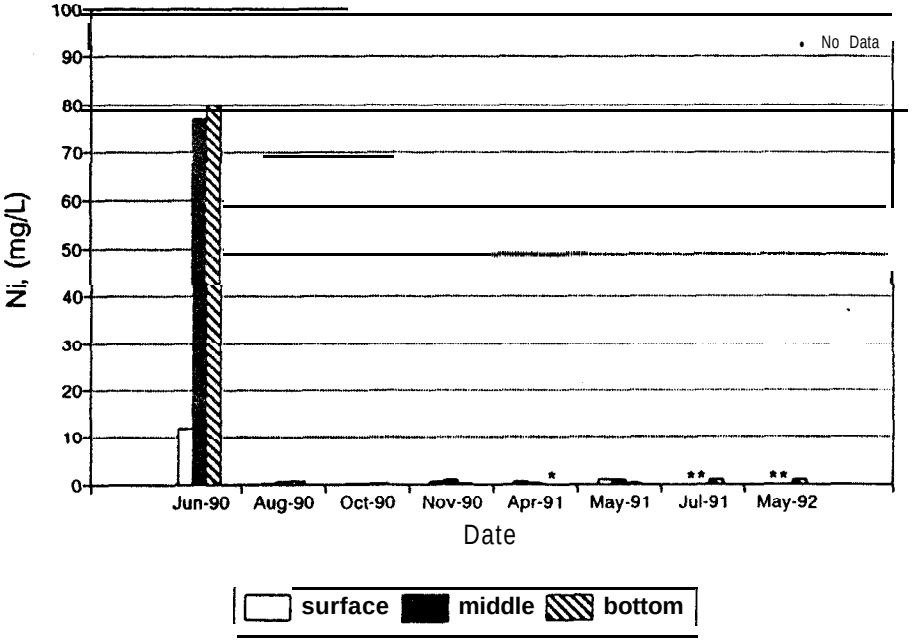


Fig. 13h: Arumator 1
Sulphur Concentration

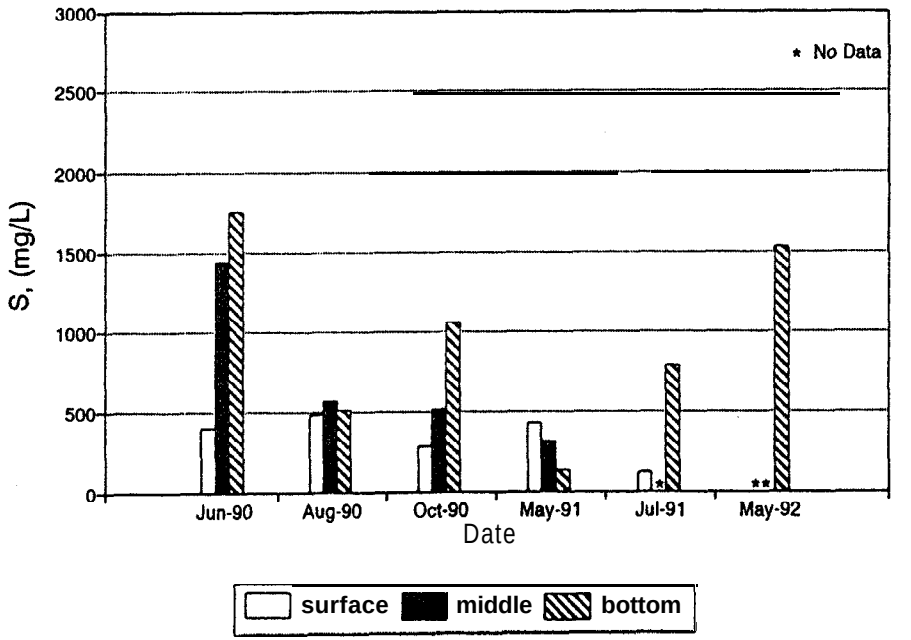
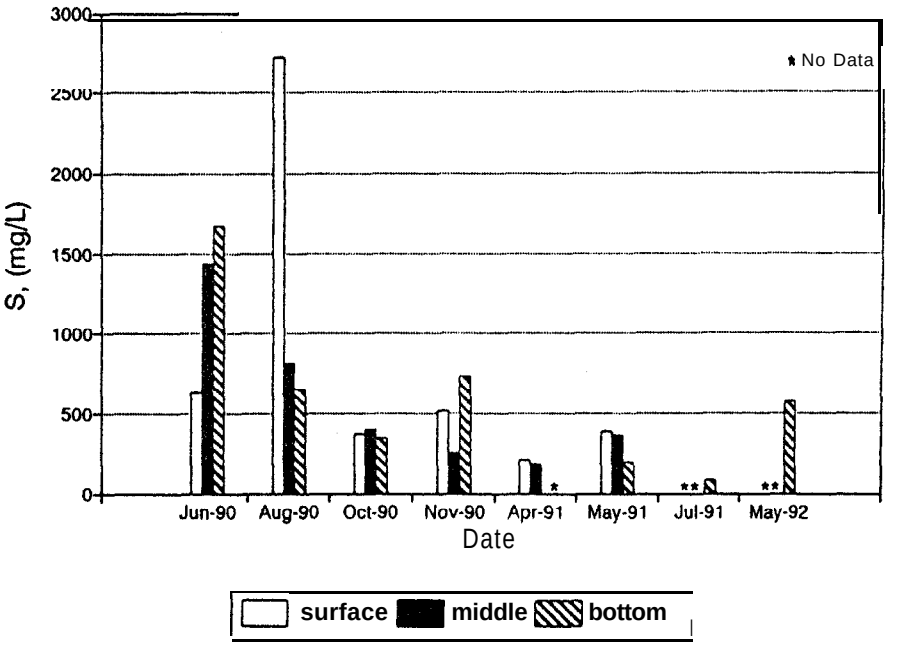


Fig. 14h: Arumator 2
Sulphur Concentration



4.4 ARUMator 3

In brief, the history of ARUMator 3 is as follows. ARUMator 3 was installed in November, 1989 with the 800 L inner sleeve (containing AMD, flax and iron) being isolated from the outer sleeve (volume 11.5 m^3). The intention was to initiate ARUM in the inner sleeve as a batch reactor. When microbial activity was evident, the inner sleeve was to be opened to the outer sleeve. The discharge from Cell 4 was to be run through the outer sleeve. In October 1990, high pH and negative E_m values were measured in the inner sleeve. Water was pumped from the inner sleeve to the outer sleeve, the inner sleeve with amendment removed, holes drilled into the top, middle and bottom, and the amendment returned.

By May 25, 1991, ARUM activity in the inner sleeve was detected again, as indicated by elevated pH and negative E_m values at the bottom. A portion of the Cell 4 discharge water was directed into the inner sleeve. On July 24 1991, elevated pH values were measured at the bottom of both inner and outer sleeves. The flow during this period averaged 0.5 L/ min. However, by August 7, pH had returned to values similar to those of water leaving Cell 4. On August 15, the system was modified. Compressed alfalfa pellets (5 kg) were added to the inner sleeve, over the old amendment. One bale of weathered flax was then added, followed by 160 pads of steel wool to the centre of the sleeve on top of the alfalfa. Water from Cell 4 was directed to the centre and middle of the inner sleeve at 0.19 Umin. Water then flowed from the inner sleeve to the outer sleeve through the drilled holes, and from there, through the exit pipe at the bottom of the outer sleeve to Stn 14. The flow from Cell 4 was cut off from October 22 1991 to May 6 1992. The conditions prevailing in 1991 were not changed in 1992, and approximately 25 % of the water leaving Cell 4 was directed through ARUMator 3.

Retention time for the inner sleeve (800 L) of ARUMator 3 with a flow rate of 0.33 L/min (mean of measured values in 1992) was 40 h. Retention time for the outer sleeve (11.5 m^3) was 24 days. Chemistry data for ARUMator 3 (Stn 14) and Stn 13 for 1992 are

summarised in Table 6. In May (shortly after the flow to the system was resumed), the pH at Stn 14 (6.27) was much higher than at Stn 13 and the E_m was negative, indicating that ARUM had continued in batch treatment. By July, the pH had dropped and the E_m was positive. During that time, the flow rate had been increased to 3 L/min, due to the good chemistry noted in May. Since the pH dropped rapidly, it is obvious that a flow rate of 3 Umin could not be maintained. After the flow was reduced, ARUM activity resumed, as the water quality was much better at Stn 14 than at Stn 13. In particular, the pH was much higher (5.98 as compared to 3.34 at Stn 13) and nickel was reduced from 5.07 to 3.38 mg/L. Using the August flow rate of 0.5 L/min into ARUMator 3, it can be estimated that the inner sleeve was removing 1.3 g Ni /day , 30.8 g S/day, 0.1 g Cu/day and 0.52 g Al/day. The performance of ARUMator 3 suggests that allowing AMD to flow through amendments is not favourable to ARUM.

Table 6: Effect of ARUMator 3 on water chemistry, Stn 13 and Stn 14 data. 1992

Station		Stn 13			Stn 14		
Date		7-May-92	21 Jul-92	26-Aug-92	7-May-92	21 Jul-92	26-Aug-92
Temp.	C	13	21.5	19.3	9.5	21.1	15.5
PH	units	3.87	5.52	3.34	6.27	3.55	5.98
Cond.	uS/cm	1310	2040	2890	1900	2500	2740
Em	mV	225	62	'408	-201	320	7
Acidity	mg/L	162.5	195	80	410	185	103
Flow	L/min	1.26	1.52	0.72	0.31	0.17	0.5
Al	mg/L	3	1	0.701	1	1.16	0.025
Ca	mg/L	235	304	322	322	326	308
cu	mg/L	2	1	0.132	1	1	0.003
Fe	mg/L	14	36.4	4.95	139	28.6	28.8
K	mg/L	16	50.3	56.2	33	49.9	51.1
Mg	mg/L	100	161	154	159	168	143
Mn	mg/L	8	6.98	8.03	IO	10.5	7.41
Na	mg/L	53	77	78.9	85	82	73
Ni	mg/L	22	8.62	5.07	21	12.1	3.38
S	mg/L	383	528	551	596	594	511
Si	mg/L	9	8.79	4.3	12	8.93	7.76

4.5 Cell 3 and Cell 4 Vertical Profiles

Curtains of flax straw were placed in Cells 3 and 4 in the spring of 1990 as a nutrient source for alkalinity-generating microbial processes (ARUM). Initially, it was thought that it might be possible to force AMD through the organic curtains, which contained the microbial consortia treating the AMD. It became clear by the middle of the third year, however, that the permeability of the organic curtains was not only difficult to quantify, but was extremely low. Thus, prior to the installation of the cattail floats, the curtains were flattened at the bottom of the cells. The ARUM community then originated on the bottom, and, after the cattail floats were added, the treated water moved slowly up through the water column.

In October 1990, in Cell 4, the first indications of the onset of ARUM activity were noted at Station 12a at the bottom. There, the pH was considerably higher than bulk water pH and redox potential was notably lower. This was only about four months after the organic material was added to the cell (Figures 15a and 15b, page 48). As was noted from earlier field tests, elevated pH pockets (localized areas) develop relatively easily, but the production of an active sediment over large areas is more complex. The measurements made at the same station for the next two years indicated however, that once a pocket has developed, its redox becomes even lower and pH remains elevated.

Chemistry data for profiles in Cells 3 (Stn 8A) and 4 (Stn 12A) are shown in Figures 16a to 16h and 17a to 17h (pages 49-52), respectively.

Elevated pHs, in the range 5 to 6, have been maintained at the bottom of Cells 3 and 4, throughout 1991 and 1992. However, by October 1992, the entire water column had achieved a pH value of 6 at Stn 8A (Figure 16a, page 49). For Stn 12A, the process was a little slower. There, by May 1992, a vertical pH profile showed values between 5 and 6, throughout the water column. Thereafter, however, the pH again declined, remaining high only on the bottom (Figure 17a, page 49).

The acidity profiles for both stations indicate that iron reduction was the prevalent alkalinity-generating process, due to the fact that the acidity was the highest at the bottom (Figures 16b and 17b, page 49).

A reduction in the redox potentials (E,) was noted throughout the water column at both stations (Figures 16c and 17c, page 50), but negative values were only measured once in August 1992, both at the surface, and at the bottom of Stn 8A. Although these trends in redox suggest that conditions for ARUM were improving, it also suggests that monitoring should continue for a longer period of time, now that the appropriate configuration has been achieved. Electrical conductivity does not change substantially along the vertical profile at either station (Figures 16 d and 17d, page 50).

Aluminum concentrations in the vertical profile reflect the increased pH values. Aluminum hydroxide starts to precipitate as the pH rises above 4. Therefore, by May 1992, most of the aluminum was removed throughout the water column at both stations (Figures 16e and 17e, page 51).

Iron, on the other hand, displays a reversal in concentration trends, as the surface concentrations were always lower than those at the bottom. As reduced iron contributes to acidity, but generates alkalinity through iron reduction, this concentration trend is expected. It should be noted that the iron concentrations in Cell 4 at Stn 12A are twice as high as those at Stn 8A in Cell 3 (Figures 16f and 17f, page 51). When the system experienced high flow rates, a great deal of iron hydroxide was deposited onto the organic material in Cell 4. Under reducing conditions, this ferric hydroxide is reduced, and redissolved. Iron is recycled and remains at the bottom of the cell. These results emphasize the need for appropriate iron hydroxide precipitation conditions in Cells 1 and 2.

Initially, nickel concentrations remained relatively constant throughout the water column, although there may have been slightly higher concentrations at the bottom of the cell.

As the ARUM system developed, nickel concentrations throughout the water column were reduced at both locations (Figures 16g and 17g, page 52).

The Makela system is dominated by sulphur in the form of gypsum, and therefore, although sulphur gradually decreases with the onset of ARUM, bottom water is always higher in sulphur than the surface water (Figures 16h and 17h, page 52). Although sulphate reduction was not measured directly, Inco personnel reported during a site inspection in 1992, that the system produced a rotten egg smell. This suggested that the pH at that time was not high enough for dissociation of hydrogen sulphide and the gas escaped from the solution. In part, this may explain the consistent reductions in sulphur noted in the vertical profiles during 1992 summer (Figures 16g and 17g, page 52).

Throughout this report, data describing the decrease in either metal concentrations or other parameters relevant to the AMD conditions have been discussed. However, as was evident from the vertical profiles, iron reduction is producing increased iron concentrations and increased acidity at the bottom of Cells 3 and 4. At the same time, given that the pH of the water is around 5, the water also has alkalinity, i.e. it is buffered. In Figures 18a and 18b (page 53), the alkalinities, which have been measured in the vertical profiles for both stations, are reported. These findings suggest that iron reduction is the main microbial process dominating the system.

Overall, water quality has improved with time (reductions in nickel, aluminum and sulphur concentrations, and elevations in pH have been noted) and the improvements have now reached the water surface. Redox potentials (E_7) have continued to decline. These data confirm that the original concept, to extend the sediment-bound microbial activity throughout the water column, is possible. Therefore, ARUM can operate even when the bulk water samples have positive redox potentials.

Fig. 15a: Stn 12A
Bottom pH

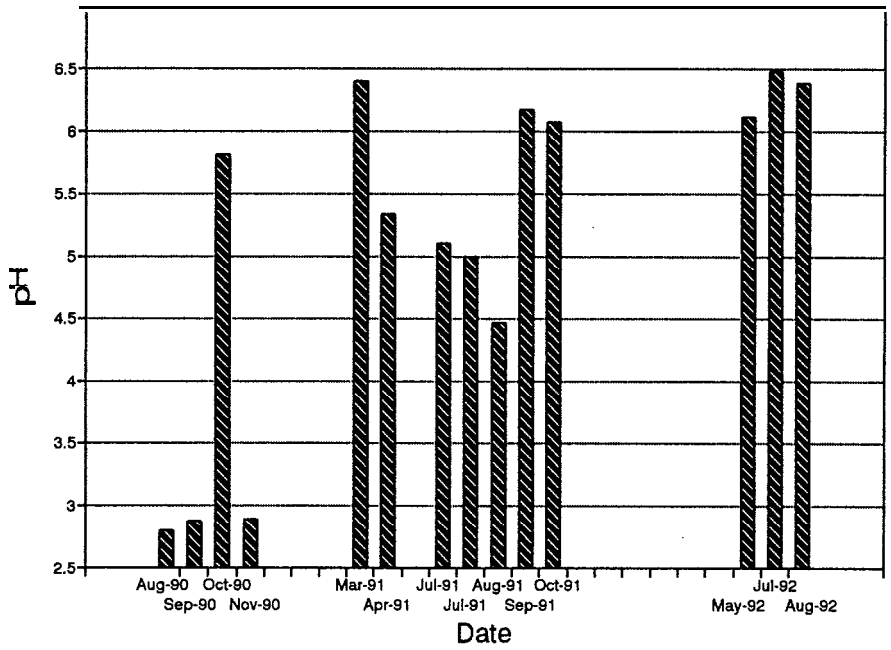


Fig. 15b: Stn 12A
Bottom Redox Potential (E7)

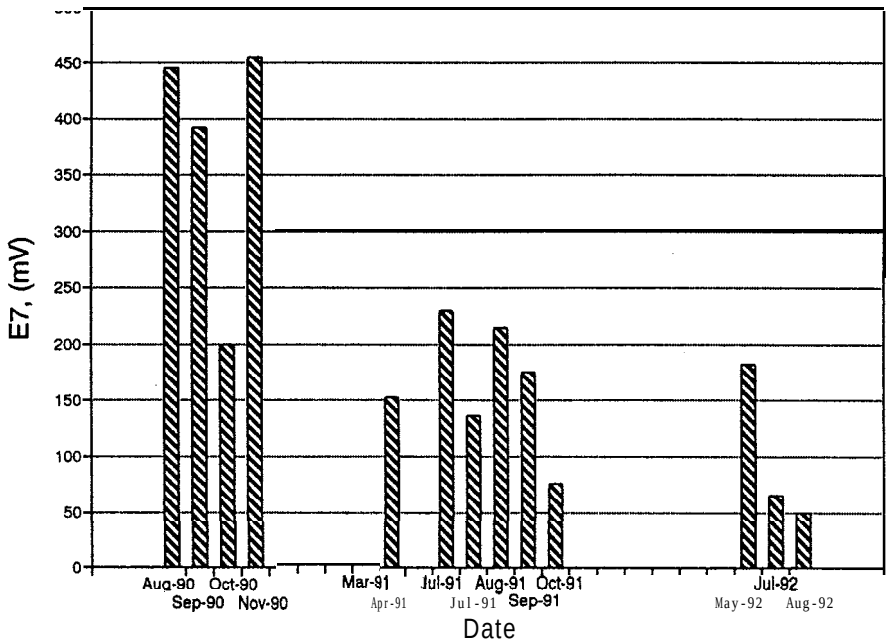


Fig. 16a: Station 8a
pH

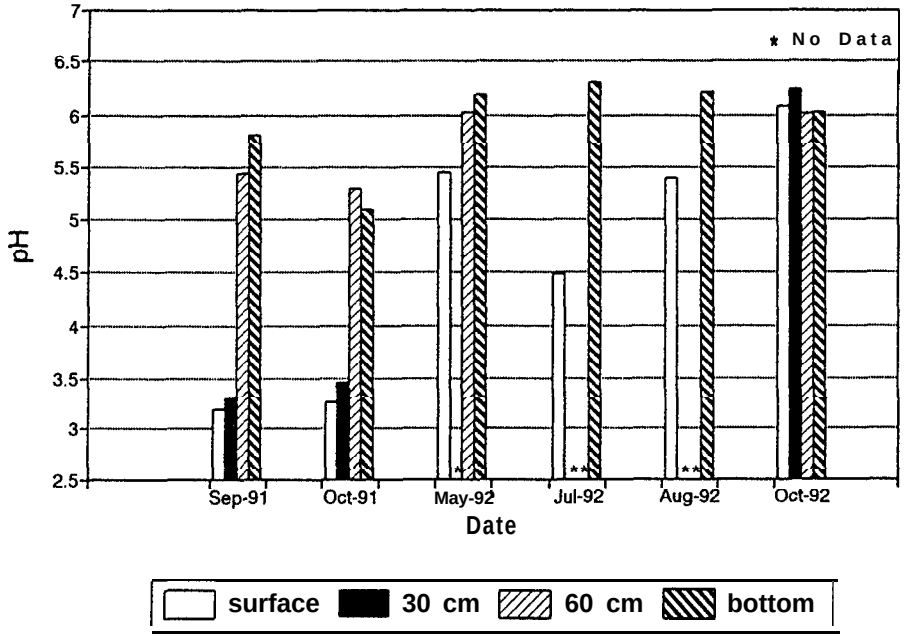


Fig. 17a: Station 12a
pH

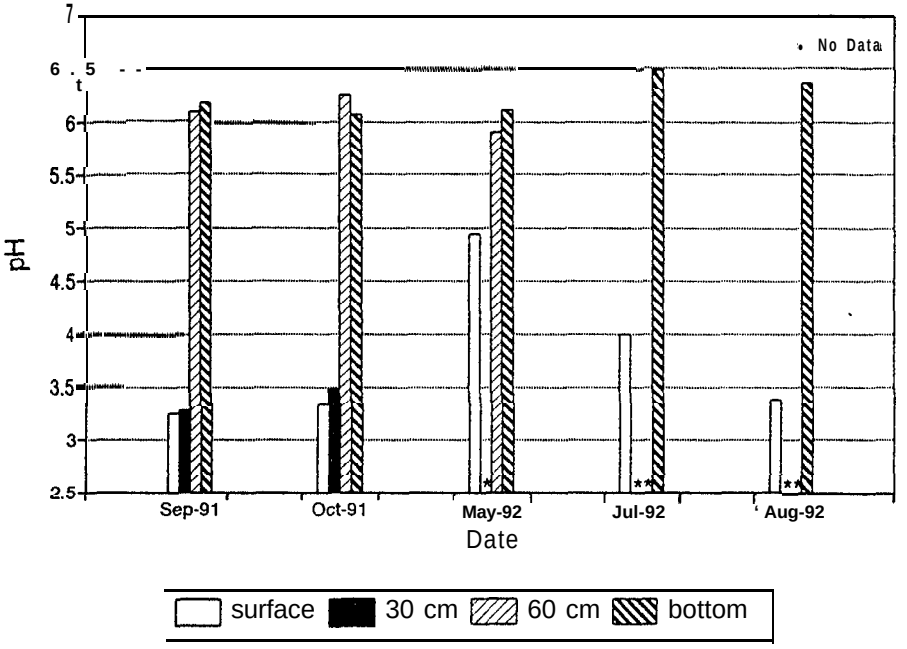


Fig. 16b: Station 8a
Acidity

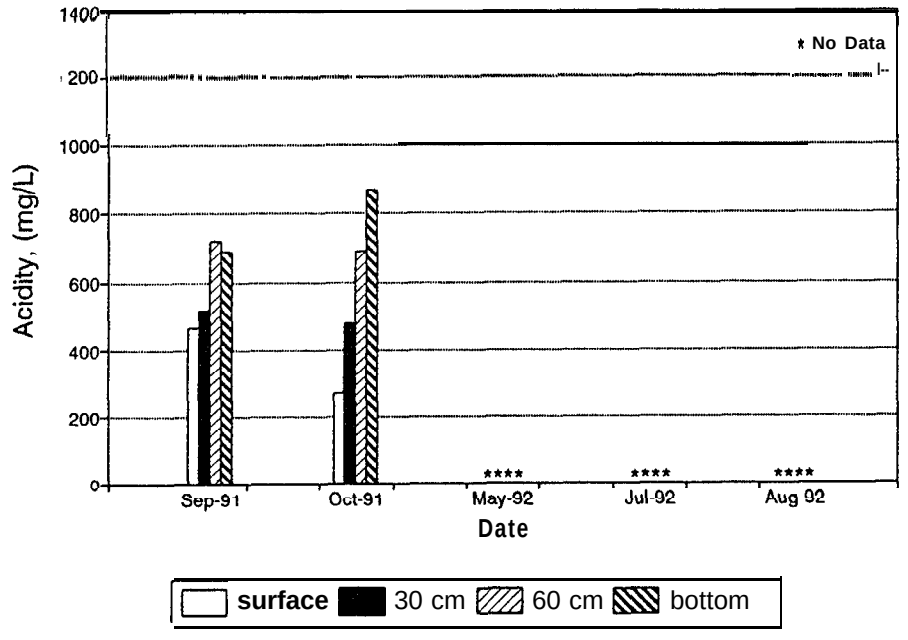


Fig. 17b: Station 12a
Acidity

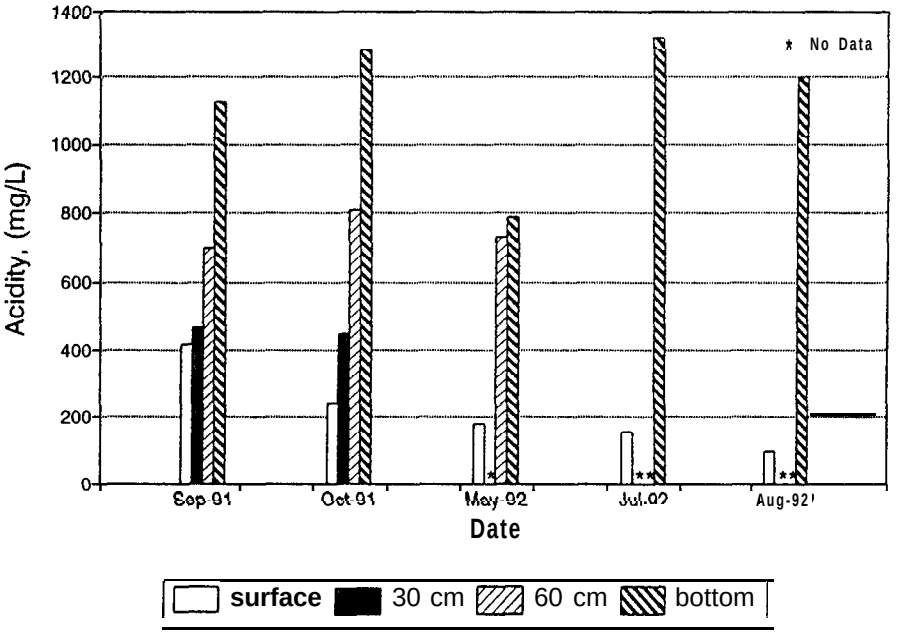


Fig. 16c: Station 8a
Redox Potential (E7)

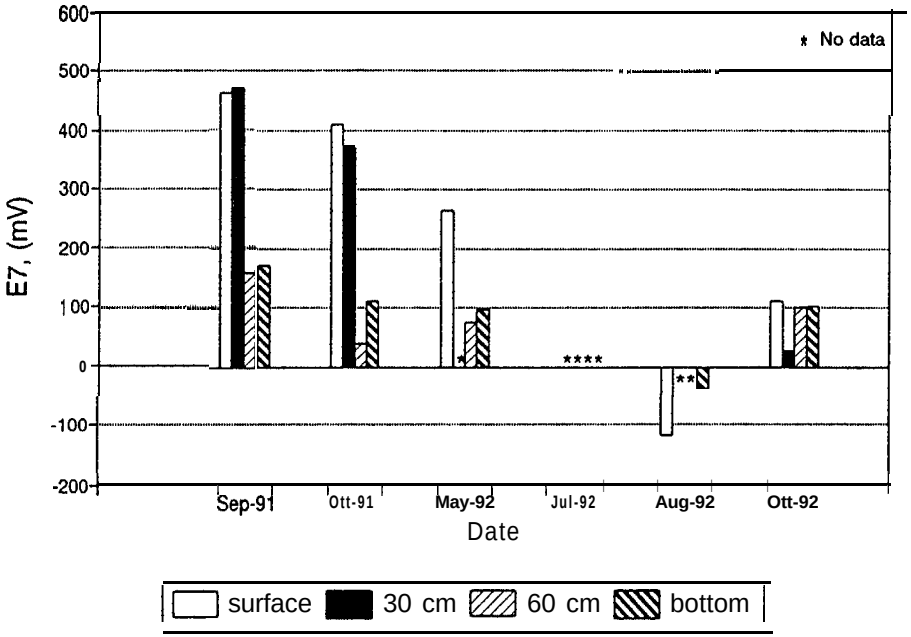


Fig. 17c: Station 12a
Redox Potential (E7)

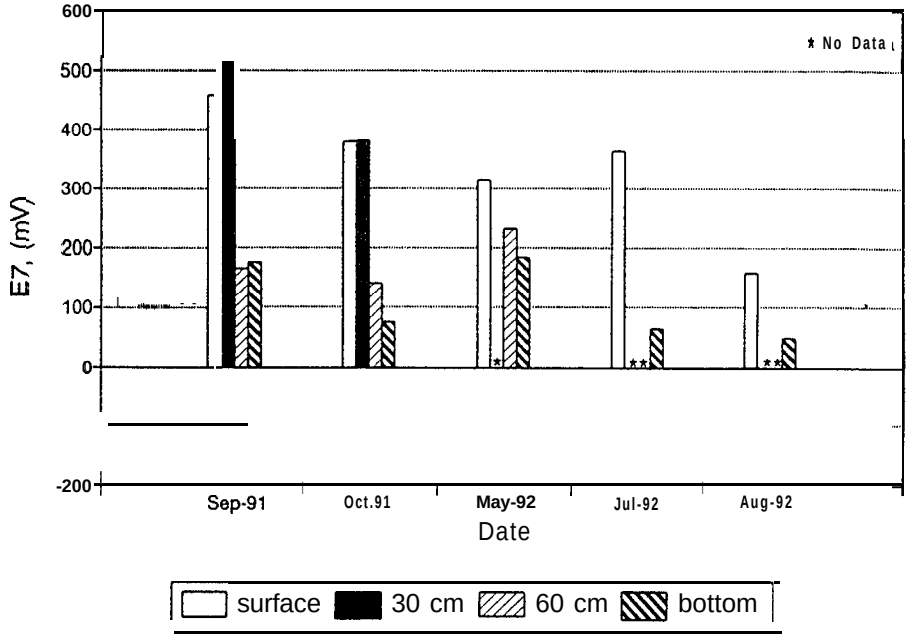


Fig. 16d: Station 8a
Conductivity

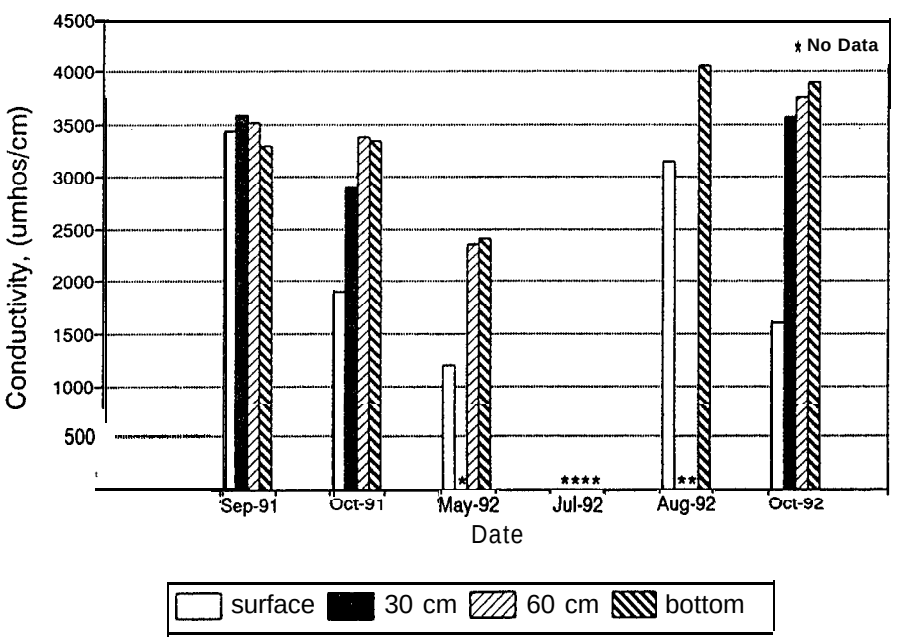


Fig. 17d: Station 12a
Conductivity

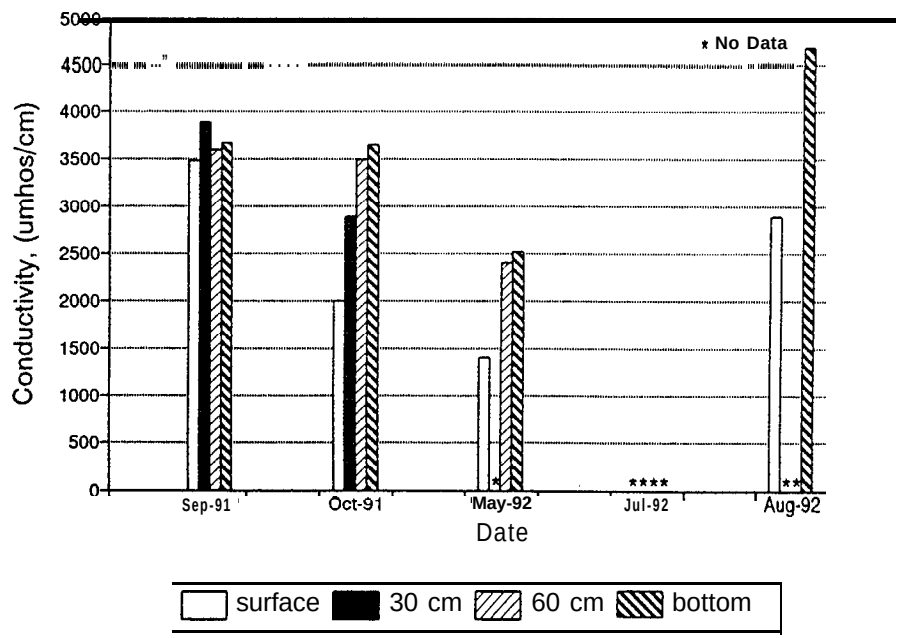


Fig. 16e: Station 8a
Aluminum Concentration

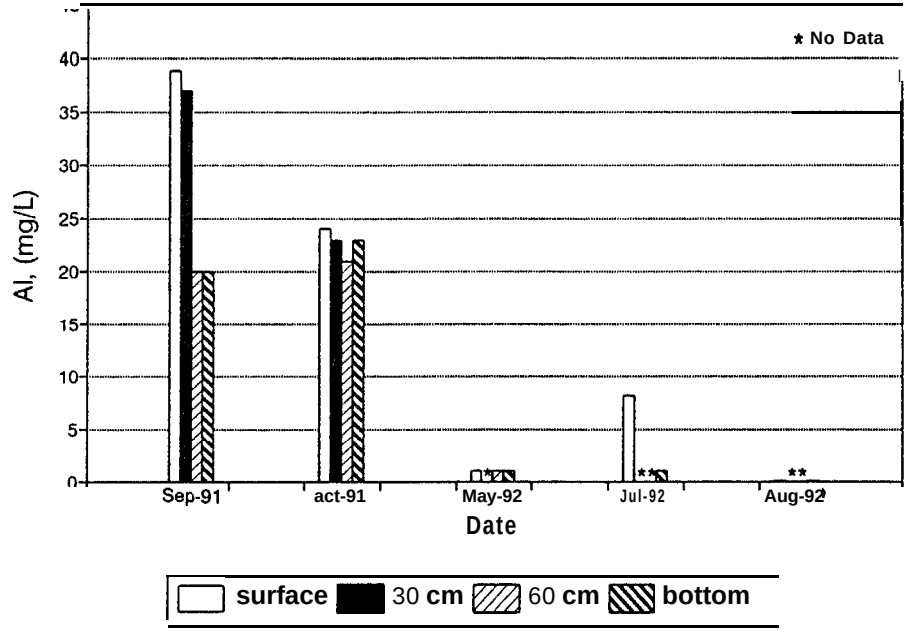


Fig. 17e: Station 12a
Aluminum Concentration

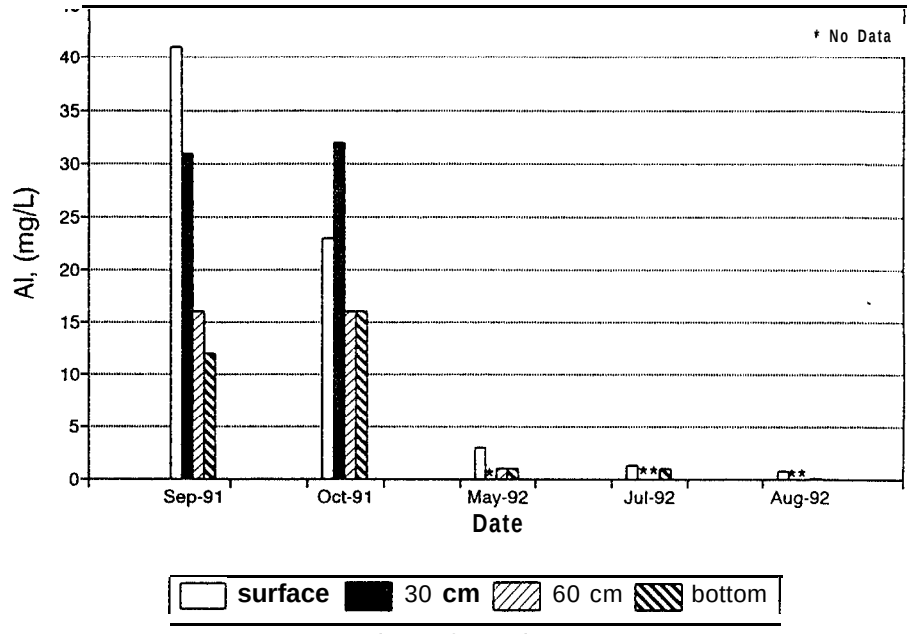


Fig. 16f: Station 8a
Iron Concentration

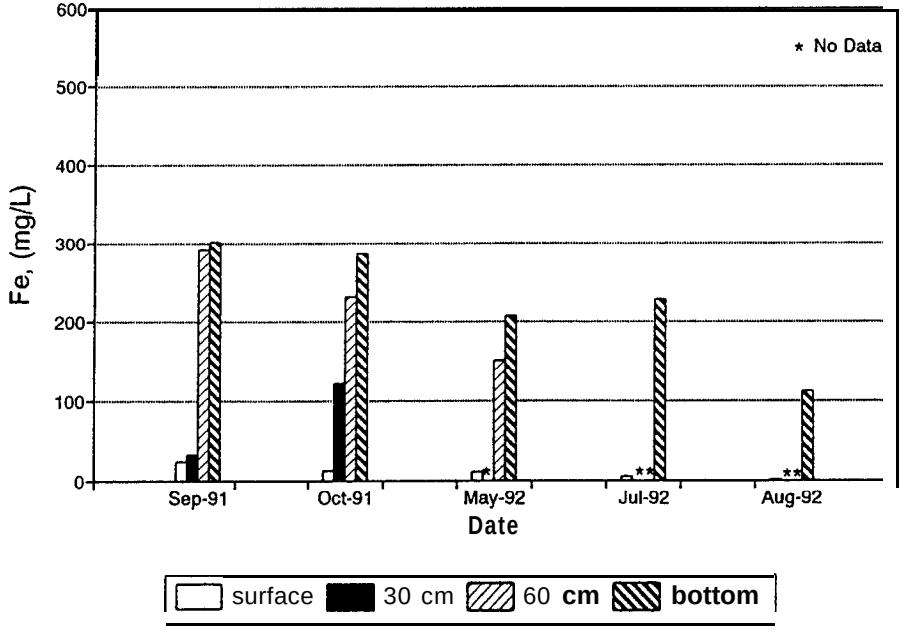


Fig. 17f: Station 12a
iron Concentration

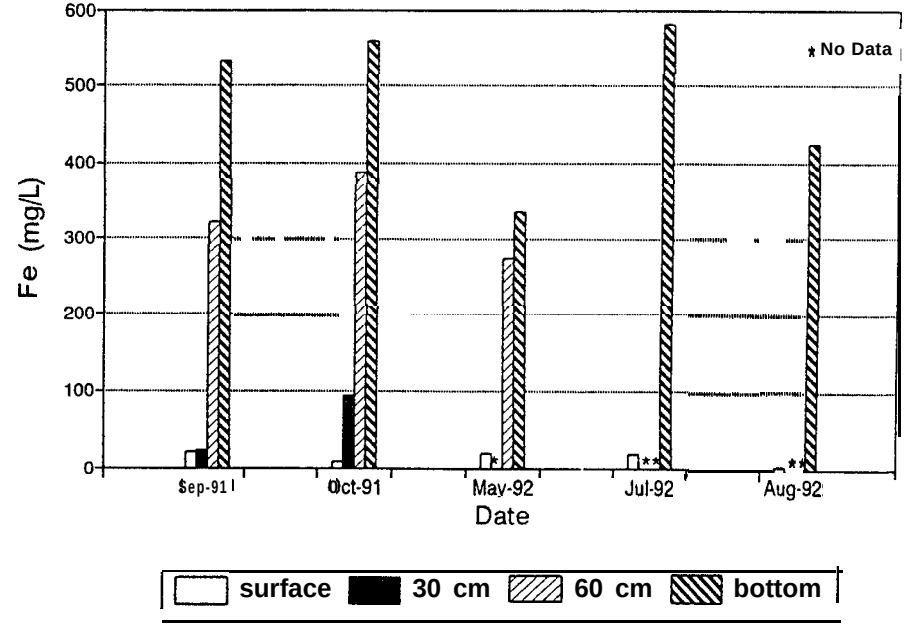


Fig. 16g: Station 8a
Nickel Concentration

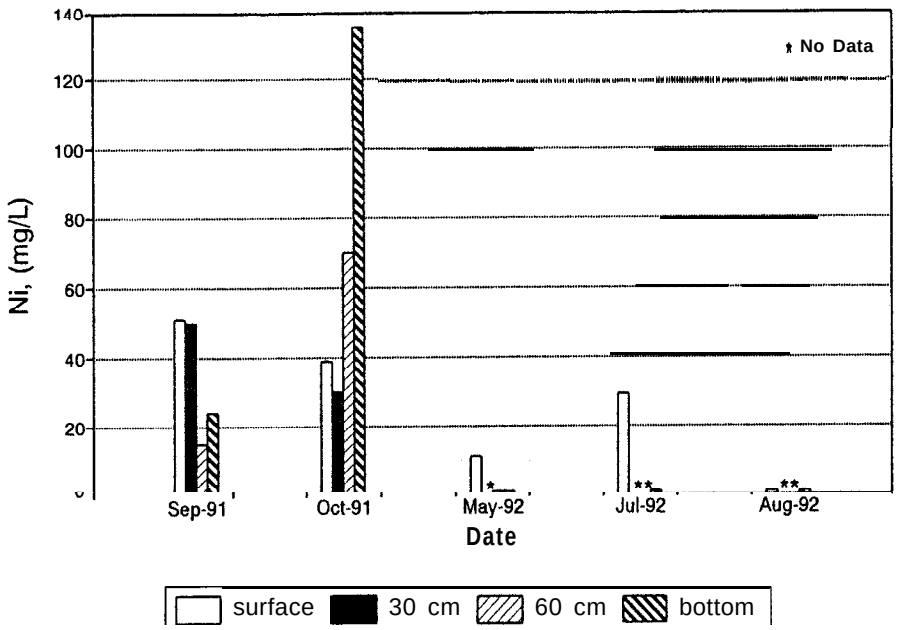


Fig. 17g: Station 12a
Nickel Concentration

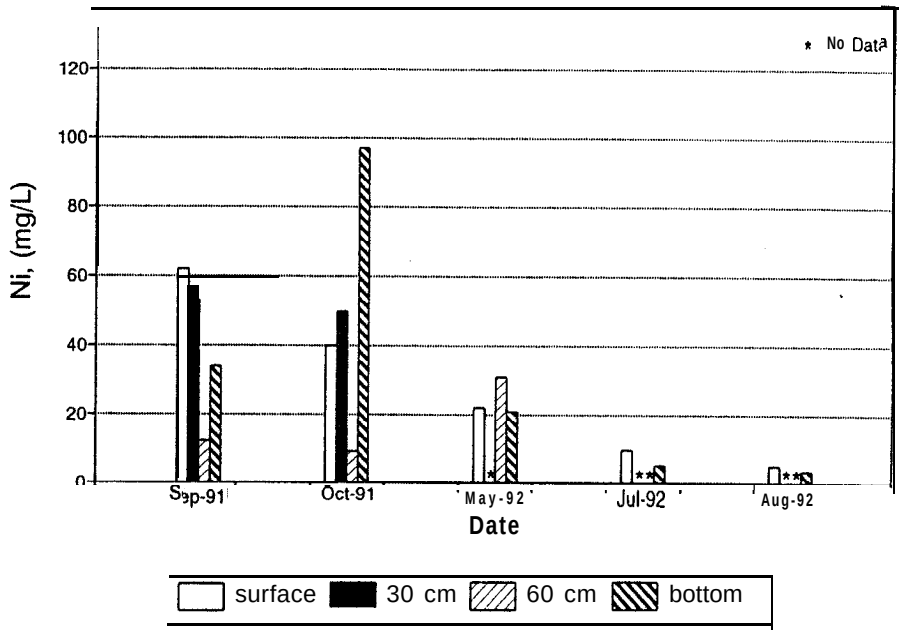


Fig. 16h: Station 8a
Sulphur Concentration

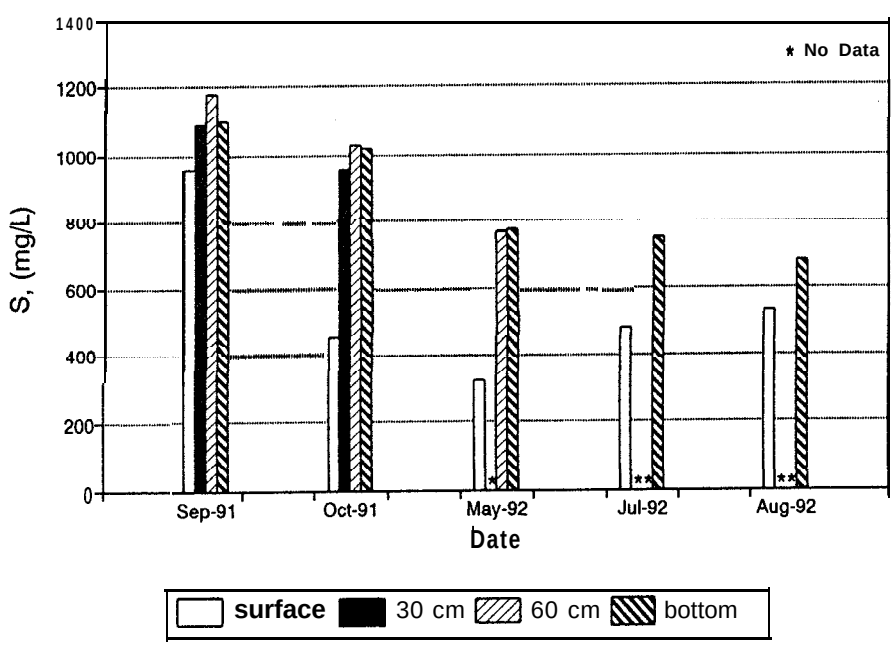


Fig. 17h: Station 12a
Sulphur Concentration

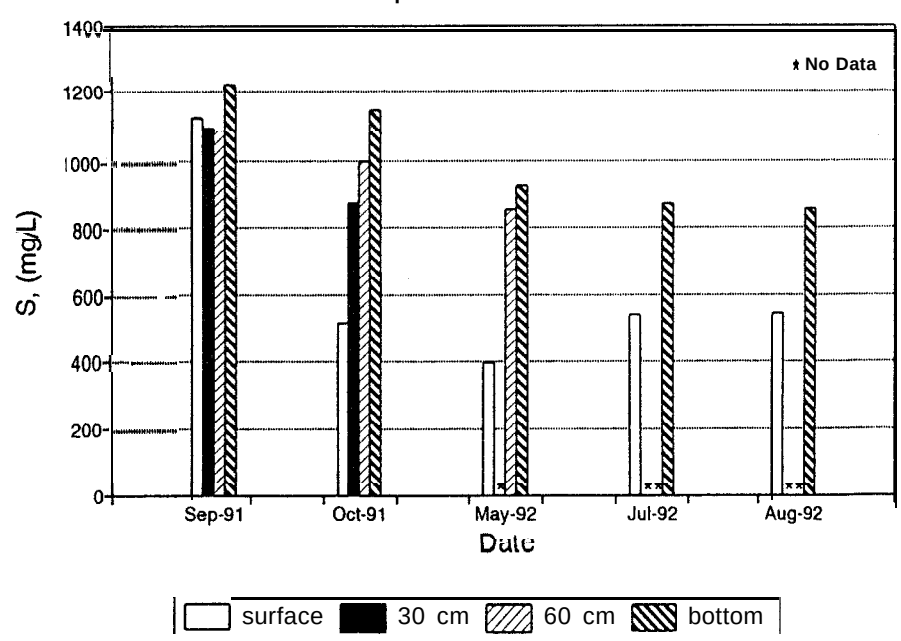


Fig. 18a: Station 8a
Alkalinity

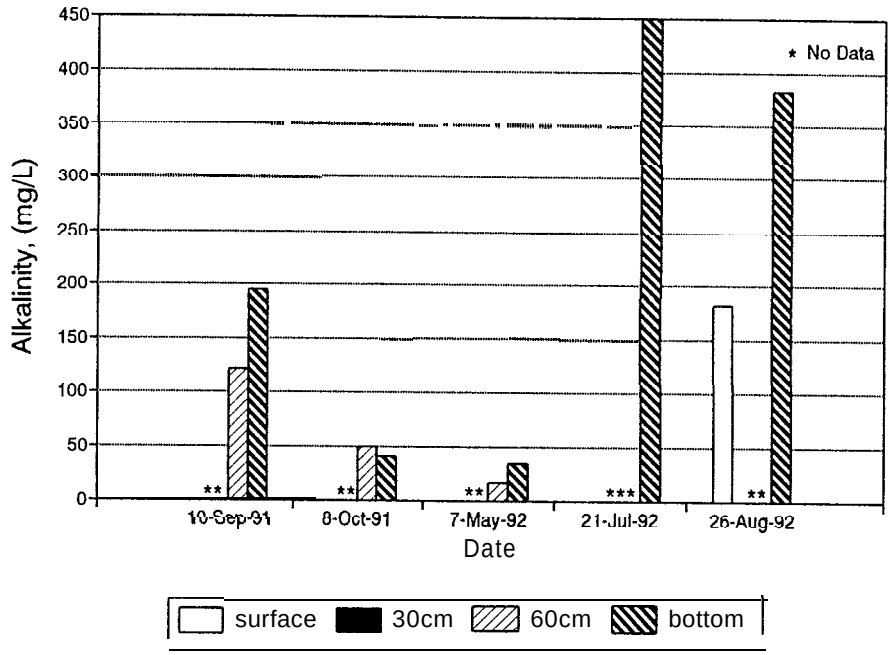
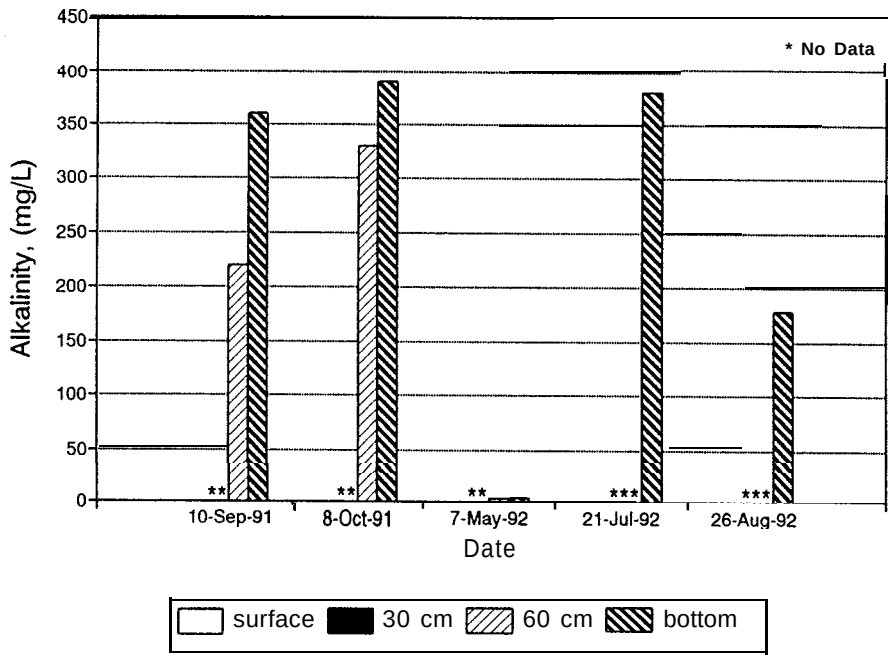


Fig. 18b: Station 12a
Alkalinity



4.6 Organic Matter Supply

The key to the microbial ecology in the ARUM sediments is the supply of carbon to the alkalinity-generating bacteria. An attempt was made to quantify decomposition, which supplies the needed food sources.

Decomposition bags were placed in Cells 3 and 4 in 1990 to determine which of a variety of amendment materials could decompose and therefore provide substrate for ARUM. Details of the work are summarized in a conference paper (M. Kalin, A. Fyson and M.P. Smith, Biohydrometallurgy, 1993 in press; see Appendix), and in the 1991 final report. Weight loss of bags in Cell 3 after one year submergence indicated that all materials tested (peat, sawdust, straw, cattails and alfalfa) may provide carbon and energy for the ARUM micro-organisms. The sequential nutritional analysis of the material retrieved from the bags after one year exposure to Cell 3 did not produced results which could be used to differentiate the suitability of different carbon sources.

Consequently, laboratory experiments were used to determine the effects of known easily-degradable organic materials on AMD and ARUM activity. Alfalfa pellets and potato waste were found to be very effective ARUM substrates. The results of the laboratory experiments are presented in Section 5. However, they were translated to the Test Cell System, where alfalfa pellets and potato waste were added to the cattail rafts in Cell 3 and the water column of Cell 4, respectively. They were added at rates determined from laboratory jar experiments. These additions have undoubtedly contributed to the dramatic improvements seen in ARUM activity and resultant water quality in Cells 3 and 4.

4.7 Cattail Rafts

Cattail rafts were established in 1991 to provide a carbon source for ARUM in the long-term. The rafts also help reduce sheet flow and suppress wave action, enhancing the generation and maintenance of reducing conditions, required for ARUM. Cattails were transplanted as seedlings and initially survived well in Cell 4 and Pond B, but growth was impaired by the low redox conditions in the water column of Cell 3. The root zone of the cattail mat must be oxidized. ARUM activity was apparent within the peat layers on all the rafts. Although ARUM activity should ultimately take hold in the root zone, it appears to be detrimental to the establishment of cattail seedlings.

In 1992, cattails were replanted and the root zone was padded with living moss, which protected the roots from the reducing conditions in the raft. Cattail establishment was well under way before the growing season was over, and growth continued into 1993.

H_2S production was noticed by smell, and pH values in the rafts were generally higher than 3. The expected ARUM activity in and under the rafts completes the demonstration of the overall ARUM concept. It is possible to establish reducing conditions throughout the water column, not just in the sediment.

Details of cattail seedling growth, development and organic matter production are summarized in Kalin and Smith (1992), provided in the Appendix.

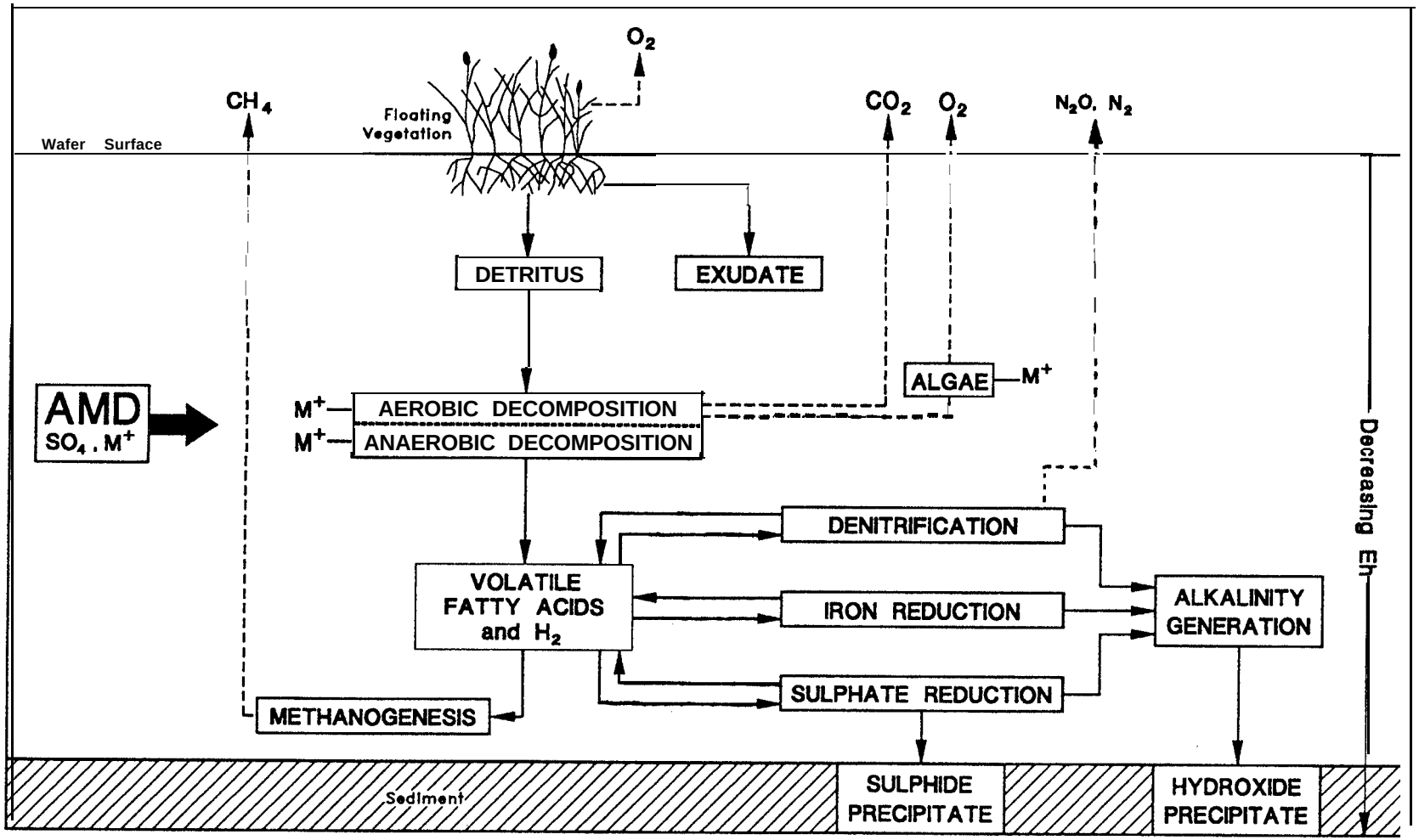
5.0 OPERATING PARAMETERS OF ARUM

The operating parameters of a process can be defined as those conditions which are required for the process to work. If the ARUM process is to function properly, both a nutrient supply and the correct chemical conditions for metal precipitation are required. Although these two sets of operational factors are discussed separately, it must be emphasised that both sets of conditions are required simultaneously.

5.1 Nutrient Supply for ARUM

In Schematic 2, the processes which take place in the ARUM sediment are depicted. In the space between the sediment and the floating cattail mat, only the most important reactions for the ARUM process are presented. The decomposition of organic matter leads to the production of volatile fatty acids, which then provide the carbon sources for denitrification, iron reduction and sulphate reduction. Those processes in turn generate alkalinity.

The treatment of AMD takes place on surfaces in the sediments and in the water column on suspended solids. Therefore, diffusion plays a major role in the operation of ARUM, in addition to the microbiology and chemistry. AMD in the water column has to come in contact with biofilms in the sediment and on particles located in the water column. AMD diffuses at rates dependent on concentrations gradients which are produced by the ARUM active sediments and the cattail rafts at the surface. The activity of the biofilms determines the rates at which the concentration gradients are established, which is dependent on the nutrient supply or the production of volatile fatty acids.



Schematic 2: ARUM treatment of AMD, from cattails to sediment.

For ARUM, nutrients are supplied from the decomposition of the organic matter provided in constructed sediments, and by the floating cattail mats. The coarse organic materials, such as hay bales and peat, provide the physical sediment structure, and potato waste and alfalfa pellets provide the readily degradable carbon supply for microbial alkalinity-generation.

The amount of organic amendment used in providing sediment structure is not readily quantifiable, as a microbial nutrient supply, as such materials will always contain a large fraction of non-biodegradable or refractory material (lignocellulose). Since the structural materials were so refractory, a supply of easily degradable carbon to the sediment had to be identified, which would sink to the bottom of the treatment cells. Potato waste and alfalfa pellets were tested and found to be suitable.

Experiments were carried out with different organic materials as structural substrates for the ARUM sediments. Changes in biodegradable components of peat, straw, sawdust and cattail litter, exposed to AMD over several time periods, did not provide reliable rates of carbon availability for ARUM. Although sulphate reduction was noted in the sediments, particularly the ARUMators, the results from the decomposition experiments could not be related to the sulphate reduction rates (Fyson et al., 1993, see Appendix).

Therefore, in order to determine the rates at which nutrients had to be supplied to sustain ARUM, experiments with potato waste and AMD were carried out in the laboratory.

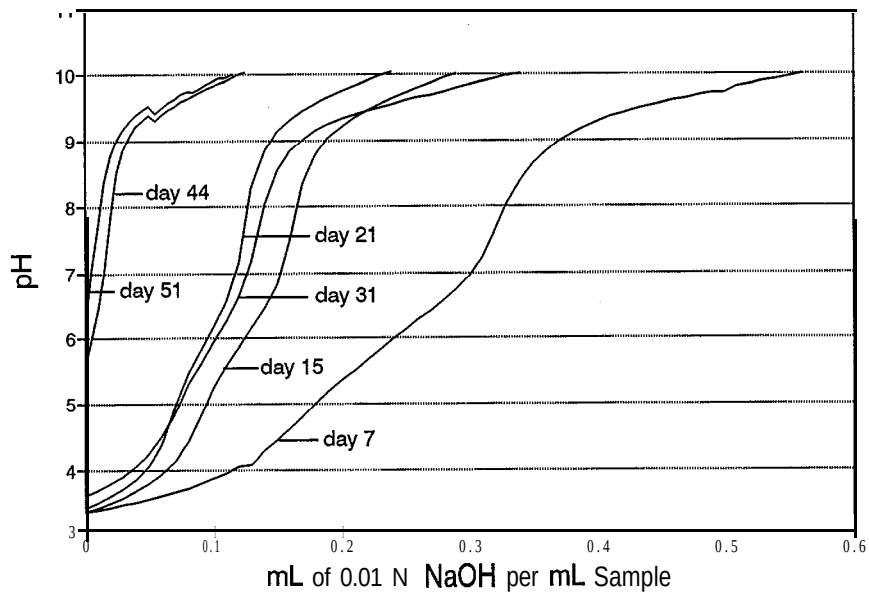
Experiments were set up with Makela Pond A water (before ARUM cells in Test Cell System) and other types of AMD to cover a wide range of AMD effluents. Potato waste (0.5 g) was added to 1 L of water in 2 L glass jars. The jars were maintained at room temperature without stirring. Acidity of samples was monitored regularly by titration of

sub-samples using 0.01 NaOH in a Metrohm Titrino Autotitrator. The amount of NaOH added to raise the pH to 8.3 can be converted to acidity by the following formula:

$$\text{Acidity as mg } CaCO_3/L = \frac{(\text{mL NaOH added} \times \text{normality of NaOH}) \times 50000}{\text{mL sample}}$$

Data obtained from the experiments with Makela Cell 4 water are presented in Figure 19. Between day 7 and day 51, acidity was reduced from 170 mg/L equiv. of $CaCO_3$ to 10 mg/L equiv. of $CaCO_3$. This equates to a removal of 1.6 millimoles of acidity. This value can be used to determine the rate at which nutrients should be supplied to the ARUM process.

Fig. 19: Makela-Potato Waste Experiment
Acidity Titration of Cell 4 Water with 0.5g/L Potato Waste



SO_4^{2-} and Fe^{3+} are the two major electron acceptors in AMD which will consume acidity (or generate alkalinity). For bacterial sulphate reduction, two moles of electron donor such as hydrogen, acetate or glucose are required for every mole of acid (H') consumed, e.g.

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

Under anaerobic conditions, the main products of decomposition of organic amendments are volatile fatty acids (acetic, propionic and butyric), sugars and hydrogen. All these can be used as energy sources (electron donors) for sulphate reduction.

For bacterial iron reduction, six to eight moles of acid (H') may be consumed for every mole of electron donor e.g.

$$(CH_2O) + 4Fe(OH)_3 \rightarrow CO, + 4Fe^{2+} + 3H_2O + 8OH^-$$

The amounts of potential electron donors required to remove the acidity of 1 L of Makela Cell 4 water, as observed in the experimental jars, can be calculated, assuming removal of acidity either by SO_4^{2-} or Fe^{3+} reduction (Table 7).

Table 7: Electron donors required to remove 1.6 mmole acidity from 1 L of Makela Cell4 water

Electron donor	Requirement to remove acidity (mg)	
	Sulphate reduction *	Iron reduction **
Acetic acid	192	12
Propionic acid	236	15
Butyric acid	282	18
Glucose	576	36

* - 2 moles electron donor per mole acid removed

** - 1 mole electron donor per 8 moles acid removed

Fewer electron donors are required for Fe^{3+} reduction per mole of electron acceptor. The potato waste used in the jar experiments (and for ARUM in the field) contains approximately 60 % starch or 300 mg of starch per jar. All of this material (300 mg) can be converted to acetic acid through acetogenesis under anaerobic conditions.

Therefore, in the experiment, more than enough potato waste was provided (0.5 g/L) to account for the acidity changes in the test water by either SO_4^{2-} or Fe^{3+} reduction.

In the jar experiment, 160 mmole of acidity was removed in 44 days. This can be converted to an equivalent acidity removal of 4.77 mg equiv. of $CaCO_3$/day or 0.32 m^3 of ARUM-active water/mg of acidity/min, a rate similar to that in the Makela ARUMators.

Nutrient requirements of ARUM can be estimated from the ability of a known amount (e.g. 0.5 g) of potato waste (or other material) to treat a known volume (e.g. 1 L) of AMD. This nutrient supply does not function if it is isolated from the sediments.

5.2 Chemical Operating Parameters

The interactions of microbial processes and the chemical conditions which lead to a functional ARUM process have been summarized in Schematic 2. However, the microbial activities required for ARUM have to be associated with the appropriate water chemistry to remove metals through precipitation.

Although the carbon supply to the microbial consortia in the ARUM sediment is essential for metabolic activity, the chemical conditions in the sediment control the biomineralization processes. The relation between microbiology and chemistry is best understood as a feedback loop. The microbiology brings about changes to the water chemistry, which in turn will change the microbial activity. The microbial activity will always be dictated by the availability of oxidants, of which oxygen is the easiest source, or that source which requires thermodynamically the least energy for its reduction.

ARUM processes can take place only when the oxygen supply is used up and the microbial consortia in the sediment are forced to utilize alternate electron acceptors such as nitrate, ferric iron or sulphate.

AMD effluents have a wide range of chemical characteristics with varying oxidant concentrations. These oxidants are required for the microbially-induced chemical reduction of iron, sulphate and other metals. Therefore, it is not unreasonable to expect that the ARUM process will use different electron acceptors in different AMDs.

For example, the alkalinity generated may be, at one stage of the ARUM process, dominated by denitrification (transforming nitrate to N_2), which would reduce the acidity of the AMD and raise the pH. The utilization of the nitrate would reduce the redox potential (E_h or E,) further and enable the system to use the next best electron acceptor (from a thermodynamic viewpoint) which would be Fe^{3+}. Iron reduction will generate alkalinity, raise the pH and reduce the redox potential still further. Such conditions will be favourable for sulphate reduction, reducing the sulphate concentration, reducing the acidity and increasing the pH. All these reactions result in changes in the concentration of electron acceptors. Thus, conditions could be such, that insufficient sulphate in the AMD may allow iron reduction to dominate in the alkalinity-generation.

The microbiology of ARUM can be predicted from the water chemistry. Nitrate, Fe^{3+} and SO_4^{2-} will be successively used up. After iron is oxidised and precipitated as $Fe(OH)_3$, sulphate will be the most abundant electron acceptor for alkalinity-generation. The redox potential of the system will be sufficiently reduced by Fe^{3+} reduction as well as fermentation reactions. However, the molar concentration of SO_4^{2-} often exceeds the molar concentration of the heavy metals, which suggests that sulphide precipitation will rarely be limited by the concentration of SO_4^{2-}, per se.

Microbial processes change water chemistry, thereby promoting or inhibiting chemical reactions. Of particular importance here is that the change in pH brought about by ARUM bacterial processes will promote or inhibit the precipitation of various metal ions. By and large, microbiology carries out or catalyses reactions that would be predicted from a thermodynamic point of view (Zehnder and Stumm 1988). The activity of

anaerobic micro-organisms enables various chemical reactions to be carried out by lowering E_h and changing pH. The chemistry of the AMD can therefore be used to predict which reactions will be carried out.

5.2.1 Metal concentration and pH

Metal removal is achieved through the formation of various forms of precipitates in the ARUM process. Metal precipitates form due to the chemistry prevailing in the solution, essentially pH, E_h and metal concentration.

In Table 8, metal hydroxide precipitates, which can be expected to form on the basis of pH and metal concentration, are summarized for different AMD seepages. For example, in a seepage with low aluminium concentrations, it can be expected that as the pH rises to 4.6, aluminum will be removed from the water as its hydroxide. If the concentrations of aluminium are higher, then a pH of only 3.7 is required for the formation of its hydroxide.

Table 8: pH Range of Metal Precipitation in Different AMDs

Metal	mg/L	Calculated pH	Real pH
Fe+3	1	3.18	3.5
	1000	2.18	2.5
*Fe+2	1	9.32	6.5
	500	7.97	5.5
Al	1	4.68	5
	500	3.78	3.5
#Zn	1	10.13	8
	500	9.23	6.5
#Cu	1	7.00	
	200	5.85	
Ni	1	8.61	
	200	7.46	
*Mn	1	9.70	
	400	8.40	

* Metal precipitates at lower pH due to oxidation reactions

Metal precipitates at lower pH due to carbonate formation

Thus, the concentration of the major metals in AMD determines the pH at which a metal precipitates and is removed from solution. Changes in the concentrations of sulphate and iron can be brought about by microbial activity. Metal removal, on the other hand, is due to the changes in concentration of metals at a certain pH. Therefore, concentrations of relevant elements and the pH of AMD have to be considered as essential chemical operating parameters.

The pH can be used as an indicator of an active ARUM process. Based on the oxidation reaction of reduced (Fe^{2+}) iron,

$$4\ Fe^{2+} + 10H_2O + 0, = 4Fe(OH)_3 + 8\ H^+,$$

the pH can be predicted from the iron concentration. If the ARUM process does not maintain reducing conditions, iron oxidizes, producing hydrogen ions and lowering pH.

The difference between the expected pH, at a given iron concentration, and the measured pH value indicates the activity of the ARUM process (Table 9). In the Makela system earlier in the project, when the ARUM process was not operational, the difference between the observed and calculated pH was quite small. After the floating cattail mats were placed on the ARUM cells, and the process became established in 1992, differences between the expected and measured pH values increased.

n of Theoretical and Measured pH Values in Makela System

Date	1990					
07-Jul		18-Jul	08-Aug	23-Aug	27-Sep	22-Nov
Stn6 pH	3.11	2.78	2.93	2.80	3.21	3.12
Stn13 pH	2.80	3.20	3.05	2.90	2.95	4.03
Stn6 calc.pH	2.64	2.36	2.14	2.13	3.40	2.56
Stnl3 calc.pH	2.15	2.05	1.95	2.00	2.31	2.15

Date	1991				1992		
	25-May	11-Jun	05-Jul	10-Sep	07-May	21-Jul	26-Aug
Stn6 pH	3.37	3.10	2.84	2.90	3.47	3.05	2.96
Stnl3 pH	3.28	3.07	3.23	3.33	3.87	5.52	3.34
Stn6 calc.pH	2.07	2.28	2.18	2.09	2.11	1.98	2.04
Stn13 calc.pH	2.05	2.08	2.01	2.01	2.11	1.97	2.03

5.2.2 Iron precipitation control

For microbial alkalinity-generation to take place, the microbes require access to the carbon sources. Since AMD seepages are frequently very high in iron, bacterial biofilms on amendments would be covered by iron hydroxide, rendering them ineffective. In such cases, it follows that the iron concentrations must decrease, prior to entering the ARUM treatment portion of the system, to prevent clogging of amendment surfaces with $Fe(OH)_3$ precipitates.

Decrease in iron concentrations can be achieved through provision of a precipitation pond. Iron precipitation will proceed naturally in most tailings seepages, as is the case at Makela, since the dominant form of iron is the reduced (ferrous) form. In the Makela seepage, as the ferrous iron oxidizes and ferric hydroxide precipitates, the seepage pH decreases from above 5, to pH 3 and lower (Table 8, page 63). If the AMD seepage is dominated by oxidized (ferric) iron, precipitation of iron has to be mediated by the addition of natural forms of iron precipitation agents.

The design of the iron precipitation ponds to feed water to the ARUM ponds requires the determination of conditions for the natural iron removal process leading to acidification of the AMD. In the Makela Test Cell System, the first two cells (Cell 1 and Cell 2) were used to determine those parameters, i.e. acidification through iron precipitation. Measurements in the first two cells can be used to derive parameters for pond sizes required for iron removal and acidification to take place.

When the tailings seepage, dominated by ferrous iron, enters Cell 1, ferric hydroxide precipitates and settles. The water acidifies according to the following reaction:

$$4Fe^{+2} + 0, + 10H_2O = 4Fe(OH)_3 + 8H^+$$

The rate of iron oxidation determines the rate at which iron precipitates, and subsequently settles, and thus, the quantity of iron removed from the water. To design the pond for optimal precipitation, the rate of iron oxidation is therefore significant.

Several research groups have worked on models to predict iron oxidation rates. The model concluded that pH, original iron concentrations, and oxygen are the important parameters controlling oxidation rates (Sung and Morgan, 1980). On the other hand, Hustwit et al. (1992), studying oxygen transfer in iron oxidation in the laboratory, found that the oxygen transfer rate in the AMD solution is the dominant factor which determines the reaction rates. The literature reviewed suggests that iron removal or oxidation might not be controlled by one parameter alone. The processes may be dependent on the specific conditions of the AMD seepage. Therefore, to arrive at the parameters for a pond design for optimal iron removal, rates are derived from the data collected in Cell 1 and Cell 2. It was not technically possible to determine oxygen transfer and iron oxidation rates during the MEND program, given the limited time available in the field.

Iron concentrations in water collected at Stn 4 in Cell 2 were compared to the inflow to Cell 1 (Stn 1). Figure 20 shows the data from 1991-1992, and Figure 21 shows data from 1990. Iron concentrations are plotted against retention time. The lines connect subsequent sampling points. No direct correlation was evident between the iron concentration and the retention time, but a symmetry existed between iron concentrations entering and leaving the system. The decrease in iron concentration through Cell 1 is a function of the original concentration, but not of the retention time. This suggests that the major factor determining iron precipitation in the Makela system is the iron concentration in the seepage as it enters Cell 1.

In Figure 22, the decrease in iron concentrations is plotted against the original incoming iron concentration. The data were assumed to linear, and a linear regression analysis performed.

Fig. 20: Makela - Cell 1 and Cell 2
Fe Concentration, June 1991 -August 1992

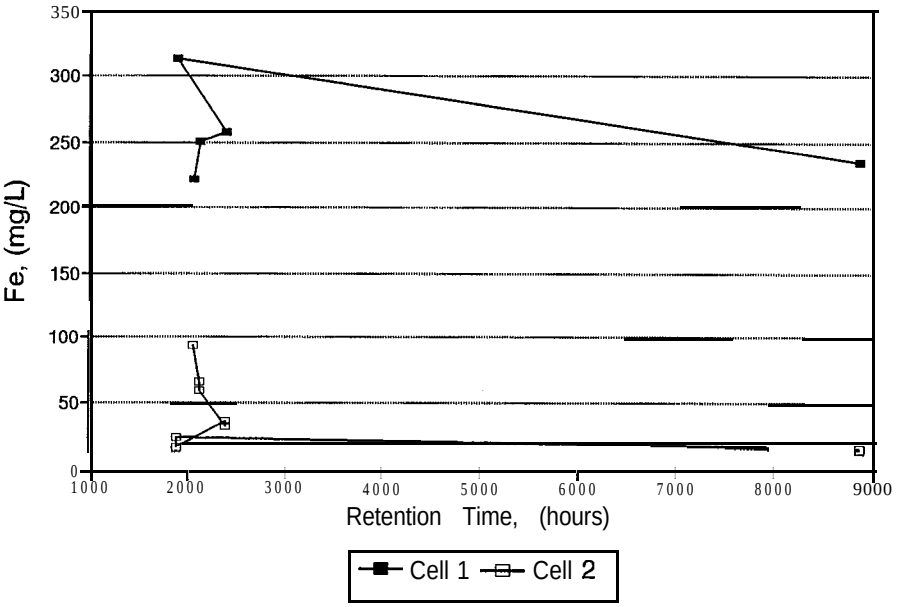


Fig. 21: Makela - Cell 1 and Cell 2
Fe Concentration, 1990

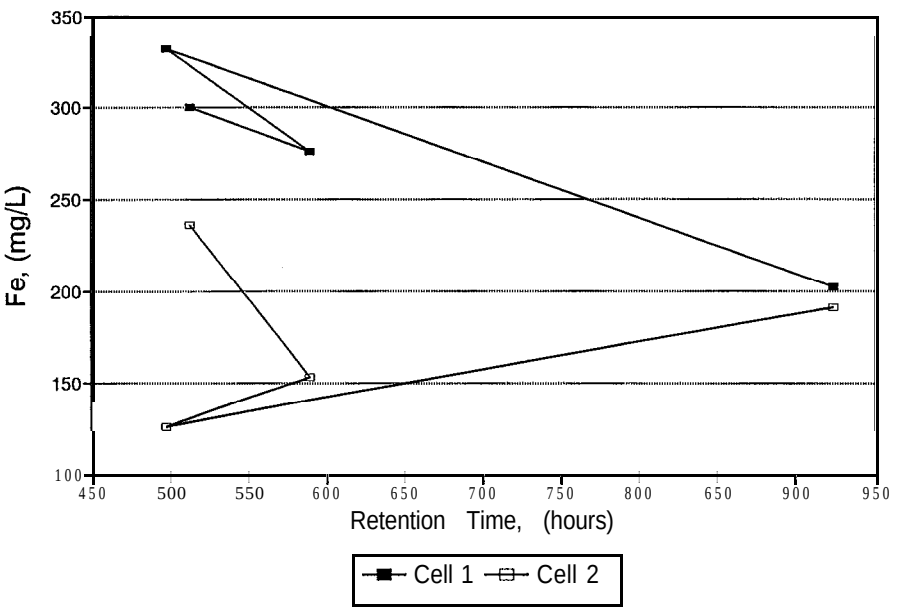
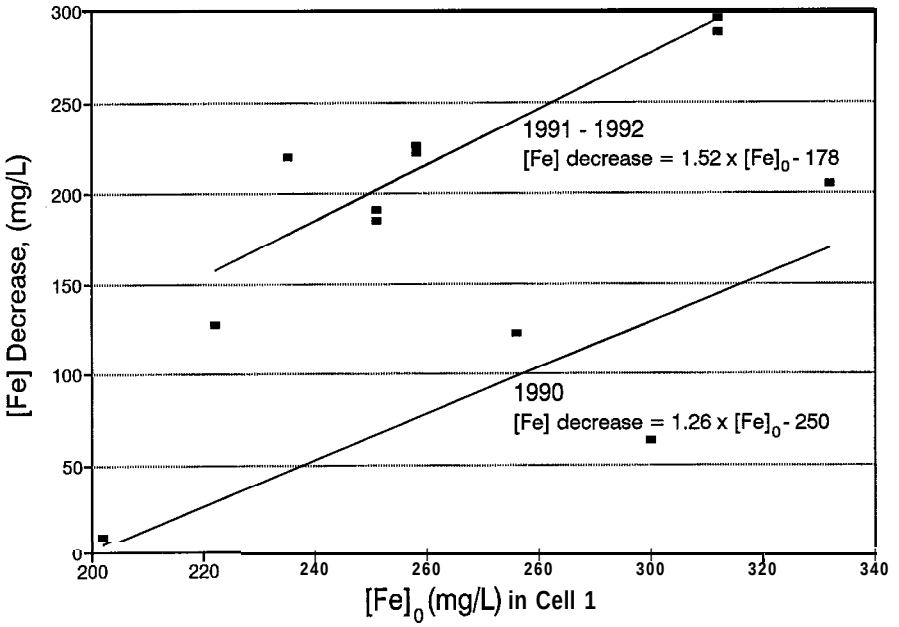


Fig. 22: Makela - Cell 1 and Cell 2
Fe Removal Rate, July 1990-August 1992



The data are shown in two sets with high (1990) and low flow rates (1991/1992). Regression analyses of the two groups give the following equations:

Fe removal(mg/L) = 1.26*Fe(mg/L) - 250	1990	r=0.71
Fe removal(mg/L) = 1.52*Fe(mg/L) - 178	1991-1992	r=0.84

The data from Figures 20, 21, 22 suggest that, in Makela Cell 1, the final iron concentration is not determined by the retention time, but by the original iron concentration. In these two cells, iron oxidation has reached an equilibrium. It can be concluded that iron oxidation has finished (reached equilibrium) after 21 days (minimum retention time measured).

These data can be used to derive a generalized minimum retention times, given the pH and iron concentration of the Makela seepage. Actual iron removal for AMD seepages which differ from those of the Copper Cliff tailings cannot be derived from the available data. However, the Makela guideline of 21 days retention, for a pond with a surface area of 128 m^2 and 1 m depth is likely applicable for all Copper Cliff seepage stations

as the old tailings dams can be expected to produce similar seepage characteristics. In Table 10, the iron precipitation pond volumes are estimated, based on the average retention time estimated for Cell 1 (79 days) and on the concentration differences with equilibrium reached after a minimum of 21 days. Pond volume based on a retention time of 79 days is much higher than that obtained using the concentration differences with a lower retention time as a design parameter.

Table 10: Pond volumes required to remove Fe from INCO seepages

	July 1992 data	
	Based on retention time	Based on precipitation rates to remove 65 % of Fe
Fe removed Stn I-4 (mg/L)	288	
Flow rate (L/min)	1.125	
Retention time (days)	79.1	21
Fe removed (mg/min)	324	
Fe removed (m3/mg/min)	0.395	
Makela Seepage (Pumping Pond)		
Flow rate (L/min)	364	
Volume required to remove 300 mg/L (m3)	43,100	11,442
Whissel Seepage		
Flow rate (L/min)	542	
Volume required to remove 300 mg/L (m3)	64,200	17,044
Pistol Seepage		
Flow rate (L/min)	417	
Volume required to remove 300 mg/L (m3)	49,400	13,115
Levack Seepage		
Flow rate (L/min)	1389	
Volume required to remove 300 mg/L (m3)	164.600	43.699

To assess the adequacy of existing seepage station size (data provided by INCO) to naturally precipitate iron, flow rates and pond areas were used to calculate retention times (Table 10). To estimate the existing pond volume, a depth of 3 m is assumed.

For the Makela seepage, a flow of 523 m^3/day collects in a pond with a surface area of 655 m^2, resulting, at a depth of 3 m, in about a 4 day retention time, too short for complete precipitation. For the Whissel Dam seepage station, the pumping pond has a surface area of 1085 m^2 with a flow rate of 780 m^3/day, which results in a retention time similar to that at Makela (4 days). For the Pistol Dam, the collection pond is larger (3972 m^2) with a flow of 600 m^3/day. This results in a retention of about 20 days. This, based on the chemical precipitation rate, would be sufficient to produce acidified water with a low enough iron concentration to enter an ARUM system.

These predictions have practical applications. The chemical characteristics of the seepage pumping stations can be determined through a sampling program. From these data, the findings of the Makela Test Cell System can be verified and adjusted to optimize iron precipitation in the seepage station pumping ponds. Although in relation to the total water volume of the Copper Cliff tailings, the seepage volumes which are recycled onto the main tailings area are relatively small. It can be considered beneficial to recycle as little iron to the tailings as possible. Less iron would be available for reduction as it enters the tailings mass and subsequent acid generation as it emerges in the seepage.

Should one of the pumping ponds have the suitable size to serve as a precipitation pond, such as the Pistol Dam seepage, it might be possible to consider scaling up the ARUM system. One of the reasons for constructing the MEND project Test Cell System in such close proximity to the Copper Cliff seepages, was that, if it worked, it might reduce the quantity of seepage which has to be recycled to the main tailings.

5.2.3 Operating parameters of sulphate and iron removal in ARUM

The ultimate goal of the ARUM process is to reduce oxidized sulphur back into sulphide, and precipitate various metal ions as secondary minerals. Therefore, in ARUM sediments, conditions have to be achieved which result in metal-sulphide precipitation, the most likely precipitate being amorphous FeS, but pyrite can form.

As AMD effluents display a wide range of characteristics and metal concentrations, an overall assessment of application to ARUM, called for an evaluation of ARUM operations under different AMD conditions.

To address this task specifically to the chemical operation of the ARUM process in different types of AMD, it is necessary to describe the complex conditions which lead to secondary mineral precipitation.

It is not possible to present a summary for each range of metal concentrations which might be encountered in different AMD streams within the context of this report. Key parameters have been summarized for a concentration range of iron and sulphur and a range of E_h conditions in which sulphate may be reduced to sulphide and produce a precipitate with iron.

The means by which this can be achieved is through the construction of E_h-concentration diagrams of iron and sulphur. These conditions are created in the ARUM sediment as indicated by E_h measurements, which together with pore water metal concentrations and pH, serve as the key indicators of ARUM process operation.

In Figures 23-la to 23-1c and Figures 23-2a to 23-2c, six diagrams are presented, which show how iron and sulphur species change with E_h in the pore water. The original AMD is considered to have a pH of 2, 5 or 7.

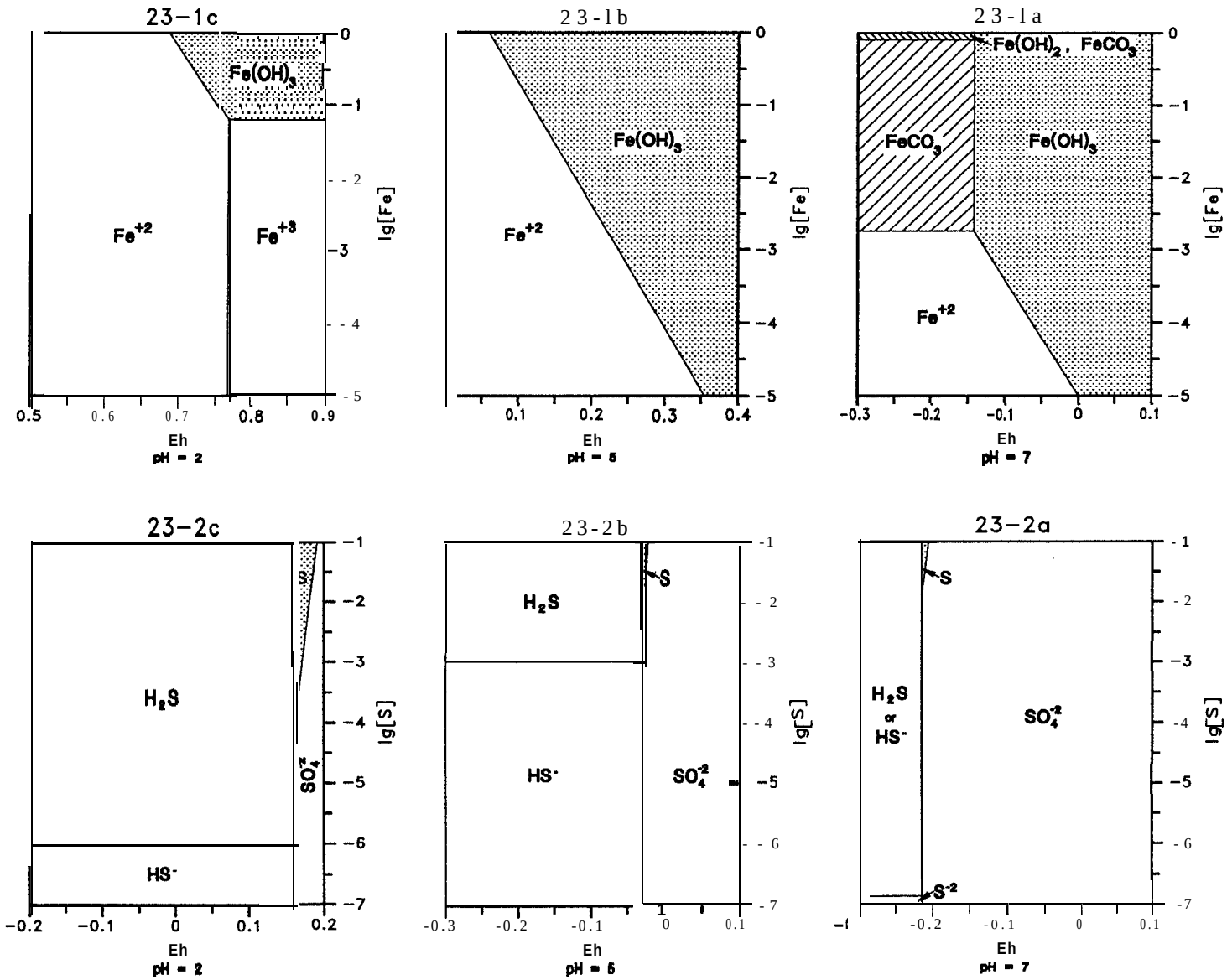


Fig. 23: Eh/pH phase diagram.

Iron may be present in AMD as either Fe^{+2} and Fe^{+3} ions. Fe^{+2} ions can precipitate as $Fe(OH)_2$ or $FeCO_3$, when Fe^{+2} concentration or pH is high. Fe^{+3} ions can also precipitate as $Fe(OH)_3$ or FeOOH (goethite) at high concentrations of Fe^{+3} or high pH. The following physical constants are used to derive the diagrams.

$Fe^{+3} + e \rightleftharpoons Fe^{+2}$	$E^0 = 0.771$ v
$Fe(OH)_3 + e \rightleftharpoons Fe^{+2} + 3OH^-$	$E^0 = 0.944$ v
$Fe(OH)_3 + e \rightleftharpoons Fe(OH)_2 + OH^-$	$E^0 = -0.56$ V
$Fe^{+2} + CO_3^{-2} = FeCO_3$	$K_{sp} = 3.5*10^{-11}$
$Fe^{+2} + 2OH^- = Fe(OH)_2$	$K_{sp} = 7.9*10^{-15}$
$Fe^{+3} + 3OH^- = Fe(OH)_3$	$K_{sp} = 6.3*10^{-38}$

The parameters used to derive the diagrams in Figure 23 were compiled from CRC Handbook of Chemistry and Physics (1970-1971), Stumm and Morgan (1981) and Kotz and Purcell (1987). E^0 is the standard redox potential of the iron electric couples and K_{sp} is the solubility product of the precipitates.

Figure 23-la (pH=7) indicates that under lower E_h conditions, (E, < -0.142 V), when molar concentration of iron [Fe"] > 1 $.74*10^{-3}$ M (97.3 mg/L), $FeCO_3$ precipitates are formed. At even higher concentrations of $[Fe^{+2}]$ (> 0.794 M (44200 mg/L)), $Fe(OH)_2$ can precipitate. When the E_h increases, Fe^{+2} is oxidized, and precipitated as $Fe(OH)_3$.

Figure 23-lb (pH=5) shows that Fe^{+2} is stable in water under most conditions. Since there are fewer OH- ions in the water compared to pH=7, it needs higher a E_h to oxidize Fe^{+2} into $Fe(OH)_3$ states. When lg[Fe] = 0 to -5, at 1 atm with a pH of 5, $FeCO_3$ and $Fe(OH)_2$ cannot be formed.

Figure 23-1c (pH=2) represents an acidic situation where Fe^{+3} ions may exist in water (pH < 2.1). At high concentrations of [Fe"'] (> 0.063 M (3533 mg/L)), Fe^{+3} precipitates

as $Fe(OH)_3$. When E_h drops below 0.77 V, Fe^{+3} is reduced to Fe^{+2}. In the presence of organic matter, iron reduction can also be carried out by micro-organisms.

In Figures 23-2a to 23-2c the E_h/pH diagrams for sulphur are described. In most AMD waters sulphur is present as sulphate ions. In the absence of oxygen, or at low oxygen concentrations (anoxic or anaerobic conditions), sulphate is reduced to elemental sulphur or sulphide species. These species include hydrogen sulphide and its ionized forms, H_2S^- and S^{-2}. The physical constants used in the diagrams are:

$SO_4^{-2} + 8H^+ + 6e \rightleftharpoons S + 4H_2O$	$E^0 = 0.357$ v
$SO_4^{-2} + 8H^+ + 8e \rightleftharpoons S^{-2} + 4H_2O$	$E^0 = 0.159$ v
$SO_4^{-2} + 9H^+ + 8e \rightleftharpoons HS^- + 4H_2O$	$E^0 = 0.252$ v
$SO_4^{-2} + 10H^+ + 8e \rightleftharpoons H_2S + 4H_2O$	$E^0 = 0.303$ V
$H_2S \rightleftharpoons H^+ + H_2S^-$	$K_1 = 1.0*10^{-7}$
$H_2S^- \rightleftharpoons H^+ + S^{-2}$	$K_2 = 1.3*10^{-13}$

K, and K_2 are ionization constants of H_2S and H_2S^-. E^0s are the standard redox potential for sulphur electric couples.

As the pH decreases, the area in the diagram where elemental sulphur can be reduced increases. This means that the probability of forming elemental sulphur also increases. The critical E_h line between sulphate and sulphide moves to higher E_h values. Thus, the conditions under which sulphides can be formed become less critical.

Hydrogen sulphide (H_2S) is a weak acid, its ionization depends on the pH of the water. At higher pHs, more H_2S molecules are ionized into HS^- and S^{-2} ions. This is the desired condition, as only ionized sulphide species can precipitate with metals. In practical terms, the ARUM system can produce H_2S, but if the pH is not high enough, and the E_h not low enough, the H_2S escapes as a gas from the treatment system. Figures 23-2a to 23-2c describe the conditions under which H_2S is formed.

When iron and sulphur co-exist in water, they may form precipitates of FeS or FeS_2.

$$Fe^{+2} + S^{-2} = FeS \qquad K_{sp} = 4.9*10^{-18}$$
$$Fe^{+2} + S^{-2} + S = FeS_2 \qquad K_{sp} = 4.9*10^{-30}$$

To find the conditions required for FeS and FeS_2 precipitation, diagrams are combined for iron and sulphur at different pH values in Figures 24 to 26.

Figure 24 graphically shows that when the pH=2, and E_h is between 190 and 156 mV, sulphate ions can be reduced to elemental sulphur. If there are ferrous ions (Fe^{+2}) and sulphide ions (S^{-2}) in the water, pyrite (FeS_2) precipitates can be formed. However, if the E_h decreases further, elemental sulphur will be reduced into sulphide (S^{2-}, HS^-, H_2S).

Figure 25 shows the conditions when the pH of the AMD is increased to 5. Pyrite formation needs lower E_hs (-0.046 to -0.066 V), and the probability of pyrite formation decreases, as expressed by the reduced area in the diagram. As pH is increased further to pH 7 (Figure 26), the probability of forming FeS_2 becomes even lower. At lower E_h (< -0.213 V), when enough S^{-2} and Fe^{+2} are present, FeS will precipitate.

Based on the above diagrams, the chemical conditions which prevail during the ARUM process and the changes which are carried out by micro-organisms, indicate that pH 7 is probably a realistic target, which can be reached with optimal operation of the process. An AMD effluent, which has been altered from a low pH value of 2, to pH 7, has already improved significantly in effluent characteristics and could be discharged to a biological polishing system (e.g. with periphytic algae) and to the environment.

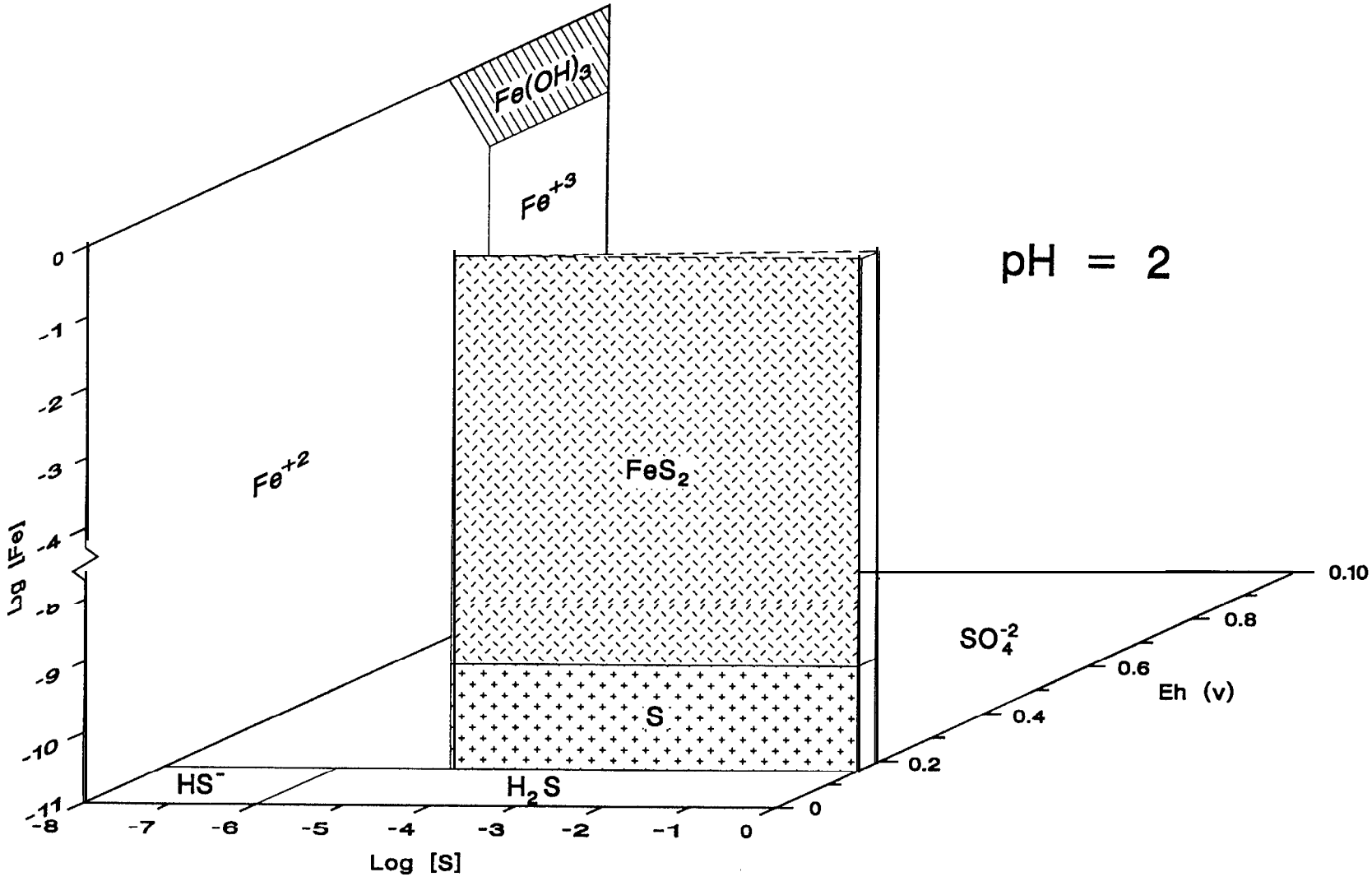


Fig. 24: Eh/S/Fe at pH 2 phase diagram.

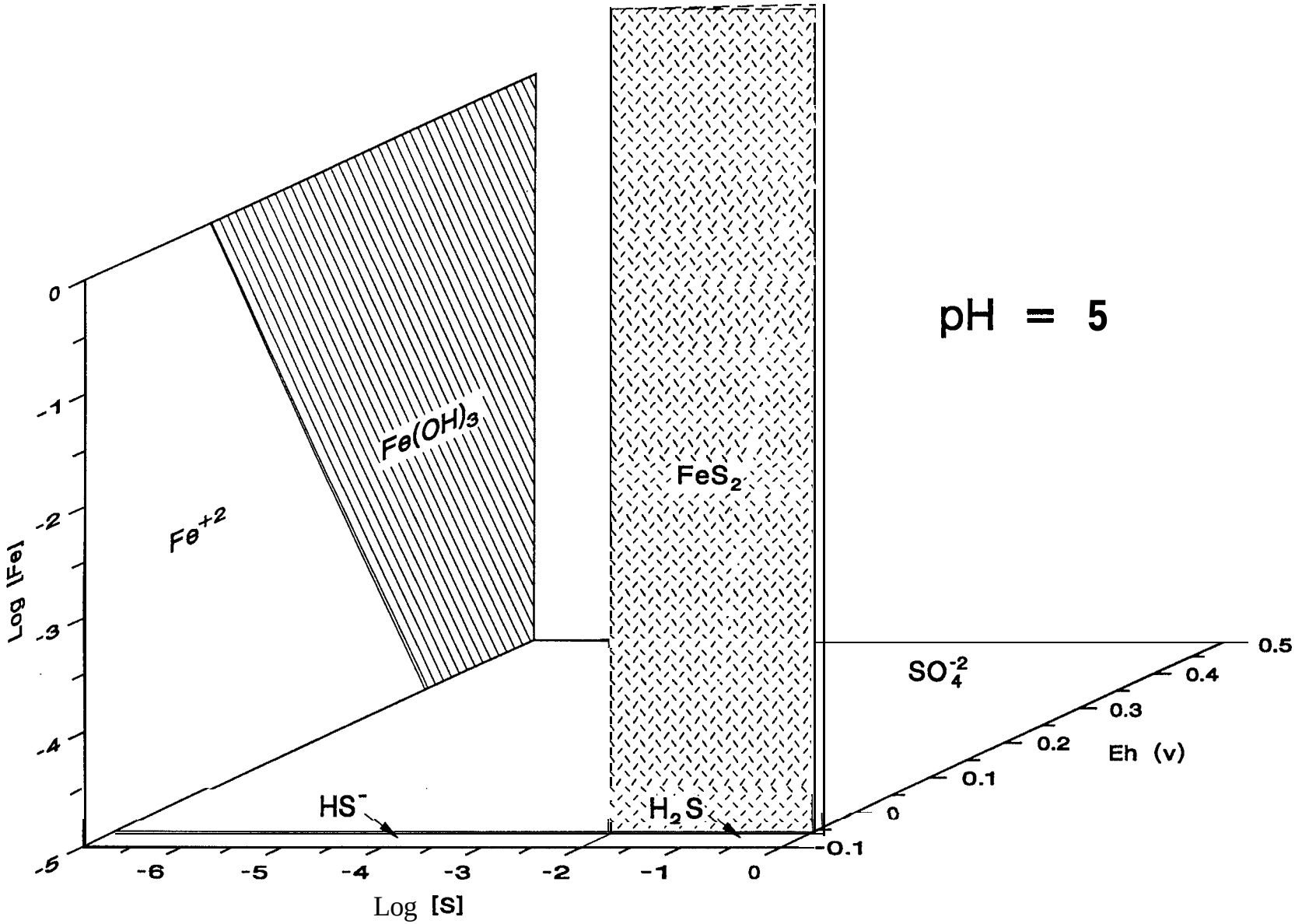


Fig. 25: Eh/S/Fe at pH 5 phase diagram.

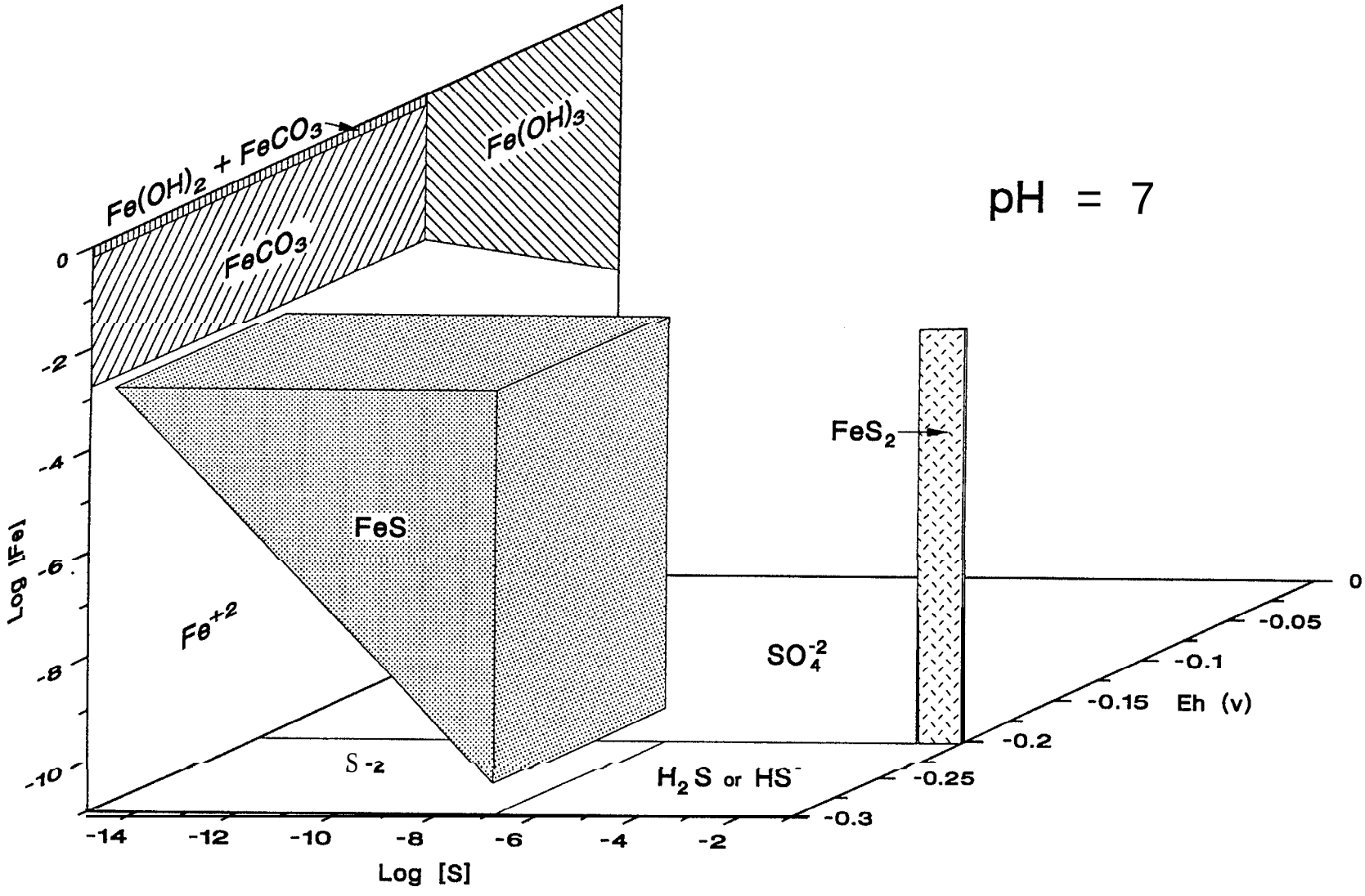


Fig. 26: Eh/S/Fe at pH 7 phase diagram.

6.0 ARUM PERFORMANCE

Through the laboratory and field experiments, it was possible to identify the conditions which lead to ARUM activity in various AMD chemistries. In principle, the sequence of events, which would lead to development of a process, would first require the identification of the system's ingredients, followed by the quantification of the required amounts for each part of the process. Once this has been completed, the process would be assembled and tested. Assessment of the performance of the process is normally carried out on an operational system, where conditions can be changed to determine how the process performs.

As might be deduced from Schematic 2 depicting the key components to the ARUM process, the interactions between the microbial processes and the physical and chemical parameters of the system are very complex. Performance tests to predict effects of particular changes on the functioning of the various parts of the system, have not yet been attempted. We do know, however, enough about of the process to assess the overall nutrient requirements of an active ARUM sediment.

The MEND contract required an assessment of the performance of ARUM in different AMD conditions. This cannot be done, based on the data of the Makela Test Cell System, since this represents only one type of AMD. In order to satisfy the contract requirements, all of the ARUM tests carried out in different AMD conditions by Boojum Research over the last 6 years have been summarized.

The experiments were carried out to provide evidence that microbial colonization of organic matter, added to various AMD types, will result in ARUM activity. A major shortcoming of all data, is the fact that under none of the contracts was it possible to do daily or even weekly sampling, due to financial restrictions. Thus, calculated process performance rates are influenced by the sampling intervals.

The chemistries of the AMDs tested are presented in Table 11. Acidities ranged from 174 to 4250 mg of $CaCO_3$ equiv./L. Similarly, large concentration ranges were found for iron, sulphur and other metals. ARUM activity was tested in the sediments of field enclosures. Only at Selminco (a coal AMD), is the complete ARUM configuration in place, with floating cattails covering the enclosure.

Table 11: AMD Chemistry

Location	System	Inflow (before) water chemistry					
		pH	Acidity (mg/L)	Alkalinity (mg/L)	Fe (mg/L)	SO4 (mg/L)	Metals (mg/L)
MAKELA	ARUMator 1	5.6	1410	161.2	164.7	2905	65 (Ni)
	ARUMator 2	5.2	983	130.8	286	3712	56.3 (Ni)
	Test Cell System	5.7	750		312	2607	37.3 (Ni)
DENISON	ARUMator A	4.8	1014	56.3	391	879	
	ARUMator B	5.1	1605	138	161	34	
VICTORIA JUNCTION	Old Bog Cells	3.3	174		14	924	43 (Zn)
SELMINCO	ARUM Enclosure	3.6	696			2247	56 (Al)
BUCHANS	LC1	3.5					30.1 (Zn)
	LC2	3.6					36.35 (Zn)
	Ponds 7-9	3.0	249		6.7	699	72 (Zn)
SELBAIE	DONUT	5.3	4250		1470	5760	569 (Zn)

Table 12 summarizes the operating characteristics of ARUM in the various test systems. The size of the systems are shown, as are the time periods over which the observed changes in water chemistry occurred. For the Makela Test Cell System, the time period is the retention time between Cells 3 and 4 as measured in July 1992. These are the same data as shown in Table 5, 10, and 11. The changes in acidity, alkalinity, SO_4^{2-} and metal ion removal are expressed in amount removed (mg) per unit volume (m^3) per unit time (minute). These units allow comparison of ARUM performance across the different systems. The acidity data are also expressed in volume required (m^3) to remove a quantity (mg) in a time period (minute). This is useful for estimating size of water bodies required to treat AMD using ARUM.

Table 12: ARUM Performance

Location	System	Period/ return time days	Volume m3	Acidity removed mg CaCO3 equiv/ m3/min	Volume Required for Acidity removal m3/mg/min	Alkalinity gained mg/m3/min	so4 removed mg/m3/min	Fe removed mg/m3/min	Metals removed mg/m3/min
MAKELA	ARUMator 1	65	0.17	6.54	0.153	9.43	6.25	0.95	0.37 (Ni)
	ARUMator 2	120	0.17	4.52	0.221	8.26	9.26	1.55	0.32 (Ni)
	*Cell 3&4	116	188	1.05	0.95		0.34		0.21 (Ni)
DENISON	ARUMator A	104	0.17	4.22	0.237	1.26	5.65	1.98	
	ARUMator B	104	0.17	3.13	0.32	1.03	0.134	4.81	
VICTORIA JUNCTION	Old Bog Cells	454	0.6	0.178	5.6		0.322	0.011	0.066 (Al)
SELMINCO	ARUM Enclosure	55	40	7.09	0.141		22.2	0.29	
BUCHANS	LC1	51	34						0.33 (Zn)
	LC2	122	36						0.18 (Zn)
	Ponds 7-9	3.56	53	2.94	0.34		1.22		1.36 (Zn)
SELBAIE	DONUT	482	0.018	5.52	0.181		2.72	0.472	0.82 (Zn)

* - based on a July 1992flow rate of 1.13 L/min

Values obtained from the test systems, in this manner, indicate that, with one or two exceptions (notably the Victoria Junction Old Bog Cells), the ARUM process performs at a rate of the same order of magnitude for all AMD types. The narrow range of these rates is not surprising, since the same microbiological processes are involved.

Although ARUM operating rates may be similar in different types of AMDs, the time required for ARUM establishment may differ. For example, for the strongest AMD treated (Selbaie B3), it took 18 months under laboratory conditions to initiate ARUM activity. Actual ARUM performance may have been better than reported in all field enclosures, since all the enclosures leaked to various unknown degrees.

In general, no tests have been carried out to determine the length of time over which ARUM activity can be sustained with low or diffuse flow conditions. At some experimental sites, where diffuse flow of AMD was treated, ARUM activity has been maintained for several years.

It was found that retention time, volume to be treated and establishment of reducing conditions in the water column are all factors which play a role in sustaining process performance. The systems compared with respect to ARUM performance were all small-scale, batch experiments, which were not set up to measure performance of a

fully-designed ARUM test system. By mid 1992, the Makela Test Cell System, was in its final configuration. Performance characteristics have been measured since then.

6.1 Amendment Requirements

Decomposition experiments were carried out to provide information on the best suitable material for the construction of sediments to supply the nutrients to ARUM (Kalin et al., 1993, see Appendix). The productivity of the floating cattail mats was quantified and estimates were made of the time required for the mat to be sufficiently buoyant to be self-supporting (Fyson et al., 1991; Kalin and Smith, 1992, see Appendix). It appears from tests carried out on floating mats established in an open pit at Buchans (shown in Plate 1) that 5 years are required to reach buoyancy.

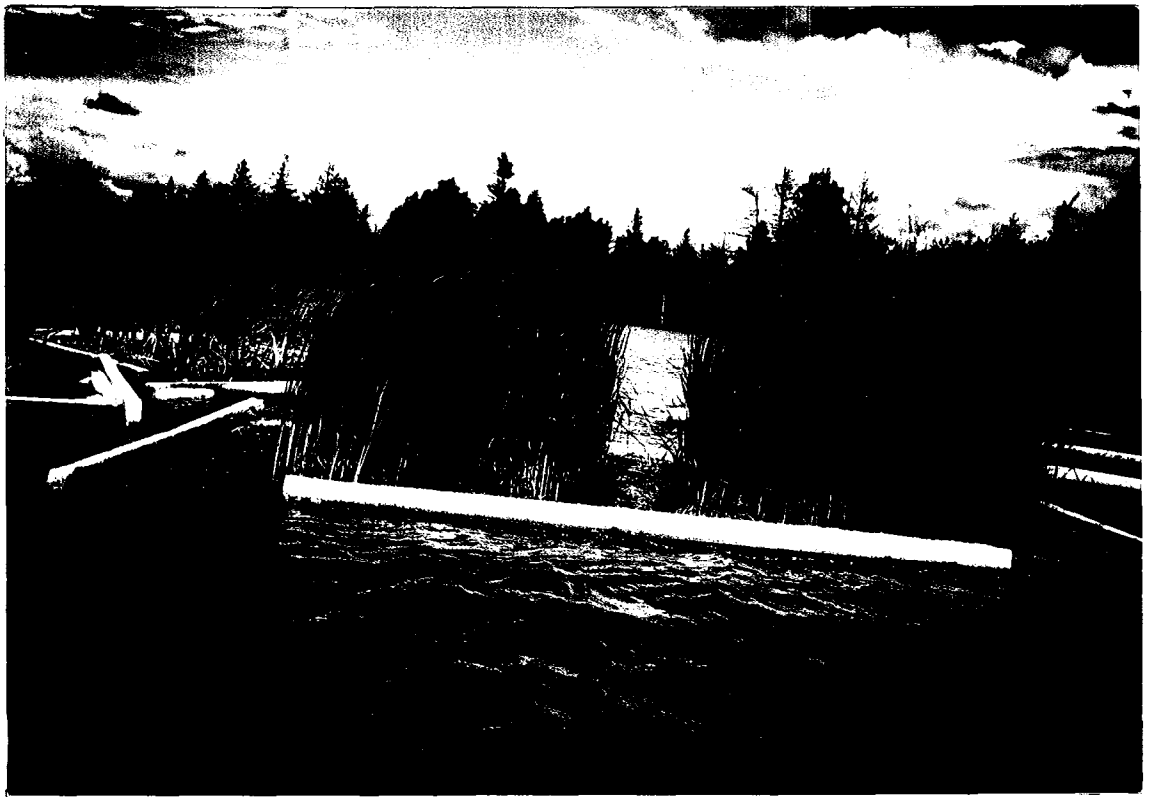


Plate 1: Floating cattail rafts on an open pit at Buchans

For each of the test systems, the amendment requirements were estimated (Table 13). As decomposition in these tests had to provide the required electron donors for sulphate reduction, systems were evaluated with respect to the rate of electron donor supply in μmol/min and μmol/min/m^3, to account for the sulphate and acidity removed at the observed rates.

Table 13: ARUM energy source requirements

Location	System	Volume	Electron donor to remove SO4 at observed rate		Energy source to remove acidity at observed rate	
		m3	umol/min	umol/min/m3	umol/min	umol/min/m3
MAKELA	ARUMator 1	0.17	21.6	127	11.1	65.3
	ARUMator 2	0.17	32.8	183	7.69	45.2
	Test Cell System	188	1336.8	7.1	196.2	1.04
DENISON	ARUMator A	0.17	20	118	7.17	42.2
	ARUMator B	0.17	0.952	5.6	5.31	31.2
VICTORIA JUNCTION	Old Bog Cells	0.6	3.96	6.6	1.07	1.78
SELMINCO	ARUM Enclosure	40	18520	463	2837	70.9
BUCHANS	Ponds 7-9	53	1348	25.4	1559	29.4
SELBAIE	DONUT	0.018	1.02	56.7	0.99	55

In some experimental systems, the values required for the calculations (sulphate and acidity concentrations) were not determined. Generally, the analytical budgets were restricted to metal determinations.

The quantity of nutrients supplied to the microbial communities, and the subsequent rates at which sulphate reduction occurred were measured in several systems (Table 9). Rates of usage of electron donors at Selminco, based on sulphate reduction, were relatively high, with 18,520 μmol/min or 463 μmol/min/m^3. This may have been due to the fact that potato waste and alfalfa generate high specific rates of sulphate reduction. When the data are expressed in μmol/min/m^3, the Makela Test Cell System had a low requirement, due to low specific rates of sulphate reduction.

The amendment rate required to sustain the observed removal rate of sulphate of 1332 μmol/min would translate for the Makela Test Cell System to an addition of about 134 kg of potato waste per year for the sediment in both Cell 3 and Cell 4.

As was discussed in previous sections, it is not surprising that the nutrient supply to drive the biological process (i.e. remove of a quantity of acidity per unit time with biology) is of the same order of magnitude, regardless of the chemistry of the effluent. This is dictated by the fact that to reduce one mole of sulphate requires two moles of carbon and an energy source.

A potential difficulty in operating the ARUM process is the provision of the carbon source as an electron donor for the process. The fact remains that decomposition of organic matter in acidic environments is slow. Although the decomposition experiments carried out have established that some fraction of the organic material placed in AMD environments is biodegradable, the rate at which it decomposed to provide carbon sources for the ARUM sediment is not known. The fact that ARUM operating rates (acidity removed per unit volume per unit time) are high in systems with only straw or hay (e.g. Makela ARUMators), indicates that these materials can support ARUM. However, they may take a longer time to establish reducing conditions than a more readily degradable material such as potato waste.

6.2 Year-round Treatment Capacity

In the previous sections, carbon supply was related to amendment placed into experimental systems. The resulting performance of the ARUM process in different AMD types was discussed. One of the questions frequently raised in connection with biological treatments is, how well they function in the winter.

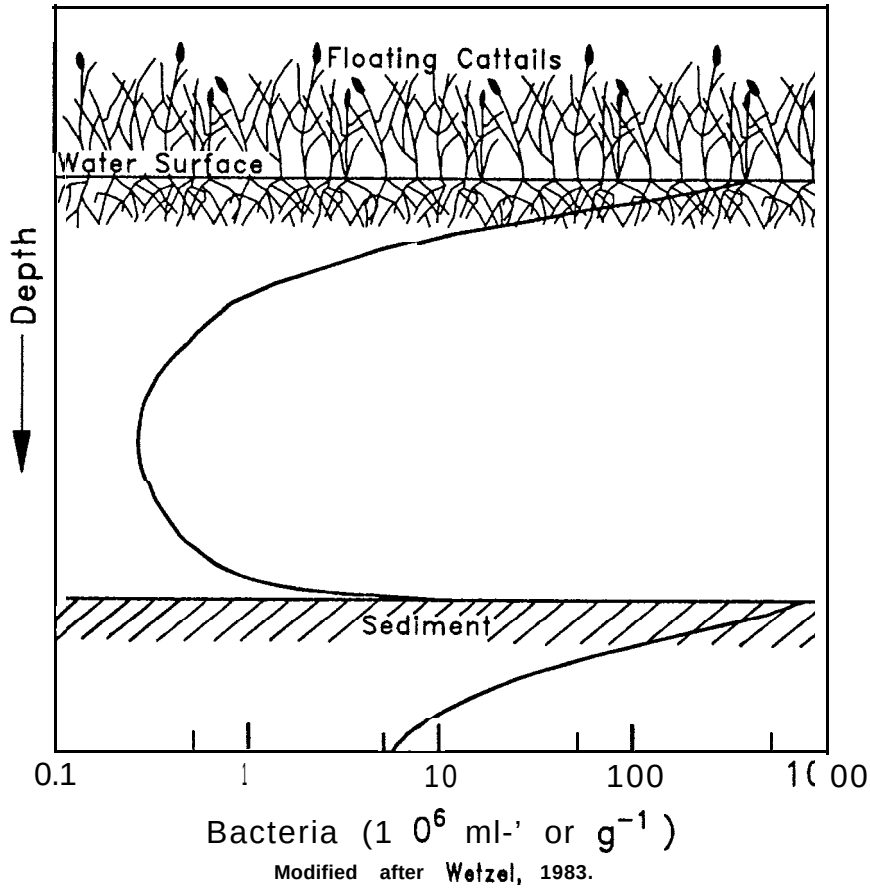
This question can only be addressed on a generic level, since no year-round data are available for a fully operational system. Several samples have been obtained during the winter (including the Makela Test Cell System) when the test systems were frozen over. After drilling through the ice, the rotten egg smell of hydrogen sulphide was often noted. However, as discussed previously, the smell of hydrogen sulphide is not indicative of an operating ARUM process. To the contrary, the smell suggests that insufficient alkalinity-generation has taken place to elevate the pH sufficiently to utilize the hydrogen sulphide in metal precipitation. Hence, the reduced sulphate species escape as a gas. This means that sulphate reduction is proceeding, but the "treatment plant" operating conditions have not been achieved. It is also possible that local re-acidification and dissolution of sulphide precipitates may have occurred, with the consequent generation of hydrogen sulphide.

The main "treatment plant" of the ARUM process is the sediment. Sediments do not freeze during the winter, as they absorb an appreciable amount of heat from the water during the summer. Likens and Johnson (1969) report that shoreline sediments of deep bog lakes are generally warmer than sediments beneath open water. ARUM sediment temperatures can be expected to be relatively stable, as the floating cattail mats will provide insulation over the treatment cell throughout the year.

During the winter, food supply to the treatment system will be reduced, as less algal biomass will be produced and less root exudates (see Schematic 2) will enter the water. Thus, the best time to add easily degradable carbon, if necessary, should be the end of the growing season.

Bacteria are distributed throughout the water column (Wetzel 1983). Bacterial numbers and activity are generally believed to correspond well with productivity of the water body. Productivity can be equated, to a degree, with metabolic activity. The same would apply to the operation of the ARUM process. Thus, winter and summer activity can be expected to differ, due to changes in productivity.

Fig. 27: Vertical Distribution of Bacteria in an ARUM Treatment System with Floating Cattails



Modified after Wetzel, 1983.

Bacterial population numbers are quite high at the sediment surface (Wetzel 1983; Figure 27). Normally, bacterial populations would decrease towards the water surface. However, floating cattail mats will act as a second sediment surface and provide additional microbial activity throughout the year, as described in Figure 27.

The floating cattail mat is therefore essential not only for the provision of reducing conditions, but also for the maintenance of an insulation layer, buffering temperature fluctuations in the treatment system. In addition, the root region of the floating cattail mat also functions as sediment, where ARUM activity will take place. Thus, bacterial population numbers are increased per unit area of pond. An ARUM system, in its proper configuration, can be expected to function throughout the year.

7.0 APPLICATIONS OF ARUM

The ARUM process is one component of an overall decommissioning plan. In general terms, applications of the process are indicated for all water bodies within the waste management area (tailings ponds, seepage collection ponds, acidified lakes, open pits) and applications can also be envisaged along tailings beaches.

As with any other process, ARUM applications require site-specific feasibility studies in which the hydrological conditions, as well as the chemical and physical characteristics of the waste management area, have to be determined. One of the most important criteria is the retention time of the water body in which ARUM sediments are to be established.

Furthermore, the process has to be given time. Time is required to establish, not only the sediment microbial activity, but also, the growth of the floating cattail mats. At present, vegetation mats are established on rafts which are expected to provide flotation after about 5 years, when the cattail roots and rhizomes have achieved sufficient biomass to reach buoyancy. Much experience has been gained with cattail germination and transplanting small cattails to the rafts. In some cases, it is now possible to define conditions to germinate and grow cattails on site.

In principle, for the ARUM process to function, a cover is usually needed. To achieve this from a practical point of view, an artificial floating cover could be used at the time of installation. Cattail populations could be established on top of the artificial floating cover which, with time, would provide the long-term sustainability of the system.

To define the time frame required for the establishment of ARUM activity (a detectable local rise in pH in sediments), data from all the experimental sites are summarized in Table 14. The placement method of the organic matter does not seem to be important in relation to the time to onset of ARUM activity. It appears that, in general, ARUM

activity can be expected one year after the placement of organic amendments. The **time until** elevated pH pockets were measured was determined by the time of the field **trip,** and by no means represents the actual onset time. Frequent sampling was never possible.

Table 14: Time required to establish ARUM (pockets of elevated pH) in field systems

Site	Location	Date Set up	Placement Method	Amendment Material	pH Original	pH Elevated	pH Elevation days
Levack	PBAC	1986	curtains	straw			365
Makela	Test cells	11/1989	ARUMators	flax	3.0	5.2	250
			curtains	flax	3.0	3.5	350
			ARUMator 3	flax	3.0	4.7	300
Stanrock	Straw Pond	1988	mat	straw	2.1	5.5	365
		5/1 990	ARUMators	flax	2.3	4.9	450
		3/1 991	honeycomb	flax/hay	2.3	4.0	218
Buchans	LC2	1989	limnocorrals	peat	3.5	4.8	290
Oriental	LC1			sawdust	3.5	4.5	140
Pits		6/1 990	curtains	hay	3.5	6.0	365
	Ponds 7-9	7/1 989	mat	hav	3.4	5.5	450
Selminco I	boa	10/1989	mat	hav	3.0	3.0	270
VJCPP	New Bog	1988	mat	hay	2.5	4.0	600
	Old Bog	1988	10 cells		3.5	6.0	300
		1988	mat		2.5	3.0	800
South Bay	Decant Pond	7/1 988		straw			680
	Mill Pond	7/1 988		straw			680
	Mill Pond	7/1 987		sawdust			680

If one were to plan an ARUM installation, it would be reasonable to expect that one year after placement of organic matter, a readily-degradable carbon source (e.g. potato waste) should be added to the sediment.

To arrive at a general framework for the application of the ARUM process, the locations of Boojum's experimental sites will be used as examples.

INCO seepages: Table 15 provides estimates of ARUM requirements to remove acidity from INCO seepages. The areas of ARUM ponds required are calculated as follows. The Test Cell System, with a flow of 1 L/min, resulted in an acidity removal rate of 0.95 m^3 of ARUM active water/mg of $CaCO_3$ equiv./min. Therefore, if a flow of 364 L/min as used for the total Makela seepage flow, it can be estimated that a volume of 173,000 m^3 of ARUM active water is required to remove an acidity of 500 mg/L equiv. of $CaCO_3$. Such a volume of pond would have a retention time of about a year, or three times the retention time of the Makela system (116 days, July 1992). If the acidity is lower, the required pond volume can be reduced. The size of an ARUM treatment pond is therefore dependent on the concentration of acidity to be removed. The size of the pond also depends on the depth of ARUM active water (Table 15).

Table 15: Pond size estimates for ARUM treatment of INCO seepages
(Volume of cells 3 + 4 = 188 m3)

	Source data					
	July 1992					
Acidity removed Stn 6-13 (mg/L)	175					
Flow rate (L/min)	1.125					
Acidity removed (mg/min)	197					
Acidity removed (m3/mg/min)	0.95					
	Flow rate (L/min)	Loading @ 500 mg/L (mg/min)	Volume required (m3)	Area required (ha) 1 m depth	3 m depth	5 m depth
Makela Seepage (Pumping Pond)	364	182000	172900	17.29	5.76	3.46
Whissel Seepage	542	27-I 000	257500	25.75	8.58	5.15
Pistol Seepage	417	208500	198100	19.81	6.6	3.96
Levack Seeeaae	1389	694500	659800	65.98	21.99	13.2

It should be noted that, to date, we have no information on the maximum depth to which reducing conditions can be established in a water body with a floating cover. Given that the Makela system is about one meter deep, a scale-up depth of 3 m does not seem unreasonable.

The areas required for the three other seepages stations are not impractical, particularly if a pond with 5 m depth could be constructed (Table 15).

The Pistol dam precipitation pond was estimated to provide sufficient retention time to precipitate most of the iron. Retention structures could be constructed in the vicinity of this seepage station to pond the annual flow of about 200,000 m^3. An area of between 4 ha to 20 ha would be required for such an ARUM treatment pond, depending on its depth.

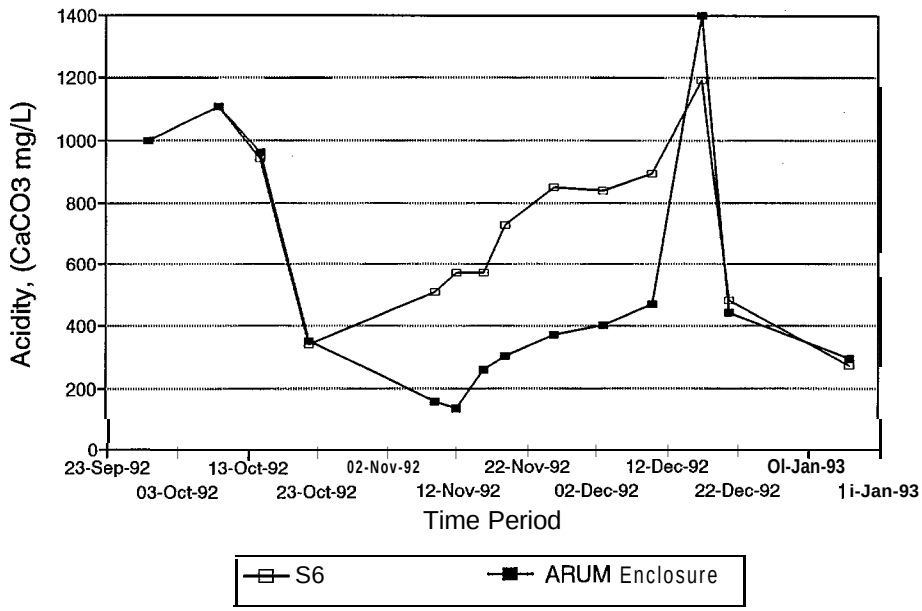
If the same scale-up considerations are used for the flows from the Levack tailings area, it is evident that a downstream ARUM system would not be feasible, as a pond required to treat this flow would need an area larger then the tailings area (45 ha) unless it were more than about 2 m deep. To apply ARUM at Levack, within the decommissioning scenario, the area of the tailings where annual precipitation could be ponded should be determined. As mine slimes have been distributed over the tailings in cells created of waste rock berms, infiltration of annual precipitation into the tailings would be minimal. Ponding would be expected, particularly in the lower portion of the tailings area, where the retention pond is presently located. This area could then be converted into an ARUM treatment pond, which would partially treat the seepage leaving the tailings area. A further application of ARUM would be the gravel pit which is located beside the tailings area. This pit likely feeds a seepage, which joins the present tailings discharge into Grassy Creek.

Some of the early test work on the development of Ecological Engineering as a decommissioning approach was carried out at the Levack site, under the RATS program. The first set of recommendations from this work resulted in the use of mine slimes to cover the tailings. Cattails and other vegetation colonized the mine slimes. Further implementation of Ecological Engineering methods, such as the ARUM process, would require the assessment of dam stability, hydrological balances for the site, and the identification of seepage pathways.

A coal pile seepage: At Selminco, a seepage from coal waste rock presently passes through a series of precipitation ponds, where phosphate rock is being tested for the removal of the iron. An experimental 10 m x 10 m ARUM enclosure was established in September 1992. Alfalfa pellets and potato waste were added to stimulate microbial activity and generate reducing conditions. Cattail rafts were placed to assist in generation of reducing conditions (and in the long-term provide carbon for ARUM).

Early results have been promising (Figure 28). In particular, the period from late September to mid November showed good acidity removal in the enclosure. The performance ceased in mid December, reportedly due to a massive rainstorm, when the water was washed out of the enclosure.

Figure 28: Selminco Summit
Acidity at S6 and in ARUM Enclosure



To estimate the scale-up of ARUM for this site, the data on acidity removal in the enclosure were used. The volume of water required and the amount or supplement of amendment required to treat the seepage using ARUM have been calculated (Table 16). The acidity reduction noted in the enclosure was used to assess the volume of ARUM-active water, which in turn determined the retention time of the system. The

existing system has a retention time which is not known, as flow control for all cells was only partly achieved by the summer of 1992.

Table 16: Selminco Summit - Requirements for ARUM

Acidity removed	560 mg/L
Volume of AE	40 m3
Acidity removed from AE	283 mg/min
Acidity removed - volume to remove unit weight per unit time	0.14 m3/mg/min
Base flow (S1) - summer conditions	94 m3/day
Acidity after removal of metals with phosphate rock	62 mg/L equiv. CaC03
Annual acidity loading	2,127 kg/year or 4.05 g/min
Volume required to remove acidity @ 450 mg/L	571 m3
Area required at 40 cm depth	0.14 ha
Area required at 1 m depth	0.06 ha
Turnover time	109 days
Area of existing ARUM cells	0.39 ha

Based on data for AE of November 16, 1992 and at S6 at time of AE set up (September 22, 1991)

Organic Carbon Requirements for Treatment of S6 Water by ARUM

Acidity after removal of metals by phosphate rock	62 mg/L or 0.62 mmol/L
Annual acidity loading with base flow of 94 m3/day assuming 1 mol energy source required for 1 mol acidity consumption by sulphate reducers	or 21,270 moles/year
Energy source required (acetic acid)	21,270 mol/year or 1.32 tonne/year
Potato waste contains 60 % starch, all degradable to acetic acid	
Potato waste required	2.20 tonnes

Based on data for AE of November 16, 1992 and at S6 at time of AE set up (September 22,1992)

Using the acidity removal rates observed in the enclosure, and the previously discussed nutrient supply requirements, it can be estimated that, for this system, on an annual basis, about 2.2 tonnes of potato waste are needed to maintain the ARUM removal rates. The average flow per day from the seepage is about 94 m^3/day (in summer).

Small open pit: At Buchans, limnocorrals (isolated water columns within lakes) were set up in the Oriental West Pit (OWP) with various amendments to establish ARUM conditions. Using the data from the limnocorral with peat, which has maintained low zinc concentrations for four years (Figure 29), quantities of amendment and required volume of water were estimated for removal of zinc and acidity for scale-up of the processes for the entire pit (Table 17).

The pit is estimated to have a volume of 66,000 m^3, and retention time of 0.5 to 1 year. The pit turns over completely as the ice is melting. The pH of the pit is about 3.5. In this case, the application of ARUM would depend on the pit volume being large enough for the rates observed in the limnocorral. Enlarging the pit is not an available option.

Utilizing the previous scale-up approach, it is apparent that the ARUM can be implemented in the whole pit, at a scale large enough to remove the acidity, but only if 33 tonnes of potato waste or 714 tonnes of peat are added.

A lake acidified by tailings seepage: Ecological Engineering is in its final stages of implementation as a decommissioning approach at a mine site in northwest Ontario. South Bay Mine, a copper/zinc operation, which was active between 1971 and 1981, generated 0.75 million tonnes of tailings, with a pyrite content of 41 % and a pyrrhotite content of 4 %. Acid generation, based on the sulphur content, is expected to continue for a minimum of 1,100 years and a maximum of 36,000 years. Boomerang Lake which receives AMD seepages from a perched tailings deposit, and from the mine/mill site, has been integrated into the waste management area as a biological polishing pond. The lake has a volume of about 1 million m^3 and contains natural sediments. For this application of ARUM, the natural sediment needs to be supplemented with a carbon source. The available data on acidity removal rates are used to estimate the amount of potato waste required to increase the sediment activity (Table 18). This application will probably not require the installation of a floating cattail cover.

Fig. 29: Buchans - Limnocorrals
Zinc Concentration of Surface Water

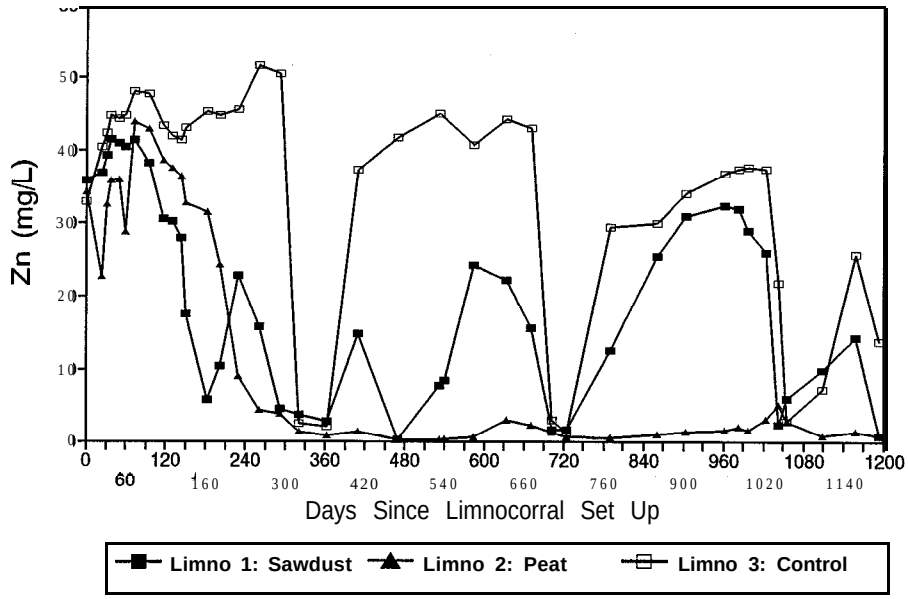


Table 17: Buchans Oriental West Pit - ARUM requirements

Volume of Oriental West Pit = 66245 m3 At a concentration of 30 mg/L, total Zn content of pit = 199 kg Removal rate in limnocorral (LC2) = 5.48 m3/mg/min Rate required to remove 199 kg in 6 months = 754.2 mg/min Volume required to remove Zn with conditions present in LC2 = 4131 m3 Amendment present in LC2 (peat) = 388 kg Peat required to remove all acidity from the pit = 714 tonnes
Total acidity of pit at 100 mg/L = 6624.5 kg equiv of CaC03 Acidity removal (based on pH and Zn2+ removal) = 68.8 mg/L Rate required to remove 6624.5 kg acidity in 6 months = 25139 mg/min Removal rate in limnocorral (LC2) = 14.1 mg/min or 0.39 m3/mg/min Volume require to remove all the acidity from the pit = 64360 m3 Therefore if ARUM conditions were established in the pit as in LC2, the pit would be sufficiently large to remove acidity. In jar experiment, 0.5 g of potato waste removed acidity from 1 L of OWP water To remove acidity from whole pit would require 66245*0.5 kg = 33.1 tonnes

Table 18: South Bay, Boomerang Lake - ARUM requirements

Mean acidity (1992) = 61 mg/L Base flow is 342854 m3/year Total acidity = 20914 kg/year = 39791 mg/min OWP acidity removal = 8.5 m3/mg/min Volume required to remove Boomerang Lake acidity = 338224 m3 Therefore the volume of the lake is sufficient to remove acidity by ARUM
Amendment required In OWP (LC 2) 388 kg/peat has fed ARUm for 4 years 388 kg peat in 36 m3 is equivalent to 10.8 kg/m3 For Boomerang Lake would require 3653 tonnes of peat For OWP, 0.5 g potato waste remove acidity from 1 L of water For Boomerang Lake, would require 338224*0.5/1000 tonnes = 16.9 tonnes

Because ARUM experimentation has not been carried out for this site, acidity removal rates from Buchans (OWP limnocorrals) were used. The calculations are based on removal of a total year's acidity loading in a period of about 150 days (time required to remove acidity in OWP limnocorral). The required volume of water with 3,653 tonnes of peat added, is almost exactly the estimated annual contaminant loadings to the lake. A similar result might be expected from 17 tonnes of potato waste.

The applications presented in this section provide a reference point for applying ARUM within the decommissioning plans of mining operations. The experimental rates obtained in the different acid mine drainage conditions provide a framework for the application of this natural, sediment-driven process. In summary, the examples of the ARUM process given should demonstrate the differences between ARUM and constructed wetlands, as well as dispell the perception that ARUM is only a treatment system for small seeps.

8.0 LIMITATIONS OF ARUM

8.1 Reducing Conditions and Available Land Area

The ARUM processes which remove acidity and metals from AMD require reducing redox conditions. ARUM establishment requires that degradable organic matter exceed the oxygen supply. Clearly, in conditions where it is not possible to establish reducing conditions, ARUM activity cannot be expected.

The hydrological conditions of a site can preclude the possibility of total confinement of the annual effluent with the required retention time. The physical layout of the waste management area can frequently be a serious limitation to the application of the process.

Planning for decommissioning ideally should be integrated during the mine development phase. If, for example, seepages are collected in a pond from a waste rock pile, ARUM could be established in the pond during operations. Floating cattail mats could be installed, at that time, on polishing ponds, which during operations, receive lime-treated effluents. The mats would also assist in controlling suspended solids in the effluent, which frequently do not meet effluent guidelines. At the time of decommissioning, the vegetation mat would already have reached buoyancy and a sediment could then be installed to initiate ARUM.

8.2 Contact Time (Retention Time, Mixing, Diffusion)

If ARUM-active sediments are established, they can only treat AMD if there is sufficient contact time between the water to be treated and the ARUM microbial consortia. Mixing characteristics in the pond will also be important for the maintenance of reducing

conditions and for the interaction of ARUM sediments with the AMD. Diffusion rates and convection are critical in water bodies with low flow rates.

If contaminated ground water enters at the bottom of a water body, which can be the case in a open pit, this physically prevents the installation of an active sediment. Near the surface of the sediment (amendment materials) diffusion rates are critical.

ARUM processes take place in biofilms on surfaces. A biofilm is a surface-bound population of bacteria within a matrix of extracellular polysaccharide. In nature, bacteria generally live in such environments, and not in stirred (and therefore uniform) culture media, as used in the microbiology laboratory. A biofilm, for example, with sulphate-reducing bacteria will be limited in size and 'treatment capacity' by concentrations and gradients within the biofilm. Thus, if the sediment becomes too compacted and diffusion to the biofilms in the pore spaces of the sediment is reduced, it can be expected that the capacity of the treatment system will be reduced.

8.3 Toxics (Heavy Metals, Organics)

Heavy metals are toxic to all living organisms, attributable to their interference with metabolic processes etc. Bacteria are generally able to tolerate low concentrations of dissolved metals in culture media. In environments with high concentrations of heavy metals, bacteria have evolved mechanisms to tolerate these conditions. At the genetic level, there is an extensive literature on heavy metal resistance genes which are often borne on extrachromosomal DNA, e.g. plasmids. Such plasmids can be exchanged with other bacteria, spreading the ability to tolerate metals to a variety of micro-organisms.

It has been noted in laboratory experiments that if the organic matter addition produces high concentrations of volatile fatty acids, the sulphate reduction ceases.

Bacteria which use metals as electron acceptors live in environments with high metal concentrations. Living within biofilms can protect bacteria (through diffusion barriers) from metals in the bulk water. The ability to establish ARUM in the strongest AMD tested (Selbaie B3 water, with 4250 mg/L $CaCO_3$ equiv. acidity, 1470 mg/L iron, 569 mg/L zinc and 5760 mg/L SO_4^{2-}) indicates that, although the strength of the AMD may increase the time to establish ARUM and reduce the rate of ARUM, it does not per se prevent ARUM establishment. Thus, presence of metals affects the onset of the process, but does not inhibit it.

8.4 Presence of Bacteria

In all ARUM field experiments, ARUM activity has been established by indigenous bacteria i.e. without inoculation. It is perhaps surprising that micro-organisms required for the various ARUM processes are present. It should be noted, however, that these processes will occur naturally near the mine site, notably in muskeg. It is also becoming established that some anaerobic bacteria are very flexible in terms of the processes they can use to provide energy. For example, the study of Coleman et al. (1993) showed that Fe^{3+} reduction was carried out in salt marsh sediments by bacteria previously described as sulphate reducers. These characteristics of bacterial populations do not per se represent a limitation, but it is strongly advised, that for each site, feasibility tests be carried out. It is not possible to state categorically that ARUM consortia will always colonize organic matter introduced to Acid Mine Drainage.

8.5 Nutrients

Research on processes in anaerobic digesters (for treatment of sewage) have provided guidelines for optimal ratios of carbon to nitrogen and carbon to phosphorus, as indicators of nutritional status of anaerobic consortia. For example, Frostell (1981)

determined an optimum carbon to nitrogen ratio of 23:1 and phosphorus to nitrogen ratio of 113:1 for fixed film reactors. The basic metabolic requirements for ARUM are likely to be similar, although no work has been done on the subject. The composition of potato waste used at Makela (data provided by McCain's) has a carbon to nitrogen ratio of 225:1 and a carbon to phosphorus ratio of 29:1. These are higher than the ratios defined by Frostell (1981). Potato waste may not be the perfect waste material to use for establishment and maintenance of high rates of ARUM. However, other amendments such as straw, peat and hay have much higher carbon to nitrogen and carbon to phosphorus ratios, and a large fraction of materials are not readily decomposed. In addition, the nitrogen and phosphorus fractions may be more readily leached out and made available than the carbon, and therefore, will be rapidly exhausted. Thus nitrogen and phosphorus supply may become limiting to ARUM. Detritus from cattails growing on the ARUM ponds is likely to provide suboptimal quantities of nitrogen and phosphorus for ARUM. Chynoweth (1987) gives a carbon to nitrogen ratio of 41 :1 and carbon to phosphorus ratio of 278:1 for cattails. The nitrogen and phosphorus content of cattails will undoubtedly depend on growing conditions. Phosphate may also be bound by organic matter and be unavailable to ARUM bacteria. Therefore, additions of these nutrients may be required infrequently to maintain high ARUM rates. It is assumed that nutrients will be recycled to and from the sediments, once the ARUM process is initiated.

9.0 ECONOMIC CONSIDERATIONS

A significant fraction of decommissioning costs results from the placement of covers on tailings and waste rock, and from the operation of a lime treatment facility for effluents. A reduction in seepage volume might be expected with cover placement. However, generally the hydraulic retention time of water contained in the waste materials is such that contaminated seepages can be expected for a long time. At most mines, a lime treatment facility operates both during and after operations to treat the effluents.

An objective of the ARUM process is the reduction in costs associated with decommissioning. Capital and operating costs of several technology trains have been recently identified in the Kilborn (1991) study, for five subsectors of mining, gold, base metal, iron, uranium and silver. The unit operation costs for the technology train 'tailings pond/ hydroxide precipitation/ polishing pond' are given for the base metal sector as $\$0.28/m^3$ for low flow (case A) and $\$0.10/m^3$ for high flow (case B) situations. For a ten year period, the accumulated annual costs would be $1.6 million in case A and $2.3 million in case B.

In the evaluated technology trains, no costs for sludge disposal are evident (Kilborn study, Appendix E, page 15), which, of course, have to be considered.

The ARUM process should be viewed as a measure which will result in reduction in the long-term costs. After five years of installation, cost for case A with a flow of 583,000 m^3/a and case B with a flow of 2.3 million m^3/a could be expected to be halved to $\$0.14/m^3$ and $\$0.05/m^3$, respectively. This is based on the fact that ARUM will reduce acidity, which, in turn, reduces lime and labour costs. After 10 years, it is reasonable to expect that an ARUM ecosystem will be self-sustaining (requiring little maintenance), resulting in a further cost reduction.

Design: The costs of designing an ARUM system at a specific site depends on the amount of information which has already been gathered regarding the hydrology and the geochemistry of the site, and seasonal changes in effluent characteristics. Generally, the essential information is available. The design phase can include field tests to select and test the most suitable, locally available, organic material for the sediment. Design costs are not expected to exceed $100,000, assuming that expert advice is sought at that stage.

Construction: These costs will vary from site to site. They will greatly depend on whether ponds need to be excavated and berms constructed for seepage retention. Excavation costs are dependant on the conditions of the terrain. If blasting is required, the costs will increase. Providing construction costs is outside Boojum's expertise.

In most applications, existing retention structures or ponds can be used. The preferred kind of retention structure would be designed so that berms are maintained in perpetuity, providing habitat for wildlife.

Special care has to be taken during establishment of an ecological system, to provide alternate food sources for herbivorous animals. For example, after the third year at a site northwestern Ontario, small cattail floats, used to test regrowth after overwintering, were decimated by muskrats. In designing the retention structures, be they existing or constructed, the final habitat configuration and its wildlife have to be taken into account.

Once the type of retention pond is determined, the next cost to be considered is the material for the floating cattail supports. Such structures are required for a period of 5 years. Floats have been built out of 2x4 lumber supported with Styrofoam, using fish-netting or burlap. This construction method is adequate for experimental purposes, or for applications where cattails mats are used as living baffles for suspended matter control. For covering large areas, other methods should be tested. For example,

geotextiles, which provide floatation such as swimming pool covers, might be considered. Cost per unit area of material for scale-up cannot be given at this stage.

The production of cattails for the rafts requires the appropriate germination conditions. Those conditions are site specific. Plants can be produced in greenhouses to a stage mature enough for transplanting to rafts at the mine site. However, work is progressing to create germination conditions on floats which would not require transplanting. This would be the most cost effective means of establishing cattails, as transplanting plants to rafts is labour intensive.

Cost estimates for plant production can be considered as the one time cost of training company personnel to germinate plants each year. A cost of about $20,000 would cover the training period. Once the site-specific conditions for plant production are established, cattail crops could be produced each year by the company.

Finally, the construction of an ARUM system has to take into account costs of sediment material. As it became evident from the work on decomposition, the choice of structural material such as peat, straw or hay is not as important as the addition of readily degradable organic material. Consideration should be given to any organic material which is available, in the vicinity of the mining operation. This could be brush cuttings from a logging operation, muskeg which is being cleared during exploration or sludges from an on-site sewage lagoon. The only material which is not recommended for use is sawdust. Even this statement has to be qualified, as some sawdust or woodchips might be useful in combination with other material.

Once the cheapest available organic materials have been identified, they should be tested for suitability as ARUM substrates. Although data are not available for different substrates, nitrogen and phosphorus content of the sediment material are important, since they will affect the rate of decomposition.

In light of these considerations, cost of sediment materials becomes minimal, compared to the shipping and handling costs. Those are site-specific and will depend on the distance and unit volume which has to be transported. For example, empty concentrate trucks returning to the site can be used to transport wood wastes or other material for the ARUM sediment. We are not in the position to give costs of this nature.

Operations: These are probably the only costs which can be readily assessed as a tonne of potato waste was sold to Boojum at US $200, packed in 50 lb bags. The freight from Maine to Toronto was $315. The material was used in many experimental systems at various sites, at a rate of 0.5 kg per m^3 of seepage water.

Potato waste is not the only material which can be considered. Easily degradable carbon sources should fulfil three criteria. The material has to sink to the sediment, the material should not dissolve, and finally it should have a reasonable microbial nutrient value as discussed previously. Materials such as alfalfa pellets are suitable.

In summary, the most substantial installation costs of an ARUM system are the construction costs of retention ponds. These are not required when existing ponds are sufficient to do the job. The next largest cost factor would be the installation of the floating support for the cattails mats along with the shipping costs for the sediment material. The chemical and hydrological information which is required for the feasibility study ranks third in cost. Operation costs would be the lowest.

Decommissioning requirements for mining operations do not appear only at the end of the operation, but can be part of the overall plan. The implementation of an ARUM system can be planned in stages, as material becomes available at the site. This would lower costs further.

10.0 CONCLUSIONS

The Test Cell System and the associated ARUMator experiments were constructed to determine the conditions under which a microbial system could ameliorate tailings seepages. The construction of the Test Cells encountered several problems, and flow control in the final ARUM configuration was only achieved in the system during 1992. In 1992, with a floating cattails cover over a microbially-active sediment, the project yielded valuable data on ARUM operating conditions.

In general, alkalinity-generation, in the presence of iron, is dominated by iron reduction. The pH of the seepage can be increased from 3 to 5 and higher. As the pH increases and Fe^{3+} becomes more limiting, sulphate reduction takes place. Concurrently, acidity is reduced and alkalinity is generated in the water.

A drop in metal concentrations in the system was not evident until 1992, when substantial decreases in nickel and sulphur concentrations through the system were apparent. As ARUM increases pH, concentrations of aluminum are reduced, since pHs over 4 facilitate aluminum hydroxide precipitation.

Both the precipitation of iron and aluminum lead to the co-precipitation of both copper and nickel. Both these metals were adsorbed onto organic amendment surfaces (and algal surfaces) which are present in Cell 3 and Cell 4.

Iron was removed in Cells 3 and 4 partly due to precipitation of Fe^{2+} sulphides. Under reducing conditions, Fe^{3+} is reduced to Fe^{2+} and, S^{2-} generated by sulphate-reducing bacteria results in precipitation of Fe^{2+} as sulphide, if the pH exceeds 6. These conditions occurred and continue to occur at the bottom of Cells 3 and 4.

From pH / E_h chemical equilibria, the removal of copper as a sulphide, requires that the solution be above pH 7. The removal of nickel requires a pH greater than 8. In the

Test Cell System, measurements of the bulk solution between the sediment and the floating cattail rafts indicate that these conditions have not yet been met. However, geochemical simulations carried out on water collected from the ARUMators have indicated that ARUM activity results in the formation of 13 different mineral species, such as sphalerite, marcasite, covellite and chalcopyrite. The ARUMators reflect the conditions in the sediment. Hence, metal removal from AMD is a combination of both chemical processes, where co-precipitation with iron and aluminum hydroxides removes some metals (Cell 1 and Cell 2), and microbially-mediated changes in the sediment remove metals as sulphides (Cell 3 and Cell 4).

Further precipitation processes are brought about through alkalinity generation which raises the pH. In summary, in the Makela Test Cell System, the ARUM process is responsible for removal of aluminum, copper, iron and nickel in Cells 3 and 4. ARUM was established throughout the water column in these cells within two months of achieving final configuration. The system is now capable of treating approximately 1440 L of seepage water per day in the spring, summer and fall. In winter, the system had to be closed, and therefore acted as a batch treatment system.

Extensive work on the use of wetlands for the treatment of AMD has been done. The ARUM process is conceptually derived from wetlands. Therefore, a comparison of the published work to the ARUM Makela Test Cell results will be useful. In Table 19a, two open field, constructed wetlands are compared to the Makela Test Cell System. The fundamental difference lies in the substrate used. Both the Big Five Tunnel site (Wildeman) and Weider's wetland in Pennsylvania employed alkaline materials as substrate, namely mushroom compost and limestone.

Makela amendments are all organic. Flax and hay bales, installed as curtains, provide the surface areas for the biofilms. Later, dry potato wastes and alfalfa pellets were added to provide easily degradable carbon sources. All systems increase the pH, but only in the case of the Makela system can this increase be attributed entirely to

Table 19a: Performance of wetland treatment systems for AMD

		@		Weider (1992)				Makela Test Cells*		
System		wetland		wetiand						
Amendment		mush comp /mix			peat	peat	mush comp	lax straw		
Limestone		+				+	+			
Vegetation		cat/sed/rus		cat		cat	cat		cat	
Volume	m3	44.06		126		126	126	188		
Flow	L/min	0.41		5.9		5.9	5.9	1	1	
Retension Time	days	75		15		15	15	168	131	
Time after setup	months	3		0-25				15 (after flow control)		
		inflow	outflow	inflow	outflow	outflow	outflow	inflow	inflow	outflow
								rec/cells	ARUM	
pH	units	2.9	6.5	2.89	2.79	3.06	3.38	5.71	3.01	4.43
Acidity	mg/L			557	559	386	189	565	330	138
Alkalinity	mg/L			0	0	0	253			
Al	mg/L			26.5	93.3	77.8	26.7	0.51	21.6	0.85
Ca	mg/L			517	548	551	627	420	339	313
cu	mg/L	0.614	co.05					0.52	4.94	0.57
Fe	mg/L	36.3	co.5	119.1	93.3	77.8	26.7	274	13	20.7
Mn	mg/L	31 .a	19.7	19.3	21.7	19.6	14.6	4.29	4.37	7.51
Na	mg/L			66.6	70.2	65.9	77.2	103	72	78
Ni	mg/L							31.3	40.9	6.85
so4	mg/L	1740	1480	3132	3169	2976	2644	2571	1905	1620

* - Mean of July an August 1992 data; @ - Machemer & Wilderman (1991)
cat - cattails, sed - sedges, rus - rushes

microbial activity. The same holds for the reduction in acidity and increases in alkalinity. Iron removal is evident in all systems, but iron concentrations in the Makela system are substantially higher than in the Weider and Wildeman systems. It is important to recognize that iron removal in the Makela system is as effective as in the other systems. Sulphate concentrations are reduced in all field systems.

Comparing the retention times of the field systems, both systems have shorter retention times than Makela Test Cells. The Weider wetland has a short retention time (15 days) and the Big Five Tunnel site retention (75 days) is about half that of the Makela system (131 days for ARUM cells; July and August 1992 avg.). The Weider data are based on performance of the system sampled during a period of 67 weeks, but the time period for monitoring data at the Big Five site could not be determined. The ARUM system at Makela has been operational since June 1992, and the data used in the comparison are the averages from July and August 1992, representing a period about 2 months. In October 1992, water samples were collected, and the pHs were (5.70, 2.94 and 6.14

for Stn 1, Stn 6 and Stn 13, respectively). Chemical analyses of the samples were not performed. The pH and E_h data (not shown) indicate that the system has functioned effectively for four months.

In Table 19b, the key publications on reactors are compared to the Makela field ARUMators. Flows through these reactors ranged from 0.055 L/min to 0.15 L/min, which is less than ARUMator 3 with 0.5 Umin. Unfortunately, many of the parameters reported are not available across all three systems. The batch configuration, reflected in the data for ARUMators 1 and 2, can be considered as more effective, supporting the conclusion that ARUM is a microbial process restricted to biofilms (on sediments and suspended particulate surfaces), and as such, is suited to flow-through conditions, but only with long retention times. The iron removal which takes place in biofilms, as well as the reductions in sulphate and the alkalinity-generation brought about by microbial systems in biofilms, will find applications-in decommissioning plans of mining operations.

able 19b: Perform lce of re :tor treatment systems for AMD

		Dvorak et al. (1991)				Kuyucek (1991)		ARUMators*		
System		'eactor 1		reactor 2		lab reactor		field reactor		
Amendment		nush comp		mush comp						
Limestone		+		+						
Volume	m3	0.6		4.5		0.0015		0.2		
Flow	L/min	0.055		0.07		0.15		batch		
Retention Time	days	5		17		7				
Time after setup	months	0.8-4.3		1.5-5.5		1.1		8-20	.	
		inflow	outflow	inflow	outflow	inflow	outflow	0 days	120 days	352 days
pH	units	3.7	6.9	6.3	6.9	2.5-3.1	6.63	5.37	6.56	7
Acidity	mg/L							1197	240	301
Alkalinity	mg/L	17	632	17	1102			146	1700	3035
Al	mg/L	7	0.2			127.4	116	1.37	0.65	3.47
Ca	mg/L	294	518	275	720			542	465	704
cu	mg/L					60.8	0.4	0.0165	0.01	0.18
Fe	mg/L	67	<0.2			180	49.8	281	0.4	1.15
Mn	mg/L			24	0.5			11.1	8.7	15.8
Na	mg/L							215	151	217
Ni	mg/L			0.85	0.03			60.6	0.22	0.6
so4	mg/L	973	712	2969	2352	3675	2300	3663	1485	567

- Mean value for p, midd and bottom samples of ARUMators and 2

As expected, the microbially-driven process is slower, if not augmented with limestone. The applications of the ARUM process have to be viewed with respect to the overall requirements of decommissioning. A retention time of one growing season has to be provided for water treatment. Treatment is most active during the summer months, at which time the water can be released, either to a biological polishing pond or to the environment.

ARUM can be installed in retention ponds (which during operations of the mine were used to collect AMD prior to lime treatment), in shallow open pits, in ponded areas on tailings piles, and in lakes which have become acidified (where sediments can be supplemented to produce increased ARUM activity). ARUM can also be used in conjunction with biological polishing ponds in seepage holding ponds.

The construction of a new ecosystem as part of waste management technique in mining operations is a slow process. The fundamental building blocks of installing an alkalinity-generating system have been identified.

ARUM can not be put together like a chemical treatment plant. The design criteria for the process do not fit well into strict engineering terms. The actual process which leads to the performance of ARUM or 'the treatment plant' will remain, to a large degree, a black box, due to the complexity of its interacting components. The experimental work to date proves that microbial activity can be stimulated and maintained in Acid Mine Drainage when appropriate conditions are provided.

Finally, the most important conclusion, which can be drawn from the findings of the Makela Test Cell System and the work carried out at other sites, is that microbial activity can convert AMD into alkaline effluents. Ecological Engineering technology can now be considered as a planning option for the closure of mining operations.

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EFFECT OF FOLIAR FERTILIZATION ON *Typha latifolia* L. GROWING IN ACID MINE DRAINAGE

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Boojum Research Limited, 468 Queen Street, East, Toronto, Ontario M5A 1T7, Canada

Abstract

Acid mine drainage (AMD) produced through microbial oxidation of mine tailings and waste-rock is a major pollution problem. Amelioration of AMD by wetlands can be anticipated if conditions are created which facilitate microbial alkalinity generation and sulphate reduction. The microbial communities require a carbon source, which dan be derived from *Typha latifolia* L. (cattail) either through release from roots into the rhizosphere or through litter decomposition.

Cattail populations are tolerant of the harsh environmental conditions created by AMD. Iron accumulates in the rhizosphere and within both rhizomes and roots. X-ray analyses of cattail root sections examined by scanning electron microscopy show that very high concentrations of iron in association with sulphur are found on the root epidermal surface.

Experiments with foliar fertilization of cattails are being carried out on acidic tailings in the Elliot Lake area (Ontario, Canada) to stimulate biomass production and hence the potential to ameliorate AMD. Results to date indicate that at the concentrations used, the fertilizer had no significant ($P \leq 0.05$) effects -on dimensions or starch content of overwintering rhizomes, parameters which we are using as indirect indicators of plant productivity. Fertilization significantly ($P \leq 0.05$) reduced the weight of roots on the rhizome sections examined.

These studies suggest that cattails grow well in AMD polluted waters and further studies will help optimise their role in reducing pollution and restoring wetland ecosystems.

Introduction

Cattails (*Typha* spp.) are widely distributed in temperate and subtropical regions of the world. In North America, cattails are found from Alaska in the north (ca 67°N) to Mexico in the south (Scoggan, 1978). They are confined to environments which remain wet throughout the year. In Canada, they may survive within thick ice layers (pers. obs.). Cattails are so effective at colonizing aquatic environments that they are often treated as weeds due to clogging of waterways or out-competing other species and reducing habitat diversity for wildlife (Linde et al 1976). The success of cattails in colonizing a wide range of aquatic situations is,

in part, attributable to the ability of roots to oxygenate the rhizosphere (Dunbarin et al 1988) where otherwise, the anaerobic conditions would prevent root growth. We are investigating the use of cattails as candidates for producing biomass and hence organic carbon in situations influenced by AMD. The sediments of wetlands, including those exposed to AMD, are low in oxygen. In these reducing conditions, anaerobic bacteria (e.g. sulphate reducers) reduce sulphate, iron and other ions in the water. These processes generate alkalinity and with increasing pH, the reduced compounds are precipitated as metal sulfides. The beneficial bacteria which carry out these processes require organic matter as a source of carbon for growth. Foliar fertilization was tested as a means of increasing cattail biomass and hence the potential for microbial alkalinity generation. The alternative of adding fertilizer directly to the substrate will result in rapid leaching of the plant nutrients. We therefore investigated foliar fertilization as a means of efficiently retaining the nutrients on the plants and hence increasing productivity.

Cattails thrive in a range of pHs and can survive high levels of pollution from mine and municipal wastes. Constructed and natural wetlands dominated by cattails have been tested for their ability to survive and indeed to reduce AMD (Sencindiver et al 1989; Brodie et al 1990; Lan et al 1990; Tarutis and Unz 1990). Cattails roots and rhizomes accumulate large amounts of iron, mainly on the surface as a plaque (Taylor et al 1984; Macfie and Crowder 1987; Crowder et al 1987). Dissolved iron is a major constituent of AMD. The ability of cattails to ameliorate the effects of AMD by removing metals from solution is not certain, but cattails can certainly survive in waters with a pH of c 2 or > 10 (Samuel et al 1988; pers. obs.) and iron concentrations of up to 150 $mg.L^{-1}$ (Samuel et al 1988; Stark et al 1988; Brodie 1990). We are investigating the ability of cattails to tolerate AMD and, with both natural and constructed wetlands, are seeking to optimise the role of this plant in ameliorating AMD.

In optimal conditions, cattails are very productive, with a total (above and below ground) annual biomass production of up to 100 $T.ha^{-1}$ (Lakshman 1987). In AMD water, growth is reduced in greenhouse conditions (Wenerick et al 1989), but there is a lack of data on cattails in the field.

Cattail biomass, particularly the underground component, is difficult to measure (Hogg and Wein, 1987). A 'population' of cattails often comprises one or a small number of clones, many shoots derived from one parent and linked by rhizomes. These rhizomes are often found at considerable and variable depths in the substrate, requiring excavation of a considerable volume of material. Parameters measured on these rhizomes can give an indirect measure of productivity (Linde et al 1976). Most of the net photosynthate of the previous summer is stored as starch in the rhizomes. Data on starch content and rhizome dimensions, together with shoot density, can give clear indications of the effects of fertilization on productivity.

Study site

Cattails were studied at two Ontario sites, the locations of which are shown in Fig 1. The work on AMD water chemistry and the accumulation of metals on root surfaces was carried out on water and cattails from seepage below the tailings dam on the Makela site near Sudbury. These tailings, which generate the AMD, are from a nickel and copper extraction operation. The foliar fertilization study was carried out on tailings at the Stanrock site, a uranium mine near Elliot Lake. Off-site control plants were dug from a roadside wetland not subject to direct influence of AMD.

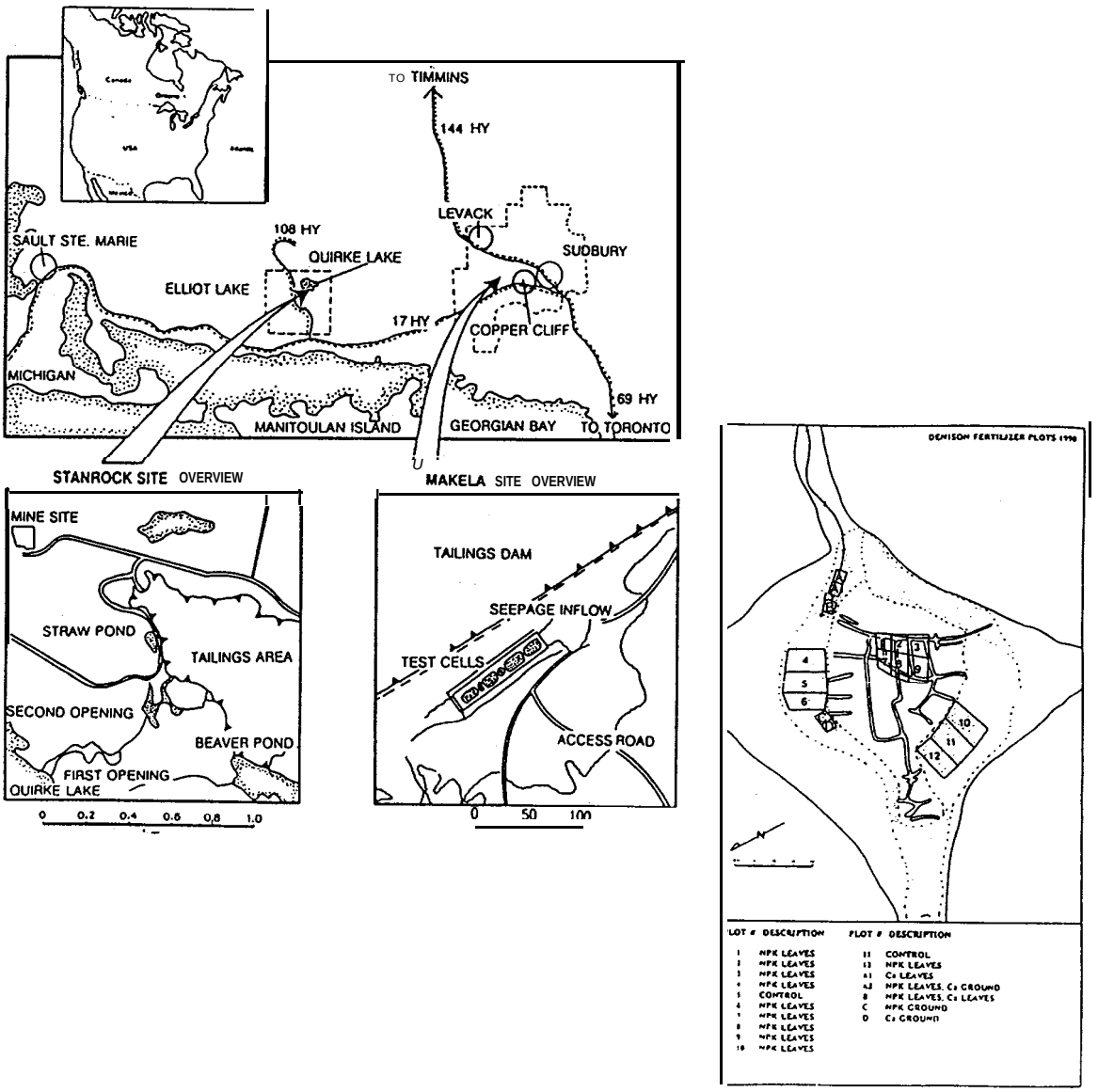

FIG 1 The location of study sites (above) and the layout of the Elliot Lake experiment

Materials and methods

Foliar fertilization experiment

Foliar fertilization was carried out on plots of cattails on the Stanrock site in 1989 and 1990. In 1990, 14-4-6 NPK ('Harvest Plus', Stoller Chemical Company) was applied to all fertilized plots from a portable sprayer on three occasions, at the beginning (May), middle (June) and end (July) of the growing season. Calcium was applied as 'This' (Stoller Chemical Company) liquid fertilizer (6 % calcium). Total applications were 87.5 $kg.ha^{-1}$ nitrogen, 25 $kg.ha^{-1}$ phosphorus and 37.5 $kg.ha^{-1}$ potassium and where applied, calcium, 85.3 $kg.ha^{-1}$.

Field sampling,

Cattails were sampled at Makela and Elliot Lake in February to March 1991. At the Elliot Lake site, holes approximately 1 m^2 were cut through the ice and snow and sediment. Cattail 'plants' (old shoot + attached rhizome and roots) were carefully excavated from the sediment. Five 'plants' were dug from each plot. Plants were photographed and kept under ice in coolers until returned to the laboratory where they were immediately frozen (-20°C).

Makela water sampling

Conductivity, Eh and pH of the stream feeding the Makela cattails were determined in the field. Inductively coupled plasmaspectroscopy (ICP) analysis was carried out by Chauncey Laboratories (Toronto, Ontario) on filtered, acidified samples.

Rhizome sample preparation

To prepare the cattails for morphological and chemical investigations, individual plants were taken from the freezer and washed under hot tap water. Rhizomes formed during the previous growing season (identified by the presence of a new, yet to emerge shoot apex) were measured for length. Rhizome and roots were separated and oven dried at 80°C Hand-cut sections of the rhizome were cut and fixed in 95 % ethanol for starch and metal staining.

Starch determination

Starch content of rhizomes was determined by a modification of the method of Neilson (1943). The rhizome and roots were ground in a Wiley mill and weighed. Distilled water was added to make a 0.67 % suspension. 2 mL of the suspension was measured and 2.7 mL of 72 % perchloric acid was added with continuous

stirring. The mixture was allowed to stand with occasional stirring for 10 minutes. A 1 mL aliquot was transferred to another beaker and 6 mL of water was added. The pH was raised to 8.3 with 6N sodium hydroxide and then lowered to 4.5 with 6 N acetic acid. Then, 2.5 mL of 2 N acetic acid was added followed by 0.5 mL of 10 % potassium iodide and 5 mL of 0.01 N potassium iodate. The solution was left for 5 minutes for colour development. This solution was then made up to 50 mL with distilled water. Absorbance at 680 nm was determined on a Coleman Junior II spectrophotometer. A standard curve was made daily using the above procedure but with potato starch ('Analar', BDH Laboratories).

Metal staining

Hematoxylin staining of hand-cut rhizome sections was carried out by the method of Pizzolato et al (1967). Fresh staining solution was prepared by adding 2 mL of a stock solution to 200 mL of phosphate buffer (pH 7) immediately before the staining procedure. For the stock solution, 1 g of hematoxylin was dissolved in 160 mL of 0.01 M phosphate buffer (pH 7).

Scanning electron microscopy

Makela cattails collected in March were washed under the tap. Roots were severed and placed in distilled water until required for SEM. 1.5 cm sections of adventitious roots were mounted on a copper specimen holder with Tissue-tek O.C.T. compound (Miles Scientific). The cryogenic preparation of roots was carried out in an EMscope SP cryo-sputter unit (EMscope Laboratories Limited, Ashford, England). The specimen holder was plunged into subcooled liquid nitrogen. Root samples were etched at -90°C for 15 minutes and then sputter coated with chromium. The samples were then viewed on a cryo-stage in an ISI model DS-130 scanning electron microscope at an accelerating voltage of 15 kV.

Results

Water chemistry measurements of the stream feeding the cattails at Makela were made in the field through the summer of 1990. In addition, ICP analyses of filtered, acidified samples of the same water were conducted. Data for 5 sampling dates are summarised in Table 1. The high total conductivity was largely attributable to sulphur (as sulphate) and to a lesser extent, calcium and iron. Concentrations of other heavy metals were low compared to AMD at many Canadian mining sites.

TABLE 1 Chemistry of Makela seepage, July-October 1990

Metal	Concentration ($mg.L^{-1}$)
Fe	202-302
S	728-1043
Mg	156-216
Mn	3.1-4.4
Zn	0.4-1.1
Cu	0.04-0.1
Al	0.1-0.4
K	75-92
P	0.5-3.3
Ca	445-579
pH	5.85-6.40
Eh	-85 - -48 mV
Conductivity	2600-3100 µmhos (20-25°C)
Acidity	600-800 $mg.L^{-1}$ equiv of $CaCO_3$

Makela root surfaces were examined by SEM and associated X-ray scanning. Fig 2a is an X-ray scan of the surface of the adventitious cattail root shown in Plate 1. Large peaks for iron are evident (the peak at around 7 KeV is also an iron peak). Smaller peaks, for sulphur, silicon and copper are also present. The chromium peaks are due to the sputter coating material (the peak at 6 KeV is a chromium peak). These peaks indicate that the coating process was successful. Several other scans were made with very similar results. A scan of a dead lateral root (Fig 2b and Plate 2) shows a similar pattern.

Following fracturing of a root, X-ray scans were made within the exodermis. The example in Fig 2c was about three cells below the epidermis. There are no iron and sulphur peaks evident in this scan. There is a peak for potassium presumably from cytoplasmic contents.

Hand-cut sections of Makela- rhizomes were examined for starch and metal distribution.. The distribution of iodine stain indicates that most starch was in the central pith of the rhizome. Starch was also present, at much lower concentrations, in the outer cortex.

Hematoxylin staining indicated the presence of heavy metals, mainly in the epidermal cell layer. The dark-blue colour probably indicates the presence of iron as this is by far the most abundant of those metals detected in the water expected to give such' a reaction. A little staining was also present. in the pith. The colour here (red-purple) was very different from the epidermal layer, indicating a different metal(s) present.

Rhizome dimensions, starch content and root dry weight data for the Elliot Lake cattail roots and rhizomes are summarised in Table 2.

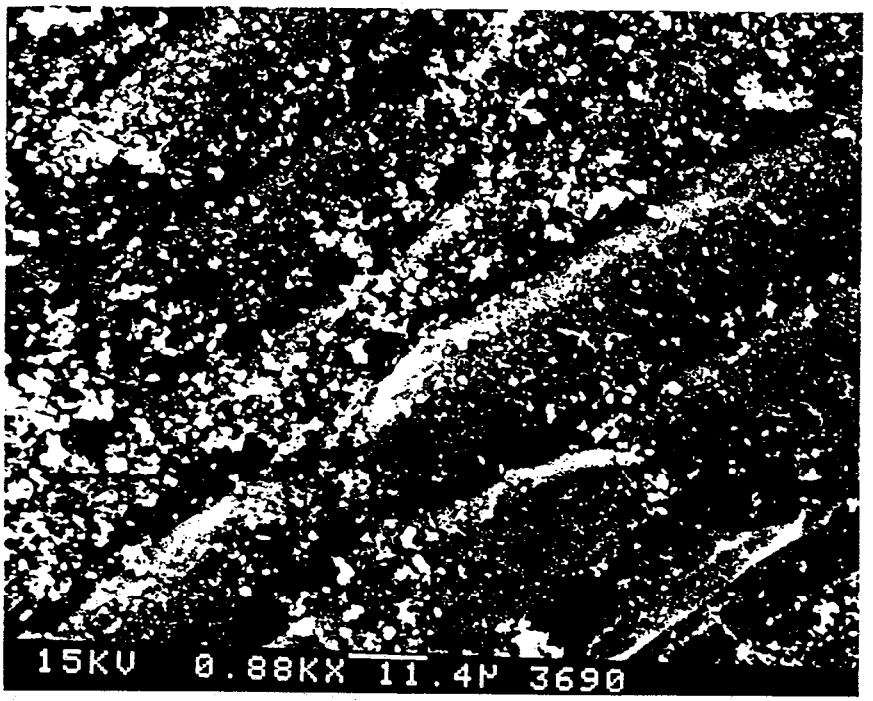

PLATE 1 Scanning electron micrograph of the surface of an adventitious root of a Makela cattail

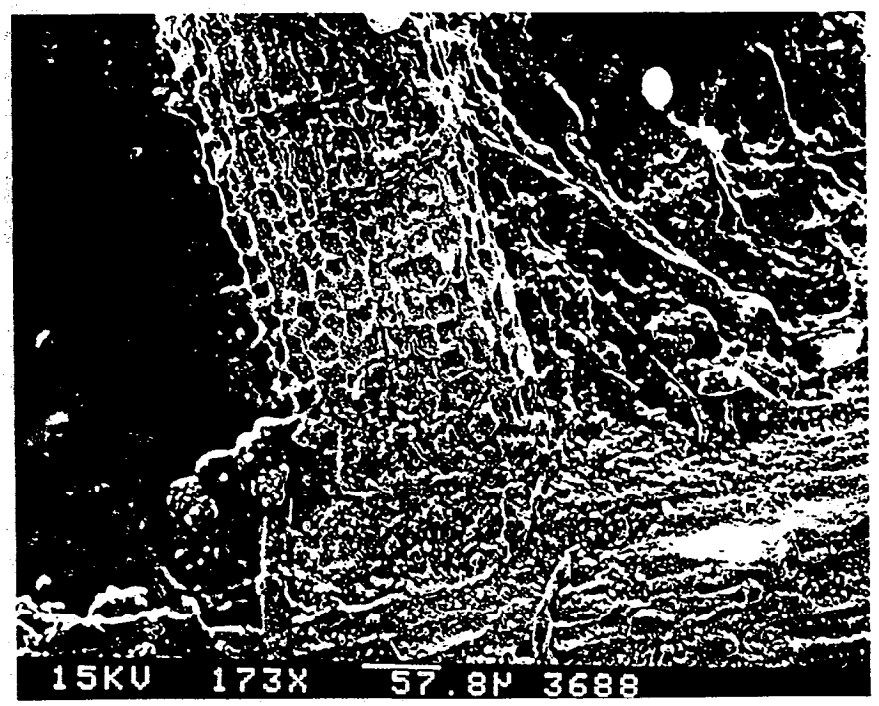

PLATE 2 A dead lateral root on a Makela cattail

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APPENDIX BOOJUM QA/QC INFORMATION

Methods for field sampling and the storage and handling of samples are summarised in MEND summary report, February 1990. The MEND document also describes methods used for determinations of pH, conductivity, acidity and alkalinity, and information on calibration, etc. The method for Eh determination is available on request. Microbiology and analytical chemistry methods carried out at Dearborn are summarised in the following MEND reports:DSS 23440-8-905/015Q (1988); DSS 03954.23440-8-9264 (June 1990); DSS 0145Q.23440-0-9065 (March 1991).

External quality control (quality assurance)

Cation/anion balances of both water and solid samples were carried out by ICP (Inductively Coupled Plasma Spectrophotometry), U.S. EPA Method No.200.7 at certified laboratories. The QA/QCs of EPL and X-Ral are available on request. To assure the validity of the results, blanks and standards were sent together with field samples. These samples were packaged and marked as per the field samples. Standards with different concentrations of metals were sent (0.1, 1, 10, 100, 1000 mg L^{-1} of metals) every few months. The composition of the standards and the procedure for sending these samples to Chauncey Laboratories are available on request. U.S. National Bureau of Standards 1645 (River Sediment) and 1571 (Orchard Leaves) samples were sent as solid standards. The quality control data for these is available on request. In 1991, 12 standards were sent to Chauncey laboratories and 24 to X-Ral. In 1992, one Al standard and 4 Boojum standards were sent to X-Ral and 18 standards were sent to EPL. The standard analyses were not consistently accurate for any of the three laboratories. These results and those of field samples were thoroughly screened on receipt and where obvious inaccuracies or anomalies were detected, the laboratory was informed, the errors explained and the samples reanalysed.

XRAL ENVIRONMENTAL
A Division of SGS Supervision Services Inc.
310 Brunel Road,
Mississauga, Ontario
Canada L4Z 2C2
Telephone: (416) 890-4880
Facsimile: (416) 890-4890

T
468 Queen Street East, Suite 400
Toronto, Ontario
M5A 1T7

Fax: (416) 861-0634
Fax: (416) 861-1086

Attention: Mr. Paul Douris

Re: Round Robin Lab Testing - QA/QC Procedures

Dear Mr. Douris:

As per your request attached please find a brief description of our QA/QC procedures.

Trusting that this meets with your requirement. If you require any additional information please do not hesitate to contact me at (416) 890-4890.

Yours truly,

Dariush Majlessi
Manager, Environmental Services

SGS

Quality Assurance Procedure

S.G.S. Supervision services have a commitment to quality and a statement of S.G.S. Quality Policy is on display at every S.G.S. Laboratory and Inspection Office.

[illegible] are inherent in any analysis performed. These procedures include a 10% level replicate analysis, blank analysis, and finally control sample analysis. Wherever possible the control sample used is a certified reference material prepared by an external agency. In situations where certified reference material is simply not available for the matrix of interest or the parameter being measured, Xral Environmental will analyze a suitably similar matrix material. Where sample volumes permit, original material is spiked with a known quantity of analyte.

Xral Environmental and its staff are active participants in the Canadian Association of Environmental Analytical Laboratories (CAEAL) Certification Program and the Association of the Chemical Profession of Ontario (ACPO) Occupational Health Chemistry Certification Program. Xral Environmental is also a regular participant in the Agriculture Canada Accredation Program which includes both round robin and check samples.

To ensure optimal operation of our analytical instruments, all of the analytical instruments used by Xral Environmental go through routine service maintenance programs.

Quality Control procedures

Great care is taken at all stages of our analysis to prevent contamination of the samples. All glassware used is carefully cleaned in accordance with the requirement of the specific certified/validated methodology used.

Reagent blank and control standards are carried out through all stages of the analysis with the samples.

EPL
ENVIRONMENT PROTECTION LABORATORIES

February 2, 1993

RECEIVED FEB 04 1993

Mr. Paul Douris
Boojum Research Ltd.
468 Queen Street East
Suite 400, Toronto, Ontario
M5A 1T7

Dear Paul:

Further to your request for information on EPL's round robin participation and QA/QC documentation, I am enclosing three separate packages.

1.0 QUALITY ASSURANCE, QUALITY CONTROL, DETECTION LIMITS

This is a precis of our QA Manual, which is at least three inches thick. This precis describes EPL's QA/QC goals and objectives as well as the laboratory applications and (p. 5) a listing of specific, routine QA/QC steps that we perform on every project. EPL is probably unique among labs in going to these lengths, and in actually providing the customer with a full QA/QC report on each project.

2.0 EPL LABORATORY CERTIFICATION

This describes our certification in Canada and the U.S., as well as a listing of the round robins we've participated in. Our performance in these round robins is always among the top 1-5 participants.

3.0 MISA ATG # CHART

A listing of EPL's methods, keyed to MISA test group, U.S. EPA method code, and EPL's method detection limits - all of which meet the MISA requirements, as well as the other existing regulatory requirements.

If you require further information please give me a call.

Yours very truly,

J.N. Bishop
Vice President

JNB/no
Enclosures

6850 Goreway Drive

QUALITY ASSURANCE, QUALITY CONTROL, DETECTION LIMITS, LIMS

QUALITY ASSURANCE PROGRAM

EPL's Quality Assurance Program (QAP) develops information which can be used to provide on indication of the need for corrections to the analytical system (QA). The QA Program measures whether or not the lab is in overall control. Quality Control (QC) becomes a subset of the QAP and evaluates the accuracy and precision of analytical data to establish the quality of data.

The foll

EPL's QA Manual is available for review upon request. An outline is attached (Appendix 1). The complete document which is several inches thick is available for viewing anytime at the EPL office.

OBJECTIVES AND GOALS

Quality Objectives

The Quality Assurance Program (QAP) assures the accuracy, precision, and reliability of the analytical data produced by EPL. Management, administrative, statistical, investigative, preventive, and corrective techniques are employed to achieve this objective through the following goals.

Quality Goals

- To develop and implement approved methods capable of meeting EPL client needs for precision, accuracy, sensitivity, and specificity.
- To ensure that all EPL staff receive training in quality technology enabling them to carry out their QAP responsibilities.
- To establish and keep under review a baseline of quality performance against which the effectiveness of quality improvement efforts are measured.
- To monitor the routine operational performance of the laboratory through participation in appropriate interlaboratory testing programs and to provide for corrective actions as necessary.
- To improve and validate laboratory methodologies by participation in method validation studies.

Quality Tactics

This section lists the tactics EPL follows to achieve the QAP goals.

- Quality activities emphasize the prevention of quality problems rather than detection and correction of problems after they occur.
- Quality cost figures are computed quarterly and reported to the President.

- All employees undergo training programs commensurate with their positions, duties, and responsibilities.
- EPL uses only published and approved methods.
- EPL retains copies of all test and analytical reports in a manner and for a period specified by regulatory or accrediting bodies.
- EPL has a comprehensive calibration program involving all instrumentation used for making analytical determinations.
- EPL uses appropriate, reagents and chemicals, certified when necessary, and appropriate calibrated glassware, certified when necessary.
- EPL establishes and maintains a total interlaboratory quality management system to assure continued precision and accuracy of laboratory results.
- EPL participates in interlaboratory testing programs on its own initiative and as prescribed by accrediting organizations.

Laboratory Facilities

EPL's state-of-the-art laboratory is located at 6850 Goreway Drive, Mississauga, Ontario L4V 1P1. Specific features include:

- A high security building with restricted access to laboratory area.
- Emergency electrical back up to essential services including, fumehoods, storage refrigerators, lab lighting etc.
- Controlled laboratory suitable for trace analysis.
- Centralized services, library with on-line data searching, centralized glassware washing, maintenance, chemical and labware stores.

Sample Management

EPL's operating policies regarding sample management are designed to ensure proper identification and storage, efficient handling and full documentation of Chain of Custody. All data are recorded in EPL's proprietary Laboratory Information System (LIMS).

- Where applicable, EPL provides precleaned containers of the type and with the preservatives specified by MOE 695/88, with full Chain of Custody

- Upon receipt of samples, EPL's Sample Receptionist documents the following information under a unique project number.

Client information:

- client name and contact
- client reference number
- date of submission, chain of custody

Sample information:

- type, amount, # of containers
- preservation type
- condition (warm, chilled, broken, ID uncertain, etc.)

- Unique lab numbers are generated for each sample.
- All of the information is documented on a Sample Receipt Record.
- Labels containing the pertinent information are generated for each container received and applied to the samples prior to storage.
- Samples are stored in a locked, segregated, walk-in refrigerator (4 o C)/freezer with emergency power back up and hard copy recording temperature charts.

Workload Management

EPL defines its analytical services from LIMS specified Analytical Test Codes. The test codes form the basis of the SOP's which outline the analytes, the sample type, detection limits the reference method and instrument operating methods, calibration standards and QC records. The use of these codes ensures that the requirements of the requested testing is clearly defined and formally documented.

- Projects are defined by assigning the specified test codes to the lab sample within a given project.

- On-line LIMS reports display the real time status of lab workstations.

- EPL follows the U.S. EPA's recommended frequency for processing QC samples. These requirements are predefined and enforced by the LIMS system.

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s recovery spike per 15 samples, as well as 1 replicate and 1 matrix spike per client within the run.

- Hardcopy worksheets containing all of the pertinent information are generated for each run.

- Signed and dated records of each laboratory activity are maintained.

- Lab staff have ready access to LIMS status reports including:

  - work in progress
  - work due dates
  - overdue work
  - project status etc.

- The LIMS audit trail documents all key events:

  - sampling date
  - date received
  - process data
  - analysis data
  - report data

QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

- All analytical services are based upon accepted (MOE, U.S. EPA) procedures and are fully validated prior to use.
- Analytical standards are prepared from neat solids or from certified solutions.
- Calibration standards are validated against external reference standards wherever possi
- Extensive use is made of Standard Reference Material (SRM) for routine procedure evaluation.
- Surrogate standards are used.
- Routine submission of blind samples is standard practice.
- Analytical sequences are predefined and ensure all results are traceable to calibration and QC data.
- Hardcopy reports displaying all of the required data are generated for each instrument analysis.
- Analytical results are determined only from instrument responses that fall within the demonstrated calibration range.
- Acceptable QC sample performance must be demonstrated prior to the authorization of data, (data are subjected to 3 levels of QC review: technician, supervisor, and manager).
- On-going method and instrument performance records are maintained for all analysis.
- A QC certificate is issued with each project. The QA/QC data reported is specific to your project, and it consists of:
  - full spike/recovery determination
  - blanks
  - standard reference material
  - replicate analysis
- Records containing all pertinent data are securely archived for seven years.
- The LIMS database is backed up daily.

Interlaboratory Comparison

- EPL is accredited by CAEAL as of June 1991.
- EPL is accredited by New York State, as of January 1992.
- EPL welcomes audits and inspections by current and potential clients.

s. A

ANALYTICAL METHODS & INSTRUMENTATION

EPL analytical methods are listed in Appendix 3. We have also included information on standard holding times and preservation methods. Methodologies specific to this contract are referenced on "Attachment A".

EPL METHOD DETECTION LIMITS

EPL follows EPA and Ontario Ministry of the Environment (MOE) analytical methods. Method Detection Limits (MDL's) are established following MOE Analytical Protocols.

Method Detection Limit (MDL) - in a given matrix and with a specific method is defined as the minimum concentration of an analyte that can be identified, qualitatively or quantitatively measured, and reported to be greater than zero at the 99% confidence level.

An MDL is a statistically defined decision point. Measured results falling at or above this point are interpreted to indicate the presence of an analyte in the sample with a specified probability, and assumes that there are no known sources of error in identification or biases in measurement.
It should be noted that when MDL estimates are developed using clean samples (i.e. reagent blanks) they represent an optimum achievable value. MDL's obtained in this fashion are useful for establishing performance criteria and allowing comparison of interlaboratory method capabilities, but are not applicable in defining the quantitation capability for other samples which introduce matrix effects. EPL MDL's have been established for the matrix being analyzed. As such, real sample MDL's can be higher than instrument detection limits. MDL's specific to this proposal are available upon request.

LIMS

Environment Protection Laboratories have a proprietary PC based LIMS system. MOE and OGS systems supplied by BMB Compuscience served as the genesis LIMS. The software

has been customized for EPL's specific needs. The system is capable of both sample/data handling and reporting as well as a data management to log workload, throughput and costing.

All major components of the LIMS have a backup which can be easily installed should the original fail. Data are stored on the host/server PC (33 Compaq 386) hard drives, and it is backed up to tape nightly.

There are tv Welcome Screen | Search | Report List requiring that the user know both a user number and a password. The software has security, restricting access to certain modules and various levels within those modules (i.e. browsing, updating, approvals).

The system has a sophisticated costing and invoicing module. Invoices and Certificates of Analysis are generated by the LIMS on completion and approval of sample analysis. Reports of Analysis are available electronically through EPL's bulletin board, in ASCII delimited files if requested.

EPL's instrument data capture module has a QC checking routine which flags QC data that fall out of tolerance. The routine also compares the present QC data to long term trends. The analyst receives a QC error report with each run of samples which lists all QC exceptions. In addition, the percent spike recovery is calculated and listed. All control charts are updated and generated each night by the LIMS. All QC data are stored in databases for long term precision and accuracy tracking.

The data undergoes three levels of approval before release to the client. Each approval is time stamped along with the LIMS usercode of the individual who approved it.

All written methods are available on the LIMS for referral by the analyst.

INTERLABORATORY COMPARISON STUDIES

EPL routinely participates in government and industry sponsored interlaboratory comparative studies. Appendix 2 lists all the studies EPL has participated in since start up. Two round robins are of special interest - the Canadian Association of Environmental Analytical Laboratories accreditation for metals and anions and the O'Connor Associates BTEX evaluation; both demonstrate that EPL's data falls in the upper decile. More recent robins such as the air filter study from the Association of the Chemical Profession of Ontario (ACPO), January 1992, and the Atmospheric Environment Studies 1991 air filter study have established EPL as a leading air analysis laboratory. EPL has also recently been certified by New York State, and their certification involved analyses of interlaboratory check samples.

EPL
ENVIRONMENT
PROTECTION
LABORATORIES INC.

EPL LABORATORY CERTIFICATION

Neither the Government of Canada nor the Provincial Government of Ontario has a formal approval [illegible] senior officials in the Canadian Federal Government and the Ontario Ministry of the Environment, and we have been approved by these agencies to perform analytical work for them.

EPL has performed extensive testing for the Province of Ontario, and has taken part in numerous interlaboratory studies for water and other materials. Our laboratory has always performed very well, and on the basis of our data quality we have been contacted by the Ontario Ministry of the Environment to perform environmental analyses.

EPL has also performed testing for several departments of the Federal Government of Canada, including Agriculture Canada and Environment Canada. Before selecting EPL, the Canadian Government examined EPL's data quality and put the lab through an extensive cross-comparison with several U.S. laboratories. The fact that both the Provincial Government of Ontario and the Canadian Federal Government have approved EPL for their work constitutes de facto acceptance of EPL's capabilities. The Ontario Ministry of the Environment has even used EPL to act as a referee laboratory to settle questions about data from different provincial government laboratories.

EPL is a member of the International Association of Environmental Testing Laboratories (IAETL), an organization made up of laboratories working on issues such as accreditation.

We are also members of the Ontario Bottled Water Association (OBWA) and the Canadian Bottled Water Association. EPL was selected by the OBWA as their laboratory of choice for 1991 and 1992.

EPL has been certified by the Canadian Association of Environmental Analytical Laboratories (CAEAL). CAEAL is the only organization in Canada that formally certifies analytical laboratories.

In the U.S., EPL has been granted Certification by New York State, through the Department of Health. EPL's certification covers bottled water, effluent, air samples and the range of other environmental analyses. Certification by New York State is regarded as primary certification by a large number of other states.

6850 Goreway Drive, Toronto, Ontario, Canada L4V 1P1 Telephone: (416) 673-3255 • FAX: (416) 673-7399

EPL
ENVIRONMENT
PROTECTION
LABORATORIES INC.

Environment Protection Laboratories Inc.
Round Robin Studies

DATE	SPONSOR	TYPE OF ANALYSIS
Jan 1990	MOE # 90-1	Cyanide / Water
Jan 1990	MOE ENV890543	Metals / Sediment
Aug 1990	MOE # 90-5	BTX and Acrylonitrile
Oct 1990	MOE # 90-6	Mercury
Nov 1990	MOE # 90040	Lithium / Water
Nov 1990	MOE Sludge	Metals / Sludge
Jan 1991	CCIW # G-1	Chlorinated Hydrocarbons / Sediment
Feb 1991	O'Connor Associates	BTX / Water
Mar 1991	AES	Metals / High Vol Filters
April 1991	CAEAL	Metals / Anions
May 1991	MOE	PCDD/DF / PUFs
May 1991	Proctor & Redfern	PAH / PCDD/DF / Metals
Oct 1991	CAEAL	Metals / Anions
Oct 1991	MOE # 91-3	Phenols by 4AAP
Nov 1991	MOE # 91-4	Oil & Grease
Nov 1991	MOE # 91-5	TOC / DOC in Water
Nov 1991	ACPO	Metals / Anions on Filters
Dec 1991	WTC	Cyanide / Water
Dec 1991	ESSO	BTX / VCM
Dec 1991	CCIW CEPA CP-3	PCDD/DF / Ampules
Jan 1992	ACPO	Metals / Anions on Filters
Feb 1992	WTC	Cyanide / Effluents
Feb 1992	State of New York	Metals / Anions / Pesticides / Volatiles
Feb 1992	Labatts	Metals / BTX
March 1992	CAEAL	Metals
April 1992	ACPO	Metals / Anions
April 1992	State of New York	Metals / Anions / Pesticides / Volatiles
April 1992	O'Connor Associates	BTEX
April 1992	Golder/Shell	BTX / EHC / Lead
July 1992	State of New York	Metals / Anions / Pesticides / Volatiles
Oct 1992	CCIW CEPA CP-4	PCDD/DF / Ampules
Nov 1992	State of New York	Metals / Anions / Pesticides / Volatiles
Nov 1992	CAEAL	Metals

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NEW YORK STATE DEPARTMENT OF HEALTH

DAVID AXELROD, M. D. *COMMISSIONER*

Expires 12:01 AM April 1, 1993
ISSUED April 1, 1992
REVISED June 30, 1992

INTERIM CERTIFICATE OF APPROVAL FOR LABORATORY SERVICE

Issued in accordance with and pursuant to section 502 Public Health Law of New York State

Lab ID No.: 11284 *Director: MR. TIM MINSHAW*

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Address : 6990 CREDITVIEW DRIVE
MISSISSAUGA ONTARIO CAN

is hereby APPROVED as an Environmental Laboratory for the category

ENVIRONMENTAL ANALYSES NON POTABLE WATER

All approved subcategories and/or analytes are listed below:

Hydrocarbon Pesticides :
4,4'-DDD
4,4'-DDE
4,4'-DDT
alpha-BHC
Aldrin
beta-BHC
Captan
Chlordane Total
delta-BHC
Dieldrin
Endrin aldehyde
Endrin
Endosulfan I
Endosulfan II
Endosulfan sulfate
Heptachlor
Heptachlor epoxide
Isodrin
Lindane
Mirex
Methoxychlor
PCNB
Toxaphene

Wastewater Miscellaneous :
Bromide
Boron, Total
Cyanide, Total
Color
Phenols
Oil & Grease Total Recoverable
Hydrogen Ion (pH)
Specific Conductance
Silica, Dissolved
Sulfide (as S)
Surfactant (MBAS)
Temperature
Organic Carbon, Total
Purgeable Aromatics (ALL)

Mineral :
Acidity
Alkalinity
Chloride
Sulfate (as SO4)
Hardness, Total
Acrolein and Acrylonitrile (ALL)
Wastewater Bacteriology (ALL)
Chlorinated Hydrocarbons (ALL)
Demand (ALL)
Wastewater Metals I (ALL)
Nitroaromatics and Isophorone (ALL)
Polynuclear Aromatics (ALL)
Phthalate Esters (ALL)
Purgeable Halocarbons (ALL)

Wastewater Metals III :
Cobalt, Total
Molybdenum, Total
Tin, Total
Thallium, Total
Nitrosoamines :
N-Nitrosodiphenylamine
N-Nitrosodi-n-propylamine
Dioxins (ALL)
Haloethers (ALL)
Wastewater Metals II (ALL)
Nutrient (ALL)
Polychlorinated Biphenyls (ALL)
Priority Pollutant Phenols (ALL)
Residue (ALL)

Lawrence S. Sturman

Lawrence S. Sturman, M.D., Ph.D., Acting Director
Herbert Wadsworth, M.D., Ph.D., Director
Wadsworth Center for Laboratories and Research

Serial No.: 12864

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NEW YORK STATE DEPARTMENT OF HEALTH

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ISSUED April 1, 1992
REVISED June 30, 1992

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Lab ID No.: 11284 *Director: MR. TIM MUNSHAW*

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S INC

MISSISSAUGA ONTARIO CAN

is hereby APPROVED as an Environmental Laboratory for the category

ENVIRONMENTAL ANALYSES/ POTABLE WATER

All approved subcategories and/or analytes are listed below:

Drinking Water Non-Metals :
- Alkalinity
- Chloride
- Color
- Fluoride, Total
- Nitrate (as N)
- Hydrogen Ion (pH)
- Solids, Total Dissolved
- Sulfate (as SO4)

D.W. Organohalide Pesticides :
- Endrin
- Lindane
- Methoxychlor
- Toxaphene

D.W. Chlorinated Acids :
- 2,4-D
- 2,4,5-TP (Silvex)

Drinking Water Metals I (ALL)
Volatile Halocarbons (ALL)

Drinking Water Bacteriology :
- Standard Plate Count

Drinking Water Trihalomethane (ALL)
Volatile Aromatics (ALL)

Lawrence S. Sturman

Serial No.: 12865

Lawrence S. Sturman, M.D., Ph.D., Acting Director
[illegible] Director
Wadsworth Center for Laboratories and Research
XV

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NEW YORK STATE DEPARTMENT OF HEALTH

DAVID AXELROD, M. D. *COMMISSIONER*

Expires 12:01 AM April 1, 1993
ISSUED April 1, 1992
REVISED June 30, 1992

INTERIM CERTIFICATE OF APPROVAL FOR LABORATORY SERVICE

Issued in accordance with and pursuant to section 502 Public Health Law of New York State

Lab ID No.: 11284 *Director: MR. TIM MUNSHAW*

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MISSISSAUGA ONTARIO CAN

is hereby APPROVED as an Environmental Laboratory for the category

ENVIRONMENTAL ANALYSES/AIR AND EMISSIONS

All approved subcategories and/or analytes are listed below:

ils I (ALL)

Serial No.: 12866

Lawrence S. Sturman

Lawrence S. Sturman, M.D., Ph.D., Acting Director
~~Herbert W. Dickerman, M.D., Ph.D., Director~~
Wadsworth Center for Laboratories and Research

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DOH-3317 (11/90)

NEW YORK STATE DEPARTMENT OF HEALTH

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Expires 12:01 AM April 1, 1
ISSUED April 1, 1992
REVISED June 30, 1992

INTERIM CERTIFICATE OF APPROVAL FOR LABORATORY SERVICE

Issued in accordance with and pursuant to section 502 Public Health Law of New York State

Lab ID No.: 11284 *Director: MR. TIM MUNSHAW*
Lab Name: ENVIRONMENTAL PROTECTION LABORATORIES INC

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is hereby APPROVED as an Environmental Laboratory for the category

ENVIRONMENTAL ANALYSES/SOLID AND HAZARDOUS WASTE

All approved subcategories and/or analytes are listed below:

Miscellaneous :
Cyanide, Total
Hydrogen Ion (pH)
Sulfide (as S)
Priority Pollutant Phenols (ALL)

Characteristic Testing :
Toxicity - Metals Only
Metals I (ALL)
Polynuclear Arom. Hydrocarbon (ALL)
Purgeable Aromatics (ALL)

Acrolein and Acrylonitrile (ALL)
Chlorinated Hydrocarbons (ALL)
Metals II (ALL)
Polychlorinated Biphenyls (ALL)
Purgeable Halocarbons (ALL)

Chlor. Hydrocarbon Pesticides (ALL)
Haloethers (ALL)
Nitroaromatics Isophorone (ALL)
Phthalate Esters (ALL)

Lawrence S. Sturman

Serial No.: 12867

Lawrence S. Sturman, M.D., Ph.D., Acting Director
[illegible] Director
Wadsworth Center for Laboratories and Research

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DOH-3317 (11/90)

CAEAL LABORATORY CERTIFICATION PROGRAM

REGISTRATION STATUS

LABORATORY: ENVIRONMEN PROTECTION LABS
REGISTRATION NO: 1380
EGISTRATION DATE: JUNE 7, 1991

PARAMETER	METHOD	AUDIT DATE	PE SCORE	STATUS
ATRIX: FRESH WATER				
CHLORIDE	ION CHROMATOGRAPHY	91.03.01	100	Y
CALCIUM - DISSOLVED	ICP	91.03.01	100	Y
CADMI	ICP	91.03.01	100	Y
COBALT - DISSOLVED	ICP	91.03.01	100	Y
CHROMIUM - DISSOLVED	ICP	91.03.01	100	Y
COPPER - DISSOLVED	ICP	91.03.01	100	Y
IRON - DISSOLVED	ICP	91.03.01	100	Y
MAGNESIUM - DISSOLVED	ICP	91.03.01	95	Y
MANGANESE - DISSOLVED	ICP	91.03.01	100	Y
NICKEL - DISSOLVED	ICP	91.03.01	100	Y
LEAD - DISSOLVED	ICP	91.03.01	100	Y
VANADIUM - DISSOLVED	ICP	91.03.01	100	Y
ZINC - DISSOLVED	ICP	91.03.01	100	Y
POTASSIUM	FLAME PHOTOMETRIC	91.03.01	80	Y
SODIUM	FLAME PHOTOMETRIC	91.03.01	100	Y
NITRATE	ION CHROMATOGRAPHY	91.03.01	90	Y
NITRATE + NITRITE	ION CHROMATOGRAPHY	91.03.01	100	Y
SULPHATE	ION CHROMATOGRAPHY	91.03.01	100	Y

PROVISIONAL REPORT

ACLAE

CAEAL

This is to certify that

Environment Protection Laboratories

6850 Goreway Drive
Toronto, Ontario

is a member of
The Canadian Association for
Environmental Analytical Laboratories

CAEAL 1992 ACLAE

Dr. John Lawrence, President

Member until: June 18, 1991

MISA ATG #	Parameter	Method Code	Method Identifier	MISA MDL mg/L	EPL MDL mg/L	EPL SRI mg/L	HIGH CAL mg/L
1	Chemical Oxygen Demand (COD)	EP011A	Potassium Dichromate/Sulphuric Acid Reflux Colourimetric Determination (HACH DR/3000)	10	5	1	100/500
2	Total Cyanide	EP022A	Acid Distillation into Alkaline Absorber Pyridine - Barbituric acid Colourimetric Determination (Hach DR/3000)	.005	.002	0.001	.1
3	pH (Hydrogen Ion)	EP031D	Combination Electrode (Hach) pH meter	.01 units	.01 units	.01	10
4a	Ammonia + Ammonium	EP042A	Alkaline Distillation into Boric Acid (Buechi 321) Nesslerization Colourimetric Determination (Hach DR/3000)	.25 (as N)	.05 (as N)	.01	2.5
4a	Total Kjeldahl Nitrogen (TKN)	EP044A	Acid Digestion - H2SO4/HgSO4/K2SO4 (Buechi 430) followed by alkaline distillation into Boric acid (Buechi 321) Nesslerization Colourimetric Determination (Hach DR/3000)	.5 (as N)	.10 (as N)	.1	2.5
4b	Nitrate plus Nitrite	EP042A	Copper-Cadmium Reduction. NED Colourimetric Determination (Hach Dr/3000)	.25 (as N)	.10 (as N)	.05	1.25
5a	Dissolved Organic Carbon (DOC)	EP051A	Filtration - 0.45u Membrane Filter, UV/ persulphate oxidation followed by Colourimetric Determination (Skalar SA 20/40)	.5 (as C)	.5 (as C)	.1	2.5
5b	Total Organic Carbon (TOC)	EP053A	UV/persulphate oxidation followed by Colourimetric Determination (Skalar SA 20/40)	5 (as C)	1.0 (as C)	.1	2.5
6	Total Phosphorous (TP)	EP062A	Acid Digestion - HNO3/H2SO4 - Ascorbic acid Colourimetric Determination (Hach DR/3000)	.1	.02	.01	1.0
7	Specific Conductance	EP071D	Conductivity Cell with Resistance Thermometer (MetrOhm) Conductometer (MetrOhm 660)	5 uS/cm	.1 uS/cm	.1 uS/cm	.1
8	Total Suspended Solids (TSS)	EP081C	Filtration - 2 um glass fibre, drying @ 103 C (Precision STM 135), Gravimetric Determination (Sartorius R200D)	2	1	1	.1
8	Volatile Suspended Solids (VSS)	EP082C	Filtration - 2 um glass fibre, ignition @ 550 C (Neytech 85P), Gravimetric Determination (Sartorius R200D)	4	1	1	.1
9	Copper * Cobalt * Nickel Silver Zinc * (Cadmium) (Lead)	EP092B	Nitric evaporation Air-acetylene AAS (TJA Smith-Hieftje 22) * A 5 fold preconcentration is carried out for Co, Cu, and Zn	.01 .02 .02 .03 .01	.005 .01 .005 .03 .005	.001 .01 .001 .01 .001	5 5 5 5 5 5 5

U.S. Department of Commerce
Juanita M. Kreps
Secretary

National Bureau of Standards
Ernest Ambler, Acting Director

National Bureau of Standards Certificate of Analysis

Standard Reference Material 1571

Orchard Leaves

This Standard Reference Material is intended for use for the calibration of apparatus and the validation and/or verification of methods used in the analysis of agricultural and other botanical materials for major, minor, and trace elements.

Certified Values of Constituent Elements: The certified values for the constituent elements are shown in Table 1. The analytical techniques used and the names and affiliations of the analysts are shown in Table 3. Certified values are based on results obtained by reference methods of known accuracy and performed by two or more analysts; or alternatively, from results obtained by two or more independent, reliable analytical methods. Non-certified values are given for information only in Table 2. All values are based on a minimum sample size of 250 mg of the dried material.

Notice and Warnings to Users:

Expiration of Certification: This certification is invalid after 5 years from the date of shipping. Should it be shown invalid prior to that time, purchasers will be notified by NBS.

Stability: The material should be kept in its original bottle and stored at temperatures between 10-30 °C. It should not be exposed to intense sources of radiation, including ultraviolet lamps or sunlight. Ideally, the bottle should be kept in a desiccator in the dark at the temperature indicated.

Use: A minimum sample of 250 mg of the *dried* material (see Instructions for Drying) should be used for any analytical determination to be related to the certified values of this certificate.

The overall direction and coordination of the technical measurements leading to this certificate were performed under the chairmanship of P. D. LaFleur. The overall coordination of the cooperative work performed by the Commission of European Communities, Joint Research Center, Ispra Establishment, Italy, was by G. Rossi of thc Chemistry Division.

The technical and support aspects involved in the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by T. W. Mears.

Washington, D.C. 20234
(January 28, 1971)
(Revised August 15, 1976)
(Revised August 31, 1977)

J. Paul Cali, Chief
Office of Standard Reference Materials

Table 1. Certified Values of Constituent Elements[a]

Major Constituents		Minor Constituents	
Element	Content Wt. Percent	Element	Content Wt. Percent
Nitrogen	2.76 ± 0.05	Magnesium	0.62 ± 0.02
Calcium	2.09 ± 0.03	Phosphorus	0.21 ± 0.01
Potassium	1.47 ± 0.03		

The uncertainties shown above include both the imprecision, expressed as the standard deviation of a single measurement, and an allowance for unknown sources of systematic error.

Trace Constituents[a]

Element	Content μg/g	Element	Content μg/g
Iro			.3
Ma	91 ± 4	Chromium	2.6 ± 0.3
Sodium	82 ± 6	Nickel	1.3 ± 0.2
Lead	45 ± 3	Molybdenum	0.3 ± 0.1
Strontium	37 ± 1	Mercury	0.155 ± 0.015
Boron	33 ± 3	Cadmium	0.11 ± 0.01
Zinc	25 ± 3	Selenium	0.08 ± 0.01
Copper	12 ± 1	Thorium	0.064 ± 0.006
Rubidium	12 ± 1	Uranium	0.029 ± 0.005
Arsenic	10 ± 2	Beryllium	0.027 ± 0.010

The uncertainties shown above are the imprecisions expressed as either two standard deviations of a single determination (commonly, but perhaps incorrectly, called the "95 percent confidence limit"), or the entire range of observed results - whichever of the two is larger. No additional allowance for the uncertainty from unknown sources of systematic error has been included, since these are considered to be small relative to the imprecision as expressed.

[a]Analytical values are based on the "dry-weight" of material (See Instructions for Drying).

Table 2. Non-certified Values for Trace Constituent Elements[a]

NOTE: The following values are not certified because they are not based on the results of either a reference method or of two or more independent methods. These values are included for information only.

Element	Content μg/g	Element	Content μg/g
Sulfur	(1900)	Cobalt	(0.2)
Chlorine	(690)	Iodine	(0.17)
Barium	(44)	Bismuth	(0.1)
Bromine	(10)	Gallium	(0.08)
Fluorine	(4.)	Cesium	(0.04)
Lithium	(0.6)	Tellurium	(0.01)

[a]Analytical values are based on the "dry-weight" of material (See Instructions for Drying).

Additional Information on Analyses: Digestion procedures should be designed to avoid loss of volatile elements such as arsenic, mercury, etc. It was found that digestion of the orchard leaves in nitric and perchloric acids was incomplete, a small residue of siliceous material remaining. This residue must be considered an integral part of this Standard Reference Material. Therefore, dissolution procedures must be capable of complete dissolution of the leaves, but must not result in losses of volatile elements.

Iron and lead in the nitric-perchloric acid soluble portion were determined to be 270 μg/g and 44 μg/g, respectively. These two values, not to be confused with the *total* material values given in Table 1, are not certified, but given for information only.

Source and Preparation of Material: The orchard leaves for this Standard Reference Material were collected and prepared under the direction of A. L. Kenworthy of Michigan State University. These leaves were hand picked from an orchard near Lansing, Michigan, and air dried. The dried leaves were ground in a [illegible]ting mac[illegible] drie[illegible] was lined fiber drums and sterilized *in situ* with 4.9 megarad of cobalt-60 radiation. The sterilization procedure was carried out at the U. S. Army Natick Laboratories under the direction of A.Brynjolfsson.

Homogeneity Assessment: The homogeneity of this material was established on the premise that the minimum sample size be 250 milligrams. Assessment of homogeneity was made using analyses for nitrogen, potassium, and magnesium. A statistical analysis of the data shows that there is evidence for a small degree of variability between samples with respect to potassium. The data for the other elements do not reveal such an effect. Statistical design and analysis of data were performed by J. Mandel of the NBS Institute for Materials Research.

Instructions for Drying: Before weighing, samples of this Standard Reference Material *must* be dried by either:

1. Drying in air in an oven at 85 °C for at least 4 hours.
2. Lyophilization using a cold trap at or below −50 °C at a pressure *not greater* than 30 Pa (0.2 mm Hg) for at least 24 hours.

NOTE: Drying at 135 °C results in large losses and discoloration and should *not* be used.

Analysts and Analytical Methods Used

Analytical Methods

A. Atomic absorption spectroscopy
B. Flame emission spectrometry
C. Gravimetry
D. Intersociety Committee Method 12204-01-68T for fluorine
E. Isotope dilution mass spectrometry
F. Isotope dilution spark source mass spectrometry
G. Kjeldahl method for nitrogen
H. Neutron activation
I. Nuclear track technique
J. Optical emission spectroscopy
K. Photon activation
L. Polarography
M. Spectrofluorimetry
N. Spectrophotometry

Analysts

Analytical Chemistry Division, National Bureau of Standards

1. T. C. Rains
2. M. S. Epstein
3. T. A. Rush
4. W. P. Schmidt
5. B. S. Carpenter
6. E. R. Deardorff
7. R. A. Paulson
8. L. P. Dunstan
9. L. J. Moore
10. E. L. Garner
13. J. W. Gramlich
14. R. Alvarez
15. K. M. Sappenfield
16. C. Mueller
17. P. J. Paulsen
18. D. A. Becker
19. T. E. Gills
20. W. D. Kinard
21. P. D. LaFleur
22. L. T. McClendon
23. H. L. Rook
26. B. T. Diamondstone
27. S. A. Wicks

Cooperating Analysts

28. Nuclear Chemistry Section, Josef Stefen Institute, Ljubljana, Yugoslavia.
    V. Ravnick, L. Kosta, A. R. Byrne, M. Dermelj
29. Chemistry Division, Standards and Reference Substances Secretariat, Commission of European Communities, Joint Research Center, Ispra Establishment, Italy.
    F. Giradi, G. Guzzi, R. Pietra, E. Sabbioni
30. L. A. Rancitelli, Pacific Northwest Laboratories, Battelle Memorial Institute, Richland, Washington.
31. J. B. Jones, Jr., University of Georgia, Athens, Georgia.
32. A. L. Kenworthy, Michigan State University, East Lansing, Michigan.
33. L. Rengan, Eastern Michigan University, Ypsilanti, Michigan.

Table 3 Methods and Analysis*

METHOD / ELEMENT	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Sb								19				25		
As	1,2							18,19,20, 22,31,34				25		27
Ba					9,11									
Be	1,3													
Bi													28	
B														
Br								19						
Cd	1,3							30				25		
Ca	1,3	1,3												
Cs								30						
Cl								19,30						
Cr					8,10			18						
Co								19						
Cu						14,16,17		19,20,29, 30		32				
F				7										
Ga								30						
I								23						
Fe	1,2					14,16,17		19,30,31				25		
Pb						14,16,17					24	25		
Li														
Mg	1,3,29							30		32				
Mn	1,3							18,20,30						
Hg	1,3					14,16,17		19,23,29, 30						
Mo					9,12			21,30						
Ni						14,16,17		30				25		
N							4,7		5					
P			4,7					30		32,33				
K	1,3	1,3						30						
Rb		1,3,29			9,11,12			30,31						
Se						14,16,17		23,30						
Na		1,3						20,30,31						
Sr					9,12									
S			5,6											
Te								23						
Th					10,12									
U					12,13			18	5					
Zn	1,3					14,16,17		18,29,30, 31		32,33				

*Numbers in body of table refer to analysts named above.

MISA ATG #	Parameter	Method Code	Method Identifier	MISA MDL mg/L	EPL MDL mg/L	EPL SRI mg/L	HIGH CAL mg/L
9 con't.	Aluminum Chromium Molybdenum * Vanadium * (Beryllium)	EP093B	Nitric evaporation Nitrous oxide - acetylene AAS (TJA Smith-Hieftje 22) * A 10 fold preconcentration is carried out for Mo and V.	.03 .02 .02 .03	.001 .0005 .02 .03	.001 .0001 .01 .01	25 25 25 25 10
	Beryllium Cadmium Lead Thallium	EP094B	HNO3 Digestion, Graphite Furnace AAS (TJA Smith-Hieftje 22/CTF 188)	.01 .002 .03 .03	.0002 .0002 .001 .002	.0001 .0001 .001 .001	.05 .05 .05 .05
10	Arsenic Selenium Antimony	EP101B	HNO3/H2SO4/HClO4 Acid Digestion Hydride Generation AAS (TJA Smith-Hieftje 22/Varian V6A 76)	.005 .005 .005	.001 .0001 .0001	.001 .001 .001	.02 .02 .02
11	Hexavalent Chromium	EP112A	Diphenylcarbazide Colourimetric Determination (Hach DR/3000)	.01	.01	.01	.5
12	Mercury	EP121B	Oxidative acid digestion Cold Vapour AAS (TJA Smith-Hieftje 22/Varian V6A 76)	.0002	.0002	.0001	.005
13	Tetra Alkyl Lead Tri Alkyl Lead	EP131A EP132B	Liquid/Liquid Extraction, Colourimetric Determination (Hach DR/3000 or Graphite Furnace) AAS (TJA Smith-Hieftje 22/CTF 188)	.002	.002	.001	10
14	Phenolics (4AAP)	EP142A	Acidic Distillation Solvent Partition/Colourimetric Determination (Hach DR/3000)	.002	.002	.001	.2
15	Sulphide	EP152A	Flocculation/Filtration, Methylene Blue Colourimetric Determination (Hach Dr/3000)	.02	.02	.01	.5

MISA ATG #	Parameter	Method Code	Method Identifier	MISA MDL ug/L	EPL MDL ug/L	EPL SRI ug/L	HIGH CAL ug/L
16	1,1,2,2-Tetrachloroethane	EP161A	Purge and Trap, GC/ELCD/PID	4.3	0.3	.1	40
	1,1,2-Trichloroethane			0.6	0.3	.1	40
	1,1-Dichloroethane			0.8	0.6	.1	40
	1,1-Dichloroethylene			2.8	0.6	.1	40
	1,2-Dichlorobenzene			1.4	0.7	.1	40
	1,2-Dichloroethane			0.8	0.7	.1	40
	1,2-Dichloropropane			0.9	0.7	.1	40
	1,3-Dichlorobenzene			1.1	0.7	.1	40
	1,4-Dichlorobenzene			1.7	0.7	.1	40
	Bromoform			3.7	0.8	.1	40
	Bromomethane			3.7	3.3	.1	40
	Carbon tetrachloride			1.3	0.2	.1	40
	Chlorobenzene			0.7	0.7	.1	40
	Chloroform			0.7	0.4	.1	40
	Chloromethane			3.7	3.5	.1	40
	Cis-1,3-Dichloropropylene			1.4	0.5	.1	40
	Dibromochloromethane			1.1	0.7	.1	40
	Ethylene Dibromide			1.0	1.0	.1	40
	Methylene Chloride			1.3	0.6	.1	40
	Tetrachloroethylene			1.1	0.8	.1	40
	Trans-1,2-Dichloroethylene			1.4	0.8	.1	40
	Trans-1,3-Dichloropropylene			1.4	0.3	.1	40
	Trichloroethylene			1.9	1.0	.1	40
	Trichlorofluoromethane			1.0	5.0	.1	40
	Vinyl Chloride			4.0	4.6	.1	40
17	Benzene	EP171A	Purge and Trap, GC/ELCD/PID	0.5	0.4	.1	40
	Toluene			0.5	0.5	.1	40
	o-Xylene/Styrene			0.5	0.5	.1	40
	m/p-Xylene			1.1	0.05	.1	40
18	Acrolein	EP181A	Purge and Trap, GC/MS using capillary column	4.0	4.0	.1	40
	Acrylonitrile			4.2	4.0	.1	40
19	Acenaphthene	EP191A	Liquid/liquid extraction capillary column GC/MS	1.3	1.0	.1	40
	5-nitro-acenaphthene			4.3	2.0	.1	40
	Acenaphthylene			1.4	1.0	.1	40
	Anthracene			1.2	1.0	.1	40
	Benz(a)anthracene			0.5	1.0	.1	40
	Benzo(a)pyrene			0.6	0.5	.1	40

MISA ATG #	Parameter	Method Code	Method Identifier	MISA MDL ug/L	EPL MDL ug/L	EPL SRI ug/L	HIGH CAL ug/L
19 con't.	Benzo(b)fluoranthene	EP191A		0.7	0.7	.1	20
	Benzo(g,h,i)perylene			0.7	0.7	.1	20
	Benzo(k)fluoranthene			0.7	0.7	.1	20
	Camphene			3.5	2.5	.1	20
	1-Chloronaphthalene			2.5	1.0	.1	20
	2-Chloronaphthalene			1.8	1.0	.1	20
	Chrysene			0.3	0.2	.1	20
	Dibenz(a,h)anthracene			1.3	1.0	.1	20
	Fluoranthene			0.4	0.3	.1	20
	Fluorene			1.7	1.0	.1	20
	Indeno(1,2,3-c,d)pyrene			1.3	1.0	.1	20
	Indole			1.9	2.0	.1	20
	1-Methylnaphthalene			3.2	1.0	.1	20
	2-Methylnaphthalene			2.2	1.0	.1	20
	Naphthalene			1.6	1.0	.1	20
	Perylene			1.5	1.0	.1	20
	Phenanthrene			0.4	0.3	.1	20
	Pyrene			0.4	0.4	.1	20
	Benzylbutylphthalate			0.6	0.6	.1	20
	Bis(2-ethylhexyl)phthalate			2.2	2.0	.1	20
	Di-n-butylphthalate			3.8	1.0	.1	20
	4-Bromophenyl phenylether			0.3	0.3	.1	20
	4-Chlorophenyl phenylether			0.9	0.8	.1	20
	Bis(2-chloroisopropyl)ether			2.2	1.0	.1	20
	Bis(2-chloroethyl)ether			4.4	1.0	.1	20
	2,4-dinitrotoluene			0.8	0.7	.1	20
	2,6-dinitrotoluene			0.7	0.7	.1	20
	Bis(2-chloroethyoxy)methane			3.5	2.0	.1	20
	Diphenylamine			14	4.0	.1	20
	N-Nitrosodiphenylamine			14	4.0	.1	20
	N-Nitrosodi-n-propylamine			3.1	2.8	.1	20
	Biphenyl			2.0	1.6	.1	20
20	2,3,4,5-Tetrachlorophenol	EP201A	Liquid/liquid extraction capillary column GC/MS	0.4	0.4	.1	40
	2,3,4,6-Tetrachlorophenol			2.8	1.5	.1	40
	2,3,5,6-Tetrachlorophenol			1.6	1.3	.1	40
	2,3,4-Trichlorophenol			0.6	0.6	.1	40
	2,3,5-Trichlorophenol			1.3	1.3	.1	40
	2,4,5-Trichlorophenol			1.3	1.0	.1	40

MISA ATG #	Parameter	Method Code	Method Identifier	MISA MDL ug/L	EPL MDL ug/L	EPL SRI ug/L	HIGH CAL ug/L
20 con't.	2,4,6-Trichlorophenol	EP201A		1.3	1.2	.1	40
	2,4-Dimethylphenol			7.3	1.0	.1	40
	2,4-Dinitrophenol			42	2.0	.1	40
	2,4-Dichlorophenol			1.7	1.7	.1	40
	2,6-Dichlorophenol			2.0	1.9	.1	40
	4,6-Dinitro-o-cresol			24	4.0	.1	40
	2-Chlorophenol			3.7	2.0	.1	40
	4-Chloro-3-methylphenol			1.5	1.5	.1	40
	4-Nitrophenol			1.4	1.3	.1	40
	m-Cresol			3.4	1.0	.1	40
	o-Cresol			3.7	1.0	.1	40
	p-Cresol			3.5	1.0	.1	40
	Pentachlorophenol			1.3	1.0	.1	40
	Phenol			2.4	1.0	.1	40
23	1,2,3,4-Tetrachlorobenzene	EP231A	Liquid/liquid extraction, florisil clean up, dual capillary column ECD/GC	0.01	0.01	0.01	20
	1,2,3,5-Tetrachlorobenzene			0.01	0.01	0.01	20
	1,2,4,5-Tetrachlorobenzene			0.01	0.01	0.01	20
	1,2,3-Trichlorobenzene			0.01	0.01	0.01	20
	1,2,4-Trichlorobenzene			0.01	0.01	0.01	20
	2,4,5-Trichlorotoluene			0.01	0.01	0.01	20
	Hexachlorobenzene			0.01	0.01	0.01	20
	Hexachlorobutadiene			0.01	0.007	0.01	20
	Hexachlorocyclopentadiene			0.01	0.005	0.01	20
	Hexachloroethane			0.01	0.01	0.01	20
	Octachlorostyrene			0.01	0.005	0.01	20
	Pentachlorobenzene			0.01	0.01	0.01	20
24	2,3,7,8-Tetrachlorodibenzo-p-dioxin	EP241A	Liquid/liquid extraction, multi component chemically modified adsorbent column clean up SIM GC/MS analysis	0.000020	0.000015	.000005	.0005
	Octachlorodibenzo-p-dioxin			0.000030	0.000025	.000005	.0005
	Octachlorodibenzofuran			0.000030	0.000025	.000005	.0005
	Total heptachlorinated dibenzo-p-dioxins			0.000030	0.000025	.000005	.0005
	Total heptachlorinated dibenzofurans			0.000030	0.000025	.000005	.0005
	Total hexachlorinated dibenzo-p-dioxins			0.000020	0.000015	.000005	.0005
	Total hexachlorinated dibenzofurans			0.000020	0.000015	.000005	.0005
	Total pentachlorinated dibenzo-p-dioxins			0.000015	0.000010	.000005	.0005
	Total pentachlorinated dibenzofurans			0.000020	0.000020	.000005	.0005
	Total tetrachlorinated dibenzo-p-dioxins			0.000015	0.000010	.000005	.0005
	Total tetrachlorinated dibenzofurans						

MISA ATG #	Parameter	Method Code	Method Identifier	MISA MDL ug/L	EPL MDL ug/L	EPL SRI ug/L	HIGH CAL ug/L
25	Oil and Grease	EP252C	Liquid/liquid extraction (Freon) Gravimetric Determination (Sartorius R200D)	1	.5	.5	50
27	Polychlorinated Biphenyls (PCB)	EP271A	Identification of Arochlors present, and total concentration, by liquid/liquid extraction, florisil clean up, capillary ECD/GC analysis	0.1	0.07	.01	5
28a	Open Characterization - Volatiles	EP281A	Purge and Trap GC/MS using capillary column	1	1	0.1	20
28b	Open Characterization - Extractables	EP282A	Liquid/liquid extraction, capillary column GC/MS	1	1	0.1	40
29	Al, Ba, Be, Bi, B, Cd, Ca, Ce, Cr, Co, Cu, Dy, Er, Eu*, Gd, Ga, Ge, Au, Hf*, Ho*, In, Ir, Fe, La*, Pb, Lu*, Mg, Mn, Mo, Nd, Ni, Nb, Os, Pd, P*, Pb, K*, Pr, pH*, Re*, Rh, Ru, Sm, Sc, Si*, Ag, Na, Sr, S*, Ta, Tb, Th, Tm, Sn, Ti, W, U*, V, Yb, Y, Zn, Zr	EP291B	Nitric digestion/aqua regia digestion Inductively Coupled Plasma (TJA ICAP 61E) Argon Emission Spectrometry	0.05	0.05	0.01	10
	Cs*, Li, Rb	EP292B	Flame Atomic Absorption (TJA Smith-Hieftje 22)	0.05	0.05	0.01	10
	As, Se, Sb	EP293B	Hydride Generation Atomic Absorption (TJA Smith-Hieftje 22/Varian VGA 76)	0.005	0.001	0.001	0.02
	Hg	EP294B	Cold Vapor Atomic Absorption (TJA Smith-Hieftje 22/Varian VGA 76)	0.0002	0.0002	0.0001	0.005
	Tl	EP295B	Graphite Furnace Atomic Absorption (TJA Smith-Hieftje 22/CTF 188)	0.03	0.002	0.001	0.05
	XXX		* A 5 fold preconcentration is carried out for Cs, Eu, Hf, Ho, La, Lu, P, K, Pt, Re, Si, S, U				
M1	Chloride	EP312A	Mercuric thiocyanate reaction Ferric Nitrate Colourimetric Determination (Hach Dr/3000)		0.5	.1	10
M2	Cyanates	EP321A	Acidic Reflux/Neutralization Nesslerization Colourimetric Determination (Hach Dr/3000)		0.05	.01	8.0
M3	Total Dissolved Solids	EP331C	Filtration - 2 ug glass fibre, evaporation @ 103 C (Precision STM 135) Gravimetric Determination (Sartorius R200D)		1	1	500
M4	Sulphate	EP342A	Ion Chromatograph Dionex Series 4500I AS4A Anion exchange column		0.5	0.1	15.0
M5	Iron	EP351A	Nitric digestion Inductively Coupled Plasma (TJA ICAP 61E) Argon Emission Spectrometry		0.005	0.001	10
M6	Thiocyanate	EP361A	Absorption (Ion Exchange Resin)/Filtration Ferric Nitrate Colorimetric Determination (Hach Dr/3000)		0.05	.01	2.0
M8	Cyanide-free	EP381A	Acidic Distillation into buffering absorber Isonicotinic Acid/Barbituric Acid Colourimetric Determination (Skalar SA 20/40)		0.02	.01	0.10

STANDARDS PREPARATION FOR CHAUNCEY LABORATORIES

March 4, 1991

M.P. Smith

Specific quantities of reagent grade chemicals were weighed in order to achieve a stock solution with 10,000 mg/L of various heavy metals commonly found in AMD.

These chemical were added to one L of distilled water with 10% HNO_3 in order to dissolve

ion of the

Using this stirring stock solution, 100 mls were removed and added to 900 mL of distilled water, in order to make a 1/10 dilution. This solution, with 1000 mg/L metals was stirred and another 100 mL were removed, and topped up to 1 L of solution, thereby making a 100 mg/L metals solution. This was rpeated twice more to make 10 mg/L and 1 mg/L solutions.

Because the chemicals in solution precipitated with dilution with distilled water, concentrated Nitric acid was again added as exactly 1 % by volume of the standard solutions. Therefore, the 1000, 100, 10 and 1 mg/L metals solutions are actually 990, 99, 9.9 and 0.9 mg/L standards.

Of the stock dilutions, six 100 ml were sampled, and placed in 100 ml white bottles waiting assaying. Three replicates of each concentration will be submitted, and three will be held back. Stock dilutions will be stored as need be. The assayer's samples are labelled accordingly:

SAMPLE NAME	ASSAY NUMBER	DESCRIPTION	Replicate
SR A-1	2496	1 mg/l metals	1
SR A-2	2497	1 mg/l metals	2
SR A-3	2498	1 mg/l metals	3
SR B-1	2499	10 mg/l metals	1
SR B-2	2500	10 mg/l metals	2
SR B-3	2501	10 mg/l metals	3
SR C-1	2502	100 mg/l metals	1
SR C-2	2503	100 mg/l metals	2
SR C-3	2504	100 mg/l metals	3
SR D-1	2505	1000 mg/l metals	1
SR D-2	2506	1000 mg/l metals	2
SR D-3	2507	1000 mg/l metals	3

Sample	2496-2498	2499-2501	2502-2504	2505-2507	STOCK
Element					
Fe	1.00	10.00	100.00	1000.00	10000.00
Cu	1.00	10.00	100.00	1000.00	10000.00
Ni	1.00	10.00	100.00	1000.00	10000.00
Co	1.00	10.00	100.00	1000.00	10000.00
Mn	1.00	10.00	100.00	1000.00	10000.00
Zn	1.00	10.00	100.00	1000.00	10000.00
NO3	2.10	21.04	210.42	2104.24	21042.43
S	1.62	16.18	161.81	1618.09	16180.86
Cl	3.02				93
P	0.33	3.26	32.61	326.15	3261.49
H	0.02	0.21	2.12	21.23	212.27
H2O	6.81	68.06	680.62	6806.17	68061.71

= 0.99 9.9 99 990

BECAUSE I ADDED 1% NITRIC ACID (69-71%) TO EACH STANDARD

PREPARATION OF STANDARD SOLUTIONS FOR ASSAYING WATER QUALITY

COMPOUND	M.W.(g)	M.W.'s g/L (Cold)		55.847 Fe	63.546 Cu	58.71 Ni	58.9332 Co	54.938 Mn	65.37 Zn	62 NO3	96.062 SO4	35.453 Cl	39.1 K	94.9714 PO4	1.01 H	18 H2O	
FeCl3	162.22	744		0.34427								0.6556					0.9999
NiSO4.7H2O	280.88	756				0.2642583					0.342					0.45	1.0552
MnSO4.H2O	169.01	985						0.32506			0.5684					0.11	1.0000
Co(NO3)2.6H2O	291.04	1338					0.20249175			0.43						0.37	1.0000
CuCl2.2H2O	170.47	1104			0.37277							0.4159				0.21	1.0001
ZnSO4.7H2O	287.54	965							0.22734		0.3341					0.44	1.0000
KH2PO4	136.09	330											0.29	0.69786	0.01		1.0000
"	desired conc.		g/L	10	10	10	10	10	10					10			
	grams added to 1 L			29.0472	26.8262	47.841935	49.3847271	30.7638	43.9865					14.3296			

PREPARATION OF STANDARD SOLUTIONS FOR ASSAYING WATER QUALITY

COMPOUND	M.W.(g)	M.W.'s (Cold)	g/L	55.847 Fe	63.546 Cu	58.71 Ni	58.9332 Co	54.938 Mn	65.37 Zn	62 NO3	96.062 SO4	35.453 Cl	39.1 K	30.9748 94.9714 PO4	1.01 H	18 H2O	
FeCl3	162.22	744	29.047218293	10								19.045					29.044711444
NiSO4.7H2O	280.88	756	47.841934934			10					16.362					21.5	47.841761199
MnSO4.H2O	169.01	985	30.763770068					10			17.485					3.28	30.764669264
Co(NO3)2.6H2O	291.04	1338	49.384727115				10			21						18.3	49.383885484
CuCl2.2H2O	170.47	1104	26.826236112		10							11.158				5.67	26.828231517
ZnSO4.7H2O	287.54	965	43.986538167						10		14.695					19.3	43.986382132
KH2PO4	136.09	330	14.329577115										4.12	10	0.21		14.32950762
	G/L		242.180	10	10	10	10	10	10	21	48.543	30.203	4.12	10	0.21	68.1	242.17914866
				Fe	Cu	Ni	Co	Mn	Zn	NO3	16.181 SO4	Cl	K	3.26149 PO4	H	H2O	242.17914866

	2496-2498	2499-2501	2502-2504	2505-2507	STOCK
Fe	1.00	10.00	100.00	1000.00	10000
Cu	1.00	10.00	100.00	1000.00	10000
Ni	1.00	10.00	100.00	1000.00	10000
Co	1.00	10.00	100.00	1000.00	10000
Mn	1.00	10.00	100.00	1000.00	10000
Zn	1.00	10.00	100.00	1000.00	10000
NO3	2.10	21.04	210.42	2104.24	21042.4
S	1.62	16.18	161.81	1618.09	16180.9
Cl	3.02	30.20	302.03	3020.29	30202.9
K	0.41	4.12	41.17	411.72	4117.24
P	0.33	3.26	32.61	326.15	3261.49
H	0.02	0.21	2.12	21.23	212.268

Standard Solutions: December 9, 1991

Sample	Fe	Cu	Ni	Co	Mn	Zn	NO3	SO4	Cl	K	PO4
A-1	1.0	1.0	1.0	1.0	1.0	0.8	2.1	4.5	3.0	0.4	1.0
A-2	1.0	1.0	1.0	1.0	1.0	0.8	2.1	4.5	3.0	0.4	1.0
A-3	1.0	1.0	1.0	1.0	1.0	0.8	2.1	4.5	3.0	0.4	1.0
B-1	10.0	10.0	10.0	10.0	10.0	7.7	21.0	45.2	30.2	4.1	10.0
B-2	10.0	10.0	10.0	10.0	10.0	7.7	21.0	45.2	30.2	4.1	10.0
B-3	10.0	10.0	10.0	10.0	10.0	7.7	21.0	45.2	30.2	4.1	10.0
C-1	100.0	100.0	100.0	100.0	100.0	77.0	210.0	452.0	302.0	41.2	100.0
C-2	100.0	100.0	100.0	100.0	100.0	77.0	210.0	452.0	302.0	41.2	100.0
C-3	100.0	100.0	100.0	100.0	100.0	77.0	210.0	452.0	302.0	41.2	100.0
D-1	1000.0	1000.0	1000.0	1000.0	1000.0	770.0	2100.0	4520.0	3020.3	412.0	1000.0
D-2	1000.0	1000.0	1000.0	1000.0	1000.0	770.0	2100.0	4520.0	3020.3	412.0	1000.0
D-3	1000.0	1000.0	1000.0	1000.0	1000.0	770.0	2100.0	4520.0	3020.3	412.0	1000.0

U.S. Department of Commerce
Juanita M. Kreps
Secretary

National Bureau of Standards
Ernest Ambler, Director

National Bureau of Standards

Certificate of Analysis

Standard Reference Material 1645

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This Standard Reference Material is intended for use in the calibration of methods used in the analysis of river sediment and materials with similar matrices. The material has been freeze dried and is now essentially free from moisture. The certified values given below are based on measurements made on a dried sample of at least 100 mg for the trace elements and for a 1-g sample for iron and chromium.

The values are based on the results of 6 to 30 determinations by the analytical techniques indicated. The estimated uncertainties include those due to sample variation, possible method differences, and errors of measurement (see Preparation and Analysis).

Element	µg/g	Element	µg/g
Cadmium[c d]	10.2 ± 1.5	Thorium[b]	1.62 ± 0.22
Copper[b c]	109 ± 19	Uranium[b]	1.11 ± .05
Lead[b d]	714 ± 28	Vanadium[a c]	23.5 ± 6.9
Manganese[b c]	785 ± 97	Zinc[a d]	1720 ± 169
Mercury[a c]	1.1 ± 0.5		Weight %
Nickel[b d]	45.8 ± 2.9	Chromium[b c]	2.96 ± 0.28
Thallium[b]	1.44 ± 0.07	Iron[a c]	11.3 ± 1.2

a. Atomic Absorption Spectrometry
b. Isotope Dilution Mass Spectrometry
c. Neutron Activation Analysis
d. Polarography

The overall direction and coordination of the technical measurements leading to certification were performed under the chairmanship of J. K. Taylor.

The technical and support aspects involved in the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by W. P. Reed.

Washington, D.C. 20234
November 16, 1978

J. Paul Cali, Chief
Office of Standard Reference Materials

(over)

Instructions for Use

The material, as received, is essentially free from moisture. In case of exposure to moisture, it should be dried without heat to a constant weight before using. Recommended procedures for drying are: (1) drying for 24 hours using a cold trap at or below −50 °C and a pressure not greater than 30 Pa (0.2 mm Hg); (2) drying in a desiccator over P_2O_5 or $Mg(ClO_4)_2$. When not in use, the material should be kept in a tightly sealed bottle and stored in a cool, dark place.

Mater ny segregation by thoroughly mixing the contents of the bottle by shaking and rolling before each withdrawal on, when taking a portion for analysis, the analyst should strive to remove as representative a sample as possible.

Preparation and Analysis

This SRM was prepared from material dredged from the bottom of the Indiana Harbor Canal near Gary, Indiana. This material was screened to remove foreign objects, freeze dried, and sieved to pass a No. 80 (180μm) screen. This material was thoroughly mixed in a V-blender, bottled, and sequentially numbered. The material has been radiation-sterilized to minimize alteration from biological activity.

Randomly selected bottles were used for the analytical measurements. Each analyst examined at least 6 bottles, some of them measuring replicate samples from each bottle. No correlation was found between measured values and the bottling sequence. The results of measurements on samples from different bottles did not appear to differ significantly from sub-samples within the bottles. Accordingly, it is believed that all bottles of this SRM have substantially the same composition. The analytical methods employed were those in regular use at NBS for certification of Standard Reference Materials, except as noted below. Measurements and calibrations were made to reduce random and systematic errors to no more than one percent, relative. The uncertainties of the certified values listed in the table include those associated with both measurement and material variability. They represent the 95 percent tolerance limits for an individual sub-sample, i.e., 95 percent of the sub-samples from a unit of this SRM would be expected to have a composition within the indicated range of values 95 percent of the time.

The following values have not been certified because either they are not based on results of a reference method, or were not determined by two or more independent methods. They are included for information only.

All values are in units of μg/g of sample, unless otherwise indicated.

Antimony	(51)	Potassium	(1.2 wt. %)
Arsenic	(66)	Scandium	(2)
Cobalt	(8)	Sodium	(0.55 wt. %)
Lanthanum	(9)		

The values listed below are based on measurements made in one laboratory, and are given for information only. While no reason exists to suspect systematic bias in these numbers, no attempt was made to evaluate such bias attributable to either the method or the laboratory. The method used for each set of measurements is also listed. The uncertainties indicated are two times the standard deviation of the mean.

Kjeldahl Nitrogen	(0.0797% ± 0.0048)
Total Phosphorus	(.051% ± .0014)

Oil and Grease (Freon)	(1.71% ± .26)
Chemical Oxygen Demand (Dichromate)	(149,400 mg/kg ± 9,000)

The methods used are:

Total Phosphorus - ASTM Method E-350.

Chemical Oxygen Demand (Dichromate) - Standard Methods for the Examination of Water and Waste Water, 14th Edition (1975), Section 508, page 550.

Oil and Grease (Freon 113 Extraction) - ibid., Section 502, page 518.

The following values are not certified, but are given to describe the matrix of the material: SiO_2 - 51%; MgO - 4%; Al_2O_3 - 4%; CaO - 4%.

H. L. Rook supervised collection, freeze drying, and homogenization of the SRM. The following members of the staff of the NBS Center for Analytical Chemistry performed the certification measurements: T. J. Brady; E. R. Deardoff; L. P. Dunstan; M. S. Epstein; R. Filby; M. Gallorini; E. L. Garner; T. E. Gills; J. W. Gramlich; R. R. Greenberg; S. H. Harrison; G. J. Lutz; L. A. Machlan; E. J. Maienthal; T. C. Rains; H. L. Rook; T. A. Rush; and W. P. Schmidt.

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BOOJUM RESEARCH LIMITED

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EFFECT OF FOLIAR FERTILIZATION ON *Typha latifolia* L. GROWING IN ACID MINE DRAINAGE

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Abstract

Acid mine drainage **(AMD)** produced through microbial oxidation of mine tailings and waste-rock is a major pollution problem. Amelioration of AMD by wetlands can be anticipated if conditions are. created which facilitate microbial alkalinity generation and sulphate reduction. The microbial communities require a carbon source, which can be derived from *Typha latifolia* L. (cattail) either through release from roots into the rhizosphere or through litter decomposition.

Cattail populations are tolerant of the harsh environmental conditions created by AMD. Iron accumulates in the rhizosphere and within both rhizomes and roots. X-ray analyses of cattail root sections examined by scanning electron microscopy show that very high concentrations of iron in association with sulphur are found on the root epidermal surface.

Experiments with foliar fertilization of cattails are being carried out on acidic tailings in the Elliot Lake area (Ontario, Canada) to stimulate biomass production and hence the potential to ameliorate AMD. Results to date indicate that at the concentrations used, the fertilizer had no significant ($P \leq 0.05$) effects on dimensions or starch content of over-wintering rhizomes, parameters which we are using as indirect indicators of plant productivity. Fertilization significantly ($P \leq 0.05$) reduced the weight of roots on the rhizome sections examined.

These studies suggest that cattails grow well in AMD polluted waters and further studies will help optimise their role in reducing pollution and restoring wetland ecosystems.

Introduction

Cattails *(Typha* spp.) are widely distributed in temperate and subtropical regions of the world. In North America, cattails are found from Alaska in the north (ca 67°N) to Mexico in the south (Scoggan, 1978). They are confined to environments which remain wet throughout the year. In Canada, they may survive within thick ice layers (pers. obs.). Cattails are so effective at colonizing aquatic environments that they are often treated as weeds due to clogging of waterways or out-competing other species and reducing habitat diversity for wildlife (Linde et al 1976). The success of cattails in colonizing a wide range of aquatic situations is,

in part, attributable to the ability of roots to oxygenate the rhizosphere (Dunbarin et al 1988) where otherwise, the anaerobic conditions would prevent root growth. We are investigating the use of cattails as candidates for producing biomass and hence organic carbon in situations influenced by AMD. The sediments of wetlands, including those exposed to AMD, are low in oxygen. In these reducing conditions, anaerobic bacteria (e.g. sulphate reducers) reduce sulphate, iron and other ions in the water. These processes generate alkalinity and with increasing pH, the reduced compounds are precipitated as metal sulfides. The beneficial bacteria which carry out these processes require organic matter as a source of carbon for growth. Foliar fertilization was tested as a means of increasing cattail biomass and hence the potential for microbial alkalinity generation. The alternative of adding fertilizer directly to the substrate will result in rapid leaching of the plant nutrients. We therefore investigated foliar fertilization as a means of efficiently retaining the nutrients on the plants and hence increasing productivity.

Cattails thrive in a range of pHs and can survive high levels of pollution from mine and municipal wastes. Constructed and natural wetlands dominated by cattails have been tested for their ability to survive and indeed to reduce AMD (Sencindiver et al 1989; Brodie et al 1990; Lan et al 1990; Tarutis and Unz 1990). Cattails roots and rhizomes accumulate large amounts of iron, mainly on the surface as a plaque (Taylor et al 1984; Macfie and Crowder 1987; Crowder et al 1987). Dissolved iron is a major constituent of AMD. The ability of cattails to ameliorate the effects of AMD by removing metals from solution is not certain, but cattails can certainly survive in waters with a pH of < 2 or > 10 (Samuel et al 1988, pers. obs.) and iron concentrations of up to 150 $mg.L^{-1}$ (Samuel et al 1988; Stark et al 1988; Brodie 1990). We are investigating the ability of cattails to tolerate AMD and, with both natural and constructed wetlands, are seeking to optimise the role of this plant in ameliorating AMD.

In optimal conditions, cattails are very productive, with a total (above and below ground) annual biomass production of up to 100 $T.ha^{-1}$ (Lakshman 1987). In AMD water, growth is reduced in greenhouse conditions (Wenerick et al 1989), but there is a lack of data on cattails in the field.

Cattail biomass, particularly the underground component, is difficult to measure (Hogg and Wein, 1987). A 'population' of cattails often comprises one or a small number of clones, many shoots derived from one parent and linked by rhizomes. These rhizomes are often found at considerable and variable depths in the substrate, requiring excavation of a considerable volume of material. Parameters measured on these rhizomes can give an indirect measure of productivity (Linde et al 1976). Most of the net photosynthate of the previous summer is stored as starch in the rhizomes. Data on starch content and rhizome dimensions, together with shoot density, can give clear indications of the effects of fertilization on productivity.

Study site

Cattails were studied at two Ontario sites, the locations of which are shown in Fig 1. The work on AMD water chemistry and the accumulation of metals on root surfaces was carried out on water and cattails from seepage below the tailings dam on the Makela site near Sudbury. These tailings, which generate the AMD, are from a nickel and copper extraction operation. The foliar fertilization study was carried out on tailings at the Stanrock site, a uranium mine near Elliot Lake. Off-site control plants were dug from a roadside wetland not subject to direct influence of A M D .

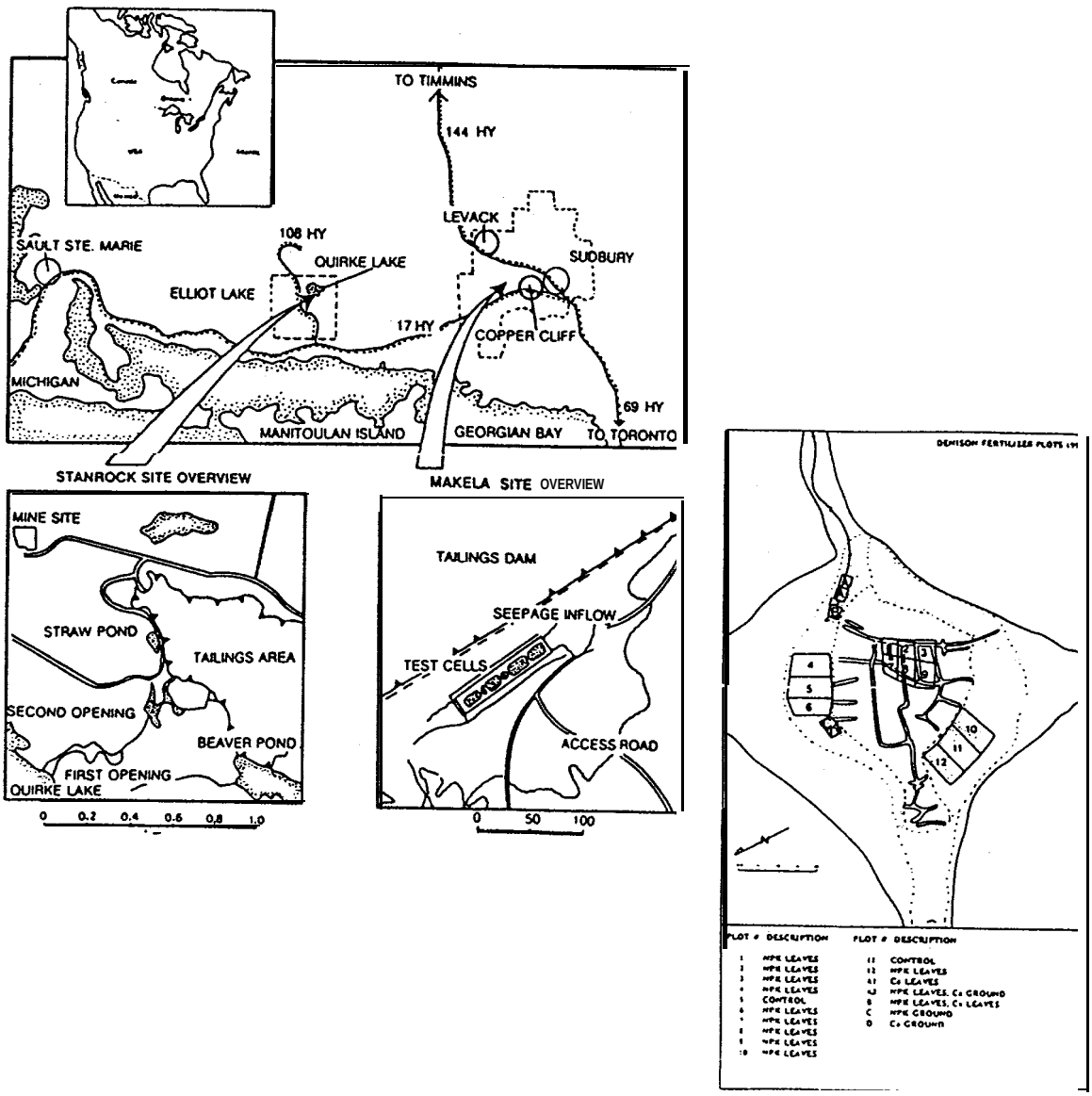

FIG 1 The location of study sites (above) and the layout of the Elliot Lake experiment

Materials and methods

Foliar fertilization experiment

Foliar fertilization was carried out on plots of cattails on the Stanrock site in 1989 and 1990. In 1990, 14-4-6 NPK ('Harvest Plus', Stoller Chemical Company) was applied to all fertilized plots from a portable sprayer on three occasions, at the beginning (May), middle (June) and end (July) of the growing season. Calcium was applied as 'This' (Stoller Chemical Company) liquid fertilizer (6 % calcium). Total applications were 87.5 $kg.ha^{-1}$ nitrogen, 25 $kg.ha^{-1}$ phosphorus and 37.5 $kg.ha^{-1}$ potassium and where applied, calcium, 85.3 $kg.ha^{-1}$.

Field sampling.

Cattails were sampled at Makela and Elliot Lake in February to March 1991. At the Elliot Lake site, holes approximately 1 m^2 were cut through the ice and snow and sediment. Cattail 'plants' (old shoot + attached rhizome and roots) were carefully excavated from the sediment. Five 'plants' were dug from each plot. Plants were photographed and kept under ice in coolers until returned to the laboratory where they were immediately frozen (-20°C).

Makela water sampling

Conductivity, Eh and pH of the stream feeding the Makela cattails were determined in the field. Inductively coupled plasmaspectroscopy (ICP) analysis was carried out by Chauncey Laboratories (Toronto, Ontario) on filtered, acidified samples.

Rhizome sample preparation

To prepare the cattails for morphological and chemical investigations, individual plants were taken from the freezer and washed under hot tap water. Rhizomes formed during the previous growing season (identified by the presence of a new, yet to emerge shoot apex) were measured for length. Rhizome and roots were separated and oven dried at 80°C Hand-cut sections of the rhizome were cut and fixed in 95 % ethanol for starch and metal staining.

Starch determination

Starch content of rhizomes was determined by a modification of the method of Neilson (1943). The rhizome and roots were ground in a Wiley mill and weighed. Distilled water was added to make a 0.67 % suspension. 2 mL of the suspension was measured and 2.7 mL of 72 % perchloric acid was added with continuous

stirring. The mixture was allowed to stand with occasional stirring for 10 minutes. A 1 mL aliquot was transferred to another beaker and 6 mL of water was added. The pH was raised to 8.3 with 6N sqdium hydroxide and then lowered to 4.5 with 6 N acetic acid. Then, 2.5 mL of 2 N acetic acid was added followed by 0.5 mL of 10 % potassium iodide and 5 mL of 0.01 N potassium iodate. The solution was left for 5 minutes for colour development. This solution was then made up to 50 mL with distilled water. Absorbance at 680 nm was determined on a Coleman Junior II spectrophotometer. A standard curve was made daily using the above procedure but with potato starch ('Analar', BDH Laboratories).

Metal staining

Hematoxylin staining of hand-cut rhizome sections was carried out by the method of Pizzolato et al (1967). Fresh staining solution was prepared by adding 2 mL of a stock solution to 200 mL of phosphate buffer (pH 7) immediately before the staining procedure. For the stock solution, 1 g of hematoxylin was dissolved in 100 mL of 0.01 M phosphate buffer (pH 7).

Scanning electron microscopy

Makela cattails collected in March were washed under the tap. Roots were severed and placed in distilled water until required for SEM. 1.5 cm sections of adventitious roots were mounted on a copper specimen holder with Tissue-tek O.C.T. compound (Miles Scientific). The cryogenic preparation of roots was carried out in an EMscope SP cryo-sputter unit (EMscope Laboratories Limited, Ashford, England). The specimen holder was plunged into subcooled liquid nitrogen. Root samples were etched at -90°C for 15 minutes and then sputter coated with chromium. The samples were then viewed on a cryo-stage in an ISI model DS-130 scanning electron microscope at an accelerating voltage of 15 kV.

Results

Water chemistry measurements of the stream feeding the cattails at Makela were made in the field through the summer of 1990. In addition, ICP analyses of filtered, acidified samples of the same water were conducted. Data for 5 sampling dates are summarised in Table 1. The high total conductivity was largely attributable to sulphur (as sulphate) and to a lesser extent, calcium and iron. Concentrations of other heavy metals were low compared to AMD at many Canadian mining sites.

TABLE 1 Chemistry of Makela seepage, July-October 1990

Metal	Concentration ($mg.L^{-1}$)
Fe	202-302
S	728-1043
Mg	156-216
Mn	3.1-4.4
Zn	0.4-1.1
Cu	0.04-0.1
Al	0.1-0.4
K	75-92
P	0.5-3.3
Ca	445-579
pH	5.85-6.40
Eh	-85 - -48 mV
Conductivity	2600-3100 µmhos (20-25°C)
Acidity	600-800 $mg.L^{-1}$ equiv of $CaCO_3$

Makela root surfaces were examined by SEM and associated X-ray scanning. Fig 2a is an X-ray scan of the surface of the adventitious cattail root shown in Plate 1. Large peaks for iron are evident (the peak at around 7 KeV is also an iron peak). Smaller peaks for sulphur, silicon and copper are also present. The chromium peaks are due to the sputter coating material (the peak at 6 KeV is a chromium peak). These peaks indicate that the coating process was successful. Several other scans were made with very similar results. A scan of a dead lateral root (Fig 2b and Plate 2) shows a similar pattern.

Following fracturing of a root, X-ray scans were made within the exodermis. The example in Fig 2c was about three cells below the epidermis. There are no iron and sulphur peaks evident in this scan. There is a peak for potassium presumably from cytoplasmic contents.

Hand-cut sections of Makela rhizomes were examined for starch and metal distribution. The distribution of iodine stain indicates that most starch was in the central pith of the rhizome. Starch was also present, at much lower concentrations, in the outer cortex.

Hematoxylin staining indicated the presence of heavy metals, mainly in the epidermal cell layer. The dark-blue colour probably indicates the presence of iron as this is by far the most abundant of those metals detected in the water expected to give such a reaction. A little staining was also present in the pith. The colour here (red-purple) was very different from the epidermal layer, indicating a different metal(s) present.

Rhizome dimensions, starch content and root dry weight data for the Elliot Lake cattail roots and rhizomes are summarised in Table 2.

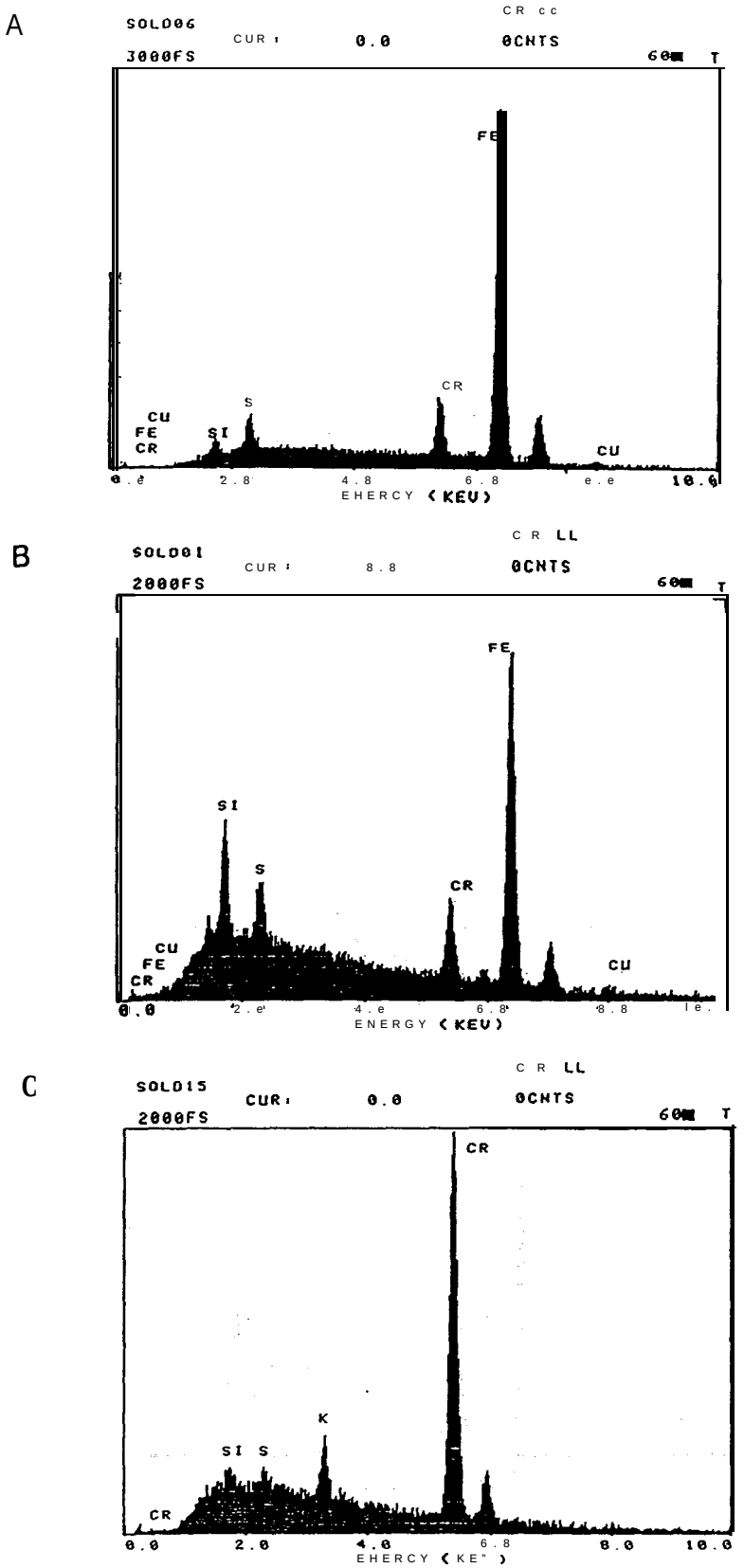

FIG 2 X ray scans of scanning electron micrographs of Makela cattail roots. A Adventitious root; B Dead lateral root; C Exodermis of adventitious root.

TABLE 2 Effects of foliar fertilization on Elliot Lake cattails. Mean ± S.E. for 5 plants

A New shoot end of rhizome;.

	Rhizome length (cm)	Rhizome diameter (mm)	Pith diameter (mm)	Root wt. (mg)	Rhizome starch (%)
Off-site control	28.8 ± 4.7	14.0 ± 0.6	8.8 ± 0.4	233 ± 100	37
On-site control	26.0 ± 4.5	12.1 ± 1.3	8.2 ± 1.0	109 ± 24	32
+NPK	27.7 ± 5.7	13.8 ± 1.1	9.4 ± 0.9	160 ± 84	33
+NPKCa	16.1 ± 3.1	12.2 ± 0.6	8.2 ± 0.3	42 ± 18	28

B Parental shoot end of rhizome

	Rhizome length (cm)	Rhizome diameter (mm)	Pith diameter (mm)	Root dry wt. (mg)	Rhizome starch (%)
Off-site control	28.8 + 4.7	12.8 ± 0.8	7.5 ± 0.7	85 ± 26	34
On-site control	26.0 ± 4.5	11.0 ± 1.1	6.6 ± 0.6	109 ± 30	37
+NPK	27.7 ± 5.7	12.4 ± 0.7	7.2 ± 0.5	28 ± 10	33
+NPKCa	16.1 ± 3.1	10.8 ± 1.1	6.2 ± 0.5	17 ± 5	28

Rhizomes were very variable in length and diameter. The foliar fertilizer had no significant effect ($P \leq 0.05$) on rhizome length, rhizome diameter, pith diameter or starch content either at the parent shoot end or the younger, new shoot end of the rhizome. Rhizome diameter and pith diameter and therefore total starch content were greater at the new shoot end. Root weight on rhizome sections from fertilized plots was significantly less ($P \leq 0.05$) than on those from unfertilized plots.

Discussion

The Makela data indicate that cattails can grow in the presence of 300 $mg.L^{-1}$ of dissolved iron. This is more than suggested in the literature (around 150 $mg.L^{-1}$) as an upper tolerance limit (Samuel et al 1988). Some AMD contains much higher concentrations of iron (pers. obs.) but cattails have not been observed by us to grow in such conditions. It should also be mentioned that the chemistry of a feeding stream as determined in this study may be very different from the interstitial water to which cattail roots and rhizomes are directly exposed. The survey by Brodie (1990) on a number of constructed wetlands suggests that the iron content of water passing through cattail-dominated wetlands can be dramatically reduced by 90 % or more, although figures vary considerably from one wetland to another.

The SEM X-ray analyses of Makela cattail roots indicate that iron is by far the most abundant metal on the root surface, but it is not possible to accurately quantify metals from such analyses. Sulphur peaks were much smaller than those for iron, although sulphur was much more abundant in the water (around 1000 $mg.L^{-1}$). This is consistent with the iron deposits being composed of oxidised iron (Fe^{3+}) in the form of ferric hydroxide as has been shown for rice (Chen et al 1980) and suggested for cattails (Taylor et al 1984). The absence of iron in exodermal cells beneath the root surface suggests that iron is only accumulated on the epidermal cell surfaces. Metals associated with the Makela roots have not been quantified. Other studies indicate that although roots and rhizomes can accumulate high concentrations (up to 5 % dry weight) of iron as plaques (Taylor and Crowder 1983), estimates of total iron on roots and rhizomes in a cattail stand suggest that only a small percentage of iron can be removed from AMD by this route.

It is probable that much greater amounts of iron and other metals are removed by precipitation as sulphides in sediments following alkalinity generation by anaerobic bacteria. Such activity requires organic matter which may be provided by cattails. Therefore, there is a need to investigate means of increasing productivity of cattails and to determine whether this increases the capacity of a wetland to ameliorate AMD. Here we report on the first phase.

The studies of Linde et al (1976) indicated that rhizome size was a good indicator of productivity. Analysis of the data suggests that the foliar fertilization procedure had no clear effects on rhizome size, pith content or starch content. At the sampling time (March), the cattails were still in an over-wintering state. Therefore, starch contents should be near a maximum and reflective of the productivity during the summer of 1990. The reduction of root growth in the presence of fertilizer is interesting and suggests that root production is stimulated by stress (such as nutrient limited) conditions. This affect however, is unlikely to have much direct effect on productivity, as roots comprise less than 10 % of total plant dry weight (Hogg and Wein 1987).

The following conclusions may be drawn from this study:

1) Cattails can grow in AMD affected water at Makela with a dissolved iron content of 300 $mg.L^{-1}$ and a total acidity of 600-800 $mg.L^{-1}$ equivalents of $CaCO_3$.

2) Iron is the predominant metal accumulated on the surface of Makela cattail roots as shown by X-ray analysis in association with SEM. Some sulphur and silicon are also present on the root surfaces.

3) Foliar fertilization of Elliot Lake cattails exposed to AMD had no significant effect ($P \leq 0.05$) on rhizome size or starch content, but significantly reduced ($P \leq 0.05$) the mass of roots on the rhizome sections sampled.

This study demonstrates that cattails can grow in AMD conditions and therefore contribute to processes which reduce water pollution. Ongoing studies will help define the conditions required to optimise the productivity of cattails in polluted mine waste waters. Many wetlands are affected by mines, either directly upon burial by tailings or indirectly through damage from AMD. Technologies to reestablish cattail populations on such sites will, therefore, contribute to the restoration of severely damaged ecosystems.

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Presented at the IAWPRC conference on 'Wetland Systems in Water Pollution Control', Sydney, NSW, Australia, November 30 1992

THE DEVELOPMENT OF FLOATING *Typha* MATS

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ABSTRACT

Continuous covers of floating *Typha* (cattail). mats in waste water polishing ponds can decrease turbulence, thereby improving settling of suspended solids. Decomposition of organic debris, released from the floating mats, maintains anaerobic conditions in the sediments. Reduced oxygen concentrations in the water column and sediments assist in stabilizing contaminants bound to settled solids.

In order to develop floating mats; the conditions under which cattail seeds could germinate on floating structures had to be addressed. Those conditions were first tested in the laboratory, then scaled-up to a greenhouse. Populations were grown from seeds in both the greenhouse, for transplant to the field, and *in situ,* using specially constructed flotation structures.

Techniques for germinating *Typha* seeds, promoting seedling development, and constructing floating rafts are discussed. During germination, substrate moisture, pH, and nutrients, both during seedling development and transplantation, have been identified as key factors governing successful population establishment. Floating *Typha* mat populations have overwintered two seasons in inactive mine open pits and on iron hydroxide sludge settling ponds:

KEYWORDS

Cattails; -'Productivity; Litter Production; Growth; *Typha*; Germination; Seedling Development; Floating Populations; Mine Wastewater.

Cattail (*Typha*) populations are extremely productive (Wetzel 1983). They have an exceptional capacity to colonize harsh environments (Kalin 1984), and they spread rapidly (Dykyjova and Kvet 1978). These characteristics make them ideal plants for wetland stabilization, and as an organic carbon supply, especially in harsh, industrial waste water polishing ponds.

Floating cattail mats are common in wetlands throughout the world (Sassar and Gosselink 1984). Mostly considered a weed problem (Sculthorpe 1967), floating mats, if deliberately planted, would provide a living cover for polishing ponds which would decrease turbulence and aeration of pond water. De-aeration of pond water would improve settling of suspended solids, enhance anaerobic sediment production, and stabilize contaminants bound to settled solids. Decomposition of organic debris, released from the floating mats (Hogg and Wein 1987a), would provide a carbon source for sediment-based, anaerobic, microbial communities.

An effective, economical means of establishing floating or rooted cattail stands over large areas can probably only be achieved- through development of cattails from seeds. The objective of this paper, then, is to describe methods of -large-scale cattail production from seeds, and early results.

METHODS

Seed Germination. *Typha angustifolia* seed heads were collected from Bluffer's Park, Scarborough, Ontario in March 1990, and stored in the refrigerator. Seeds were separated from seed head parts by fluffing in warm, dilute-detergent water. The detergent assisted the wetting of the pericarp, which resulted in spontaneous release of the seed. As seeds are more dense than water, they sink to the bottom, while the remaining debris floats.

Seeds were planted in three types of trays in the laboratory, which varied the amount of soil moisture and fertilizer. The first set-up, termed the Gradient Seedbeci, consisted of growing seedlings on an inclined rack set in a plastic tray. The tray was filled with water so that half of the wire seedbed was submerged.

The second set-up used a suspended rack in a water-filled tray, called the Suspended Seedbed. The bottom of the rack contacted the water surface, maintaining seedbed moisture. However, with water loss from the trays by evapotranspiration, the degree of, saturation varied over the two day period between waterings.

The third set-up was a flbating rack (Floating Seedbed). The Styrofoam frame maintained the soil surface 1-2 mm above the water surface. Besides soil moisture the following factors were also considered. Humidity was enhanced in half of the treatments by injecting water mist periodically into the growth chamber during each light period. A 1.5 mL L^{-1} dose of fungicide (No-Damp; Plant Products; 2.5% Benzoxine) was applied to the water supply of half of the treatments (3.3 L each) prior to planting.

A low dose (1.3 g L^{-1}) of fertilizer (Flowering Plant fertilizer; 15:30:15; Plant Products) was applied to one-third of the treatments. A high dose (2.6 g L^{-1}) of fertilizer was applied to another one-third, and the last third received no fertilizer.

All together, 36 factors were considered. In each treatment, six replicates of approximately 100 seeds were planted. Water levels were adjusted every second day with tap water to compensate for water losses due to evapotranspiration. A total of 36 treatments were set up.

A summary 'of the growth experimental factors is shown below (Table 1):

WATER DEPTH	GRADIENT/MOIST/FLOATING
HUMIDITY	HIGH/LOW
FUNGICIDE	ADDED/NOT ADDED
FERTILIZER	HIGH/LOW/NONE

Greenhouse Germination. Germination of cattail seeds on a larger scale was initiated in a specially-constructed greenhouse. The greenhouse contained 8 large wooden boxes (2.4 x 1.2 x 0.6 m) which were filled half-full with tap water. Cattails were planted in seedling trays floating on the water in the boxes. In all, 16 trays per box and 8 boxes, covering 24 m^2, were set up in the greenhouse.

In the first seeding trial, the soil in the trays contained two layers: a base layer, 4 cm thick, composed of a sandy till and a second 4 cm thick layer of local peat.

In the second trial, the soil also contained two layers, but, the upper layer was composed of commercial peat and agricultural limestone, which when mixed, and wetted in the boxes, gave pHs in excess of 5.5.

Trial 3 took place the following year (1991) and repeated the procedures used in trial 2.

The greenhouse was maintained at a minimum of 22° C. Water levels were maintained by addition of tap water. Ambient sunlight, through the translucent plastic sheeting, provided illumination.

Seeds from 42 seed heads, collected and prepared as above, were mixed with 32 litres (by moist volume) of the peat. A thin layer of seed-peat mixture was applied over each tray surface.

After planting, the water in-the each box was given 0.2 g L^{-1} of fertilizer (30:15:30; plant food, Plant Products).

In Situ Development. In 1990; twenty rafts, 1.8 x 3.6 m in size, were constructed from timber, Styrofoam and netting. Ten rafts were placed on an acidic mine pit (pH 3.5, Zn 35 mg L^{-1}), and ten rafts were placed on a circum-neutral mine pit (pH 6.5, Zn 25 mg L^{-1}).

After 40 days growth (appx. 15 cm in height), seedlings from the greenhouse were transplanted to the floating rafts. Rafts received either slow-release fertilizer (Nutricote, 19:6:12; Plant Products) and/or bone meal, or no nutrient supplements.

In October, 1991, 30 ‘x 30 cm blocks of *Typha* biomass were cut from the 1990 and 1991 seedling rafts for determination of the above and below substrate surface standing biomass. The fraction of the raft area covered ‘by the *Typha* population was recorded in order to correct for heterogeneity of colonization success over the raft. The blocks were sorted into six categories: green shoots, brown shoots, rhizomes, roots, seed heads and biomass of other species. Samples were dried and weighed.

In addition, blocks of *Typha* were cut from a Scarborough, Ontario natural, sediment-bound population (pH 7), and an artificial floating population located in an acid mine drainage pumping pond (pH 2.5 - 5.0) in Sudbury, -Ontario.

Germination and Seedling Establishment in the Laboratory. After 16 days, approximately 12,600 seedlings had germinated and survived the 36 treatments. However, after 46 days, only approximately 7,700 seedlings, or 61% survived.

Trkatments which provided moisture, either through 'suspension or floating trays, gave the best results (2200 seedlings) compared to the Gradient Seedbed (1740 from beach, and 800 for submerged soils, after 46 days).

The addition of fertilizer apparently reduced germination rates. Humidity had no significant effect on germination. Fungicide appeared to have had a slightly negative effect on germination.

TABLE 2 Seed Germination Rates

FACTOR	NUMBER OF SEEDLING	
	16 DAYS	46 DAYS
WATER LEVEL		
BEACH	3090	1740
WATERLINE	1430	650
SUBMERGED	1530	800
SUSPENDED	3620	2290
FLOATING	2290	2230
FERTILIZER		
NONE	5070	3170
LOW	4460	2630
HIGH	3110	1920
HUMIDITY		
LOW	6290	3800
HIGH	6260	3720
FUNGICIDE		
NONE	7360	4000
PRESENT	5280	3720

Germination in the Greenhouse. Soil used in the first trial was composed of local peat without adequate buffering. The pH of soil slurries taken in the greenhouse indicated that the soil was quite acidic (3.5-4.5). Very few of the cattails germinated.

Soil in the second trial was composed of commercial peat. It was mixed with agricultural limestone, so that pHs were above 5.5. The seedling population that grew in this soil grew evenly over all eight seed bed flats.

In Situ Cattail Growth. Within the first growing season, the seedling growth was much greater with the application of both bone meal and fertilizer, than with either supplement alone. Seedlings without supplements fared very poorly (Figure 1).

Most of the seedlings over-wintered and produced new shoots the following spring. These second season cattails were monitored throughout the summer. Biomass distributions of cattails grown in different treatments are described below.

The dry weight (are metre. These values were multiplied by the fraction of population coverage recorded for each raft. These data, as well as data for the mature (> 10 years) *Typha angustifolia* population in Scarborough, Ontario (which served as the seed source population), and the floating population established on an acid mine drainage pumping pond in Sudbury, Ontario are presented in Figure 1.

This graph clearly shows that the standing *Typha* biomass in the second year of growth was significantly greater than first year biomass on the circum-neutral pit, but still far less than the biomass produced in a mature cattail stand (as exemplified by the Scarborough population). The two Ontario populations, one natural and the other artificially grown on rafts, had much longer growing seasons than those grown on the mine pits in Newfoundland. In addition, the pumping pond population was heavily fertilized. As cattail rhizomes can live up to four years (Hogg and Wein 1987a), maximum accumulation of below ground biomass in the form of roots and rhizomes on floating populations cannot be expected for approximately two more growing seasons.

A large difference in biomass is evident between the circum-neutral and acidic pit second year populations, indicating that acidity or pH may affect cattail growth. Figure 1 also presents data for standing biomass according to nutrient supplement for the 1990 seedling populations in their second year. The combined effect of fertilizer and bone meal addition at the time of transplant, compared to fertilizer or bone meal alone, is still evident after the second growing season.

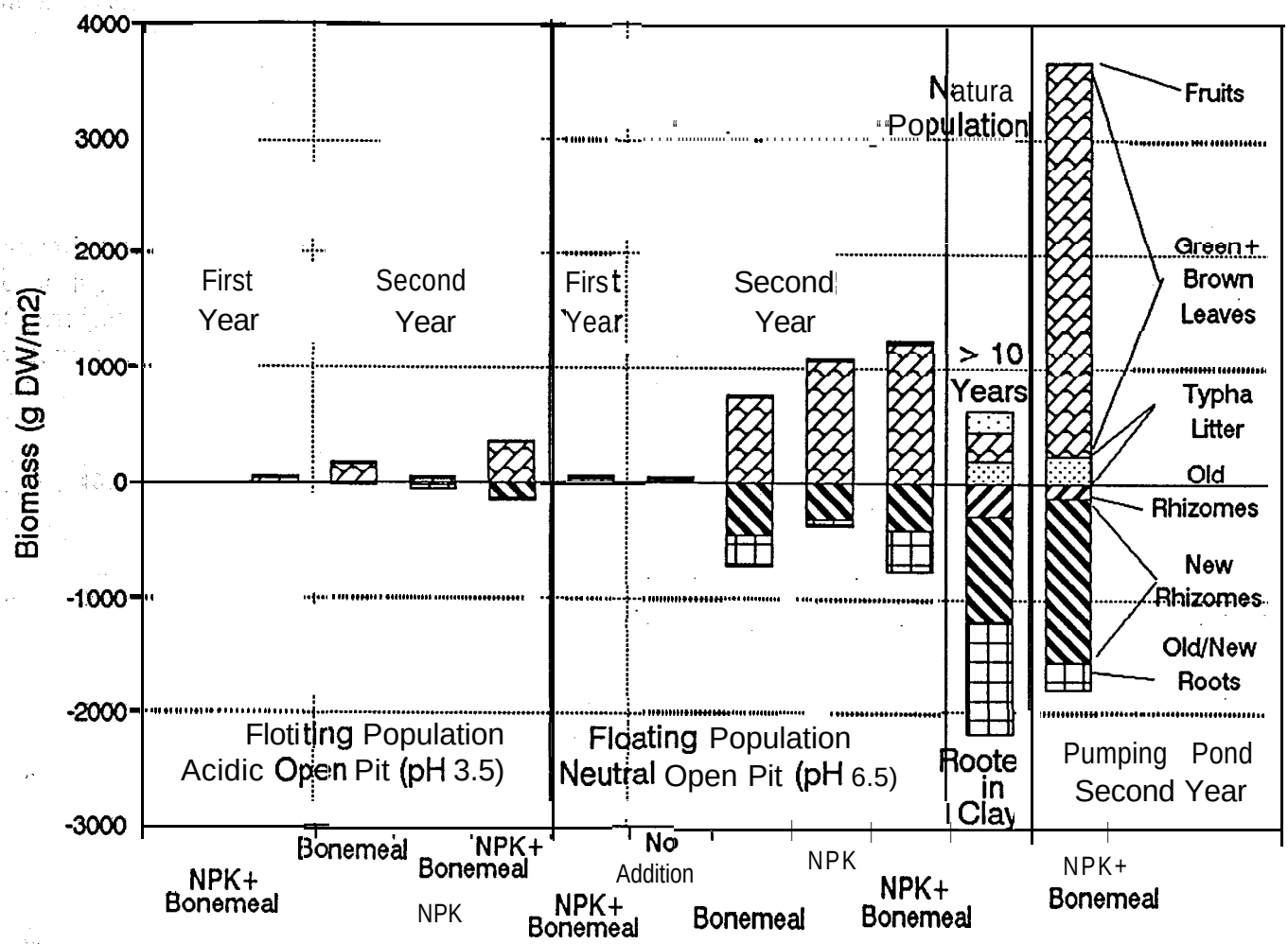

Figure 1: *Typha* biomass in both floating and natural populations.

The development of methods for establishing floating *Typha* mat populations over large areas of waste water polishing ponds must pass several milestones. First, seeds must be viable. Second, after germination, initial growing conditions for seedlings must be determined. Third, optimal conditions for plant maturation and subsequent biomass accumulation must be addressed. Finally, the population structure and development, which is required for mat populations become positively buoyant, must be defined.

Seed Viability. In the work reported here, and in all subsequent germination trials, the viability of *Typha angustifolia* seeds, collected in winter, were typically greater than 90%. In fact, according to McNaughton (1966), *Typha* seeds are already viable when still on the inflorescence, prior to complete plant senescence and the winter cold period.

Seed Germination and Early Seedling Development. A moist, but not wet, seedbed appeared optimal for seed germination. From the laboratory data, the highest germination rate was observed in the beach treatment of the Gradient Seedbed, as well as the Suspended and Floating Seedbeds. The poorest germination rate was observed in the below water treatments.

Saturation of seeds has been determined necessary for germination by other workers (Bedish 1967; Weller 1975; Leck and Graveline 1979). The moist conditions provided during the work presented here were likely sufficient for saturation of the seeds.

The laboratory germination data indicate that seed germination was inhibited by increasing concentrations of fertilizer. While low concentrations of fertilizer are adequate during establishment, seedling development was greatly enhanced by fertilizer addition during the rapid growth phase (data not shown). Therefore, to promote seedling growth, low levels of fertilizer should be added after germination. Variation in air humidity does not appear to affect either seed germination or seedling establishment in the laboratory. The addition of fungicide did not -promote seedling germination, nor did it maintain a higher number of seedlings after establishment. Seedling establishment has been reported to be dependent upon a similar set of conditions, such as moisture, light and temperature (Yeo 1964, Bedish 1967; Weller 1975; Sharma and Gopal 1979), but is also sensitive to water and sediment chemistry (McMillan 1959; Kadlec and Wentz 1974).

Overall, the moisture regime during germination and early seedling development appears to be the most important physical variable. Floating seed beds, where a moist, but not wet, water regime is maintained, provide. the most appropriate conditions for germination and seedling establishment.

Floating cattail mat development can only be considered a successful technique if these populations ultimately become buoyant and self-supporting. In order to predict the time frame which is required for maintenance, biomass measurements were obtained. These biomass data can be compared to measurements on existing floating cattail mats, which are self-buoyant.

Positively Buoyant Mat Populations. *All Typha* mats established during the present study used artificial structures to provide buoyancy. These rafts were intended to float populations until the mat structure became self buoyant. This can be anticipated, given examples of naturally buoyant *Typha* stands reported in the literature (Lieffers 1983; Hogg and Wein 1987a).

Hogg and Wein (1987b) examined factors affecting the overall buoyancy of natural floating *Typha* mats. On a square metre basis, they reported that live and dead organic solids (-8.1 kg m^{-2}), attached mineral soil (- 6.4 kg m^{-2}) and above ground shoots and litter (-3 kg m^{-2}) all contributed to a negative buoyancy (-17.5 kg m^{-2}). However, gas within living components of *Typha* biomass (+3.6 kg m^{-2}), and gas trapped within the organic material comprising the mat (+40 mg m^{-2}) yielded an overall net buoyancy of +26.3 kg per square metre of *Typha* mat.

Establishment o[illegible]n production of gas below and within the mat, and sustained entrapment of gas within the mat. The artificial flotation structures used during this study did not capitalize on additional buoyancy provided by gas generated with the mat. New designs must incorporate gas entrapment techniques, if flotation is to sustained over the long term.

Net Organic Matter Production. The standing biomass values for the circum-neutral and acidic pit second year populations were used for estimating the rate of organic matter deposition to the raft substrate and underlying water body.

Hogg and Wein (1987a) estimated that, in a floating Typha population in New Brunswick, all of the annual standing biomass of above ground shoots, 23% of shoot bases, 25% of rhizomes and 30% of dead roots were deposited each year within the floating mat.

Extrapolating these fractions to the second year population standing biomass from both pits, 291 g m^{-2} yr^{-1} litter will be deposited in the acidic pit, while 1,148 g m^{-2} yr^{-1} will be deposited in circum-neutral pit. This translates to 1.35 tonnes of *Typha* litter/acidic pit/year, and 22.4 tonnes *Typha* litter/neutral pit/year available for 'microbial decomposition. However, until the pit populations are at least four years old, the fractions of deposition suggested by Hogg and Wein (1987a) cannot be cannot be expected to be achieved. The actual values. may well prove to be site-specific. Meanwhile, standing biomass can be expected to increase as the populations mature; and therefore, higher rates of annual deposition can be anticipated than can be calculated given current standing biomass. Typha Population Development Following Seedling Transplant. Wide variation in standing biomass according to location and treatment was observed by the end of the second growing season (Figure I). In those treatments which included fertilizer addition, accumulation of above and below ground biomass was 7 to 33 times greater than in treatments which did not receive fertilizer. Populations in fertilizer-supplemented treatments in the neutral pit accumulated nearly 4 times amount of above and below ground biomass, compared to nutrient-supplemented treatments in the acidic pit.

The above 'ground biomass of *Typha* after two years in the neutral pit was twice that of the older (>10 years) natural, sediment- bound population, but below ground biomass was only one-third that of the natural population. Given that rhizomes, the below ground storage organs, may live up to four years (Hogg and Wein 1987a), maximum below ground biomass can be expected for to increase for several years.

Interestingly, the above ground biomass of the *two* year old *Typha* stand on rafts in the seepage collection pond (pH 2.5 - 5.0) was greater for both the fertilized stands in the neutral pit and the natural, sediment-bound population, Again, the below ground biomass is expected to increase for several years.

Of the total -above' and below ground biomass produced each year by a mature population, a large fraction of the below ground biomass overwinters for regenerating the population the next spring, while most above ground biomass is lost as litter. The biomass of this litter represents the net productivity of the population. Cattail stands populating sewage waste water treatment wetlands, with adequate nutrients, circum-neutral pHs, as well as background metal concentrations, routinely have productivities in excess of 3000 g m^{-2} yr^{-1} (Kadlec 1990). Nutrient-supplemented sediment-bound *Typha* populations in artificial wetlands have been reported to produce up to 2570 g m^{-2} yr^{-1} (Andrews and Pratt 1978).

The seepage collection pond floating *Typha* mat population in the present study had an above ground productivity comparable to that in the literature cited above. Similar productivities can therefore be expected for 'nutrient-supplemented floating populations introduced to waste water ponds, compared. to natural, sediment-bound *Typha* populations. Overall, the above and below ground biomass data indicate that nutrient supplements are required at the time of transplant, especially when establishing floating mat populations in oligotrophic, acidic waters. Once established, however, populations take longer than 2 years to mature. Calculations based on two year old plants, while promising, will not represent mature, floating cattail stands.

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