

**BIOLOGICAL CYCLING OF ARSENIC
IN SEDIMENTS IMPACTED BY
GOLD MILL EFFLUENTS**

SHORT TITLE: ARSENIC CYCLING IN SEDIMENTS

**RESEARCH AREA: MULTI-MEDIA CONTAMINANTS
AND BIOTECHNOLOGY RESEARCH**

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AFFILIATION: BOOJUM RESEARCH LTD.

**SUBMITTED TO: ONTARIO MINISTRY OF
THE ENVIRONMENT**

JANUARY 14, 1992

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SECTION 3 CORPORATE PROFILE AND LIST OF PUBLICATIONS

1.0 INTRODUCTION

Effluent treatment from gold milling requires the removal of heavy metals, cyanide and arsenic. The waters are generally alkaline and hence this presents a technical problem regarding the choice of a removal process.

Conventional water treatment processes for arsenic removal include chemical oxidation (chlorination), coagulation - flocculation, liming, filtration, activated carbon adsorption and, ultimately, processes such as demineralizing by reverse osmosis or ion exchange.

In principal, therefore, it is possible to select a treatment technology which will produce the desired effluent quality; however, the costs associated with the treatment technology are frequently prohibitive. The cost effectiveness of a treatment process is therefore equally important. With increasing environmental awareness, the treatment technologies do not only have to be economic and technically effective, but consideration has to be given to the long-term stability of the resultant sludge. In addition, the decommissioning of the these facilities, along with the disturbed areas, has to be considered.

The CREM task force has evaluated these technologies for their effectiveness. For arsenic, liming or softening was considered the most effective method which also removes any heavy metals. These methods produce sludges which represent an environmental problem.

Therefore, better and more environmentally acceptable treatment processes have to be developed.

1.1 Problem Definition

Existing waste water treatment technologies can not adequately meet Certificate of Approval waste water discharge objectives. Dickenson Mines Limited, on a number of occasions, has exceeded MOE guidelines for arsenic, total metals and cyanide at its final discharge point from Balmer Lake. It is suspected that sediments in the lake, which have been impacted by gold mill effluents, are contributing to this problem. Specifically, the sediment, through biomethylation processes, will liberate arsenic from the sediments to the overlaying water.

A NRC study in 1978 (Effects of Arsenic in the Canadian Environment) pointed out the overall lack of understanding with respect to the interactions of arsenic with other forms of pollutants in the environment. Further research is needed on the immobilization of arsenic in the environment. While in the past 10 years it has been shown that arsenic does not concentrate in the food chain, many other aspects of sediment-water-arsenic interactions remain unclarified (Baudo et al. 1990 and Landner et al. 1989).

2.0 OBJECTIVES

Year 1: The relevant organic arsenic species in sediments from Balmer Lake will be identified. The microbial/fungal activity which result in biomethylation will also be addressed, with reference to the most recent literature.

Year 2: Quantification of biomethylation which takes place in the sediments will be performed, and related to total arsenic loadings in Balmer Lake.

Year 3: Biotechnological processes which could be utilized to inhibit the biomethylation will be identified.

3.0 TECHNICAL BACKGROUND

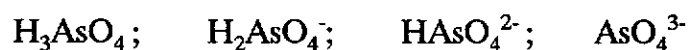
In order to address the objectives set out above, the technical background and rationale for the microbiological work is presented. A review of the geochemistry of arsenic describes the inorganic behaviour of arsenic in the context of gold mining. Finally, a review of methods examining arsenic speciation in sediments is presented. These three technical components, combined, have to be utilized to develop a biotechnological solution, which could result in eliminating the release of arsenic into surface waters from contaminated sediments.

3.1 Microbiological Aspects of Arsenic

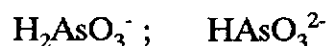
An overview of the arsenic cycling in natural waters is given in SCHEMATIC 1. An important chemical process is illustrated in this schematic; in the water column, arsenic can be methylated by fungi and bacteria. This schematic also provides a summary of possible microbial interactions with arsenic compounds. Both arsenic oxidation and reduction is mediated by bacteria. These interactions provide the theoretical basis for the potential biological removal or reduction of arsenic from waste water, through enhancement of biomineralization processes.

Arsenic is a toxic metalloid found in all natural waters at concentrations generally less than 0.05 mg/L (CREM). Arsenic can be found in four oxidation states; +5, +3, 0 and -3. The oxidation state of arsenic is dependent on pH and redox. However, arsenic in the oxidation state of 0, or in the metal state, is very rare. Arsenic has an oxidation state of -3 only when redox is very low.

Hence, in oxygenated waters arsenic is found as:



Under mildly reducing conditions, arsenic is found as:



3.1.1 Removal of arsenic by bacterial reduction and subsequent volatilization

Some bacteria, such as *Pseudomonas* and *Alcaligenes* spp., are able to reduce arsenate (AsO_4^{3-}) to arsenite (AsO_2^-), to dimethylarsine ($\text{AsH}(\text{CH}_3)_2$) under anaerobic conditions (Cheng and Focht 1979). Dimethylarsine and related compounds are volatile and may be lost to the atmosphere. The arsenite ion (As^{3+}) is much less toxic to organisms than the more oxidised arsenate (As^{5+}) form. It is, therefore, desirable to generate reducing conditions in order to remove arsenic from the water, through loss of arsine gas to the atmosphere, precipitation of arsenic as sulphide and maintenance of remaining arsenic in a reduced, less toxic form.

Sediment samples will be added to media containing arsenic, in order to enrich the system with anaerobic arsenic-tolerant bacteria. Bacterial cultures (40 ml tubes) will be prepared anaerobically. The following media will be used:

- 1) The basic media for SRB's (e.g. *Desulfovibrio*) developed by Pfennig et al. (1981).
- 2) For methanogenic bacteria (e.g. *Methanobacterium*), the carbonate buffered mineral medium of Widdel (1986).
- 3) For denitrifying bacteria (e.g. *Pseudomonas fluorescens*), a nitrate broth (Focht and Joseph 1973).

Bacteria will be isolated and maintained in culture. These isolates will subsequently be used to develop inocula for studying behaviour of As in water/sediment microcosms.

3.1.2 Biological polishing with algae

AsO_4^{3-} is a phosphate analogue and is accumulated by organisms through phosphate transport systems. Tolerance to AsO_4^{3-} is through an ion-specific efflux system, or a highly specific phosphate uptake mechanism. In the presence of AsO_4^{3-} and phosphate, the blue-green alga *Synechococcus leopoldiensis* accumulates very little As (Budd and Craig 1980). Addition of phosphate to seepage waters can stimulate the growth of such algae in the presence of AsO_4^{3-} . Such algal blooms may reduce concentrations of nitrate and metal ions through biological polishing.

Nitella spp. are members of the Characeae, and are utilized in the Chara Process. In certain geothermal lakes in the vicinity of Rotorua, natural populations of *Nitella* have been found with elevated arsenic concentrations (Fish 1963; 59 ppm). In other rivers in New Zealand, *Nitella* has also been found with elevated As content (182 ppm on a dry weight basis; Reay 1972). In Canada, Kalin and Smith (1987) have reported concentrations of As in *Chara vulgaris* as high as 1000 ppm in populations among gold tailings seepages with no more than 0.9 mg/l As.

3.1.3 Arsenic in the environment

Arsenic is a toxic chemical and has been used as essential trace element in medicine. It is suspected that arsenic is either a carcinogen, co-carcinogen or promoter. Experiments have showed that both animals and humans have a biochemical mechanism for oxidizing As^{3+} to As^{5+} and then methylating these compounds to methyl arsonic acid (MAA) and finally dimethyl arsinic acid (DMAA). Since these methylated compounds are 1/100 as toxic as As^{3+} , the process can be termed as a detoxification.

The present drinking water standards, promulgated in the National Interim Primary Drinking Water Regulations, has a "Maximum Contaminant Level" of 0.05 mg/L for arsenic. This standard is based on the assumption that, at an average water intake of two liters per day, arsenic intake from water would not exceed 100 micrograms per day (36.5 mg/year). In soils, 1 to 200 ppm arsenic is considered a normal level to support plant growth.

3.2 The Geochemistry of Arsenic in Gold Milling

The geochemistry of arsenic is summarized below:

As_4 : yellow; boiling point 633°C ; atomic radius 1.21Å; first ionization potential 10.0 eV; most common oxidation states 3-, 3+, 5+.

The following compounds occur of arsenic.

As^{3-}

Hydride : AsH_3 - arsine, extremely poisonous gas
Metal arsenides: Na_3As , K_3As etc.; hydrolysis produces arsine

As^{3+} and As^{5+}

Arsenic trihalides : AsF_3 , AsCl_3 , AsI_3 , AsBr_3
Arsenic pentahalides: AsF_5 etc.
Arsenic sulphides : As_4S_3
 As_4S_4 - red/yellow (realgar)
 As_2S_3 - yellow (orpiment)
 As_2S_5 - yellow

Oxides and oxy-acids: As_2O_3 - arsenious acid
(AsO_3^{3-} - arsenite ion)
 As_2O_5 - arsenic oxide
 H_3AsO_4 - ortho-arsenic acid
(AsO_4^{3-} - arsenate ion)

The following arsenic minerals are known to occur:

Primary minerals:

Arsenopyrite	FeAsS (also called mispickel)
Enargite	Cu_3AsS_4
Orpiment	As_2S_3
Realgar	AsS or As_2S_2
Smaltite	CoAsS_2
Nicolite	NiAs ,

and other, less common minerals; also substitutions in pyrite, galena, sphalerite and other sulphides.

Secondary minerals:

Adamite	$\text{Zn}_2(\text{OH})\text{AsO}_4$
Olivenite	$\text{Cu}_2(\text{OH})\text{AsO}_4$
Pharmacolite	$\text{HCaAsO}_4 \cdot 2\text{H}_2\text{O}$
Scorodite	$(\text{Fe}, \text{Al})\text{AsO}_4 \cdot 2\text{H}_2\text{O}$

and other, less common secondary minerals.

Smelter Product (and in burning coal mines):

Arsenolite As_2O_3

The geochemical behaviour of arsenic is summarized after (Hem, 1985; Levinson, 1980).

Aqueous phase: stable arsenate and arsenite oxy-anions; As also forms complexes, but their exact nature is poorly known. pH-Eh diagrams indicate that monovalent arsenate anion H_2AsO_4^- would be expected to dominate at pH between 3 and 7, the divalent arsenate ion HAsO_4^{2-} at pH from 7 to 11. Mildly reducing conditions would favour uncharged arsenite, HAsO_2^0 . Ferric and other metal arsenates have low solubilities; data on solubility of arsenites are sparse. Common analytical procedures report "Total As", without species differentiation.

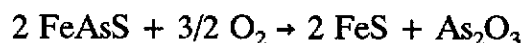
Natural aqueous concentrations: up to 40 mg/L in Steamboat Springs, Nevada.

Barriers: sulphide; adsorption on (or co-precipitation with) limonite.

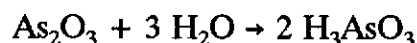
Volatile compound: arsine, in volcanic gases.

3.2.1 Chemical aspects of waste water

Laguitton (1976) discussed removal of As from gold-mine waste waters. The prime source of As in mine wastes is arsenopyrite. Its sensitivity to oxidation is similar to that of pyrite. In gold processing, contamination of both air and water by arsenic occurs commonly after roasting of gold-bearing arsenopyrite:



The arsenious oxide (arsenolite) forms a finely divided powder, which can enter the environment in two ways. Part of it escapes as "fumes" with the combustion gases; gold-bearing particles are trapped by electrostatic precipitation and leached with cyanide, which is responsible for most of the solubilization of As. The solubility of arsenious oxide, forming arsenious acid,



is enhanced by the high pH associated with the cyanide treatment. Highly concentrated waste solutions can contain as much as 3000 ppm As.

Oxidation of arsenopyrite in mining waste exposed to air can also release arsenic; As from this source appeared in well waters in Nova Scotia with concentrations up to 5 ppm (Hem 1985, p.145).

3.2.2 The chemistry of arsenic removal from waste water

The removal of arsenic from waste waters can be achieved by:

- a) precipitation of calcium-arsenate [$\text{Ca}_3(\text{AsO}_4)_2$] through lime addition, or precipitation of arsenic sulphide through Na_2S addition; liming is the most economical treatment, although it requires carefully controlled oxidation from As^{3+} to As^{5+} (using chlorine or sodium hypochlorite), maintenance of $\text{pH} > 12$, and filtration; simple aeration also works, but it is slow and likely inefficient in large-scale operation; if As concentrations below 0.5 ppm is required, addition of phosphate should be considered;
- b) adsorption on solid phase, e.g. ion-exchange resin or activated charcoal; cost would be prohibitive;
- c) trapping in "gelatinous" precipitate, commonly Fe or Al hydroxide; precipitates may be subject to redissolution when conditions become acidic.

Wagemann (1977) tested 14 metals (Al, Ba, Ca, Cd, Co, Cu, Cr, Fe, Mg, Mn, Ni, Pb, Sr and Zn) for their potential to control total As in freshwater; four (Ba, Cr, Cu and Fe) were possible candidates.

3.3 Chemical Analysis of Arsenic Compounds

There are several methods to determine arsenic in systems, such as neutron activation analysis, X-ray fluorescence spectroscopy and atomic absorption spectrometry. They generally report total arsenic concentrations.

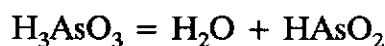
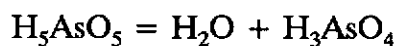
The silver diethyldithiocarbamate method can determine the inorganic arsenic by, first, reduction with zinc/HCl to arsine, then complexing with silver diethyldithiocarbamate, then measurement colorimetrically at 540 nm. Employing the pH-dependent reduction to methylarsine to arsenate by sodium borohydride, arsenite and arsenate have been determined separately. By this method, 0.1 ug can be determined.

The Standard Reference Certified for arsenic is show below:

Name	$\mu\text{g/g}$
Trace Elements in Water	0.076+/-0.007
Urban Particulate Matter	115+/-10
Trace Elements in Coal (Bituminous)	9.3+/-1
Trace Elements in Fuel Oil	0.11
Trace Elements in Coal (sub-bituminous)	0.42+/-0.15
Trace Elements in Coal (Fly ash)	145+/-15
River Sediment	66
Estuarine Sediment	11.5

Arsenic has four possible valence states in the nature. They are +5, +3, 0 and -3. The natural existence of pure arsenic is very rare. The -3 valence compounds are very unstable and easily oxidized.

Inorganic compounds, such as arsenite (3+) and arsenate (5+), usually have different forms according to the environmental pH value.



Both arsenite and arsenate can be reduced to hydrogen arsine, AsH_3 , which can then form a complex with silver diethyldithiocarbamate. The complex can be detected by the absorbency at 410 nm. The detection threshold is 0.1 ug. Thioarsenite (H_3AsS_4) and thioarsenate (HAsS_2) have the similar activities as arsenite and arsenate.

The organic compounds, arsine (R_3As) and arsone (R_5As), can also can be reduced to AsH_3 .

According the above properties of arsenic, we may identify the arsenic species in water and plants. The analytical routine is as follows:

1. Separate the inorganic and organic arsenic components by chemical extraction.
2. The arsenite, arsenate, thioarsenite and thioarsenate compounds in inorganic layers can be tested by colourimetric method of silver diethyldithiocarbamate complexing. The reduction at different pH's can separate the arsenite and the total arsenic compounds.
3. The arsenite and thioarsenite are in As^{3+} compounds, while arsenate and thioarsenate in As^{5+} compounds. Identification of distinct compounds with the same oxidation state can be performed using mass spectroscopy methods.
4. The arsine and arsone compounds in organic layer can be detected by mass spectroscopy, as well as NMR spectroscopy.

Grabinski (1981) separated As^{3+} , As^{5+} , arsenate, arsenite and methylated arsenic acids using cation-anion exchange columns (9 cm AG1-X8 and 26 cm AG50W-X8). Fractions from such columns could be analyzed for As by the silver dithiocarbamate colourimetric method, after reduction of the arsenic compounds to arsine.

4.0 TASK DEFINITION

Milestones

# 1	Literature summary; sediment collection;	Jul 31/92
# 2	Arsenic species identification in sediment;	Sep 30/92
# 3	Microbial/fungal activities identified;	Nov 30/92
# 4	Data analysis and reporting;	Jan 30/93
# 5	Collection of sediments in Balmer Lake;	Apr 30/93
# 6	As discharge and sediment loadings;	Jul 31/93
# 7	Biomethylation rates during/after ice melt;	May 30/93
# 7	Biomethylation rates during early fall;	Sep 30/93
# 8	Data analysis and reporting;	Jan 30/94
# 9	Laboratory biomethylation tests;	May 30/94
# 10	Balmer L. field inhibition tests;	Sep 30/94
# 11	Report on potential processes;	Dec 30/94

YEAR 1 TASKS

Sediment cores will be collected by Dr. D.Mchaina from Balmer Lake and shipped to Boojum Research Limited. The organic and inorganic arsenic species in the sediment will be separated through extractive methods which are well established. Microbiological work to identify *Pseudomonas fluorescens*, *Methanobacterium methanogen*, and *Desulfovibrio vulgaris*, the bacterial species involved in transformation and biomethylation of arsenic species, will be carried out.

Anticipated results: By determining the speciation of As, and identifying the As species and microbial populations, it will be possible to determine which bacterial groups and which

arsenic species are the major contributors to the total As to the water column in Balmer Lake.

YEAR 2: TASKS

As microbial activity in the sediments can be expected to vary seasonally, two sampling campaigns are planned. Sampling stations will be marked with buoys so that the same location can be monitored again in the fall. At least 30 sampling locations will be selected, with reference to discharge points from both mines. Available monitoring data will be utilized to arrive at approximations of sediment loading over the years. Seasonal variation in As species and microbial populations will be carried out, based on the findings in the first year.

Anticipated results: The significance and magnitude of the As biomethylation process in contaminated sediments and its contribution to the total dissolved As in Balmer Lake water will be determined.

YEAR 3: TASKS

The removal of arsenic from water can be achieved by precipitation of calcium arsenate ($\text{Ca}_3(\text{AsO}_4)_2$) with the application of lime, or precipitation of arsenic sulphide through

addition of NaS_2 . Biotechnological methods to remove arsenic from water, through stimulation of bacterial sulphate reduction, and/or through biological precipitation of calcium arsenate on the surface of algae (*Chara* Process) may be feasible. Tests addressing these potential processes will be carried out in the laboratory and in Balmer Lake.

Anticipated results: The foundation of a biotechnological solution for treatment of As in water bodies contaminated by gold mill effluents, particularly at the time of decommissioning of waste management areas, will be established.

5.0 REFERENCES

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SECTION 2 BUDGET

YEAR 1:

Labour

M. Kalin, 18 days @ \$268/day	4,824
J.Y. Liu, 30 days @ \$158/day	4,740
A. Fyson, 30 days @ \$180/day	<u>5,400</u>
TOTAL LABOUR	appx. 15,000

Expenses

Supplies and Equipment	<u>2,550</u>
	1,800
TOTAL COST FOR YEAR 1	appx. \$17,550

YEAR 2:

Labour

M. Kalin, 30 days @ \$268/day	8,040
J.Y. Liu, 60 days @ \$158/day	9,480
A. Fyson, 42 days @ \$180/day	<u>7,560</u>
TOTAL LABOUR	appx. 25,000

Expenses

2 Field trips @ approx \$3,000 ea.	6,000
Assays	3,000
Supplies	3,500
Miscellaneous (telephone, courier. film)	<u>2,500</u>
TOTAL EXPENSES	15,000
TOTAL COST FOR YEAR 2	appx. \$40,000

YEAR 3:

Labour

M. Kalin, 10 days @ \$268/day	2,680
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J.Y.Liu, 23 days @ \$158/day	3,634
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A. Fyson, 20 days @ \$180/day	<u>3,600</u>
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TOTAL LABOUR	appx. 10,000
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Expenses

2 Field trips @ \$3,000 ea.	6,000
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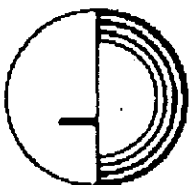
Assays	2,000
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Supplies	2,000
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Miscellaneous (telephone, courier, film)	<u>2,450</u>
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TOTAL EXPENSES	12,540
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TOTAL COST FOR YEAR 3	\$22,450
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DICKENSON MINES LIMITED

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January 13, 1992

The President
Boojum Research Limited
468 Queen Street East
Suite 400
Toronto, Ontario, Canada
M5A 1T7

Attention: M. Kalin

Dear Margarete:

RE: BIOLOGICAL CYCLING OF ARSENIC IN SEDIMENT RECEIVING GOLD MILL EFFLUENT

Dickenson Mines Limited is highly interested in the above study. Your proposed study will address many issues that are of interest to us. The research project will have the following benefits:

- . Provide an assessment of the total arsenic contamination and accumulation in sediments.
- . Provide information on the physical characteristics of sediment which are related to a propensity to sorb arsenic.
- . Provide more information on biomethylation mechanisms of arsenic.

The ability to understand the physical, chemical and biological characteristics of the sediments receiving gold mill effluent is an important consideration for the development of sound control measures.

To this end, Dickenson Mines Limited supports the project's terms of reference and will participate to accomplish the objectives. Our assistance with engineering, site investigations, accommodation, local transport and communication services while on site(Phone and Fax) will be offered to the best of our ability.

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January 13, 1992

M. Kalin

We look forward to participating in this project in the near future. If you need any further assistance, please don't hesitate to call Dr. David Mchaina, our Environmental Engineer.

Your truly,

DICKENSON MINES LIMITED



B. E. BRIED, P. Eng.
Mine Manager

BEB/jb

CURRICULUM VITAE

Margarete Maria Kalin
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Toronto, Ontario
M4X 1E6
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EDUCATION

Ecole Benedict, Bienne, Switzerland, Commercial Diploma - 1964
Agricultural Biochemical Technologist, Geigy Ltd., Basel,
Switzerland - 1965-1969
University of Waterloo - 1970-1971
University of Toronto, B.Sc. (Biology) - 1972-1974
University of Toronto, M.Sc. (Entomology) - 1975-1977

PROFESSIONAL EXPERIENCE

1982-	Research Director and President of Boojum Research Limited. Management of the company and Principal Investigator of all contracts related to Ecological Engineering and Biological Polishing.
1977-1984	University of Toronto, Institute for Environmental Studies Research Associate and Principal Investigator of all university contracts. Research on uranium mill tailings, chemical and ecological characteristics, and long-term rehabilitation of the waste sites.
1974-1977	University of Toronto, Department of Zoology Graduate Student, Research and Teaching Assistant, and Laboratory Technician (part-time). Research in social entomology, glandular development in Heteroptera by scanning electron microscopy, and reproductive biology in mice.
1973-1974	University of Waterloo, Departments of Biology and Earth Sciences Student and Research Assistant. Research on fossil insects in arctic soils, identification of insect fractions.
1972-1973	McGill University, Department of Biology Research Assistant. Department of Biochemistry Laboratory Technician (part-time). Genetics of Aspergillus sp. and research on enzyme kinetics.

1969-1972 University of Zurich, Switzerland, Department of Zoology
Laboratory Technician.
Research on neuro-physiology of insect vision, desert ants
and bees.
Swiss Federal Institute of Technology, Zurich, Switzerland,
Department of Zoology
Laboratory Technician.
Research on repair mechanisms in X chromosomes of
Drosophila melanogaster.

PUBLICATIONS

Refereed Journals

1971 - 1991 Senior author - 6.
 Junior author - 2.

Conference Proceedings

1981 - 1991 Senior or sole author - 35
 Junior author - 5

Contract Reports at University of Toronto

1978 - 1986 Senior and sole author - 12 (including one Royal
 Commission)
 Joint author - 1

Book Chapters

1988 Senior author
1989 Sole author

MEMBERSHIPS AND ASSOCIATIONS

American Association for the Advancement of Science
American Society for Surface Mining and Reclamation
American Society for Testing and Materials
BIOMINET
BIOQUAL
Canadian Association on Water Pollution Research & Control (CAWPRC)
Canadian Institute of Mining and Metallurgy
Canadian Land Reclamation Association - Presently serves as Vice-
President
Canadian Mineral Processors (CMP)
Canadian Nuclear Society
Reclamation Technology (RECTEC)
Society for Ecological Restoration (SERM)
Soil and Water Conservation Society

PUBLIC RELATIONS ACTIVITIES

Standing Committee on Energy, Mines and Resources, selected as speaker for the House of Commons Session, November 6, 1991, as an "expert" in the field of Ecological Engineering.

IAEA served as Canadian Observer and Chairman of Workshop Session on Radium and Uranium Tailings, Rio de Janeiro, February, 1984.

Radio interviews on CBC-LaRonge, regarding Uranium City work during 1981 and 1982. "Low-level radwaste disposal opinions sought." ECO/LOG, Volume 10, No.6, February 19, 1982. "Uranium waste a growing problem."

IAEA served as Canadian Observer for Workshop in Ottawa, Ontario, May 17-21, 1982.

Resides on Editorial Board for The Journal of Ecotechnology, Elsevier Science Publishers, N.Y., N.Y.

VHS Video Documentary on Ecological Engineering, sponsored by CANMET.

NEWSPAPER ARTICLES

Toronto Sunday Star, Perspective, December 19, 1982.

"ICP Users" Bulletin, July 25, 1983.

"Tailings management: A long-term problem?" CNS Bulletin, January/February 1984.

"Biologist advises working with nature in abandoned uranium mill tailings." Nuclear Fuels, December 5, 1983.

"Mining with Microbes", by Keith Debus in Technology Review, August/September 1990, published by MIT, Massachusetts.

MITEC Newsletter, Vol. 3, No. 4, April 1991. Boojum Research Limited is Feature Organization.

LECTURES AND COURSES

McGill University, 1991. Department of Mining and Metallurgical Engineering.

University of Toronto, 1989. Division of Geo-engineering, Guest Lecturer for the Geological and Mineral Engineering Program.

Guest lecturer, "Professional Development Seminar on Water in Mineral Processing."

CIM, 1991, taught course "Mining and the Environment." Course was offered at Vancouver, B.C. and Red Lake, Ontario and will soon be available in the United States. Course notes are currently being published.

ANDREW FYSON - CURRICULUM VITAE

EDUCATION:

1985 - 1990

Post doctoral research with Prof. Ann Oaks FRSC.

Department of Botany

University of Guelph

Enhancement of corn growth by legume soils - funded by NSERC
(Strategic Grant Program StrN 040)

- 1) **Legume growth factor.** Greenhouse and field experiments (Agriculture Canada, Harrow) have characterized microbial factors from legume soils which enhance corn growth. A long term aim is to produce inocula which substitute for rotation effects. In the greenhouse, legume soil inocula give substantial (up to 8 fold) increases in early corn growth. the role of vesicular arbuscular mycorrhizal (VAM) fungi and rhizosphere bacteria have been investigated. A growth response has been produced in the field.
- 2) **Root-soil interactions.** Structural relationships of the rhizosphere have been examined by cryo SEM.
- 3) **Colonization of corn seedling roots** by plant growth promoting rhizobacteria from seed borne inocula (fluorescent pseudomonads-porr colonizers; azospirilla-good colonizers).
- 4) **VAM fungi.** Isolation characterization and identification. Use of pigment production by infected roots to quantify and locate infections.

1981-1984 Post doctoral research with prof. J.E. Beringer, Rothampstead Experimental Station, UK.

Rhizobium and VAM inoculants - project funded by AGC (Agricultural Genetics Company).

- 1) ***Rhizobium* inoculants** - developed technology for production of peat based soybean inoculants (Test marketing and field testing when I left).
- 2) **Multi-seeded pellets** - technology for production of peat pellets containing white clover seed, fertilization, rhizobia and VAM fungi for broadcast sowing in poor pastures.
- 3) ***Rhizobium* collection.** Collected, maintained and determined DNA plasmid profiles of isolates. Characterized by patterns of heavy metal tolerance on minimal medium.
- 4) **Hydroponic VAM inoculum** - In collaboration with a lettuce grower, used lettuce roots in nutrient flow culture as substrate for bulk production of VAM inoculum.
- 5) **Land Restoration.** Field experiments tested use of legumes inoculated with

rhizobia and VAM fungi to assist in rapid restoration of gravel pit land to agriculture

1977 - 1981 **PhD with Dr. Janet Sprent, University of Dundee, 'Low temperature effects on nodulation and nitrogen fixation in *Vicia faba* L.'**

Vicia faba L. (faba bean) is a legume crop of increasing importance in eastern Scotland. It is sown in the fall or spring, the fall crop yielding more and maturing earlier in most years. This study assessed the contribution of nitrogen fixation in the fall and early spring to this advantage. Growth chamber and field studies (SCRI, Invergowrie) examined development and functioning of root nodules at low temperatures (10°C).

1974 -1977 BA hons . Botany, Oxford University 2(i) - top 20%

TEACHING EXPERIENCE

- 1) Demonstration of labs and field courses (Dundee)
- 2) Supervision of technicians, summer students and honors project students
- 3) Teaching final years honors/graduate course on plant physiology at McMaster University, Hamilton, Ontario, 1986 and 1987.

OTHER EXPERIENCE

Expedition to Nepal. As a graduate, I participated in an expedition (through Durham University) to set up a management plan for the LangTang national Park in the Himalayas. I collected and identified plants and studied usage of plant resources by the local people.

Librarianship. Worked for six months in children's library and a teacher's library where I assembled materials for primary school projects.

OTHER INTERESTS

Running. Active member of Burlington Runners. Help organize races (5 km to 100 km - Nanisivak, Baffin Island, N.W.T.)

Natural History. Have collected data for local flora. Studied ecology of threatened plant species

PUBLICATIONS

Published Papers

Fyson, A. and Oaks, A. 1990. Growth promotion of maize by legume soils. *Plant and Soil*, **122**, 259-266.

Fyson, A., Kerr, P., Lott, J.N.A. and Oaks, A. 1988. The Structure of the Rhizosphere of maize seedling roots - a cryogenic electron microscopy study. *Can. J. Bot.*, **66**, 2431-2435

Fyson, A. and Oaks, A. 1987. Physical factors involved in the formation of soil sheaths on corn seedling roots. *Can. J. Soil Sci.*, **12**, 591-600.

Sutherland, J.M., Fyson, A. and Raven, J.A. 1984. Growth of grain legumes in the north of Scotland. *Fabis Newsl.*, **12**, 12-13.

Andrews, M., Box, R., Fyson, A. and Raven, J.A. 1984. Source sink characteristics of carbon transport in *Chara hispida*. *Plant, Cell and Environ.*, **7**, 683-687.

Fyson, A. and Sprent, J.I. 1982. The development of primary nodules on *Vicia faba* L. grown at two temperatures. *Ann. Bot.*, **50**, 681-692.

Fyson, A. and Sprent, J.I. 1980. A light and scanning electron microscope study of stem nodules in *Vicia faba* L.J. *Exp. Bot.*, **31**, 1101-1106.

In Preparation/Submitted

Fyson, A. and Oaks, A. Colonization of corn seedling roots by fluorescent-pseudomonads and zospirilla. *Phytopath.* (submitted)

Fyson, A., Stone, J.A. and Oaks, A. Management of a poorly drained clay loam in southwestern Ontario - effects of rotation forage soils on the growth of corn in a greenhouse assay. *Plant and Soil* (submitted)

Oaks, A. and Fyson, A. (Invited review) Nitrogen metabolism in roots. *Bioscience* (in preparation).

Fyson, A., Stone, J.A. and Oaks, A. Effects of 22 rotation of forages on the growth of corn on a poorly drained clay loam southwestern Ontario. *Can. J. Plant Sci.*

CURRICULUM VITAE

DR. JING-YAO LIU

EDUCATION:

1986-1990: University of Western Ontario
London, Ontario
PhD program in Chemistry

Thesis Title: Solvents Effects on the photoinduced
Intramolecular Electron Transfer

Supervisor: Professor J.R. Bolton

1979-1981: Graduate Institute
Chinese Science and Technology University, Academic
Sinica
Beijing, China
MSc. in Chemistry

Thesis Title: Conformation analysis of the complex
between boric acid monosaccharide

Supervisor: Professor Li-jing Jiang

1986-1990: Graduate Student at the University of Western Ontario

WORK PROJECT: Solvent Effects on the Photoinduced Electron
Transfer Process

Experience

1. Synthesize molecules linked by porphyrin, amino acid and quinone compounds, using MS, NMR and Two dimensional proton-proton decoupling spectrum to identify the compound structures.
2. Measure the fluorescence lifetime of compounds by laser and hydrogen flash light with single photon counting technique.

Papers Published

Intramolecular Photochemical Electron Transfer, J. Chem. Soc., Faraday Trans.1, 85(5), 1027-1041, 1989.

Solvent, Temperature and Bridge dependence of

Photoinduced Intramolecular Electron Transfer. Submitted to "Electron Transfer in Inorganic, Organic and Biological Systems in Advances in Chemistry, Sers. Amer. Chem. Soc., 1990.

Bridge and Solvent Dependence of Electron Transfer in Covalently Linked porphyrin-Peptide-Quinone Compounds. Submitted to J. Phys. Chem.

Temperature Dependent Electron Transfer Rate Constant in Different Solvents. Paper will be submitted.

Feature of PAQ Fluorescence Lifetime and Its Conformation in Different Solvents. Paper will be submitted.

Heavy Atom Effect in Electron Transfer Process. Paper will be submitted.

1981-1986: Research Assistant,
 Institute of Photographic Chemistry, Academic Sinica,
 Beijing, China

WORK PROJECT: Photophysical properties study of Chinese medicine-hypocrellin.

Experience

1. Measure the fluorescence and phosphorescence quantum yields of Hypocrellin using Perkin-Elmer Emission Spectrometer; measure the polarization of the fluorescence and measure the pK_a values for Hypocrellin at the ground state and at the excited state.

Papers Published

Photophysics of Hypocrellin - Investigation of the Absorption and Emission Spectrum. Photographic Science and Photochemistry, 1, 36-41, 1986.

Photophysics of Hypocrellin - Intramolecular Proton Transfer and Ground and Excited State Acidity Study. Science Bulletin, 14, 1077-1080, 1985

WORK PROJECT: Study of manufacturing a new reagent in sunblock skin protection cream

Experience

Synthesize a Photosensitive compound.

WORK PROJECT: Study of aggregation properties of long chain molecules in DMSO-water system

Experience

Separate the photoreaction products by HPLC and HPGC techniques. measure aggregation numbers in micellar system, measure exciplex emission spectrum.

1981-1986: Graduate Student,
Chinese Science and Technology University

WORK PROJECT: Conformation analysis of the complex between boric acid and monosaccharides.

Experience

determine the conformation of monosaccharides in alkaline solution by NMR INDOR and proton exchange technique. Determine the conformation and configuration of the complex boric acid and monosaccharides by NMR spectrum and measure the equilibrium constant of the complex by pH titration.

Paper Published

NMR Study of D-Glucose in Alkaline Solution, Acta Chimica Sinica 41(4), 359-362, 1983.

Analysis of Glucose NMR Spectrum - Application of Indor Technique and Exchange Theory, Molecular Science and Chemical Research, 4, 117-120, 1983.

Discussion of the complexes of Monosaccharides and Boric Acid, Organic Chemistry, 6, 427-433, 1984.

1991: BOOJUM RESEARCH LIMITED

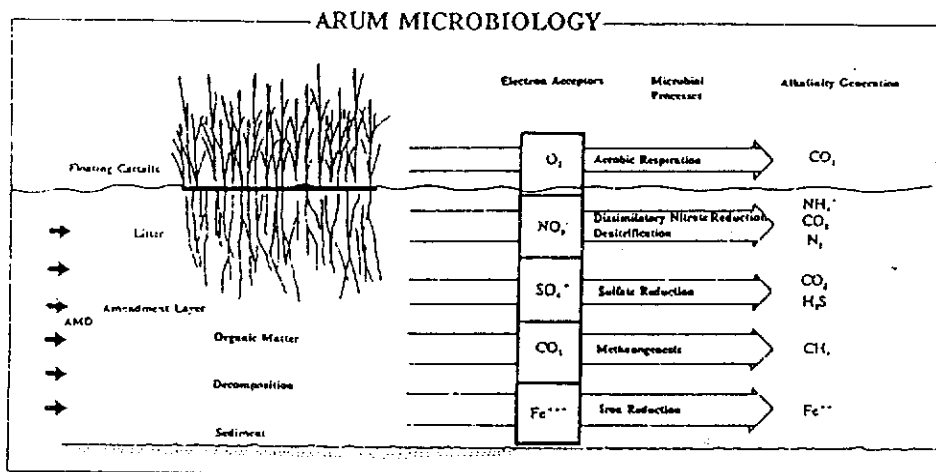
WORK PROJECT: Elucidation of chemical reactions responsible for alkalinity generation in the ARUM microbiological system.

Qualitative analysis of organic and inorganic chemicals in Acid Mine Drainage

Derivation of energy transfer process for the above chemical reactions.

Determination of factors influencing reaction rates

Elucidation of kinetics of reactions involved



*ENHANCING NATURAL TREATMENT SYSTEMS
THROUGH ECOLOGICAL ENGINEERING*

WHAT WE DO

ECOLOGICAL ENGINEERING

The Technology

Ecological Engineering and Biological Polishing methods are low cost, natural solutions to decommissioning waste management areas in the mining industry. The technology uses indigenous ecosystems, where aquatic and emergent plants, and microbial communities are assisted by engineering measures to improve water quality. These methods are ideal solutions to decommissioning because of their long-term stability and low capital and maintenance costs. The self-sustaining characteristics of Ecological Engineering technology rest in natural biomineralization, which are promoted through the application of Boojum's technology.

Applications of the Technology

Every mine has a finite life and at the end of its operation the mill and mine wastes remain. Ecological Engineering concepts integrated at the beginning of mine development and operation will lead to the most effective application of this technology. Boojum technology has been developed on inactive mine sites in Canada. Acidic seepages from tailings dams and reduction of suspended solids and metals in mine and mill effluents are areas where this technology can be applied cost effectively. Demonstration projects are underway for the decommissioning of drainage basins of uranium mines and abandoned coal dumps, pyritic Cu/Zn tailings, and for inactive open pits at a polymetallic mine.

Ecological Engineering Processes

Three processes are under active development:

Ecological Engineering measures are those which use site-specific, hydro-biogeochemical conditions in and around indigenous ecosystems in conjunction with the three processes below. In this manner, a reduction of contaminant release and an improvement of the water quality leaving the waste management area is achieved. This technology is being applied at a number of mines from Saskatchewan to Newfoundland.

1. **The ARUM Process** (Acid Reduction Using Microbiology) is used in the treatment of acidic seepages. Sulphate-reducing, iron-reducing, and other bacteria generate alkalinity using cattail litter and other organic substrates as a carbon source.
2. **Biological Polishing** is a process which uses attached filamentous algae in acid or alkaline conditions, for the removal of metals and precipitates in polishing ponds.
3. **The Chara Process** utilizes underwater meadows of charophytes for removal of metals, radionuclides, and suspended solids in alkaline waste waters.

HOW WE CAN HELP YOU

Boojum Research has the practical expertise to carry out a wide range of activities across a broad cross-section of environmental conditions. The overall strategy employed by the company has three stages: a pre-feasibility study is carried out for a specific site, followed by an experimental test phase to determine the site-specific conditions, upon which the test results are translated for implementation on a larger scale.

These three steps can be used to address the problems in:

- dry or wet, acid or alkaline tailings areas
- seepage control (acid or alkaline); and
- polishing or settling ponds.

The techniques developed by Boojum Research can be used during mine/mill operations as routine reclamation methods and, finally, as abandonment or close-out procedures.

Biological systems are site-specific, and many parameters have to be evaluated on-site. This enables Boojum to tailor R&D activities for rapid transfer of technology.

OUR APPROACH

Boojum Research approaches the commercial exploitation of its technologies in a manner similar to that employed by industrial software developers. Potential clients are presented with a plan whereby:

- i) Boojum Research, for a small fee, will do tests on-site and identify the appropriate site specific technology.
- ii) A second phase demonstration covering about 30-40% of the site will be proposed, along with a techno-economic assessment. The costs for this phase will be borne by the client, with possible minor contributions from governments.

- iii) During the third phase, full-scale implementation will be proposed and executed if the results in phase two are sufficiently encouraging.
- iv) Upon completion of phase three, Boojum will provide periodic examination of the site for an annual fee. Boojum's fee is based on the savings achieved by using Boojum's expertise instead of conventional methods.

OUR ENVIRONMENTAL STRATEGY

The major market for Boojum Research services is the mining industry around the world. Boojum has identified two major points in its strategy. The first is education. The expectation is that a better informed client who has made commitments to the technology over a reasonable period of time, will appreciate the technologies' advantages through:

- i) Emphasis on economics. It is less expensive to set up natural ecosystems to clean waste water than to operate chemical treatment plants in perpetuity.
- ii) Emphasis on 'environmental goodwill'. The 'green' movement is increasingly forcing regulators to tighten regulations and increase penalties for environmental pollution.

This education strategy is implemented through continued participation in BIOMINET, the CIMM, Canadian Mineral Processors, MEND Program and through joint projects with CANMET and Industry. Boojum Research Ltd. has also presented a number of papers at various conferences and will continue to publish scientific/technical papers on Ecological Engineering and Biological Polishing.

The marketing strategy is based on technology demonstration. Showing that these techniques will work is the most effective proof for clients.

WHO WE ARE

COMPANY

Boojum Research Ltd. is a Canadian company incorporated in 1982 and has pioneered the application of biological solutions to environmental problems. The company's mission is to develop innovative, durable and low cost environmental management solutions, based on ecological engineering and biological polishing, which can be effectively applied to help the mining and chemical industries, among others, to comply with the most stringent environmental regulations. The company's headquarters are at 468 Queen St. E, Suite 400 in Toronto, Canada, where offices and laboratories are located, although Boojum typically conducts most experiments and demonstration projects at clients' sites.

Boojum Research Ltd. operates with a core of seven trained professionals in association with the Boojum team of companies. The company provides the required experience in geology, microbiology, geochemistry, hydrology, engineering, mining and metallurgy.

BOOJUM STAFF

M. Kalin M.Sc. - President and Research Director
Margarete founded the company and pioneered the application of Ecological Engineering and Biological Polishing to the mining industry. Since 1978, she has worked in the ecology of uranium tailings sites in Canada and has written and co-authored numerous publications detailing her research.

M.P. Smith, M.Sc. - Vice-President of Operations
Martin has worked with Boojum as a Research Associate since the inception of the company in 1982. He has recently become our Vice President of Operations. Martin specializes in the Chara Process, a biological alkaline waste water treatment system, and is now addressing organic matter production for the ARUM process.

W.N. Wheeler, Ph.D. - Plant Physiologist
Bill has extensive experience in aquatic plant physiology, with specializations in aquaculture and ecophysiology, as well as large-scale manipulation of biological systems. His expertise is in plant/nutrient interactions and biohydrodynamics. He is assisting in the development of the biological polishing process.

A. Fyson, Ph.D. - Microbial Ecologist
Andrew is a microbiologist and plant physiologist from Oxford University. His expertise and specialization in plants and microbes assist in the development of the ARUM process and other applications of microbiology.

Shen Lu Ph.D. - Soil Scientist

Shen joined Boojum in the summer of 1991 after completing his Ph.D. in soil science, with a specialization in plant nutrition and plant/soil relationship. His focus in Boojum's ecological engineering projects will be plant nutrition and growth in the biological polishing process.

Jing-Yao Liu Ph.D. - Chemist

Jing-yao Liu is a Ph.D. chemist from the University of Western Ontario. Her expertise is in photophysics and electron transfer process theory. Her work at Boojum entails the chemical analysis of AMD water treatment and further research into the ARUM Process.

W. Poliszot, M.Sc. - Programmer/System Analyst

Wieslaw has extensive experience in computer software development and its applications to geophysical and hydrogeological exploration. Wieslaw is responsible for Boojum's data management and computer modelling.

A. Unwin B.E.S. - Lab/Field Operations Manager

Alan has recently graduated from University of Waterloo with a degree in Environmental Studies. He is responsible for Laboratory maintenance and safety.

C. Lendrum, B.Comm. - Administrator

Clyde joined the company early in 1991. He has spent several years working as an administrator in the mining industry in Canada and now acts as our financial planner and personnel administrator.

P.A. Douris, B.A. - Executive Assistant

Paul is responsible for all administrative issues and the implementation of new administrative procedures typically associated with a developing organization.

THE BOOJUM TEAM OF COMPANIES & ASSOCIATES

J. Cairns, M.Sc. — *Microbiologist*

Jim is with Dearborn Environmental, and has collaborated with Boojum since 1987 on the study of microbial ecology required to develop the ARUM Process. In the laboratory, he studies those microbial processes which generate alkali and reduce sulphate in the complementary field tests.

T. Edwards, B.Eng. — *Metallurgical and Mining Advisor*

Tim is with Edwards Consulting, and since 1984 has provided Boojum with the necessary expertise in mining and milling operations, making Ecological Engineering a realistic option for waste management in the mining industry.

R.O. van Everdingen, Ph.D. — *Hydrologist/Geochemist*

Robert is with Van Everdingen Research Specialties Ltd., and he has worked with Boojum Research for several years addressing the geochemical problems related to acid generation in ecologically engineered projects. His expertise in sulphur chemistry, his background in isotope work and the chemical processes of precipitation, combined with his expertise in hydrology, make him essential to all process development.

Richard Vladars, B.E.S. - *Cartographer/ Graphics*
Richard is an Environmental Studies graduate with extensive experience as a cartographer. He is responsible for the production of maps and illustrations and is currently working on a map inventory system for Boojum.

Ewa Poliszot M.Sc. - *Mathematician/Statistician*

Ewa's background is mathematics. She worked in applied mathematics at the University of Warsaw using methods of statistical analysis. She is currently assisting Boojum in the organization of a database for its research.

Mieczyslaw Wanat M.Sc. — *Field Hydrologist/Geologist*

Mike joined Boojum during the summer of 1991. He brings to Boojum four years experience as a geologist in a copper mine in Poland. His present role within our company is that of Field Hydrologist/Geochemist and works under the supervision of R. van Everdingen.

1984/5

Rio Algom Ltd./ Denison Mines Ltd.:

Investigation of the Chara Process for effluent polishing. Phase 1: field test program.

INCO Ltd.: A feasibility study on the use of the Chara process, and an assessment of reclamation by Ecological Engineering for the Levack tailings.

Alcan International: The potential use of passive polishing for cyanide removal with the Chara Process and indigenous biota.

Alcan International: Diatoms in waste sites and an assessment of the ease of culturing.

Energy Mines and Resources, CANMET: The Chara Process - biological economization of mill effluent treatment: potential applications and preliminary results.

1985/6

Ministry of Natural Resources, Ontario: Kam-Kotia tailings - reclamation by Ecological Engineering. Phase 1: feasibility evaluation.

Department of Indian and Northern Affairs: A synthesis and analysis of information on inactive hardrock mining sites in the Northwest Territories and the Yukon.

NRC/Rio Algom Ltd./Denison Mines Ltd.: The use of the Chara Process in effluent polishing - Phase 1: Growth characteristics.

INCO/CANMET: Reactive Acid Tailings Program: Ecological Engineering - test of concepts and assumptions on Levack, year 1: ecology.

Falconbridge Ltd./CANMET: Reactive Acid Tailings Program: Ecological Engineering: An experimental approach to building a bog or wetland, year 1: seed sources and transplant methods.

CANMET: Acidophyllic aquatic mosses as biological polishing agents.

1986/7

INCO Ltd., Levack Mines: Ecological Engineering research, Phase II: Implementation of experiments for close-out conditions.

BP Resources, South Bay Mines - Ecological Engineering research, Phase 1: Implementation of close-out experiments.

BP Resources, South Bay Mines - Ecological Engineering research, Phase II.

CANMET: Growth dynamics of naturally colonizing Characeae in abandoned tailings ponds.

Department of Indian and Northern Affairs: Ecological Engineering for gold and base-metal operations in the Northwest Territories.

Denison Mines Ltd.: Development of an Ecological Engineering close-out concept.

1987/8

INCO Ltd., Levack Mines: Ecological

Engineering research, phase III.

NRC/CANMET: Biological polishing of alkaline effluents.

Kidd Creek Mines Ltd.: Ecological Engineering and biological polishing systems for the Kidd Creek Met site.

BP Resources, South Bay Mines: Ecological Engineering research, phase III: close-out implementation.

Cape Breton Development Corp.: Implementation of biological polishing experiments for run-off from coal waste.

INCO, Sudbury Operations: Microbiological reduction process of acid tailings/seepages, phase I: organic matter supply.

ASARCO Inc.: Phase I: Feasibility of Ecological Engineering for the Buchans waste management area.

Eldorado Resources Ltd./CAMECO: Feasibility of using the Chara Process in the Rabbit Lake watershed.

1989/90

ASARCO Inc.: Buchans Nfld: Testing and implementation of Ecological Engineering and biological polishing during close-out of the Buchans waste management area.

Department of Transport, Halifax, Canada: Site assessment and development of a design for constructed wetlands for the mitigation of acid mine drainage from Halifax International Airport.

ASARCO, Montana: Assessment of abandoned mine workings in the Rocky Mountains.

Curragh Resources: Ecological Engineering - feasibility for the Faro Mine site.

Les Mines Selbaie: Application of Ecological

Engineering methods during operations.

C.I.L.: Ecological Engineering applied to phosphate tailings decommissioning.

CAMECO: Implementation and monitoring of the Chara Process in the Rabbit Lake watershed.

Cape Breton Development Corp./CANMET: Morphological aspects of cattail root development in acidic conditions.

MEND (Denison/INCO/Environment Canada/CANMET): The development of the ARUM process at a seepage on the Copper Cliff Tailings.

Cape Breton Development Corp.: Implementation of Ecological Engineering at the Selminco Coal Dump.

1990/91

ASARCO Inc./CANMET Buchans NFLD., Algal Bio-Polishing of Zinc

Cape Breton Development Corporation/CANMET: A Study of Algal establishment and growth in Acid Mine Drainage Seeps and ponds

CAMECO Corporation: Decommissioning of Rabbit Lake Drainage Basin Using Ecological Engineering

Ecological Engineering in the Rabbit Lake B-Zone Pit.

DENSION MINES LIMITED
Treatment fo Acidic Seepages Employing Wetland Ecology and Microbiology

Selbaie Mines:
Applications of Ecological Engineering methods during operations, Phase II.

LIST OF PAPERS

- 1 -

- 1991 Fyson, A., M.W. English and M. Kalin, "Effect of Foliar Fertilization on the Accumulation of Iron by *Typha latifolia* L. Proceedings of the Eighteenth Annual Conference on Wetlands Restoration and Creation, 1991.

- 2 -

- 1991 Kalin, M., J. Cairns and W.N. Wheeler, "Biological Alkalinity Generation in Acid Mine Drainage." Proceedings of the Second International Symposium on the Biological Processing of Coal, 1991.

- 3 -

- 1991 Cairns, J.E., R. McCready and M. Kalin, "Integrated field and laboratory experiments in ecological engineering methods for acid mine drainage treatment." Proceedings of the Second International Conference on the Abatement of Acidic Drainage, 1991, pp.409-425

- 4 -

- 1991 Kalin, M., "Biological alkalinity generation in acid mine drainage." Proceedings of the 23rd Annual Meeting of the Canadian Mineral Processors, Paper No. 9, 1991.

- 5 -

- 1991 Kalin, M., "Ecological engineering applied to base metal mining wastes for decommissioning." Proceedings 1990 BIOMINET Annual Meeting, 1991.

- 6 -

- 1991 Kalin, M., J. Cairns and R. McCready, "Ecological engineering methods for acid mine drainage treatment of coal wastes." Resources, Conservation and Recycling, 5, 1991.

- 7 -

- 1991 Kalin, M., R.W. Scribailo and W.N. Wheeler, "Acid mine drainage amelioration in natural bog systems." Proceedings 1990 BIOMINET Annual Meeting, 1991.

- 8 -

- 1991 Kalin, M. and M.P. Smith, "Biological amelioration of acidic seepage streams. "Proceedings of the Second International Conference on the Abatement of Acidic Drain 1991, pp. 355-368.

- 9 -

- 1991 Wheeler, W.N., M. Kalin and J. Cairns, "The ecological response of a bog to coal acid mine drainage - deterioration and subsequent initiation of recovery." Proceedings of the Second International Conference on the Abatement of Acidic Drainage, 1991, pp. 449-464.

- 10 -

- 1990 Kalin, M., "Ecological engineering applied to base metal mining wastes." Proceedings of the Canadian Land Reclamation Association (CLRA), 15th Annual Meeting, Acidity and Alkalinity in Terrestrial and Aquatic Reclamation, 1990, pp. 105-112.

- 11 -

- 1990 Kalin, M., "Ecological engineering applied to base metal mining wastes." In, Acid Mine Drainage - Designing for Closure (J.W. Gadsby, J.A. Malick, and S.L. Daly, editors), p.407-414. Papers presented at the 1990 GAC/MAC Joint Annual Meeting, Vancouver.

- 12 -

- 1990 Kalin, M., R.O. van Everdingen and R. McCready, "Ecological Engineering: Interpretation of hydrogeochemical observations in a sulfide tailings deposit", Proceedings of the 92nd Annual General Meeting of the CIM, 1990.

- 13 -

- 1990 Kalin, M. and R. Scribalo, "Ecological responses of bog vegetation receiving coal acid mine drainage." Proceedings of the Use of Constructed Wetlands in Water Pollution Control, 24-28 September 1990, Cambridge, UK.

- 14 -

- 1989 Kalin, M., J. Cairns and R. McCready, "Ecological engineering methods for acid mine drainage treatment of coal wastes." Proceedings of the Bioprocessing of Fossil Fuels Workshop, Tysons Corner, Virginia, August 8-10, 1989, pp.208-222.

- 15 -

- 1989 Kalin, M., "Ecological engineering applied to base metal and uranium mining wastes." Proceedings of the 1989 Biohydrometallurgy Conference, Jackson Hole, Wyoming, August 13-18, 1989, pp. 363-368.

- 16 -

- 1989 Smith, M.P. and M. Kalin, "Biological Polishing of Mining Waste Waters: Bioaccumulation by the Characeae." Proceedings of the 1989 Biohydrometallurgy Conference, Jackson Hole, Wyoming, August 13-18, 1989.

- 17 -

- 1989 Kalin, M., R.O. van Everdingen and G. Mallory, "Ecological engineering measures developed for acid-generating waste - The close-out of a decant pond." Proceedings of the International Symposium on Tailings and Effluent Management, Halifax, Nova Scotia, August 20-24, 1989, pp. 195-204.

- 18 -

- 1989 Kalin, M., M. Olaveson and B. McIntyre, "Phytoplankton and Periphyton Communities in a Shield Lake Receiving Acid Mine Drainage in NW Ontario." Proceedings of the Fifteenth Annual Aquatic Toxicity Workshop: November 28-30, 1988, Ed. R. Van Coillie et al., 1989. pp. 166-187.

- 19 -

- 1989 Kalin, M., M.P. Smith and R.O. van Everdingen, "Ecological engineering measures developed for acid generating waste from a copper/zinc concentrator in Northern Ontario." Proceedings of the Conference "Reclamation, A Global Perspective," Calgary, Alberta, August 27-31, 1989, pp. 661-672.

- 20 -

- 1988 Kalin, M., "Ecological engineering and biological polishing methods to economize waste management." Proceedings of the 20th Annual Meeting of the Canadian Mineral Processors, Ottawa, Ontario, January 19-21, 1988, pp. 302-318.

- 21 -

- 1988 Kalin, M., "Ecological engineering and biological polishing: Its application in close-out of acid generating waste management areas." Proceedings of the 1988 Mine Drainage and Surface Mine Reclamation Conference, Sponsored by the ASSMR, Pittsburgh, Pennsylvania, April 19-21, 1988, p. 399.

- 22 -

- 1988 Kalin, M. and M.P. Smith, "Biological polishing: The Chara Process." Proceedings of the 13th CLRA Convention, Ottawa, Ontario, August 7-10, 1988, pp. 87-95.

- 23 -

- 1988 Kalin, M. and R. Scribailo, "Biological treatment of acid mine drainage." 1988 BIOMINET Annual Meeting, Calgary, Alberta, November 2, 1988, pp. 57-68.

- 24 -

- 1988 Vandergaast, G., B. Phillips, M. Kalin and M. Smith, "Application of ecological engineering at a uranium mining facility in northern Saskatchewan." Proceedings of the International Symposium on Uranium and Electricity: The Complete Nuclear Fuel Cycle, Sponsored by The Canadian Nuclear Society, Saskatoon, Canada, 1988.

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January 14, 1992.

Bak Chauhan
Ministry of the Environment
Research and Technology Branch
135 St. Clair Ave. W., 12th floor
Toronto, Ont.,
M4V 1P5

Dear Mr. Chauhan;

Please find enclosed our proposal entitled, "*Biological Cycling of As in Sediments Impacted by Gold Mill Effluents*". The original and five copies of the application form are also enclosed.

We trust that we have completed all the requirements as you outlined in your Environmental Research Grants, General Application Guidelines, November 1991. After reviewing your document on Strategic Areas, we will inform you as to which strategic area this proposal corresponds.

We look forward to hearing from you.

Sincerely,

A handwritten signature in dark ink, appearing to read "M. Kalin". The signature is fluid and cursive, with a large, stylized "M" and a trailing flourish.

Margarete Kalin
President, Research Director