IMPACT OF AIR POLLUTANT MIXTURES ON FOREST VEGETATION AND SOILS

by

P.A. ADDISON

A.A. KHAN

S. L'HIRONDELLE

F. THERIAULT

Northern Forest Research Centre

Canadian Forestry Service

for

ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM

Project RMD - 80 - 24

April 1982

TABLE OF CONTENTS

TABLE OF CONTENTS (CONCLUDED)

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

This report describes both the accomplishments to date and the long-term plans of the joint project between the Toxic Substances Program of the Canadian Forestry Service and the Research Management Division of Alberta Environment.

No evidence of impact on jack pine physiology was found when concentrations of the dominant pollutants from Oil Sands operations equivalent to 104 years of soluble disposition were added to intact soil cores. Evidence indicates that the surface litter layer or LFH horizon plays a dominant role in protecting both the mineral soil and established plants from pollutant effects either through an improved nutrient balance or by complexing the pollutants. Several major cations increase in solubility with the addition of SO_4 and nutrient depletion may occur in field situations. Metal pollutants did not enhance the solubility of cations as has been reported elsewhere.

The lichen *Evernia mesomorpha* responded to SO_2 at lower levels than reported elsewhere and demonstrated that it was indeed sensitive to this pollutant. Further work is required to clarify the response of this lichen's physiology and sulphur uptake during fumigation with SO_2

ACKNOWLEDGEMENTS

This research project RMD-80-24, was funded by the Alberta Oil Sands Environmental Research Program which was established to fund, direct, and co-ordinate environmental research in the Athabasca Oil Sands area of northeastern Alberta. The authors thank J.I. Ridgway and F. Radford for technical assistance.

1. PROJECT PLAN

Industrial effluents discharged into the atmosphere in a number of locations have a real, imagined, or potentially deleterious effect on adjacent trees and other plant life. Government agencies and the general public have expressed concern. In addition, industrial groups are apprehensive as to restrictions which may be applied to their installations. Regulatory agencies in many instances lack essential scientific information describing cause and effect relationships between a variety of pollutants and environmental impact. Provincial government agencies, industry and the public have requested involvement by the Canadian Forestry Service in the environmental problem in the form of cooperative research programs, detection and assessment surveys, and advisory services.

In order to put the cooperative program between the Canadian Forestry Service, Environment Canada and the Research Management Division, Alberta Environment into perspective, and to show how the long-term objectives of the program will be met, the following report includes both a description of the accomplishments during 1981-82 and a definition of the objectives for the next four years. To date, descriptive studies on the pattern and impact of pollutant deposition have been carried out in the Athabasca Oil Sands area (Addison and Baker 1979, Addison 1980a, b) and initiated in west-central Alberta near two sour gas plants (Kennedy and Addison 1981). Both laboratory (Malhotra and Khan 1979, 1980; Malhotra and Addison 1979, Malhotra *et al.* 1980) and field studies on soils, vascular plants and lichens have resulted in biomonitoring techniques (Addison and Puckett 1980) some of which show promise for future application while others need further definition (Addison 1982). Studies on the natural environment and biotic factors that influence pollutant uptake by, or impact on boreal forest plant species have been initiated (Addison *et al.* 1981, Khan *et al.* 1981). Preliminary results indicated that further study particularly on mixtures of pollutants and elemental sulphur are required before the capability to predict pollutant impact to forest components can be reached.

The objectives of this project are to

1. Determine the impact of pollutant mixtures on native soils and their ability to support vegetation.

2. Determine the effect of mixtures of gaseous pollutants on the previsible and visible symptoms of boreal forest plant species.

These two objectives effectively divide the project into the direct or gaseous (Objective 2) and indirect or soil ameliorated (Objective 1) effects of pollutants characteristic of oil sands operations on boreal forest plant species. The goals arising from the objectives culminate in three major products from the research which are:

1. To provide provincial government and industry with an estimate of the long-term implications of continued deposition of pollutant mixtures.
2. To provide federal and provincial regulatory agencies with scientific data for setting better air quality standards for single and mixed pollutants.
3. To assess the capabilities of lichens as biomonitors of ecological effects caused by air pollutants.

The specific goals (Table 1) that must be accomplished to provide these products are outlined in Figure 1. The expected time-frame is also included.

The following two reports describe activities during 1981-82 related to the above-stated objectives.

Table 1. Project goals of the Toxic Substances Program to 1986.

Goal	Description
1	Determine the rate of pollutant deposition by examining its spatial variation and the factors that influence deposition to the forest floor.
2	Determine the effect of pollutants in the soil solution on vascular plant functioning.
3	Determine the influence of the soil litter on the availability and mobility of toxic elements.
4	Determine the influence of pollutants on soil biological processes relating to nutrient cycling.
5	Determine field soil pollutant content; variability, horizontal and vertical distribution.
6	Develop a predictive capability as to the fate of forest soils and vegetation at current and anticipated rates of pollutant deposition from major industrial areas.
7	Determine the influence of SO_2 concentration and the duration of fumigation on jack pine physiology and growth.
8	Develop methods to determine previsual and transitory effects of pollutants on plant functioning.
9	Determine the influence of SO_2 concentration and time of exposure on the activity of the epiphytic lichen *Evernia mesomorpha*.
10	Partition pollutant effects in jack pine into biophysical and biochemical components.
11	Determine the influence of NO_2 on the response of jack pine to SO_2.
12	Compare vascular plant and lichen responses to SO_2.

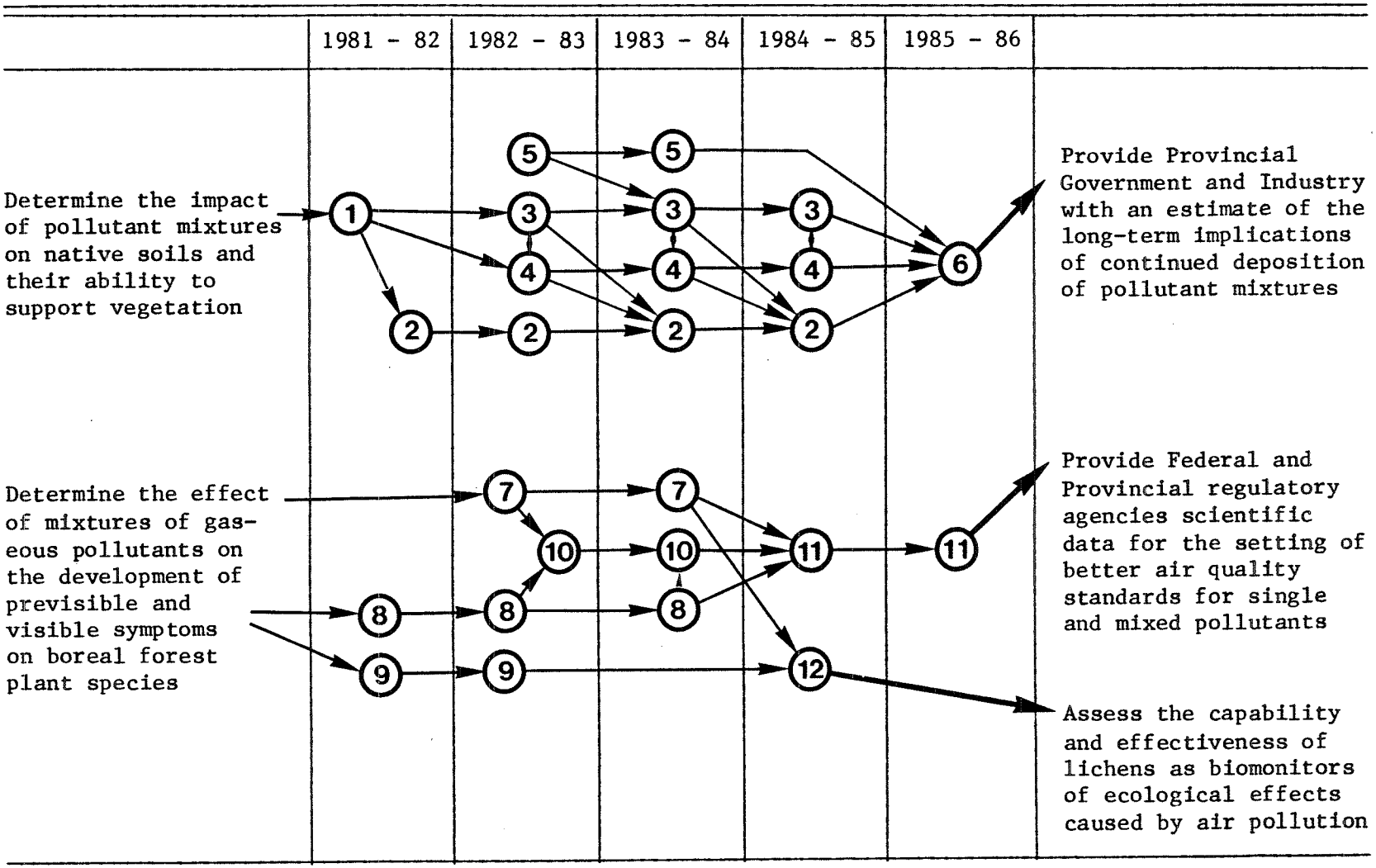

Figure 1. Outline of the interaction between project goals in the Toxic Substances Program 1981 - 1986. Details of the various goals are in Table 1.

2. IMPACT OF POLLUTANT DEPOSITION TO SOIL ON JACK PINE PHYSIOLOGY

2.1 INTRODUCTION

In the Athabasca Oil Sands area in northeastern Alberta, very little obvious damage to the forest ecosystem has been related to air pollution from Suncor Inc. and Syncrude Canada Ltd. (Addison 1980a). Suncor has been operating at its present location (ca. 30 km north of Fort McMurray) since 1967 and for much of that time has emitted about 150 $t.d^{-1}$ of S (Shelfentook 1978). In addition, particulates containing Al, Fe, V, and Ni have been emitted to the atmosphere at the rate of approximately 40 $t.d^{-1}$ (Shelfentook 1978). Syncrude Canada Ltd., when it is in full production is expected to emit about 130 $t.d^{-1}$ of S and 110 $t.d^{-1}$ of NO_x. Because of the magnitude and the combination of types and form of the emissions, the potential for serious air pollution impact on the forest ecosystem is very great.

In order to address this problem, a study was designed to determine the impact of pollutant mixtures on native soils and their ability to support dominant tree species of the area to develop a predictive capability with respect to long-term effects of industrial emissions on the forest system in this area.

2.2 MATERIALS AND METHODS

2.2.1 Seeds

Jack pine (*Pinus banksiana* Lamb.) seeds were collected from mature trees in the Anzac area (30 km south of Fort McMurray). The seeds had a 97% viability.

2.2.2 Soil Cores

Intact soil cores (15 cm diameter x 20 cm depth) were excised from a relatively uncontaminated site in the Athabasca Oil Sands area. Details of the site and its soil characteristics have been described (Addison *et al.* 1981).

2.2.3 Experiments with Soil Cores

In experiments 1 and 2 intact soil cores were used. Details of core preparation, pollutant application, seeding and general maintenance of the seedlings have been described (Addison *et al.* 1981).

2.2.4 Experiments with Reconstituted Soil Systems

For these experiments, soil was removed from the columns and the LFH and mineral horizons were separated. Each horizon sample was then well mixed. The sand was passed through an 18 mesh sieve to remove stones and dead roots. Two sets of 40 plastic pots (12.5 cm diameter) were than packed with sand. The sand in one set was topped with a layer (1-1.5 cm) of LFH, while in the other it was left bare. The pots of both the sets were soaked with water for several days. Each pot was then seeded with 15 jack pine seeds. The pots were covered with a clear plastic sheet to prevent drying of the soil surface. Upon germination the plastic sheet was removed and the seedlings were allowed to grow under greenhouse conditions (Addison *et al.* 1981). After two weeks the number of seedlings established was counted and the pots were thinned to 4 seedlings per pot. After 5 weeks of seedling growth, applications of the water soluble forms of pollutants (V, Ni, SO_4, NO_3; see Table 2, Addison *et al.* 1981) and their mixtures were started in each set. The pollutants were applied to the top of the soil once each week (Table 2). The untreated (control) pots received an equal volume of deionized water. Each treatment consisted of 3 pots. All the pots were watered on alternate days with a measured volume of water. Seedlings were harvested at intervals and analyzed for various biochemical and physiological processes.

2.2.5 Biochemical and Physiological Analysis

Peroxidase and ribulose diphosphate carboxylase enzymes were extracted from needles and assayed as described earlier (Malhotra and Khan 1979). Protein content of the enzyme extracts were determined according to Lowry *et al.* (1951) after precipitation with 10% trichloroacetic acid.

Table 2. Deposition of pollutants to homogenized soils with and without an LFH horizon.

Working solutions concentration	Form	pH
Ni - 70 mg/L	$Ni(NO_3)_2 \cdot 6H_2O$	5.3
V - 454 mg/L	V_2O_5	5.5
SO_2 - 3992 mg/L	H_2SO_3	3.5
NO_3 - 1490 mg/L	HNO_3	3.5

Treatment	Deposition per week (mg)
V	11.6
Ni	1.75
SO_2	98.05
NO_3	37.25
V + SO_2	11.6, 98.05
V + NO_3	11,6, 37.25
V + Ni	11.6, 1.75
Ni + SO_2	1.75, 98.05
Ni + NO_3	1.75, 37.25
SO_2 + NO_3	98.05, 37.25

Shoot growth was measured either as height (mm) or as total dry weight per seedling. For dry weight the needles were dried in an air circulating oven at 80°C for 24 h.

Initiation and development of visible symptoms on the needles were recorded weekly.

2.2.6 Soil Analysis

After the final harvesting of seedlings from the soil core (Exp. 1), the free water from each soil core was removed by suction. The water was then analyzed for pH and various elements. Element analysis was done by Inductively Coupled Argon Plasma Atomic Emission Spectrometry after acidification with HNO_3.

After removing the free water, each soil core was removed from its column and each horizon was sampled. Samples are currently being prepared for chemical analysis.

2.3 RESULTS AND DISCUSSION

2.3.1 Biochemical and Physiological Analysis of Jack Pine Seedlings Grown in Soil Cores Treated with Various Pollutants and their Mixtures

Jack pine seedlings grown for 29 weeks in pollutant treated and control soil cores (Exp. 1) were analyzed for peroxidase activity and shoot growth. The results (Table 3) show that the amount of the pollutants applied did not produce any significant effect on either of the measurements. Furthermore, visible symptoms of pollutant toxicity were not observed in any treatment. A similar lack of pollutant response was reported in seedling establishment, peroxidase activity and shoot growth during the intitial period (9 and 17 weeks) of plant growth (Addison *et al.* 1981). Since no significant changes in plant responses were observed in this experiment, the concentration of applied pollutants was doubled in the second experiment and the effects on plant responses at two stages of seedling growth were examined. Analysis after 23 weeks of growth showed that the peroxidase activity of seedlings treated with pollutants was not significantly different than the control (Table 4).

Table 3. Biochemical activity and growth of jack pine seedlings grown in soil cores after 29 weeks of exposure to various concentrations of pollutants and pollutant mixtures characteristic of Oil Sands operations. Values are means of 5 replicates with their standard deviations.

Treatment[a]	Peroxidase units / mg protein	Growth height (mm)
Vanadium		
Low	1.2 ± 0.2	98.2 ± 10.2
Medium	1.5 ± 0.6	86.6 ± 13.1
High	1.3 ± 0.2	90.5 ± 10.6
Nickel		
Low	1.6 ± 0.3	91.7 ± 13.4
Medium	1.6 ± 0.3	92.9 ± 11.7
High	1.3 ± 0.4	93.2 ± 27.0
Aluminum		
Low	1.0 ± 0.3	87.2 ± 17.3
Medium	1.6 ± 0.4	83.0 ± 10.0
High	1.4 ± 0.4	98.8 ± 22.4
Sulphate		
Low	1.3 ± 0.5	92.6 ± 11.4
Medium	1.5 ± 0.6	96.6 ± 19.8
High	1.2 ± 0.1	86.8 ± 12.1
Mixtures		
V + Ni Medium	1.0 ± 0.4	89.7 ± 15.2
V + SO Medium	1.4 ± 0.3	96.8 ± 11.0
Ni + SO Medium	1.5 ± 0.4	85.3 ± 16.1
V + Ni + SO Medium	1.2 ± 0.4	79.9 ± 10.0
Control	1.4 ± 0.5	89.1 ± 5.5
Treated Control	1.3 ± 0.6	104.2 ± 12.8

a - analysis of 29 week seedlings. Treatments represent a single application of soluble forms of the pollutants to the LFH horizon at rates of Low - 13 years, Medium - 26 years and High - 52 years.

b - a unit of peroxidase enzyme = ΔO.D. of 0.001 min^{-1} at 485 nm.

Table 4. Biochemical activity and growth of jack pine seedlings grown in soil cores after 23 and 40 weeks of exposure to 104 years (equivalent) of deposition of various pollutants and their mixtures. Values are means of 5 replicates with their standard deviations.

Treatment[a]	Peroxidase Units / mg protein		Growth g dry weight / seedling	
	1	2	1	2
Control	2.8 ± 0.7	6.7 ± 2.1	0.35 ± 0.14	3.47 ± 1.53
V	2.6 ± 0.3	7.6 ± 3.9	0.15 ± 0.07	2.99 ± 1.64
Ni	2.9 ± 1.6	7.6 ± 2.2	0.19 ± 0.04	3.07 ± 0.96
SO_4	2.2 ± 0.4	8.5 ± 3.5	0.33 ± 0.11	3.23 ± 2.87
V + Ni	3.0 ± 1.1	8.2 ± 2.9	0.20 ± 0.10	2.48 ± 1.01
V + SO_4	-	6.9 ± 3.6	0.25 ± 0.01	3.28 ± 3.26
V + NO_3	-	6.1 ± 4.1	-	2.64 ± 1.26
Ni + SO_4	3.1 ± 1.0	8.2 ± 1.3	0.15 ± 0.05	1.91 ± 0.70
Ni + NO_3	-	6.3 ± 1.2	-	3.15 ± 1.89
SO_4 + NO_3	-	7.7 ± 2.9	-	4.07 ± 1.63
V + Ni + SO_4	2.9 ± 0.7	7.3 ± 2.1	0.26 ± 0.05	3.34 ± 1.07
V + SO_4 + NO_3	-	6.7 ± 3.9	-	3.39 ± 1.05
Ni + SO_4 + NO_3	-	7.4 ± 2.5	-	2.82 ± 1.45
V + Ni + SO_4 + NO_3	-	6.3 ± 3.2	-	3.12 ± 0.41

1 - after 23 weeks of growth
2 - after 40 weeks of growth
a - Treatments represent a single application of the equivalent of 104 years of deposition in soluble form to the LFH horizon.

The shoot growth in some treatments (V, Ni, V + Ni) appeared to be less than the control. This may be due either to an initial absorption of pollutants by roots near the LFH layer or a natural variation in the nutrient content and depth of LFH in the cores. The differences, however, were not statistically significant ($p > 0.05$). Further analysis of these seedlings at 40 weeks of growth also showed no significant effect of any pollutant or any interaction of pollutants in mixtures (Table 3). It appears from these experiments that at the concentrations of pollutants used, the pollutants were effectively complexed with soil organic matter in the form of insoluble and stable complexes (Schnitzer and Khan 1972). It has been shown that the organic components of the soil (humic acid and fulvic acid) interact with metal ions by various mechanisms (ion exchange, surface adsorption, chelation and coagulation). For complexing the metals a number of functional groups present in the organic matter may be involved (Schnitzer and Khan 1972; Zunino and Martin 1977) and so the stability and the nature of the metal complexes will depend on complexing groups involved. A number of other soil related factors (pH, ionic strength and the amount of the clay) can also influence the nature and stability of the complexes (Slavek and Pickering 1981). The availability and the toxicity of the applied pollutants to plants grown in soil, therefore, can be limited by number of such soil factors. Since peroxidase activity in jack pine seedlings was shown to be markedly increased upon hydroponic treatment with low concentrations of V and Ni (Malhotra and Khan 1979, 1980), a lack of such response in the soil core seedlings would suggest that these plants were protected from the initial toxic effects of the pollutants due to binding in the LFH layer. Miles and Parker (1979) showed that Cd applied to the surface of the soil was bound in the top (2.5 cm) layer of the soil. Recently, Chang and Broadbent (1982) found that soluble metals applied to Yolo silt loam soils were quickly converted to more insoluble forms and this was independent of the level of metal added. We suggest that the LFH layer of the soil cores plays a similar role. These results are therefore of considerable significance in explaining a lack of initial injury to forest species in the field, where low levels of these and other pollutants may be deposited on the forest floor.

2.3.2 Effect of Various Pollutants and their Mixtures on Biochemical and Physiological Activities of Jack Pine Seedlings Grown in Soil Systems with and without LFH

The results in the preceding section suggest that the organic matter (LFH) in the soil cores may have influenced plant responses by interacting with the applied pollutants and rendering them unavailable to plant roots in the mineral horizons. Since, in the field, erosion of the LFH layer is not uncommon (primarily owing to industrial activity) it was necessary to examine and compare plant responses to the pollutants in soils with and without LFH. In contrast to the soil core experiments, the application of pollutants in this study was on a continual basis.

Initial analysis (8 weeks) showed that in general the seedlings grown in soils with LFH had significantly ($p < 0.01$) higher enzyme activities and shoot growth than in those grown without LFH (Table 5).

In the soil system with LFH, the peroxidase and RuDP Carboxylase activities were generally higher in the treated seedlings than in the control (Table 4) but the differences were not significant ($p > 0.05$). In plants grown without LFH a similar trend was observed for peroxidase whereas activity of RuDP Carboxylase remained either unchanged or was lower in the treated seedlings than the controls.

In general it can be suggested that during the early stage of seedling growth, when the concentration of the pollutants was not high in either soil system, plant responses were not significantly affected. After 15 weeks (11 applications) of growth, plants in the soil with an LFH were not significantly affected by any treatment ($p > 0.05$), however, in the soils lacking LFH a number of treatments produced significant changes in plant responses (Table 6). Application of V significantly reduced RuDP Carboxylase activity ($p < .001$) as was observed by Malhotra and Khan (1980) in hydroponically grown jack pine.

A significant ($p < 0.05$) effect on carboxylase activity and shoot growth was also observed in the NO_3 treatment, but the effect was positive and indicated a fertilizer effect of NO_3 on the plant response.

In the soil without LFH, mixtures of two pollutants produced plant responses that were different from those induced by individual pollutants (Table 6). For example in V containing pollutant mixtures,

Table 5. Initial effects (8 weeks) of various pollutants and their mixtures on the biochemical and physiological activities of jack pine seedlings grown in soils with and without LFH. Values are means of three replicates.

Treatment[a]	Peroxidase[b] Units / mg protein		RUDP Carboxylase[c] Units / mg protein		Growth mg ODW/seedling	
	+LFH	-LFH	+LFH	-LFH	+LFH	-LFH
V	3.3	1.7	8.0	5.0	88.9	34.9
Ni	2.1	2.4	7.7	6.0	118.0	29.9
SO_4	2.8	1.9	8.0	5.0	89.8	38.1
NO_3	3.2	1.9	9.6	7.0	78.3	37.1
V + Ni	3.5	1.9	8.0	5.0	65.5	27.8
V + SO_4	3.3	2.4	9.0	5.0	93.1	30.3
V + NO_3	3.3	1.8	8.7	7.0	82.0	27.8
Ni + SO_4	2.6	2.2	7.0	7.0	78.7	36.5
Ni + NO_3	4.5	1.8	7.0	7.0	95.2	40.0
SO_4 + NO_3	3.6	1.6	7.0	8.0	88.4	41.8
Control	2.6	1.6	6.3	7.0	86.7	31.0

a - soluble forms of elements applied. Applications were started 5 weeks after seed germination. Plant responses were analysed after 8 weeks (4 aplications).

b - a unit of peroxidase = Δ O.D. of 0.001 min^{-1} at 485 nm.

c - a unit of RuDP Carboxylase = 10^4 cpm (^{14}C) HCO_3 incorporated into acid stable products.

Table 6. Effects of various pollutants and their mixtures after 15 weeks growth on various biochemical and physiological activities of jack pine seedlings grown on soils with and without LFH. Values are means of three replicates.

Treatment	Peroxidase units		RUDP Carboxylase per mg protein		Growth mg ODW/seedling	
	+LFH	-LFH	+LFH	-LFH	+LFH	-LFH
V	5.8	5.4	6.0	1.7[c]	188.7	48.8
Ni	4.4	3.5	8.0	4.5	218.5	62.8
SO_4	4.6	4.3	5.6	3.7	225.4	60.1
NO_3	4.6	5.8	8.0	5.8[a]	212.0	98.2[a]
V + Ni	4.3	4.1	6.8	5.4	169.6	84.8[a]
V + SO_4	4.7	5.4	5.6	3.2[b]	185.5	44.3
V + NO_3	6.2	5.7	7.8	5.3	180.8	105.0[a]
Ni + SO_4	5.3	4.7	7.4	6.5[b]	248.1	100.4[a]
Ni + NO_3	3.7	5.2	7.0	4.1	166.0	80.9
SO_4 + NO_3	4.7	6.4	7.1	6.2[b]	230.2	170.0[b]
Control	4.7	4.5	7.7	4.8	208.1	55.9

a - mean is significantly ($p<0.05$) different from the control.
b - mean is significantly ($p<0.01$) different from the control.
c - mean is significantly ($p<0.001$) different from the control.

the inhibitory effect of V on RuDP Carboxylase was antagonized by other pollutants. A significant ($p < 0.05$) and positive effect was observed on shoot growth in V + Ni and V + NO_3 treatments. Since the Ni salt used was $Ni(NO_3)_2$, a positive effect may be due to NO_3 and not due to Ni, as was observed with NO_3 alone. Similar statistically significant effects were also observed in Ni + SO_4 treatments on RuDP Carboxylase ($p < 0.05$). The effects appeared antagonistic on RuDP Carboxylase and synergistic on shoot growth. Again the positive effects in this treatment may be attributed to NO_3[from $Ni(NO_3)_2$] and SO_4 acting as fertilizers. This appeared to be the case since a mixture of SO_4 + NO_3 produced the greatest positive effect on both plant responses (Table 6).

In general, it can be suggested from our limited data that in situations where plants are growing on nutrient deficient sand soils (with no LFH cover) some pollutants individually can be very toxic to plants but in mixtures with other pollutants their phytotoxic effects may be delayed, altered and even masked by the other pollutant(s). This experiment is still in progress and further changes in the physiological and other plant responses are being monitored and will be reported in future.

2.3.3 Effect of Various Pollutants and their Mixtures on Soluble Cations and Sulphur in the Soil Cores

The concentration of various major cations and sulphur in the water phase of the soil cores was estimated in order to assess the effect of added pollutants on the solubility of these elements. The results in Table 7 showed that the pH of the soil water was not affected significantly by any treatment. It must be pointed out that in this experiment the pH of the applied pollutants was 5.0 for metals (V, Ni, Al) and 2.0 for SO_4 (Addison *et al.* 1981). It would appear that in all cases the mean pH value was higher than the pH of the applied pollutants. It has been shown recently that litter leachates of acid rain treated forest soils had considerably higher pH than the incident rain (Lee and Weber 1982) indicating the influence of litter components

Table 7. Effect of various pollutants and their mixtures on major cations and sulphur content in the soil solution of the soil cores. Values are means ± 95% confidence limits.

Treatment	pH	Concentration (ppm)			
		Ca	Mg	K	S
Vanadium					
Low	5.5±0.1	6.5± 6.4	1.1± 1.6	5.4± 6.3	7.6± 7.2
Medium	5.6±0.4	8.4± 2.3	1.3± 0.8	7.6± 4.9	9.2± 4.6
High	5.1±0.6	7.3± 3.3	0.9± 0.4	9.6± 9.2	10.7± 4.9
Nickel					
Low	5.7±0.1	10.4± 7.1	1.7± 1.3	7.6± 4.2	15.6±10.4
Medium	5.2±0.3	12.4±10.0	2.3± 1.9	9.1± 7.5	18.5±14.5
High	5.7±0.4	7.3± 3.6	1.4± 0.7	6.0± 2.7	10.7± 7.3
Aluminum					
Low	5.4±0.3	6.7± 2.2	1.0± 0.4	5.4± 2.4	9.4± 5.0
Medium	5.6±0.2	6.8± 3.5	1.2± 0.8	5.9± 2.3	8.4± 3.5
High	5.7±0.6	6.6± 4.2	1.1± 0.6	6.7± 5.2	7.3± 4.9
Sulphate					
Low	5.5±0.2	22.5±10.0	4.2± 1.6	7.8± 3.1	29.8±13.6
Medium	5.3±0.3	29.7±14.6	6.2± 3.1	13.5± 9.7	33.4±22.3
High	5.1±0.4	47.2±29.1	11.4±11.2	23.4±15.3	70.7±47.5
Mixtures					
V + Ni	5.5±0.4	6.8± 6.7	1.2± 1.3	10.3± 9.6	9.8± 8.2
V + SO_4	5.2±0.9	41.9±17.9	8.3± 2.6	21.2± 7.7	44.1±29.4
Ni + SO_4	5.3±0.2	25.1±23.8	6.2± 8.7	12.9± 6.3	37.0±33.2
V + Ni + SO_4	5.4±0.3	26.5±15.6	5.2± 3.7	20.8±10.3	30.7±16.9
Control	5.5±0.1	8.1± 1.9	1.4± 0.3	6.1± 1.3	10.6± 2.0
Treated Control	5.5±0.3	5.4± 2.6	0.8± 0.6	9.5± 4.7	6.8± 1.4

in partially neutralizing the applied acid. This may also be the case in our experiment.

In the water phase the contents of major cations (Ca^{2+}, Mg^{2+} and K^{+}) were significantly higher in SO_4 treated soil cores than the control soil cores. Similar increases were also observed in mixtures containing SO_4. The amount of total S in the water phase was also increased in treatments containing SO_4 either individually or in mixtures. The presence of higher amounts of these cations in the water phase of SO_4 treated soils may be attributed to low (acidic) pH of the applied SO_4 solution. It has been shown that acid precipitation accelerates leaching of Ca^{2+} and Mg^{2+} from the forest and mineral soils (Overrein 1972, Abrahamsen *et al.* 1977, Haman 1977). A similar leaching of major cations (Ca^{2+}, Mg^{2+} and K^{+}) and related increase in SO_4 content has been reported recently from soils of hardwood forests upon sulphuric acid rain (Lee and Weber 1982).

The results in Table 7 also showed that addition of various metal pollutants did not significantly alter the amounts of the major cations and sulphur in the soluble water phase. This would suggest that at the concentrations of the metals used the added metals had no effect on soluble nutrients. The amount of applied metal pollutants in the water phase was not in the detection limits of the analysis indicating that the applied metal pollutants were bound in the LFH layer and were not in the soluble form, and therefore not available to plant roots. This may explain an apparent lack of plant responses in the soil core experiments. Furthermore, since our system was a closed type in which leaching of nutrients and applied pollutants was not allowed, any effects on plant responses due to nutrient cycling or loss cannot be demonstrated. A closed type system was designed to maximize interaction of applied pollutant(s) with plant roots. Effects related to leaching of nutrients and cations as a result of various treatments remains to be elucidated.

Detailed soil analyses, which are in progress at present, are expected to provide essential information about any chemical changes in the treated cores as well as the location of applied pollutants in the soil horizons.

2.4 CONCLUSIONS

Application of soluble forms of various pollutants and their mixtures to soil cores did not produce significant changes in the biochemical and physiological processes of jack pine seedlings grown in these cores. Since some of these pollutants have been shown to be phytotoxic to jack pine in hydroponic experiments (Malhotra and Khan, 1979, 1980), it would appear that at the concentrations used, the applied pollutants were rendered unavailable to the plant seedlings by the LFH horizon. The absence of detectable levels of metal pollutants in soil water indicates that the soluble forms of the applied pollutants were converted to more insoluble forms and therefore not available for root uptake from the mineral soil. It is suggested that at low and moderate concentrations of the pollutants, the LFH layer may initially act as an effective filter. This was evident in latter experiments where initial application of pollutants to soil with and without LFH produced different effects on biochemical and physiological responses of jack pine seedlings. In pots containing a top layer of LFH, addition of pollutants did not produce a significant effect on plant responses. In soil pots containing no LFH layer, however, application of a number of pollutants (individually or in mixtures) produced both significant inhibitory and stimulating effects on plant responses. The LFH horizon also appears to enhance nutrient availability which may, in turn, influence the impact of pollutants on the plants. Jack pine grown in a soil with an LFH horizon were significantly larger and showed no effects of pollutant addition; including the stimulation of growth by the addition of NO_3 and SO_4.

3. TIME AND CONCENTRATION EFFECTS OF SO_2 ON LICHEN PHYSIOLOGY

3.1 INTRODUCTION

Numerous studies of lichen distribution around industrial developments have demonstrated that lichens are susceptible to atmospheric pollution, particularly SO_2 (James 1973). It is surprising that in spite of the large number of studies that have been carried out on the influence of air pollution on lichen distribution, very little attention has been paid to the actual response of this group of organisms to the primary pollutant, SO_2. Most studies on the resistance of lichens to SO_2 have used either the photosynthetic system or chlorophyll content as a physiological measure of plant health. The studies have typically dealt with either very high concentrations of SO_2 (Pearson and Skye 1965, Rao and LeBlanc 1966, Nash 1973) or aqueous SO_2 (Hill 1971, Puckett *et al.* 1973). Only a very few studies have tried to determine the sensitivity of lichens under controlled conditions at low gaseous SO_2 concentrations (Türk *et al.* 1974, Wirth and Türk 1974, Malhotra and Khan 1980, Khan *et al.* 1981).

Comparisons between lichen response and responses of either a vascular plant or the ecosystem as a whole have never been made in any quantitative manner. Presently, doubt is being raised as to the usefulness of lichens for early warning biomonitors of air pollution and in the determination of long-term ecosystem changes. In response to this deficiency, this study was designed to determine the response of lichens to air pollutants and to determine the factors that influence the response. A comparison can then be made with known responses of vascular species and the effectiveness of lichens as biomonitors ascertained. Measurements at the biochemical and physiological levels have been used effectively to detect vascular plant responses to air pollutants (Malhotra and Khan 1979, 1980), and this approach to lichen response is directly comparable.

In 1980-81, efforts were concentrated in technique development related to environment and fumigation facilities using only SO_2 as an air pollutant. In 1981-82, the fumigation facilities that were developed

were used to determine the interrelationship between the duration of fumigation and the concentration of pollutant.

3.2 MATERIALS AND METHODS

3.2.1. Plant Material

Branches of jack pine (*Pinus banksiana* Lamb.) supporting a lichen community dominated by *Evernia mesomorpha* Nyl., *Usnea* spp., *Parmelia sulcata* Tayl. and *Hypogymnia physodes* (L.) W. Wats. were collected from a relatively pollution-free area near Fort MacKay in northeastern Alberta. Branches were collected air dry and stored in the laboratory in polyethylene bags at -15°C in the dark. The branches were brought to room temperature (20°C) but left to dry before the experiment.

Tissue of *E. mesomorpha* was separated from the branches and 16 samples of approximately 0.5 g were selected and suspended on spring steel wire loops. Each sample was soaked thoroughly and blotted dry before its placement in the experimental chamber.

3.2.2 Fumigation Conditions

The fumigation conditions were such that both fungal and algal cells of the lichen were fully hydrated throughout the fumigation period. Air was passed through 2-30 cm water columns and 1 L min^{-1} was delivered to both the control and the fumigation cuvette. The cuvettes (12 x 23 x 16 cm) were insulated with 2.5 cm of plastic foam and a temperature between 24 and 26°C was maintained in the dark. Samples of *E. mesomorpha* (8 replicates) were placed in both the control and fumigation cuvettes and various concentrations of SO_2 (0.1, 0.2, 0.4, 0.8 and 1.2 ppm) for selected durations (1, 2, 4 and 8 h) were maintained in the fumigation cuvette. The air in each cuvette was mixed with a propeller-type fan with the motor mounted outside of the cuvette and temperature was monitored with a copper-constantan thermocouple and potentiometer. After the designated time, the samples were removed from the cuvettes and each sample was divided into two portions for the measurement of ^{14}C incorporation and sulphur content.

3.2.3 Analysis of Response

The phytosynthetic CO_2 fixation was measured according to the procedure described by Khan *et al.* (1981).

3.2.4 Sulphur Content

Tissue samples were dried at 80°C for 42 h and then frozen in liquid nitrogen (-196°C). The frozen samples were ground to approximately 100 mesh in a cold mortar and pestle. Approximately 0.1 g of tissue was mineralized by oxygen flask combustion (Chan 1975). Analysis of sulphur content was accomplished by an Inductively Coupled Argon Plasma Atomic Emission Spectrometer (ICAP-AES).

3.3 RESULTS AND DISCUSSION

The results are not yet complete and, in some cases, experiments will need to be duplicated to confirm the results. In general, the response of fully hydrated *E. mesomorpha* to SO_2 is well defined if either a high concentration is given (0.8 or 1.2 ppm) or if sufficient time (i.e., 8 h) is allowed for the pollutant gas to react (Table 8). The effect of SO_2 on the photosynthetic response of *E. mesomorpha* appeared to be similar to that of *E. prunastri* (Türk *et al.* 1974), but *E. mesomorpha* could be slightly more sensitive since 0.2 ppm of SO_2 for 8 h elucidated a similar response to that of 0.2 ppm for 14 h in the case of *E. prunastri*. It is impossible to make absolute comparisons, however, since there were differences in the experimental conditions. Tomassini *et al.* (1977) developed a model for estimating the threshold of injury in *Cladina rangiferina* owing to SO_2. When this model was applied to *E. mesomorpha* at several times of exposure, the model consistently estimated threshold concentrations about 0.2 ppm high. This difference can only relate to either inherent errors in converting from aqueous to gas phase of SO_2 in the model or a difference in sensitivity between *E. mesomorpha* and *C. rangiferina*. Both of these lichen species have been classified as being sensitive to SO_2.

The response of *E. mesomorpha* to SO_2 measured to date is not consistent and the means have high random errors (see 95% confidence limits, Table 8) at low SO_2 concentrations and at times shorter than

Table 8. Response of ^{14}C incorporation in *E. mesomorpha* to various concentrations and duration of exposure to SO_2. Values are means of % reduction from the control ± 95% confidence limits.

Time (h)	Concentration (ppm) 0.1	0.2	0.4	0.8	1.2
1	30.4 ± 22.2	5.7 ± 12.8	37.8 ± 10.3	41.2 ± 12.7	66.9 ± 16.0
2	6.9 ± 32.9	10.1 ± 8.0	12.5 ± 14.3	66.2 ± 27.2	89.9 ± 4.9
4	-18.9 ± 20.3	-19.5 ± 12.6	44.5 ± 14.3	80.1 ± 9.3	95.5 ± 2.7
8	14.2 ± 15.2	53.2 ± 15.7	74.8 ± 10.9	83.2 ± 12.8	99.1 ± 1.1

8 h. It is anticipated that errors stem from both the experimental system and the analytical technique. Further experimental work should resolve the difficulties and should permit a mathematical representation of the response of this lichen to SO_2.

Sulphur content of the fumigated and control lichen tissue has only been partially completed (Table 9). Even with only the lowest two concentrations analyzed, increases in sulphur content with time of exposure are evident. Once experiments on the lichen response of ^{14}C incorporation are completed, a comparison can be made between gaseous pollutant uptake and biological response.

Table 9. Increase in sulphur content with fumigation for various durations at various concentrations of SO_2. Values are means of the difference between control and treated tissue of *E. mesomorpha* ± 95% confidence limits.

Time (h)	Concentration (ppm) 0.1	0.2	0.4	0.8	1.2
1	-14.1 ±137.8	28.9 ± 42.5			
2	104.1 ± 32.8	63.7 ± 48.3		NOT	
4	63.6 ± 20.8	359.1 ± 50.4		AVAILABLE	
8	140.8 ± 48.9	319.4 ± 41.3			

4. REFERENCES CITED

Abrahamsen, G., R. Horntvedt, and B. Tveite. 1979. Impacts of acid precipitation on coniferous forest ecosystems. Water, Air, Soil Pollut. 8:57-73.

Addison, P.A. 1980a. Baseline condition of jack pine biomonitoring plots in the Athabasca Oil Sands area, 1976-77. Prep. for the Alberta Oil Sands Environmental Research Program. Environ. Can., Can. For. Serv. AOSERP Rep. 98. 43 pp.

_____. 1980b. Ecological bench-marking and biomonitoring for detection of airborne pollutant effects on vegetation and soils. Prep. for Alberta Oil Sands Environmental Research Program. Environ. Can., Can. For. Serv. AOSERP Rep. 111. 48 pp.

_____. 1982. Biomonitoring in the Athabasca Oil Sands area of Alberta: Progress and Pitfalls. *in* H.S. Sandhu, J.R. Clements and B.L. Magill (eds.). Symposium Workshop on Acid Forming Emissions in Alberta and their Ecological Effects. Edmonton, March 1982.

Addison, P.A. and J. Baker. 1979. Interim report of ecological bench-marking and biomonitoring for detection of airborne pollutant effects on vegetation and soils 1975 to 1978. Prep. for the Alberta Oil Sands Environmental Research Program. Environ. Can., Can. For. Serv. AOSERP Rep. 46. 38 pp.

Addison, P.A., A.A. Khan, J. Baker, S.S. Malhotra, F. Theriault, F. Radford, and J.I. Ridgway. 1981. Effect on mixed pollutants on soil-plant microcosms. Interim research report. Prep. for Alberta Oil Sands Environmental Research Program. Environ. Can., Can. For. Serv. 20 pp.

Addison, P.A. and K.J. Puckett. 1980. Deposition of atmospheric pollutants as measured by lichen element content in the Athabasca Oil Sands area. Can. J. Bot. 58:2323-2334.

Chan, C.C.Y. 1975. Determination of total sulphur in vegetation by a turbidimetric method following oxygen-flash combustion. Anal. Lett. 8:655-663.

Chang, F.H. and F.E. Broadbent. 1982. Influence of trace metals on some soil nitrogen transformations. J. Environ. Qual. 11:1-4.

Haman, F. 1977. Effects of percolating water with graduated acidity upon the leaching of nutrients and changes on some chemical properties of mineral soils. J. Sci. Agric. Soc. Finl. 49:250-257.

Hill, D.J. 1971. Experimental study of the effect of sulphite on lichens with reference to atmospheric pollution. New. Phytol. 70:831-836.

James, P.W. 1973. Introduction. Pages 1-43. *in* B.W. Ferry, M.S. Baddeley and D.L. Hawksworth (eds.) Air pollution and lichens. Univ. of Toronto Press, Toronto, Canada. 389 pp.

Kennedy, K.A. and P.A. Addison. 1981. Monitoring of plant ecological effects of sour gas processing in west-central Alberta. Interim report to Canterra Energy Company, Ltd. Edmonton, Alberta. 8 pp.

Khan, A.A., S.S. Malhotra, F. Radford, and P.A. Addison. 1981. Effects of SO_2 on lichen physiology. Prep. for the Alberta Oil Sands Environmental Research Program. Environ. Can., Can. For. Serv. 8 pp.

Lee, J.J. and D.E. Weber. 1982. Effects of sulphuric acid rain on major cation and sulphate concentrations of water percolating through two model hardwood forests. J. Environ. Qual. 11:57-64.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem. 193:265-275.

Malhotra, S.S. and P.A. Addison. 1979. Interim report on symptomology and threshold levels of air pollutant injury to vegetation, 1975-1978. Prep. for the Alberta Oil Sands Environmental Research Program. Environ. Can., Can. For. Serv. AOSERP Rep. 44. 13 pp.

Malhotra, S.S., P.A. Addison, and A.A. Khan. 1980. Symptomology and threshold levels of air pollutant injury to vegetation, 1979-80. Prep. for the Alberta Oil Sands Environmental Research Program. Environ. Can., Can. For. Serv. AOSERP Rep. 109. 17 pp.

Malhotra, S.S. and A.A. Khan. 1979. Interim report on physiology and mechanisms of airborne pollutant injury to vegetation 1978-79. Prep. for the Alberta Oil Sands Environmental Research Program. Environ. Can., Can. For. Serv. AOSERP O.F.2. 23 pp.

_____. 1980. Physiology and mechanisms of airborne pollutant injury to vegetation 1979-80. Prep. for the Alberta Oil Sands Environmental Research Program. Environ. Can., Can. For. Serv. AOSERP Rep. 110. 49 pp.

Miles, L.J. and G.R. Parker. 1979. The effect of soil added cadmium on several plant species. J. Environ. Qual. 8:229-232.

Nash, T.H. 1973. Sensitivity of lichens to sulphur dioxide. The Bryologist 76:333-339.

Overrein, L.N. 1972. Sulphur pollution patterns observed: leaching of calcium on forest soil determined. Ambio. 1:145-147.

Pearson, L. and E. Skye. 1966. Air pollution ffects pattern of photosynthesis in *Parmelia sulcata*, a corticolous lichen. Science 148:1600-1602.

Puckett, K.J., E. Nieboer, W.P. Flora, and D.H.S. Richardson. 1973. SO_2: its effect on photosynthetic ^{14}C fixation in lichens and suggested mechanism of phytotoxicity. New Phytol. 72:141-154.

Rao, D.N. and F. LeBlanc. 1965. Effects of SO_2 on the lichen algae with special reference to chlorophyll. The Bryologist 69:69-75.

Schnitzer, M. and S.U. Khan. 1972. Humic substances in the environment. Marcel Dekker, Inc. New York, New York. 327 pp.

Shelfentook, W. 1978. An inventory system for atmospheric emissions in the AOSERP study area. Prep. for the Alberta Oil Sands Environmental Research Program by SNC Tottrup Services Ltd., Edmonton, Alberta. AOSERP Rep. 29. 58 pp.

Slavek, J. and W. Pickering. 1981. The effect of pH on the retention of Cu, Pb, Cd, and Zn by clay-fulvic acid mixtures. Water, Air and Soil Pollut. 16:209-221.

Tomassini, F.D., P. Lavoie, K.J. Puckett, E. Nieboer, and D.H.S. Richardson. 1977. The effect of time of exposure to sulphur dioxide on potassium loss from and photosynthesis in the lichen, *Cladina rangiferina* (L.) Harm. New Phytol. 79:147-155.

Türk, R., V. Wirth, and O.L. Lange. 1974. CO_2-Gaswechsel-Untersuchungen zur SO_2-Resistenz von Flechten. Oecologia 15:33-64.

Wirth, V. and R. Türk. 1975. Zur SO_2-Resistenz von Flechten verschiedener Wuchsform. Flora 164:133-143.

Zunino, H. and J. Martin. 1977. Metal-binding organic molecules in soil: 1. Hypothesis interpreting the role of soil organic matter in the translocation of metal ions from rocks to biological systems. Soil Sci. 123:65-76.