Physiology and Mechanisms of Airborne Pollutant Injury to Vegetation 1979-80

Project LS 3.3

December 1980

Alberta
ENVIRONMENT

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ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM

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Physiology and Mechanisms of Air-borne Pollutant Injury to Vegetation, 1979-80

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The Hon. J.W. (Jack) Cookson
Minister of the Environment
222 Legislative Building
Edmonton, Alberta

Sir:

Enclosed is the report "Physiology and Mechanisms of Air-borne Pollutant Injury to Vegetation, 1979-80.

This report was prepared for the Alberta Oil Sands Environmental Research Program, through its Land System, under the Canada-Alberta Agreement of February 1975 (amended September 1977).

Respectfully,

W. Solodzuk, P.Eng.
Chairman, Steering Committee, AOSERP
Deputy Minister, Alberta Environment

PHYSIOLOGY AND MECHANISMS OF AIR-BORNE
POLLUTANT INJURY TO VEGETATION
1979-80

DESCRIPTIVE SUMMARY

The present research was sponsored to study the effects of various combinations of gaseous and heavy metal pollutants on the native forest vegetation of the Alberta oil sands area.

Relatively little is known about the effects of pollutant mixtures on biochemical and physiological processes of plants. Studies on this subject are essential for determining injuries at the subcellular level, since this type of injury precedes the expression of visible symptoms of injury.

The epiphytic lichen, *Evernia mesomorpha*, suffered irreversible injury to nearly all biochemical functions studied after 3 to 7 d fumigation with 0.34 ppm of SO_2. Controlled fumigation of the vascular species of plants [jack-pine (*Pinus banksiana* Lamb), paper birch (*Betula papyrifera* Marsh), green alder (*Alnus crispa* Ait.) Pursh] with low levels of SO_2 and NO_2 resulted in appreciable changes in the various biochemical functions; in many cases, such exposures did not produce any visible symptoms of pollutant injury. The metal pollutants, vanadium and nickel, also proved to be highly toxic to various metabolic processes in jack pine and paper birch.

This report has been reviewed and accepted by the Alberta Oil Sands Environmental Research Program.

W.R. MacDonald
Director

PHYSIOLOGY AND MECHANISMS OF AIRBORNE POLLUTANT INJURY TO VEGETATION, 1979-80

by

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Canadian Forestry Service

for

ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM

Project LS 3.3

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ABSTRACT

Several important biochemical functions in an epiphytic lichen, *Evernia mesomorpha*, in response to controlled SO_2 exposures were studied. Appreciable changes in these functions were observed even at a very low SO_2 concentration (0.1 ppm), suggesting that epiphytic lichens are probably one of the most sensitive species to SO_2 exposures. Fumigation of these lichens at 0.34 ppm SO_2 for 3 to 7 d caused irreversible injury to nearly all the biochemical functions studied.

Controlled fumigation of vascular species with low levels of SO_2 and NO_2 caused appreciable changes in several biochemical and physiological functions; in many cases such exposures did not produce any visual symptoms of pollutant toxicity. Biochemical indicators such as peroxidase and glycollate oxidase exhibited a synergistic response and ribulose diphosphate carboxylase an additive response to an SO_2-NO_2 mixture in alder. These enzymes, when examined from pine and birch, produced little or no additional response to the mixture compared to that produced by either pollutant individually.

Metal pollutants such as vanadium and nickel were very toxic to various cellular processes. The maximum biochemical response was obtained by the metals alone followed by SO_2. The pollutant mixture (metal + SO_2) did not produce much more additional response than that caused by metals alone.

Biochemical and physiological methods developed in the laboratory were utilized for detecting previsual air pollutant injury to jack pine and highly sensitive epiphytic lichens in the oil sands area. The biochemical functions examined showed no significant differences between the sites at different distances from the pollutant source. The lack of response is considered to be due to the ability of vegetation to recover its metabolic functions between the rare incidents of heavy fumigation. It is possible that the minor pollutant effects, if any, may be masked by the natural variability between sites.

ACKNOWLEDGEMENTS

This research project LS 3.3 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 P.A. Hurdle, M. Grandmaison and F. Radford for technical assistance.

1. INTRODUCTION

The oil sands extraction plants near Fort McMurray in northeastern Alberta release a variety of emission elements into the atmosphere that may have actual or potentially deleterious effects upon forest vegetation and soils in the area. A major portion of earlier research (Malhotra 1979; Malhotra and Khan 1979) on the physiological and biochemical impacts of airborne emissions on forest vegetation has been limited to individual pollutants. Considerable necessary background information on phytotoxicity of several major individual pollutants has been obtained, and this information has a direct application in many instances. Since the pollutant impact on vegetation under field conditions is a reflection of total emissions from a source, there is considerable concern about potentially dangerous mixtures of emission elements such as sulphur dioxide (SO_2), nitrogen oxides (NO_x), vanadium (V), and nickel (Ni).

Several studies have indicated that the pollutant response differs when plants are exposed to more than one pollutant (Bennett et al. 1975; Skelly et al. 1972); such response may be additive, antagonistic, or synergistic in nature. Tingey et al. (1971) have reported for several species a threshold level of injury after 4 h fumigation with 2 ppm nitrogen dioxide (NO_2) and 0.5 ppm SO_2. The injury developed at much lower concentrations, however, when the same species were exposed to a mixture of NO_2 and SO_2 (0.05 to 0.25 ppm of each gas for 4 h). Bennett and Hill (1974) reported that SO_2 in the presence of NO_2 in concentrations similar to those allowed under ambient air quality standards would suppress considerably CO_2 uptake rates of sensitive species even if exposed under favourable growing conditions. Sulphur dioxide and NO_2 have been shown to produce a synergistic response in apparent photosynthetic rates of alfalfa (White et al. 1974). Relatively little is known, however, about the effects of pollutant mixtures on important biochemical and physiological process of plants. Such studies are essential to determine hidden injuries at the subcellular and biochemical levels, because the expression of visible injury is generally preceded by alterations in cellular and subcellular functions.

At present there is also a serious lack of essential scientific information relative to the effects of various combinations of gaseous and heavy metal pollutants on native forest vegetation of the oil sands of Alberta. In order to provide information that will permit the forest managers to assess air pollution impacts and to make recommendations as appropriate, a long-term research study was initiated with the following objectives:

1. To describe and measure the physiological, biochemical, and visible impacts of SO_2 on epiphytic lichens, as they are some of the most sensitive vegetation species of the oil sands area;
2. To conduct controlled environmental research to detect and describe the physiological and visible impacts of SO_2, NO_2, V, and Ni and their various combinations on selected vascular species of the oil sands area; and
3. To analyze the vascular and lichen species collected from the "impingement" and relatively "clean" areas to assess the effects of emissions from the industrial operations near Fort McMurray.

2. MATERIALS AND METHODS

Most of the procedural details have been described in previous reports (Malhotra 1979; Malhotra and Khan 1979) and were followed without major modifications. If any change was necessary, it is described in the appropriate sections of this report.

2.1 PLANT MATERIAL AND GROWTH CONDITIONS

2.1.1 Vascular Plants

Plant seedlings of jack pine (*Pinus banksiana* Lamb.), paper birch (*Betula papyrifera* Marsh), and green alder (*Alnus crispa* Ait., Pursh) were grown from their seeds (collected from the Fort McMurray area) in pots containing peat moss as described earlier (Malhotra 1979; Malhotra and Khan 1979).

In experiments dealing with heavy metals, jack pine seedlings were grown hydroponically as described before (Malhotra and Khan 1979). Solutions of nickel (nickel acetate) and vanadium (ammonium vanadate) were used in experiments dealing with metals. The pH of the stock solutions of both metal salts was adjusted to the pH of the nutrient solution (pH 5.5) prior to being mixed with the nutrient solution.

2.1.2 Epiphytic Lichens

Epiphytic lichens were collected from jack pine dominated forest stands in areas near Fort MacKay, Alberta (Figure 1). The lichen samples were collected with the dead branches on which they were growing. The branches bearing lichens were collected from a height of about 1.5 m in a relatively open jack pine stand. Although all lichens present on the collected branches remained undisturbed during controlled fumigations in the laboratory, only the samples of *Evernia mesomorpha* were selectively harvested for analyses. The lichen samples were almost dry at the time of collection in the field. They were brought to the laboratory in the same condition in dark polyethylene bags and were kept in a cold room ($-15^{\circ}C$) in the dark until used.

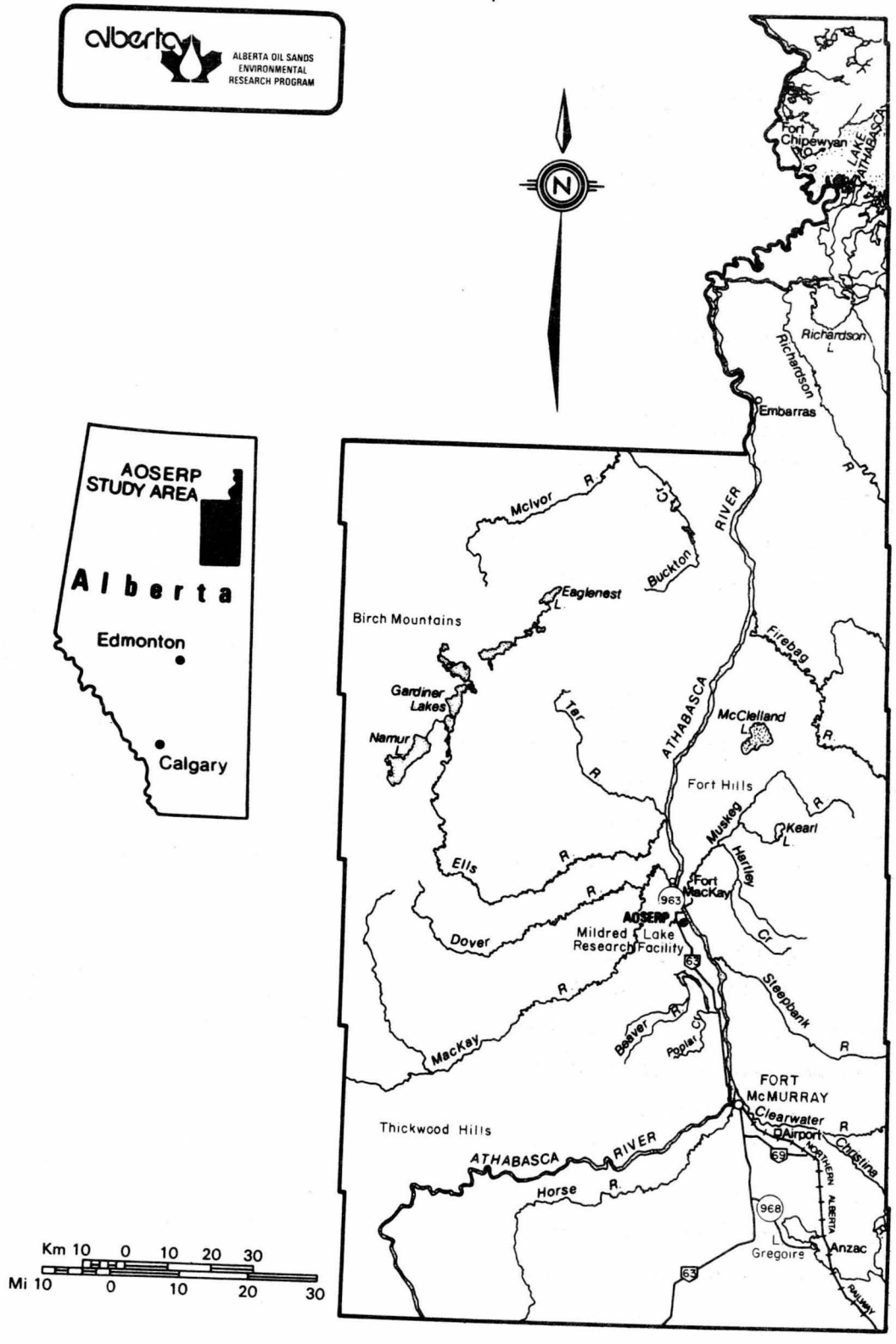

Figure 1. Location of the AOSERP study area.

2.2 GASEOUS TREATMENTS

2.2.1 Sulphur Dioxide in Combination with Nickel and Vanadium

For studying the effects of SO_2 in the mixtures with metals (Ni and V), 3 to 4 mo old, hydroponically grown jack pine seedlings were used. These seedlings were placed in high flow cuvettes under conditions described earlier (Malhotra and Khan 1979). In these experiments, the following four sets of plants in hydroponic solutions were used: Set 1--control (seedlings without any pollutant treatment); Set 2--metal (seedlings treated hydroponically with test metal alone); Set 3--SO_2 (seedlings fumigated with 0.34 ppm SO_2 alone); Set 4--metal + SO_2 (seedlings treated hydroponically with test metal and fumigated with 0.34 ppm SO_2). The seedlings were exposed to metal alone (Set 2) for 7 d or to SO_2 alone (Set 3) for 3 d. In the combination (Set 4), after 4 d of treatment with metal, the seedlings were concurrently treated with metal and SO_2 for another 3 d. Roots and foliage of these seedlings were harvested at the end of a specified interval. Visible symptoms, if any, were recorded both on roots and foliage, and the tissues were analyzed for various metabolic functions.

2.2.2 Sulphur Dioxide in Combination with Nitrogen Dioxide

In these experiments, plant seedlings grown in peat moss pots were used. The jack pine seedlings were 3 to 4 mo old, while birch and alder seedlings were 5 to 6 mo old. In each experiment, seedlings of uniform age and growth were randomly selected and divided into four sets (at least six seedlings per set). One set served as control (untreated), while the other three sets received: SO_2 (0.34 ppm); NO_2 (concentration as specified under results and discussion); and SO_2 (0.34 ppm) + NO_2 (concentrations as specified).

These four sets of plants were transferred to four high-flow cuvettes (Malhotra and Khan 1979) and the cuvettes then were placed in a controlled environment growth chamber, where the plants were maintained at 20°C under approximately 450 micro-Einstein$\cdot m^{-2}\cdot S^{-1}$ ($\mu E\cdot m^{-2}\cdot S^{-1}$) light

intensity (mixture of incandescent, high pressure sodium, and multivapour lamps) with 18 h photoperiod and 75% humidity. Plants were preconditioned for 2 to 3 d before the fumigations were started. The foliage from the seedlings was harvested at different intervals (specified in the tables) after the start of fumigations and used as experimental material.

The SO_2 and NO_2 concentrations were controlled by computerized manifolds. Concentration of NO_2 in the fumigation cuvette was measured by an SM-400 derivative spectrometer, while SO_2 was measured by a Monitor Labs' sulphur monitor model 8450.

2.2.3 Fumigation of Lichens with SO_2

Samples of air-dried lichens on small branches were taken out from cold stored material and left overnight in controlled environment growth chambers for preconditioning. Before the start of SO_2 treatment, the lichen branches were divided randomly into two groups. One group was used as a control set (untreated) and the other for SO_2 treatment. The lichen samples of both sets were moistened by a fine spray of distilled water at the start of the experiment. Branches containing moistened lichen samples were then mounted horizontally between holders in control and SO_2 cuvettes. The cuvettes were placed in controlled environment chambers and maintained at $20^{\circ}C$ and 80 $\mu E \cdot m^{-2} \cdot s^{-1}$ with a 16 h photoperiod. Humidified air (80 to 85%) was passed through the cuvettes at a flow rate of 10 L/min. The dimension and operation of the cuvettes has been described (Malhotra 1979). Low light and medium air flow were used to avoid very rapid drying of the lichen samples. In all the experiments, the schedule for wetting the lichen samples was maintained at 0800 and 1600 each day. Representative samples of lichens were harvested at specified intervals and processed for assaying various metabolic functions.

2.3 PREPARATION OF ENZYME EXTRACTS FROM VASCULAR PLANTS

Details of tissue sampling and preparations of enzyme extracts for jack pine needles and leaves of alder and birch were the same as described in the previous report (Malhotra and Kahn 1979).

2.4 ENZYME ASSAYS

The activities of acid phosphatase, peroxidase, ribulose diphosphate carboxylase, and glycollate oxidase were assayed by procedures described earlier (Malhotra 1979; Malhotra and Khan 1979).

2.5 LIPID BIOSYNTHESIS

2.5.1 Jack Pine

The incorporation of [1-^{14}C] acetate into polar lipids of jack pine needles was measured as described before (Malhotra 1979).

2.5.2 Lichens

Lipid biosynthesis in lichen tissues was also measured as described for jack pine. The only modification was that the lichen tissue was first preincubated with distilled water (5 mL) at 30°C for 10 min. Total lipids from the tissues were extracted by the method of Folch et al. (1957) as modified for jack pine. Radio thin layer chromatography of the total lipids revealed various radioactive components, which were identified by matching with authentic standards.

2.6 PROTEIN BIOSYNTHESIS

The biosynthesis of proteins in lichen tissues was measured by the incorporation of [U-^{14}C] leucine into proteins. This was done by preincubating the lichen sample (0.1 g) in distilled water (5 mL) for 10 min at 30°C. After this initial incubation, [U-^{14}C] leucine (1.5 μCi) was added to the solution and the sample was further incubated for 4 h at 30°C with constant shaking under 150 $\mu E \cdot m^{-2} \cdot s^{-1}$ light source. After the incubation, the tissue was separated by filtration and was washed thoroughly with distilled water. The labelled tissue was homogenized in 0.1 M (molar) phosphate buffer (pH 7.0) and the proteins precipitated with trichloroacetic acid (final conc. 10%). The label in the protein was measured after first washing the protein residue with 10% TCA (twice) and 80% acetone (once) and then dissolving in 0.5 M NaOH.

2.7 PHOTOSYNTHESIS (CO_2-FIXATION)

The photosynthetic CO_2-fixation in lichen samples was measured by using a [^{14}C] sodium bicarbonate ($NaH^{14}CO_3$) solution. The CO_2-fixation was measured by first preincubating the lichen sample (0.1 g) in distilled water (3 mL) at 30°C for 10 min; this was followed by an incubation in the presence of $NaH^{14}CO_3$ (1.5 μCi), with light (150 $\mu E \cdot m^{-2} \cdot S^{-1}$) for 2 h. After incubation, the reaction was stopped by adding 1 mL of formic acid and 16 mL of absolute ethanol. The contents were brought to a boil and, after cooling, they were homogenized for 1 min with a Polytron homogenizer. The contents were filtered on a Büchner funnel and the residue was washed several times with hot 80% ethanol. Aliquots of the filtrate were mixed with a little formic acid (0.1 mL) in scintillation counting vials and flushed with a stream of N_2 on a hot water bath to almost dryness. The contents of the vials were then dissolved in a small amount of distilled water and the fixed CO_2 was measured by assaying [^{14}C].

2.8 POTASSIUM AND MAGNESIUM EFFLUX

Potassium and Mg^{2+} efflux from the lichen tissues was measured by by incubating the sample (0.1 g) in distilled water (8 mL) with gentle shaking at 30°C for 3 h. After the incubation the sample was filtered and washed with distilled water. The filtrate and washings were brought to 10 mL, and an appropriate aliquot was used to measure K^+ (flame photometer) and Mg^{2+} (atomic absorption spectrometer) contents.

2.9 MEASUREMENT OF RADIOACTIVITY

The incorporation of radioactive [^{14}C] compounds in various processes described in previous sections was measured by mixing the [^{14}C] products in a scintillation vial with a scintillation counting fluid (0.4 g Omnifluor dissolved in a mixture of 30 mL ethanol and 70 mL toluene). Counting was done with a Nuclear Chicago Isocap/300 Liquid Scintillation Counter.

2.10 DRY WEIGHT AND ELEMENTAL ANALYSES

The sample dry weight was measured by oven drying the fresh tissues of vascular plants or lichens at 80°C for 24 h. For elemental

analyses, the dried samples were ground to a fine powder and a portion of the powder was used for oxygen flask combustion (Chan 1975). In the combusted samples, the analyses of Ca, K, V and Ni were done by atomic absorption spectometry, while S was determined by the method of Carson et al. (1972).

2.11 BIOCHEMICAL AND PHYSIOLOGICAL ANALYSES ON FIELD SAMPLES

Jack pine branches were collected in mid-June in a southeast gradient from the Suncor plant at 2.8 km ("maximum impingement" area), 4.2 km ("intermediate impingement" area) and 8.3 km ("relatively clean" area). Needles in their second year of growth were used for the laboratory analyses (Malhotra and Khan 1979).

Samples of epiphytic lichens, *Evernia*, were collected in early September from the above three areas as well as from areas near the Mildred Lake camp and Fort MacKay (Figure 1). The collection and storage methods of the lichen sample were the same as those described in an earlier section. These lichen samples were analyzed in the laboratory for their biochemical and physiological functions by the methods described in earlier sections.

3. RESULTS AND DISCUSSION

Since each sample comprised a mixture of tissues from eight to 10 plant specimens, the experimental data represent averages of large populations of individuals. Except as otherwise specified, the experiments for each investigation were conducted several times, and their trends were found to be reproducible. In spite of the careful sampling, biological variability on a day-to-day basis was difficult to eliminate. To overcome this problem, each experiment was designed to include a control (plant material not treated with any pollutant). A comparison of results between control and treated plants provided meaningful data on pollutant response (% of control). Such treatment of data was absolutely necessary for comparing results between the experiments. Once a trend for the pollutant response was established, a single concentration for each pollutant was selected in order to keep the experiment to a manageable size for statistical treatment. Several extractions were made from the plant material (eight to 10 plants for each extraction) in each treatment in order to determine statistical significance.

The reasons for studying the impact of air pollutants (individually and in combination) on certain specific biochemical and physiological functions have been discussed in previous reports (Malhotra 1979; Malhotra and Khan 1979).

3.1 EFFECTS OF SO_2 ON EPIPHYTIC LICHENS

3.1.1 $^{14}CO_2$-Fixation, Protein Biosynthesis, Acid Phosphatase, and K^+ and Mg^{++} Efflux

Earlier work (Malhotra 1979; Malhotra and Khan 1979) has indicated that processes of $^{14}CO_2$-fixation, lipid biosynthesis, acid phosphatase activity, and magnesium efflux rates are some of the most sensitive events to SO_2 in the vascular species. A study, therefore, was designed to examine some of these responses within epiphytic lichens that are one of the most sensitive vegetation groups in the Athabasca Oil Sands area. Such examination might provide a reliable and effective early warning system against air pollution injury to the forest ecosystem.

As shown in Table 1, $^{14}CO_2$-fixation and protein synthesis rates declined dramatically with increasing lengths of lichen exposure to 0.1 ppm SO_2 concentration. Protein synthesis was shown to be more sensitive to SO_2 than $^{14}CO_2$-fixation, especially after long exposure times. It is important to note that even 2 d of exposure at such a low concentration resulted in about 15% inhibition in the two rates. The increase in inhibition rates of the two processes appeared to be related to sulphur content of the lichen tissues, especially after long SO_2 exposures (29 d). Results on photosynthesis similar to those of this study have also been reported by Hill (1971) for another fruticose lichen, *Usnea subfloridana*.

At a little higher SO_2 concentration of 0.34 ppm, the inhibitory response in $^{14}CO_2$-fixation and protein synthesis was much faster and more pronounced (Table 2). There was considerable decline in the activity of both processes even after 2 h exposure time; 7 d fumigation resulted in almost their total inhibition. The loss of activity of both processes was related to tissue sulphur content up to 3 d fumigation. On Day 7, even though there was almost a total loss of activity, the tissue sulphur content dropped appreciably. The decrease in sulphur may be due to a net loss of sulphur gases from severely injured lichen tissues. After 7 d fumigation, the injury was advanced to such a stage that the activity of these processes did not recover appreciably upon transferring the treated plants to a SO_2-free atmosphere for a week. Sulphur content of the SO_2-treated tissues (nearly dead lichens) also showed no recovery in clean air, similar to that of the two processes.

Previously, K^+ (Puckett et al. 1977) and Mg^{++} (Malhotra 1977) release from the SO_2-treated plant tissues had been used as a measure of SO_2 phytotoxicity. The magnitude of both these responses showed a dependence on SO_2 concentration, the length of exposure, and the specific sensitivity of the vegetation species. An attempt was made, therefore, to study the effect of 0.34 ppm SO_2 (for various lengths of time) on an epiphytic lichen, *Evernia mesomorpha*. On Day 7 of fumigation, K^+ efflux almost doubled whereas there was very little or no increase in the Mg^{++} efflux. Both, however, increased considerably

Table 1. The effect of long term exposure to 0.1 ppm SO_2 on the $^{14}CO_2$-fixation and protein biosynthetic rates of *Evernia mesomorpha*.[a]

Length of Exposure (days)	$^{14}CO_2$-Fixation	Protein Biosynthesis	S Content
	% of Control		
2	85.2	85.5	113.6
15	65.3	35.2	115.4
29	32.6	11.6	133.6

[a] The lichen samples were moistened twice daily (at 0800 and 1600) by lightly spraying with water.

Table 2. The effect of 0.34 ppm SO_2 on several metabolic activities of *Evernia mesomorpha* and their recovery in SO_2-free atmosphere.

Length of Exposure	$^{14}CO_2$-Fixation	Protein Biosynthesis	K^+ Efflux	Mg^{++} Efflux	S Content
	% of Control				
2 h	72.4	40.7	-	-	120.0
1 d	65.0	28.3	100.0	100.0	129.5
3 d	27.6	9.2	-	-	157.5
7 d[a]	5.4	1.8	200.0	106.6	129.4
SO_2-free, 7 d[a]	16.5	3.6	245.6	181.8	130.4

[a] Recovery period in an SO_2-free atmosphere started 7 d after fumigation. The lichen samples were moistened twice daily (at 0800 and 1600) by lightly spraying with water; this practice was continued even during the recovery period in an SO_2-free atmosphere.

after 7 d of recovery period in an SO_2-free atmosphere. The increase in Mg^{++} efflux was much larger than the one for K^+ efflux during the recovery period. This increased loss of these elements during the recovery period is attributed to further destruction of cellular membrane and cell wall barriers. These results, therefore, suggest that 3 to 7 d of SO_2 fumigation at 0.34 ppm concentration can cause irreversible injury to various metabolic activities of sensitive lichen species such as *Evernia*. Such response, however, would be dependent upon the environmental conditions such as temperature, light, and moisture during SO_2 exposures.

3.1.2 Lipid Biosynthesis

It has been reported that very low concentrations of SO_2, which usually do not produce any visual symptoms of toxicity, cause marked changes in the lipid biosynthesis of jack pine needles (Khan and Malhotra 1977; Malhotra and Khan 1978). Since lipids are essential constituents of cellular membranes, changes in their composition by SO_2 (Khan and Malhotra 1977) were considered to be the primary reason for adverse effects of SO_2 on cellular membranes (Malhotra 1976). In view of this and many other important roles of lipids, the authors attempted to investigate the effect of SO_2 on lipid biosynthesis in *Evernia*, one of the vegetation species most sensitive to air pollution.

Long-term exposure of *Evernia mesomorpha* to low levels of SO_2 (0.1 ppm) caused considerable reduction in the biosynthesis of some of the major membrane glycerolipids of this lichen, namely, phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), diphosphatidyl diglycerol (DPDG), and triacyl glycerol (TAG) (Table 3). The inhibition of lipid biosynthesis on Day 29 of fumigation was much more pronounced than on Day 15. The reduction in biosynthetic capacity appeared to be related to the sulphur content of the SO_2 treated tissues. The inhibition was almost completely reversed after transferring the SO_2-treated lichens to an SO_2-free atmosphere. In the clean atmosphere, even the sulphur content of treated lichens dropped back to nearly original levels, suggesting that the inhibition and recovery of lipid biosynthetic

Table 3. The effect of long exposure to 0.1 ppm SO_2 on the lipid biosynthesis of *Evernia mesomorpha* and its recovery in SO_2-free atmosphere.

Length of Exposure (days)	PC[a]	PE[b]	DPDG[c]	TAG[d]	S Content[e]
	% of Control				
15	92.5	84.4	74.6	77.0	115.4
29[f]	54.0	55.2	72.7	43.7	133.6
SO_2-free, 8 d[f]	100.0	94.6	100.0	86.2	90.6

[a] PC = phosphatidyl choline

[b] PE = phosphatidyl ethanolamine

[c] DPDG = diphosphatidyl diglycerol

[d] TAG = triacyl glycerol

[e] S Content = sulphur content

[f] Recovery period in an SO_2-free atmosphere started 29 d after fumigation. The lichen samples were moistened twice daily by lightly spraying with water; this practice was continued even during the recovery period in an SO_2-free atmosphere.

ability is directly related to SO_2 toxicity. In clean air, such metabolic recovery would be due to utilization of accumulated sulphur, a part of which may be lost in the form of volatile compounds. O'Hare and Williams (1975) have shown that a considerable amount of sulphur in lichens exists in the form of volatile sulphur compounds. It appears that lichens, similar to vascular species (Malhotra and Khan 1978), have a remarkable ability to recover from SO_2-related metabolic injury provided they are fumigated with low levels of SO_2 for no more than 3 to 4 wk.

Exposure of these lichens at 0.34 ppm SO_2 concentration for up to 7 d, however, can cause a severe decline in the biosynthesis of all major lipids examined (Table 4). On Day 3, biosynthesis of all lipids was inhibited by more than 50%. This response was inversely related to tissue sulphur content for the first 3 d of fumigation. On Day 7, in spite of a drop in sulphur content from Day 3, there was a dramatic reduction in biosynthesis of all lipids; maximum reduction occured in DPDG (96%) followed by PC, PE, and TAG (all approximately 90%). Such changes in lipids would alter their composition in lichen membranes; this would ultimately have an adverse effect on membrane permeability and other related functions. Even though there was a slight recovery in the lipid biosynthetic capacity of SO_2-treated lichens after transferring them to clean air for 7 d, there was virtually no change in the tissue sulphur content (7 d fumigation vs 7 d fumigation followed by 7 d clean air).

The lichen data clearly indicate that at low concentrations (0.1 ppm), SO_2 can have a deleterious effect on the lichen metabolism. Such an effect can be almost fully recovered by transferring plants to clean air following 1 mo SO_2 exposure time. It appears that at low SO_2 concentrations lichens have an excellent ability to detoxify SO_2 Türk et al. 1974). At high concentrations (0.34 ppm), however, the injury to various biochemical and physiological functions can be much more severe and not fully recoverable. Under field conditions, exposure of lichens to relatively high concentrations of SO_2 under moist conditions for relatively short periods of time can be expected to cause severe phytotoxicity problems.

Table 4. The effect of 0.34 ppm SO_2 on lipid biosynthesis of *Evernia mesomorpha* and its recovery in SO_2-free atmosphere.

Length of Exposure (days)	PC[a]	PE[b]	DPDG[c]	TAG[d]	S Content[e]
	% of Control				
1	79.0	66.7	53.3	62.6	129.5
3	49.4	39.0	37.0	37.1	157.5
7[f]	10.7	9.8	4.3	10.2	129.4
SO_2-free, 7 d[f]	51.2	37.2	25.0	38.8	130.4

[a] PC = phosphatidyl choline

[b] PE = phosphatidyl ethanolamine

[c] DPDG = diphosphatidyl diglycerol

[d] TAG = triacyl glycerol

[e] S Content = sulphur content

[f] Recovery period in an SO_2-free atmosphere started 7 d after fumigation. The lichen samples were moistened twice daily by lightly spraying with water; this practice was continued even during the recovery period in an SO_2-free atmosphere.

3.2 EFFECTS OF SO_2, NO_2, AND THEIR MIXTURE ON JACK PINE, PAPER BIRCH, AND GREEN ALDER ENZYME SYSTEMS

3.2.1 Ribulose Diphosphate Carboxylase, Glycollate Oxidase, and Peroxidase in Jack Pine Needles

As shown in Table 5, ribulose diphosphate carboxylase and glycollate oxidase activities declined appreciably after 48 h exposure of jack pine seedling to 0.34 ppm SO_2. This response was very similar to the one reported earlier (Malhotra and Khan 1979). Peroxidase activity, on the other hand, increased after SO_2 fumigation. Unlike ribulose diphosphate carboxylase and glycollate oxidase activities, peroxidase activity has been shown to be increased dramatically by SO_2 treatment prior to the onset of tissue necrosis (Keller 1974; Malhotra and Khan 1979). The response of all three enzymes appeared to be related to the tissue sulphur content (Table 5).

Nitrogen dioxide at 2.0 ppm concentration for 48 h exposure produced a stimulatory response in all three enzymes (Table 5); the maximum stimulation was in glycollate oxidase (74%) followed by peroxidase (26%) and ribulose diphosphate carboxylase (12%). Ashenden (1979) has reported a short-term stimulation of transpiration rate after fumigation of *Phaseolus vulgaris* with NO_2 alone.

When both gases were used as a mixture, the ribulose diphosphate carboxylase activity remained unchanged over that of the control. It appears that NO_2 in a mixture protected carboxylase activity from inhibitory effects of SO_2. The stimulation in glycollate oxidase activity dropped somewhat over that produced by NO_2 alone; this drop was due solely to SO_2. Since glycollate oxidase is involved in photorespiration, an increase in its activity would imply a drop in photosynthesis or net growth. It appears that NO_2 alone, as well as in a mixture with SO_2, would influence plant productivity by accelerating photorespiration. The mixture of SO_2 and NO_2 caused an increase in peroxidase activity over that of either gas; the increase appeared to be additive. It is suggested that metabolic processes associated with increased peroxidase activity (aging, senescence) would be greatly accelerated by SO_2-NO_2 mixtures.

Table 5. The effect of 0.34 ppm SO_2 and 2.0 ppm NO_2 individually and as a mixture on jack pine needle enzymes.

Treatment[a]	RuDPC[b]	GO[c]	PO[d]	S Content[e]
	Activity·g^{-1} dry wt			mg·g^{-1} dry wt
Control	28.4 ± 0.7 (100.0)[f]	15.4 ± 2.6 (100.0)	108.5 ± 6.5 (100.0)	0.9
SO_2	17.6 ± 1.1 (62.0)	11.2 ± 1.4 (72.7)	151.9 ± 6.0 (140.0)	1.8
NO_2	32.3 ± 0.3 (112.2)	26.8 ± 1.4 (174.0)	137.2 ± 3.3 (126.5)	1.1
SO_2 + NO_2	28.6 ± 0.8 (100.7)	22.6 ± 1.4 (146.8)	177.1 ± 9.2 (163.2)	1.4

[a] Pine seedlings were fumigated for 48 h.

[b] RuDPC = ribulose-1, 5-diphosphate carboxylase (X 10^5 cpm).

[c] GO = glycollate oxidase (ΔOD/min).

[d] PO = peroxidase (ΔOD/min).

[e] S Content = sulphur content.

[f] The values in parenthesis denote percentage of the control activity and ± values represent standard deviation.

3.2.2 Ribulose Diphosphate Carboxylase, Glycollate Oxidase and Peroxidase in Paper Birch Foliage

Fumigation by SO_2 (0.34 ppm for 72 h) caused a reduction of about 50% in the activity of all three enzymes tested (Table 6). This response corresponded with a sharp increase in tissue sulphur content. Unlike pine seedlings (Table 5), birch foliage exhibited a drop even in peroxidase activity (Table 6). Such a response is attributed to general interveinal and marginal chlorosis and desiccation of most foliage.

Fumigation by NO_2 (0.2 ppm for 72 h) did not produce any visual symptoms of toxicity. The enzyme activities, however, dropped considerably even in the absence of visual symptoms of injury; the maximum inhibition occured in glycollate oxidase (56%) followed by ribulose diphosphate carboxylase (36%) and peroxidase (30%).

The mixture of the two gases did not produce any appreciable additional response in ribulose diphosphate carboxylase over that produced by either gas individually. The inhibition of glycollate oxidase by gaseous mixture was appreciably less then that produced by these gases individually. This response occurred in spite of the tissue sulphur level having doubled during the gas mixture treatment. It appears that the interaction of the two pollutants in a mixture may be responsible for this effect. The stimulation of peroxidase activity occurred only when the plants were fumigated with the mixture; this response appeared to be antagonistic in nature. The stimulatory response in peroxidase occurs in the absence of severe toxicity symptoms (Malhotra and Khan 1979) and is attributed mainly to the presence of SO_2 in the mixture.

3.2.3 Ribulose Diphosphate Carboxylase, Glycollate Oxidase, and Peroxidase in Green Alder Foliage

When used individually, SO_2 and NO_2 produced a substantial alteration in the activities of all three enzymes, ribulose diphosphate carboxylase and glycollate oxidase being inhibited and peroxidase being stimulated (Table 7). The mixture of the two gases produced a further pronounced response in all three enzymes. This increased

Table 6. The effect of 0.34 ppm SO_2 and 0.2 ppm NO_2 individually and as a mixture on paper birch foliage enzymes.

Treatment[a]	RuDPC[b]	GO[c]	PO[d]	Visual Symptom	S Content[e]
	% of Control				$mg \cdot g^{-1}$ dry wt
Control	100.0	100.0	100.0	-[f]	1.0
SO_2	53.4	53.4	45.6	+[g]	2.3
NO_2	63.6	43.5	70.0	-	0.8
$SO_2 + NO_2$	61.4	69.5	145.4	±[h]	2.0

[a] Plants were fumigated for 72 h.

[b] RuDPC = ribulose-1,5-diphosphate carboxylase.

[c] GO = glycollate oxidase.

[d] PO = peroxidase.

[e] S Content = sulphur content.

[f] - = no symptoms, healthy leaves.

[g] + = interveinal and marginal chlorosis on most leaves, foliar desiccation.

[h] ± = spotty chlorosis and brown discoloration.

Table 7. The effect of 0.34 ppm SO_2 and 0.2 ppm NO_2 individually and as a mixture on green alder foliage enzymes.

Treatment[a]	RuDPC[b]	GO[c]	PO[d]	Visual Symptom	S Content[g]
	% of Control				$mg \cdot g^{-1}$ dry wt
Control	100.0	100.0	100.0	-[d]	1.0
SO_2	69.0	74.9	129.2	-	2.2
NO_2	59.2	89.7	135.3	-	1.5
$SO_2 + NO_2$	38.8	52.3	554.7	+[f]	2.9

[a] Plants were fumigated for 24 h.

[b] RuDPC = ribulose-1,5-diphosphate carboxylase.

[c] GO = glycollate oxidase

[d] PO = peroxidase.

[e] - = no symptoms, healthy leaves.

[f] + = slight interveinal chlorosis.

[g] S Content = sulphur content.

response is synergistic in nature for peroxidase and glycollate oxidase (Table 7). Wellburn et al. (1976) and Ashenden (1979) have reported similar synergistic effects of SO_2 and NO_2 on enzymic activities and in transpiration rates.

There were no visual symptoms of toxicity produced either by SO_2 or NO_2 individually and only a slight intervenial chlorosis was produced by the mixture. It can be concluded that SO_2-NO_2 mixtures can cause a synergistic deleterious effect on alder even in the absence of any severe visual symptoms of toxicity.

These results clearly suggest that alder seedlings are much more sensitive to the gaseous mixture (SO_2 + NO_2) than either jack pine or birch seedlings. The response of these enzymes to individual pollutants and their mixture differed considerably in jack pine, birch, and alder, suggesting that the biochemical sensitivity is neither pollutant nor species specific. Rabe and Kreeb (1979) have attributed this to genetic variability in species.

3.3 PHYTOTOXIC EFFECTS OF VANADIUM, SO_2, AND THEIR MIXTURE

3.3.1 Polar Lipid Biosynthesis in Jack Pine Needles

Both phospholipids (PI, PC, PE, and PG) and glycolipids (DGDG, MGDG, and ESG) (see Table 8 for their full names) were inhibited by 10, 50, and 100 ppm vanadium (Table 8). At 100 ppm concentration, there was almost total inhibition of all lipids analyzed; at 50 ppm concentration the inhibition ranged from 60 to 75%. The inhibition in lipid biosynthesis at 50 and 100 ppm concentration is considered due mainly to severe injury to root tissues (dark brown discoloration of root tips and damage to root hairs), which ultimately resulted in desiccation of the foliage. Vanadium at 10 ppm concentration caused a marked reduction in the biosynthesis of PG and MGDG, the two glycerolipids characteristic of chloroplast membranes (Mudd and Garcia 1975). The inhibition in biosynthesis of other lipids, however, was not as severe. No visual injury to roots and foliage could be

detected at this concentration. When the seedlings were treated with SO_2 alone (which does not result in visual injury to roots and desiccation of the foliage), there was considerable inhibition in the biosynthesis of all lipids (Table 8).

Vanadium at 10 ppm concentration in the presence of 0.34 ppm SO_2 produced an additive inhibitory response on lipid biosynthesis but at increased metal concentration the response of vanadium and SO_2 mixture was very similar to the one obtained with vanadium alone (Table 8). In the mixture study, the lack of additional response, by combinations of vanadium (50, 100 ppm) and SO_2 over that of vanadium alone may be the inability of the desiccated foliage (with at least partly closed stomata) to take up appreciable amounts of SO_2. This point is further strengthened by the observation that there was only a slight increase in the sulphur content of tissues treated with the mixture over the ones treated only with vanadium (Table 9).

In order to keep the experiments within manageable size for statistical treatment, only one concentration was used for both vanadium (50 ppm) and SO_2 (0.34 ppm) individually as well as in combination (Table 10). The results show that, in general, the lipid biosynthetic response to individual pollutants was significantly different from that of the control tissues but they were not consistently different from each other. Generally, the effect produced by the pollutant mixture was statistically different from that of either control or SO_2 but not from that of vanadium alone. These results suggest that lipid biosynthesis may be used as a very sensitive indicator of vanadium and SO_2 phytoxicity.

3.3.2 Acid Phosphatase, Glycollate Oxidase, Peroxidase, and Ribulose Diphosphate Carboxylase in Jack Pine Needles

Increasing concentrations of vanadium (10 to 100 ppm) markedly reduced the activities of acid phosphatase, glycollate oxidase, and ribulose diphosphate carboxylase (Figure 2). As expected (Malhotra and Khan 1979), peroxidase activity increased considerably up to 50 ppm vanadium and then declined at 100 ppm concentration,

Table 8. The effect of vanadium, SO_2, and their combination on polar lipid biosynthesis in jack pine needles.

Polar Lipid[a]	Without SO_2			With 0.34 ppm SO_2			
	V10[b]	V50	V100	V0	V10	V50	V100
	% of Control						
PI	86.7	37.9	10.6	62.1	47.5	31.3	14.6
PC	75.5	25.5	6.0	83.0	47.7	21.4	11.6
PE	93.0	41.7	9.6	66.8	80.3	27.0	13.5
PG	51.8	31.1	8.1	85.7	42.8	24.9	9.8
DGDG	81.9	35.7	8.6	64.8	49.5	25.7	13.8
MGDG	53.9	32.0	5.3	65.9	28.8	16.6	8.7
ESG	71.0	35.5	9.7	71.4	60.2	32.8	13.5

[a] PI = phosphatidyl inositol; PC = phosphatidyl choline; PE = phosphatidyl ethanolamine; PG = phosphatidyl glycerol; DGDG = digalactosyl diglyceride; MGDG = monoglactosyl diglyceride; ESG = esterified sterol glycoside.

[b] The number after V (vanadium) represents ppm of metal in solution. Pine seedlings were either hydroponically treated with vanadium for 7 d or with SO_2 for 3 d. In the combination experiment, after 4 d of hydroponic treatment with vanadium, the seedlings were concurrently treated with the two pollutants for another 3 d.

Table 9. The effect of 50 ppm vanadium and 0.34 ppm SO_2 on polar lipid biosynthesis in jack pine needles[a].

Polar Lipid[b]	Control	SO_2	V	SO_2 + V
		X 10^4 cpm·g^{-1} dry wt[c]		
PI	8.8 ± 1.2[d]	6.0 ± 0.7	5.7 ± 1.4	4.4 ± 0.7
PC	59.8 ± 5.5	44.5 ± 3.1	25.5 ± 5.6	17.0 ± 1.3
PE	26.5 ± 1.2	16.0 ± 1.2	13.0 ± 3.7	8.2 ± 0.8
PG	53.1 ± 3.9	35.4 ± 2.4	18.7 ± 3.8	15.3 ± 3.1
DGDG	24.8 ± 4.3	14.5 ± 3.7	12.4 ± 3.5	8.5 ± 1.8
MGDG	36.7 ± 0.2	24.3 ± 6.7	17.7 ± 1.6	9.4 ± 0.4
ESG	28.2 ± 1.6	18.8 ± 4.1	15.1 ± 2.1	9.7 ± 1.7

[a] Pine seedlings were either hydroponically treated with vanadium for 7 d or with SO_2 for 3 d. In the combination experiment, after 4 d of hydroponic treatment with vanadium, the seedlings were concurrently treated with the two pollutants for another 3 d.

[b] PI = phosphatidyl inositol; PC = phosphatidyl choline; PE = phosphatidyl ethanolamine; PG = phosphatidyl glycerol DGDG = diagalactosyl diglyceride; MGDG = monogalactosyl diglyceride ESG = esterified sterol glycoside.

[c] cpm·g^{-1} dry wt = radioactivity in counts per min (cpm) per gram dry weight of tissue.

[d] Standard deviation.

Table 10. The effect of 50 ppm vanadium and 0.34 ppm SO_2 on the activity of several enzymes in jack pine needles.

Treatment[a]	RuDPC[b]	GO[c]	PO[d]	S Content[e]
	Activity·g^{-1} dry wt			mg·g^{-1} dry wt
Control	46.6 ± 3.2[f]	21.2 ± 0.8	160.7 ± 18.2	1.7
SO_2	40.9 ± 0.8	16.0 ± 1.0	216.8 ± 11.4	2.1
Vanadium	28.4 ± 1.2	11.8 ± 1.6	300.4 ± 26.0	1.7
SO_2 + Vanadium	23.3 ± 0.6	10.0 ± 1.6	262.5 ± 2.9	2.1

[a] Pine seedlings were either hydroponically treated with vanadium for 7 d or with SO_2 for 3 d. In the combination experiment, after 4 d of hydroponic treatment with vanadium, the seedlings were concurrently treated with the two pollutants for another 3d.

[b] RuDPC = ribulose-1, 5-diphosphate carboxylase (X 10^5 cpm).

[c] GO = glycollate oxidase (ΔOD/min).

[d] PO = peroxidase (ΔOD/min).

[f] Standard deviation.

which was indicative of tissue necrosis at high concentration. In the presence of 0.34 ppm SO_2, acid phosphatase and peroxidase activities exhibited an initial stimulation and then declined to almost the same level as produced by 100 ppm vanadium alone. The pollutant mixture containing 0.34 ppm SO_2 and 50 ppm vanadium, however, produced an increased response in acid phosphatase and peroxidase over that produced by 50 ppm vanadium alone. On the other hand, exposure of vanadium treated plants (10 to 100 ppm vanadium) to 0.34 ppm SO_2 did not produce much additional response in glycollate oxidase and ribulose diphosphate carboxylase (Figure 2). In general, the response of various enzymes to increasing concentrations of vanadium (in hydroponic solution) with and without SO_2 can be related to a parallel increase in vanadium.

Table 11 shows a statistical treatment of enzymes (ribulose diphosphate carboxylase, glycollate oxidase, and peroxidase) response to only one concentration of each pollutant (50 ppm vanadium and 0.34 ppm SO_2). Vanadium at 50 ppm concentration was shown to produce a larger response in the activity of all three enzymes than 0.34 ppm SO_2 (Table 11). The changes in enzymic activities due to SO_2 and vanadium treatments individually were statistically different from each other as well as from the control. The pollutant mixture containing SO_2 and vanadium, however, produced very little or statistically insignificant change in enzymic activities over those produced by vanadium alone (Table 11). Response of all three enzymes to SO_2 is related to sulphur content of pine needle tissues.

The data suggest that vanadium and SO_2 both have great potential to cause damage to jack pine in the oil sands area; however, there does not appear to be much additional (additive or synergistic) biological response for the vanadium-SO_2 mixture over the response produced by them individually.

3.3.3 Elemental Analyses

The effect of vanadium and SO_2 on sulphur, potassium, and calcium content of both pine roots and foliage is shown in Table 11. As discussed earlier, the sulphur content of both tissues did not

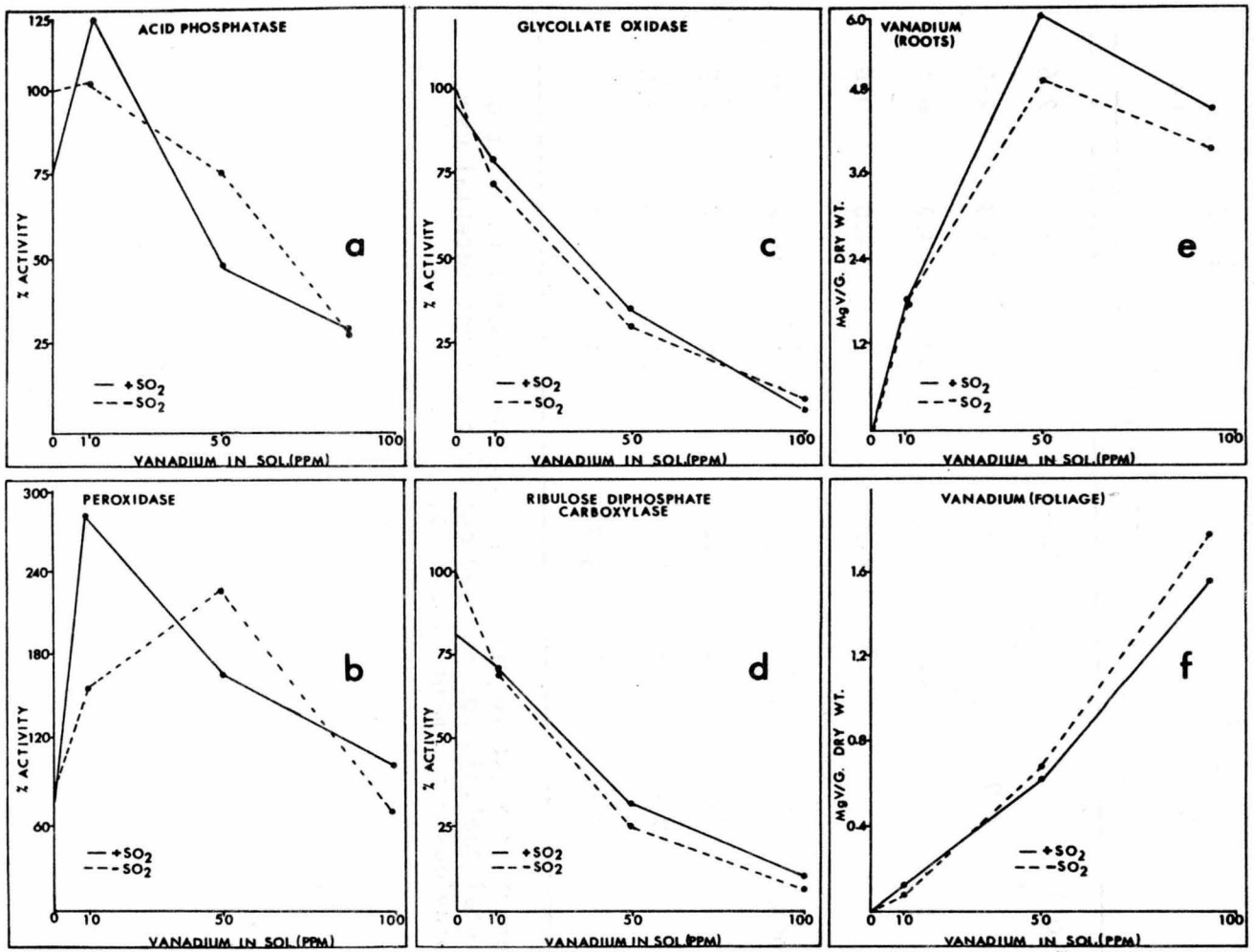

Figure 2. The effect of vanadium alone and in combination with SO_2 on jack pine enzymes and metal uptake by plant tissues.

Table 11. Elemental analysis of total sulphur, potassium, and calcium in the needles and roots of jack pine seedlings exposed to vanadium and SO_2.

Treatment	Needles			Roots		
	S	K	Ca	S	K	Ca
	$mg \cdot g^{-1}$ dry wt					
None (Control)	2.0	7.9	2.9	2.7	18.8	2.6
V10[a]	2.0	8.7	2.9	2.2	14.3	2.9
V50	2.4	12.7	3.2	1.7	4.2	10.1
V100	2.1	12.4	2.9	1.8	4.0	9.8
SO_2 (0.34 ppm)	2.4	10.1	2.9	3.1	19.8	2.1
+V10	2.1	9.0	3.0	2.6	8.7	7.5
+V50	2.6	11.5	4.2	2.2	4.0	9.6
+V100	2.6	13.2	3.7	2.0	3.5	7.7

[a] The number after V (vanadium) represents ppm of metal in solution. The elemental analysis was carried out on the plant material used for the previous experiment (Table 8).

increase appreciably after the mixture (SO_2 + vanadium) treatment over that of only vanadium treatment. Increasing concentrations of vanadium alone, however, caused a general increase in foliar potassium and a decrease in root potassium (Table 11). A similar general trend for potassium was also observed in both roots and foliage of seedlings treated with the mixture (vanadium + SO_2). Increasing concentrations of vanadium alone and in combination with SO_2 did not produce any appreciable change in the calcium content of the foliage but sharply increased its content in the roots. Treatment with vanadium also caused a reduction in the sulphur content of the roots. It is suggested that vanadium can cause toxicity to vegetation by bringing about an imbalance in the nutrients status of both the foliage and the roots.

3.4 PHYTOTOXIC EFFECTS OF NICKEL, SO_2, AND THEIR MIXTURE

3.4.1 Polar Lipid Biosynthesis in Jack Pine Needles

As shown in Table 12, the increasing concentrations of nickel produced a progressive reduction in the biosynthesis of both phospholipids and glycolipids. There was almost a total loss in the biosynthetic capacity of all lipids at 100 ppm nickel concentration. As reported earlier, sulphur dioxide alone inhibited lipid biosynthesis and the response appeared to be related to the sulphur content of the fumigated tissues (Tables 12 and 15). The pollutant mixtures containing nickel and SO_2 caused a reduction in lipid biosynthesis very similar to that produced by nickel alone.

Table 13 shows a statistical treatment of lipid biosynthetic response to only one concentration of each pollutant (50 ppm nickel and 0.34 ppm SO_2) and their mixture. When used individually, SO_2 and nickel produced responses in lipid biosynthesis that, in general, were statistically different from each other as well as from the control (Table 13). The pollutant mixture response was different from that of either control or SO_2 but not from that of nickel alone. The data clearly indicate that nickel can cause a significant inhibition of the important mechanism of lipid biosynthesis.

Table 12. The effect of nickel alone and in combination with SO_2 on polar lipid biosynthesis in jack pine needles.

Polar Lipid[a]	Without SO_2			With 0.34 ppm SO_2		
	Ni10[b]	Ni50	Ni100	Ni0	Ni10	Ni50	Ni100
	% of Control						
PI	37.2	20.7	5.7	63.6	33.0	19.5	10.0
PC	34.2	14.5	6.3	70.9	32.0	26.2	9.6
PE	39.8	22.1	9.6	77.9	41.0	33.1	13.7
PG	32.8	18.8	6.6	71.2	30.8	29.0	13.7
DGDG	36.0	11.4	4.2	61.6	30.2	11.6	6.9
MGDG	30.9	11.2	3.9	61.2	34.1	14.3	7.9
ESG	55.7	33.1	8.4	89.9	55.7	32.1	15.0

[a] PI = phosphatidyl inositol; PC = phosphatidyl choline; PE = phosphatidyl ethanolamine; PG = phosphatidyl glycerol; DGDG = digalactosyl diglyceride; MGDG = monogalactosyl diglyceride; ESG = esterified sterol glycoside.

[b] The number after Ni (nickel) represents ppm of metal in solution. Pine seedlings were either hydroponically treated with nickel for 7 d or with SO_2 for 3 d. In the combination experiment, after 4 d of hydroponic treatment with nickel, the seedlings were concurrently treated with the two pollutants for another 3 d.

Table 13. The effect of 50 ppm nickel and 0.34 ppm SO_2 on polar lipid biosynthesis in jack pine needles.[a]

Polar lipids[b]	Control		SO_2		Ni		SO_2 + Ni	
	x 10^4 cpm·g^{-1} dry wt[c]							
PI	8.6	0.1[d]	6.7	0.1	3.5	0.3	3.7	0.2
PC	54.0	1.8	47.9	3.6	21.9	1.7	19.2	4.4
PE	27.0	2.6	13.3	0.5	11.7	1.1	12.9	1.5
PG	45.7	5.0	35.5	5.6	13.8	0.5	14.5	2.0
DGDG	27.5	2.0	17.3	4.0	8.7	0.7	6.0	1.6
MGDG	36.9	2.0	24.8	0.4	8.9	1.0	6.8	1.8
ESG	22.7	1.0	14.5	4.1	9.9	2.9	8.1	3.3

[a] Pine seedlings were either hydroponically treated with nickel for 7 d or with SO_2 for 3 d. In the combination experiment, after 4 d of hydroponic treatment with nickel, the seedlings were treated concurrently with the two pollutants for another 3 d. The S values are the same as in Table 13.

[b] PI = phosphatidyl inositol; PC = phosphatidyl choline; PE = phosphatidyl ethanolamine; PG = phosphatidyl glycerol; DGDG = diagalactosyl diglyceride; MGDG = monogalactosyl diglyceride; ESG = esterified sterol glycoside.

[c] Radioactivity in counts per min (cpm) per gram dry weight of the tissue.

[d] Standard deviation.

3.4.2 Acid Phosphatase, Glycollate Oxidase, Peroxidase, and Ribulose Diphosphate Carboxylase in Jack Pine Needles

In general, increasing concentrations of nickel (10 to 100 ppm) caused a progressive alteration in the activities of acid phosphatase, glycollate oxidase, peroxidase, and ribulose diphosphate carboxylase (Figure 3). The response patterns to nickel alone and the nickel-SO_2 mixture were essentially similar to those reported in the previous section for vanadium and the vanadium-SO_2 mixture. The extent of enzymatic response appeared to be related to the level of nickel in the foliage and roots of jack pine seedlings (Figure 3).

Table 14 shows a statistical treatment of enzymes (ribulose diphosphate carboxylase, glycollate oxidase, and peroxidase) to only one concentration of each pollutant (50 ppm nickel and 0.34 ppm SO_2). All three enzymes responded more adversely to nickel (50 ppm) than SO_2 (Table 14) and the inhibitory effect of nickel on ribulose diphosphate carboxylase and glycollate oxidase activities was more than that of vanadium. The changes in the activities of all three enzymes due to SO_2 and nickel were statistically different from each other as well as from the control (Table 14). The enzymatic response caused by the SO_2 and nickel mixture was very small and statistically insignificant as compared to that produced by nickel alone; the mixture response, however, was statistically different from that produced by SO_2 and the control. The enzymatic response by SO_2 appeared to be related to the sulphur content of pine foliage. The sulphur content of the pine seedlings treated with nickel, however, remained almost unchanged from that of control (Table 14). Since nickel caused a much more severe wilting of the pine foliage than vanadium, it is suggested that low levels of sulphur in the pine seedlings treated with the pollutant mixture (SO_2 + nickel) may be due to closure of stomata and thus their inability to take up SO_2 from the atmosphere (compare Tables 14 and 10).

It is suggested that nickel when taken up by the vegetation can be highly toxic even at very low concentrations. This toxicity, however, does not appear to increase further in the presence of SO_2.

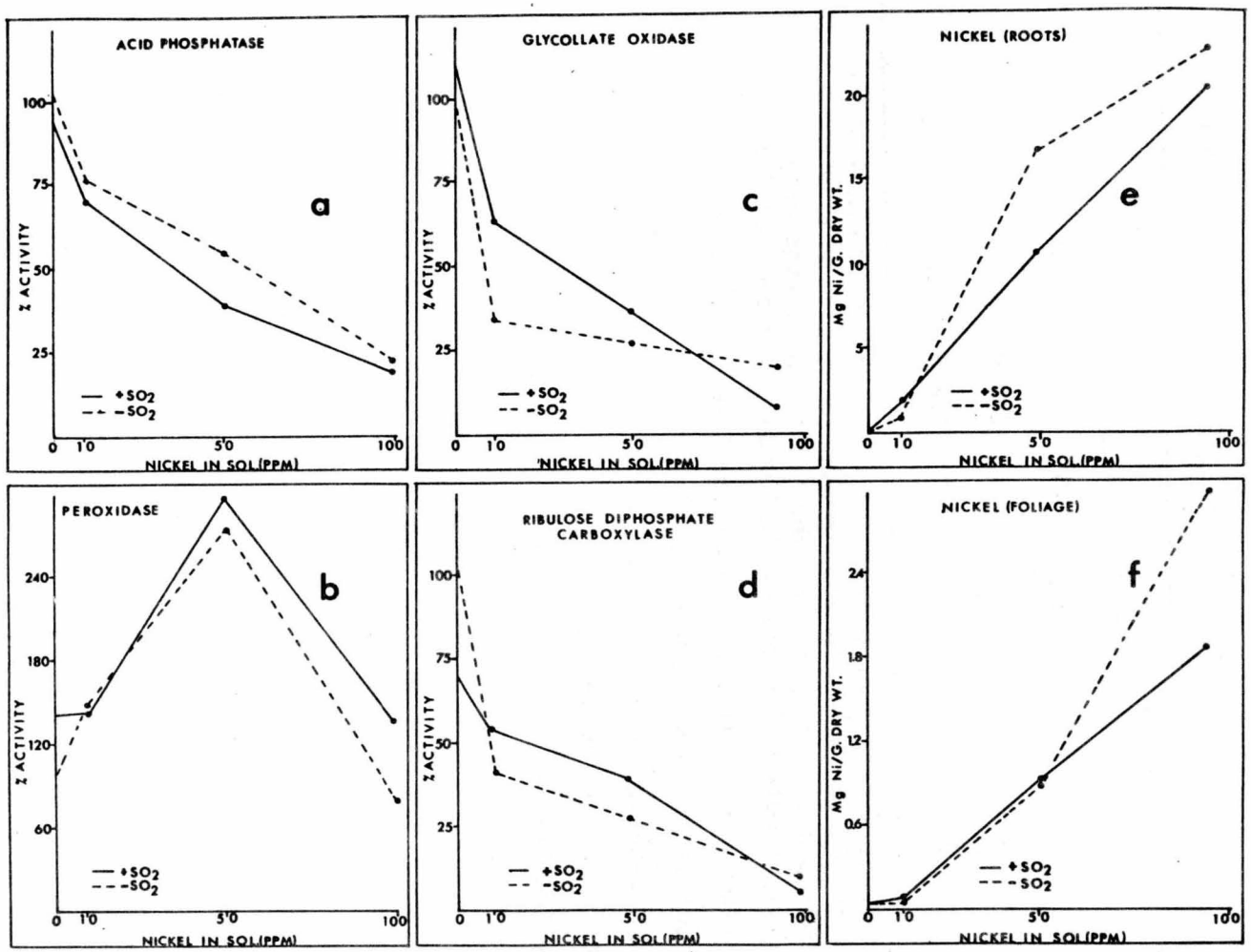

Figure 3. The effect of nickel alone and in combination with SO_2 on jack pine enzymes and metal uptake by plant tissues.

Table 14. The effect of 50 ppm nickel and 0.34 ppm SO_2 on the activity of several enzymes in jack pine needles.[a]

Treatment	RuDPC[b]	GO[c]	PO[d]	S Content[e]
	Activity·g^{-1} dry wt			mg·g^{-1} dry wt
Control	44.2 ± 2.9[f]	23.6 ± 1.3	168.5 ± 1.4	1.2
SO_2	39.3 ± 2.1	9.2 ± 0.6	172.0 ± 1.0	2.1
Nickel	15.4 ± 0.4	3.0 ± 0.5	248.0 ± 2.2	1.2
SO_2 + Nickel	18.6 ± 1.7	2.2 ± 0.5	286.5 ± 12.1	1.3

[a] Pine seedlings were either hydroponically treated with nickel for 7 d or with SO_2 for 3 d. In the combination experiment, after 4 d of hydroponic treatment with nickel, the seedlings were concurrently treated with the two pollutants for another 3 d.

[b] RuDPC = ribulose-1,5-diphosphate carboxylase (x 10^5 cpm).

[c] GO = glycollate oxidase (ΔOD/min).

[d] PO = peroxidase (ΔOD/min).

[e] S Content = sulphur content.

[f] Standard deviation.

3.4.3 Elemental Analyses

In general, increasing concentrations of nickel produced a response in potassium and calcium content of pine tissues (Table 15) similar to the one produced by vanadium (Table 11). It is suggested, therefore, that nickel can be highly phytotoxic in nature. In addition to production of a strong inhibitory effect on biochemical processes, its phytotoxicity is due to its ability to cause a severe imbalance in the nutrient make up of the vegetation. Recently, Koontz and Berle (1980) have shown that exposure of corn and bean roots to silver ions produced a similar kind of imbalance on the uptake of some of the nutrients, including calcium and sulphur.

3.5 PHYSIOLOGICAL AND BIOCHEMICAL ANALYSES OF FIELD SAMPLES

Assessment of air pollution injury to jack pine and epiphytic lichens at three sites in a southeast gradient from the Suncor plant (two closest sites being in the "impingement" zone and the third distant site in a relatively "clean" area) was carried out using some of the biochemical and physiological methods developed in this laboratory for detecting previsual injury of air pollution toxicity. No significant differences in any of the metabolic responses were observed between the sites. This may be due either to the low levels of SO_2 experienced in these areas or the ability of the vegetation to recover its metabolic functions between the rare incidents of heavy fumigation. The levels of sulphur in jack pine were shown to be near the lower end of the normal sulphur range for such species, and those in epiphytic lichens were also low but highly variable. Other reasons for not being able to detect injury to field specimens may be low levels of tissue sulphur and the transient nature of SO_2 injury probably brought about by the ability of the vegetation to detoxify the toxic species of sulphur (conversion of HSO_3^- and $SO_3^=$ to $SO_4^=$). It must be pointed out that some of these biochemical techniques have proved quite useful in detecting air pollution injury to vegetation near an air pollution emission source that has been releasing much larger quantities of emission elements over a considerably longer period of time than the oil sands source (unpublished data). In these areas, there appeared to be a good relationship between the pollutant content of the tissues and the biochemical responses.

Table 15. Elemental analysis of total sulphur, potassium, and calcium in the needles and roots of jack pine seedlings exposed to nickel and SO_2.

Treatment	Needles			Roots		
	S	K	Ca	S	K	Ca
	$mg \cdot g^{-1}$ dry wt					
None (control)	3.3	11.7	4.7	3.8	22.3	3.8
Ni10[a]	-	-	-	2.7	9.2	6.4
Ni50	3.6	13.4	5.2	1.0	3.5	5.9
Ni100	4.1	19.0	5.2	0.4	1.8	4.9
SO_2 (0.34 ppm)	4.3	11.1	4.3	4.0	17.5	3.9
+ Ni10	-	-	-	3.0	10.6	6.2
+ Ni50	4.2	13.2	5.1	1.0	3.2	6.8
+ Ni100	4.2	17.2	4.8	0.5	1.6	4.5

[a] The number after nickel (Ni) represents ppm of metal in solution. The elemental analysis was carried out on the plant material used for the previous experiment (Table 12).

4. CONCLUSIONS AND RECOMMENDATION

Controlled exposure studies have shown that sensitive metabolic processes within epiphytic lichens (one of the forest species most sensitive to air pollution) are altered considerably after fumigation with low levels of SO_2. The essential processes of photosynthesis, protein and lipid biosynthesis, and potassium and magnesium efflux are all affected adversely even at fairly low SO_2 concentrations. In clean air, the recovery of metabolic processes impaired by SO_2 is possible provided the lichens are exposed to low levels of SO_2 (0.1 ppm) for only a limited period of time (3 to 4 wk). It is evident from the fumigation experiments that metabolic injury can occur prior to visual symptoms of pollutant toxicity. Such metabolic alterations would eventually lead to detrimental effects on growth and morphology of lichen tissues. For example, a severe decline in photosynthesis would limit the availability of metabolites essential for the survival of both the algal and fungal components of lichens. Therefore, prolonged exposure of sensitive lichen species to even low levels of SO_2 could disturb their delicate symbiotic relationship; this could lead to changes in their growth, distribution, and cover patterns in the polluted areas. Long-term exposure of even less sensitive vegetation to industrial emissions may result in deleterious effects.

The measurement of biochemical response of lichens, therefore, provides an effective early warning system to detect SO_2 injury to vegetation prior to the development of visible symptoms of toxicity.

Controlled fumigation of vascular species with low levels of SO_2 and NO_2, either singly or in combination, lead to the following important conclusions:

1. Each pollutant produced marked changes in biochemical functions of plants which would eventually be reflected in the growth and yield of vegetation.
2. Biochemical response to a given pollutant varied from one species to another. For example, upon fumigation with NO_2, the enzymes ribulose diphosphate carboxylase (involved in the process of photosynthetic CO_2 fixation) and glycollate oxidase (involved in the process of photorespiration) were stimulated in jack pine but inhibited in paper birch

and green alder. Peroxidase (involved in aging, senescence, nutrient transport, etc.), on the other hand, was stimulated in jack pine and green alder but inhibited in paper birch. An increase in the activity of ribulose diphosphate carboxylase could be beneficial provided it is not accompanied by a large increase in the activity of glycollate oxidase. In jack pine, the increase in ribulose diphosphate carboxylase activity upon NO_2 fumigation was accompanied with a much higher increase in glycollate oxidase activity. Such events would result in a net loss in the photosynthetic activity and growth. On the other hand, a decrease in ribulose diphosphate carboxylase activity (as is the case with all three plant species fumigated with SO_2 and paper birch and green alder exposed to NO_2) would produce similar response due to decreased fixation of CO_2 during photosynthesis.

3. Pollutant mixtures produced effects that were: (a) additive (activities of peroxidase and glycollate oxidase in jack pine and ribulose diphosphate carboxylase in green alder); (b) synergistic (activities of peroxidase and glycollate oxidase in alder); and (c) antagonistic (activities of peroxidase and glycollate oxidase in paper birch). In general, it is obvious that a mixture of SO_2 and NO_2 gases is more phytotoxic than these pollutants individually.

Metal pollutants such as vanadium and nickel also proved to be highly toxic to various metabolic processes. The response produced by the vanadium-SO_2 mixture is very similar to that produced by nickel-SO_2. The pollutant mixtures (metal + SO_2) did not produce much more additional response than that caused by metals alone. It is suggested that in jack pine either of the two metals alone can cause plant injury provided they are present in the soil in a readily available form. Increased soil acidity can increase the availability of such metals to the plants. Once the acidity of soil increases beyond a certain point by extensive fumigations of an area with sulphur and nitrogen gases,

the metals alone can be expected to cause severe toxicity to vegetation. In the oil sands area where the heavy metals do not seem to be in readily available form as yet, any injury to vegetation, if observed, may be attributed to the major pollutant SO_2.

Physiological and biochemical techniques developed in this laboratory have the capability to detect air pollutant (individually as well as in combination) injury to vegetation prior to the development of visible injury symptoms. When these techniques were used on vegetation from the oil sands area, however, no significant differences were observed in any of the physiological or biochemical responses. It is concluded that the vegetation in the oil sands area has either a good capacity to assimilate pollutants such as SO_2 and NO_x into its normal metabolism or it can recover its normal function between the rare incidents of heavy fumigation. Since several biochemical functions responded to air pollutants in vegetation growing in another area, around a much larger emission source than in the oil sands area, it is suggested that the species tested in the oil sands area currently do not suffer from any persistent air pollutant injury. Also, there appears to be no immediate danger of metal toxicity to the native vegetation as the soil pH appears to be well above the levels that cause metal solubilization.

A major portion of our research to date on the impact of airborne emissions from the oil sands industry on forest vegetation has been limited to individual pollutants such as SO_2, NO_2, vanadium, and nickel. Necessary background information is now available on individual pollutants as well as a few pollutant mixtures characteristic of the oil sands operation in relation to certain dominant species of the native vegetation. This information has a direct application in many instances.

It is expected that there will be more future oil sands development resulting in relatively higher levels of oxides of nitrogen and sulphur, and heavy metals such as nickel and vanadium. In view of the fact that pollutant impact on vegetation is a reflection of pollutant mixtures, the effect of which is not-well known, there is a need for further research to determine the impact of such mixtures on a wide

variety of oil sands vegetation and soils. It is suggested that further studies on pollutant mixtures be undertaken under both controlled environment "pollution chamber" and field conditions. The vegetation response should be measured in terms of pollutant mixture impact on seed germination, plant growth, physiological and biochemical processes, and visible injury symptoms. The information thus obtained, along with the rates of pollutant deposition on soils, will enable the management to assess the eventual fate of soils and vegetation at the present and proposed levels of emissions.

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