EFFECTS OF SO_2 AND HEAVY METALS ON PINUS BANKSIANA

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SUMMARY

Exposure of pine seedlings to solutions of heavy metals (vanadium and nickel) either singly or in combination with SO_2 resulted in the uptake of metal, biochemical disturbances and production of severe visual injury symptoms. Individual metal pollutants such as vanadium and nickel and SO_2 proved to be highly toxic to various metabolic processes (glyco - and phospholipids biosynthesis, activities of ribulose diphosphate carboxylase, peroxidase, glycollate oxidase and acid phosphatase). In general, the pollutant mixtures (metal + SO_2) did not produce much more additional response than that caused by metals alone. The response of various biochemical functions to each pollutant appeared to be related to the pollutant uptake by treated tissues.

INTRODUCTION

The oil sands extraction plants in northeastern Alberta (Canada) release a variety of emission elements such as SO_2, vanadium and nickel into the atmosphere. These emissions may have actual or potentially deleterious effects upon forest vegetation. Several studies have indicated that the pollutant response differs when plants are exposed to more than one pollutant (Bennet *et al.* 1975; Skelley *et al.* 1972); such responses may be additive, antagonistic, or synergistic in nature. At present there is a serious lack of essential information relative to the effects of various combinations of SO_2 and heavy metals on native forest vegetation of northeastern Alberta. A study was therefore initiated to conduct controlled environmental research to describe and measure the biochemical and visual impacts of SO_2, vanadium, and nickel and their various combinations on *Pinus banksiana*, a dominant conifer species of northeastern Alberta.

MATERIALS AND METHODS

Pollutant Treatment

For studying the effects of SO_2 in the mixtures with nickel and vanadium, 3 to 4-month-old hydroponically grown jackpine seedlings were used. These seedlings were exposed to SO_2 and metals in high flow microchambers under conditions described earlier (Malhotra and Khan, 1980). 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 foliar tissues were then analyzed for various metabolic functions.

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 1980).

Lipid Biosynthesis

The incorporation of [$1^{-14}C$] acetate into pine needle lipids was measured as described before (Malhotra and Khan 1978).

Dry Weight and Elemental Analyses

The sample dry weight was measured by oven drying the fresh tissues of vascular plants at 80° 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 for vanadium and nickel was done by atomic absorption spectrometry, while sulphur was determined by the method of Carson *et al.* (1972).

RESULTS

Vanadium and nickel caused severe injury to root tissues (dark brown discoloration), which ultimately resulted in desiccation of the foliage. SO_2 alone did not produce such a response; combination of metal and SO_2 produced injury symptoms similar to those of metals alone.

Effect of Vanadium, SO_2 and Their Mixture on Enzymes

Vanadium and SO_2 when applied individually increased the activity of peroxidase and decreased the activities of ribulose diphosphate carboxylase, glycollate oxidase, and acid phosphatase. The mixture containing 0.34 ppm

TABLE 1

Effect of vanadium and SO_2 on the activities of several enzymes in pine needles [a]

Treatment	Peroxidase	Ribulose diP Carboxylase	Glycollate Oxidase	Acid Phosphatase	Elemental Uptake S	Elemental Uptake V
	Activity, % of Control				µg/G dry wt.	
Control	100.0	100.0	100.0	100.0	1700	- [b]
SO_2 (0.34 ppm)	134.9	87.7	75.5	73.7	2100	-
Vanadium (50 ppm)	186.9	60.9	55.7	77.4	1700	700
Vanadium + SO_2 (50 ppm) (0.34 ppm)	163.3	50.0	47.2	46.5	2100	600

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

[b] - undetectable

TABLE 2

Effect of vanadium and SO_2 on the biosynthesis of pine needle lipids [a]

Treatment	Polar Lipids [b]						
	PI	PC	PE	PG	MGDG	DGDG	ESG
	% of Control						
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0
SO_2 (0.34 ppm)	68.2	74.4	60.4	66.7	66.2	58.5	66.7
Vanadium (50 ppm)	64.8	42.6	49.0	35.2	48.2	50.0	53.5
Vanadium + SO_2 (50 ppm) (0.34 ppm)	50.0	28.4	30.9	28.0	25.6	34.3	34.4

a Same as in Table 1

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

TABLE 3

Effect of nickel and SO_2 on the activities of several enzymes in pine needles [a]

Treatment	Peroxidase	Ribulose diP Carboxylase	Glycollate Oxidase	Acid Phosphatase	Elemental uptake S	Ni
	Activity, % of Control				µg/dry wt.	
Control	100.0	100.0	100.0	100.0	1200	-[b]
Nickel (50 ppm)	147.2	34.8	12.7	62.2	1200	870
Nickel + SO_2 (50 ppm) (0.34 ppm)	170.0	42.1	9.3	71.8	1300	910

[a] Same as in Table 1 except nickel was used instead of vanadium.

[b] Same as in Table 1

TABLE 4

Effect of nickel and SO_2 on the biosynthesis of pine needle lipids [a]

Treatment	Polar Lipids [b]						
	PI	PC	PE	PG	MGDG	DGDG	ESG
	% of Control						
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Nickel (50 ppm)	40.7	40.6	43.3	32.4	24.1	31.6	51.0
Nickel + SO_2 (50 ppm) (0.34 ppm)	43.0	35.6	47.8	34.0	18.4	21.8	41.8

[a] Same as in Table 1 except nickel was used instead of vanadium.

[b] Same as in Table 2.

SO_2 and 50 ppm vanadium produced a very little increase in response of ribulose diphosphate carboxylase and glycollate oxidase, a substantial increase in response of acid phosphatase and a decreased response in peroxidase over that produced by 50 ppm vanadium alone (Table 1).

Effect of Vanadium, SO_2 and Their Mixture on Lipid Biosynthesis

Biosynthesis of both phospholipids (phosphatidyl inositol (PI), phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), and phosphatidyl glycerol (PG)) and glycolipids (digalactosyldiglyceride (DGDG), monogalactosyldiglyceride (MGDG), and esterified sterol glycoside (ESG)) was inhibited by SO_2 and vanadium when applied individually (Table 2).

Vanadium (50 ppm) in the presence of SO_2 (0.34 ppm) produced a response that was consistantly higher than that of the control or SO_2 but not always significantly different from that of vanadium alone (Table 2).

Effect of Nickel, SO_2 and Their Mixture on Enzymes

Nickel (50 ppm) produced a substantial increase in the activity of peroxidase and a marked reduction in the activities of ribulose diphosphate carboxylase, glycollate oxidase and acid phosphatase (Table 3).

The nickel (50 ppm) and SO_2 (0.34 ppm) mixture produced a further increase in response of peroxidase while other enzymes remained almost unaffected as compared to their activities with nickel alone (Table 3).

Effect of Nickel, SO_2 and Their Mixture on Lipid Biosynthesis

Nickel (50 ppm) when used individually resulted in a significant decline in biosynthesis of all lipids (Table 4).

The response in the presence of nickel (50 ppm) and SO_2 (0.34 ppm) was consistantly different from that of control or SO_2 (Table 4) but not always significantly different from that of nickel alone.

DISCUSSION

At low concentrations (10-50 ppm) of both vanadium and nickel, peroxidase activity was markedly stimulated. Electrophoretic studies indicated that the increased response was due to synthesis of new isozymes and increased production of the existing ones. SO_2, on the other hand, produced an increased peroxidase response due only to increased synthesis of existing isozymes. Kinetic experiments with purified ribulose diphosphate carboxylase and glycollate oxidase from untreated seedlings showed that vanadium and nickel inhibition of these enzymes was nonspecific. In the case of carboxylase, the metals appeared to interfere with the activation

mechanism of the enzyme and possibly through their effects on -SH groups (Khan and Malhotra - unpublished results)

In general, vanadium and nickel appeared to have more deleterious effect on the activities of various enzymes than SO_2. The response of various enzymes to SO_2, vanadium, and nickel can be related to an increase in sulphur, vanadium, and nickel content of the treated tissues.

The pollutant mixture containing 0.34 ppm SO_2 and 50 ppm of either vanadium or nickel produced little additional response in enzymatic activities over that of vanadium and nickel alone. Such a response is suggested to be due to foliar desiccation and a substantial stomatal closure caused by metal treatment which eventually interfered with SO_2 uptake.

Vanadium, nickel and SO_2 when used individually inhibited biosynthesis of both phospholipids (PI, PC, PE and PG) and glycolipids (DGDG, MGDG, and ESG). These are generally membrane lipids out of which MGDG, DGDG and PG are characteristic of chloroplast membranes (Mudd and Garcia 1975). The galactolipids (MGDG and DGDG) have been implicated in chloroplast structure and function (Shaw *et al.* 1976). Alterations in membrane lipids would, therefore, affect a variety of metabolic functions.

It is suggested that in plants the uptake of these metals and SO_2 can alter metabolic processes which in turn results in a progressive development of visual injury symptoms.

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