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Influence of Vanadium on Soil Phosphatase Activity[1]

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ABSTRACT

Acid phosphatase activity of an organic soil (spruce needle mor) from southern Sweden was significantly reduced by vanadium in concentrations occurring in urban and suburban areas and due largely to fuel oil combustion. The inhibiting power of different forms of added vanadium (100 mg/kg dry weight) decreased in the order $Na_3VO_4 > NaVO_3$, $VOSO_4 > V_2O_5$. Na_2SO_4 and NaCl did not influence the activity. Above 100 mg/kg dry weight, phosphatase activity was inversely proportional to the logarithm of the added $NaVO_3$ concentration.

Additional Index Words: enzyme, inhibition.

The biological effects of V resulting from its emission to the atmosphere on combustion of fuel oils have attracted little attention as an environmental problem. Though greatly variable, the V concentration of fuel oils averages about 30 mg/kg and may be much higher in certain oils (1). Part of this V is emitted to the atmosphere on combustion and deposited on the ground, particularly in densely inhabited areas where it can accumulate in such materials as mosses, lichens, litter, and humus (5). According to recent measurements from southern Sweden (unpublished), the V concentration of spruce needle mor is 50-100 mg/kg dry weight, or sometimes even greater, in and around urban areas, but only 10-20 mg/kg in sparsely inhabited regions.

The principle steps in litter decomposition and humus formation are often governed by soil enzymes. These are mainly of microbial origin and may accumulate extracellularly in the soil. The mineralization of organic phosphorus is largely governed by phosphatases and is of major importance in maintaining the productivity of ecosystems. Little seems to be known about the effects of V on soil phosphatase activity. That metavanadate in vitro may suppress acid phosphatase activity in certain microorganisms has been demonstrated with *Aspergillus orizae* (2) and *Neurospora crassa* (4). The objective of the present paper is to determine whether V, in concentrations occurring in the environment, exerts a significant influence on the acid phosphatase activity of mor soils.

METHODS

Fresh needle mor (pH_{H_2O}, 5.0; loss on ignition, 91-92%) from a *Picea abies* (L.) H. Karst plantation in southern Sweden was sampled in May 1973, sifted through a 5-mm mesh, and carefully mixed. After determination of water content, 15 subsamples of the fresh mor, each containing 1.0 g dry weight, were transferred to 100-ml volumetric flasks. Sodium metavanadate ($NaVO_3$) was added to five flasks in an amount yielding a V concentration of 1,000 mg/kg dry weight. Five flasks were supplied with an equimolar amount of NaCl and five flasks with distilled water to serve as controls. Microbial activity was suppressed by the addition of 2 ml toluene. After 30 minutes, 10 ml phenyl disodium orthophosphate ($C_6H_5 \cdot Na_2PO_4 \cdot 2\ H_2O$); 6.75 g/liter aquous solution; and 10 ml of a HAc-NaAc-buffer, pH 5.0, were added and the flasks incubated at 22 ± 0.2C for 180 ± 1 minute. Phenol concentrations were determined colorimetrically according to Hoffmann (3).

To measure the influence of different vanadate concentrations on phosphatase activity, mor was incubated at concentrations of 0-2,000 mg V/kg, added as $NaVO_3$. Five replicates of all 12 concentrations were run. Mor was also stored aerobically in a humid chamber at 70% of its water-holding capacity and at 20C after addition of 0, 100, and 500 mg V/kg as $NaVO_3$. Eight replicates were used at each concentration. The phosphatase activity of the samples was determined as above after storage for 6 months.

Another sampling was performed in the vicinity of the previous site in early December 1973. After pretreatment as described above, vanadium pentoxide (V_2O_5), vanadyl sulphate ($VOSO_4$), sodium metavanadate ($NaVO_3$), or sodium orthovanadate (Na_2VO_4) was added to five replicates in amounts yielding 100 mg V/kg dry weight. Also, sodium sulphate (amount equimolar to $NaVO_3$) was supplied to five replicates and distilled water to an additional five controls. On incubation the acetate buffer was replaced by 10 ml distilled water. The pH was determined electrometrically in the filtrates; pH differences between different treatments were small and not statistically significant. The influence on the color development of standard solutions from salts or compounds used was tested but found to be negligible.

Total V of the mor samples was analysed by atomic absorption spectrophotometry (acetylene-nitrous oxide flame) after wet combustion with $HNO_3/HClO_4$ (4:1). Throughout, means ± standard deviation are given when five replicates were run.

RESULTS

The original total V concentration of the mor was 19-22 mg/kg dry weight in the spring sample and 24-25 mg/kg dry weight in the winter sample. The addition of 1,000 mg V/kg as $NaVO_3$ decreased phosphatase activity to 31.8 ± 1.6% of the controls (12.63 ± 0.34 mg phenol/g dry weight per 3 hours. The addition of an equimolar amount of NaCl did not measurably influence the activity (97.4 ± 4.9% of controls, difference not significant).

Graded concentrations of $NaVO_3$ added to the mor material reduced the activity to 80.5 ± 5.5% with 30 mg V/kg, to 59.5 ± 2.1% with 50 mg V/kg, and to 52.5 ± 3.2%

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Table 1—Soil phosphatase activity, calculated as percent of non-treated controls, at 100 mg V/kg dry weight with various forms of vanadium, and at equimolar concentration of Na_2SO_4. No buffer added; pH of the filtrates 6.19–6.22 of both controls and treatments

	Percent of controls, mean ± standard deviation (n = 5)†
Control	100.0 ± 5.9 a
V_2O_5	87.4 ± 2.0 b
$NaVO_3$	74.8 ± 3.5 c
Na_3VO_4	71.3 ± 5.4 c
$VOSO_4$	77.7 ± 3.3 c
Na_2SO_4	99.3 ± 3.2 a

† Significant differences ($p < 0.01$) between the means are indicated by different lettering.

with 100 mg V/kg. Above 100 mg V/kg the activity was inversely proportional to the logarithm of the V concentration.

This high sensitivity to metavanadate was confirmed by storage of samples for 6 months at 0, 100, and 500 mg V/kg, added as $NaVO_3$. In the samples stored with 100 mg V/kg, the activity was 70.6 ± 7.9% of the activity of controls (11.68 ± 0.88 mg phenol/g dry weight per 3 hours) stored without added V. The activity of the samples stored with 500 mg V/kg was only 48.5 ± 2.9%. In this, as well as in the previous experiments, an acetate buffer of pH 5.0 was introduced during the 3-hour incubation.

The influence of various V forms was tested without the use of an incubation buffer. The pH of the filtrates was higher (6.19–6.22) but was not influenced by the additions. All forms of V tested (100 mg/kg dry weight) caused a statistically significant reduction of phosphatase activity (Table 1). The influence of V_2O_5 was less than that of $VOSO_4$, $NaVO_3$, or Na_3VO_4 ($p < 0.001$), whereas the influence of Na_3VO_4 was greater than that of $VOSO_4$ ($p < 0.05$). The addition of Na_2SO_4 in amounts equimolar to $NaVO_3$ did not influence the activity.

CONCLUSIONS

In the study by Nicholas and Commissiong (4), complex formation between vanadate and the substrate they used for phosphatase activity measurements (p-nitrophenyl phosphate) was presumed to cause the inhibition. However, Courtois and Anagnostopoulos (2) considered the inhibition to be due to a combination between enzyme and vanadate. The former explanation seems unlikely or incomplete in the light of the present study, because a depression of the activity occurred at V concentrations much too low to account for the necessary blocking of the introduced substrate. One further possibility is the formation of a vanadate-organic phosphate complex, which may have an inhibitory action on phosphatase activity.

It may be concluded that V, in concentrations occurring in the soil of urban and suburban areas, as well as in the vicinity of certain metal processing industries, may suppress soil phosphatase activity. Due to the occurrence of pollutants other than V, however, it may be difficult in empirical studies to distinguish possible effects of V on the phosphatase activity from the influence of other pollutants.

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