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Effects of Herbicides and Fumigants on Microbial Activities in Soil

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Bacteria, actinomycetes and fungi occupy a unique position in biological cycles and are essential for plant growth and soil fertility. The importance of their many roles has been well established. Bollen (1961), Elliot et al. (1972), Tu and Miles (1976) showed that a change in their activities may affect crop yield; yet little is known about the effects of pesticides on beneficial soil microbes. This paper reports the effects of six herbicides and three fumigants on microbial activities in a sandy loam soil.

MATERIALS AND METHODS

The soil used in the experiment was a medium texture sandy loam from Southwestern Ontario. Samples were taken to a depth of 15 cm from soils with no history of pesticide treatment. The bulk sample was sifted through a 2-mm mesh. The soil had 4.42% organic matter, 0.24% Kjeldahl-N, 2.7% clay content and pH of 7.8.

Nine pesticides with minimum of 92% purity were used in the study (Table 1). With the exception of the fumigants, DD, dichloropropene and Vorlex, the required amounts of herbicides at 10 µg/g were applied to the soil using a carrier sand as reported previously (Tu 1970). Fumigants at rate of 100 µg/g were injected directly into and mixed with the soil. Controls, with soil only, were included within all tests. Soil moisture was maintained at 60% moisture-holding capacity. The mixed and control samples were set up in 0.236 litre milk bottles, which were closed with 0.0381 mm (1.5 mil) thick polyethylene film and incubated at 28 ± 1°C for appropriate periods i.e. 1 wk for ammonification, 2 and 3 wk for nitrification, 1 and 2 wk for denitrification, and 4 wk for sulfur oxidation. Changes occurring in the mineralization of soil nitrogen, and sulfur compounds were determined by methods reported previously (Tu 1970).

Determinations were made for triplicate samples and results were expressed on the oven-dry basis.

To study the effect of pesticides on denitrification, 20g portions of soil samples were weighed into 100-mL serum bottles containing KNO_3 (500 µg nitrate-N/g soil) equipped with gas tight butyl-rubber serum stoppers and sealed with an aluminum seal using a hand crimper. The activity of the soil to denitrify nitrate and nitrite was studied by determining the amounts of N_2O-N evolved in the presence of acetylene (C_2H_2) to inhibit reduction of N_2O to N_2 when samples were incubated anaerobically under flood after treatment at 28°C and analyzed for N_2O. A control which received no chemical treatment was included. Gas analysis was carried out by a Varian model 3700 gas chromatograph equipped with a thermal conductivity detector and a Varian model 9176 recorder. A column 2.74 m length by 2 mm internal diameter was packed with Porapak Q (50-80 mesh) operated at temperatures of 70°C for injector, 100°C for column oven, 120°C for detector and 150°C for filament-temperature (Smith and Dowdell 1973), and with helium carrier-gas at flow rate of 23 mL/min. Retention time for N_2O was 42 sec at range of 0.5 and attenuation of 16. Corrections were made for N_2O solubility. Peak area for N_2O was directly proportional to its concentration over the range used in all assays. The capacity of soil samples to reduce NO_3^--N to N_2O provided presumptive evidence of denitrification.

Table 1. Chemicals used in this study

Chemicals	Source	Trade name or code number	Purity (%)
2,4-D	Shell	D 50	98
Dicamba	Velsicol	Banvel	99
Glyphosate	Monsanto	Roundup	100
Paraquat	Chipman	Gramoxone	95
Picloram	Dow Chemical	Tordon	100
Simazine	Ciba-Geigy	Princep	100
DD[a]	Dow Chemical	Vidden D	100
Dichloropropene[b]	Dow Chemical	Telone	92
Vorlex[c]	Nor-Am	Trapex	100

a. 1,3-dichloropropene, 1,2-dichloropropene and related C_3 hydrocarbons mixture.
b. 1,3-dichloropropene and related C_3 hydrocarbons.
c. 80% 1,3-dichloropropene and related C_3 hydrocarbons and 20% methylisothiocyanate.

RESULTS AND DISCUSSION

The influence of different pesticides varied and showed no pattern consistent with length of time of incubation.

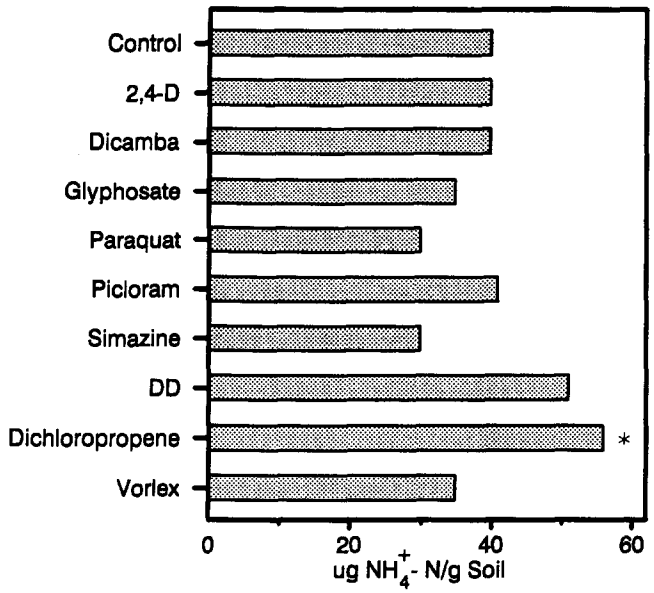

Figure 1. Soil ammonification as related to pesticide treatments. *Significantly different from control at p = 0.05.

With the exception of dichloropropene, none of the pesticides affected microbial ammonification of organic nitrogen indigenous to soil (Figure 1). A significant stimulatory effect on activities of soil ammonifiers was observed with treatment of dichloropropene after one week.

The biological formation of nitrite and nitrate from compounds containing reduced nitrogen is the second stage of the nitrogen cycle in soil. The importance of the nitrifying microorganisms rests on their capacity to produce the nitrate which is the major nitrogen source assimilated by higher plants. This reaction is mainly carried out by Nitrosomonas sp. and Nitrobacter sp.. As compared to "control" with the exception of paraquat and Vorlex, all pesticide treatments affected nitrification of ammonium from soil organic nitrogen during 2 wk incubation. However, no inhibition was observed after 3 wk incubation in all treatments. The recovery of nitrification after 3 wk suggests that nitrifying organisms recover and nitrification proceeds in a normal fashion.

Microbial denitrification, the reduction of NO_3^- and NO_2^- into nitrous oxide (N_2O) or nitrogen gas (N_2) which is lost from soil into the atmosphere, represents a net loss of nitrogen to microorganisms and plants. The process

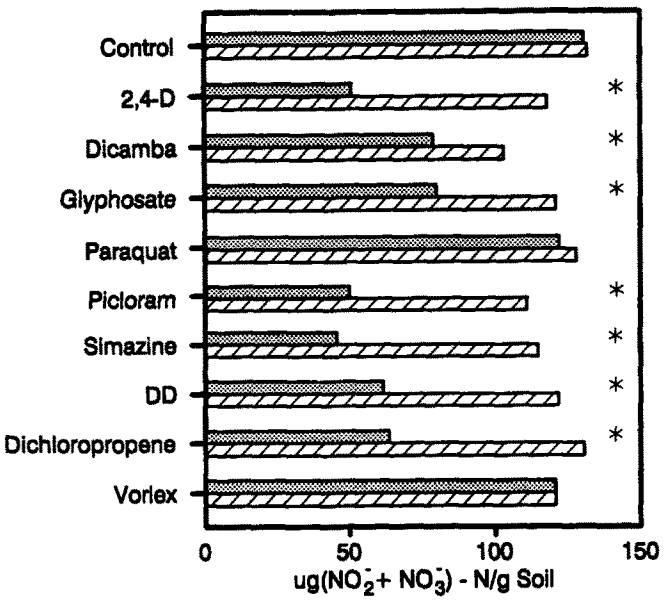

Figure 2. Effect of pesticide treatments on soil nitrification in sandy soil after two-(darkened bars) and three-(slashed bars) weeks incubation. * Significantly different from control at p = 0.05.

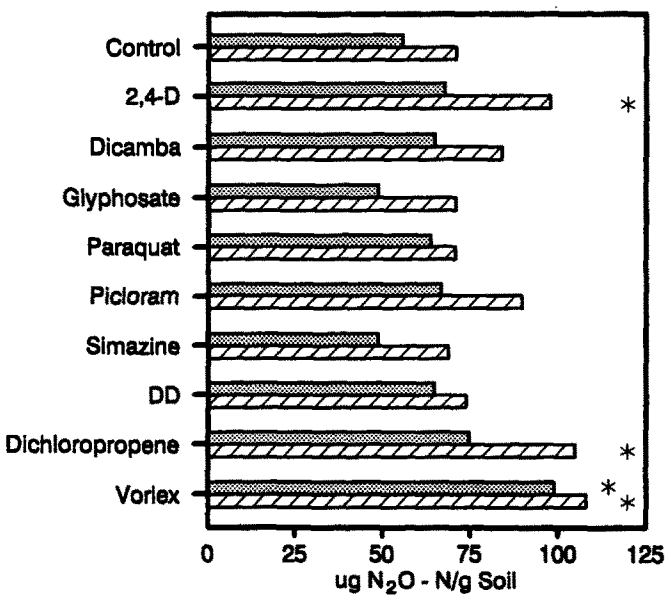

Figure 3. Soil microbial activities in denitrification as related to pesticide treatments in sandy soil after one-(darkened bars) and two-(slashed bars) weeks incubation. * Significantly different from control at p = 0.05

is influenced by soil aeration, moisture, organic matter, acidity and temperature. The major mechanism of nitrogen volatilization and probably the most common means whereby N_2O and N_2 are evolved is by microbial denitrification. Denitrification is known to take place under anaerobic conditions. The active species are largely limited to the genera Pseudomonus sp.,Achromobacter sp. Bacillus sp. and Micrococcus sp (Alexander 1961). Although denitrification is a common and important activity in soil, it is the least investigated. The N_2O evolution from the soil anaerobic assay system (Figure 3) indicated that the pesticides used in the experiment are non-toxic to denitrifying microorganisms.

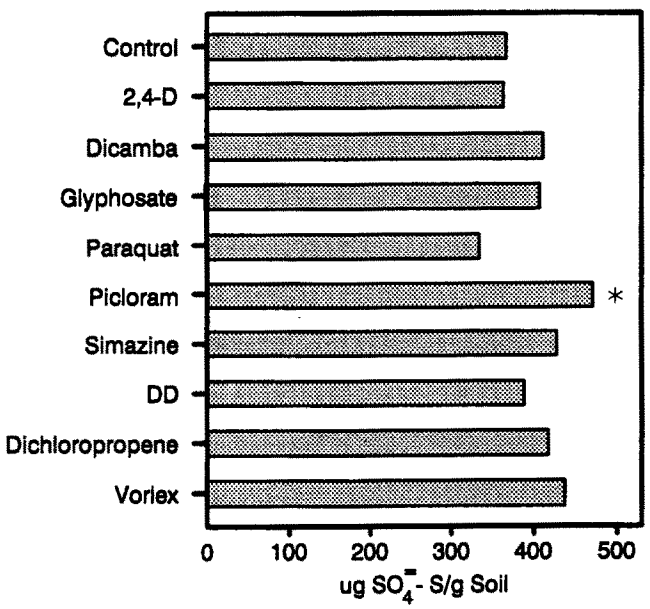

Figure 4. Effect of pesticides on sulfur oxidation of elemental sulfur(1,000 µg/g) at 28°C after 4 weeks incubation in sandy soil. *Significantly different from control at p = 0.05.

A stimulatory effect was observed with treatments of 2,4-D and dichloropropene after 2 wk, and Vorlex for 1 and 2 wk incubation. This may indicate that metabolites of pesticides, 2,4-D, dichloropropene or Vorlex may also be a substrate for certain microorganisms at the conditions of this experiment.

Oxidation of incorporated elemental sulfur ranged from 334 µg $SO_4^=$-S/g soil with reduction of 9% in paraquat compared to control and increased to 473 µg with 29% more in picloram samples compared to the control.

Sulfur enters the soil in the form of plant residues, animal wastes, chemical fertilizers and rain water. A large part of the sulfur in the soil profile is in combination with organic matter. The inorganic sulfate concentration is invariably low (Alexander 1961). Little is known of the chemistry of humus sulfur, but the soil organic fraction probably contains amino acid derivatives of proteins of microbial cells and plant tissues (Russell and Russell 1961).

The pesticide treatments were observed in many experiments to have significant effects on the microbial activities but the microorganisms recovered rapidly. These effects were not drastic but minor in nature. There is little evidence to suggest that these pesticide treatments have any prolonged deleterious effect on the soil microbial activities.

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REFERENCES

Alexander M (1961) Introduction to soil microbiology. John Wiley and Sons Inc. New York.

Bollen WB (1961) Interactions between pesticides and soil microorganisms. Ann Rev Microbiol 15:69-92.

Elliot JM, Marks CF, Tu CM (1972) Effect of nematicides on Pratylenchus penetrans, soil microflora, and flue-cured tobacco. Can J Plant Sci 52:1-11.

Russell EW, Russell EJ (1961) Soil conditions and plant growth. 9th ed. Longmans, Green and Co. Ltd. London.

Smith KA, Dowdell RJ (1973) Gas chromatographic analysis of the soil atmosphere: Automatic analysis of gas samples for O_2, N_2, Ar, CO_2, N_2O and C_1 - C_4 hydrocarbons. J Chromatograph Sci 11:655-658.

Tu CM (1970) Effect of four organophosphorus insecticides on microbial activities in soil. Appl Microbiol 19:479-484.

Tu CM, Miles JRW (1976) Interactions between insecticides and soil microbes. Residue Rev 64:17-65.