Bull. Environ. Contam. Toxicol. (1993) 51:30–39

Bulletin of Environmental Contamination and Toxicology

Influence of Ten Herbicides on Activities of Microorganisms and Enzymes in Soil

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Treatment of soil with chemicals to control soil pests can result in alterations in microbial populations, activities of microflora and enzymes important to soil fertility (Bollen 1961; Tu and Bollen 1968; Tu and Miles 1976). Widespread use of synthetic pesticides and increasing quantities of herbicide residues in agricultural soils (Dustman and Stickel 1966) have led to concern about the effects of these chemicals on non-target soil microorganisms (Bollen 1961; Tu and Miles 1976). Because of the persistence of the herbicides in soil and estuary systems, newer chemicals are being substituted wherever possible. It is important to know if these alternative herbicides have any pronounced influence on soil microbial activities. This paper summarizes results of laboratory studies on the effects of 10 herbicides on microbial and enzymatic activities in a soil.

MATERIALS AND METHODS

The soil used was a loamy sand, a typical agricultural soil in southwestern Ontario. Samples were collected randomly to a depth of 15 cm and the bulk sample was passed through a 2-mm sieve and analyzed for chemical and physical characteristics. The soil had 3.2% organic matter, 0.29% Kjeldahl-N and pH value of 7.6. Procedures for chemical and physical properties of the soil have been reported previously (Tu 1970). Technical grade herbicides used are listed in Table 1.

Herbicides were applied to the soil at 10 μg active ingredient per g of soil using a carrier sand as described elsewhere (Tu 1970). Nitrapyrin at 30 μg and autoclaved soils (steam sterilized at 15 lb pressure at 121°C for 7 hr daily for a period of 4 days and oven-dried at 105°C for 4 hr) were included to compare the effects of the treatments on soil microbial and enzyme

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Table 1. Herbicides used in the study

Chemicals	Source	Trade name	Purity
Allidochlor	Monsanto	Randox-T	100.0
Bentazon	BASF	Basagran	99.5
Chlorbromuron	Ciba-Geigy	Maloran	99.5
Diclofop	Hoechst	Hoegrass	99.0
Diuron	Dupont	Karmex	99.0
EPTC	Stauffer	Eptam	100.0
Ioxynil	May & Baker	Actril	89.5
Monolinuron	Niagara	Afesin	99.0
Nitrofen	Rohm & Haas	Niclofen	85.0
Propazine	Ciba-Geigy	Primatol P	100.0

activities with those of herbicides. Untreated controls were included with all tests. To determine changes in microbial populations, the treated soils were placed in 236-mL milk bottles, which were closed with 1.5 mil thick polyethylene film. Moisture was maintained at 60% moisture holding capacity of the soil. Samples were subjected to soil dilution plate method, using sodium albuminate agar (Waksman and Fred 1922) for bacteria and actinomycetes, and rose bengal streptomycin agar (Martin 1950) for fungi, and plates were incubated in the dark at 28_0C for appropriate periods.

To study the changes in oxidation of ammonium from soil organic nitrogen and organic sulfur, samples were determined for nitrification and sulfur oxidation. Nitrite was analyzed by a diazotization method with sulphanilic acid, α-naphthylamine hydrochloride and sodium acetate buffer (Tu and Bollen 1968) and nitrate was determined by a phenoldisulphonic acid method (Harper 1924). Sulphate was determined turbidimetrically (Tu and Bollen 1968).

To determine soil denitrification activities, 20g soil samples were placed in 100 ml serum bottles and 500 ppm KNO_3-N were added, stoppered with red butyl rubber stoppers and sealed with aluminum seals using a hand crimper. Samples in the serum bottles were evacuated and flushed for 20 min and back-filled with helium to 1 atmosphere three times and 10 mL of gas phase was replaced with the same volume of acetylene (C_2H_2). Samples were then incubated at 28°C for 1 and 2 wk in the dark. Gas chromatographic analysis of N_2O was carried out with a Varian model 3700 gas chromatograph equipped with a thermal conductivity detector and maintained at 70°C for injector; 100°C for column oven; 120°C for detector oven, and 150°C for filament temperature (Smith and Dowdell 1973). The column (2.74 mx2mm id) was packed with 80-100 mesh Porapak Q. Helium was a carrier-gas

operated at a flow rate of 23 mL/min. Retention times for N_2O and N_2 were 42 and 150 sec respectively. Corrections for N_2O solubility were made. Peak area for N_2O was directly proportional to its respective 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.

In soil respiratory studies, triplicate samples of treated and untreated soil (8 g) were placed in Warburg flasks. After equilibration at 30°C for half hour, oxygen consumption was measured at intervals for 96 hr using a Gilson differential respirometer.

Activities of soil enzymes were determined at 1 and 3 d for amylase and 1 and 2 d for invertase. Triplicate samples of 2 g soil were allowed to stand with 0.6 mL toluene for 15 min before incubating with 4 mL acetate-phosphate buffer (0.5 M acetic acid - 0.5 M Na_2HPO_4) at pH 5.5 and 5 mL solution of 2% starch or 5% sucrose. After shaking, the samples were placed in an incubator at 28°C. Controls with or without added substrate were included. Enzyme activities were determined for the reducing sugar using the Prussian blue method of Folin and Malmros (Kolmer et al. 1951). Values for hydrolysis of starch or sucrose by soil enzymes were corrected for the reducing sugars produced on incubation of soil with toluene and buffer without added substrate. Reducing sugars produced were estimated as glucose.

Soil dehydrogenase activity was measured by incubating the soil at 28°C with 2,3,5-triphenyltetrazolium chloride (TTC) for the formation of formazan (2,3,5-triphenyl-tetrazolium formazan) (TTF)(Casida et al. 1964). Activity of soil urease was determined using a steam distillation method (Bremner and Keeney 1966) after 2 and 14 d at 28°C. To test the effects of the treatments on phosphatase activity, 1 g soil in 20-mL serum bottles was treated with p-nitrophenyl disodium orthophosphate and the hydrolysis was determined after 2h (Tabatabai and Bremner 1969). All data were expressed on an oven-dry basis and were averages of triplicate determinations. Data were subjected to analysis of variance, and Duncan's multiple range test was used to determine the level of significance among means.

RESULTS AND DISCUSSION

The effect of the different treatments on microbial populations was summarized in Table 2. Plate counts indicated that both bacterial and fungal counts were affected with treatments of most herbicides after 1 wk, while a stimulatory effect was evident with nitrofen after 2 wk. Although the dilution plate procedure

Table 2. Effect of different treatments on microbial populations in soil.

Treatment	Bacteria ($x10^{-5}$) Incubation period (wk) 1	2	Fungi ($x10^{-3}$) Incubation period (wk) 1	2
Control	199 a*	87 cde	56 a	19 bcd
Autoclaving	1 f	1 f	1 g	1 e
Nitrapyrin	191 ab	143 ab	48 ab	24 bc
Allidochlor	152 bc	94 cd	44 abc	19 bcd
Bentazon	128 cde	82 cde	17 f	13 d
Chlorbromuron	105 de	56 e	17 f	16 cd
Diclofop	157 abc	80 cde	27 def	19 bcd
Diuron	144 bcd	78 cde	24 ef	16 cd
EPTC	152 bc	85 cde	33 cde	17 bcd
Ioxynil	166 abc	72 de	47 ab	22 bcd
Monolinuron	121 cde	90 cde	23 ef	21 bcd
Nitrofen	161 abc	163 a	38 bcd	38 a
Propazine	147 bcd	94 cd	29 def	26 b

* Mean values within each column followed by the same letter are not different significantly at 5% level.

reflects the selective influence of the medium, favours dominant propagules, and can only be taken as an indication of what could occur in soil, it neverthless represents a practical basis for comparing pesticides tested during the same study. Autoclaving resulted in inhibition of the microbial populations throughout the experiment.

Inhibitory effect on nitrification was observed with treatments of autoclaving and nitrification inhibitor, nitrapyrin throughout the experimental period (Table 3). Stimulatory effect on nitrification of ammonium-N mineralized from soil organic nitrogen after 1 wk was observed with many treatments, and with allidochlor and diuron after 2 wk.

Mineralization and oxidation of soil native organic sulfur were not influenced by the presence of herbicides (Table 3). Oxidation of the indigenous soil sulfur ranged from 0.2% with autoclaving to 142% with propazine after 4 wk and to 173% with ioxynil after 8 wk. With the exception of autoclaving, none of the treatments depressed sulfur oxidation.

Soil gaseous N loss from KNO_3 into atmosphere occurs primarily as N_2O and N_2 as a result of reductive process (denitrification) and the presence of C_2H_2 permits measurements of N_2O accumulation in soil. The employment

Table 3. Different treatments on microbial activities in a soil.

Treatment	Nitrification $\mu g(NO_2^- + NO_3^-)$-N/g		S-oxidation $\mu g SO_4^=$-S/g		Denitrification $\mu g\ N_2O/g$	
	Incubation period (wk)					
	1	2	4	8	1	2
Control	12 g*	80 bcd	58 abc	51 bc	12 def	44 cd
Autoclaving	1 i	3 h	1 d	6 d	1 g	3 d
Nitrapyrin	1 i	13 g	62 abc	61 abc	12 def	201 abc
Allidochlor	89 c	95 a	47 c	80 ab	6 ef	165 abc
Bentazon	140 a	84 b	58 abc	39 c	138 abc	141 abcd
Chlorbromuron	77 d	71 def	54 bc	65 abc	112 bcd	127 abcd
Diclofop	61 ef	68 def	52 bc	61 abc	144 ab	191 abc
Diuron	66 e	91 a	75 ab	74 ab	111 bcde	64 cd
EPTC	58 f	80 bcd	51 bc	83 ab	61 bcdef	271 a
Ioxynil	106 b	75 cdef	55 bc	88 a	23 def	260 ab
Monolinuron	9 gh	82 bc	45 c	78 ab	11 def	118 abcd
Nitrofen	12 g	74 def	37 c	87 a	8 def	102 bcd
Propazine	10 g	72 def	82 a	60 abc	10 def	161 abc

* Mean values within a column followed by the same letter do not differ significantly at 5% level.

of C_2H_2 blockage of N_2O to N_2 permits accurate quantification of N_2O and hence the denitrification potential of the soil (Yoshinami and Knowles 1976). Although denitrification is a common and important activity in soil, it is the least investigated. The effect of different treatments on denitrification over 1 and 2 wk is presented in Table 3. With the exception of autoclaving, none of the treatments affected N_2O formation in the soil. However, a stimulatory effect on N_2O formation was observed with bentazon and diclofop after 1 wk, and with EPTC and ioxynil after 2 wk incubation. Although biological fixation of atmospheric N_2 by heterotrophic bacteria does not appear to be important, there is evidence that fixation by photosynthetic cyanobacteria, symbiotic nitrogen fixers and incorporation of nitrogen by rain, plantseeds and fertilizer may contribute significantly for the nitrogen input (Powlson and Jenkinson 1990).

The influence of different herbicides on microbial activities varied showing no pattern consistent with time of incubation. Soil microbial respiration as indicated by oxygen consumption is an index of the activity of microflora involved in soil indigenous organic matter decomposition. The effect of different treatments on respiration over 96 hr was significantly different from that of control sample and is presented in Figure 1. All chemical treated samples consumed greater amount of oxygen than those of controls. The lowest response from autoclaved soil was 58 $\mu L/g$. The behavior of limited oxygen consumption in autoclaved soil has been noted by other workers (Birch 1960; Stevenson 1956; Tu 1970; 1977).

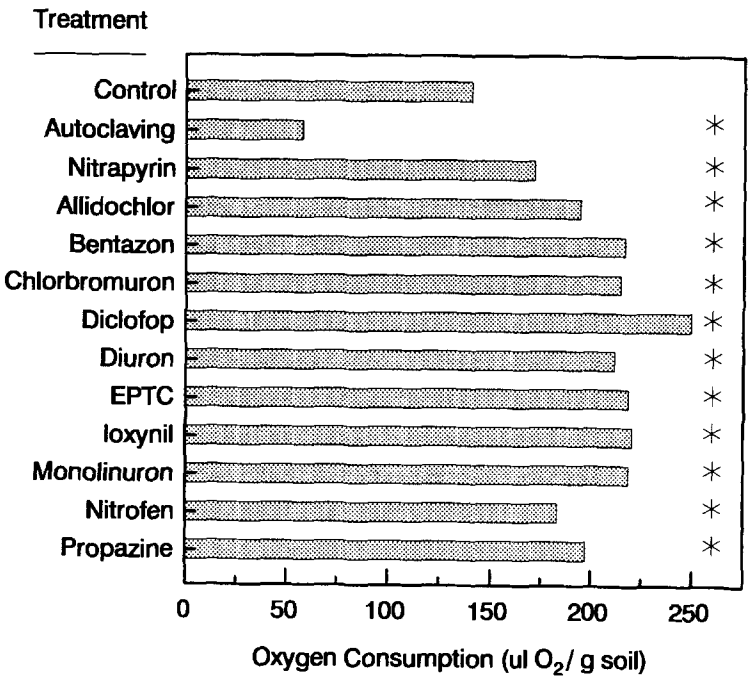

Figure 1. Microbial respiration as related to different treatments of a soil after 96 hr. * Significantly different from control at 5% level.

Amylase is an enzyme which catalyzes the hydrolytic depolymerization of polysaccharides. All treatments inhibited amylase activities after 1 day incubation (Table 4), while none of the treatments inhibited amylase activity after 3 days. Invertase activity was consistently greater than that of amylase. Allidochlor, bentazon, chlorbromuron, monolinuron, nitrofen and propazine reduced invertase activity at 1 day (Table 4), while all herbicide treatments were equal to that of the control after 2 days. Autoclaving inhibited formation of reducing sugar. Drobnik (1955) reported that amylase is inducible in a soil with high organic carbon content which usually exhibits greater activities in amylase (Ross 1965) and invertase (Hofmann and Braunlich 1955). Soil dehydrogenase system has a role in the initial stage of oxidation of soil organic matter. With the exception of autoclaving and allidochlor 7 and 21 days following treatment, and nitrofen after 7 days, none of the treatments inhibited dehydrogenase activity (Table 5). Formazan production in samples treated with diclofop and ioxynil was significantly greater after 7 days incubation.

Table 4. Activities of amylase and invertase as related to different treatments of soil.

Treatment	Amylase mg glucose/g soil Incubation period (Days)		Invertase	
	1	3	1	2
Control	36 b*	32 bc	127 a	167 bcde
Autoclavin	38 ab	42 ab	45 g	55 f
Nitrapyrin	23 def	38 b	117 b	201 a
Allidochlor	25 cdef	26 c	94 ef	153 de
Bentazon	19 f	29 c	103 cde	167 bcde
Chlorbromuron	23 def	29 c	112 bc	178 abcd
Diclofop	26 cde	31 bc	131 a	183 abc
Diuron	26 cde	27 c	127 a	184 abc
EPTC	27 cde	29 c	127 a	164 bcde
Ioxynil	28 cd	32 bc	126 a	152 e
Monolinuron	29 cd	31 bc	117 b	160 cde
Nitrofen	30 c	27 c	91 f	147 e
Propazine	25 cdef	30 c	104 cd	166 bcde

* Within each column, mean values followed by the same letter are not significantly different at 5% level.

Soil urease is the enzyme that catalyzes the hydrolysis of urea to ammonia and carbon dioxide. Due to the increased use of urea as a fertilizer in agriculture ,

Table 5. Effect of different treatments on activities of enzymes in a soil.

Treatment soil	Dehydrogenase mg Formazan/g soil Incubation period (Days)		Urease 100 $\mu g(NH_4^+ -N)/g$	
	7	21	2	14
Control	25 cd*	56 ab	14 bcd	36 ab
Autoclaving	10 g	23 d	6 e	5 c
Nitrapyrin	25 cd	50 b	15 abcd	37 a
Allidochlor	21 f	42 c	16 abcd	35 ab
Bentazon	25 cd	53 ab	13 d	35 ab
Chlorbromuron	27 abc	57 a	18 a	29 bc
Diclofop	28 ab	56 ab	16 abcd	35 ab
Diuron	26 bcd	58 a	14 bcd	32 ab
EPTC	26 bcd	54 ab	18 a	36 a
Ioxynil	29 a	56 ab	16 abcd	35 ab
Monolinuron	27 abc	56 ab	17 abc	34 ab
Nitrofen	23 ef	50 b	15 abcd	36 a
Propazine	25 cd	55 ab	14 bcd	35 ab

* Mean values within each column followed by the same letter are not different significantly at 5% level.

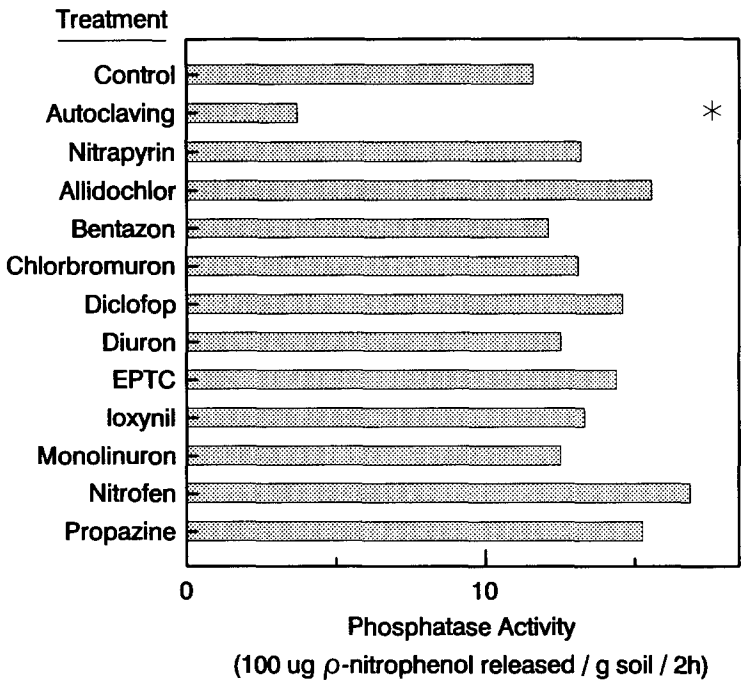

Figure 2. Activity of phosphatase as related to treatment of a soil with different chemicals and autoclaving after 96 hrs. * Significantly different from control at 5% level.

soil urease has attracted a great deal of attention. Treatment of the sandy soil with autoclaving reduced urease activity throughout the experiment while a stimulatory effect was observed with chlorbromuron and EPTC for 2 days.

Phosphatase in soils is largely responsible for mineralization of organic phosphorus and is of major agricultural and economic importance (Cosgrove 1967). Hydrolysis of an incorporated substrate, p-nitrophenyl disodium orthophosphate, by phosphatase (Figure 2) was equal to that of control in the sandy soil. Autoclaving was inhibitory after 2 h incubation.

Every one of these indices should be used in conjunction with other tests to lead to a better understanding of the effects in the soil ecosystem. It is inappropriate to attempt to use only one or two indices as general means of estimating biological or enzymatic activity in a soil system (Ausmus 1973).

The present study indicated that the chemicals at the levels tested did not drastically reduce the activities of microbes and enzymes in the soil and did not have deleterious effect on soil fertility important to plant growth.

Acknowledgment. The author gratefully acknowledges the technical assistance provided by Mr. G. Hietkamp.

REFERENCES

Ausmus BS (1973) The use of the ATP assay in terrestrial decomposition studies. Bull Ecol Res Comm (Stockholm) 17:223-234

Birch HF (1960) Nitrification in soils after different periods of drying. Plant and Soil 12:81-98

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

Bremner Jm, Keeney DR (1966) Determination and isotope-ratios analysis of different forms of nitrogen in soils. 3. Exchangeable ammonium, nitrate, and nitrite by extraction-distillation methods. Proc Soil Sci Soc Amer 30:577-582

Casida LE Jr., Klein DA, Santoro T (1964) Soil dehydrogenase activity. Soil Sci 98:371-376

Cosgrove DJ (1967) Metabolism of organic phosphates in soil. In: McLaren AD, Peterson GH (eds), Soil biochemistry, Vol. 1, pp.216-228. Marcel Dekker, N.Y.

Drobnik J (1955) The hydrolysis of starch by the enzymatic complex of soils. Folia Biol 1:29-40

Dustman EH, Stickel LF (1966) Pesticide residues in the ecosystem. In:ASA special publication No.8, Pesticides and their effects on soils and water. Soil Sci Soc Amer Inc. Madison, Wisconsin, PP109-121.

Harper HJ (1924) The accurate determination of nitrates in soils. Ind Eng Chem Anal Edit 16:180-183

Hofmann E, Braunlich K (1955) The saccharase contents of soils as affected by various factors of soil fertility. Z. Pflanzen Dung Bodenk 70:114-123

Kolmer LA, Spaulding EH, Robinson HW (1951) Approved laboratory technique, 4th ed, Appleton-Century-Crafts, Inc. pp 987-988

Martin JP (1950) Use of acid rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci 69:215-233

Powlson DS, Jenkinson DS (1990) Quantifying inputs of non-fertilizer nitrogen into an agro-ecosystem. In: Harrisen AF, Inerson P and Heal OW (eds), Nutrient cycling in terrestrial ecosystems. Elsevier Applied Science Ltd., N. Y., pp.56-68.

Ross DJ (1965) A seasonal study of oxygen uptake of some pasture soils and activities of enzymes hydrolysing sucrose and starch. Soil Sci 16:73-85.

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. Chromatographic Sci 11:655-658
Stevenson IL (1956) Some observations on the microbial activity in remoistened air-dried soils. Plant and Soil 8:170-182
Tabatabai JJ, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1:301-307
Tu CM (1970) Effect of four organophosphorus insecticides on microbial activities in soil. Appl Microbiol 17:479-484
Tu CM (1977) Influence of physical treatments on acetylene reduction (nitrogen fixation) in soils. Comm Soil Sci Plant Anal 8:605-613
Tu CM, Bollen WB (1968) Effect of paraquat on microbial activities in soils. Weed Res 8:28-37
Tu CM, Miles JRW (1976) Interaction between insecticides and soil microbes. Residue Rev 64:17-65
Waksman SA, Fred EB (1922) A tentative outline of the plate method for determining the number of microorganisms in the soil. Soil Sci 14:27-28
Yoshinari T, Knowles R (1976) Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochem Biophys Res Commu 69:605-710

Received August 10, 1992; accepted December 22, 1992.