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Effect of Three Newer Pesticides on Microbial and Enzymatic Activities in Soil

C. M. Tu

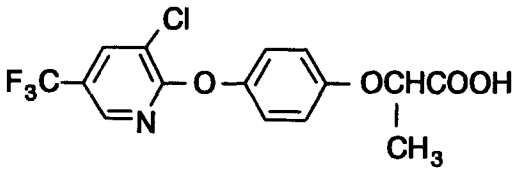
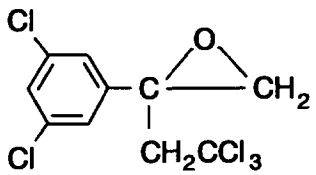
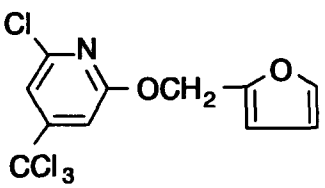
Research Centre, Agriculture Canada, 1400 Western Road, London, Ontario, N6G 2V4, Canada

Many studies (Bollen 1961; Karanth et al 1975; Tu and Miles 1976) indicate that pesticides have little effect on microbial and enzyme activities related to soil fertility. Because of the persistence of some of these compounds in soil and the development of pesticide resistance by soil pests, i.e. weeds and plant pathogens, other chemicals are being used whenever possible. It is of importance to know if these alternative pesticides have any pronounced influence on the activities of soil microorganisms and enzymes. This paper reports the effects of three newer pesticides on activities of microorganisms, enzymes and on populations of indigenous microorganisms in a sandy loam.

MATERIALS AND METHODS

A sandy loam (2.85% organic matter, 0.19% Kjeldahl nitrogen, pH 7.6, 43% moisture-holding capacity) of southwestern Ontario was collected from the surface 15 cm and sifted through a 2-mm mesh screen. Soil organic matter was determined by chromic acid titration (Walkley and Black 1934). The pH was measured in a 1:5 soil:water suspension using a Corning model 10 glass-electrode pH meter. The procedures for microbial, chemical and physical analyses of the soil were reported previously (Tu 1970). Pesticides tested were herbicides, haloxyfop (99.7%) and tridiphane (99.5%), and a fungicide, pyroxyfur (EC72%) (Table 1). These were applied to the soil at 10 ug active ingredient (AI) per gram of soil using a carrier sand (Tu 1970). A broad spectrum germicide, mercuric chloride at 80 ug/g, a nitrification inhibitor, nitrapyrin at 40 ug/g, and autoclaved soil (steam sterilized at 121^0 C for 7h daily for 5 days and oven-dried at 105^0 C for 8h) were included for comparison. Untreated controls with soil only were included with all tests. All data are expressed on an oven-dry basis and are averages of triplicate determinations. The mixtures and controls were set up in 0.236 litre milk bottles, which were closed with

Table 1. Pesticides used in the study*.

Common name	Chemical name and structure	Half life $t_{\frac{1}{2}}$
haloxyfop	2-{4-[(3-chloro-5-trifluoromethyl)-2-pyridinyl]oxy]phenoxy}propanoic acid. 	55days
tridiphane	2-(3,5-dichlorophenyl)-2-(2,2,2-trichloroethyl)-oxirane 	28days
pyroxyfur	2-chloro-6-(2-furanylmethoxy)-4-(trichloromethyl)pyridine 	24hrs

* Humburg(1989), Schmitthenner and Kroetz (1982).

0.0381mm (1.5 mil) thick polyethylene film. The required amounts of technical herbicides, haloxyfop and tridiphane, were dissolved in a distilled acetone-pentane solvent mixture (1:1; v/v) while formulated fungicide, pyroxyfur was suspended in water. Both technical and formulated materials were mixed separately with a carrier sand. After the solvent mixture had evaporated from the technical samples, both the treated sands were incorporated with soil. Moisture was maintained at 60% of the soil moisture-holding capacity.

Changes in populations and activities of soil microflora in mineralization of soil nitrogen, nitrification and

sulfur oxidation were determined by the methods reported previously (Tu 1970). Samples were incubated at 28^0 C for 1 and 3 wks for biomass and nitrification, and 2 and 4 wks for sulfur oxidation studies. In the soil respiration experiments, oxygen consumption in pesticide-treated and untreated soils was determined at 30^0 C for 96h using 8-g samples of soil in a Warburg flask (Tu 1970). Data were analyzed statistically using analysis of variance.

Dehydrogenase activity was measured by the formation of formazan (2,3,5-triphenyl tetrazolium formazan) after incubating the soil samples at 28^0 C in a system containing 2,3,5-triphenyl tetrazolium chloride (TTC) (Casida et al 1964). Soil phosphatase was evaluated by hydrolysis of p-nitrophenol disodium orthophosphate (Tabatabai and Bremner 1969). Effects of the pesticides on soil urease activity were assessed after 1 wk incubation using steam distillation (Bremner and Keeny 1966). Nitrogenase activity was determined by acetylene reducing capacity using gas chromatography (Tu 1978). Adenosine 5'-triphosphate (ATP) content was analysed after 1 and 2 days with a luminometer model 1070 (Tu 1982). An internal standard for correction of extraction loss was also used. In addition to the controls, soil samples without substrates were included to measure endogenous formation of enzymatic reactions.

RESULTS AND DISCUSSION

An analysis of variance on data summarized in Table 2 revealed no significant difference in bacterial numbers after 1 and 3 wks incubation of soil with pesticides at 10 ug/g. All treatments inhibited fungi for 1 wk and stimulatory effects were observed with tridiphane, pyroxyfur and nitrapyrin after 3 wks. Autoclaving was inhibitory to both microbes throughout the experiment.

The effect of pesticides on nitrification is summarized in Table 3. The nitrification with different treatments is too small to be important after 1 wk, perhaps due to a limited population of nitrifiers; however, there were significant differences between treatments after 3 wks incubation; haloxyfop, pyroxyfur and autoclaving were inhibitory to nitrification. Once the pesticide has been dissipated, the treated soil often responds to inoculation although natural contamination would ultimately overcome the deficiency.

Sulfur oxidation was extensive in soils (Table 3). Decreases in pH were in line with acidity developed by the oxidatuion. Thiobacillus thiooxidans is largely responsible for oxidation of sulfur in soils, but T. thioparus and T. denitrificans metabolize free sulfur as

Table 2. Changes in microbial populations with different treatments of a sandy soil.

Sample	Bacteria ($x10^3$) Incubation Period (wks) 1	3	Fungi ($x10^3$) 1	3
Control	64	290	46	27
Autoclaving	1*	1*	1*	1*
$HgCl_2$	72	410	7*	27
Nitrapyrin	49	352	9*	53*
Haloxyfop	73	318	14*	34
Tridiphane	65	358	9*	57*
Pyroxyfur	60	351	14*	87*

* Significantly different from control at p=0.05.

Table 3. Effect of different treatments on microbial activities in a sandy soil.

Sample	Nitrification ug(NO_2^-+NO_3^-)-N/g Incubation Period (wks) 1	3	S-oxidation ug $SO_4^=$-S/g 2	4
Control	0.01	5.17	291	587
Autoclaving	0.01	0.10*	264	281*
$HgCl_2$	0.01	3.52	294	570
Nitrapyrin	0.01	6.32	257	434
Haloxyfop	0.10	0.53*	191	314*
Tridiphane	0.03	4.16	220	526
Pyroxyfur	0.22	0.68*	341	613

* Significantly different from control at p=0.05

well. Experimental results indicated that haloxyfop was inhibitory to sulfur oxidation after 4 weeks, as was autoclaving.

A comparison of oxygen consumption in the control soils with that in the soil of different treatments showed a vigorous response to the treatments, indicating an active heterotrophic microflora in each case (Fig. 1). Haloxyfop, pyroxyfur and $HgCl_2$ significantly increased decomposition of the native organic matter, while treatment with autoclaving decreased it.

The measurement of dehydrogenase activity in soil is utilized to obtain correlative information on the

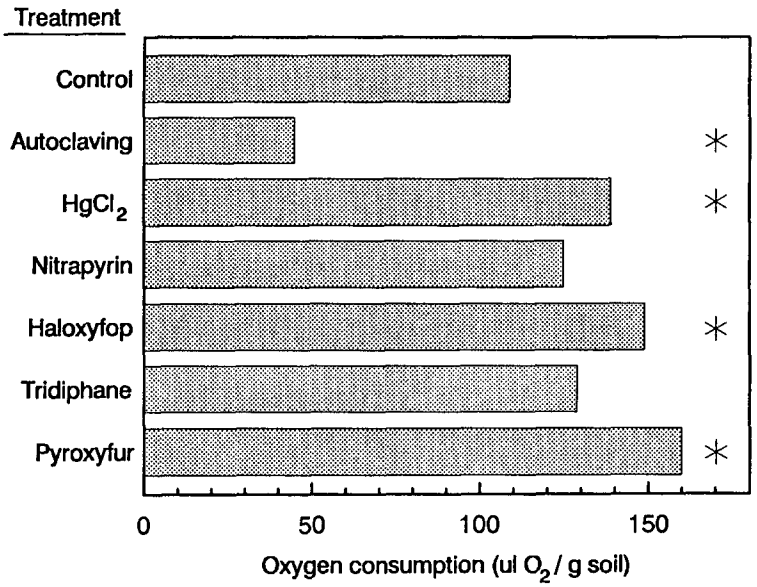


Figure 1. Changes in oxygen consumption of soil microorganisms as related to different treatments in sandy soil after 96 h. * Significantly different from control at p=0.05.

biological activities of microbial populations in soil rather than on the enzyme itself. Fertile and cultivated soils exhibit high dehydrogenase activity (Skujins, 1973). Although a nonbiological TTC reduction occurs in samples at temperatures higher than 65^0 C (Casida et al 1964) the formazan release at the temperature used in this assay, 28^0 C is due to biological activity only. Active dehydrogenases are considered to exist in soils as integral parts of intact cells and dehydrogenase activities are thought to reflect the total range of oxidative activities of the soil microflora. Neverthless dehydrogenase activities do not consistently correlate with numbers of microorganisms in soils or with rates of soil oxygen consumption (Skujins 1973). No inhibitory effect on dehydrogenase activity was observed with different treatments throughout the 7 days experiment (Table 4). Although autoclaving exhibited greatest TTC reduction in samples resulted from nonbiological reduction (Casida et al 1964).

Greaves and Webley (1964) have demonstrated that phosphatase is an exocellular enzyme, to be excellent mineralizer of organic phosphorus in culture media and is produced by a large number of soil microbes. Several

Table 4. Effect of different treatments on enzyme activities in a sandy soil.

Sample	Dehydrogenase Formazan mg/g		Phosphatase p-nitrophenol 100ug/g	Urease NH_4^+-N 100ug/g
	Incubation Period			
	2d	7d	2h	7d
Control	2	4	22	39
Autoclaving	127*	140*	9*	34*
$HgCl_2$	16*	28*	15	39
Nitrapyrin	8*	15*	20	16*
Haloxyfop	17*	33*	21	37
Tridiphane	15*	30*	20	39
Pyroxyfur	12*	22*	20	38

* Significantly different from control at p=0.05.

other workers (Geller and Dobrotvorska 1960) have shown that it accumulates as a result of microbial activity. Kotelev and Mekhtieva (1961) indicated that in certain soils the phosphatase activity of soil, rhizosphere bacteria and actinomycetes was greater than that of fungi. No significant effect on activities of phosphatase was observed with pesticides (Table 4). However, autoclaving was inhibitory to phosphatase activities. Phosphatase activities correlated with bacterial populations in samples.

Urease catalyzes the hydrolysis of urea to carbon dioxide and ammonia. It is widely distributed in plants and microorganisms and has been detected in soils. Soil urease activity tends to correlate with organic carbon and cation-exchange capacity (Dalal 1975). Studies of the effect of different treatments on urease activities in soils (Table 4) have shown that with the exception of autoclaving and nitrapyrin treatments, urease was not inhibitory after 7 days.

The capacity of soil samples to reduce C_2H_2 to C_2H_4 provides evidence for potential N_2 fixation (Hardy et al 1973). The effect of pesticides on C_2H_2 reduction to C_2H_4 by nitrogenase was measured after 2 and 7 days in soil. The values shown in Table 5 have been corrected for endogenous formation of C_2H_4 from the soils. None of the treatments affected C_2H_2 reduction in the soil as relative to the controls. Autoclaving caused a significant increase in C_2H_2 reduction in the soils. Heating of soils and remoistening results in liberation of soluble organic matter (Paul and Tu 1965) and reducing substances

Table 5. Effect of different treatments on nitrogenase activities and level of ATP in sandy soil.

Sample	Nitrogenase nM(C_2H_2-->C_2C_4)/g Incubation Period (days)		ATP changes ug ATP/g Incubation Period (days)	
	2	7	1	2
Control	2.5	3.8	3.9	7.0
Autoclaving	16.7*	10.5*	0.3*	1.5*
$HgCl_2$	1.6*	5.8	0.8*	1.4*
Nitrapyrin	2.3	7.4	2.8	5.9
Haloxyfop	2.6	4.3	4.4	6.8
Tridiphane	2.7	6.5	3.2	14.0*
Pyroxyfur	3.2	5.1	3.7	7.8

* Significantly different from control at p=0.05.

(Dahr 1959) necessary for C_2H_2 reduction. Such non-enzymatic nitrogen fixation in soil has been reported by some workers (Brill 1977; Tu 1977).

Adenosine 5'-triphosphate occurs in all living cells and is a useful indicator of life in soil. In dead cells, ATP is rapidly decomposed as is extracellular ATP in soil (Jenkinson and Oades 1979). The ATP contents of the soils determined by the luciferin-lucifurase method, ranged from 0.3 ug ATP/g in an autoclaved soil after 1 day to 14 ug ATP/g in tridiphane treated soil after 2 days incubation (Table 5). Contents of ATP were significantly greater in tridiphane treated soil than that of control soil after 2 days incubation. Due to the heavy metal, $HgCl_2$ has strong coagulative activity and is a possible germicidal agent, ATP decreased by 80% in $HgCl_2$ treated soils throughout the experiment. Extensive literature on the microbiotoxicity of Hg is available due to its common commercial use.

The general conclusion which can be drawn from this study is that the three pesticides, haloxyfop, tridiphane and pyroxyfur had no permanent drastic effect on microbial populations, activities and enzymes, and had a pronounced stimulatory effect on dehydrogenase. In contrast to the stability of the pesticides to microbial attack, the possibility of microbial degradation of the pesticides in soil is indicated.

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