Bull. Environm. Contam. Toxicol. 24, 13-19 (1980)

Influence of Pesticides and Some of the Oxidized Analogues on Microbial Populations, Nitrification and Respiration Activities in Soil*

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In recent years, due to the widespread use of organic pesticides, increasing quantities of pesticide residues have built up in agricultural soils (EDWARDS 1966, UPCHURCH 1966) and pesticide resistance by soil pests has developed (BROWN 1978). Many pesticides have been developed as replacements for the more persistent chemicals. Several studies (BOLLEN 1961, TU and MILES 1976, WAINWRIGHT 1978) indicate that pesticides have little effect on microbial equilibrium and biochemical changes in soils. A group of organophosphorus chemicals that includes phorate and terbufos, is characterized by the presence of the $-CH_2-S-R$ group that is oxidized in animals, insects, plants and in soils to form the corresponding sulfoxide and sulfone analogues. Since they differ only by R=ethyl and t-butyl respectively, they and their thioether oxidative products were included in this experiment. It is of importance to know the effects of some alternate pesticides and their analogues on beneficial soil microbes. Their effects on microbial populations, nitrification and respiration activities in a loamy sand were determined in the present study.

MATERIALS AND METHODS

The soil used throughout the experiment was a loamy sand of medium texture, common in southwestern Ontario, having nitrogen content of 0.05%; organic matter, 2.7%; sand, 83%; silt, 9%; clay content, 8%; moisture holding capacity, 49%; initial inorganic nitrogen content: NH_4^+-N, 10 µg/g; NO_2^--N, 0.05 µg/g; NO_3^--N, 11.6 µg/g and pH value of 7.4, as determined by the methods described previously (TU 1970).

The fresh soil was collected to a depth of 15 cm and was sifted through a 2-mm mesh screen before use. With exception of the fumigants, chemical purity of the insecticides and the analogues (Table 1) was of analytical grade. An antibiotic, streptomycin; a fungicide, maneb; a nitrogen stabilizer, nitrapyrin [2-chloro-6-(trichloromethyl)pyridine]; and autoclaved soil (steam sterilized at 121 C for 7 h daily for a period of 5 days and oven-dried at 105 C for 8 h) were included for comparative

* Contribution No. 766, Research Institute, Agriculture Canada, London, Ontario.

0007-4861/80/0024-0013 $01.40

purposes. Untreated soil samples were included with all tests. Insecticides and their analogues were applied to the soil at 5 µg per gram of soil using a carrier sand (TU 1970). Similarly, maneb and nitrapyrin were applied at 100 µg/g. Streptomycin was applied directly to the soil at 100 µg/g. The fumigants, Telone II® and Telone C-17® were also injected directly into the soil.

Changes in populations of soil microflora were determined by plating on sodium albuminate agar (WAKSMAN and FRED 1922) for bacteria and rose bengal-streptomycin agar (MARTIN 1952) for fungi. Plates were incubated at 28 C. The arithmatic means of the triplicate counts were used to calculate numbers of microorganisms per gram soil.

Nitrification of ammonium from soil organic nitrogen was measured using the soil perfusion technique (KAUFMAN 1966). Fifty-gram soil sample mixed with pesticides- or nitrapyrin-carrier sand was supported between glass wool in the upper soil tube and was perfused under positive air pressure for 6 weeks with 300 ml of solution. The perfusate was analyzed weekly for nitrite- and nitrate-nitrogen by methods reported previously (TU 1970).

Triplicate samples of the pesticide-treated and untreated soils were placed in Warburg flasks for studies on microbial activities in oxygen consumption. After equilibration at 30 C for 1 h, oxygen consumption was measured at intervals for 72 h using a Gilson differential respirometer. Two replications of each treatment were studied. The values shown in Figure 2 have been corrected for the oxygen consumption of the control soils.

All results are expressed as the oven-dry weight of soils.

RESULTS AND DISCUSSION

The influence of different pesticides varied, showing no pattern consistent with time of incubation. With the exception of permethrin, none of the insecticides showed an inhibitory effect on bacterial and fungal colony counts in the soil (Table 1). A significant stimulatory effect on bacterial numbers was observed with treatments of terbufos sulfoxide and Telone C-17, and with phorate and terbufos after one week and with Dowco 275 and permethrin after 2 weeks' incubation. Similar results on fungal populations were obtained with terbufos sulfone and Dowco 275 after 2 weeks. Maneb and autoclaving significantly reduced microbial populations in soils throughout the experiment.

Nitrification, the biological oxidation of reduced nitrogen to nitrite and nitrate from soil organic or inorganic compounds, is the second stage of the nitrogen cycle in soil. This reaction is carried out mainly by Nitrosomonas sp. and Nitrobacter sp. Based on production of $(NO_2^- + NO_3^-)$-N, the response of nitrifiers to the treatments can be classified into four groups (Figure 1). (A) shows the pesticides, Telone C-17, Telone II and oxamyl

TABLE 1

Influence of treatments on colony counts of microorganisms in a sandy loam.

Treatment	µg/g	Bacteria($X10^5$/g soil) Incubation time (week) 1	Bacteria($X10^5$/g soil) Incubation time (week) 2	Fungi($X10^3$/g soil) Incubation time (week) 1	Fungi($X10^3$/g soil) Incubation time (week) 2
Control	0	42	38	26	30
Streptomycin	100	48	30	-	-
Maneb	100	28*	28*	16*	20*
Autoclaving	0	0*	0*	0*	0*
Phorate	5	64*	43	24	30
Phorate sulfone	5	55	45	28	36
Phorate sulfoxide	5	61	40	20	30
Terbufos	5	73*	55	24	25
Terbufos sulfone	5	63	48	27	50*
Terbufos sulfoxide	5	92*	59*	24	35
Dowco 275[a]	5	48	60*	24	50*
Triazophos	5	48	40	28	25
Oxamyl	5	42	55	20	35
Permethrin[b]	5	30*	58*	18*	32
Telone II[c]	60.8/ha	58	42	28	25
Telone C-17[d]	60.8/ha	65*	74*	20	30

* Significantly different from control at P=0.05.

a. Phosphorothioic acid, O,O-diethyl-O-(6-fluoro-2-pyridyl)ester.

b. FMC 33297, 3-phenoxybenzyl(±)-3-(2,2-dichlorovinyl)-2,2-dimethlcyclopropane-carboxylate.

c. 1,3-dichloropropene 92% and related C_3 hydrocarbons.

d. 1,3-dichloropropene and related C_3 hydrocarbons 83% and chloropicrin 17%.

had no significant effect on nitrification during 6 weeks' incubation. (B) shows inhibitory effects of treatments with triazophos, autoclaving and nitrapyrin on nitrification. (C) illustrates that nitrifiers obviously required 2 to 3 weeks of adaptation. However, after 3 weeks, most samples treated with different insecticides, Dowco 275, phorate sulfoxide, phorate sulfone, permethrin, terbufos sulfoxide, terbufos sulfone, phorate and terbufos recovered from the insecticidal effects. At 6 weeks, the nitrification levels were 5 to 20 $\mu g(NO_2^- + NO_3^-)$-N/g soil. The recovery of nitrification probably results from the increased activity of a few resistant species (DOMSCH 1970) and also from a breakdown of the chemicals (BARTHA et al. 1967). (D) indicates stimulatory effects of streptomycin on activities of nitrifiers in the process.

Respiration, as indicated by oxygen consumption, is an index of the activity of microflora involved in soil organic matter decomposition. The effect of different treatments on respiration is presented in Figure 2. With the exception of autoclaving, none of the treatments suppressed the vigorous uptake of oxygen by soils. The

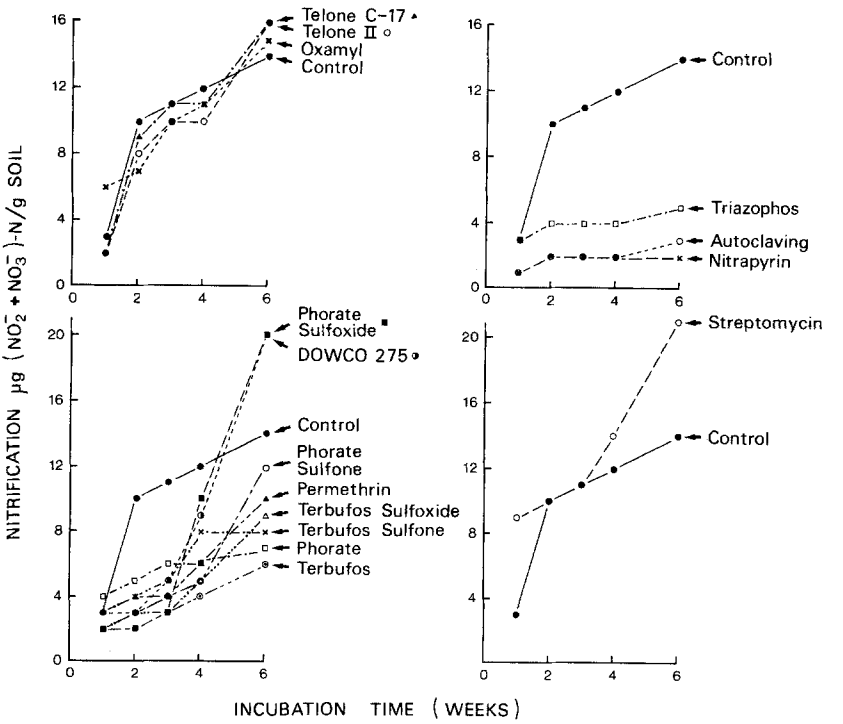

FIGURE 1. Effect of different treatments on nitrification in a sandy loam.

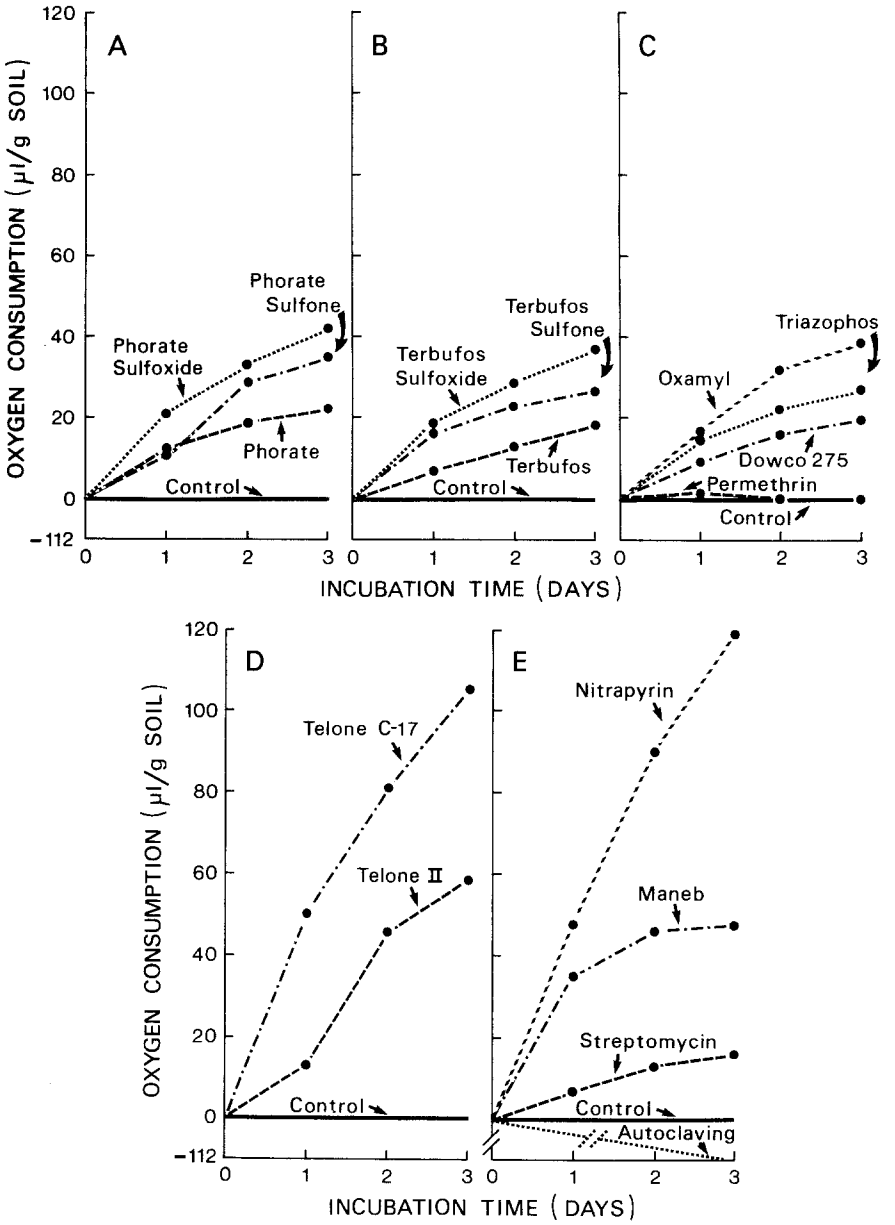

FIGURE 2. Changes in microbial respiration as related to treatments of a sandy loam. A. Phorate and oxidized analogues; B. Terbufos and oxidized analogues; C. Pesticides; D. Fumigants; E. Different treatments.

pesticide treatments stimulated uptake of oxygen by soils. The oxidative analogues of phorate and terbufos consumed greater amount of oxygen than the parent materials (Figure 2, A and B). The amount of consumed oxygen, 143 µl/g of soil, was obtained with control soil. Maximum oxygen consumption occurred with nitrapyrin (263 µl/g) and Telone C-17 (249 µl/g). The increased oxygen consumption with treatments possibly resulted from the oxidation of pesticides by soil microorganisms. In previous studies, a soil treated with fensulfothion at a higher concentration consumed more oxygen from the decomposition of native organic matter in the treated than in the control soils (TU 1972). Oxygen consumption increased with increasing concentration of the insecticide in soil with and without supplemented glucose-C (TU 1972, 1973). Miles et al. (1979) demonstrated that fensulfothion disappeared rapidly in the non-sterilized mineral and organic soils, with the appearance of an oxidized metabolite, fensulfothion sulfone, and it declined to non-detectable levels after 8 weeks. They therefore concluded that an essential part of the decomposition of fensulfothion in the soils was due to the activity of microorganisms. A synergistic action of soil microorganisms involving Arthrobacter sp. and Streptomyces sp. in the degradation of diazinon was demonstrated (GUNNER and ZUCKERMAN 1968). It is apparent that no one species of microbes is solely active and accountable for degrading pesticides in the soil.

The pesticide treatments were observed in many experiments to have significant effects on the microbial populations and activities but the microorganisms recovered rapidly. These effects were not drastic but minor in nature when compared with those reference chemicals or treatments, such as autoclaving. There is little evidence to suggest that these pesticide treatments have any prolonged deleterious effect on the soil microbial activities.

ACKNOWLEDGMENT

The technical assistance of Mr. G. Hietkamp is gratefully acknowledged.

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