Bull. Environm. Contam. Toxicol. 20, 212-218 (1978)

A Screening Technique for Assessing Effects of Pesticides on Population and Activities of Non-target Soil Microorganisms*

C. M. Tu

Research Institute, Agriculture Canada, University Sub Post Office, London, Ontario N6A 5B7, Canada

Due to the wide spread use of organic pesticides, increasing quantities of pesticide residues have reached in agriculture soils. Experience with the organochlorine insecticides has shown that some may have far-reaching ecological side effects (TU and MILES, 1976). These materials have been restricted in use, but many new chemicals, primarily organophosphorus, carbamate, and synthetic pyrethroid insecticides, are being substituted. Prior to the registration of new materials studies are conducted to determine their efficacy, persistency, movement, effects on non-target aquatic organisms and wild life etc. Little consideration is given to the effects they have on the population and the activities of non-target microorganisms important to fertility in the soil. Several studies (BOLLEN, 1961; TU and MILES, 1976) have shown that pesticides have some effects on microbial activities. To evaluate the safe and effective use of these chemicals, a screening technique for the effect of the pesticides on non-target microorganisms in soils is desirable. Most of the screening work done so far has been directed toward determining microbial mineralization of soil organic nitrogen, respiration, oxidation of soil organic sulfur, and changes in populations of microorganisms. Because these methods are complex and time-consuming, it would not be feasible to do them all on all experimental pesticides prior to registration. There is, therefore, a need to devise a simple screening technique capable of yielding a preliminary assessment of the effects of pesticides on soil microorganisms and microbial activities. Hopefully such a technique could be incorporated into protocols for registration of pesticides. The objective of this study was to devise such a simple screening procedure.

MATERIALS AND METHODS

Soil samples were collected to a 15-cm depth and screened through a 2-mm sieve. Some of the chemical and physical properties of the soils are shown in Table 1.

Two insecticides, dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8a-octahydro-1,4-endo, exo-5,8-dimethanonaphthalene) and chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) were applied at 20 µg active ingredient per gram of soil. The fumigant, Vorlex (20% methyl isothiocyanate and 80% chlorinated C_3 hydrocarbons including 1,3-dichloropropene), was applied at 650

* Contribution No.690, Research Institute, Agriculture Canada, London, Ontario.

0007-4861/78/0020-00212 $01.40

TABLE 1

Properties of three soils

Determination	Loamy sand	Sandy loam	Clay loam
pH	6.5	7.6	7.9
Kjeldahl nitrogen (%)	0.08	0.21	0.20
Organic carbon (%)	0.41	4.74	1.68
Moisture-holding capacity (%)	38.00	66.10	53.00

µg/g by direct injection. Samples treated with streptomycin (2,4-diguanidine-3,5,6-trihydroxy-cyclohexyl-5-deoxy-2-0-2-deoxy-2-methylamino-α-glucopyconosyl-3-formyl pentano furanoside sulfate), N-Serve (98.6% 2-chloro-6-trichloromethyl pyridine), maneb (manganous ethylene bisdithiocarbamate) at 100 µg/g and pasteurization were also prepared to compare their effects on soil microbial activity with those of the pesticides. The required amounts of pesticides, dieldrin, chlorpyrifos, N-Serve, and maneb were applied to the soil using a carrier sand as described before (TU, 1970). The streptomycin was mixed thoroughly into the soil. Soil was pasteurized by autoclaving at 15 lb of steam presure at 121°C for 7 hr daily for a period of 4 days and oven-dried for 3 hr at 105°C.

To determine changes in microbial populations, the treated soil samples were incubated five weeks at 28°C in 236-ml milk bottles, which were closed with 0.038-mm thick polyethylene film. The moisture was maintained at 60% of the moisture-holding capacity. Microbial populations were determined after 1,3, and 5 weeks by the dilution plate method using sodium albuminate agar for bacteria and rose bengal-streptomycin agar for fungi as reported previously (TU and BOLLEN, 1968). An untreated control was included. Results reported are the averages of three plates from each of triplicated samples.

Nitrification of $(NH_4)_2SO_4$-N added at 200 µg/g was measured using the soil perfusion technique (KAUFMAN, 1966). The ammonium sulfate was mixed thoroughly into 50-g of pesticide or N-Serve treated soils. The soil sample was supported between glass wool in the upper soil tube and was perfused under positive air pressure for 10 weeks with 250 ml of solution. The perfusate was analysed weekly for nitrite and nitrate-nitrogen by methods described before (TU and BOLLEN, 1968).

Oxygen consumption was measured at 30°C for a period of 4 days using a Gilson differential respirometer. Eight-gram (oven-dry weight) portions of each soil in a 15-ml Warburg flask containing 0.15 ml of 20% KOH and a filter-paper strip in the center well were used. Three replications of sample were studied. The values shown in graphs have been corrected for nitrification and oxygen consumption of untreated soils. All results are expressed as the oven-dry weight of soils.

RESULTS AND DISCUSSION

The ecological significance of any temporary change in numbers of soil microorganisms, activities in nitrification and respiration can only be judged when the role of groups of soil microbes and

TABLE 2

Effect of various treatments on microbial populations in three soils

Treatment		Bacteria(X10^5/g soil) Incubation time (weeks)			Fungi (X10^3/g soil) Incubation time (weeks)		
	(µg/g)	1	3	5	1	3	5
				Loamy sand			
Control		83	120	94	25	18	13
Pasteurization		0*	0*	0*	0*	0*	0*
Streptomycin	100	78	71*	66*	-	-	-
Maneb	100	-	-	-	23	17	14
Dieldrin	20	81	101	92	21	18	14
Chlorpyrifos	20	92	111	88	26	16	16
Vorlex	650	95	103	97	18	20	20
				Sandy loam			
Control		308	108	88	61	58	38
Pasteurization		0*	0*	1*	0*	0*	1*
Streptomycin	100	283	92	94	-	-	-
Maneb	100	-	-	-	33*	16*	33
Dieldrin	20	338	126	104	37*	38*	51*
Chlorpyrifos	20	308	113	133	34*	25*	43
Vorlex	650	456*	117	106	16*	18*	32
				Clay loam			
Control		16	81	240	7	16	6
Pasteurization		0*	0*	1*	0*	0*	0*
Streptomycin	100	18	21	228	-	-	-
Maneb	100	-	-	-	5	13	5
Dieldrin	20	24	242*	306	5	45*	18*
Chlorpyrifos	20	15	22	293	5	5	13
Vorlex	650	28*	254*	806*	8	20	19*

* Significantly different from control($P < 0.05$)

their activities within the terrestrial ecosystem is properly defined and quantified.

The effect of different pesticides on soil microflora in 3 soils is summarized in Table 2. None of the pesticides showed an inhibitory effect on bacterial colony counts in the sandy and clay loams. Streptomycin at 100 µg/g reduced bacterial populations significantly in the loamy sand for 5 weeks. Low concentrations of streptomycin have been demonstrated to inhibit completely the growth of a soil bacteria in pure culture in laboratory media (PRAMER and STARKEY, 1962). SOULIDES et al. (1962) demonstrated that streptomycin in clay loams is unstable and degraded microbiologically. It appears that streptomycin can persist in loamy sand for sufficient time to have a microbiological effect. All pesticides inhibited to some extent the growth of fungi in the sandy loam for 3 weeks, however, none of the pesticide suppressed

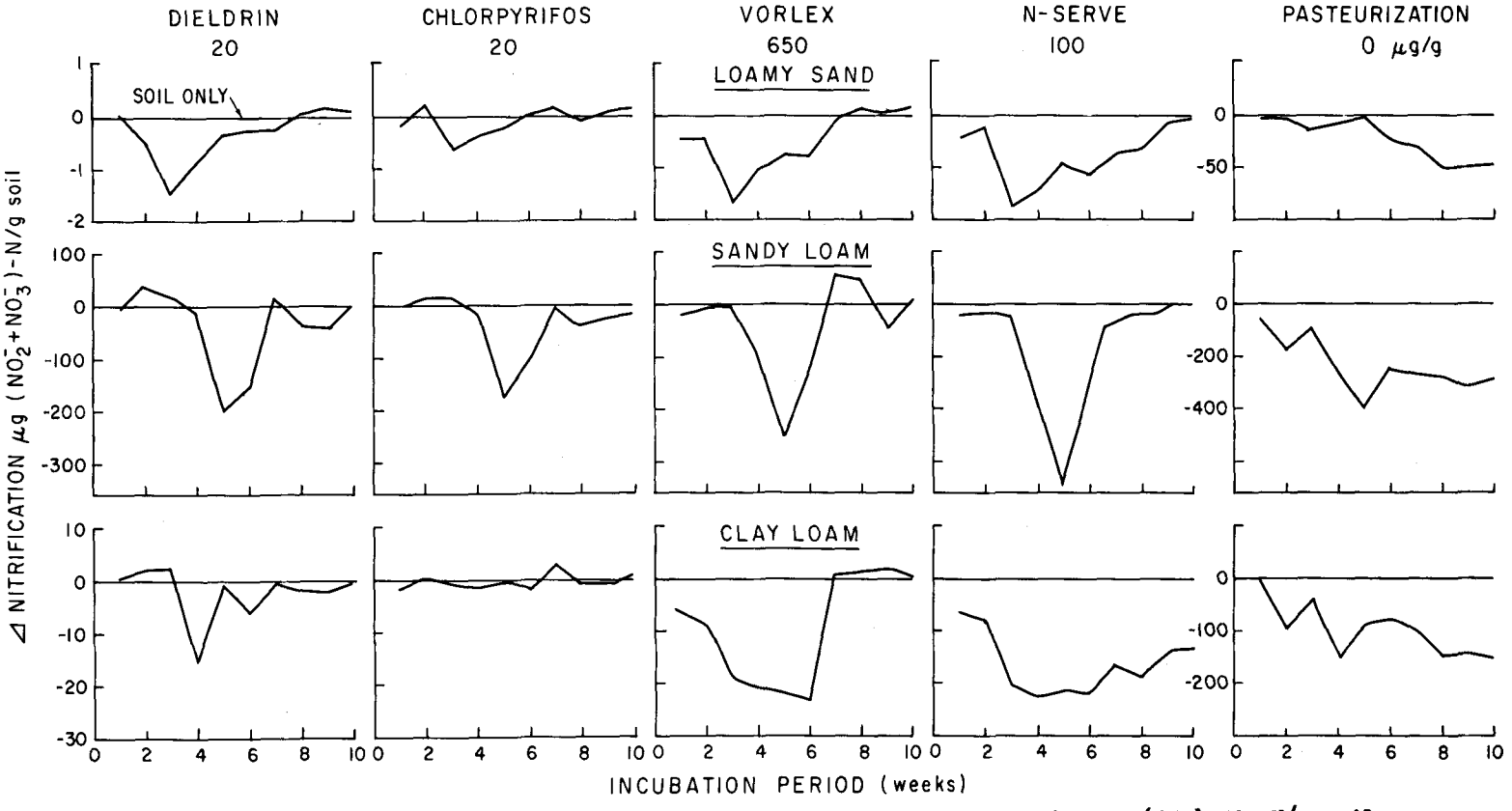

FIGURE 1. Nitrification in soils after various treatments with the addition of 200 µg $(NH_4)_2SO_4$-N/g soil.

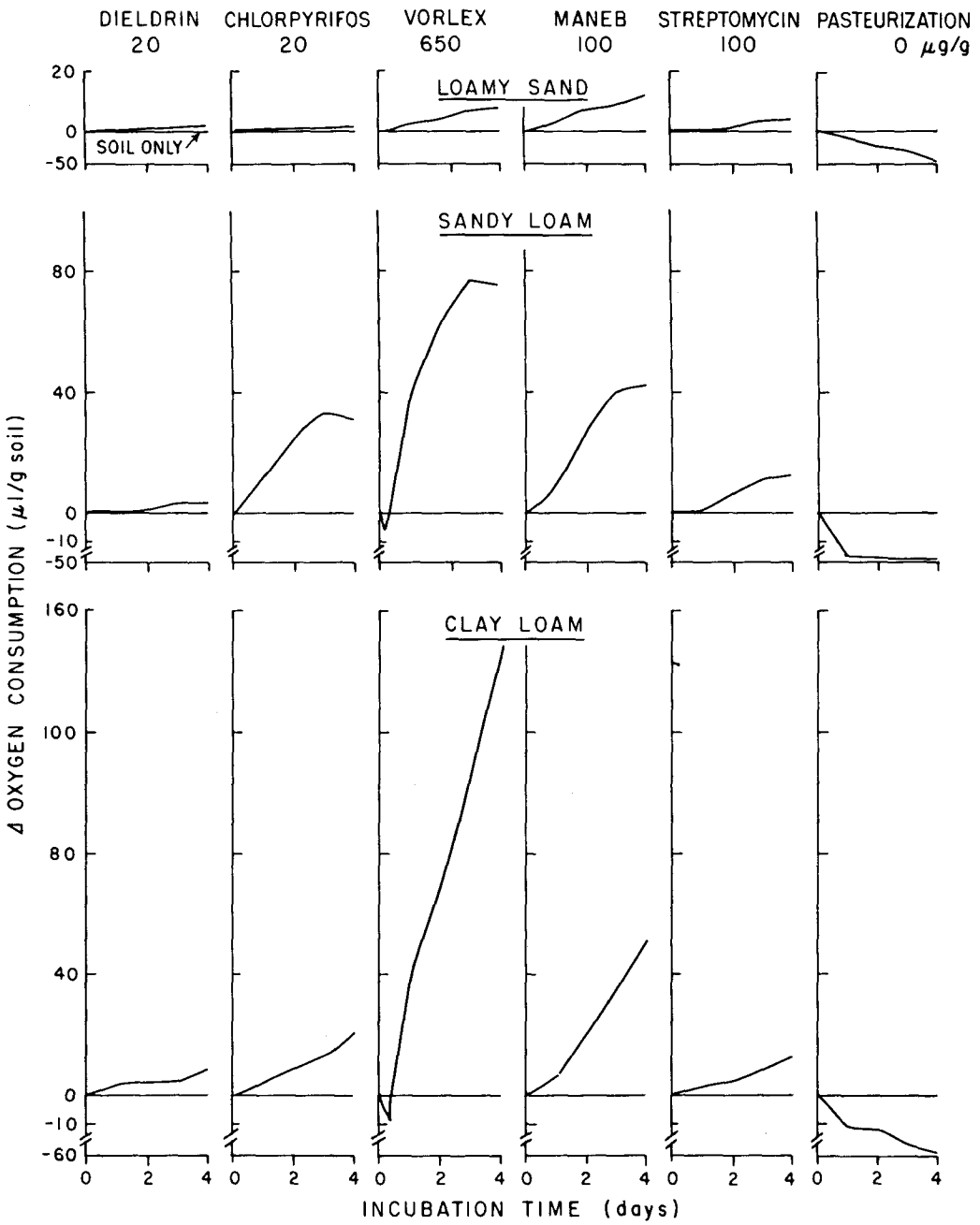

FIGURE 2. Effect of various treatments on soil microbial respiration.

the fungal population in the loamy sand and the clay loam. By contrast, populations of bacteria and fungi in pasteurized soils were significantly lower than those in the controls.

Treatments with the pesticides inhibited nitrification to various degrees (Figure 1). Nitrate originally present was immobilized in the loamy sand after 3 weeks and in the sandy loam after 5 weeks. Nitrification recovered after 7 weeks in the pesticide treated loamy sand and sandy loam. N-Serve showed greater inhibition in nitrate production in all soils, however, nitrification was not affected drastically by chlorpyrifos in the clay loam, and by the pesticides after 7 weeks in sandy and clay loams. A greater effect was observed with pasteurization in all soils.

The oxygen consumption from the decomposition of soil native organic matter was not affected by the treatment of pesticides in 3 soils for 4 days (Figure 2). A greater oxygen consumption was observed in the Vorlex and maneb treated soils. Vorlex reduced respiration in the loams in the early stages of incubation, however, the recovery was fast after 6 hr incubation. More than 40 µl oxygen/g of soil were consumed by soil microbes in the maneb treated loams. Neither the insecticide, dieldrin, itself nor the metabolites of this insecticide could have directly contributed significantly to the extent of oxygen consumption which could have come from the trace amount of carbon introduced in the chemical formulation. In the loamy sand, respiration was not affected by dieldrin, chlorpyrifos or streptomycin treatments. None of the chemicals appeared to drastically reduce oxygen consumption as compared to the effect of pasteurization.

The biological activity in soil is dependent to a large extent on the physical and chemical characteristics of the soils under study. All results reported here were obtained using agriculturally important soils in southwestern Ontario of low buffer capacity. Although, some stimulatory effects of pesticides on microbial numbers and activities in three soils were observed, these effects were not drastic or sufficient to be considered deleterious to soil microorganisms and their activities important to soil fertility. The present technique utilized for studying the pesticidal effects on soil microbial activities is found to be effective, simple and equally satisfactory to the previous methods (TU,1970; TU and BOLLEN, 1968) employed.

SUMMARY

A simplified technique for assessing the effect of pesticides on non-target soil microorganisms was developed. Changes in microbial population determined by the dilution plate method, in nitrification by the soil perfusion technique, and in oxygen consumption using a differential respirometer are the only measurements required. A comparison of the effects observed for three pesticides, dieldrin, chlorpyrifos and Vorlex, in three soil types with those produced by an antibiotic, a fungicide, a nitrification inhibitor and steam pasteurization of the soil clearly demonstrate the effectiveness of the simple technique.

ACKNOWLEDGMENT

The technical assistance provided by Mr. G. Hietkamp is gratefully acknowledged.

REFERENCES

BOLLEN, W.B.: Annu. Review Microbiol. 15, 69 (1961).
KAUFMAN, D.D.: Weeds 14, 90 (1966).
PRAMER, D. and R.L. STARKEY: Soil Sci. 94, 48 (1962).
SOULIDES, D.A., L.A. PINCKAUD, and F.E. ALLISON: Soil Sci. 94, 239 (1962).
TU, C.M.: Appl. Microbiol. 19, 479 (1970).
TU, C.M., and W.B. BOLLEN: Weed Res. 8, 28 (1968).
TU, C.M., and J.R.W. MILES: Residue Reviews 64, 17 (1976).