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Persistence of Eight Organophosporus Insecticides in Sterile and Non-Sterile Mineral and Organic Soils

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Insecticide degradation in soil may be chemical and/or biological in nature. Organochlorine (OC) insecticides were considered initially to be very resistant to biological degradation. Subsequently, it was found that, depending on the specific compound, environmental conditions, and agronomic practices, microbial degradation could play an important role in degrading these insecticides in soil. Organophosphorus (OP) insecticides are generally less persistent in soil and early studies suggested that they degraded primarily by chemical hydrolysis. Later work indicated that soil microorganisms were capable of metabolizing a number of OP insecticides such as chlorpyrifos, diazinon, dichlorvos, dimethoate, fonofos, malathion, methyl parathion, and mevinphos (e.g. FLASHINSKI and LICHTENSTEIN 1974, GETZIN and ROSEFIELD 1968, LICHTENSTEIN and SCHULZ 1964, MATSUMURA and BOUSCH 1966). Much of the research on OP insecticides has been in vitro making it difficult to judge the relative importance of biological versus chemical degradation under practical conditions. In vivo studies have been hampered by problems associated with the preparation of sterile soil and maintenance of sterility for any length of time. Nevertheless some of the definitive studies on microbial degradation of insecticides in soil have involved comparisons of rates of insecticide degradation in sterile and non-sterile soils (e.g. LICHTENSTEIN and SCHULZ 1960, 1964, GETZIN and ROSEFIELD 1968, WALKER and STOJANOVIC 1973). The literature on interactions between soil microbes and insecticides has been documented in a number of comprehensive reviews (e.g. ALEXANDER 1965, BOLLEN 1961, BROWN 1978, EDWARDS 1966, SETHUNATHAN 1973, TU and MILES 1976, WILLIAMS 1977).

In recent years a number of OP insecticides have been developed as soil insecticides as replacements for the more persistent OC insecticides. Some are moderately persistent in soil (HARRIS 1972) and there is recent evidence that residues of some OP insecticides are accumulating in organic soils (HARRIS et al. 1977, MILES and HARRIS 1978, MILES et al. 1978). It was the objective of this study to examine the persistence of some of these insecticides in a mineral and an organic soil and to determine the role of soil microorganisms in their degradation.

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MATERIALS AND METHODS

A sandy loam (2.9 organic matter, 46% moisture holding capacity, pH 8.0 and an organic soil (48.7% OM, 166% MHC, pH 7.6) were used. Bulk samples were sieved through a 10-mesh screen and aliquots of moist soil representing 70 g oven-dry wt were placed in 280 ml glass milk bottles. A sufficient number was prepared to provide triplicate samples for analyses at each sampling interval. Samples for sterilization were closed with aluminum foil, autoclaved at 1.05 Kg/cm^2 at 121°C for 7 h daily for 5 days, and then oven-dried for 3 h at 105°C. Insecticides (analytical grade) dissolved in pentane were aseptically added to, and mixed with sterile soil. The pentane was evaporated while samples were held in an inoculation hood. Since the addition of pentane can effect partial sterilization of the soil (WAKSMAN 1932) insecticides were applied to non-sterile soils in a different fashion to maintain full microbial activity. Insecticides dissolved in pentane were added in appropriate amounts to pulverized quartz sand. After evaporating the solvent the treated sand was added to the soil (0.5 g sand/100 g oven-dry soil) and the sample mixed to obtain homogeneity. (Untreated carrier sand was added also to sterile soils before sterilization). Insecticide treatments were at a theoretical level of 10 ppm (based on the oven-dry wt of the soil). Insecticide-free sterile and non-sterile controls were included. Sample bottles were closed with 0.038 mm (1.5 mil) thick polyethylene film (RICHARDSON 1975) and incubated in darkness at 28°C. Soil moisture was maintained at 60% MHC. Samples were removed for determination of microbial activity and insecticide residues 0, 1, 2, 4, 8, 12, 16, 20, and 24 wk after treatment.

Changes in the microflora population in both sterile and non-sterile soils were determined by the soil dilution plate technique, using sodium albuminate agar for bacteria and actinomycetes (WAKSMAN and FRED 1922) and rose bengal-streptomycin agar for fungi (MARTIN 1950.

To extract the insecticides, 100 ml acetone were added to the soil in each sample bottle and the latter placed in an ultrasonic bath for 15 min. The contents were quantitatively transferred to a 455 ml narrow-neck bottle and 100 ml 1:1 benzene:hexane were added. The bottle was sealed with a tin foil-lined cap and tumbled end-over-end for 30 min. The supernatant was filtered through glass wool into a 1 liter separatory funnel. Acetone was removed with 2 washes (50 and 100 ml) of distilled water and the benzene:hexane extract dried with anhydrous Na_2SO_4. Avg. % recoveries were: chlorfenvinphos, 95; chlorpyrifos, 81; diazinon, 100; ethion, 95; fensulfothion, 85; fonofos, 82; parathion, 77; and trichloronat, 85%. Extracts were analyzed by GLC without cleanup. Gas chromatographs and operating parameters were described by MILES et al. (1978). All columns were operated at 180°C except when analyzing for fensulfothion (200°C).

RESULTS AND DISCUSSION

As compared to untreated soil, none of the insecticides had any significant long-term effects on populations of bacteria or fungi in either of the non-sterile soils. Because of this the mass of plate count data obtained is not presented here. Microbial populations in the non-sterile soil declined over the 24 wk period, e.g. in the sandy loam bacteria in all samples averaged 135 and 21 x 10^5/g soil after 1 and 24 wk, respectively. Corresponding values for fungi were 52 and 7 x 10^3/g soil, respectively. In the organic soil, bacterial counts at 1 and 24 wk were 94 and 26 x 10^5/g soil and fungi 19 and 7 x 10^3/g soil, respectively. In the sterile soils no significant bacterial (>300/g soil) or fungal (>50/g soil) activity was noted until the 16th wk. By the 24th wk most "sterile" samples were contaminated with bacteria and fungi.

Soil pH was determined for each sample at every sampling interval. Individual pH values are not presented here as there were no significant differences. Overall avg, 1, and 24 wk pH values for the various soils were: non-sterile sandy loam 8.0, 8.0, 8.0; sterile sandy loam 7.7, 7.5, 7.7; non-sterile organic soil 7.5, 7.5, 7.4; sterile organic soil 6.8, 6.9, 6.8; respectively. Maximum variation between 9 samples (8 insecticide treatments + control) was for sterile sandy loam at 16 wk, i.e. 7.79±0.23.

In the non-sterile sandy loam ethion was the most persistent >fonofos=trichloronat>chlorfenvinphos>fensulfothion>parathion> chlorpyrifos=diazinon (Table I). The insecticides were all more

TABLE I

Persistence of 8 OP insecticides in sterile and non-sterile sandy loam and organic soil.

	Wk incubation to 50% remaining[1]				Wk incubation to 5% remaining[2]	
	Sterile		Non-sterile		Non-sterile	
Insecticide	Sandy loam	Organic soil	Sandy loam	Organic soil	Sandy loam	Organic soil
Diazinon	12½	6½	<1	2	1	7
Chlorpyrifos	17	>24	<1	2½	1	8
Parathion	>24	>24	<1	1½	3	10
Fensulfothion	>24	>24	<1	1	4	6
Chlorfenvinphos	>24	>24	<1	1	5	9
Trichloronat	>24	>24	1½	4	20	>24
Fonofos	>24	>24	3	4	20	20
Ethion	>24	>24	7	8	>24	>24

1), 2) 50 and 5%, respectively, of initial concentration. The theoretical initial concentration for each insecticide was 10 ppm. Actual treatment levels were determined 2 h after addition of the insecticides.

persistent in the non-sterile organic soil as compared to the sandy loam with the order of persistence being ethion=trichloronat>fonofos> chlorpyrifos>parathion>chlorfenvinphos>diazinon>fensulfothion. These results will not necessarily be representative of the relative persistence of these insecticides under field conditions. While the experimental conditions were conducive to chemical and microbial degradation of the insecticides in soil, the fact that the sample jars were covered with polyethylene film (which allowed air but not water exchange) would reduce the opportunity for insecticide volatilization, an important factor under field conditions.

All of the insecticides were more persistent in sterile as compared to non-sterile soils (Fig. 1 and 2)(Data points shown are averages of triplicate sample analyses with upper and lower marker levels). With exception of diazinon, the insecticides were all more persistent in the sterile organic soil as compared to the sterile sandy loam.

Diazinon degraded quickly in the non-sterile sandy loam, with only 5% of the initial concentration present 1 wk after treatment (Fig. 1, Table I). In non-sterile organic soil it declined to a similar level in 7 wk. Although more persistent in sterile soils, significant degradation occurred over 24 wk. Diazinon was unique, in that it was more persistent in sterile sandy loam than in sterile organic soil. Soil pH may influence the degradation of some insecticides (FLASHINSKI and LICHTENSTEIN 1975) but it probably was not a factor in the degradation of diazinon in the sterile organic soil (pH 6.8) since diazinon is stable to hydrolysis at pH 6.0 (KONRAD et al. 1967). Chlorpyrifos also degraded quickly in non-sterile soils, especially in the sandy loam, where 95% disappeared in 1 wk (Fig. 1, Table I). It was more persistent in sterilized soils but, like diazinon, significant degradation occurred over 24 wk.

Parathion degraded quickly in non-sterile soils, but was very persistent in sterile soils (Fig. 1, Table I). There was little variation in residue levels between replicates in the non-sterile soils (as was the case with all insecticides). There was more variation between replicates with parathion (and the other insecticides) in sterile soils. Microbial contamination of the sterile soils does not account for this variation, e.g. sterility in the parathion-treated sandy loam and organic soil was maintained until the 24th and 16 wk, respectively. Fensulfothion disappeared rapidly in the non-sterile soils, with the appearance of the only metabolite detected in this study, i.e. fensulfothion sulfone (Fig. 1, Table I). It increased in concentration for 2 wk and then declined to non-detectable levels in 8-12 wk. More sulfone was produced in the non-sterile organic soil than in the sandy loam. No sulfone was detected in sterile soils.

Chlorfenvinphos, trichloronat, fonofos, and ethion were more persistent in the non-sterile soils than the former insecticides (Fig. 2, Table I). Five percent of the initial chlorfenvinphos concentration was still present in the sandy loam and organic soil after 5 and 9 wk, respectively. It was very persistent in the sterile soils for 20 wk. Residue levels declined markedly in

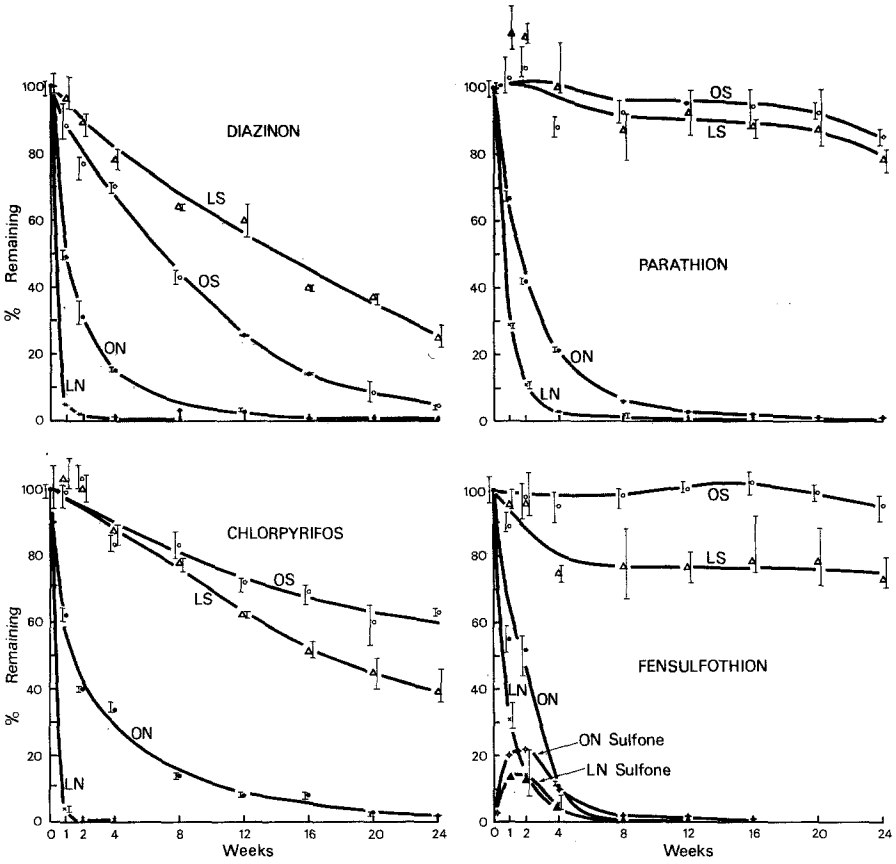

Figure 1. Persistence of diazinon, chlorpyrifos, parathion and fensulfothion in sterile (OS) and non-sterile (ON) organic soil and sterile (LS) and non-sterile (LN) sandy loam.

both sterile soils at 24 wk, which illustrates the point made above regarding microbial contamination of the later samples, i.e. chlorfenvinphos began to degrade as the soils lost their sterility. Trichloronat, in the sterile soils, showed a steady decrease with time and the relative difference in its persistence in the sandy loam and organic soil was not as great as with the other insecticides. Fonofos was more persistent than trichloronat in the sterile soils in the early stages of the study. Marked degradation occurred between the 20-24th wk which correlated with development of microbial activity beginning in the 16th wk.

Ethion was the most persistent of the 8 insecticides in the non-sterile soils. It was very persistent also in the sterilized soils, although residues declined in the organic soil at 24 wk which may have been due to development of microbial activity at that time. The longer persistence of ethion agrees with field data (MILES et al. 1978) which indicated that it is one of the most persistent OP insecticides in soil.

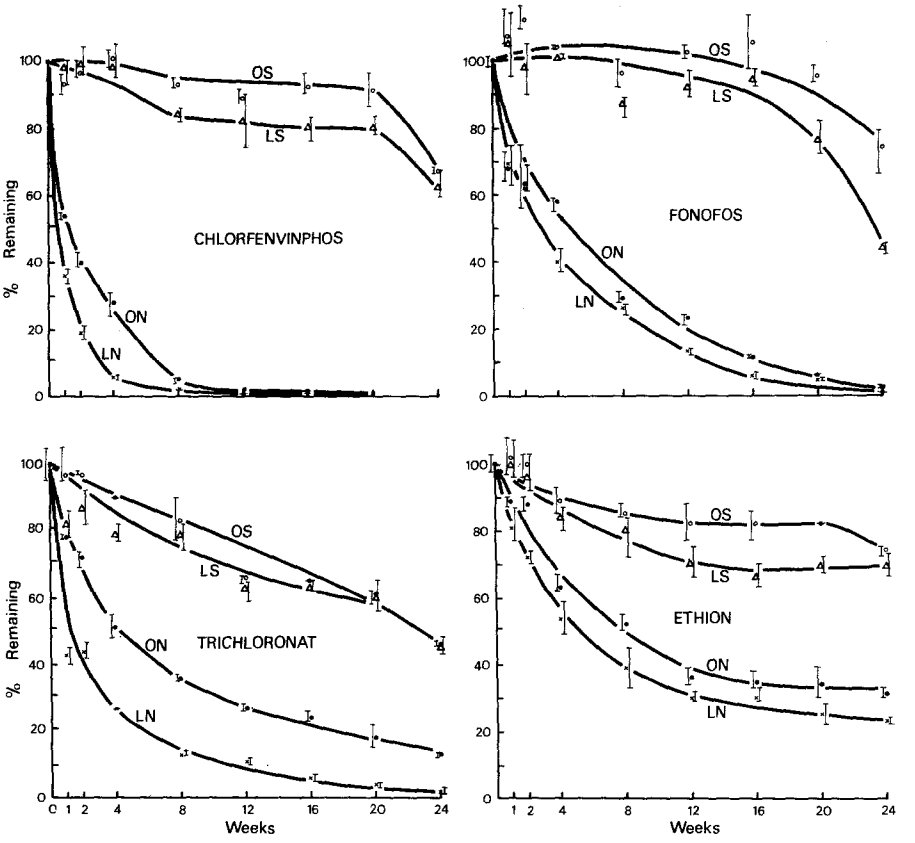

Figure 2. Persistence of chlorfenvinphos, trichloronat, fonofos, and ethion in sterile (OS) and non-sterile (ON) organic soil and sterile (LS) and non-sterile (LN) sandy loam.

As noted, previous studies have established that residues of OP insecticides in soil may be subject to chemical and/or microbial degradation. The latter has often been considered to play a secondary role. In this study chemical degradation appeared to have a major role in the degradation of diazinon, chlorpyrifos, and trichloronat and to be of minor importance in degradation of the other insecticides. Microbial degradation played a major role in the degradation of parathion, fensulfothion, chlorfenvinphos, fonofos and ethion. Although the role of microorganisms in the degradation of OP insecticides in soil has been recognized in the past, its importance may have been underestimated.

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