Degradation of linuron in soils as influenced by different organic amendments and surfactants

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ABSTRACT

A study has been made of the influence of contrasting organic amendments and two surfactants on the rate and pathway of linuron degradation in soil. Degradation of the herbicide and the presence of its metabolites were assessed in unamended and amended soil under non-sterilized and sterilized conditions. The results pointed to an inhibition of degradation by liquid humic amendment and peat, an enhanced microbiological degradation by city refuse compost and an inhibition of microbiological degradation by the surfactants, which at the same time favoured chemical degradation of the herbicide. These results demonstrate that the organic amendments and surfactants modify the rate and pathway of linuron degradation in soil. These modifications could be valuable when using simultaneously organic amendments and pesticides in agricultural practices and when using surfactants in technologies for the prevention of pollution and the recovery of soil polluted by hydrophobic organic compounds.

INTRODUCTION

The use of organic amendments and organic residues in agricultural practices has increased considerably in recent years. On the other hand the development of physico-chemical techniques based on the use of surfactants for the prevention of pollution and the recovery of soils polluted by hydrophobic organic compounds is also on the increase. In view of the importance of soil organic matter for the adsorption and mobility of hydrophobic organic compounds in soils, much research has been carried out on the effects that the addition of such organic materials to the soil may have on these processes. However, although such additions may modify the chemical and biological conditions of the soil, little attention has been paid to the effect of amendments and surfactants on the rate and pathway of pesticide degradation.

Linuron is a herbicide belonging to the phenylurea group that is widely used in different types of cultivation. Its degradation in the soil may occur through biochemical, chemical and photochemical mechanisms, giving the following transformation products: N-(3,4-dichlorophenyl-N'-methyl-urea) (M1), N-(3,4-dichlorophenyl-N'-methoxy-urea) (M2), N-(3,4-dichlorophenyl-urea) (M3), and 3,4-dichloro-aniline (M4) (Maier & Härtel 1981). As a result of these processes, its persistence in the soil varies considerably and depends on the characteristics of the soil and the environmental conditions under which it is applied (Walker 1976; Walker & Welch 1991). Studies on the adsorption and mobility of linuron in soils have shown that soil organic matter content is the main parameter involved in these processes (Sánchez-Camazano *et al.*, 2000). Additionally, studies of the effects of agricultural organic

amendments and surfactants (Iglesias-Jiménez *et al.*, 1997) on the adsorption of linuron by soils have been carried out. However, to date the effects of the addition of agricultural organic amendments, city refuse compost, and surfactants to the soil on the persistence and degradation of linuron have not been addressed.

In this work, we studied the effect of three organic amendments and two surfactants on the rate and pathway of linuron degradation in soil. The degradation of the herbicide and the presence of its metabolites were assessed in unamended and amended soil under non-sterilized and sterilized conditions.

MATERIALS AND METHODS

The surface horizon (0-15 cm) of a sandy loam soil (<2 mm fraction) with the following characteristics: pH 7.5; organic matter 0.67%; clay 18.1%; silt 15.5% and sand 64.0% was used.

The organic amendments employed were the following: a city refuse compost (CRC) from the urban solid waste treatment plant at Valdemingomez (Madrid, Spain), two commercial organic amendments used in agricultural practices, Grün Garant peat (P) (Deutsche Torfgesellschoft GmbH, Saterland Scharrel, Germany), Humimag liquid humic amendment (LHA) (Braker Laboratories, S. L. Valencia, Spain), and two surfactants, sodium dodecyl sulfate (SDS), an anionic surfactant, and tetradecyltrimethylammonium bromide (TDTMA), a cationic surfactant. Both surfactants were supplied by Aldrich Chemical Co. (Milwaukee, USA). The total organic carbon contents of the amendments were as follows: 28.1% for CRC; 34.7% for P; 22.1% for LHA; 49.9% for SDS; and 60.6% for TDTMA.

Linuron (>99% technical purity) was supplied by Riedel de Häen (Hannover, Germany) and the M1, M2, M3, and M4 (>98% technical purity) metabolites of linuron were supplied by AgrEvo (Valencia, Spain). Linuron is a solid hydrophobic pesticide with a water solubility of 81 mg/litre at 25 °C and a Kow of 1010 (Tomlin 1995).

Fifty grams of unamended soil (as control) and fifty grams of soil amended with the different organic amendments at doses equivalent at 15 t/ha (as total carbon) were placed in covered aluminium cups. The degradation of linuron was studied in non-sterilized and sterilized amendment-soil mixes to elucidate the mechanisms of degradation. Non-sterilized mixes were prepared by directly mixing the organic amendment with the soil, and sterilized mixes were obtained by autoclaving the mixes twice at 120°C for 30 min each time. The sterilized samples were handled aseptically prior to and during the incubation. Before incubation, the water content was adjusted with distilled water to 80% of the field capacity and this moisture content was maintained weekly with distilled water.

Methanol (3 ml) containing 1mg/ml of linuron was then added to the cups and the treated cups were incubated under temperature conditions of 28 ±2°C. At time intervals between 0 and 210 days, three replicates of 5 g were taken and shaken for 24 h with 10 ml of methanol for residue analysis. The standard relative deviations of the results obtained for the replicates were always less than 5%.

The concentrations of linuron and its metabolites in the methanol solution were determined by hplc by the method described previously by Sánchez-Martín *et al.*, (1996) for linuron determination. The apparatus used was a Waters chromatograph (Waters Assoc., Milford, USA) equipped with a model 600E multisolvent delivery system attached to a model 717 autosampler, a model 996 photodiode array detector, and a Millennium 2010 chromatography manager data acquisition and processing system. The detection limits of linuron and its metabolites were 0.010μg/ml. The extraction efficiency measured previously with soil samples spiked with different amounts of linuron was >92%.

RESULTS AND DISCUSSION

Figure 1 shows the degradation kinetics of linuron in the soil unamended and amended with the different organic materials under non-sterilised and sterilised conditions as estimated from the amounts of non-degraded linuron measured in methanol extracts from the samples during the incubation. Fitted to first-order kinetics, the data obtained allow calculation of the rate constant (K) of linuron degradation and the half-life (DT50) of the herbicide. Table 1 shows the K and DT50 values for the linuron degradation in unamended soil and soil amended with the different organic materials.

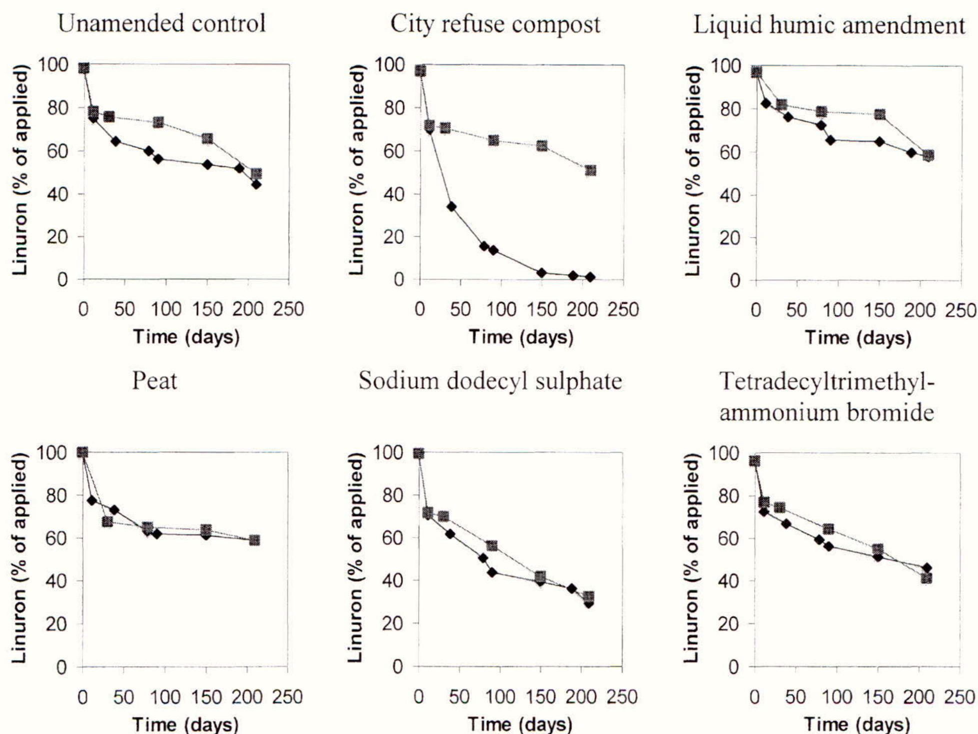

Figure 1. Degradation kinetics of linuron in unamended and amended soil samples under nonsterilized (—◆—) and sterilized (—■—) conditions.

In the non-sterilized conditions, the DT50 of linuron in the unamended soil was 248 d^{-1} and increased in the soils amended with LHA (344 d^{-1}) and with P (333 d^{-1}). It decreased considerably in the soil amended with SDS (147 d^{-1}) and decreased dramatically in the soil amended with CRC (34 d^{-1}). In the samples of sterilized soils, unamended and amended with CRC, LHA and P, the half-life of linuron increased with respect to that of the non-sterilized soil samples. However, in the soil amended with surfactants, the DT50 of linuron either did not vary (SDS) or decreased slightly (TDTMA).

Table 1. Rate constants (K) and half-life (DT50) for linuron degradation in unamended and amended soil samples.

	Non-sterilized		Sterilized	
Samples	K (d^{-1})	DT50 (d)	K (d^{-1})	DT50 (d)
Soil (S)	$27.9 \cdot 10^{-4}$	248	$25.0 \cdot 10^{-4}$	277
S-CRC	$203 \cdot 10^{-4}$	34	$22.0 \cdot 10^{-4}$	314
S-LHA	$20.1 \cdot 10^{-4}$	344	$19.2 \cdot 10^{-4}$	360
S-P	$20.8 \cdot 10^{-4}$	333	$19.4 \cdot 10^{-4}$	357
S-SDS	$46.9 \cdot 10^{-4}$	147	$46.6 \cdot 10^{-4}$	147
S-TDTMA	$29.5 \cdot 10^{-4}$	234	$34.0 \cdot 10^{-4}$	204

Table 2 shows the type and concentrations of the metabolites originated by the degradation of linuron in the non-sterilized and sterilized samples at the different incubation times. In the unamended, non-sterilized soil, the metabolites (M1, M2, M3 and M4), described as products of the microbial degradation of the herbicide (Maier & Härtel 1981), were found. Following sterilization of the soil, only the M4 metabolite was detected. Since microbiological activity will disappear after sterilization, and since M4 can also be originated by chemical hydrolysis of linuron (Maier & Härtel 1981), this metabolite could be attributed to a chemical degradation of the herbicide. These observations, together with the increase in the DT50 value after sterilization, point to the existence of both degradation mechanisms, chemical and microbiological, in the natural soil.

In the non-sterilized soil amended with CRC the M2, M3 and M4 metabolites were detected. After sterilization none of the metabolites was detected. Moreover, the DT50 of linuron in the sterilized soil was 9-fold higher than that of the non-sterilized soil. These findings show that the process of biological degradation of the herbicide is favoured strongly in the presence of CRC due to the strong microbial activity of this organic matter as compared with that of the natural soil and also to the differences in the nature of the microbial population (Perucci 1990).

No degradation products were detected in the samples of soil amended with LHA and P, either under sterilized or non-sterilized conditions. These samples had the highest values for the DT50 of linuron. These organic amendments probably adsorbed the molecules of linuron strongly, inhibiting the biological degradation of the herbicide by soil microorganisms. The inhibition of the degradation of different herbicides adsorbed by the organic components of the soil has been reported by several authors (Alexander 1994). However, despite the absence of metabolites a slight increase in the DT50 value can be seen in the sterilized samples, demonstrating a small loss of the herbicide by microbial mineralisation in the non-sterilized samples.

Table 2. Linuron metabolites (μg/g) in samples of unamended and amended soil.

Samples	Incubation time (d)	Non-sterilized M 1	Non-sterilized M 2	Non-sterilized M 3	Non-sterilized M 4	Sterilized M 1	Sterilized M 2	Sterilized M 3	Sterilized M 4
Soil (S)	0	nd	nd	nd	nd	nd	nd	nd	nd
	12	0.03	nd	md	0.06	nd	nd	nd	nd
	39	0.03	nd	nd	0.05	nd	nd	nd	0.03
	79	nd	nd	nd	0.04	-	-	-	-
	90	nd	nd	nd	0.03	nd	nd	nd	0.02
	150	0.02	nd	nd	0.02	nd	nd	nd	0.03
	188	nd	0.44	nd	0.04	-	-	-	-
	210	nd	0.96	0.04	0.08	nd	nd	nd	0.03
S-CRC	0	nd	nd	nd	nd	nd	nd	nd	nd
	12	nd	9.60	nd	nd	nd	nd	nd	nd
	39	nd	9.80	nd	0.19	nd	nd	nd	nd
	79	nd	8.70	0.09	0.03	-	-	-	-
	90	nd	8.38	0.68	nd	nd	nd	nd	nd
	150	nd	0.90	1.36	nd	nd	nd	nd	nd
	188	nd	0.78	1.86	nd	-	-	-	-
	210	nd	0.96	3.00	nd	nd	nd	nd	nd
S-SDS	0	nd	nd	nd	nd	nd	nd	nd	nd
	12	nd	nd	nd	0.34	nd	nd	nd	0.09
	39	nd	nd	nd	0.80	nd	nd	nd	0.19
	79	nd	nd	nd	2.66	-	-	-	-
	90	nd	nd	nd	2.96	nd	nd	nd	0.30
	150	nd	nd	nd	2.60	nd	nd	nd	0.28
	188	nd	nd	nd	1.60	-	-	-	-
	210	nd	nd	nd	3.68	nd	nd	nd	0.38
S-TDTMA	0	nd	nd	nd	nd	nd	nd	nd	nd
	12	nd	nd	nd	0.19	nd	nd	nd	0.03
	39	nd	nd	nd	1.06	nd	nd	nd	0.05
	79	nd	nd	nd	2.72	-	-	-	-
	90	nd	nd	nd	2.24	nd	nd	nd	0.02
	150	nd	nd	nd	2.44	nd	nd	nd	0.72
	210	nd	nd	nd	3.28	nd	nd	nd	1.22

nd = not detected

\- = not determinated

In the samples amended with both surfactants in non-sterilized and sterilized conditions, M4 was detected as the only transformation product and the DT50 of linuron was always lower than in the unamended soil. The DT50 of linuron remained unmodified in the soil amended with SDS, and decreased slightly in that amended with TDTMA, after sample sterilization. The effect of surfactants on the degradation of pollutant organic compounds has not been fully elucidated. Thus, whereas some authors have reported the ability of surfactants to inhibit soil microorganisms capable of metabolising organic compounds, others have indicated the capacity of surfactants to increase the desorption of the compounds and hence increase its availability for biological degradation by soil microorganisms (Aronstein *et al.,* 1991).

However, the similar DT50 values of linuron in the non-sterilized and sterilized samples would indicate that chemical rather than microbiological degradation is the major degradation process in the soil. Nevertheless the presence in the extracts of the metabolite 3,4-dichloroaniline, which is characteristics of the chemical hydrolysis of linuron, also indicates that the presence of the surfactants studied here favoured the chemical degradation of linuron.

The results obtained in this work, which demonstrate the potential of organic amendments and surfactants for affecting rates of linuron degradation in soil, could be valuable when using simultaneously organic amendments and pesticides in agricultural practices and when using surfactants in technologies for the prevention of pollution and the recovery of soil polluted by hydrophobic organic compounds.

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Degradation of pesticides in combination and their effect on soil microbial activity

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ABSTRACT

The effects of combinations of pesticides (chlorpyrifos, chlorothalonil, fenamiphos) on their degradation rates and on some soil microbial characteristics were studied. There were few interactions between chlorpyrifos and fenamiphos, but the presence of chlorothalonil always resulted in reduced degradation rates of the other compounds. Chlorothalonil also caused a significant reduction in soil microbial biomass and enzyme activities (phosphatase and dehydrogenase).

INTRODUCTION

Most studies of the environmental fate of pesticides are done with single applications of one compound. However, in practice, different pesticides are used to protect the crop from weeds, pathogens and insect pests, and repeated application of individual pesticides is a common occurrence. This is particularly the case in tropical regions with most horticultural crops and with major cash crops like cotton and sugarcane. It is important therefore that studies are made of possible interactions between pesticides when they are applied in combination to soils. The objectives of the experiments reported here were:

1. To investigate the effects of repeated application of pesticides to soil on their degradation rates.
2. To study the possibility of interactions between pesticides in terms of degradation rates when applied in combination.
3. To measure the effects of the chemicals, alone and in combination, on key simple soil microbial properties.

MATERIALS AND METHODS

Pesticides, soils and residue analysis

The compounds chosen for study were chlorpyrifos, chlorothalonil and fenamiphos which are a widely used insecticide, fungicide and nematicide respectively. Commercial formulations of chlorpyrifos and chlorothalonil and analytical grade fenamiphos were used throughout these studies. The soils (sandy loam) were collected from the experimental farm at Silwood Park,

Ascot, UK. Top-soil samples were collected from the 0-10 cm layer at three separate locations approximately 10 m apart to provide three true replicates. They were partially air dried overnight and sieved to pass a 3-mm mesh, and their moisture contents and water holding capacity were determined. One sub-sample (1 kg) of each of the replicate soils was treated with the pesticides chlorpyrifos, chlorothalonil (both suspended in water), or fenamiphos (solution in methanol) to give a concentration of 10 mg a.i./kg dry soil. Other subsamples of each replicate soil were treated with the pesticides at a rate of 10 mg/kg in the combinations:

chlorpyrifos + fenamiphos

chlorpyrifos + chlorothalonil

fenamiphos + chlorothalonil

chlorpyrifos + fenamiphos + chlorothalonil.

All samples treated with the fenamiphos solution in 3 ml methanol were left for 3 to 4 h for the solvent to evaporate. Distilled water was added to all soils to adjust the final moisture contents to 40% of water holding capacity. One set of three replicate samples without pesticide treatment and one set receiving 3 ml of methanol were kept as controls. Soils were mixed by hand initially and then passed through a 3 mm mesh sieve after which samples were incubated at 20°C. The treated soils were sampled periodically for 98 d and analyzed for concentration of pesticides and their metabolites. Once 50% of a specific pesticide had disappeared or 30 d after first treatment (whichever came later), the soil was retreated with another dose of the appropriate chemical or combination of chemicals at 10 mg/kg. A second re-treatment was done 30 days after the first re-treatment irrespective of residue concentration in the soil. Pesticides and their metabolites were extracted from soil with acetonitrile:water (90:10) by shaking for 1 h on a wrist action shaker. The concentration of pesticides and their metabolites were measured by HPLC using Kontron series 300 equipment. The column used was Lichrosorb- RP 18 (250mm x 4mm; Merck) with an isocratic mobile phase at a flow rate of 1ml/min.

Microbial studies

Dehydrogenase phosphatase and total microbial biomass were measured after 30, 60, 90 days from the first pesticide application in all treatments. Soil dehydrogenase and phosphatase activities were measured using the method of Tabatabai (1982). The method of Mele & Carter (1996) was used to determine total microbial biomass.

RESULTS

Pesticide persistence

Degradation of the pesticides in the various treatments approximated to first-order reaction kinetics. Table 1 shows correlation data, together with first-order rate constants and half-lives derived from the dissipation data for each treatment. The half-lives of chlorpyrifos alone and in combination with fenamiphos were about 63 and 64 days respectively in the first treatment, and these were extended to 118 and 193 days respectively when chlorothalonil was included. The

degradation rate of chlorpyrifos alone and in combination with fenamiphos was not affected in the second treatment, but the suppression of the degradation rate in the presence of chlorthalonil in the second treatment was much more pronounced with half-lives, 165 and 224 day respectively (Table 1). In the case of fenamiphos there was a slowing of the rate of degradation in all treatments with repeated application, which was particularly pronounced with the third treatment. In all treatments, fenamiphos was first transformed into fenamiphos sulfoxide and then to fenamiphos sulfone. These transformations into the oxidation products were slowed considerably when the soil was treated with chlorothalonil or with chlorpyrifos plus chlorothalonil (data not shown).

In terms of total toxic residues (TTR) the suppressive effect of chlorothalonil was similar whether by itself or in combination with chlorpyrifos. The transformation rate of fenamiphos was not affected by the presence of chlorpyrifos alone. The calculated half-lives for chlorothalonil (Table 1) show that the degradation rates of the second and third applications were slower than that of the first in most of the treatments. There appeared to be a suppression of chlorothalonil degradation when applied in combination with chlorpyrifos, but this was less marked in the triple combination of chlorothalonil plus chlorpyrifos plus fenamiphos.

Table 1. Correlation coefficients (R^2), first order rate constants (k, day^{-1}) and half-lives (HL, days) for pesticide degradation

	First treatment			Second treatment			Third treatment		
Pesticide treatment	R^2	k	HL	R^2	k	HL	R^2	k	HL
Chlorpyrifos									
CHP	0.979	0.011	63	0.984	0.012	60			
CHP + FEN	0.976	0.011	64	0.986	0.010	68			
CHP + CHTH	0.963	0.006	118	0.971	0.004	165			
CHP + FEN + CHTH	0.889	0.004	193	0.864	0.003	224			
Fenamiphos (parent compound)									
FEN	0.965	0.083	8.4	0.962	0.083	8.4	0.964	0.029	24
FEN + CHP	0.983	0.071	10	0.965	0.083	8.4	0.977	0.028	25
FEN + CHTH	0.984	0.059	12	0.999	0.042	17	0.929	0.018	40
FEN + CHP + CHTH	0.979	0.055	13	0.990	0.036	19	0.966	0.020	36
Chlorothalonil									
CHTH	0.923	0.056	12	0.997	0.035	20	0.949	0.033	21
CHTH + CHP	0.844	0.052	13	0.930	0.028	25	0.605	0.012	60
CHTH + FEN	0.853	0.052	13	0.982	0.050	14	0.943	0.027	26
CHTH + CHP + FEN	0.945	0.057	13	0.961	0.040	17	0.940	0.028	25

CHP=Chlorpyrifos; FEN=Fenamiphos; CHTH=Chlorothalonil

Microbial Studies

Results from the measurement of soil dehydrogenase, phosphatase and total microbial biomass activity at 30, 60 and 90 days after the first treatment are shown in Figure 1. A marked reduction in dehydrogenase activity was observed in all treatments involving chlorothalonil. Suppression of dehydrogenase activity was up to 50% in soil treated with chlorothalonil alone or with chlorothalonil plus chlorpyrifos. Fenamiphos alone or in combination with chlorpyrifos had no adverse effect on dehydrogenase activity, however chlorpyrifos alone had a small suppressive effect. Soil phosphatase activity was suppressed by the application of chlorothalonil alone or in combination with chlorpyrifos.

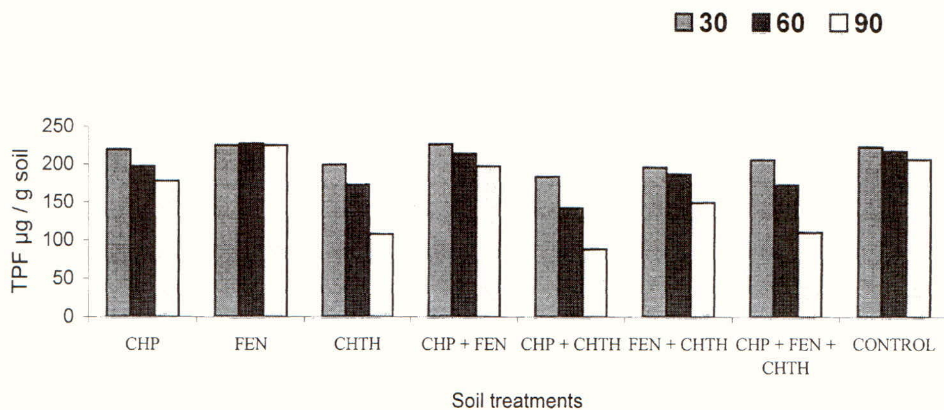
Application of fenamiphos gave a significant increase in soil phosphatase activity whether alone or in combination with chlorpyrifos. Small reductions in phosphatase activity were observed in the chlorpyrifos treatment especially after 90 days. All treatments involving chlorothalonil (alone or in combination with other pesticides) had significant effects on microbial biomass after 60 and 90 days. Fenamiphos alone or in combination with chlorpyrifos had no effect on microbial biomass. There was a small reduction in biomass in soil treated with chlorpyrifos after the second application.

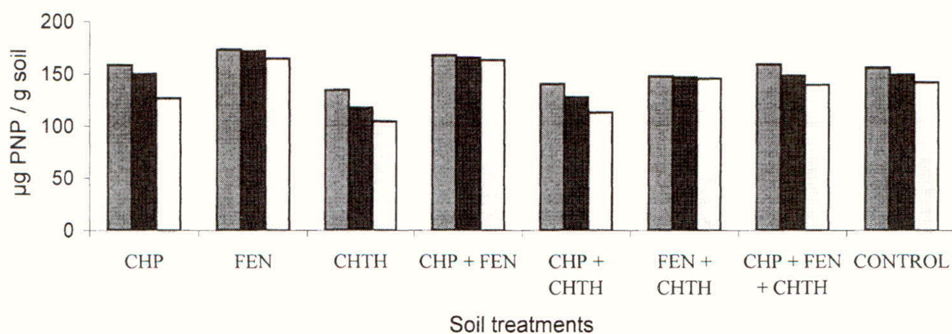
DISCUSSION

The results of the present study with the individual pesticides were broadly consistent with those reported previously. However, the present results extend those observed previously by inclusion of the pesticide combinations. The half-life of chlorpyrifos when applied alone was 64 days which is very similar to that reported by Racke *et al.,* (1990). The rate of chlorpyrifos dissipation was not affected by the presence of fenamiphos. However degradation of chlorpyrifos was suppressed in the presence of chlorothalonil, and this reduction in the rate of chlorpyrifos degradation was substantial even with the first application.

Chlorothalonil has been reported previously to suppress its own degradation when applied repeatedly (Motonaga *et al.*, 1998). When all three pesticides were applied together, the rate of chlorpyrifos degradation was suppressed further, indicating that there were synergistic interactions between the pesticides. An additive effect cannot explain the results since fenamiphos had no effect on chlorpyrifos degradation when applied in the absence of chlorothalonil (Table 1).

Fenamiphos degradation rate was the same when applied individually or in combination with chlorpyrifos. Repeated application of fenamiphos to this soil did not result in induction of fenamiphos accelerated degradation contrasting with the previous findings of Smelt *et al.,* (1996). However, the rate of transformation was reduced when treated with chlorothalonil or the chlorothalonil plus chlorpyrifos combination. Dissipation rates of total toxic residues (TTR) were also considerably reduced when the nematicide was incubated in soil in combination with chlorothalonil or with chlorothalonil plus chlorpyrifos. These effects were solely the result of the presence of chlorothalonil since chlorpyrifos had no effect on fenamiphos degradation.





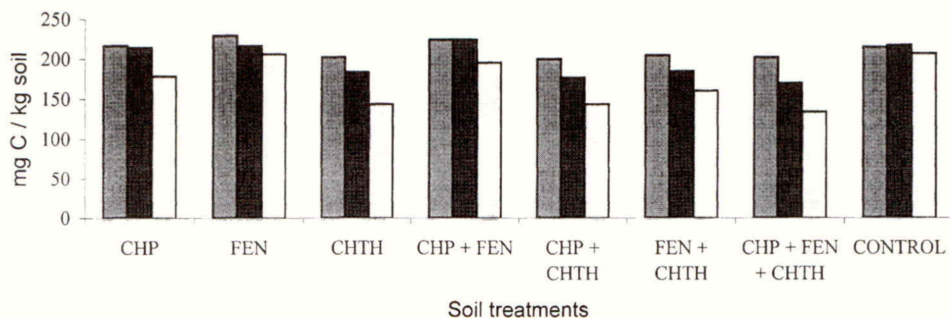


Figure 1. The effect of pesticide combinations on (a) dehydrogenase activity (b) phosphatase activity and (c) total microbial biomass after 30, 60 and 90 days. CHP = chlorpyrifos; FEN = fenamiphos; CHTH = chlorothalonil

The degradation of chlorothalonil when applied alone was suppressed by repeated application, which is in agreement with earlier findings of Motonaga *et al.*, (1998). None of the pesticide combinations had any effect on chlorothalonil degradation in the first treatment. However a slight reduction in dissipation rate was observed following the second application in combination with chlorpyrifos.

In the present study all of the measured microbial characteristics were adversely effected by chlorothalonil treatment when applied individually or in combination with the other pesticides. Chlorpyrifos and fenamiphos had little significant effect on the microbial properties investigated. These findings are in agreement with the previous study by Motonaga *et al.*, (1998) which demonstrated that soil respiration was suppressed following the application of chlorothalonil.

The present results provide an additional dimension to the study of environmental fate and ecotoxicology of pesticides and suggest that significant interactions can occur when combinations of different pesticides are applied repeatedly to soils. In our experiments, the combinations of pesticides examined were limited, and the studies were done in a single soil type. Further studies are needed with other pesticide combinations and in other soils.

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