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Effects of DDT and its metabolites on soil algae and enzymatic activity

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Abstract The persistence of DDT [1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane] and its metabolites in soil, their toxicity to soil algae, and effects on microbial activities were studied in laboratory microcosms for 45 days. In non-sterile soils, removal of DDD [1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethane] and DDE [1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethylene] was less than 3%, while 4–8% of applied DDMU [1-chloro-2,2-*bis*(*p*-chlorophenyl)ethylene], DDA [2,2-*bis*(*p*-chlorophenyl)acetic acid] and DDT were lost. Added DDOH [2,2-*bis*(*p*-chlorophenyl)ethanol] was more labile, as 60% was degraded during the same period. Soil microalgae were not measurably affected by the compounds tested at 10–50 mg kg^{-1}, but at 100 mg kg^{-1} soil, DDD, DBP (*p,p′*-dichlorobenzophenone) and DDA significantly reduced their growth. Phosphatase activity was not affected by DDT and its metabolites at the concentrations tested ($\leq$50 mg kg^{-1}), but all compounds inhibited dehydrogenase activity at concentrations of 50 mg kg^{-1} soil. The toxic effects of DDT and its metabolites were dose-related.

Key words DDT · Metabolites · Persistence · Toxicity · Microalgae · Soil enzymes

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Introduction

Since the 1940s, DDT [1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane] has been used worldwide to control arthropod disease-vectors and pests (Laws 1981). DDT residues are now widely distributed and persistent environmental contaminants. The US Environmental Protection Agency has recognised this by listing these compounds as priority pollutants (Keith and Telliard 1979). Although use of DDT is now banned in most developed countries, its continued use in developing nations is significant (Hussein et al. 1994). Widespread agricultural use of kelthane (which contains 14% DDT isomers) has also contributed to environmental contamination (Florida Department of Environmental Regulation and Agriculture and Consumer Services 1984). Formation of DDE [1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethylene], DDD [1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethane], DDMU [1-chloro-2,2-*bis*(*p*-chlorophenyl)ethylene], DDOH [2,2-*bis*(*p*-chlorophenyl)ethanol] (Mitra and Raghu 1988; Agarwal et al. 1994) and DDA [2,2-*bis*(*p*-chlorophenyl)acetic acid] (Xu et al. 1994) in DDT-contaminated soils has been reported, and photolytic transformation of DDE to DDMU and DBP (*p,p′*-dichlorobenzophenone) has also been shown (Maugh 1973). Photo-oxidation and volatilisation are probably responsible for the rapid loss of DDT and its residues from tropical soils (Hussein et al. 1994), but in temperate climates DDT residues can persist for longer periods. The predominant residues detected in aerobic soils are usually DDE, parent DDT and, to a lesser extent, DDD (Boul 1995).

The effects of DDT, DDE and DDD on aquatic biota have been widely studied (Day 1991), but no information has been published on the effects of DDT and its metabolites towards enzymatic activities and algal populations in soil. Microalgae are ubiquitous and form an important component of the soil ecosystem (Megharaj et al. 1993), while soil enzymes released by a wide variety of biota play an important role in soil organic

matter degradation and nutrient recycling (Tu 1980). Interference of DDT and its residues with the normal activities of microalgae and soil enzymes were therefore examined.

Materials and methods

Soil

Soil which had not previously received any DDT applications was collected from the Winchmore Research Station, Canterbury, New Zealand from a depth of 0–8 cm. The soil was air-dried and sieved (2 mm). The soil (Lismore Silt loam) had pH 5.3; organic matter, 3.5%; total nitrogen, 0.27%, fine sand, 38.7%; coarse sand, 10.3%; silt, 33.0%; clay, 18.0%.

Test chemicals

All chemicals were analytical grade from Aldrich, Michigan, USA except DDE, which was purchased from Janssen, Geel, Belgium.

Soil microcosms

The persistence of DDT and its metabolites was compared in autoclaved and unautoclaved soil samples under non-flooded conditions (Megharaj et al. 1993). Soil samples (5 g) were introduced into sterile culture tubes (30 ml) fitted with Teflon-covered screw caps. One set of microcosms was sterilised by autoclaving three times at 120 °C for 1 h at 1-day intervals. The soil samples were then treated with the compounds of interest dissolved in acetone to a final concentration in soil of 100 mg kg^{-1}. The acetone solvent (20 μl) was then allowed to evaporate and soils were rigorouly mixed (Megharaj et al. 1997a). Soil samples were maintained at 50% water content to maintain unflooded conditions (Megharaj et al. 1993). Microcosms were incubated at room temperature (20 ± 2 °C) under diffuse ambient laboratory light.

Extraction and residue analysis

For both autoclaved and unautoclaved treatments duplicate microcosms were extracted immediately after the experiment was set up (time 0). Further duplicates were extracted after 45 days. Microcosms were extracted twice with equal volumes (5 ml) of methanol by shaking on an orbital shaker for 3 h followed by vigorously vortexing and centrifugation (6000 *g*, 15 min) to remove particulate matter. Prior to a second extraction soil samples were treated with sulfuric acid to release any bound residues (Agarwal et al. 1994). Extracts were pooled, and DDT metabolites determined by HPLC on an Econosil C-8 column (Alltec, Deerfield, Ill.) using an isocratic elution phase (80:19:1 methanol/deionised water/acetic acid, v/v/v) as described previously (Megharaj et al. 1997b). Detection was by UV absorbance at 235 nm and peak areas were integrated using ICI DP800 software (GBC, Scientific, Auckland, New Zealand) and quantitated by comparison with authentic external standards. Retention times of DDT and its metabolites DDD, DDE, DDMU, DBP, DDA and DDOH were 10.2, 7.8, 11.3, 9.8, 6.0, 5.1 and 5.1 min respectively. Recoveries of these compounds in autoclaved controls were >97% with a lower detection limit of <0.5 mg kg^{-1} soil.

Toxicity to the soil algal population

The toxicity of DDT residues to algae was examined in microcosms similar to those described above except that compounds were added to final concentrations of 0, 10, 50 and 100 mg kg^{-1} soil. Soil samples treated with acetone only served as controls. After 45 days, incubation, triplicate soil samples were withdrawn for population estimation of microalgae by the most-probable number (MPN) method (Muralikrishna and Venkateswarlu 1984; Megharaj et al. 1986).

Assay of enzyme activities

Dehydrogenase activity (as an indirect measure of microbial activity) in soils was determined following the method of Casida et al. (1964) by reduction of 2,3,5-triphenyltetrazolium chloride (TTC). Each soil sample (5 g) was treated with 0.05 g $CaCO_3$, 1 ml of 3% (w/v) TTC and incubated for 24 h at 37 °C. The triphenyl formazan formed was extracted from the reaction mixture with methanol and assayed at 485 nm with a Spectronic 20D spectrophotometer (Milton Roy, USA). Acid phosphatase activity was determined by the method of Tabatabai and Bremner (1969). Soil samples (1 g) were mixed with 0.25 ml toluene, 4 ml modified universal buffer (pH, 6.5) and 1 ml of *p*-nitrophenyl phosphate solution (0.115 M) and incubated for 1 h at 37 °C. Reaction was stopped by addition of 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH. The reaction product was recovered by filtration through Whatman no. 2 filter paper. The *p*-nitrophenol (PNP) in the filtrate was determined colorimetrically at 410 nm.

Results and discussion

Stability of DDT and its metabolites in soil

When soil samples were autoclaved prior to addition of DDT and its metabolites recovery of all compounds after 45 days of incubation was similar to initial (time 0) recoveries (Table 1). In unautoclaved soils residual DDD, DDE and DBP levels were virtually identical to autoclaved controls after incubation, while levels of DDT, DDMU and DDA decreased only slightly (Table 1). DDOH was most rapidly degraded in unautoclaved soils with only 40% remaining at the end of the incubation period. These results are consistent with the established persistence of these residues. Similar decreases in levels of DDT and DDE were reported by Boul (1996) in experiments conducted with soil from the same site. Boul (1996), however, noticed that a significant portion of the residues were bound to the soil. It is assumed that the sulfuric acid treatment used during extractions in this study released these residues, but the

Table 1 Persistence of DDT and its metabolites in soil. Values are means of duplicate determinations. Initial (time 0) recoveries of these compounds in autoclaved soils were >97%

Treatment (50 mg kg^{-1} soil)	% recovery, 45 days after treatment	
	Autoclaved	Unautoclaved
DDT	98.6	92.8
DDD	99.0	97.2
DDE	98.9	98.9
DBP	98.4	98.2
DDA	98.5	93.0
DDMU	99.1	95.6
DDOH	97.3	40.2

effectiveness of the treatment was not directly assessed.

Microbial activity is the main factor responsible for degradation of most pesticides in soils. Slow abiotic transformation of DDT to DDD and DDE in soils under temperate climates has been reviewed (Boul 1995).

Toxicity to soil algal population

Viable count estimates (MPN), along with their 95% confidence limits of the total soil algal population in the presence of increasing concentrations of DDT or its metabolites are presented in Table 2. DDT and its metabolites at 10 or 50 mg kg^{-1} soil did not measurably affect the composition and numbers of soil algae after 45 days of incubation. There was no apparent effect of DDT, DDE, DDMU and DDOH on the algal population even at the highest levels tested (50 or 100 mg kg^{-1} soil). A large reduction in algal population size was observed, however, in the presence of DDD or DBP at this level. The apparent toxicity of DDT and its metabolites to soil algae followed the order: DDT $\leq$ DDE $\leq$ DDMU $\leq$ DDOH $<$ DDA $<$ DDD $<$ DBP. The inhibitory effects of DDD and DBP increased with dose.

Of particular interest in the study was the change in species composition of the soil algal population after application of DDT or its metabolites. The predominant algae in untreated soil belonged to five species of Chlorophyta and two of Cyanophyta. Consistently recovered algae were *Chlorella* sp., *Chlorococcum* sp., *Cylindrocystis* sp., *Gloeocystis* sp., *Scenedesmus* sp., *Anabaena* sp. and an unidentified unicellular cyanobacterium. The cyanobacteria present in untreated soil were sensitive to DDT and its residues, whereas the unicellular green algae *Chlorella* sp. and *Scenedesmus* sp. became dominant when soils received high pesticide doses (Table 3). Similar phenomena have been observed in organophosphate (monocrotophos and quinalphos) treated soils (Megharaj et al. 1986, 1988). Algal species vary widely in their response to toxic pollutants and DDT has been shown to be selectively toxic to sensitive marine algal species (Mosser et al. 1972b). Alteration of phytoplankton species composition in marine communities by Polychlorinated biphenyls and DDT of sufficient magnitude to potentially affect the structure and functioning of the ecosystem has also been reported (Mosser et al. 1972a, b). To our knowledge, however, this is the first report that DDT alters the composition of native algal species in a soil ecosystem.

DDT has been shown to inhibit marine phytoplankton photosynthesis (Wurster 1968) and growth of *Chlorella fusca* in pure culture at concentrations above 0.1 mg l^{-1} (Goulding and Ellis 1981). Clegg and Koevenig (1974) showed that four organochlorine insecticides, including DDT, significantly reduced the ATP content in

Table 2 Effect of DDT and its metabolites on algal populations (MPN, $\times 10^3$ g^{-1} soil) in soil. Figures in parentheses represent higher and lower fiducial limits, respectively, of each MPN at the 95% confidence level. *ND* Not determined, *NA* not applicable

Treatment	Concentration (mg kg^{-1} soil)			
	0	10	50	100
Untreated	153.7 (282.2, 83.7)	NA	NA	NA
DDT		153.7 (282.2, 83.7)	153.7 (282.2, 83.7)	153.7 (282.2, 83.7)
DDD		153.7 (282.2, 83.7)	153.7 (282.2, 83.7)	11.7 (21.6, 6.4)
DDE		153.7 (282.2, 83.7)	153.7 (282.2, 83.7)	153.7 (282.2, 83.7)
DBP		153.7 (282.2, 83.7)	114.9 (211.0, 62.6)	5.4 (9.9, 4.0)
DDA		153.7 (282.2, 83.7)	114.9 (211.0, 62.6)	114.9 (211.0, 62.6)
DDMU		153.7 (282.2, 83.7)	153.7 (282.2, 83.7)	ND
DDOH		153.7 (282.2. 83.7)	153.7 (282.2, 83.7)	ND

Table 3 Qualitative occurrence of soil microalgae at the end of 45 days as influenced by the treatment of DDT and its metabolites. − absent, + common, ++ abundant

Organism	Treatment (mg kg^{-1} soil)														
	Untreated	DDT		DDD		DDE		DBP		DDA		DDMU		DDOH	
	0	10	100	10	100	10	100	10	100	10	100	10	50	10	50
Anabaena sp.	++	+	−	−	−	+	−	−	−	+	−	+	−	+	−
Chlorella sp.	++	+	++	+	+	++	+	+	−	++	+	++	+	++	−
Chlorococcum sp.	+	+	+	++	+	++	+	+	+	+	+	−	+	++	+
Cylindrocystis sp.	+	+	−	+	−	+	−	−	−	+	+	+	+	+	+
Gloeocystis sp.	+	++	+	+	−	+	−	+	−	++	−	+	+	+	+
Scenedesmus sp.	++	++	+	+	+	+	+	+	−	+	+	++	+	++	+
Unicellular cyanobacterium	+	−	−	−	−	+	−	−	−	+	−	+	−	+	−

algae, suggesting the effect of these compounds may be on photophosphorylation.

Chlorinated hydrocarbons can be highly persistent in the environment. Where degradation does occur, products may be more stable and sometimes more toxic than the parent compound (Stratton 1984). The environmental impact of a xenobiotic compound may not, therefore, be limited by the toxicity of the parent chemical. Studies of the inhibition of cyanobacterial growth by aldrin, dieldrin and their transformation products indicated that the transformation products can be at least as inhibitory as the parent compound (Batterton et al. 1971). Growth of *Anacystis nidulans* was markedly inhibited by DDT, but the metabolites DDD and DDE were more toxic (Boush and Matsumura 1975). Our results are consistent with these findings. Our observation that microalgae and cyanobacteria were not inhibited by DDT and its metabolites in soil at levels up to 50 mg kg^{-1} may be due to low availability of the compounds to the microbiota. It is possible that the adsorptive/protective effect of the soil environment could protect sensitive microorganisms from any inhibitory effect of pesticide residues. The finding that a large proportion of the DDT residues are bound to soil components (Boul 1996) suggests that the concentrations of these residues available to algae in soils may be lower than the amounts added would indicate.

Effect on soil enzymes

Dehydrogenase activity is a useful indicator of the overall microbial activity of soils and has been recommended as a measure of the side effects of agrochemicals (Gerber et al. 1991), as it is likely to be affected by pesticide application (Cervelli et al. 1978). Phosphatase is an exocellular enzyme produced by a large number of soil microbes, and is involved in release of inorganic phosphate from organically bound phosphate and is important for plant nutrition (Speir and Ross 1975; Dick and Tabatabai 1986). The effect of DDT and its metabolites on dehydrogenase and phosphatase activity in the soil samples was therefore determined (Table 4). None of the metabolites examined inhibited dehydrogenase activity at 10 mg kg^{-1}, whereas at 50 mg kg^{-1} all measurably reduced formazan formation (Table 4). No effect of DDT and its metabolites on phosphatase activity was observed, however, even at 50 mg kg^{-1}.

Ko and Lockwood (1968) have also reported inhibitory effects of DDT and DDD on soil microorganisms. DDD was found to have a broader antimicrobial spectrum and greater toxicity to microorganisms than DDT. The current study supports this observation, as our results (Table 4) show that dehydrogenase activity is more sensitive to DDD than DDT. Since the higher levels of DDT residues (50 and 100 mg kg^{-1} soil) used in this study are in excess of those that would commonly have arisen at field application rates (Boul et al. 1994), it is not clear whether the soil microorganisms are endangered by these residues in the field. Notwithstanding this reservation, a simple mass/mass ratio of contaminant to soil may not adequately describe the exposure of microbes to pollutants, since hydrophobic contaminants may accumulate in microbial cells. Megharaj et al. (1997b) found up to 91% of the DDE added to a culture medium incubated with bacteria to be associated with the cellular fraction. The interference of the residues with algal species composition at levels of 10 mg kg^{-1} soil is, however, more significant. Such levels have frequently been reported, particularly in horticultural soils. Effects on the microflora in such soils can therefore be expected and are undesirable.

In summary, the results of the present study are relevant in view of the continued usage of DDT in developing countries and the continued presence of its residues in developed nations. DDT residues can impact both qualitatively and quantitatively on soil algal populations and also affect other microbial processes as indicated by their affect on soil enzymatic activity. These off-target effects of pesticide use are of unknown significance and warrant further study.

Table 4 Effect of DDT and its metabolites on soil enzymatic activities. Values represent mean of triplicate determinations. All the values are expressed as percentages in relation to controls

Treatment (mg kg^{-1} soil)	% Dehydrogenase activity	% Phosphatase activity
Untreated	100.0bc*,[1]	100.0cd[2]
DDT		
10	101.8bc	99.6cd
50	59.8e	105.4bc
DDD		
10	111.6a	99.0cd
50	48.7fg	100.9cd
DDE		
10	115.3a	118.1a
50	62.5e	102.6cd
DBP		
10	104.8abc	115.5ab
50	78.9d	101.7cd
DDA		
10	99.8bc	107.8bc
50	59.0ef	92.6d
DDMU		
10	97.3c	98.5cd
50	53.8ef	99.1cd
DDOH		
10	110.0bc	100.9cd
50	44.7g	98.4cd

* Means ($n=3$) in each column followed by the same letter are not significantly different from each other ($P \leq 0.05$) according to Duncan's new multiple range test

[1] 29 μg formazan formed per gram of soil after 24 h incubation with TTC

[2] 70 μg PNP per gram soil formed after 1 h incubation with *p*-nitrophenyl phosphate

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