Bull. Environ. Contam. Toxicol. (1998)60:585-591
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Bulletin of Environmental Contamination and Toxicology

Long Term DDT Pollution in Tropical Soils: Effect of DDT and Degradation Products on Soil Microbial Activities Leading to Soil Fertility

J. Mitra, K. Raghu

Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai 400 085, India

Received: 19 November 1997/Accepted: 16 January 1998

The use of highly persistent organochlorine compound DDT (1,1'bis(p-chlorophenyl) 2,2,2-trichloroethane) in tropics for public health and agricultural purposes is almost unavoidable. In India restricted use of DDT was recommended for certain crops till recently. DDT degrades faster in warm and moist tropical soils compared to temperate ones. Faster disappearance is due to more volatilisation and biodegradation processes (Samuel and Pillai, 1988; Zhengsheng and Haibo, 1994). Rapid disappearance of DDT from Indian soil with the accumulation of DDD (1,1'bis-(parachlorophenyl) 2,2-dichloroethane) in flooded and DDE (1,1'bis-(parachlorophe- nyl)2,2-dichloro ethylene) in unflooded soils was reported earlier and the rate of degradation was found to be directly proportional to the increase in temperature, moisture and microbial activities in the soil (Mitra and Raghu, 1978). Agricultural practices influence the DDT degradation and the nature of residues in the soil (Guenzi and Beard 1968; Nash et al. 1973; Raghu et al. 1983; Boul 1994; Spencer et al. 1996). Perfect and co-workers (1979) reported faster decline of yield in cultivated soil with DDT treatment and this was attributed to rapid loss of fertility in DDT treated soil. Similar effect was also observed by us with chilli (Capsicum annuum) when grown in fields with a history of DDT treatment earlier (Mitra and Raghu, 1996). The exact mechanism by which DDT reduces soil fertility is not known. Hence, studies on the long term effect of DDT in Indian soils and the effect of DDT and its major degradation products on soil microbial activities leading to soil fertility were taken up.

MATERIALS AND METHODS.

Red sandy and Black clay soils commonly occurring in this region were collected from the surface horizon (10 cm) of cultivated fields. Characteristics of two soils are shown in Table 1. Soil samples were air-dried and passed through a 2mm sieve. 20 g soils in triplicate in glass tubes were used for each treatment throughout the study. Anaerobic condition in flooded soil (F) was created by adding water to the soil 7cm above soil surface and the moisture content of unflooded (UF) or aerobic soil was maintained around 40-60% MHC (Mitra and Raghu, 1978).

Analytically pure p-p'isomers of DDT, DDD; and DDE (Analabs) were used for

Correspondence to: J. Mitra

Table 1. Characteristics of soils used in this study

Soil Characteristics	Soil Types	
	Red sandy	Black clay
pH	6.8	7.6
O.C. (%)	0.7	0.9
Sand (%)	51.6	20.4
Silt (%)	23.4	17.8
Clay (%)	25.0	61.8
Dehydrogenase Activity (μg TTF g^{-1} soil)*		
UF	0.018	0.022
F	0.09	0.12

*16 hour incubation period

this study. DDT, DDD or DDE dissolved in acetone at 5 and 50ppm (mg Kg^{-1} soil) final concentration were mixed thoroughly with small amount of air dry soil samples separately Acetone was evaporated from soil and was mixed with the corresponding bulk soil. Soils treated with acetone only was served as control. ^{14}C DDT was obtained from Amersham International Plc. Buckmghamshire, England. Long term DDT pollution was studied in black clay soil in the laboratory under out door condition using uniformly labelled ^{14}C DDT (final sp. activity 2.9 µCi $umole^{-1}$). Soil samples containing 5ppm DDT in triplicate were incubated for 18 months under continuous flooding (F) and alternate cycles of flooding and unfIooding (F/UF), conditions prevailing in low land cultivation system. Soils were extracted with petroleum ether as per Mac Rae et al. (1967) and were analysed by Gas Liquid Chromatography (GLC) and two dimensional Thin Layer Chromatography (2-D TLC) following Mitra and Raghu (1986a). Long term effect of MgO was studied using red sandy and black clay soils contaminated with DDT at 5ppm level under dry and alternate cycles of moist (60% MHC) and dry conditions of soil. Soil samples were treated with 4% MgO following Nash et al. 1973. Soils were extracted after 2 years and the extract were analysed by GLC. The values were corrected for percent recovery against 0 day extracts.

The microbial studies were carried out in black clay soil under flooded and unflooded conditions for 6weeks simulating field conditions. Samples in triplicate from each treatment at intervals were removed for analysis. The different groups of soil micro-organisms were enumerated by soil dilution plate technique (Johnson et al., 1959) using soil extract agar (Allen, 1957), glycerol aspergine agar (Conn, 1921), Martin's Agar (Martin, 1950), Ashby's nitrogen free mannitol phosphate agar (Clark, 1965) and yeast mannitol agar media (Clark, 1965) for bacteria, actinomycetes, fungi, Azotobacter and Rhizobium respectively. Congo-red (0.25%) solution was used to identify the Rhizobium population.

Soil respiration was studied for 5 weeks using 50g black clay soil treated with DDT, DDD or DDE in 250 ml Erlenmeyer flasks under flooded (2 cm of water above soil surface) and unflooded conditions (60% MHC) following the method of Kale and Raghu (1989). Different soil enzymes studied were dehydrogenases (Casida et al., 1964), acid phosphatase, alkaline phosphatase, phosphotriesterase, arylsulfatase (Bremner and

Tabatabai, 1969) and phosphodiesterase (Browman and Tabatabai 1978).

RESULTS AND DISCUSSION

The long term effect of DDT pollution in continuously flooded (F) and intermittently flooded (FAJF) soils are shown in Table 2a and 2b. It shows 20-25% added ^{14}C activity was recovered after 18 months. DDT degraded faster in F compared to F/UF soil. Less

Table 2a. Long term degradation of DDT in Black clay soil

Fractions	0 day	18 months	
	F	F	UF
Nonpolar fractions	88.60	11.80	10.59
Polar fractions	5.80	2.72	5.70
Bound residues	5.50	5.70	9.05
Total ^{14}C DDT recovered	99.90	20.20	25.34

* Different components of nonpolar fraction are shown in Table 2b

DDT was recovered from F compared to the F/UF soil (Table 2b). In F soil the major degradation product was DDD while in F/UF soil both DDD and DDE were present at much lower level.. The latter observation is interesting as both aerobic and anaerobic conditions prevailing under such situation, and formation of both the metabolites is probably reflective of conditions in rain fed agriculture which is typical in many parts of India. The degradation of DDD was faster in F/UF soil as the ratio between DDD to other subsequent degradation products was 11.60%: 13.10% i.e. <l while in flooded soil was it was 53.90% : 30.24% i.e. ~2.

Our earlier studies showed under unflooded condition MgO increased DDE formation considerably in all soil typos collected from different parts of India. DDE formation was directly proportional to the soil pH (Mitra and Raghu, 1986b). MgO is an important constituent of commercial lime and is recommended for agriculture in acid soils. It is also recommended for soils with low exchangeable magnesium and which are sandy in nature. DDE being more persistent and toxic compared to DDT, is not desirable to accumulate in soil. Hence, the long term effect of MgO on DDE formation was studied in dry and intermittently moist (dry and moist) red sandy and black soils. These conditions commonly prevail in agricultural lands. Results presented in Table 3 show more DDE in dry MgO amended red sandy and black clay soils compared to the dry unamended soils. Unamended soils on the other hand show more DDE in intermittently moist soils compred to the dry soils, More DDE in unamended moist soils could be due to microbial degradation of DDT to DDE along with the chemical degradation in these soils. Reverse was seen in MgO amended soils where less DDE was formed in intermittently moist soils compared to the respective dry soils. It could be due to the lack of microbial degradation in wet MgO amended soils showing high pH (above 9.0) which did not support microbial growth in such soils.

The effect of DDT on microbial activities in tropical soils was studied by several workers. However, informations regarding the effects of the accumulatory products on microbial activities in these soils are lacking. Present study shows that DDD, the

major anaerobic degradation product of DDT inhibits actinomycetes and fungi in flooded and unflooded black clay soils at 5ppm level even after 6 weeks. Azotobacter and Rhizobium on the other hand showed stimulation even at 50 ppm level during the later periods of incubation (Table 4).

Table 2b. Percent distribution of ^{14}C activity in different components of nonpolar* fraction shown in Table 2a

Componenets	0 day	18 months	
		F	F/UF
DDT	85.61	9.18	64.04
DDD	2.54	53.90	11.60
DDE	1.01	4.08	10.41
Kelthane	1.08	0.33	0.38
Others + unidentified metabolites	9.8	30.2	13.1
Total	100.04	96.59	99.53

*DDM (bis(p-chlorophenyl)methane, DBH (4-4,dichlorobenzhydrol), DBP (4-4,-dichloroenzophenone), DDOH (1,1′-bis(p-chlorophenyl)2-hydroxyethane

Table 3. Long term (2yr) effect of MgO on DDE formation in soil

Soil	Treatment	% Recovery	
		DDE	DDT
Red Sandy	Dry/unamended	2.7	68.3
Red Sandy	Dry/amended	49.5	31.7
Red Sandy	Int. moist/unamended	6.2	90
Red Sandy	Int. moist/amended	40.7	65.7
Black Clay	Dry/unamended	10.3	92.2
Black Clay	Dry/amended	64.5	53.4
Black Clay	Int. moist/unamended	14.9	85.2
Black Clay	Int. moist/amended	46.1	59

The effects of DDT, DDD and DDE on soil respiration in black clay soil was studied for5 weeks under flooded and unflooded conditions. CO_2 evolved was measured at intervals. Table 5 shows the results of 2days and 5 weeks only. Inhibition of CO_2 evolution was shown initially with all treatments in flooded soils which was mitigated at 5 ppm level during the later part of incubation. Under unflooded condition no inhibition was observed rather the rate of respiration in treated soils was found to be more compared to the untreated or control soils. The effects of DDT and metabolites on different soil enzymes were studied in black clay soil under flooded and unflooded conditions for 5 weeks. Only the results of 0-day (overnight incubation) and 5-week are presented in Table 6. Inhibition in enzyme activities was observed in flooded and unflooded soils mostly during the initial stages. Inhibition was less in unflooded soils compared to flooded ones. The inhibitory effect in dehydrogenase and acid phosphatase activity in unflooded soil and arylsulfatase activity in flooded soils was seen with all the chemicals even after 5 weeks. Dehydrogenase activities in unflooded soils after 5 weeks were 2.1, 2.9 and 2.7 with DDT, DDD and DDE at 5ppm level respectively against 6.0 mg Formazan formed g^{-1} control soil and acid phosphatase activities were 0.53, and 0.55

Table 4. Effect of DDD (μg g^{-1}) on soil microbial numbers

Organism /g soil	Incubation (weeks)	Flooded soil			Unflooded soil		
		0	5	50	0	5	50
Bacteria	2	32 ± 15	6 ± 0	31 ± 9	13 ± 3	13 ± 2	55 ± 7
$X10^6$	6	17 ± 2	16 ± 3	57 ± 6	3 ± 1	4 ± 2	31 ± 8
Actinomycetes	2	7 ± 1	5 ± 2	3 ± 2	50 ± 24	14 ± 5	9 ±1.0
$X10^5$	6	7 ± 2	3 ± 1	1 ± 0	120 ± 20	28 ± 10	8.0 ± 1.0
Fungi	2	12 ± 5	30 ± 3	9 ± 2	600 ± 80	100 ± 10	230 ± 20
$X10^4$	6	50 ± 3	20 ± 3	3 ± 0	230 ± 30	50 30	200 ± 30
Azotobacter	2	120 ± 9	32 ± 12	100 ± 20	80 ± 3	61 ± 3	278 ± 49
$X10^5$	6	69 ± 3	110 ± 1	370 ± 70	43 ± 22	301 ± 40	319 ± 43
Rhizobium	2	140 ± 30	20 ± 1	64 ± 10	110 ± 28	70 ± 3	204 ± 47
$X10^5$	6	100 ± 40	230 ± 50	350 ± 60	61 ± 29	200 ± 33	320 ± 40

with DDD and DDE respectively at 5ppm level against 0.63 μ mole p-nitrophenol released/g control soil. The levels of arylsulfatase activities in flooded soils after 5 weeks were 0.30, 0.27 and 0.32 with DDT, DDD and DDE (5ppm) amended soils respectively against 0.41μmole p-nitrophenol released/g control soil. Others, however, showed enzyme activities at par with the control soils or exceeded the control values. Above studies on soil respiration and soil enzyme activities gives an indication of over all microbial activities and the elemental cyclic processes in the soil. Dehydrogenases are group of enzymes involved in the the oxidation reduction reactions of organic compounds in a living cell and are significantly correlated with the oxygen uptake in the living cells. Phosphatases and arylsulphatases on the other hand are the enzymes responsible for the transformation and degradation of nucleic acids/other phosphatic compounds and organic sulfates respectively in the soil .Inhibitory effect of DDT and its metabolites on these processes would certainly affect mineralization of complex organic matters in the soil which in the long run it may lead to loss of fertility of soil resulting in nonavailability of mineral nutrition to the plants and subsequently poor productivity of crop plants in cultivated lands.

Table 5. Effect of DDT and metabolites on microbial respiration in black clay soil

Treatment	Concentration	mg CO_2 evolved/50g soil			
		Flooded Soil		Unflooded Soil	
	ppm	2 d	5 wk	2 d	5 wk
C		11.80	116.6	14.3	144.10
DDT	5	7.39	123.18	14.78	169.05
	50	8.31	108.00	17.55	148.76
DDD	5	7.40	120.72	26.83	176.63
	50	6.47	107.08	20.33	153.23
DDE	5	10.62	126.92	19.86	168.09
	50	8.32	115.39	29.57	199.64

Table 6. Effect of DDT and its major degradation products on soil enzyme activities

Enzymes	ppm	0 d						5 wk					
		Flooded			Unflooded			Flooded			Unflooded		
		T	D	E	T	D	E	T	D	E	T	D	E
1	5	-	-	-	±	±	±	±	±	±	-	-	-
	50	-	-	-	±	±	±	±	±	±	-	-	-
2	5	x	x	±	-	-	-	±	x	x	±	x	x
	50	±	x	±	-	-	-	±	x	x	x	x	±
3	5	-	-	-	-	-	-	±	±	±	±	-	-
	50	-	-	-	-	-	-	±	±	±	-	-	-
4	5	-	-	±	-	-	-	x	x	x	x	x	-
	50	-	-	-	-	-	-	x	x	x	x	x	x
5	5	x	x	x	±	x	x	x	x	x	±	±	±
	50	-	-	-	-	x	x	x	x	x	±	±	±
6	5	x	-	-	±	±	±	-	-	-	±	±	±
	50	-	-	-	x	-	-	-	-	-	±	±	±

T: DDT, D: DDD, E: DDE ± : Stimulation, - : Inhibition, x : No effect

1. Dehydrogenase, 2. Alkaline Phosphatase, 3. Acid Phosphatase, 4. Phosphodiesterase, 5. Phosphotriesterase, 6. Arylsulfatase.

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