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Accelerated Biodegradation of γ-Hexachlorocyclohexane (γ-HCH) in a Flooded Alluvial Soil Retreated with γ-HCH or Its Metabolite 1,2,4-Trichlorobenzene (TCB)

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Accelerated biodegradation of soil-applied pesticides upon their repeated application is the result of soil-pesticide-microbe interaction and can undermine the efficacy of the pesticides under consideration (Sethunathan et al. 1971; Felsot 1989; Racke 1990). Enhanced microbial degradation is usually defined as a phenomenon in which adapted soil microorganisms make use of the pesticide or its degradation products as an energy or nutrient source leading to decreased persistence of the pesticide (Somasundaram and Coats 1990). Hexachlorocyclohexane (HCH), an organochlorine insecticide widely used in India, has recently been shown to be degraded faster upon its repeated application to nonflooded (Wada et al. 1989) and flooded rice fields (Bhuyan et al. 1992) due to selective proliferation of HCH-degrading aerobic bacteria (Bhuyan et al. 1993). Aerobic degradation of HCH includes stepwise dechlorination followed by ring opening (Imai et al. 1991). In the aerobic degradation of γ-isomer of HCH 1,2,4-trichlorobenzene (TCB) was identified as a metabolite (Imai et al. 1991; Adhya et al. *unpublished data*). We report here enhanced degradation of γ-HCH in a flooded alluvial soil retreated with γ-HCH and/or its metabolite, TCB.

MATERIALS AND METHODS

Technical formulation of γ-HCH (98.6% purity) and 1,2,4-TCB (99%, purity) were obtained from M/s Lachat Chemicals, Wisconsin, USA and M/s Aldrich Chemical Company, Milwaukee, USA, respectively. Acetone solutions of γ-HCH and TCB were applied at 10 µg/g soil to 5 kg of an alluvial soil (Typic Haplaquept with a sandy clay loam texture containing 25.9% clay, 21.6% silt and 52.5% sand, pH 6.3, organic carbon 0.65% and total N 0.06%, with no history of HCH use) contained in earthenware pots (25.5 x 9.5 cm). Two series of pots (unplanted and planted to rice) were maintained under four treatments *viz.* (1) pots retreated with γ-HCH, (2) pots retreated with TCB, (3) pots retreated with both γ-HCH and TCB and (4) untreated control amended with only acetone. 21 d old seedling of rice plants (*cv.* Lalat) planted in pots represented the planted series. All the pots were maintained in the greenhouse under flooded condition with a standing water level of 5 ± 1 cm above the soil. The first application of HCH or TCB or both was made 10 days after flooding the pots. A second application was made 15 d later(25 d after flooding) and subsequent applications were made at 40 (50 d after flooding)

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and 55 d (65 d after flooding) respectively.

At regular intervals, a portion (1 g) of the soil was removed from both sets (unplanted and planted) and a suspension was prepared in sterile distilled water (10 mL) in presterilized test tubes (200 x 25 mm). Portion (10 mL) of the mineral salts medium (Bhuyan et al. 1992) supplemented with γ-HCH (5-6 μg/mL) was inoculated with 1 mL of soil suspension from both sets and incubated under aerobic condition in a shaker at room temperature (28 ± 2° C). Uninoculated medium served as control. At periodic intervals 1 to 2 mL portions of the inoculated or uninoculated medium were withdrawn aseptically from each duplicate set and the γ-HCH remaining in the medium was determined by gas-liquid chromatography (glc) after extraction with hexane.

γ-HCH extracted in hexane was analyzed in a Varian gas chromatograph model 3400 equipped with ^{63}Ni detector and a metal column (2 m length, 1/8" OD) packed with 3% OV-17 on Chrom WHP 80/100 mesh. Column, injector and detector were maintained at 220°, 240° and 240°C respectively with a flow rate of the carrier gas (95% argon in 5% methane) at 20 mL/min. Under these conditions, the retention time for γ-HCH was 2.42 min with an average recovery ranging between 95 and 98%. The residues of γ-HCH were quantified using a standard curve linear over 0.5 to 3.0 ng.

RESULTS AND DISCUSSION

γ-HCH disappeared more rapidly from the soil suspension retreated twice with γ-HCH than from the control soil never exposed to γ-HCH (Table 1). Enhanced biodegradation of γ-HCH in HCH acclimatized flooded soil has been reported earlier (Bhuyan et al. 1992, 1993) and was attributed to the selective enrichment of γ-HCH degrading microbial population. In the present study also, entire amount of γ-HCH was degraded within 10 d. Prior exposure of the soil to TCB, although accelerated the degradation of γ-HCH, the enhancement was marginal and comparatively more pronounced in planted flooded soil. Interestingly, soils preexposed to a combined application of γ-HCH and TCB resulted into a greater acceleration of γ-HCH degradation and entire amount of γ-HCH was lost within 5 d. TCB has been detected as a metabolite during the degradation of γ-HCH in pure culture of γ-HCH degrading bacteria *S. paucimobilis* (Adhya et al. *unpublished data*). It is possible that pretreatment of soil with both γ-HCH and TCB resulted into acclimatization of bacteria to a greater extent than only γ-HCH.

Since there was an indication of enhancement of γ-HCH degradation in soils preexposed to TCB, we further probed the degradation of γ-HCH in soils retreated with TCB. Results indicate a clear cut enhancement of degradation of γ-HCH in both unplanted and planted soils retreated with TCB (Table 2) and this effect was related to the degree of preexposure. Thus, soils retreated with TCB four times

Table 1. Degradation of γ-hexachlorocyclohexane (γ-HCH) in a mineral salts medium inoculated with suspension from unplanted or planted flooded soil treated twice with γ-HCH, 1,2,4-trichlorobenzene (TCB) or both

Incubation (days)	γ-HCH recovered (μg/mL) from the mineral salts medium inoculated with suspension from soils								
	Uninoculated	Untreated		Pretreated with					
				γ-HCH		TCB		γ-HCH + TCB	
		Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted
0	5.3 ± 0.1	4.9 ± 0.3	4.8 ± 0.3	5.6 ± 0.3	4.7 ± 0.3	5.2 ± 0.3	4.9 ± 0.1	4.1 ± 0.4	4.2 ± 0.2
5	5.0 ± 0.3	4.4 ± 0.1	4.5 ± 0.1	2.2 ± 0.2	3.0 ± 0.1	4.6 ± 0.4	4.0 ± 0.2	0	0
10	3.9 ± 0.1	3.6 ± 0.1	3.8 ± 0.3	0	0	3.9 ± 0.1	3.1 ± 0.3	0	0
15	3.3 ± 0.1	3.1 ± 0.2	3.2 ± 0.1	0	0	2.0 ± 0.4	0.9 ± 0	0	0

Average of two replicate observations ± mean deviation

Table 2. Degradation of γ-hexachlorocyclohexane (γ -HCH) in a mineral salts medium inoculated with suspension from unplanted or planted flooded soil retreated with 1,2,4-trichlorobenzene (TCB)

Incubation (days)	γ-HCH recovered (μg/mL) from the mineral salts medium inoculated with suspension from soils								
	Uninoculated	Untreated		Pretreated with TCB					
				2nd application		3rd application		4th application	
		Unplanted	Planted	Unplanted	Planted	Unplanted	Planted	Unplanted	Planted
0	5.6 ± 0	5.3 ± 0.3	5.0 ± 0.2	4.9 ± 0.1	5.2 ± 0.3	4.8 ± 0.4	4.8 ± 0.4	4.9 ± 0.1	5.0 ± 0.1
5	5.0 ± 0.1	4.9 ± 0.1	4.8 ± 0.2	4.0 ± 0.2	4.6 ± 0.4	2.1 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	1.8 ± 0.1
10	3.9 ± 0.2	3.9 ± 0.3	4.1 ± 0.1	3.1 ± 0.3	3.9 ± 0.1	1.8 ± 0.1	1.6 ± 0.2	1.5 ± 0.3	0
15	3.3 ± 0.1	2.8± 0.2	3.6 ± 0.1	1.9 ± 0	1.1 ± 0.4	0.6 ± 0	0	0	0

Average of two replicate observations ± mean deviation

degraded γ-HCH faster and entire amount of γ-HCH was metabolised within 10 d in planted pots and within 15 d in unplanted pots. Though TCB was recorded as a metabolite in soils preexposed to only γ-HCH, no TCB could be detected in soils preexposed to either γ-HCH + TCB or TCB alone (data not shown).

The data presented in this study demonstrate that degradation of γ-HCH can be enhanced in an ecosystem following the application of its metabolic product. Accelerated degradation of several organophosphorus (Adhya et al. 1987; Misra et al. 1983) and carbamate (Rajagopal et al. 1986) insecticides upon repeated application of their hydrolysis products has been demonstrated earlier. TCB is the dechlorination product of γ-HCH and has been found to be mineralized further in the cultures of γ-HCH degrading bacteria (Imai et al. 1991). Pesticide degradation products are capable of conditioning soils for enhanced degradation of their parent compounds and could play an important role in the induction of enhanced degradation of select pesticides (Somasundaram and Coats 1990). It is possible that TCB or some of its further degradation products can enhance the degradation of γ-HCH by stimulating microbial population that in turn can metabolize γ-HCH.

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