

Degradation of soil-sorbed isomers of hexachlorocyclohexane in soils under flooded conditions

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Abstract

Isomers (alpha-, beta-, delta- and gamma-) of hexachlorocyclohexane (HCH) were first allowed to get sorbed individually to three soils (alluvial, Pokkali and Kari) and the soils with the respective sorbed HCH-isomer were incubated under flooded conditions for 30 days. HCH-isomers remaining in the soil were extracted with acetone-hexane at 10-day intervals and then quantified by gas-liquid chromatography. In alluvial soil with sorbed HCH-isomers, alpha- and gamma-isomers were readily degraded and beta- and delta-isomers resisted degradation. All the four isomers sorbed to Kari soil were recalcitrant to degradation. What is particularly interesting is the fairly more rapid degradation of thermodynamically stable beta- and delta-isomers of HCH sorbed to Pokkali soil than soil-sorbed alpha- and gamma-HCH under flooded conditions. Pokkali soil with high organic matter content exhibited a high sorption of all the four isomers with relatively little desorption. Within 10 days after submergence, the redox potential reached -170 and -208 mV in alluvial and Pokkali soils, respectively indicating the onset of anaerobiosis in both soils. Despite high sorption and low desorption, all the four isomers of HCH sorbed to Pokkali soil were readily degraded under anaerobic conditions. Conversely, despite relatively high desorption, sorbed delta- and beta-HCH resisted degradation in flooded low organic alluvial soil. Rapid degradation of HCH-isomers in Pokkali soil, despite high sorption and low desorption, may be due to increased microbial activity in this organic soil and ability of microorganisms to penetrate into soil aggregates. Evidence suggests that anaerobic biodegradation of soil sorbed HCH-isomers in flooded soils may not be necessarily related to their desorption from the soil.

Introduction

Agricultural chemicals, applied directly to the soil or reaching the soil after foliar application, are subjected to some degree of sorption to the soil, depending on the soil characteristics, pesticide molecule and cultural practices (moisture regime for instance) used in agriculture. High soil sorption, coupled with low desorption, may decrease the availability of pesticides for biodegradation processes in soils and in highly contaminated sites. Sorption may impede or enhance the degradation of organic chemicals, based on studies with aerobic soils. Low bioavailability of pesticides has often been cited as one of the main reasons for the failure of many bioremediation technologies using micro-organisms in field situations, albeit their proven ability to degrade the pesticides in enrichment and axenic cultures. Generally, sorption-desorption of pesticides determines their bioavailability for degradation (Ogram *et al.*, 1985; O'Loughlin *et al.*, 1988; Robinson *et al.*, 1990; Mueller *et al.*, 1992) and toxicity to target pests and nontarget organisms. In soils with high sorption and very low desorption capabilities, pesticides may not be easily available for biodegradation. For instance, carbofuran, sorbed to a low organic alluvial soil exhibiting low sorption and high desorption, was readily degraded by an active enrichment culture, but resisted degradation when sorbed to organic-rich Pokkali soil with high sorption and very little desorption (Singh and Sethunathan, 1992). Likewise, a *Sphingomonas paucimobilis* could readily degrade gamma- and beta-HCH, sorbed to alluvial soil, under nonflooded conditions; but in organic rich Pokkali soil with sorbed HCH-isomers, gamma-isomer was aerobically degraded by this bacterium while degradation of sorbed beta-isomer in inoculated Pokkali soil was not considerable (Sreedharan, 1995). But, according to a more recent study, a *Pseudomonas* sp. degraded 23% more of the sorbed (to montmorillonite-Al(OH)_x complex) 2,4-D than could be accounted for by desorption from this complex in noninoculated controls (McGhee *et al.*, 1999).

That alpha-, beta-, delta- and gamma-isomers of hexachlorocyclohexane (HCH) can undergo very rapid degradation in predominantly anaerobic ecosystems such as flooded soils and lake sediments is well known (Raghu and MacRae, 1966; Castro and Yoshida, 1971; Sethunathan *et al.*, 1998). In almost all studies on their persistence in flooded soils,

HCH-isomers were applied directly to the soil in solution or in solid form prior to soil submergence or few days after soil submergence (Raghu and MacRae, 1966; Castro and Yoshida, 1971; Sethunathan and MacRae, 1969). Although aerobic biodegradation of soil-sorbed carbofuran and HCH-isomers has been demonstrated in nonflooded soils inoculated with an enrichment culture and a *Sphingomonas paucimobilis*, respectively, there is no information on the degradation of soil-sorbed HCH-isomers or any other pesticides in predominantly anaerobic flooded soils. In the present study, the degradation of HCH-isomers (alpha-, beta-, delta- and gamma-), individually sorbed to three soils differing in physico-chemical characteristics, was examined under predominantly anaerobic flooded soil conditions.

Materials and methods

Soils

Three soils of varying physico-chemical characteristics (Table 1) from rice growing tracts were used in the study. A deltaic alluvial soil from the experimental farm of Central Rice Research Institute, Cuttack and two acid sulphate soils, Pokkali and Kari, from Kerala were used. After collection, soils were air-dried and sieved through a 2-mm mesh and stored in polyethylene bags at room temperature.

Table 1. Physico-chemical characteristics of the soils used

Location	Soil	pH	Organic matter (%)	Clay (%)	Silt (%)	Sand (%)
CRRI, Cuttack, Orissa	Alluvial	6.05	1.44	25.6	12.6	61.8
Cochin, Kerala	Acid sulphate Pokkali	5.32	4.21	45.6	7.8	45.6
Karmadi Alleppey, Kerala	Acid sulphate Kari	2.98	5.83	33.6	12.8	53.6

Pesticides

The technical formulations of alpha-, beta-, delta- and gamma-isomers of HCH (99.2% purity) were obtained from Lachat Chemicals, Mequon, Wisconsin, USA.

Sorption-desorption of HCH-isomers in aerobic soils

Aqueous solutions of alpha-, delta- and gamma-HCH-isomers were prepared by equilibrating respective isomer of HCH in distilled water for 48 h and then sterilising by passing through a 0.45 mm Millipore filter. Since the solubility of beta-HCH is very low (<1 ppm), it was directly added to the soil in 0.1 ml of acetone.

To study sorption-desorption of HCH-isomers in soils, 10 g of air-dried and sieved soils (alluvial, Pokkali and Kari) were equilibrated with 12.5 ml of an aqueous solution of the respective HCH-isomer in separate screw capped hard plastic tubes (10 cm x 2.6 cm) for 2 h in a shaker as described by Wahid and Sethunathan (1979). After 2 h of equilibration, the soil slurry was centrifuged at 8,000 rpm for 20 min. The supernatant was decanted and replaced with an equal volume of distilled water and shaken for 1 h. The soil slurry was again centrifuged, supernatant decanted and replaced with an equal volume of distilled water and again equilibrated for 1 h. Whatever amount of HCH-isomer remained in the soil after such two rinses was considered as sorbed, After each centrifugation, the supernatant was analysed for the respective isomer of HCH by gas liquid chromatography (glc), after extraction with hexane to determine the amount of HCH-isomer desorbed.

Degradation of soil-sorbed isomers of HCH under predominantly anaerobic flooded conditions

After the initial sorption and then desorption of HCH-isomers in soils as mentioned above, the soil samples with sorbed HCH-isomers were transferred from the plastic tubes to presterilised test tubes (200 mm x 25 mm) with 20 ml of sterile water and incubated at room temperature (28 ± 2°C). At periodic intervals, samples from duplicate tubes were extracted with hexane-acetone mixture and were analysed by glc.

Extraction of HCH residues from the soil

The procedure for the extraction of HCH residues was the same as reported earlier (Raghu and MacRae, 1966) with slight modification. The soil samples were transferred to 250-ml volumetric flasks with the help of 50 ml of acetone and shaken for 2 h on a rotary shaker. After 2 h of equilibration with acetone, 20 ml of hexane were added and contents shaken for 1 h followed by the addition of 2% solution of sodium sulphate to make up to 250 ml.

Gas-liquid chromatography (glc)

HCH-isomers, extracted from soil, were analysed in a Varian gas chromatograph model 3400, equipped with Ni⁶³ detector and a metal column (2 m length, 1/8" OD) packed with 3% OV-17 Chrom WHP 80/100 mesh. Column, injector and detector were maintained at 220, 240 and 240°C with a flow rate of carrier gas (95% argon in 5% methane) at 20 ml. min⁻¹. Under these conditions, the retention time was 1.90 min for alpha-HCH, 2.42 min for gamma-HCH, 2.67 min for beta-HCH and 3.12 min for delta-HCH. HCH residues were quantified using a standard curve, which was linear over a range of 0.2 ng to 3.0 ng.

Measurement of pH and redox potential (Eh) of soils

For determination of Eh and pH of the soil samples, 40-g soil samples were placed in 100-ml beakers and flooded with 50 ml of distilled water. Beakers were covered with polyethylene sheet (provided with few pin holes for gaseous exchange) and incubated at room temperature. At periodic intervals, duplicate beakers were removed for pH and Eh measurement. The redox potential was measured with a Barnant-20 digital ORP meter fitted with a platinum electrode. The electrode was placed in the reduced zone of the flooded soil and the potential was read in milliVolt (mV). After Eh measurements, soils were stirred thoroughly, allowed to stand for about 20-25 min and the pH measured. Variations between duplicate values never exceeded 20 for redox potential and 0.2 for pH.

Results and discussion

In earlier reports on the anaerobic instability of HCH-isomers in flooded soils, isomers were added to the soil in solution or in solid state. An experiment was conducted to determine the degradation of HCH-isomers, sorbed to aerobic soil and then incubated under flooded conditions. HCH-isomers (alpha-, beta-, delta- and gamma-) were allowed to get sorbed to three soils (alluvial, Pokkali and Kari) individually and the soils with respective sorbed isomer were incubated under submerged conditions as described in Materials and methods section. HCH-isomers in the soils were extracted with acetone-hexane at 10-day intervals and then analysed by glc.

Only Pokkali soil effected fairly rapid degradation of all the four isomers including the thermodynamically stable beta- and delta-isomers in the sorbed state under flooded conditions (Tables 2-5).

Table 2. Degradation of soil-sorbed alpha-HCH in alluvial, Pokkali and Kari soils under flooded conditions

Incubation (days)	µg alpha-HCH recovered.g ⁻¹ soil		
	Alluvial	Pokkali	Kari
0	1.84 ± 0.0	3.64 ± 0.16	3.42 ± 0.06
10	1.81 ± 0.18	4.64 ± 0.08	3.49 ± 1.07
20	1.07 ± 0.02	2.97 ± 0.18	2.35 ± 0.13
30	0.48 ± 0.06	0.65 ± 0.01	2.05 ± 0.03

Table 3. Degradation of soil sorbed gamma-HCH in alluvial, Pokkali and Kari soils under flooded conditions

Incubation (days)	$\mu\text{g gamma-HCH recovered.g}^{-1}\text{ soil}$		
	Alluvial	Pokkali	Kari
0	4.80 \pm 0.50	6.45 \pm 0.20	7.95 \pm 0.10
10	1.55 \pm 0.05	2.85 \pm 0.15	7.50 \pm 0.50
20	0.25 \pm 0.05	0.97 \pm 0.03	7.25 \pm 0.30

Table 4. Degradation of soil-sorbed beta-HCH in alluvial, Pokkali and Kari soils under flooded conditions

Incubation (days)	$\mu\text{g beta-HCH recovered.g}^{-1}\text{ soil}$		
	Alluvial	Pokkali	Kari
0	10.50 \pm 0.15	11.55 \pm 0.15	13.00 \pm 0.30
10	10.50 \pm 0.75	13.60 \pm 0.80	14.70 \pm 0.30
20	10.50 \pm 0.00	7.05 \pm 1.55	14.42 \pm 0.58
30	6.95 \pm 0.15	2.12 \pm 0.38	14.25 \pm 1.25

Table 5. Degradation of soil-sorbed delta-HCH in alluvial, Pokkali and Kari soils under flooded conditions

Incubation (days)	$\mu\text{g delta-HCH recovered.g}^{-1}\text{ soil}$		
	Alluvial	Pokkali	Kari
0	16.00 \pm 1.00	18.06 \pm 1.70	16.8 \pm 0.07
10	15.55 \pm 1.30	11.55 \pm 0.15	17.8 \pm 0.70
20	15.90 \pm 1.20	8.25 \pm 2.25	15.8 \pm 2.40
30	12.05 \pm 0.35	0.50 \pm 0.50	13.9 \pm 0.40

On the other hand, alpha- and gamma-isomers appeared to undergo more rapid degradation under flooded conditions than beta- and delta-isomers when sorbed to alluvial soil. Degradation of all the four isomers sorbed to Kari soil was, however, not considerable. What is particularly interesting is the fairly rapid degradation of all the four HCH-isomers sorbed to organic matter rich Pokkali soil, despite high sorption of all the four isomers with relatively little desorption (Table 6).

Table 6. Sorption-desorption of HCH-isomers in alluvial and Pokkali soils

Soil used	HCH-isomer	HCH sorbed (%)	% HCH		Total
			First rinse	Second rinse	
Alluvial					
	alpha	88.6	9.67	6.74	16.41
	beta	95.1	4.91	5.98	10.89
	gamma	92.7	7.76	7.90	15.66
	delta	81.9	17.47	14.83	32.20

Pokkali					
	alpha	96.7	3.87	3.49	7.36
	beta	98.8	0.63	0.58	1.21
	gamma	96.4	2.63	2.80	5.43
	delta	97.6	1.45	0.91	2.36

Sorbed beta-HCH was degraded faster in Pokkali soil than in alluvial soil (Table 4). According to available literature (Ogram *et al.*, 1985; O'Loughlin *et al.* 1988; Robinson *et al.*, 1990; Mueller *et al.*, 1992), sorption-desorption determines the availability of the pesticides in solution for biodegradation. However, despite low desorption, all the four isomers sorbed to Pokkali soil were readily degraded under anaerobic conditions [as indicated by the drop in redox potential to -208 mV within 10 days after submergence (Table 7)] in flooded soil.

Table 7. Changes in the pH and Eh of various soils after flooding

Incubation (days)	Alluvial		Pokkali		Kari	
	pH	Eh (mV)	pH	Eh (mV)	pH	Eh (mV)
0	6.05	+ 239	5.3	+ 226	2.98	+ 392
10	6.51	- 170	6.8	- 208	3.25	+ 272
20	7.11	- 200	7.5	- 292	3.55	+ 221
30	7.01	- 174	7.9	- 183	3.58	+ 228

It is not clear whether in flooded soil used in this study, beta-HCH was desorbed slowly due to concentration gradient during 30-day incubation period and was thus available in solution for degradation. More recently, more 2,4-D than could be accounted for by desorption from a montmorillonite-Al(OH)_x complex was degraded aerobically by a *Pseudomonas* sp. (McGhee *et al.*, 1999). The biodegradation rate may exceed the desorption rate if microorganisms at the surface of the sorbent immediately use the chemical as it becomes available and before it is released to the bulk solution phase, where it can be analysed by conventional analytical methods (Scow, 1993). Rijnaarts *et al.* (1991) found that in soil slurries, bacteria could penetrate into soil aggregates and effectively degrade alpha-HCH. Probably, in organic rich Pokkali soil with high sorption and low desorption characteristics, bacteria may have penetrated into the soil aggregates and caused rapid degradation of beta-HCH.

The higher the organic matter content in the soil the greater is the sorption of the chemical to the soil and least the desorption of the chemical from the soil. This may retard the biodegradation of the chemical in organic rich soil. On the other hand, biodegradation rate can be higher in organic rich soil than in a soil with low organic matter content, because organic soils can be microbially more active than low organic soils and biodegradation rate is a function of population density of microorganisms and their degrading capabilities. Very rapid degradation of sorbed HCH-isomers including beta-isomer in Pokkali soil, despite high sorption and low desorption, is attributed to the increased microbial activity in this organic soil and ability of microorganisms to penetrate into soil aggregates.

It may be mentioned that among all the three soils used, only Pokkali soil effected rapid degradation of all the four isomers of HCH upon submergence of soil with sorbed isomers; alpha-, gamma-isomers were degraded faster than delta- and beta-isomers in flooded alluvial soil after sorption. The increased persistence of soil-sorbed delta- and beta-HCH in flooded alluvial soil is not clear. All the four sorbed isomers persisted in flooded Kari soil, probably because of adverse conditions for anaerobic microbial activity in this soil with low pH and high redox potential (Table 7) even after 30 days of submergence. In contrast, within few days after soil submergence, both alluvial and Pokkali soils registered near neutral pH and very low redox potential. Evidently, microbially active alluvial and Pokkali soils were rapidly reduced upon submergence and thereby promoted anaerobically mediated degradation of HCH-isomers. It is well known that HCH-isomers, including the thermodynamically stable beta-isomer, can undergo very rapid degradation by dominant anaerobic microorganisms in a predominantly anaerobic flooded soil (MacRae *et al.*, 1967, 1969). In most of these earlier investigations, HCH-isomers were applied to the flooded soils in free state as solid or in solution. Although beta- and gamma-isomers, applied to flooded soil in free state or in solution,

are biodegradable in flooded soil, beta-HCH appeared to be more persistent than gamma-HCH (Siddaramappa and Sethunathan, 1975). Thus, in 41 days, gamma-HCH reached negligible levels in Pokkali soil under flooded conditions, as compared to a recovery of more than 32% of the initially added beta-HCH during the corresponding period. But, in the present study, HCH-isomers were sorbed to the soil prior to their submergence. According to the earlier reports (in free state, Siddaramappa *et al.*, 1995) and the present study (soil-sorbed), HCH-isomers, both in sorbed and free state, could undergo rapid degradation in predominantly anaerobic flooded soil; but, in flooded Pokkali soil, beta-HCH was degraded faster in sorbed state (Table 3, this study) than when applied in free state (Siddaramappa and Sethunathan, 1975). Moreover, gamma-HCH was degraded distinctly faster than beta-HCH when applied in free state to flooded alluvial soil (Siddaramappa and Sethunathan, 1975). Interestingly, sorbed beta-HCH was degraded faster in Pokkali soil than in alluvial soil while a reverse trend was noticed when applied in free state. Evidence suggests increased bioavailability of even strongly sorbed HCH-isomers in organic rich soils such as Pokkali soil used in this study leading to their rapid degradation by the indigenous microorganisms.

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