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Effect of Isomers of Hexachlorocyclohexane on N_2O Production in Nonflooded and Flooded Soil, Unamended or Amended with Urea

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There is concern over the increasing concentrations of greenhouse gases, such as CO_2, CH_4 and N_2O in the atmosphere. Current atmospheric concentration of N_2O is around 310 ppbv; but, it is increasing at 0.2-0.3% year^{-1} (Conard 1996). Global warming potential of N_2O is about 250 times greater than that of CO_2. N_2O is produced in agricultural soils essentially by microbially mediated nitrification and denitrification reactions. N_2O is a transitory intermediate in both nitrification and denitrification reactions. In essentially oxic upland agricultural soils, N_2O emissions from nitrification of ammonium-based fertilizers can be significant. In wetland rice soils where oxygen concentration is limited, denitrification is the important means of N_2O production. But, in rice soils, the surface soil and the rice rhizosphere are oxygen-rich and can therefore support nitrification. Simultaneous nitrification-denitrification leads to significant loss of nitrogen from rice paddies in gaseous forms, N_2 and N_2O. Certain cultural practices, used in agriculture, seem to inhibit or stimulate the emission of N_2O from agricultural soils. Generally, mitigation options for CH_4 promote N_2O emissions. For instance, mid-season drainage of rice fields, as practiced in Japan, may stimulate N_2O emissions while retarding CH_4 emissions (Wassmann et al. 1993). Application of carbofuran stimulated the autotrophic nitrification of ammonium in simulated oxidized surface of a flooded soil (Ramakrishna et al. 1978) and in rice rhizosphere soil suspensions (Ramakrishna and Sethunathan 1982). Concomitantly, carbofuran stimulated N_2O production in nonflooded soil samples held at 60% water holding capacity (Singh et al. 1999). In contrast, an isomeric mixture of the organochlorine, hexachlorocyclohexane (HCH) or the individual isomers (α, β, γ, δ) inhibited CH_4 emission from flooded soils and rice fields (Sathpathy et al. 1997). Likewise, the isomeric mixture of HCH inhibited autotrophic nitrification of ammonium to nitrate (Ray et al. 1980) and N_2O production (Singh et al. 1999) in nonflooded soil held at 60% water holding capacity. In the present study, we examined the effect of individual isomers and an isomeric mixture of HCH on N_2O production in nonflooded and flooded soil samples, unamended and amended with urea. Also, the effect of repeated applications of HCH on N_2O production in flooded soil was studied.

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MATERIALS AND METHODS

An alluvial soil (pH, 7.5; organic carbon, 0.6%; total nitrogen 0.034%), collected from 0-5 cm surface layer of the experimental farm of Indian Agricultural Research Institute, New Delhi was used in this study. Soil was air-dried, ground, sieved (< 2 mm) and stored in plastic bags at room temperature prior to use.

HCH isomers (α, β and γ) all of 99.1% purity, were obtained Lachat Chemicals, Mequon, Wisconsin, USA. A technical formulation (an isomeric mixture containing (72.0% α–, 1.0% β- and 15.5% γ-, crystalliszed from a commercial formulation of HCH obtained from Hindustan Insecticides Limited, Pune, India) was also used in this study.

To 10-g soil samples, placed in 120-ml sterile serum bottles, were added adequate quantity of distilled water or an aqueous solution of urea at 50 $\mu g\ g^{-1}$ soil to provide 60% water holding capacity (WHC) or flooded conditions. For flooded conditions, soil to water ratio was 1:1.25. Both nonflooded and flooded soil samples were then treated with individual HCH isomers (α, β and γ) or technical formulation of an isomeric mixture of HCH at 5 $\mu g\ g^{-1}$ soil in 10 μl of acetone. Soil samples, not treated with urea and HCH, but receiving distilled water or acetone served as control. Soil in each bottle was thoroughly mixed for uniform distribution of urea and HCH. Serum bottles were closed with rubber septa and the contents incubated at 35°C ± 1°C. At regular intervals, the headspace of the incubation bottles was analyzed for N_2O in Hewlett Packard gas chromatograph fitted with Ni^{63} and a Porapak N column. The operating conditions were : column : 50°C; detector : 250°C and carrier gas (95% argon + 5% methane) flow : 30 ml min^{-1}. Under these conditions, the retention time for N_2O was 1.9 min.

To study the effect of repeated additions of HCH isomers on N_2O production in flooded soil, 100-g portions of the air-dried soil were flooded with 125 ml of distilled water in plastic beakers (9 cm x 9 cm). After 10 days, soil samples were fortified with individual isomers (α, β and γ) or technical isomeric mixture in 0.1 ml of acetone at 5 $\mu g\ g^{-1}$ soil at 10-day intervals. Soil samples receiving only 0.1 ml of acetone at 10-day intervals served as control. At 30 days after flooding, soil samples received 3 applications of HCH. After 15 days of third application of HCH, water was decanted from the beakers and the beakers left undisturbed for 24 h. Soil cores from the beakers were then transferred to sterile serum bottles using 13 mm polyvenyl chloride (PVC) tubes. Soil samples in serum bottles (pretreated thrice in beakers with HCH) were again treated with respective HCH isomers or technical formulation in 10 μl of acetone. Soil samples receiving only 10 μl of acetone served as control. Serum bottles were closed with rubber septa and then incubated at 35°C ± 1°C. At 10, 20 and 30 days after incubation, headspace of serum bottles was analyzed for N_2O by gas chromatography.

After analysis of headspace for N_2O, NH_4^+ and NO_3^- in soil samples from first experiment were extracted by shaking the contents in serum bottles with 50 ml of 2 M KCl on a rotary shaker for 30 min. The soil-water suspension was then allowed to settle and filtered through a Whatman No. 42. The filtrate was then analyzed for NH_4^+ using Nessler's reagent and NO_3^- using phenol 2,4-disulfonic acid method and 6 N NH_4OH (Jackson 1967).

RESULTS AND DISCUSSION

Soil samples amended with urea always produced more N_2O than did the unamended soil samples, irrespective of the moisture regime [nonflooded (60% water holding capacity) and flooded] in the presence and absence of HCH isomers (Tables 1 and 2). Generally, urea effected almost a 2-fold increase in the headspace concentration of N_2O over that of soil samples, not treated with urea. Individually applied isomers (α, β and γ) of HCH or its isomeric mixture (technical formulation) caused a distinct inhibition of N_2O production in soil samples held under nonflooded conditions, both in the presence and absence of urea (Table 1). But, the inhibitory effect of HCH (individual isomers or the isomeric mixture) was more pronounced in soil samples fortified with urea at 50 $\mu g\ g^{-1}$ soil than in soil samples, not treated with urea. After 30-day incubation, net reduction in the headspace concentration of N_2O in soil samples treated with

Table 1. N_2O produced in an alluvial soil treated[1] with hexachlorocyclohexane isomers (individually or in mixture) under nonflooded conditions

Incubation (days)	Nitrogen applied	N_2O produced (ng g^{-1} soil)				
		Untreated	α	β	γ	Mixture
10	-Urea	110.3	71.4	40.6	27.9	36.1
	+Urea[2]	243.5	170.3	100.2	58.1	85.5
20	-Urea	439.8	245.3	163.0	121.3	147.5
	+Urea	753.1	300.1	207.1	131.1	183.9
30	-Urea	856.3	450.0	318.1	203.7	289.4
	+Urea	1632.0	583.8	397.4	259.2	343.7

[1]Soil samples were treated with HCH isomers or their mixture (technical formulation) at 5 $\mu g\ g^{-1}$ soil.
[2]Urea was applied at 50 $\mu g\ g^{-1}$ soil.

Table 2. N_2O produced in an alluvial soil treated[1] with hexachlorocyclohexane isomers (individually or in mixture) under flooded conditions

Incubation (days)	Nitrogen applied	N_2O produced (ng g^{-1} soil)				
		Untreated	α	β	γ	Mixture
10	-Urea	27.0	47.4	46.4	42.8	46.7
	+Urea[2]	34.2	54.2	59.0	54.2	64.0
20	-Urea	82.0	106.4	94.1	113.9	86.9
	+Urea	119.0	177.0	149.1	169.9	132.8
30	-Urea	142.4	223.1	219.7	247.5	155.0
	+Urea	217.5	455.5	446.5	477.0	273.1

[1]Soil samples were treated with HCH isomers or their mixture (technical formulation) at 5 μg g^{-1} soil.
[2]Urea was applied at 50 μg g^{-1} soil.

Table 3. NH_4^+ and NO_3^- recovered from alluvial soil samples treated with hexachlorocyclohexane (individually or in mixture) under nonflooded conditions

Treatment	NH_4^+-N (μg g^{-1} soil)		NO_3^--N (μg g^{-1} soil)	
	-Urea	+Urea	-Urea	+Urea
Untreated	1.0	1.35	0.91	1.38
α-HCH	1.60	2.25	0.92	1.55
β-HCH	1.75	4.65	0.93	1.35
γ-HCH	2.80	6.05	0.94	0.90
Isomeric mixture	1.70	4.35	1.02	1.01

individual isomers or the isomeric mixture of HCH over that in HCH-free samples ranged from 50-75% in the presence and absence of urea.

Nonflooded soil samples treated with HCH isomers or their isomeric mixture accumulated distinctly more ammonium than did the soil samples not treated with HCH (Table 3), concomitant with their inhibitory effect on N_2O production (Table1). Accumulation of the ammonium in HCH-treated soil samples under nonflooded conditions was more pronounced in the presence of urea than in its absence. Evidently, HCH isomers (individual or isomeric mixture) inhibited autotrophic nitrification of ammonium (formed from mineralization of soil organic matter and hydrolysis of urea), leading to decreased N_2O production and increased accumulation of ammonium. Accordingly, nitrate accumulation was inhibited by HCH isomers, but not in proportion to the accumulation of ammonium.

Soil samples, not treated with HCH isomers, produced more N_2O under nonflooded conditions (Table 1) than under flooded conditions (Table 2). Low N_2O production in flooded soil may be due to predominantly anaerobic conditions, not congenial for nitrification. As in nonflooded soil, urea stimulated the production of N_2O under flooded conditions. Interestingly, application of HCH isomers, individually or as the technical mixture, appeared to increase N_2O production in flooded soil samples over that in soil samples without HCH, both in the presence and absence of urea (Table 2). At 30 days, individually applied HCH isomers or the technical mixture increased N_2O production in flooded soil samples by 2-fold or more over that in control samples without HCH isomers in the presence of urea (Table 2), in contrast to their inhibitory effect under nonflooded

Table 4. NH_4^+ and NO_3^- recovered from alluvial soil samples treated with hexachlorocyclohexane (individually or in mixture) under flooded conditions

Treatment	NH_4^+-N (μg g^{-1} soil)		NO_3^-- N (μg g^{-1} soil)	
	-Urea	+Urea	-Urea	+Urea
Untreated	0.59	1.03	0.91	0.57
α-HCH	0.55	1.10	0.93	0.63
β-HCH	0.60	0.98	0.95	0.67
γ-HCH	0.62	0.99	0.94	0.59
Isomeric mixture	0.57	1.09	0.97	0.61

conditions (Table 1). HCH is known to retard the drop in redox potential of a flooded soil and maintain it under more oxidized conditions (Pal et al. 1980).

Enhanced N_2O production in flooded soil samples treated with HCH isomers may therefore be due to relatively more aerobic conditions, favorable for nitrification. However, the amount of ammonium nitrogen (μg g^{-1} soil) recovered from flooded soil samples at 30 days ranged between 0.55 and 0.62 in the absence of urea as compared to 0.98 to 1.1 in the presence of urea, whether unamended or amended with HCH (Table 4).

The amount of nitrate nitrogen (μg g^{-1} soil) ranged between 0.57 to 0.97, irrespective of HCH and urea application (Table 4). Flooded soil samples (Table 4) accumulated distinctly less ammonium than nonflooded soil samples (Table 3), especially in the presence of urea.

Table 5. Effect of repeated applications of hexachlorocyclohexane isomers (individually or in mixture) on N_2O production in flooded alluvial soil

Incubation (Days)[1]	No. of HCH application	μg N_2O produced g^{-1} soil				
			Pretreated with			
		None	α	β	γ	Mixture
	0	0.98				
10	3		0.30	0.57	2.26	1.36
	4		0.29	0.66	2.65	0.26
	0	3.67				
15	3		0.91	1.25	3.50	3.37
	4		0.81	2.32	3.97	1.02
	0	6.21				
20	3		1.18	1.55	5.23	3.93
	4		0.89	3.38	5.92	1.51

[1]Days after last HCH application

The effect of repeated application of isomeric mixture or individual isomers of HCH on N_2O production in flooded soil samples was examined. In contrast to the stimulation of N_2O production in flooded soil samples by HCH after first application (Table 2), repeated applications of HCH (individual isomers or mixture) to flooded soil samples generally led to substantial inhibition of N_2O

production (Table 5). Thus, soil samples, receiving 3 or 4 applications of HCH individually or in mixture accumulated less N_2O in 20 days after the last application than did the soil samples never treated with HCH. Evidently, HCH can be inhibitory to N_2O production also in flooded soil, as in nonflooded soil (Table 2), but after its intensive use.

HCH is known to inhibit the autotrophic nitrification of ammonium to nitrate in simulated oxidized surface layer of a flooded soil (Ray et al. 1980). Likewise, application of HCH isomers (individually or technical isomeric mixture) to the soil held under nonflooded conditions inhibited the nitrification of ammonium to N_2O. In flooded soil with dynamic aerobic-anaerobic interface coexisting, HCH isomers stimulated the accumulation of N_2O after the first application, but inhibited it after 2 or 3 applications.

According to the data presented in this study, the effect of HCH isomers on N_2O production in soils is governed by the moisture regime, nitrogen application and the intensity of their use.

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