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Effect of Endosulfan on Methane Production from Three Tropical Soils Incubated Under Flooded Condition

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Methanogenesis, an important transformation reaction in the biogeochemical cycling of C under anaerobic conditions, results in the formation of CH_4 - a greenhouse gas implicated in global warming (Dickinson and Cicerone 1986). Predominantly anaerobic flooded rice paddies are considered as one of the major anthropogenic sources of atmospheric CH_4 (Houghton et al. 1996). Projected increase in rice production (IRRI 1992), is anticipated to result in a higher CH_4 emission from this highly productive ecosystem. Intensive rice growing necessitates use of pesticides to protect the crop from pests and thereby achieve higher yields. Most of these agricultural chemicals, applied directly to the soil or eventually reaching the soil upon foliar application, may affect microbial transformations of importance to soil fertility (Rao et al. 1992) and the environment. While some of the insecticides like DDT (McBride and Wolfe 1971) and isomeric mixture of hexachlorocyclohexane (HCH) (Satpathy et al. 1997) inhibited CH_4 production, carbofuran stimulated the process (Kumaraswamy et al. 1998). In a laboratory investigation, we studied the effect of a commercial formulation of the commonly used insecticide Endosulfan (l,4,5,6,7,7-hexachloro-8,9,10-trinorborn5-en-2,3-ylenedimethyl sulphite) on CH_4 production and CH_4-producing bacteria in three tropical soils incubated under flooded conditions.

MATERIALS AND METHODS

Three soils from rice-growing tracts of India, widely varying in their physicochemical characteristics (Table 1), were used in the study. The soils were air-dried, ground, sieved (< 2mm) and stored at 4°C till use. Incubation method of Wang et al. (1993) as modified by Adhya et al. (1998) was used in studies on CH_4 production. Portions (5 g) of the soil samples were placed in B-D Vacutainer tubes (13 mL capacity) (Becton-Dickinson and Co., NJ, U.S.A.). Commercial formulation of endosulfan (‘Thiodan EC’ Endosulfan 35% a.i., Hoechest India Ltd., Mumbai, India) was dissolved in acetone and added to the soil to provide concentrations of 5, 10 and 50 µg/g soil. The tubes containing unamended soil receiving only acetone served as control. The tubes, kept open overnight for the evaporation of acetone, were flooded with sterile distilled water at 1:1.25 soil and water ratio. After flooding, the tubes were stoppered with rubber septa and kept in

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a BOD incubator (30 ± 2°C) in the dark upto 40 d. To estimate CH_4 production in the soils, the tubes were shaken for 10s on a vortex mixer to release soil-trapped CH_4, if any, and 5 mL of the headspace gas was analyzed for CH_4 by gas chromatography. On every sampling day four soil tubes from each treatment were sacrificed for the estimation of CH_4.

CH_4 was estimated in a Shimadzu GC-8A gas chromatograph equipped with FID and a Porapak N column. The column and detector were maintained at 70 and 110°C, respectively. The gas samples were injected through a sample loop (3 mL) with the help of an on-column injector using a multiport valve (VICI AG, Schenkon, Switzerland). The GC was calibrated before and after each set of measurement using 5.38, 9.03 and 10.8 µL CH_4/mL in N_2 (Scotty(R) II analyzed gases, M/s Altech Associates Inc., USA) as primary standard and 2.14 µL CH_4/mL in air as secondary standard. Under these conditions, the retention time of CH_4 was 0.65 min and the minimum detectable limit was 0.5 µL/mL.

Samples (40 g) of the soil, unamended or amended with endosulfan, contained in 100 ml beakers, were flooded with 50 ml of sterile distilled water to provide the same soil:water ratio as used for incubation studies for CH_4 production. The redox potential of duplicate soil samples was measured by inserting a combined platinum-calomel electrode (Barnant Co. IL, USA) and measuring the potential difference in mV (Pal et al. 1979). After measuring the redox potential, the pH of the soil samples was determined by a digital pH meter with a Calomel glass electrode assembly.

Methanogenic bacterial population was enumerated following anaerobic culture tube technique (Kasper and Tiedje 1982). The tubes were incubated at 28 ± 2°C for 30 days. Detection of CH_4 in the headspace of culture tubes was considered as evidence for the presence of methanogens and the population was counted by MPN (most probable number) method (Alexander 1982).

RESULTS AND DISCUSSION

CH_4 production in alluvial soil was low up to 15 d and increased thereafter. Application of endosulfan led to an inhibition in CH_4 production in flooded alluvial soil which was related to the concentration of endosulfan (Fig.1). Thus, mean CH_4 production was inhibited by 58, 51 and 97% over unamended control following endosulfan application at 5, 10 and 50 µg/g soil respectively. Redox potential was higher in endosulfan-amended soil (Table 2) suggesting retardation of soil reduction process in endosulfan-amended soil. Application of HCH, another organochlorine insecticide, prevented a drop in soil redox potential (Pal et al. 1979) and also CH_4 production in flooded rice soils (Satpathy et al. 1997). CH_4 production is optimum at low redox potential. Probably, high redox potential in endosulfan-amended soil had an adverse effect on CH_4 production in alluvial soil.

Table 1. Physico-chemical characteristics of soils used in the study

Location	Soil type	Taxonomic group	pH	Organic carbon (%)	Total N (%)	EC (ds/m)	CEC (meq/100g)	SO_4^{-2} (µg/g)	Soil separates		
									Clay (%)	Silt (%)	Sand (%)
Cuttack	Alluvial	Haplaquept	5.85	0.83	0.09	0.35	18.6	10.2	25.6	12.6	61.8
Sukinda	Laterite	Haplustult	6.87	0.62	0.04	1.10	6.0	92.0	14.6	10.6	74.8
Pokkali	Acid sulfate	Sulphaquept	3.90	4.86	0.21	5.01	19.2	1090.7	45.6	7.8	46.6

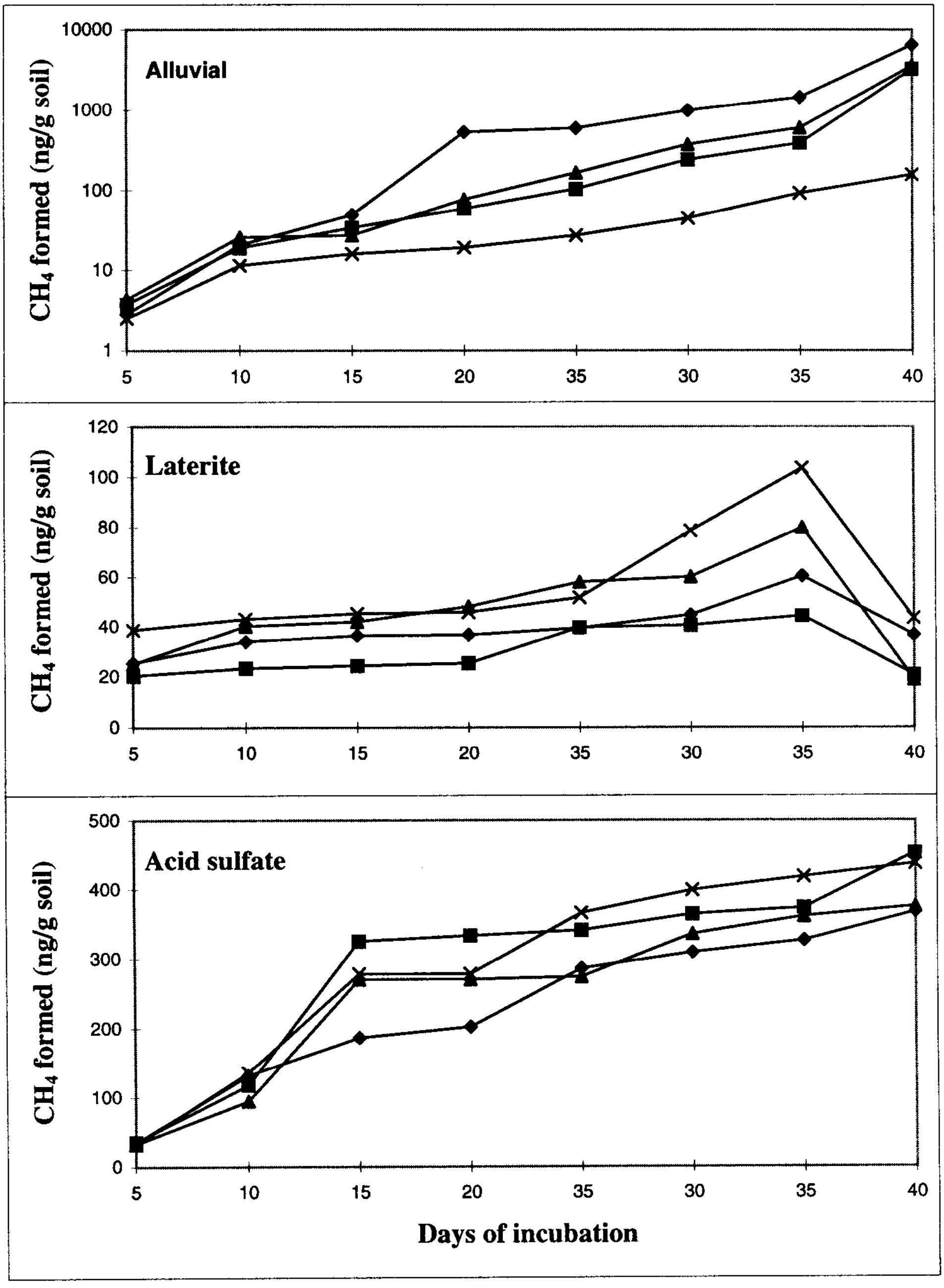

Figure 1. Effect of endosulfan on methane production in three tropical soils under flooded conditions (◆---◆none; ▲---▲5 µg; ■--■ 10µg; x----x 50 µg)

Table 2. Changes in redox potential and pH of a flooded alluvial soil treated with endosulfan

Endosulfan concentration	Days after flooding													
	0		5		10		15		20		25		30	
(µg/g soil)	Eh	pH	Eh	pH	Eh	pH	Eh	pH	Eh	pH	Eh	pH	Eh	pH
None	241^{a}	5.88^{a}	-128^{b}	6.85^{a}	-204^{d}	6.87^{b}	-219^{d}	6.93^{a}	-196^{c}	6.89^{b}	-203^{c}	7.04^{a}	-174^{a}	7.02^{b}
5	241^{a}	5.88^{a}	-124^{b}	6.86^{a}	-198^{c}	6.88^{b}	-206^{c}	6.94^{a}	-191^{bc}	6.87^{b}	-207^{c}	6.99^{ab}	-186^{b}	7.08^{a}
10	241^{a}	5.88^{a}	-123^{b}	6.87^{a}	-165^{a}	6.89^{b}	-147^{a}	6.92^{a}	-188^{b}	7.23^{a}	-174^{a}	7.03^{a}	-179^{a}	7.04^{b}
50	241^{a}	5.88^{a}	-118^{a}	6.90^{a}	-177^{b}	6.92^{a}	-197^{b}	6.95^{a}	-170^{a}	7.23^{a}	-185^{b}	6.95^{b}	-185^{b}	7.12^{a}

Mean of two replicate observations; in a column, means followed by a common letter are not significantly different at $P < 0.05$ by Duncan's Multiple Range test (DMRT).

CH_4 production in flooded laterite and acid sulfate soils was not considerable as compared to that of alluvial soil during 40 d incubation under flooded conditions. During 40 d incubation, peak CH_4 production (µg/g soil) in soils not amended with endosulfan amounted to 6493 in alluvial, 60 in laterite and 367 in acid sulfate soil. Since CH_4 production was low in laterite and acid sulfate soils, the impact of amendment with endosulfan was less pronounced and often conflicting in these soils. Thus, in laterite soil, CH_4 production was inhibited at 5 µg/g level, but stimulated at higher application levels of 10 and 50 µg/g. This finding is contrary to the commonly held belief that the chemically induced inhibition of microbially mediated processes are more pronounced at higher concentrations than at lower concentrations of the chemical applied (Wainright 1979). In the acid sulfate soil, however, CH_4 production was stimulated at all the application levels of endosulfan over that of unamended control (Fig 1). Unlike in alluvial soil, redox potential and pH measurements (data not shown) in laterite and acid sulfate soils did not indicate definite trends to ascribe reasons for the stimulatory effects of endosulfan on CH_4 production.

Populations of methanogenic bacteria were enumerated at 20 and 40 d of incubation. In the alluvial soil, total methanogenic bacterial population was inhibited by endosulfan and the degree of inhibition was related to the rate of endosulfan application (Table 3). Interestingly, the population of methanogenic bacteria following endosulfan application was marginally stimulated in laterite and acid sulfate soils, especially at 40 d. This increase in methanogenic bacterial population might have led to higher CH_4 production in these two soils.

Table 3. Changes in the methanogenic bacterial population in flooded soils treated with endosulfan

Endosulfan concentration (µg.g^{-1}soil)	Methanogenic bacteria (MPN × 10^4/g soil)					
	Alluvial soil		Laterite soil		Acid sulfate soil	
	20 d	40 d	20 d	40 d	20 d	40 d
None	0.25	0.43	0.11	0.30	0.11	0.35
5	0.20	0.33	0.10	0.25	0.27	0.22
10	0.14	0.32	0.10	0.28	0.07	0.46
50	0.04	0.14	0.12	0.34	0.06	0.78

MPN : Most probable number

Chlorinated hydrocarbons like methylene chloride, chloroform and carbon tetrachloride inhibit methanogenesis (Bauchop 1967). Chloroform effected a total inhibition in CH_4 production in paddy soil without affecting the glucose turnover (Krumback and Conrad 1991). Soil type and properties are important factors in determining the effect of a pesticide on soil processes. In the present study, endosulfan, an organochlorine insecticide, differentially affected CH_4 production in three types of soils. Most pesticides, used in agriculture and public health are seldom toxic to soil microbial processes and environmental safety when applied at recommended levels and intervals. Our study indicates that certain commonly used pesticides can affect important soil microbial processes.

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