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Effects of HCH, Carbaryl, Benomyl, and Atrazine on the Dehydrogenase Activity in a Flooded Soil

K. Chendrayan and N. Sethunathan
Central Rice Research Institute, Cuttack-753006, India

Soil dehydrogenase activity is often used as a measure of the metabolic activity of microorganisms in the soil. Recently, we found that the dehydrogenase activity in a soil significantly increased upon flooding (CHENDRAYAN et al. 1980). While the effects of pesticides on the soil dehydrogenase activity have been studied extensively under nonflooded conditions (CERVELLI et al. 1978), virtually no information is available with respect to flooded soils. The present paper reports the effect of some commonly used pesticides on the dehydrogenase activity in a flooded soil.

MATERIALS AND METHODS

Commercial formulations of the following pesticides were used: 5% granular HCH (hexachlorocyclohexane), 50% wettable powder of carbaryl, 50% wettable powder of benomyl (Benlate) and 5% wettable powder of atrazine.

An air-dried alluvial soil (pH, 6.4; organic matter, 0.65%) was sieved through a 2-mm sieve. Ten-gram portions of the soil contained in test tubes (15- x 150-mm) were flooded with 12.5 mL of distilled water to provide 3 cm standing water column over the soil. Commercial formulations of the pesticides at the rates of 1, 10 and 100 µg active ingredient/g soil were added to the soil as water suspension at the time of flooding and the soil samples incubated at 28 ± 3°C for different periods. Soil without pesticides served as control.

Soil dehydrogenase activity in duplicate samples of each treatment was assayed by TTC (2,3,5-triphenyl tetrazolium chloride) reduction method (CASIDA et al. 1964). One milliliter of 6% aqueous solution of TTC and 0.1 g of $CaCO_3$ were added to the flooded soil system at the end of desired incubation period and after thorough mixing, the contents were incubated at 37°C for 24 h. Reduced triphenyl formazan formed from TTC was extracted with methanol and quantified by measuring the absorbance at 485 nm.

For determining the redox potential, 40-g portions of the soil samples with and without pesticides were flooded with distilled water in 100-mL beaker for 4, 10 and 20 days. The potential was measured with a portable redox meter (TOA Electronics Ltd., Tokyo, Japan) fitted with a compound platinum and calomel electrode as described earlier (PAL et al. 1979).

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TABLE 1

Effect of pesticides on the dehydrogenase activity in a flooded soil

Treatment	Pesticide (µg/g)	Triphenyl formazan (mg/10 g/24 h) Days after flooding 4	10	20
No pesticide				
Nonsterile	-	1.18	6.25	16.1
Autoclaved	-	n.d.	n.d.	0.10
Irradiated	-	n.d.	n.d.	0.39
HCH	1	0.82	6.82	17.0
	10	0.36	3.60	16.2
	100	0.12	0.16	0.12
Carbaryl	1	1.28	7.0	16.8
	10	1.12	6.17	16.5
	100	0.72	3.36	10.2
Benomyl	1	1.29	6.07	16.0
	10	0.14	1.23	7.02
	100	0.03	0.04	0.05
Atrazine	1	1.0	6.10	16.8
	10	1.24	5.98	17.3
	100	0.93	6.58	17.6

n.d. - not determined

RESULTS AND DISCUSSION

The data in Table 1 showed that no appreciable reduction of TTC occurred in flooded soil sterilized by autoclaving (121°C for 1 h for 3 days) or irradiation with ^{60}Co (4 Mrads for 4 h). But, in nonsterile soil with no added pesticide, the triphenyl formazan increased significantly with incubation under flooding as a result of pronounced dehydrogenase activity of flooded soil in agreement with our earlier report (CHENDRAYAN et al. 1980). Interestingly, the addition of commercial formulation of benomyl, HCH and carbaryl, but not atrazine, to the flooded soil at the time of flooding prevented the accumulation of dehydrogenase activity (in terms of the formazan formed). Benomyl was the most effective in inhibiting the dehydrogenase activity, followed by HCH and carbaryl.

TABLE 2

Effect of pesticides on the redox potential of a flooded soil

Treatment	Pesticide (µg/g)	Redox potential (mV) Days after flooding 4	10	20
No pesticide	-	+ 15	- 80	-120
HCH	1	- 10	- 90	-130
	10	+ 70	- 60	-120
	100	+140	+120	+100
Carbaryl	1	- 10	- 90	-120
	10	- 10	- 80	-110
	100	0	- 80	-120
Benomyl	1	+ 15	- 90	-130
	10	+ 90	+ 80	- 20
	100	+150	+140	+100
Atrazine	1	+ 10	- 90	-120
	10	+ 10	- 90	-130
	100	- 20	- 80	-130

Many of the dehydrogenases are anaerobic in nature (ORTEN & NEUHAUS 1970). For instance, dehydrogenases have been implicated in the reduction of iron (BROMFIELD 1954), a dominant reaction in a flooded soil (PONNAMPERUMA 1972). Redox potential is a semi-quantitative index of the status of soil reduction. Whether a relationship existed between the redox potential and the inhibition of the dehydrogenase activity by the pesticides was, therefore, examined. The potentials decreased rapidly in soil samples without any pesticide (Table 2); but the addition of commercial formulation of benomyl (at 10 and 100 µg AI/g) and HCH (only at 100 µg AI/g) retarded the drop in redox potentials. The inhibition of dehydrogenas activity by benomyl and HCH was probably associated with high potentials. However, the inhibition of dehydrogenase activity was not necessarily related to high potentials since carbaryl inhibited the activity despite low potentials.

In another experiment, soil was first reduced by flooding for 15 days and then treated with commercial formulation of benomyl and HCH (100 µg AI/g soil) for 10 days followed by dehydrogenase assay. No appreciable inhibition of the dehydrogenase activity occurred when benomyl or HCH was added to the

soil pre-reduced by flooding (Table 3) as compared to the striking inhibition of its accumulation in soil samples treated at the time of flooding (Table 1). This would indicate that these pesticide formulations do not inhibit the dehydrogenase activity already accumulated in the soil over a period of flooding prior to pesticide exposure. At the same time the low level of dehydrogenase activity in soil treated with the formulation of either benomyl or HCH followed by incubation under

TABLE 3

Effect of HCH and benomyl (100 µg/g) on the dehydrogenase activity after application to a soil pre-reduced by flooding*

Treatment	Triphenyl formazan (mg/10 g/24 h)	Redox potential (mV)
No pesticide	9.0	-100
HCH	9.98	-110
Benomyl	6.75	-100

*Soil was flooded for 15 days and then treated with pesticides for 10 days prior to dehydrogenase assay.

flooding (Table 1) is probably due to their inhibitory action on the proliferation of microorganisms with dehydrogenase activity. Whether this inhibition was due to the pesticide molecule or other ingredients in the commercial formulation is not clear from the present study.

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