Degradation of Parathion in Flooded Acid Soils

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The persistence of parathion (O,O-diethyl O-(p-nitrophenyl) phosphorothioate) in five acid soils under flooded conditions was investigated. The insecticide degraded faster in soils which had a higher organic matter content. Fastest degradation occurred in an acid sulfate soil with an organic matter content of 12.2%, apparently due to microbial participation. Repeated additions of parathion to an alluvial soil enhanced its hydrolysis to p-nitrophenol. Heat treatment of the parathion-hydrolyzing enriched culture from the alluvial soil retarded its activity, indicating the role of biological agents in the hydrolysis. A *Bacillus* sp. capable of readily decomposing p-nitrophenol as a sole carbon source was isolated from parathion-amended flooded alluvial soil.

A substantial portion of rice-growing areas in India is comprised of acid soils. A commercial formulation of parathion (O,O-diethyl O-(p-nitrophenyl) phosphorothioate) (Folidol E.C.) is extensively used for the control of insect pests in rice. Parathion degraded faster in near neutral soils under flooded conditions than under nonflooded conditions (Sethunathan and Yoshida, 1973). However, little information is available regarding the fate of this insecticide in acid soils under flooded conditions. The present paper aims at investigating the persistence of parathion in certain acid soils under flooded conditions and the role of microorganisms in its degradation.

MATERIALS AND METHODS

Soils. Four acid soils from Kerala, India, acid sulfate (peaty soil locally known as "Kari"), lateritic soil, saline soil (locally known as "Pokkali"), and swampy soil and an alluvial soil from the institute farm were used in this study. The characteristics of these soils are given in Table I. The soils were air-dried, passed through a 2-mm sieve, and 20-g samples were placed in test tubes (25 mm × 200 mm). An aqueous solution of about 1000 ppm of parathion was prepared from 46.7% Folidol E.C. (Bayer). One milliliter of this solution was added to each tube and the soils were flooded with 24 ml of distilled water. The soils were incubated at room temperature (28–32°). Two replicates were removed at every sampling for residue analysis.

Extraction Procedure. Parathion residues in the soils were extracted with chloroform–diethyl ether (1:1) following the methods described earlier for diazinon (Sethunathan and Yoshida, 1969). The solvent fraction was evaporated to dryness in a beaker at room temperature. The residues were redissolved in 2 ml of methanol and then transferred to a glass vial. The solvent was again evaporated to dryness and the residues were finally dissolved in 1 ml of methanol for further analysis by thin-layer chromatography (tlc).

Thin-Layer Chromatography. The tlc method used for the separation of parathion and p-nitrophenol was essentially the same as used earlier for diazinon (Sethunathan and Yoshida, 1969). The silica gel G layers used in this study were 300 μ thick. The plates were developed for a distance of 15 cm in chloroform–acetone (7:1). Parathion was determined after alkaline hydrolysis to p-nitrophenol, which was quantitated by colorimetry. The silica gel above the samples and opposite to the authentic standard parathion was scraped and carefully transferred to a test tube. One milliliter of 2.5 N NaOH was added to each tube and the tubes were placed in a water bath for 1 hr. After cooling, the volume was made up to 25 ml. The solution was centrifuged at 2500 rpm for 10 min to remove the silica gel and the supernatant was read against the appropriate blank in a Klett–Summerson colorimeter,

employing a filter of 420 mμ. The amount of parathion in the samples was calculated by multiplying the values of p-nitrophenol by 2.094. Percent recovery ranging from 79 to 86% was obtained by this method.

Biological Degradation. To determine whether the degradation in acid sulfate soil was biological or chemical, 20-g aliquots of soils were autoclaved at 15 lb for 1 hr consecutively for 3 days. Parathion (300 μg) dissolved in 0.1 ml of ethanol was added to 20-g samples of autoclaved and nonautoclaved soils and the soils were flooded with 20 ml of sterile distilled water. Parathion residues in the soils were extracted as described earlier.

Enhanced Biological Hydrolysis in Alluvial Soil after Repeated Additions. One milliliter of an aqueous 1000-ppm solution of parathion prepared from Folidol E.C. was added to alluvial soil at 2-week intervals. The soils were flooded with 24 ml of distilled water.

In a test to determine whether the hydrolysis in institute farm soils observed after two or three additions of parathion was biological or chemical, the standing water of the soils which received three applications and showed hydrolytic activity after the third addition was removed to a flask. One aliquot of this standing water (enriched culture) was autoclaved at 15 lb for 15 min. Two-milliliter aliquots of autoclaved and nonautoclaved enriched culture were added to 20-g samples of flooded alluvial soil amended with aqueous parathion solution. Parathion residues in the soils were extracted with chloroform–diethyl ether and analyzed for parathion, as described earlier. p-Nitrophenol in the soil extracts was separated by tlc and determined colorimetrically after its elution in 0.1 N NaOH. No interference from colored soluble organic matter was noticed at the p-nitrophenol spot.

Isolation of p-Nitrophenol Decomposing Bacterium. Serial dilutions of the standing water of the alluvial soil that received three repeated applications of parathion were prepared. Mineral growth medium (Sethunathan and Pathak, 1971) amended with 20 ppm of p-nitrophenol was inoculated with the standing water diluted to 1/10,000 its original concentration. Individual colonies were transferred to a sterile mineral solution containing 20 ppm of p-nitrophenol as the sole carbon source. A bacterium was found to possess the ability to decompose p-nitrophenol, as indicated by decolorization of the medium. This isolate was further purified.

In a test to determine the ability of the bacterium to decompose p-nitrophenol as sole carbon source, 50-ml aliquots of a sterile mineral solution (Sethunathan and Pathak, 1971) contained in 250-ml Erlenmeyer flasks were inoculated with the bacterial suspension prepared from 3-day-old cultures. Uninoculated media served as controls. At each sampling, a 20-ml aliquot from each flask was removed and the residues from the 20-ml aliquot were extracted by shaking thrice with 20 ml of chloroform–diethyl ether (1:1). The solvent fraction was pooled in a beaker and evaporated to dryness. The residues were then dissolved in methanol as described earlier and separated by

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Table I. Characteristics of Soils Used in the Experiment[a]

Soil	pH			Electrical conductivity, Mmho/cm	Organic matter, %	Total nitrogen, %
	Initial		28 Days after flooding			
	(H₂O) 1:2.5	NKCl				
Acid sulfate	3.15	3.1	3.65	4.60	12.20	0.270
Saline	3.15	3.1	4.10	15.10	5.90	0.237
Swampy	3.50	3.4	4.60	9.00	3.20	0.164
Laterite	5.25		6.70	0.02	0.95	0.008
Alluvial	5.50		6.40	1.50	0.80	0.060

[a] Data are provided by V. O. Kuruvila, Central Rice Research Institute, Cuttack-6, India.

Table II. Persistence of Parathion in Acid Soils

Soil	Parathion recovered, μg/20 g of soil			Half-life, days	
	0[a]	14[a]	28[a]	14-day analysis	28-day analysis
Acid sulfate	838	262	104	8.32	9.27
Saline	916	375	180	10.83	11.89
Swampy	916	433	128	12.91	9.83
Laterite	785	537	273	25.45	18.31
Alluvial	812	499	291	19.85	18.85

[a] Days after incubation.

tlc. The p-nitrophenol from silica gel was extracted in 10 ml of 0.1 N NaOH and the silica gel was removed by centrifugation. The amount of p-nitrophenol in the supernatant was quantitatively determined by colorimetry, as described earlier.

RESULTS AND DISCUSSION

Degradation in Soils. The degree of parathion degradation in soil depended on soil properties. The insecticide degraded faster in soils which had a higher organic matter content. Parathion degraded very rapidly in acid sulfate soil (Table II) which had the highest organic matter but slowly in lateritic and alluvial soils with low organic matter content. The rate of decomposition appeared to be directly related to organic matter levels in the soils. When calculated from 14-day residues, the half-life ($t\frac{1}{2}$) values for parathion in different soils were 8.3 days for acid sulfate soil, 10.8 days for saline soil, 12.9 days for swampy soil, 19 days for alluvial soil, and 25.3 days for lateritic soil. At 28 days, although the trend was different, again the insecticide degraded faster in acid sulfate soil and least in lateritic and alluvial soils.

Parathion is chemically most stable at low pH values (Suffet and Faust, 1972). In lateritic and alluvial soils, the pH increased to near neutrality after 28 days of flooding and the insecticide degradation was slow. Again, highly acid conditions were recorded even after 28 days of submergence in acid sulfate, saline, and swampy soils in which parathion degradation was rapid. Evidently soil factors other than pH were involved in the degradation of parathion in these acid soils. In a test to find out whether the rapid degradation in acid sulfate soil was biological or chemical, more rapid degradation of parathion occurred in nonautoclaved soil than in autoclaved soil (Table III). After 28 days of incubation, 61.7% insecticide was recovered from autoclaved soil, whereas only 9.5% remained when the soil was not autoclaved. This indicated that rapid degradation of parathion in acid sulfate soil was caused by biological action, although general microbiological activity is considered to be low in soils of extremely low pH. However, Subramoney (1960) reported sulfide formation in this organic soil following flooding due to the activity of sulfate-reducing microorganisms present in the soil. Perhaps these microorganisms also assist in the rapid reduction of the nitro group of parathion. Intense reduc-
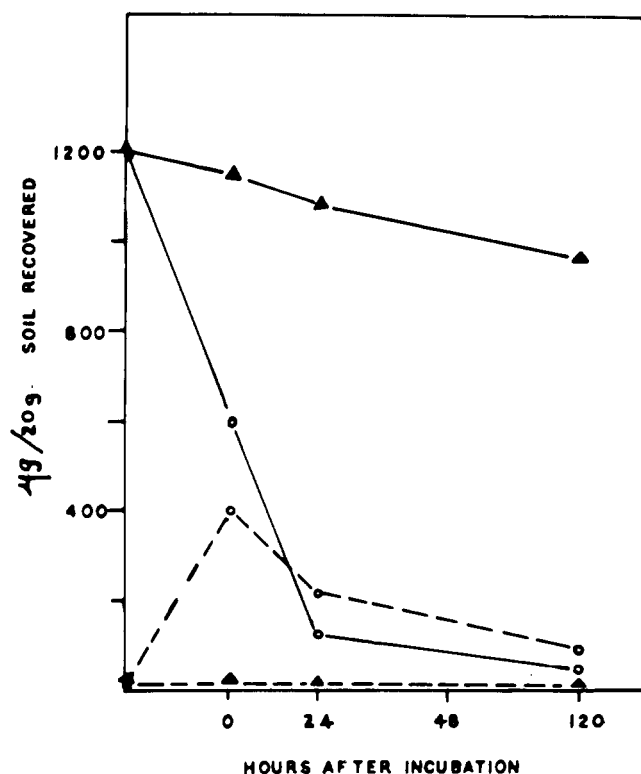

Figure 1. Biological hydrolysis of parathion (——) to p-nitrophenol (-----) in flooded alluvial soil (O, inoculated with nonautoclaved enriched culture; ▲, inoculated with autoclaved enriched culture).

tion following anaerobic decomposition of organic matter in this peaty soil might also accelerate the conversion of parathion by sulfate reducers. The ability of methane-forming and sulfate-reducing bacteria to reduce DDT to DDD when inoculated to an anaerobic sludge has been demonstrated earlier (Hill and McCarty, 1967). Further studies on the isolation of parathion degraders and metabolic products from this organic soil are underway.

Enhanced Biological Hydrolysis in Alluvial Soil after Repeated Applications. It has been reported earlier that diazinon undergoes rapid biological hydrolysis in flooded rice fields, particularly after its repeated applications (Sethunathan and Pathak, 1972). The present experiment was aimed at finding out whether repeated applications of another organophosphate, parathion, to flooded soils would likewise influence the hydrolysis of parathion. After the third addition of parathion to alluvial soil under flooded conditions, the standing water above the soils in all the three replicates turned yellow; in one replicate the standing water turned yellow following the second addition. The experiment was repeated and this phenomenon was observed in the standing water of two of the three replicates following the third addition. This indicated that parathion was rapidly hydrolyzed in flooded soil following its repeated applications to p-nitrophenol, which gives a yellow reaction at pH 5.2 and above. Further evidence of p-nitrophenol formation was provided by tlc analysis.

To test whether enhanced parathion hydrolysis observed in earlier experiments was biological, autoclaved and nonautoclaved aliquots of the soil solution (enriched culture) from a flooded alluvial soil that received three applications of parathion at 2-week intervals were added to alluvial soil amended with parathion solution. Within 48 hr of incubation, the standing water of the soils inoculated with nonautoclaved enriched culture turned yellow. Analysis by tlc indicated the formation of p-nitrophenol in soils inoculated with nonautoclaved enriched culture (Figure 1). However, p-nitrophenol formed was unstable and its con-

Table III. Persistence of Parathion in Autoclaved and Nonautoclaved Acid Sulfate Soil under Flooded Conditions

Incubation, days	Parathion recovered, μg/20 g of soil	
	Autoclaved	Nonautoclaved
0	251	251
14	163	80
28	155	24

Table IV. Degradation of p-Nitrophenol by a Bacillus sp. Isolated from Parathion-Amended Alluvial Soil

Incubation, hr	p-Nitrophenol recovered, ppm	
	Inoculated	Uninoculated
0	12.3	14.3
24	3.0	13.0
48	0	13.5

centration in the soil decreased after 24 hr. In soils inoculated with autoclaved enriched culture, no appreciable degradation of parathion occurred during the 120-hr incubation period; also p-nitrophenol was not detected. Lichtenstein and Schulz (1964) reported that degradation of parathion in nonflooded soils proceeded either by hydrolysis or by reduction of the nitro group, depending on the microbial population. Hydrolysis of parathion by resting cells and cell-free extracts of a Flavobacterium sp., isolated from diazinon-amended rice fields, has been demonstrated recently (Sethunathan and Yoshida, 1972).

Bacterial Degradation of p-Nitrophenol. p-Nitrophenol formed by hydrolysis from parathion is toxic and its accumulation in soils might cause a pollution hazard. The fall in its concentration in the soil (Figure 1) indicated that it is rapidly broken down in flooded soils. A bacterium was isolated from flooded alluvial soil enriched by repeated additions of parathion. The bacterium identified as Bacillus sp. was tested for its ability to decompose p-nitrophenol. The bacterium readily decomposed p-nitrophenol (Table IV), but no other metabolite could be detected in the thin-layer chromatogram.

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Total Toxic Aldicarb Residues in Weeds, Grasses, and Wildlife from the Texas High Plains Following a Soil Treatment with the Insecticide

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Aldicarb residues in weeds, grasses, and wildlife following a soil application of the granular insecticide in dryland and irrigated fields were investigated by a gas chromatographic–flame photometric detector (gc–fpd) analysis. Residues of aldicarb and/or its metabolites (as the sulfone) were detected in 80% of the grass and weeds collected from treated areas and in 83% of samples from untreated sections of these fields. No detectable residues were found in samples from adjacent untreated, noncultivated areas outside the treated fields. In irrigated fields residues were detected in approximately 73% of samples from treated areas, 38% of samples from untreated sections of treated fields, and 31% of samples from adjacent untreated, noncultivated areas outside the treated fields. Residues in grasses and weeds gradually decreased with time and with plant growth, which created a dilution factor. Residues were detected in only one of the wildlife samples collected in and around the treated fields. This was an oriole showing 0.07 ppm of aldicarb and/or metabolites.

During 1971 a 10% granular formulation of aldicarb, 2-(methylthio)propionaldehyde O-(methylcarbamoyl)oxime, also known as UC-21149 and Temik, was tested as a broad spectrum insecticide for the control of a variety of cotton insects. In previous investigations by Andrawes et al. (1971), ^{14}C-labeled aldicarb residues remaining in the soil from an earlier experiment were found to translocate into crabgrass, tomato, and potato plants. This work was not pursued, however, and additional data on persistence and translocation were necessary. Further information was also needed to determine if residues appeared in birds and mammals which may feed on seeds and plants in the treated areas.

This report deals with aldicarb residues found in weeds, grasses, and wildlife collected in and adjacent to areas receiving a soil application of the 10% granular insecticide (aldicarb 10G) at a rate of 15 lb/acre (1.5 lb actual) in dryland and irrigated fields.

EXPERIMENTAL SECTION

Type of soil, application rate, and method of application were described in a previous paper (Woodham et al.,

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