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Parathion Degradation in Submerged Rice Soils in the Philippines

The persistence of parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate) in four Philippine rice soils was investigated in submerged and upland conditions. The parathion in the soils after incubation at 30° was extracted with a hexane–acetone mixture and the amount was determined by gas chromatography. The insecticide disappeared more rapidly from submerged soils than from upland soils. In submerged soils parathion was reduced to aminoparathion (*O,O*-diethyl *O*-*p*-aminophenyl phosphorothioate). Autoclaving of the soils increased the persistence of parathion under submerged conditions, indicating microbial participation in its degradation. Parathion degraded faster in flooded soil inoculated with parathion-hydrolyzing *Flavobacterium* sp. than in uninoculated soil.

Recent studies indicate that several chlorinated hydrocarbon insecticides persist for years in upland soils but readily break down if the soil is submerged (Castro and Yoshida, 1971; Yoshida and Castro, 1970). Likewise diazinon, an organophosphate, appears to degrade rapidly in submerged soils (Sethunathan and MacRae, 1969).

The fate of the insecticide parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate) in submerged soil is not completely understood, however. Lichtenstein and Schulz (1964) studied the persistence of parathion in upland soils. According to them, a high soil moisture content and the presence of soil microflora favored the degradation of parathion in soils. The major pathway of parathion breakdown in soils (Lichtenstein and Schultz, 1964), lake sediments (Graetz *et al.*, 1970), and microorganisms in pure culture (Mick and Dahm, 1970; Zukerman *et al.*, 1970) involves nitro reduction to its amino compound, aminoparathion (*O,O*-diethyl *O*-*p*-aminophenyl phosphorothioate).

This paper reports a study aimed at comparing the rates of decomposition of parathion in upland and in submerged soils. The effect of *Flavobacterium* sp., which had been shown earlier to hydrolyze diazinon and parathion (Sethunathan and Yoshida, 1972), on the metabolism of parathion in submerged soil was also studied.

MATERIALS AND METHODS

Persistence in Submerged and Upland Soils. We investigated the persistence of parathion in four Philippine soils: the air-dried soils Maahas clay (pH 6.6, organic matter 2.0%, total N 0.14%); Luisiana clay (pH 4.7, organic matter 3.2%, total N 0.21%); a clay loam from Pila (pH 7.6, organic matter 1.5%, total N 0.09%); and Casiguran sandy loam (pH 4.8, organic matter 4.4%, total N 0.2%). Air-dried samples of each soil were passed through a 2-mm sieve. Twenty grams of soil was placed in test tubes (25 × 180 mm) and 25 ml of distilled water was added to half of the soil samples to obtain a flooded soil condition under standing water 5-cm deep. To simulate upland conditions 4 to 6.5 ml of distilled water was added to the other samples to keep the soil moisture at field capacity. Parathion (98.76%, City Chemical Corporation, New York, N. Y., U.S.A.) dissolved in 50 μl of acetone was introduced into the soil in each tube at a concentration of 3000 ppm. The soils were incubated in an incubator at 30°.

For each treatment three replicate tubes were withdrawn for residue analysis at the start of the experiment, 7 and 14 days after incubation. To extract parathion from the soils we used the method used for diazinon (Sethunathan *et al.*, 1971). This method gave recoveries of 91 to 96%. The hexane layer was suitably diluted before being injected into a gas chromatograph equipped with a cesium bromide detector. The temperature setting was 215° for the column and the detector and was 225° for the injector port. The other gas chromatographic conditions were the same as those reported for analysis of diazinon (Sethunathan *et al.*, 1971). Under these conditions, the retention time for parathion was 3.03 min and for aminoparathion was 2.48 min.

Biodegradation of Parathion. To determine the role of microorganisms in degrading parathion in submerged soil, the rates of degradation in autoclaved and nonautoclaved lots of four soils were compared. The soils were autoclaved at 121° for 1 hr on each of three successive days to reduce microbial population. The parathion was placed in an aqueous solution (instead of dissolving the parathion in acetone) and passed through a Millipore filter (pore size, 0.45 μ). Twenty-five milliliters of the filtered solution (8 ppm) was then added to 20 g of autoclaved and nonautoclaved soils. The residues in three replicate tubes for each treatment were analyzed at the start of the experiment and at 10 days after incubation. Nonautoclaved soil undergoes rapid reduction following flooding primarily due to intense microbiological activity; however, a thin surface layer remains oxidized. These distinct zones were absent in flooded autoclaved soils, indicating reduced microbial activity.

In earlier work we found that a species of *Flavobacterium* isolated from paddy water of a field previously treated with diazinon was highly efficient in hydrolyzing diazinon and parathion (Sethunathan and Yoshida, 1972). To test the ability of this species to metabolize parathion in submerged soil, the bacterium was grown for 4 to 7 days on modified Wakimoto agar (International Rice Research Institute, 1970) and then suspended in sterile distilled water. One milliliter of the suspension was added to tubes containing 20 g of Maahas clay and the soil was flooded with 25 ml of 8-ppm parathion solution; 1 ml of sterile distilled water was added to the controls instead of the bacterial suspension. The soil samples were then incubated at 30°. Three tubes for each treatment were removed

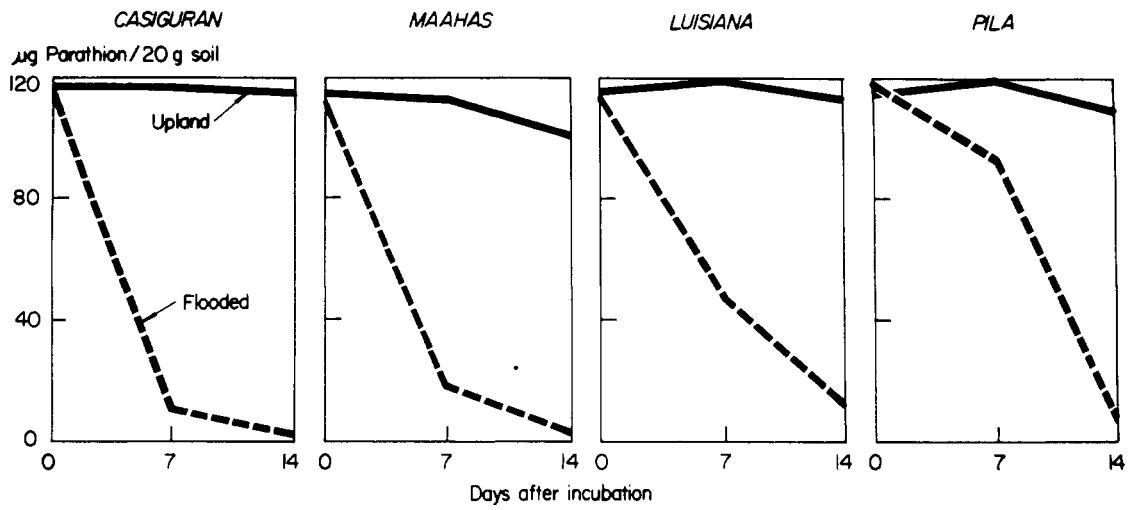

Figure 1. Degradation of parathion in four upland and submerged soils in the Philippines.

immediately after incubation, and at 5 and 10 days after inoculation. The residues were analyzed by gas–liquid chromatography after hexane–acetone extraction.

RESULTS AND DISCUSSION

During the 2-week incubation period, no appreciable degradation of parathion occurred in any of the four soils under upland conditions (Figure 1). In contrast, most of the insecticide added to these soils under submerged conditions rapidly decomposed. During the first 7 days of incubation, the rate of degradation was greater for the Casiguran soil, which had high organic matter content. By 14 days after incubation, however, most of the parent insecticide had disappeared from all the four soils under submerged conditions. Recently, lindane (Yoshida and Castro, 1970) and DDT, and methoxychlor and heptachlor (Castro and Yoshida, 1971) were also found to decompose rapidly in Casiguran sandy loam. A high organic matter content in this soil appeared to favor the anaerobic decomposition of organochlorine insecticides because it enhances the reducing conditions in the soil.

Gas chromatograms of hexane extracts revealed that aminoparathion was formed during the degradation of parathion in submerged soils. Aminoparathion was not detected in upland conditions and there was no appreciable degradation during the 2-week incubation. No other intermediate appeared on the gas chromatogram. Lichtenstein and Schulz (1964) found that in upland soils aminoparathion appeared to be the major metabolite of parathion breakdown. In our experiments the submerged conditions enhanced the formation of aminoparathion, probably because anaerobic conditions favor the reduction of the nitro group in parathion to the amino form. The high organic matter content in Casiguran sandy loam seems to favor nitro reduction, as is evident from the rapid disappearance of parent parathion (Figure 1) accompanied by the formation of aminoparathion. No attempt was made, however, to estimate the amount of aminoparathion formed.

To determine the role of biodegradation in the rapid loss of parathion from flooded soils, the persistence of parathion in autoclaved soil samples was compared to its persistence in nonautoclaved soil samples. Parathion disappeared more rapidly from nonautoclaved flooded soil samples than from autoclaved flooded soil samples (Table I). Half-life values for parathion in nonautoclaved Maahas, Pila, Casiguran, and Luisiana soils were 6.2, 12.3, 1.6, and 9.0 days, respectively; the corresponding values in autoclaved soils were 72.5, 68.0, 37.7, and 80.2 days. These results strongly indicate that soil microorganisms are involved in the rapid breakdown of parathion in flooded soils. Biodegradation of parathion in soils has been reported earlier (Lichtenstein and Schulz, 1964).

Table I. Persistence of Parathion in Four Submerged Soils

	Parathion recovered (μg per 20 g of soil)							
	Casiguran		Maahas		Luisiana		Pila	
Treatment	0[a]	10	0	10	0	10	0	10
Autoclaved	143	120	143	130	144	132	144	130
Nonautoclaved	152	2	156	51	144	67	145	83

[a] Days after incubation.

Table II. Parathion Degradation in Submerged Maahas Clay after Inoculation with *Flavobacterium* sp.

Incubation, days	Parathion recovered (μg per 20 g of soil)	
	Inoculated	Uninoculated
0	191	211
5	2	79
10	0.1	22

It was reported earlier that a diazinon-degrading *Flavobacterium* sp., isolated from water of a diazinon-treated rice paddy, exhibited an exceptionally high capacity to hydrolyze parathion to *p*-nitrophenol in pure culture (Sethunathan and Yoshida, 1972). In a test to determine whether submerged soils inoculated with this bacterium could readily decompose parathion, the insecticide degraded faster in inoculated soil than in uninoculated soil (Table II). The half-life value for parathion in the inoculated soil was 0.76 days and that in the uninoculated soil was 3.5 days. The standing water of the inoculated soil turned yellow within 24 hr after inoculation, indicating the formation of *p*-nitrophenol. Thin-layer chromatographic analysis of the soils (Sethunathan and Yoshida, 1972) confirmed its identity. Gas chromatographic analysis showed that aminoparathion was formed as a major metabolite of parathion breakdown in uninoculated controls; it was not detected in inoculated soils. These results suggest that *Flavobacterium* sp., with its high capacity to hydrolyze parathion, offers a powerful tool in decontaminating the parathion-polluted environment or containers.

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Nature of Carboxin (Vitavax)-Derived Bound Residues in Barley Plants

More than 70% of the unextractable residue derived from [^{14}C]carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) in barley leaves was liberated by hot dimethyl sulfoxide. The ^{14}C residue so released was identified as carboxin (30%) and its sulfoxide (70%). The theory of lignin complex formation for detoxification through immobilization by plants is further indicated.

In a previous paper (Chin *et al.*, 1970), the possible existence of a highly insoluble complex of carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) with lignin was reported. Further identification, however, was not successful because of the relative instability of carboxin to hydrolysis. Hot dimethyl sulfoxide (DMSO) is known to be a good solvent for lignin (Weise and Niclas, 1967) and has been used in this work for releasing the insoluble [^{14}C]carboxin derived residues from barley plants. We also report here the results of our study of the nature of the DMSO-solubilized portion of the insoluble residue.

EXPERIMENTAL SECTION

Plant Materials. Two-hundred barley seeds were treated with hetero-ring-tagged [^{14}C]carboxin by applying an acetone solution of the chemical with a micropipette on each seed. Similarly, 200 seeds were treated with phenyl-tagged [^{14}C]carboxin and the same amount was left untreated. The seeds were treated at the rate of 3 oz of active ingredient per 100 lb of seed (corresponding to 0.25 μCi at 1 mCi/mM). Seeds were planted and grown in the greenhouse and the plants were harvested 30 days later. The first two leaves with the lower stem (about 1.5 in.) were combined, dried at 50°, ground, and extracted in a Soxhlet extractor for 8 hr with a mixture of benzene–acetone–methanol (1:1:1, v/v). The ^{14}C residues remaining in the extracted plant materials were considered to be insoluble and used for DMSO extraction.

DMSO Extraction. One-half gram of extracted plant material was stirred in a stoppered Erlenmeyer flask with a magnetic stirrer with 20 ml of DMSO for 4 days at room temperature and 5 hr at 80°. After cooling, the mixture was filtered through a coarse glass filter. The weight of plant material was reduced by this extraction to 0.3 g. A third extraction (hot) caused no significant reduction in tissue weight and removed only small amounts of ^{14}C.

Identification of Residues. After DMSO was evaporated to dryness at 85° and 15 mm, the ^{14}C-containing residue was taken up in methanol and analyzed by thin-layer chromatography on Eastman Kodak silica gel sheets No. 6061 in chloroform (A), acetone (B), and acetone–methanol (4:1, v/v) (C). For comparison, a DMSO plant extract obtained from untreated plants was fortified with labeled carboxin and its sulfoxide and used as reference standard. After development, the radioactive components were detected by autoradiography; the radioactive spots on the chromatogram were cut out and counted by liquid scintillation. The R_f values for carboxin and its sulfoxide were: in solvent A, 0.82 and 0.63; in solvent B, 0.88 and 0.70; and in solvent C, 0.91 and 0.84. In each solvent, complete resolution between components was obtained. To determine if the sulfoxide is not formed by chemical oxidation, a 1% solution of carboxin in DMSO was heated for 4 hr at 75–80°, as given in the extraction procedure. No detectable amounts of the sulfoxide were found when analyzed on thin layers in solvent A.

Determination of ^{14}C. All quantitative measurements of ^{14}C were made on the Beckmann LS-100 liquid scintillation spectrometer in a PPO-containing toluene–cellosolve based counting solution. The total radioactivity in plant tissue before and after DMSO extraction was determined by wet oxidation (Mahin and Lofberg, 1966) and liquid scintillation counting.

RESULTS AND DISCUSSION

By extraction of the ^{14}C residue-containing plant material with hot DMSO, about 74% of the total insoluble ^{14}C was solubilized for both ^{14}C-labeled substrates (Table I). Thin-layer chromatography showed that the DMSO-soluble part of ^{14}C residue in plants consists of two components. When compared with standard reference compounds in chloroform, acetone, and acetone–methanol, identical R_f values and shapes of spots as for carboxin and carboxin sulfoxide were obtained in each solvent. After autoradiography of the chromatograms, the two spots corresponding to carboxin and its sulfoxide were cut out and radioassayed by liquid scintillation counting. The ratio of carboxin to its sulfoxide was found to be 3:7 for both the phenyl and hetero-tagged compounds.

About 10% of the radioactivity from the DMSO extracts remained at the origin during thin-layer chromatography. When these radioactive areas were eluted from the gel and rechromatographed, carboxin and its sulfoxide were the principal compounds detected. They were present in the same ratios as in the original chromatogram. This indicated artificial retention by extraneous polar plant materials. A fortified sample from untreated plants gave similar results, confirming that the baseline spot was an artifact.