Bull. Environ. Contam. Toxicol. (1987) 38:381–387

Environmental Contamination and Toxicology

Persistence of Phorate in Different Soils with and without Amendments and Its Degradation by a *Pseudomonas* sp.

M. Venkatramesh, V. Agnihothrudu,[1] M. S. Mithyantha,* and S. C. Tripathi

Rallis Agrochemical Research Station, 21 & 22, Peenya Industrial Area Phase II, Bangalore 560058, India

The use of chemicals for the control of agricultural pests is an established practice in farming systems throughout the world today. The environmental hazards of these pesticides has become an alarming problem and the degradation and detoxification of these chemicals is a major field of research. The vast majority of the 500 or so active pesticides available today can be biologically degraded by microorganisms in the soil.

During a systematic study of the effects of pesticides on the soil microflora and their activities (Venkatramesh 1986) it was observed that the persistence of phorate in soils and the changes in the populations of a Pseudomonas sp. were interrelated. We report here these findings.

MATERIALS AND METHODS

Four soils were used in this study viz., red (Ustalf), laterite (Ustox), black (Ustert) and alluvial (Aquents). All the soils were collected from areas of no known history of pesticide use and from the top 0–30 cm. Some of the important physical and chemical characteristics of these soils are given in Table 1.

The soils were sieved and 100 g samples were dispensed into polypropylene bags measuring 15 x 10 cm. Each sample was then thoroughly mixed with the appropriate amount of phorate 10% granules (Cyanamid India Ltd.) to give a final concentration of 50 and 100 ppm. Where the soils were amended with fertilizers, ammonium sulphate

1 Present Address: Specialist Consultant 18/1, Jayamahal Extension I Main Bangalore 560042 INDIA.
* Correspondence and reprint requests

Table 1. General physical and chemical characteristics of the soils used in the study

PROPERTY	RED	LATERITE	BLACK	ALLUVIAL
Textural class	Sandy loam	Sandy clay loam	Clay	Clay
Max. water holding capacity	21.6%	38.5%	46.6%	43.9%
Field Capacity	14.0%	24.0%	36.0%	33.0%
pH	6.4	7.6	8.4	7.7
Organic Carbon %	0.4	1.9	0.5	1.7
Mineral N %	0.01	0.01	0.01	0.01
C.E.C. me/100g	11.3	14.6	77.2	30.9

solution (500 ppm- N) and groundnut oil cake powder (1.5 g) were also added. All treatments were replicated thrice. Moisture level was maintained at field capacity and the bags were stapled. At various intervals over a period of sixty days, samples were drawn for the various analyses.

Ten gram samples (wet weight) of soil was drawn from each treatment after mixing the soil in each pouch thoroughly. The samples from the three replicates was pooled together and a further 10 g sample was taken for plating. Another 10 g sample was taken for moisture determination. The samples for plating were passed through a dilution series of up to 10^{8} and the appropriate dilutions used for enumerating the microbial populations. For bacteria a dilution of 10^{5} and Thornton's agar medium were used. Standard procedures were followed for plating (Johnson et al., 1959). Bacterial colonies were counted five days after incubation at 30 C. Pseudomonas sp. were identified by the fluorescent nature of the colonies on these plates and were recorded separately. Initially, the colonies were isolated and grown on King's basal medium which is specific for Pseudomonas to confirm the identity of the colonies.

The soil remaining in the pouches after drawing the samples for plating was used for residue analysis. Phorate was extracted and analysed by a modification of the method described by Zweig and Sherma (1972) and estimated on a Varian 3700 gas chromatograph equipped with a thermionic specific detector. The GC conditions used for the analysis were: Column used was 5% OV-101 on 60-80 mesh chromosorb G, 50 cm long, 0.3 cm dia; temperatures set (°C) were column 200, detector 230, and injector 220; gas flow rates were (cc/min) nitrogen (carrier) 30, hydorgen 4 and air 140; attenuation was 4 x 10^{-11}; range of standards used for calibration was 40

to 200 ng; and retention time was 1.5 min.

The residue from the extraction step was dissolved in 5-15 ml acetone and 1-2 µl injected for residue analysis. The sample peak heights were calibrated against a standard curve. In order to identify the metabolite peak appearing in the soil extracts, analytical standards of the two metabolites viz., phoratoxon and phorate sulfoxide were also injected under similar conditions as for phorate.

All the data were analysed statistically by the factorial method, the factors being soil, treatment and time of sampling after treatment (interval).

RESULTS AND DISCUSSION

The dissipation of phorate from soils showed a definite treatment effect and phorate sulfoxide was the major metabolite formed. This is represented in Fig. 1. The fertilizer amendments altered the dissipation of phorate (Tables 2a) and these effects varied between soils and between the amendments. In all the soils, except laterite, ammonium sulphate retarded degradation

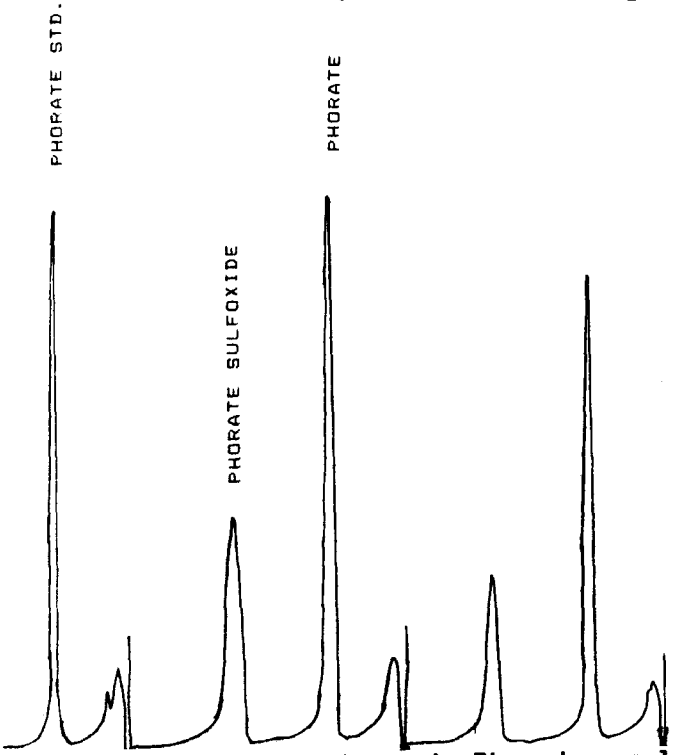

Figure 1 GC Plots of Phorate and Phorate sulfoxide along with the Standards

while groundnut oil cake hastened it. While the effect due to ammonium sulphate was not significant statistically in any of the soils, that due to groundnut oil cake was significant in red and laterite soils which recorded 40 and 51 % lower residues respectively after sixty days. In all other cases even though there is a 7-28 % variation in the residue levels over the unamended treatments with the same concentration of phorate, there is no statistical significance. This may be due to the fact that the variations reported here are represent an average of all variations occurring in the sixty days period. This fact is strenghthened by the observation that in the case of treatment versus interval interaction (Table 2b), significant variations were noticed.

These variations in the degradation rates showed a good interrelationship with the populations of a fluorescent Pseudomonas sp. In the unamended soils the populations of this bacterium increased with the increasing pesticide concentration in all the soils except black soil where there was no effect (Table 3a). In the case of the amendments, ammonium sulphate recorded lower numbers than control in all soils and this reduction ranged from 16-53 %. However, statistical significance was noticed only in the case of interactions (Table 3b) indicating that the effects varied depending on soil type and interval. Groundnut oil cake amendment recorded a statistically significant stimulation up to the 25th day and by the 60th day there was no effect.

In the present study, phorate degraded to undetectable levels in 25-40 days in all the soils though in red and laterite soils it persisted up to 60 days at 100 ppm. The amendments altered these degradation rates and in some cases were statistically significant. The major metabolite detected in the analysis was phorate sulfoxide and its level increased with the increase in degradation of phorate. Thus the highest level of this metabolite was seen in groundnut oil cake amended soils. The sulfoxide was quite stable and so increased with time. There are no reports in the literature on the effect of amendments on the degradation of phorate. However, Chapman et al. (1982) reported the enhanced degradation of phorate in natural soils when compared to sterilized soils and phorate sulfoxide was the major metabolite.

In the present study Pseudomonas sp. increased with increasing concentration of phorate. Further, it was also seen that soils amended with ammonium sulphate recorded very low populations of this bacterium with subsequent slow, degradation while groundnut oil cake amended soil with 100 ppm phorate showed a remarkable increase in the growth of this bacterium with a rapid

Table 2a Residues of phorate in different soils with and without amendments (mg phorate/g soil)

SOIL	TREATMENT	DAYS AFTER INCUBATION				
		3	10	25	40	60
RED	Control	ND	ND	ND	ND	ND
	50ppm	0.043	0.010	0.005	ND	ND
	100ppm	0.100	0.026	0.018	0.021	0.013
	100ppm+$(NH_4)_2SO_4$	0.112	0.034	0.023	0.022	0.007
	100ppm+GN oil cake	0.092	0.007	ND	ND	ND
LATE-RITE	Control	ND	ND	ND	ND	ND
	50ppm	0.030	0.005	0.006	0.007	ND
	100ppm	0.084	0.023	0.020	0.018	0.017
	100ppm+$(NH_4)_2SO_4$	0.078	0.030	0.024	0.019	0.017
	100ppm+GN oil cake	0.064	0.010	ND	ND	ND
BLACK	Control	ND	ND	ND	ND	ND
	50ppm	0.024	0.005	ND	ND	ND
	100ppm	0.083	0.015	0.006	0.008	ND
	100ppm+$(NH_4)_2SO_4$	0.093	0.024	0.009	0.015	0.005
	100ppm+GN oil cake	0.075	0.009	ND	ND	ND
ALLU-VIAL	Control	ND	ND	ND	ND	ND
	50ppm	0.055	0.012	ND	ND	ND
	100ppm	0.120	0.027	0.014	0.011	ND
	100ppm+$(NH_4)_2SO_4$	0.121	0.028	0.025	0.021	0.006
	100ppm+GN oil cake	0.133	0.019	ND	0.006	ND

SEm+/- = 0.02

$CD_{0.05}$ for interval = 0.06 $CD_{0.05}$ for treatment = 0.06
$CD_{0.05}$ for treatment x interval interaction = 0.04

Table 2b Two way table for treatment x interval interaction

INTERVAL / TREATMENT	3	10	25	40	60	TOTAL
Control	0.00	0.00	0.00	0.00	0.00	0.00
50 ppm	0.15	0.03	0.02	0.02	0.01	0.23
100 ppm	0.39	0.09	0.06	0.06	0.04	0.64
100 ppm+$(NH_4)_2SO_4$	0.40	0.12	0.08	0.08	0.04	0.72+
100 ppm+GN Oil Cake	0.36	0.05	0.01	0.01	0.00	0.43-
TOTAL	1.30	0.29	0.17	0.17	0.09	

'+' indicates significant increase over 100 ppm alone and '-'indicates significant decrease over 100 ppm alone

Table 3a Effect of phorate on Pseudomonas sp populations in the soil (* Average of 3 replicates)

SOILS	TREATMENTS	COLONY COUNT (X 10^5)* DAYS AFTER INCUBATION				
		3	10	25	40	60
RED	Control	16.0	7.2	4.5	1.2	3.3
	50ppm	42.4	24.2	24.1	11.1	17.8
	100ppm	70.1	39.8	31.6	15.0	26.7
	100ppm+$(NH_4)_2SO_4$	14.8	3.8	1.1	1.2	0.0
	100ppm+GN oil cake	774.9	231.1	85.4	19.2	48.3
LATE-RITE	Control	72.3	53.4	57.0	63.6	60.2
	50ppm	49.8	67.8	62.3	86.7	61.0
	100ppm	69.0	66.2	91.0	89.9	57.0
	100ppm+$(NH_4)_2SO_4$	72.8	62.9	68.0	42.1	11.6
	100ppm+GN oil cake	627.4	460.3	194.2	125.6	76.2
BLACK	Control	16.6	18.0	17.8	19.6	15.1
	50ppm	23.4	19.9	14.2	7.2	8.0
	100ppm	21.0	26.5	8.2	14.3	8.4
	100ppm+$(NH_4)_2SO_4$	23.4	9.9	4.1	1.4	4.4
	100ppm+GN oil cake	341.1	194.1	13.7	0.0	4.4
ALLU-VIAL	Control	70.0	16.5	15.3	30.9	9.8
	50ppm	78.0	22.5	37.5	36.0	16.3
	100ppm	63.5	17.4	23.1	22.6	10.3
	100ppm+$(NH_4)_2SO_4$	46.2	17.9	1.7	0.9	0.5
	100ppm+GN oil cake	345.5	820.7	13.1	0.0	18.7

Table 3b Two way table for treatment x interval interaction

INTERVAL / TREATMENT	3	10	25	40	60	TOTAL
Control	175	95	95	115	88	568
50 ppm	194	134	138	141	103	710
100 ppm	224	150	154	142	102	772+
100 ppm+$(NH_4)_2SO_4$	157	94	75	46	17	389
100 ppm+GN Oil Cake	2089+	1706+	306+	145	83	4329+
TOTAL	2839	2179	768	589	393	

SEm+/- = 92.25 $CD_{0.05}$ for treatment= 258.03
$CD_{0.05}$ for soil= 295.77 $CD_{0.05}$ for interval= 258.03
$CD_{0.05}$ for treatment x interval interaction = 197.0

'+' indicates significant increase over control

degradation when compared to unamended soils with the same amount of phorate. Therefore, it seems likely that this Pseudomonas sp. is responsible for the degradation of phorate. In an earlier study, Ahmed and Casida (1958) reported that while the yeast Torulopsis utilis and the green alga Chlorella pyrenoidosa were able to degrade phorate in pure culture, the bacteria Pseudomonas fluorescens and Thiobicillus thiooxidans were not able to oxidise the pesticide but were able to hydrolyse it.

In the present study it can be seen that after ten to twenty days the populations of Pseudomonas dropped drastically in the groundnut oil cake amended soils and there was a rapid increase in the level of phorate sulfoxide indicating the toxicity of this metabolite to the bacterium.

The data presented here strongly suggest that the Pseudomonas sp. isolated is able to degrade phorate in the soil and the major metabolite is phorate sulfoxide. Further the effects of the amendments on this bacterium are reflected on the degradation of phorate in soils having these amendments.

Acknowledgements: MVR would like to thank Dr. J.C. Edward for his helpful discussions and Prof. M.V. Nayudu, Botany dept., Sri Venkateswara University, Tirupati for his help.

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Received 26 June, 1986; Accepted 24 August, 1986