Regd.

D.O.No. 3199

Dr. N.Sethunathan,
Soil Microbiologist.

Central Rice Research Institute
Cuttack-6, (Orissa).

Dated the 4th March, 1978.

Dear Dr. Murty,

Please refer to your letter of 14.2.1978 concerning the progress report of IAEA projects. IAEA supported the following research projects in the Soil Microbiology Section.

i) Isotope -tracer aided studies on the fate and significance of insecticides used for the control of rice pests under conditions of Indian Agriculture (under Dr. N.Sethunathan) from 1972-1976.

Project No.1179

ii) Pesticide - soil microflora interactions in aquatic and soil environments with special emphasis to rice and paddy ecosystems (under Dr. N.Sethunathan) from October 1,1977 to date.

Project No.2089 - New Programme

iii) Nitrogen fixation by free-living microorganisms in tropical rice soils (under Dr. V.Rajaramamohan Rao) from August 1976 to date.
Project No.1804.

The first project terminated in 1976 and the progress reports for this project as published by IAEA are enclosed. IAEA started a new 'L' programme on pesticide-soil biota interactions in 1977 and under this new 'L' programme, the second project started functioning on October 1, 1977 which hopefully will continue for 3 or 4 years.

As a part of this new programme we have initiated studies toward developing ^{14}C-labelled substrate techniques (for eg. very dilute ^{14}C-glucose, followed by $^{14}CO_2$ assay) as an indicator of soil capacity for desirable transformations and residue degradation in predominantly anaerobic flooded soil system. It is too early to report on the progress of this new project yet; we hope to gather useful information on the above aspect within 6 months. The third project (on nitrogen fixation) started functioning from August,1976 and the progress report of this project is also attached herewith.

^{14}C-labelled pesticides are not available in our country. Collaboration with IAEA assisted us in initiating studies on pesticide residue problems in rice ecosystem in India for the first time with the help of ^{14}C-labelled pesticides supplied by IAEA under the contract. Likewise, IAEA provided N 15-analytical facilities in its laboratory at Vienna facilitating the analysis of N 15 samples from our nitrogen-fixation programme.

We hope that continued collaboration with IAEA in the above programmes would contribute toward a better understanding of pesticide-residue problems and nitrogen fixation in rice soils under tropical conditions.

With kind regards,

Yours sincerely,

Sethunathan

(N.Sethunathan)

Dr. B.R.Murty,
Project Director,
Nuclear Research Laboratory,
IARI, New Delni-12.

iii)

PROGRESS REPORT OF PROJECT NO. 1804

Nitrogen fixation by free-living microorganisms in tropical rice soils (Dr. V. Rajarama Mohan Rao: Principal Investigator)

During previous year nitrogen-fixing organism and their nitrogen-fixing efficiency was studied. Also, the influence of rice straw amended to soil at two moisture levels on the nitrogen fixation and population of different nitrogen-fixing organisms was studied. The occurrence of symbiotrophic nitrogen fixing association and nitrogen fixing Spirillum in Indian rice soils of varying characteristics was reported for the first time.

During the year under report nitrogen-fixing activity of various Indian rice soils as influenced by organic amendments and water regimes, and pesticides was demonstrated employing Kjeldahls' method and N-15 techniques. Further investigations on factors influencing nitrogen fixation by Spirillum were carried out. The results are summarized below :

Nitrogen fixation in rice soils

Alluvial, laterite, acid saline and two unique acid sulphate saline soils pokkali and kari from Kerala were used in the present study. Some characteristics of the five soils used in this study are listed in Table-1. One set of the soil samples placed in small vials was flooded with water and the other set was held moist (maintained at 50% water holding capacity WHC) representing flooded and nonflooded conditions respectively. The flooded and nonflooded soils were amended with (w/w) 0.5% rice straw, 0.5% glucose 0.5 and 1.0% malate to study the influence of organic substrates on nitrogen fixation. The soil samples were then transferred to a desiccator wrapped with black paper to prevent algal growth. The desiccator was then sealed and the air was evacuated by vacuum and then filled with the premixed gas phase containing N_2: Ar (30:70). The soil samples were thus incubated under anaerobic condition for a period of 30 days at 30°C in dark. Initial and final nitrogen content of triplicate soil samples was determined following microKjeldahl method. Nitrogen fixation in soils was calculated by the differential analysis of the initial and final nitrogen content.

For heterotrophic nitrogen fixation by free-living bacteria available carbon substrate is an essential prerequisite, which is more often the major limiting factor in soils. Our data (table 2) show that soil submergence accelerated the indigenous nitrogen fixation, at least, in alluvial and laterite soils. However, in acid sulphate saline kari and pokkali soils more nitrogen fixation occurred under nonflooded conditions. Highest indigenous nitrogen fixation was noticed in a laterite soil under both flooded and nonflooded conditions.

Under flooded conditions, addition of rice straw stimulated nitrogen fixation only in kari soil as compared to the effect of glucose in enhancing nitrogen fixation in laterite and pokkali soils. Malate addition led to an increase in nitrogen fixation in three soils viz., alluvial, pokkali and kari soils.

Under nonflooded conditions, application of rice straw, malate and glucose was effective in enhancing nitrogen fixation in alluvial and laterite soils (Table 2). In other soils addition of organic sources had no influence on nitrogen fixation. Our data demonstrate that organic sources differed as to their effect on the extent of nitrogen fixation depending on the moisture regime and soil type. The observed variation in the values of nitrogen fixation in soils might be due to the soil type and presence or absence of activate nitrogen-fixing microflora.

Appreciable amounts of nitrogen was fixed in the two unique acid sulphate saline kari and pokkali soils despite their high acidity and salinity. It is interesting to note that in these two soils greater nitrogen fixation was observed under nonflooded conditions indicating the role of aerobic and microaerophilic nitrogen-fixing organisms. Surprisingly, nitrogen fixation was considerably suppressed in an acid saline Karapadam soil, eventhough, the carbon substrates were provided. However, the indigenous N_2-fixing activity in this soil was appreciable under flooded conditions.

15-N studies

In another experiment employing the 15-N labelled nitrogen gas, the nitrogen fixation in alluvial soil has been determined. Soil samples contained in glass vials were waterlogged and then transferred to a desiccator wrapped with black paper to prevent algal growth. Evolved CO_2 was absorbed in 40% KOH placed in the desiccator at the beginning of the experiment. The desiccator was then sealed and the air evacuated by vacuum and then filled with the gas mixture nitrogen and Argon containing 58 atom% $^{15}N_2$. The system was then incubated at 28°C for 30 days. The soil samples after Kjeldahl digestion and distillation were subjected to 15-N analysis. In our studies atom % excess in soil samples was in the range of 0.664. Based on the data the net nitrogen fixation in this soil was about 10 kg N/ha/30 days. The experiments with other soils are in progress.

Effect of pesticides on nitrogen fixation in soil

The effect of certain commonly used pesticides on nitrogen fixation in flooded soils was studied. Technical grade Benomyl, carbofuran and BHC at 5 ppm level were applied to flooded soil system and incubated for a period of 30 days. Alluvial, laterite and acid-sulphate pokkali soils were used in the study. The preliminary results showed that application of carbofuran at 5 ppm greatly stimulated nitrogen fixation in laterite and alluvial soils (Table 3). Application of benomyl to above soils also stimulated nitrogen fixation. Nitrogen fixation was not stimulated in -BHC amended soils, instead a drastic suppression was evident. These results are being confirmed employing 15-N technique.

Studies on nitrogen fixing Spirillum from rice soils

The widespread occurrence of nitrogen-fixing *Spirillum* sp. in various soils was reported during last year. Further studies on this interesting organism were made during this year. The dynamics of nitrogen fixation by *Spirillum* isolates as influenced by flooding and rice straw amendment on different days of incubation was investigated. A decrease in the nitrogen-fixing potential was noticed (Table 4) in cultures obtained from soils incubated for prolonged periods under both flooded and nonflooded conditions. Most active and effective nitrogen fixation was noticed in cultures isolated from soils incubated for 5 days. High nitrogen fixation was noticed in cultures originating from rice straw-amended soils under flooded conditions, excepting the cultures from an extremely acid sulphate saline kari soil. Enrichment cultures obtained from rice straw-amended nonflooded alluvial and laterite soils exhibited greater nitrogen fixing efficiency than cultures from unamended soils. The cultures from limed kari soil exhibited higher nitrogen fixing potential than cultures from unamended soil under nonflooded conditions. These results suggest that *Spirillum* isolates from various soil types widely differ in their nitrogen-fixing potential. Despite acidic and saline conditions in acid sulphate soils, pokkali and kari, the isolates possessed appreciable nitrogen fixing activity indicating their acid and salt tolerance. Sensitivity of *Spirillum* to prolonged moisture regime is another interesting feature of these potential nitrogen-fixing organisms. Also certain soil amendments

yielded cultures with increased nitrogen-fixing ability.

Population of Spirillum in soils

A method for counting the Spirillum from soils has been standardized. Serial 10 - fold dilutions were prepared from the soil samples and 1-ml of each dilution was inoculated to the semi-solid malate medium in 5 replicate tubes. Positive identification of Spirillum sp. was recorded when there was the formation of typical white pellicle of few mm below the surface of the semi-solid malate medium within 24 h at 30°C. Microscopic examination of the pellicle revealed the most characteristic forms with curved rods of varying sizes containing refractive lipid bodies. The cells were extremely active with a typical spiral movement. Most probable numbers were calculated by using the probability tables.

Employing the above method, the population of Spirillum sp. was estimated in different soil types under rice cultivation and the data on the effect of rice straw amendment and water regime on the population density are presented in Table 5. All the soil types investigated contained this organism with maximum numbers in laterite soil and minimum in an extremely acid sulphate saline kari soil. Rice straw amendment to flooded alluvial laterite and acid sulphate pokkali soils considerable enhanced the population of Spirillum sp. In contrast, rice straw amendment to kari soil had no effect on Spirillum population, at least, in soils incubated for 5 days. The population of Spirillum sp. greatly increased in this soil following liming.

Spirillum sp. from rice roots

The associate symbiosis of Spirillum sp. with rice roots has been established in our studies. In a field experiment a high yielding, semi dwarf rice cultivar (CR1.13-3241) was grown during the dry season under irrigated conditions with 0, 20, 40, 60, 80 and 100 kg N/ha applied as ammonium sulphate. Fresh roots from respective treatments, collected at different stages of crop growth, were thoroughly washed under running tap water and cut into pieces of 0.5-1.0 cm which were placed in 20 ml sterile improved semi-solid malate medium for the isolation of Spirillum sp. The culture was subsequently transferred (3-4 subcultures) to fresh semi-solid malate medium. Nitrogen fixation in these multiple-transfer cultures was estimated. Spirillum sp. isolated from fresh roots receiving no fertilizer exhibited an overall low efficiency in terms of nitrogen fixation (Table 6). From such roots, a gradual decrease in the nitrogen-fixing efficiency with respect to plant age was evident in cultures isolated at different stages of plant growth. However cultures isolated from roots receiving combined nitrogen, in general, showed greater efficiency. The highest doses of combined nitrogen did, however, suppress the nitrogen fixing potential of Spirillum sp. Thus, Spirillum sp. isolated from roots which received above 60 kg N/ha were less efficient, throughout the growth of the rice plant, than cultures isolated from roots fertilized with lower levels of combined nitrogen.

Effect of soil redox potential (Eh) and pH on the enrichment culture activity

In order to determine whether N_2-fixing efficiency of Spirillum cultures in malate medium was related to the (Eh) redox potential and the pH of the soil samples prevailing at the time of transfer, Eh of the soil samples after appropriate amendments and incubation (5, 10, 20, and 40 days) was measured with a portable redox meter model RM-1F (TOA Electronics Ltd., Tokyo, Japan) fitted with a compound platinum and calomel electrode type GC-211. After Eh measurements, the pH of the soil sample was determined. There appeared to be a certain degree of relation between soil pH and the N_2-fixing activity of enrichment cultures obtained from respective soils. Thus, enrichment cultures originating from soils with higher pH values exhibited greater N_2 fixation, with the exception of cultures from flooded alluvial soil with pH above 7.0 (Table 7).

A relationship existed between the in vitro N_2-fixing efficiency of Spirillum enrichment cultures in malate medium and the redox status of the soil samples at the time of transfer to malate medium for enrichment. Enrichment cultures originating from flooded soils of relatively low potentials (-50 to -150 mV), showed higher N_2 fixing efficiency in vitro; cultures isolated from soils with higher potentials were less efficient with respect to N_2 fixing activity. For example, Pokkali soil characterized by more rapid decline in potentials upon flooding as compared to other soils harboured Spirillum cultures possessing highest N_2-fixing activity. The soil potentials decreased with increasing incubation under flooded conditions. Again, the increased efficiency of cultures from rice straw amended alluvial laterite and Pokkali soils than in unamended soils was apparently related to the accelerated drop in potential by rice straw in flooded soils.

With regard to the unique acid sulphate saline kari soil no such relation seems to exist. Highest N_2-fixing activity was noticed in cultures obtained from soils having Eh values of +100 mV. Rice straw amendment to this soil did not appreciably lower the Eh potential and also the enrichment cultures from amended soil exhibited lower N_2-fixing activity than the cultures from unamended soil. The pronounced effect of rice straw in lowering the Eh of the soils and the occurrence of Spirillum sp. with high N_2 fixing activity under such situations indicate the major role of soil amendments in Spirillum mediated N_2 fixation in natural environments.

Publications/Symposia

1. Charyulu, P.B.B.N., C.Ramakrishna and V.Rajaramamohan Rao (1977). Nitrogen fixation by Spirillum from rice soils as influenced by rice straw and moisture content. Presented at the 17th Annual Conference of Association of Microbiologists of India, Madurai.

2. Rao, V.R. (1977). Effect of organic and mineral fertilizers on Azotobacter in flooded rice fields. Curr. Sci. 46: 118-119.

3. Rajaramamohan Rao, V. Charyulu, P.B.B.N., Nayak, D.N., and Ramakrishna, C., (1977). Nitrogen fixation by free-living organisms in tropical rice soils. Presented at International Symposium and conference at the limitations and potentials or the Biological nitrogen fixation: Brasilia, Brazil.S. America. July 18-21 (in press).

4. Nayak, D.N., and V.Rajaramamohan Rao (1977). Nitrogen fixation by Spirillum sp from rice roots. Archievs of Microbiology ~~(in press)~~ 153, 359.

5. Rajaramamohan Rao, V. (1978). Nitrogen economy of rice soils in relation to nitrogen fixation by heterotrophic microorganisms. Review article. National symposium on increasing rice yield inkharif.

Communicated to Journals

6. Charyulu, P.B.B.N., Ramakrishna, C. and Rajaramamohan Rao, V. Occurrence of nitrogen fixing Spirillum sp. in Indian rice soils. Sent to OIKOS for publication.

7. Charyulu, P.B.B.N., Ramakrishna, C. and Rajaramamohan Rao, V. Occurrence of symbiotrophic nitrogen-fixing associations in Indian rice soils. Sent to Soil Biol. Biochem. for publication.

8. Charyulu, P.B.B.N., Rajaramamohan Rao, V. Heterotrophic nitrogen fixation in rice soils as influenced by carbon substrates and moisture content. Sent to Il Riso for publication.

9. Charyulu, P.B.B.N., and Rajaramamohan Rao V. Nitrogen fixation in enrichment cultures S
soil. Sent to Can. J.

Table 1 : Characteristics of the soils

Characteristics	Alluvial	Laterite	Acid sulphate saline (Pokkali)	Acid sulphate saline (Kari)	Acid saline (Karapadam)
pH[a]	6.2	5.2	4.2	3.0	5.0
Organic matter (%)	1.61	3.25	8.21	27.81	7.4
Total nitrogen (%)	0.09	0.09	0.24	0.36	0.24
Electrical conductivity (M mhos/cm)[b]	0.6	0.2	8.5	15.0	6.3

[a] measured as 1:1.25 soil-water suspension

[b] Electrical conductivity of saturation extract of the soil determined in conductivity meter.

Table 2 : Influence of carbon substrates on nitrogen fixation in rice soils under flooded and nonflooded conditions

Soil type	amendment		mg of nitrogen fixed/g soil/30 days	
			Flooded condition	Nonflooded condition
Alluvial	-		128	12
	+ rice straw	(0.5%)	127	102
	+ glucose	(0.5%)	110	112
	+ malate	(0.5%)	193	110
	+ malate	(1.0%)	198	nd
Laterite	-		1085	870
	+ rice straw	(0.5%)	1090	1220
	+ glucose	(0.5%)	1335	1090
	+ malate	(0.5%)	997	1185
	+ malate	(1.0%)	1090	nd
Acid sulphate saline (Pokkali)	-		163	437
	+ rice straw	(0.5%)	140	320
	+ glucose	(0.5%)	584	448
	+ malate	(0.5%)	268	455
	+ malate	(1.0%)	705	nd
Acid sulphate saline (kari)	-		70	292
	+ rice straw	(0.5%)	303	293
	+ glucose	(0.5%)	72	270
	+ malate	(0.5%)	110	163
	+ malate	(1.0%)	-	nd
Acid saline (Karapadam)	-		105	-
	+ rice straw	(0.5%)	5	-
	+ glucose	(0.5%)	-	17
	+ malate	(0.5%)	35	-

- = No nitrogen fixation; nd = not determined.

Table 5 : Spirillum population in flooded rice soils

Soil type	- Population x 10^{-4}/g soil Period of incubation(days) 5	10
Alluvial	92	5.4
Alluvial + RS*	160	9.2
Laterite	160	160
Laterite + RS*	230	160
Acid sulfate saline (Pokkali)	92	160
Acid sulfate saline (Pokkali) + RS*	250	160
Acid sulfate saline (Kari)	0.002	0.07
Acid sulfate saline 'Kari) + RS*	0.002	0.024
Acid sulfate saline - 1 **	22	0.2

RS* - Soils amended with 0.5% (w/w) rice straw

1** - Soil limed with 0.6 g $CaCO_3$/20 g soil

Population was estimated by MPN method.

Table 6 : Nitrogen fixing efficiency of Spirillum sp. isolated from roots of rice plants fertilized with different levels of combined nitrogen.

Combined nitrogen applied (kg/ha)	mg of nitrogen fixed/g of malate by cultures of Spirillum sp. Age of the plant(days) 40	55	65	78
0	3.92	1.68	1.38	1.10
20	3.98	2.38	5.96	2.38
40	3.17	4.91	5.00	3.47
60	2.24	4.34	3.10	3.61
80	3.10	2.95	2.66	1.79
100	2.80	3.48	1 30	2.38

Table 7 : Nitrogen-fixing activity of Spirillum enrichment cultures from rice soils

Soil type	Condition of incubation (10 days)	pH of soil	Enrichment culture activity
Acid sulphate saline (Kari)	Nonflooded	3.2	507
	Flooded	3.3	347
Acid sulphate saline (Pokkali)	Nonflooded	5.8	850
	Flooded	6.8	688
Laterite	Nonflooded	6.5	504
	Flooded	6.6	497
Alluvial	Nonflooded	6.2	406
	Flooded	7.2	256

PROGRESS REPORT OF THE PROJECT NO. 1179

Please refer to our publications listed at the end of this article for more details.

Reprint from

"TRACE CONTAMINANTS OF
AGRICULTURE, FISHERIES AND FOOD
IN DEVELOPING COUNTRIES"

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1976

PESTICIDE-SOIL MICROFLORA INTERACTIONS IN FLOODED RICE SOILS

N. SETHUNATHAN, R. SIDDARAMAPPA,
T.K. SIDDARAME GOWDA, K.P. RAJARAM,
S. BARIK, V.R. RAO
Central Rice Research Institute,
Cuttack, Orissa,
India

Abstract

PESTICIDE-SOIL MICROFLORA INTERACTIONS IN FLOODED RICE SOILS.

Isotope studies revealed that gamma and beta isomers of HCH (hexachlorocyclohexane) decomposed rapidly in nonsterile soils capable of attaining redox potentials of −40 to −100 mV within 20 days after flooding. Degradation was slow, however, in soils low in organic matter and in soils with extremely low pH and positive potentials, even after several weeks of flooding. Under flooded conditions, endrin decomposed to six metabolites in most soils. There is evidence that biological hydrolysis of parathion is more widespread than hitherto believed, particularly under flooded soil conditions. Applications of benomyl (fungicide) to a simulated-oxidized zone of flooded soils favoured heterotrophic nitrification.

INTRODUCTION

Insect pests seriously limit the yields of rice, a vital food crop in India and other Asian countries. In recent years, there has been a steady increase in the use of pesticides in agriculture. In India, insecticides constitute 66% of all the pesticides used in agriculture in terms of quantity. Despite the widespread use of commercial formulations of parathion, HCH isomers and endrin for controlling common rice insects, little is known about the fate of these insecticides in soils under conditions of Indian agriculture. Isotope studies were initiated to determine the fate and significance of these insecticides in Indian rice soils under flooded conditions. Most of the work summarized in this paper refers to the studies with carbon-labelled insecticides. In the experiments concerning parathion hydrolysis in flooded soils, nonlabelled parathion was used and the insecticide was quantitated colorimetrically after its alkaline hydrolysis to *p*-nitrophenol. Also, the effect of a fungicide, benomyl, on heterotrophic nitrification in a simulated oxidized zone of a flooded soil was studied.

1. HEXACHLOROCYCLOHEXANE (HCH)

HCH (also known as BHC – benzene hexachloride) exhibits extreme stability in nonflooded soils and other aerobic environments and is used increasingly in Indian agriculture because the raw material needed for HCH production is in surplus. Commercial formulations of HCH generally contain the alpha, gamma, beta and delta isomers of which the gamma isomer is the most insecticidal and the beta isomer the most persistent. Entry of beta-HCH into the food chain has caused concern in Japan leading to the restricted use of HCH formulations in agriculture. Isotope studies in our laboratory [1] revealed that both gamma and beta isomers of HCH decomposed rapidly in alluvial and laterite soils and in a unique acid sulphate, saline soil of Kerala, India,

TABLE I. DEGRADATION OF [^{14}C] GAMMA AND BETA ISOMERS OF HCH[a] IN DIFFERENT SOILS UNDER FLOODED CONDITIONS AS A FUNCTION OF REDOX POTENTIAL

Incubation (days)	Soils				
	Alluvial	Laterite	Pokkali	Kari	Sandy
	Gamma-HCH[b] recovered (counts/min X 10^4/20 g soil)				
0	51.7 (57.5)	52.2 (59.1)	44.9 (49.9)	43.3 (45.1)	49.4 (55.6)
20	2.3 (12.4)	19.4 (28.1)	25.3 (31.3)	31.5 (32.0)	47.2 (52.9)
41	0.8 (2.9)	1.3 (8.2)	2.1 (8.6)	24.8 (25.0)	42.4 (44.4)
	Beta-HCH[b] recovered (counts/min X 10^4/20 g soil)				
0	71.8 (75.9)	75.6 (81.6)	63.4 (66.1)	49.6 (50.7)	79.9 (82.6)
20	16.2 (25.1)	45.2 (62.3)	58.9 (62.0)	42.0 (42.4)	73.5 (79.9)
41	1.9 (4.3)	2.9 (11.4)	20.6 (23.1)	36.0 (36.7)	65.5 (70.8)
	Redox potentials (mV)				
0	+ 235	+ 255	+ 250	+ 380	+ 220
20	− 120	− 50	− 80	+ 160	+ 150
41	− 145	− 75	− 100	+ 165	+ 45

[a] Gamma and beta isomers were applied to the soils in ethanol.
[b] Gamma and beta isomers recovered after separation of residues in chloroform-diethyl ether fraction. Figures in parenthesis represent total radioactivity recovered in the chloroform-diethyl ether fraction.

locally known as pokkali, under flooded conditions (Table I). The degradation rate of these isomers was considerably retarded in the autoclaved samples of these soils, thus indicating microbial participation in their rapid loss. By contrast, gamma- and beta-HCH persisted in a sandy soil and in another acid sulphate soil of Kerala, locally known as kari, even after 41 days of flooding.

The extent of degradation of gamma- and beta-HCH in different soils was related to the redox potentials (Eh) attained by the soils following flooding [1]. In alluvial, laterite and pokkali soils possessing exceptional capacity to decompose both HCH isomers, the redox potentials declined to values of −50 to −145 mV within 20 days of flooding. The low Eh associated with HCH degradation in flooded nonsterile soils supports the reported role of anaerobic microorganisms in their degradation [2]. On the other hand, the highly oxidized conditions in sandy (+45 mV) and kari (+165 mV) soils, even after 41 days of flooding, prevented the degradation of HCH isomers in these soils. Moreover, microbial activity in these soils is expected to be low because of the low content of organic matter in sandy soil and the highly acidic conditions in kari soil. However, liming the kari soil was not effective in enhancing the degradation of the gamma and beta isomers despite favourable conditions of pH (near neutral) and Eh (negative potentials) for microbial activity in limed soils. Apparently, this unique acid sulphate soil lacked appropriate microorganisms involved in HCH degradation.

The data demonstrate that the gamma and beta isomers are unlikely to cause serious soil residue problems in microbially active soils capable of attaining Eh of −40 to −100 mV within days after flooding. Moreover, the addition of rice straw – a common cultural practice in rice culture (Table II) – or the incorporation of HCH in a carrier such as ethanol [1] would further accelerate the Eh drop because of simultaneously enhanced microbiological activity and this in turn would favour degradation of the insecticide.

TABLE II. EFFECT OF RICE STRAW ON THE DEGRADATION OF [^{14}C]-GAMMA-HCH IN ALLUVIAL SOIL UNDER FLOODED CONDITIONS

Incubation (days)	Rice-straw-amended soil		Unamended soils	
	Eh (mV)	counts/min X 10^4/20 g soil Gamma-HCH[a]	Eh (mV)	counts/min X 10^4/20 g soil Gamma-HCH[a]
0	+ 205	2.3 (3.5)	+ 220	1.8 (2.8)
3	+ 135	2.0 (3.5)	+ 160	2.2 (3.7)
7	− 5	1.0 (1.6)	+ 90	2.1 (2.6)
11	− 40	0.8 (1.6)	+ 90	2.1 (2.6)
20	− 160	0.1 (0.3)	− 90	2.0 (2.1)
30	− 160	Trace (0.1)	− 100	0.4 (0.7)

[a] Gamma-HCH was applied to the soils in aqueous solution.
Figures in parenthesis represent radioactivity recovered in the chloroform-diethyl ether fraction.

TABLE III. VOLATILIZATION OF [^{14}C]-GAMMA-HCH FROM WATER OF FLOODED, NONFLOODED AND SOIL-FREE SYSTEMS

Incubation (days)	Open vials				Sealed vials			
	Soil-free water	Flooded		Nonflooded alluvial	Soil-free water	Flooded		Nonflooded water
		Alluvial	Sandy			Alluvial	Sandy	
	Radioactivity recovered (counts/min X 10^4 vial)							
0	15.9	15.0	15.4	13.6	15.9	15.0	15.4	13.7
5	4.5	6.8	4.4	11.3	15.1	13.1	14.9	13.0
	Water evaporated (g)							
5	12.9 (49)	11.8 (29)	11.4 (29)	3.7 (4)	0 (49)	0 (29)	0 (29)	0 (4)

Figures in parenthesis represent the amount of water (ml) initially added.

Also of interest to tropical agriculture is the observation that substantial loss of gamma-HCH can occur by volatilization from the standing water of flooded soils. Thus, gamma-HCH was lost more rapidly from flooded soils contained in open vials than from those in closed vials during 5-day incubation (Table III); this indicates volatilization. Such volatile loss of gamma-HCH, before the flooded soils attain negative potentials, would render a portion of the applied insecticide unavailable for anaerobic biodegradation processes in the soil.

2. ENDRIN

The fate of endrin in eight Indian rice soils was investigated under flooded conditions by means of radiotracer techniques. Some of the characteristics of the soils used in the studies on HCH and endrin persistence are given in Table IV. Endrin decomposed rapidly in most soils except in a sandy soil (Table V). Interestingly, the most rapid degradation of endrin occurred in pokkali soil

TABLE IV. CHARACTERISTICS OF THE SOILS USED IN THE EXPERIMENTS

Characteristics	Soils							
	Alluvial (1)	Alluvial (2)	Laterite	Laterite clay loam	Red loam	Acid sulphate, saline (pokkali)[a]	Acid sulphate, saline (kari)[b]	Sandy
Location	Central Rice Research Institute, Cuttack-6, Orissa	Sakhigopal, Orissa	Pattambi, Kerala	Bastar, Madhya Pradesh	Chiplima, Orissa	Tellichery, Kerala	Vechoor, Kerala	Badagara, Kerala
pH[c]	6.2	4.8	5.0	5.0	5.9	4.2	3.0	6.0
Electrical conductivity[d]	0.6	1.80	0.2	0.81	0.46	8.5	15.0	0.4
Organic matter (%)	1.61	2.38	3.25	1.24	1.89	8.21	27.81	0.02
Total nitrogen (%)	0.09	0.10	0.09	0.09	0.06	0.24	0.36	0.002

[a,b] These unique acid sulphate, saline-rice soils are known by the local names pokkali and kari.

[c] A 1 : 1.25 soil-water suspension was used for the pH measurements.

[d] Electrical conductivity (mmho/cm) of saturation extract of the soil was determined in a conductivity meter.

TABLE V. PERSISTENCE OF ENDRIN IN DIFFERENT RICE SOILS UNDER FLOODED CONDITIONS

Incubation (days)	Endrin recovered[a] (counts/min × 10^4/20 g soil)							
	Alluvial (1)	Alluvial (2)	Laterite	Laterite clay loam	Red loam	Pokkali (acid sulphate, saline)	Kari (acid sulphate, saline)	Sandy
0	48.8 (68.7)	40.7 (69.0)	48.3 (65.0)	68.8 (102.4)	58.3 (90.1)	35.2 (59.2)	39.0 (58.1)	67.5 (100.3)
25	32.6 (61.4)	16.2 (54.4)	20.4 (73.7)	44.7 (70.6)	58.1 (67.9)	8.4 (29.8)	28.6 (40.8)	58.1 (79.5)
55	5.3 (41.4)	2.3 (41.0)	2.4 (36.5)	23.4 (55.4)	20.2 (48.9)	2.2 (33.6)	12.0 (28.7)	41.5 (68.6)

[a] [^{14}C]-endrin recovered by TLC from the spots with R_f values identical to analytical-grade reference compound. Figures in parenthesis represent total radioactivity recovered in the chloroform-diethyl ether phase.

TABLE VI. RECOVERY OF PARATHION AND ITS METABOLITES AFTER APPLICATION OF [^{14}C]-PARATHION TO FLOODED RICE-STRAW-AMENDED AND UNAMENDED ALLUVIAL SOILS

Incubation (days)	Radioactivity recovered[a]											
	Rice-straw-amended soil						Unamended soil					
	Parathion		Aminoparathion		Unidentified (R_f 0,21) metabolite[b]		Parathion		Aminoparathion		Unidentified (R_f, 0.1) metabolite[c]	
	Counts/min[d]	%	Counts/min[d]	%	Counts/min[d]	%	Counts/min[d]	%	Counts/min[d]	%	Counts/min[d]	%
0	10500	100.0	–	–	–	–	9500	100.0	–	–	–	–
3	1130	10.8	1000	9.5	860	8.2	5700	60.0	–	–	–	–
7	510	4.9	370	3.5	1920	18.3	4860	51.1	110	1.1	340	3.6
14	260	2.5	60	0.6	–	–	180	1.9	20	0.2	2300	24.2
28	130	1.2	10	0.1	–	–	150	1.6	10	0.1	–	–

[a] Residues in the solvent extract of the soils were analysed for intact parathion after separation by TLC. Radioactivity recovered at the start was considered as 100% to compare the relative loss of radioactivity at subsequent samplings.

[b] Unidentified metabolite (R_f, 0.21) was not detected in unamended soil; in rice-straw-amended soil, radioassay for unidentified metabolite was made only for 3 and 7 days.

[c] Unidentified metabolite (R_f, 0.01) was detected as a polar metabolite only in unamended soil; analysis was made only at 7 and 14 days.

[d] Counts/min per 20 g soil recovered.

despite its high salt content [3]. Although in most soils endrin declined to low levels in 55 days, the decrease in the total radioactivity partitioned in the solvent fraction was not as striking as that of endrin, thus indicating the stability of endrin metabolites. Radioautography revealed that endrin was converted to six metabolites in most soils within 25 days except in sandy and kari soils; but, these metabolites persisted even after 55 days. Three compounds were detected in sandy soil and four compounds were formed in kari soil. Studies also revealed that the degradation rate of endrin in laterite, alluvial and pokkali soils was retarded considerably by autoclaving; this indicates microbial participation. Moreover, endrin was converted to six metabolites in nonsterile samples of these soils as compared to three compounds in sterile soils. These breakdown products of endrin formed by chemical and biological processes appeared to persist even after 55 days.

3. PARATHION

The degradation of parathion in flooded soils proceeds by nitro-group reduction to amino-parathion and by hydrolysis at the P-O-C linkage to *p*-nitrophenol and diethylthiophosphoric acid. Microorganisms are involved in both reactions. Contrary to the common belief that nitro-group reduction is the major pathway of microbial metabolism of parathion in soil, water and pure cultures, rapid hydrolysis of parathion by biological action occurred in several soils under flooded conditions, particularly after repeated applications [4]. Also, bacteria capable of hydrolysing parathion and degrading its hydrolysis product, *p*-nitrophenol and other nitrophenols, have been isolated from parathion-amended flooded soils and then characterized. A *Pseudomonas* sp. hydrolysed parathion and then liberated nitrite from *p*-nitrophenol [5]. Another *Pseudomonas* sp. was unable to hydrolyse parathion, but it converted *p*-nitrophenol to an intermediate, 4-nitro-catechol, which persisted in pure cultures but not in soils. A *Bacillus* sp. liberated nitrite from *p*-nitrophenol and not from intact parathion. Interestingly, the same bacterium also produced nitrite from *m*-nitrophenol which is known for its extreme resistance to biodegradation.

The effect of organic matter on parathion degradation in flooded soils depended on the pathway involved. Thus, the addition of organic matter enhanced the nitro-group reduction of parathion to aminoparathion and an unidentified metabolite possessing a P = S bond and an ethoxy side chain (Table VI). In contrast, organic substrate inhibited the biological hydrolysis of parathion in flooded soils inoculated with parathion-hydrolysing enrichment culture [6]. Studies revealed that a water-extractable and heat-resistant factor that inhibited the biological hydrolysis of parathion developed in rice-straw-amended soil under flooded conditions; but in amended soils incubated at 25, 50 and 75% water-holding capacity (WHC), the factor failed to develop (Table VII). Molecular oxygen provided by stirring the flooded soils (Table VII) and potassium nitrate (Table VIII) retarded the formation of the toxic factor. Both free oxygen and potassium nitrate maintain the potentials of a flooded soil at a high level. These results indicate that a factor that inhibits the biological hydrolysis of parathion accumulates and persists during anaerobic decomposition of rice straw in flooded soils. The inhibition of parathion hydrolysis by organic matter is unlikely to cause any residue hazard since nitro-group reduction is enhanced at the same time.

4. HETEROTROPHIC NITRIFICATION IN BENOMYL-AMENDED SOILS

In a study on benomyl-soil microflora interactions in a simulated oxidized zone of a flooded soil, application of 5000 ppm of benomyl together with ammonium sulphate increased bacterial numbers in an alluvial soil. Nitrite was produced from the ammonium in benomyl-amended soil, though in small amounts. Nitrite disappeared later; but nitrate was not detected. No nitrite was detected in unamended soil. Also, the predominant bacterium, isolated from benomyl-amended soil and identified as a *Pseudomonas* sp., oxidized the ammonium to nitrite in a medium containing

TABLE VII. INHIBITION OF BIOLOGICAL HYDROLYSIS OF PARATHION BY WATER EXTRACTS OF RICE-STRAW-AMENDED ALLUVIAL SOIL INCUBATED UNDER DIFFERENT WATER REGIMES

Incubation after inoculation[a] (h)	Water content, % WHC												Flooded						Unamended		
	25			50			75			100			Still			Shaking					
	P[b]	PN	N	P	PN	N	P	PN	N	P	PN	N	P	PN	N	P	PN	N	P	PN	N
36	107	10	1.0	105	7	1.0	132	0	0	134	0	0	132	0	0	99	8	t	122	3	t
48	60	13	2.0	82	9	1.9	113	6	t	128	1	0	132	0	0	71	11	1.5	97	5	1.1
60	0	3	4.9	40	19	3.4	23	29	1.3	109	3	t	120	0	0	36	19	4.3	44	14	3.9
84	0	2	4.8	0	4	3.8	0	4	3.7	105	3	t	116	0	0	23	2	3.0	4	1	5.1

[a] Water extracts of soils were inoculated with parathion-hydrolysing enrichment culture.
[b] Initial quantity of parathion recovered, 134 μg/7 ml incubation mixture.

P Parathion, μg/7 ml incubation mixture.
PN *p*-Nitrophenol, μg/7 ml of incubation mixture.
N Nitrite-N, μg/7 ml of incubation mixture.
t Traces.

TABLE VIII. REVERSAL OF INHIBITORY EFFECT OF RICE STRAW ON THE HYDROLYSIS OF PARATHION BY NITROGEN SOURCES

Treatment	μg/20 g soil recovered:			
	2 days after inoculation[a]		3 days after inoculation[a]	
	Parathion[b]	*p*-Nitrophenol	Parathion	*p*-Nitrophenol
Rice straw	370	23	176	33
Rice straw + KNO_3	228	120	53	103
KNO_3	334	70	202	117
Unamended	211	145	15	224

[a] 2 and 3 days after inoculation represent 4 and 5 days after flooding respectively.
[b] Initial quantity of parathion recovered, 620 μg/20 g soil.

TABLE IX. FORMATION OF NITRITE FROM PEPTONE BY *Pseudomonas* sp. ISOLATED FROM BENOMYL-AMENDED SOIL

Medium +	μg nitrite/50 ml medium recovered		
	Incubation (days)		
	0	4	10
N-Serve	0	17	43
AM	0	20	66
Benomyl	0	9	27
Unamended	0	15	49

N-Serve, AM and benomyl were incorporated in the medium at 10 ppm in 0.1 ml ethanol.

glucose [7]. Moreover, oxidation of the ammonium to nitrite occurred in benomyl-amended soil as well as in bacterial cultures even in the presence of N-Serve or AM at concentrations known to inhibit autotrophic nitrification (Table IX). These studies revealed that benomyl applications to flooded soils stimulated the proliferation of heterotrophic nitrifying bacteria. Nitrification, in general, is not desirable in anaerobic environments, such as flooded soil, in view of the great instability of nitrite and nitrate and consequent loss of nitrogen in gaseous forms through denitrification. Moreover, intermediary products such as hydroxylamine, 1-nitrosoethanol and nitrite reported in heterotrophic nitrification by an *Arthrobacter* sp. are known to be toxic. Accumulation of such products even in small amounts in benomyl-amended soils, although not demonstrated in this case except for nitrite, may pose an indirect environmental pollution hazard in addition to the direct toxicity of the fungicide.

REFERENCES

[1] SIDDARAMAPPA, R., SETHUNATHAN, N., Persistence of gamma-BHC and beta-BHC in Indian rice soils under flooded conditions, Pestic. Sci. **6** 4 (1975) 395.

[2] SETHUNATHAN, N., Microbial degradation of insecticides in flooded soil and in anaerobic cultures, Residue Rev. **47** (1973) 143.

[3] GOWDA, T.K.S., SETHUNATHAN, N., Persistence of endrin in Indian rice soils under flooded conditions, ~~Submitted to~~ J. Agric. Food Chem. 24 (1976) 750.

[4] BARIK, S., SETHUNATHAN, N., Biological hydrolysis of parathion in aquatic environments, Unpublished data (1975).

[5] SIDDARAMAPPA, R., RAJARAM, K.P., SETHUNATHAN, N., Degradation of parathion by bacteria isolated from flooded soil, Appl. Microbiol. **26** (1973) 846.

[6] RAJARAM, K.P., SETHUNATHAN, N., Effect of organic sources on the degradation of parathion in flooded alluvial soil, Soil Sci. **119** (1975) 296.

[7] GOWDA, T.K.S., RAO, V.R., SETHUNATHAN, N., Heterotrophic nitrification in simulated oxidized zone of a flooded soil amended with benomyl, ~~Submitted to~~ Soil Sci. 123 (1977) 171.

Printed by the IAEA in Austria

PROGRESS REPORT OF THE PROJECT NO. 1179.
Please refer to ~~the~~ our (✓) articles listed at the end of this article for more details.

Reprint from

"ORIGIN AND FATE
OF CHEMICAL RESIDUES IN FOOD,
AGRICULTURE AND FISHERIES"

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1975

PERSISTENCE AND MICROBIAL DEGRADATION OF PARATHION IN INDIAN RICE SOILS UNDER FLOODED CONDITIONS

N. SETHUNATHAN, K.P. RAJARAM,
R. SIDDARAMAPPA
Department of Soil Microbiology,
Central Rice Research Institute,
Cuttack,
India

Abstract

PERSISTENCE AND MICROBIAL DEGRADATION OF PARATHION IN INDIAN RICE SOILS UNDER FLOODED CONDITIONS.

Parathion degradation in Indian rice soils under flooded conditions and the role of native or added organic matter have been studied. Parathion was rapidly hydrolysed after its repeated addition to an alluvial soil in contrast to the common pathway of reduction. A Pseudomonas sp., isolated from flooded alluvial soil by enrichment culture, readily hydrolysed parathion with the formation of nitrite from the hydrolysis product, p-nitrophenol. Organic matter amendments to flooded soil enhanced reduction of the nitro group of [^{14}C-ethoxy] parathion to aminoparathion and an unidentified metabolite with a P=S bond and ethoxy label. The hydrolytic pathway, on the other hand, was apparently inhibited by the presence of organic matter.

INTRODUCTION

High temperature and humidity, which are typical of the tropical environment, favour the proliferation of insects that are harmful to the rice plant. Furthermore, new management practices, such as the optimum use of fertilizers to exploit the high yield potential of recently developed nitrogen responsive rice varieties, also create environmental conditions that favour the build-up of harmful insects [1]. Therefore, the need for plant protection in India has assumed great significance with the recent widespread introduction of high yielding rice varieties. In India, insecticides constitute 66% of the usage of all pesticides in terms of volume and 80% in terms of value (Dhawan, C.L., Pesticides Association of India, unpublished). Folidol, a commercial formulation of parathion, is extensively used in India as a 0.04% foliar spray at 14-d intervals for the control of common rice pests. However, parathion sprayed on the foliage is often washed off by frequent showers during the monsoon when most rice is grown and thereby contaminates the soil environment.

Studies were initiated in our institute to follow the fate of parathion in certain Indian rice soils under flooded conditions and to determine the role of microorganisms in its degradation. The results of these experiments are summarized in this report. More details of this work together with the procedures used are reported elsewhere [2-5]. In the initial experiments, non-labelled parathion was used; subsequently, [^{14}C-ethoxy] parathion was employed for confirmation.

TABLE I. HALF-LIFE OF PARATHION IN FLOODED SOILS

Soil	pH		Organic matter (%)	Half-life (d)
	Initial	28 d after flooding		
Acid sulphate	3.15	3.65	12.2	8.32
Saline	3.15	4.10	5.9	10.83
Swampy	3.50	4.60	3.2	12.91
Laterite	5.25	6.70	0.95	25.45
Alluvial	5.50	6.40	0.8	10.85

TABLE II. RADIOACTIVITY RECOVERED AFTER APPLICATION OF [^{14}C]-PARATHION TO FLOODED ACID SULPHATE SOIL

Incubation (d)	Per cent radioactivity recovered			
	Water	Solvent	Soil	Total
0	0.5	81.3	10.6	82.4
1	0.5	84.1	13.2	97.8
5	0.2	68.2	20.9	89.3
10	0.2	65.0	32.2	97.4
20	0.2	29.4	31.3	61.3
30	0.5	16.1	45.6	62.2

DEGRADATION IN SOILS

The degree of parathion degradation in five Indian acid soils under flooded conditions depended on soil properties. The insecticide degraded faster in soils which had a higher organic matter content. Parathion degraded rapidly in acid sulphate soil (Table I) which had the highest organic matter content.

Isotope studies confirmed the rapid loss of parathion applied to flooded acid sulphate soil. [^{14}C-Ethoxy] parathion was applied to flooded acid sulphate soil and the residues were extracted with chloroform-diethyl ether at periodic intervals. Radioactivity in the various fractions is presented in Table II. Radioactivity in the solvent phase decreased with incubation. There was a net loss of about 38% radioactivity during 30 days of incubation. Analysis of the solvent extract by thin-layer chromatography (TLC) and subsequent counting confirmed that [^{14}C]-parathion was rapidly decomposed

after 10 days of flooding in acid sulphate soil. A radiochromatogram of the solvent extract revealed only parathion; no other radioactive metabolite was detected.

Parathion is chemically most stable at low pH values [16]. 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 sulphate, 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 sulphate soil was biological or chemical, more rapid degradation of parathion occurred in non-autoclaved soil than in autoclaved soil [3]. After 28 days of incubation, 62% 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 sulphate soil was caused by biological action although general microbiological activity is considered to be low in soils of extremely low pH. Recently, a fungus, Penicillium waksmani, isolated from flooded acid sulphate soil by enrichment culture technique, tolerated concentrations of parathion as high as 2500 ppm (Rao and Sethunathan, unpublished). Also, the insecticide was metabolized by the fungus.

ENHANCED BIOLOGICAL HYDROLYSIS IN ALLUVIAL SOIL AFTER REPEATED APPLICATIONS

It has been reported earlier [7] that diazinon is hydrolysed rapidly by a biological action in flooded rice fields, particularly after its repeated applications. Repeated applications of another organophosphate, parathion, to flooded alluvial soil likewise accelerated 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 [3]. The experiment was repeated and this phenomenon was noticed in two of the three replicates following the third addition. This indicated that parathion was hydrolysed rapidly in flooded soil following its repeated applications to p-nitrophenol.

To test whether the enhanced parathion hydrolysis was biological, autoclaved and non-autoclaved aliquots of the soil solution (enrichment culture) from a flooded alluvial soil that received three applications of parathion were added to alluvial soil amended with parathion. Within 48 h of incubation, the standing water of the soils inoculated with non-autoclaved enrichment culture turned yellow. Analysis by TLC indicated the formation of p-nitrophenol in soils inoculated with non-autoclaved enrichment culture [3]. Heat treatment of the enrichment culture retarded the hydrolysis, indicating microbial participation in the enhanced hydrolysis. Hydrolysis of parathion in pure culture by a Flavobacterium sp., isolated from diazinon-amended rice fields, has been reported recently [8]. Such rapid hydrolysis of parathion in flooded soil by a biological action and in pure culture by Flavobacterium sp. was surprising since the principal route for detoxication of parathion in non-flooded soil, and in pure cultures, is by reduction of the nitro group to form aminoparathion.

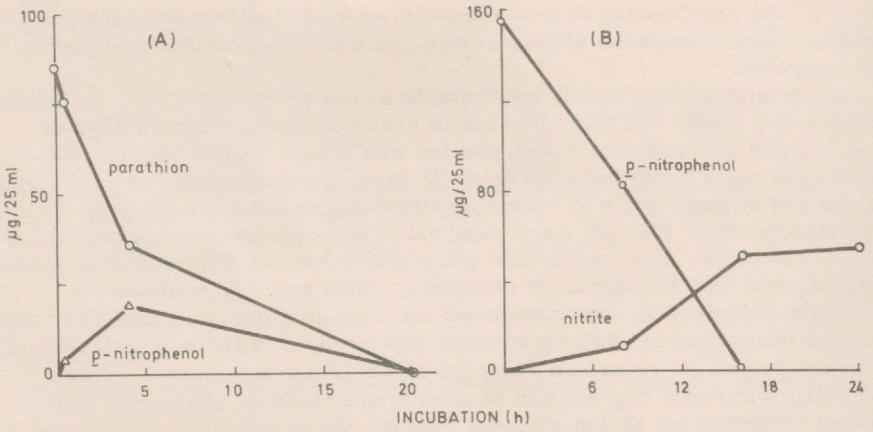


FIG.1. Conversion of parathion to p-nitrophenol (A) and then to nitrate (B) by Pseudomonas sp. isolated from flooded soil [5].

BACTERIAL ROLE IN THE ENHANCED HYDROLYSIS

Enrichment culture capable of hydrolysing parathion was prepared by repeated additions of the insecticide to flooded alluvial soil. In one case, a dilution ($\times 10^4$) of this enrichment culture was mixed directly with modified Wakimoto agar medium [9] and incubated. Individual colonies of bacteria developing on the medium were tested for parathion-hydrolysing and p-nitrophenol-degrading activity. A Bacillus sp. capable of utilizing p-nitrophenol was isolated.

To isolate parathion-hydrolysing bacterium, maximum dilution technique – a method successfully used earlier in isolating a diazinon-hydrolysing Flavobacterium sp. from diazinon-amended rice soils [9] – was employed. Serial dilutions were made from the enrichment culture and each dilution was tested for parathion-hydrolysing activity by incubating it with a mineral solution supplemented with parathion. Dilutions (up to $\times 10^6$) exhibited parathion-hydrolysing activity. The hydrolysing principle after diluting 10^6-fold was multiplied by repeated transfers in a parathion-containing mineral solution. After incubation following the third transfer, the incubation mixture was streaked on modified Wakimoto agar. Individual colonies appearing on the medium were transferred to mineral solution + parathion. A bacterial isolate P-6 hydrolysed parathion as indicated by the formation of yellow colour which, however, disappeared later. The bacterial isolate P-6 was Gram-negative, rod and aerobic and was identified as a non-fluorescent species of Pseudomonas (multivorans?).

When Pseudomonas sp. was incubated with parathion, the insecticide was degraded rapidly. At 4 h, about 50.3 µg of added parathion was hydrolysed and 19.4 µg of p-nitrophenol was recovered as the major metabolite (Fig.1). No other metabolite could be detected in the thin-layer chromatogram of the solvent extract. At 20 h, however, both parathion and its hydrolysis product, p-nitrophenol were not detected in the incubation

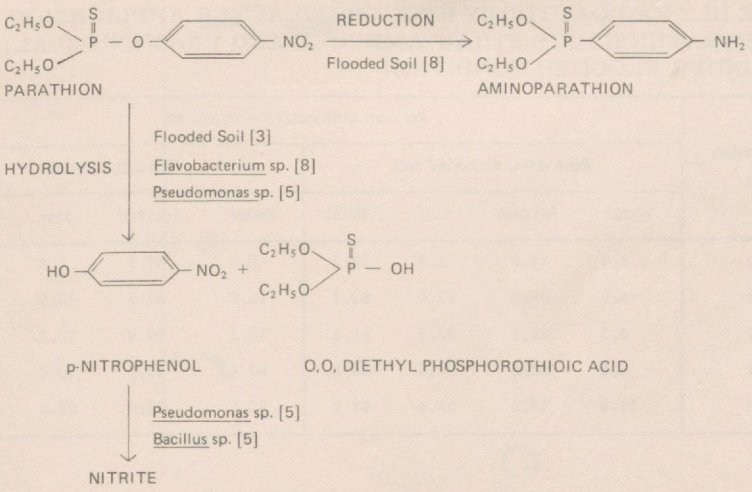


FIG.2. Suggested pathways of microbial metabolism of parathion in flooded soil.

mixture. When the bacterium was grown in a nitrogen-free medium with p-nitrophenol as sole carbon source, nitrite nitrogen was readily formed from the organic nitro molecule (Fig.1). The amount of nitrite formed was proportional to the amount of p-nitrophenol degraded. In uninoculated control, nitrite was not formed. Bacillus sp., also isolated from parathion-hydrolysing enrichment culture, was not able to hydrolyse parathion; the bacterium metabolized p-nitrophenol leading to the formation of nitrite [5]. Various nitrophenols are known to be metabolized by bacteria liberating nitrite in the process [10-12]. Similarly, both Pseudomonas sp. and Bacillus sp. used in our study formed nitrite from p-nitrophenol; the former, in addition, possessed enzyme(s) to hydrolyse parathion.

Parathion metabolism in plant and insect systems via oxidation, reduction of nitro group, or by hydrolysis is well known [13]; but stepwise degradation of this insecticide in microorganisms is not well understood [14]. The major pathway of parathion metabolism in soils and pure cultures appeared to be nitro group reduction. Now, biological hydrolysis of parathion has been clearly demonstrated in flooded soil [3] as well as in pure cultures of Flavobacterium sp. [8, 15] and Pseudomonas sp. (Fig.1). Degradation of parathion by Flavobacterium sp. ceased at the p-nitrophenol stage whereas Pseudomonas sp. decomposed parathion past p-nitrophenol to an inorganic end product, nitrite (Fig.1). Based on the results summarized in this report, the following mechanism for parathion metabolism in flooded soil is proposed (Fig. 2).

EFFECT OF ORGANIC MATTER ON NITRO GROUP REDUCTION

Addition of rice straw to the flooded soil enhanced the nitro group reduction of parathion [4]. The rate of parathion breakdown increased with

TABLE III. RADIOACTIVITY RECOVERED AFTER APPLICATION OF [^{14}C]-PARATHION RICE STRAW AMENDED AND UNAMENDED ALLUVIAL SOIL UNDER FLOODED CONDITION

Incubation (d)	Per cent radioactivity recovered							
	Rice straw amended soil				Unamended soil			
	Water	Solvent	Soil	Total	Water	Solvent	Soil	Total
0	3.4	87.0	2.7	93.1	9.4	67.7	2.8	79.9
3	4.2	36.9	21.0	60.1	13.2	55.3	10.9	79.4
7	5.1	33.1	22.6	60.8	15.1	59.9	13.1	88.1
14	5.0	35.5	28.3	68.8	58.4	12.2	19.4	90.0
28	17.3	16.5	33.4	67.2	39.5	11.6	21.4	72.5

increasing concentrations of rice straw. In the earlier study, parathion degraded faster in soils with higher native organic matter (Table I).

Isotope studies were conducted to follow the enhanced metabolism of parathion in flooded soil amended with rice straw. The radioactivity in the solvent phase decreased rapidly in rice straw amended soil as compared with unamended soil, particularly between 0 and 3 d (Table III). The radioactivity declined from 87 to 37% in 3 d in amended soils and then finally to 17% in 28 d. In unamended soil, no appreciable loss in radioactivity in the solvent phase occurred until 7 d, but at 14 d radioactivity in the solvent phase decreased rapidly with a corresponding increase in the radioactivity in the water phase remaining after solvent extraction. Thus, between 7 and 14 d the radioactivity in the solvent phase of the unamended soil declined from 60 to 12%, whereas in the water phase the radioactivity increased from 15 to 53% during the same period. Even at 28 d, 40% of the radioactivity was found in the water soluble fraction. These studies indicated that, in unamended soil, parathion was converted to water soluble metabolites between 7 and 14 d. In amended soils, despite rapid breakdown of parathion, the radioactivity in the water phase was low, but 40% of the applied radioactivity was unaccounted for, perhaps due to the formation of volatile end products.

A radioautochromatogram of the solvent phase revealed enhanced formation of aminoparathion and an unidentified metabolite in rice straw-amended soil as early as 3 d (Fig. 3). The unidentified metabolite apparently possessed the ethoxy label and P=S bond. No other metabolite was recorded in the radioautograph of the solvent phase. In unamended soil, parathion degradation was slow until 7 d and then its concentration declined rapidly. The rapid decline in insecticide levels resulted in a sharp rise in the radioactivity of the water phase (Table III). Aminoparathion was formed in unamended soil, but in smaller amounts when compared with amended soil. A radioautograph of the water phase (after re-extraction of the acidified water phase with solvent) revealed a clear radioactive spot close to the origin (Fig. 3) in the unamended soil. This polar metabolite was not detected in the amended soil.

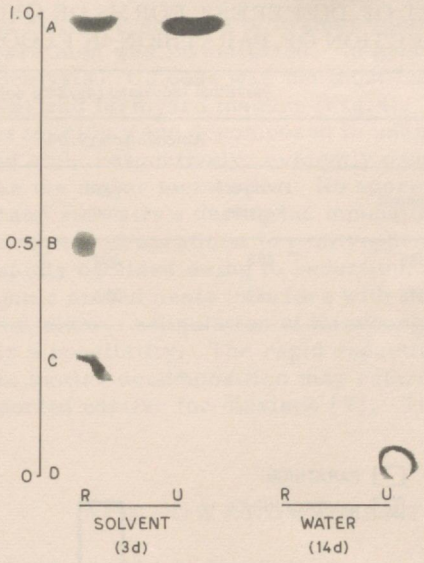


FIG.3. Radioautograph of [^{14}C]-parathion and its metabolites formed in rice straw-amended and unamended flooded alluvial soil [2]. (R: rice straw-amended; U: Unamended; A: Parathion; B: Aminoparathion; C: Unidentified metabolite; D: Unidentified polar metabolite).

Sources of organic matter that generally get incorporated in tropical rice soils are rice residues, farmyard manure and algal crust. The effect of these sources on parathion reduction in flooded soil was investigated. The per cent carbon and nitrogen was 40:0 for glucose, 34.0:0.59 for rice straw, 24.1:1.51 for farmyard manure and 12.8:1.37 for algal crust.

Organic sources accelerated the degradation of parathion in flooded soil. Easily available energy source, glucose, was the most effective in stimulating the degradation with a loss of 99% of the applied insecticide within 3 d (Table IV). The degradation followed the order glucose > rice straw > algal crust farmyard manure > unamended soil. The thin-layer chromatogram of the soil extract revealed the enhanced reduction of parathion to aminoparathion and an unidentified metabolite in all the amendments as noticed earlier with rice straw amendment. The rapid reduction of soil components in flooded soil following organic matter amendments apparently favours the reduction of parathion. Thus, high carbon sources, glucose and rice straw, caused maximum degradation of parathion via reduction. However, despite the higher carbon content in the farmyard manure than in the algal crust, the latter amendment was more stimulatory. Studies are underway to measure the redox potentials of flooded soils amended with organic sources used in this experiment. Enhanced degradation of certain chlorinated hydrocarbon insecticides in flooded soils with high native organic matter content and in soils amended with organic sources has been reviewed recently [16].

TABLE IV. EFFECT OF DIFFERENT FORMS OF ORGANIC MATTER ON NITRO GROUP REDUCTION OF PARATHION IN FLOODED SOIL

Incubation (d)	Parathion recovered (μg/20 g Soil)				
	Amendments (0.5%)				
	Control	Farmyard manure	Algal crust	Rice straw	Glucose
0	628	628	594	630	630
3	276	189	155	89	7

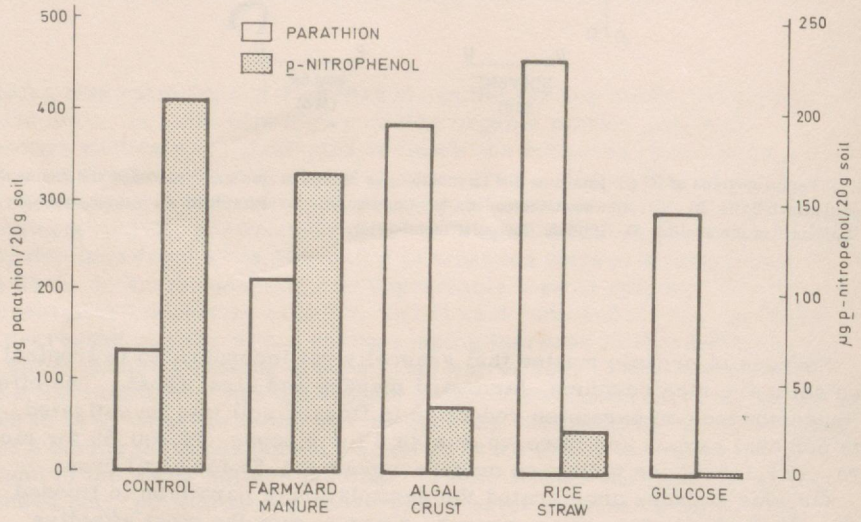


FIG.4. Inhibition of parathion hydrolysis by organic sources in flooded soil during 48 h [2].

EFFECT OF ORGANIC MATTER ON HYDROLYSIS

The effect of rice straw on the biological hydrolysis of parathion in flooded soil was investigated. In unamended soil inoculated with the parathion-hydrolysing enrichment culture, parathion was readily hydrolysed to p-nitrophenol [4]. In contrast, additions of rice straw increased the persistence of parathion in the inoculated soil in proportion to the concentration of rice straw. p-Nitrophenol was not formed in the inoculated soil amended with rice straw, indicating the inhibitory effect of organic matter source on the hydrolysis.

The effect of different sources of organic matter on the hydrolytic degradation of parathion was investigated. In general, organic sources inhibited the hydrolysis. Glucose was the most inhibitory, followed by rice straw, algal crust and farmyard manure (Fig. 4). Within 16 h, 78 and 61% of the applied insecticide were decomposed in unamended and farmyard manure-amended soil, respectively, evidently owing to p-nitrophenol which was recovered as the major metabolite. No appreciable degradation occurred with algal crust and rice straw during the incubation period. Glucose inhibited the hydrolysis of parathion to p-nitrophenol, but the insecticide concentration rapidly declined owing to reduction.

How the organic amendments interfere with the biological hydrolysis of parathion is not clear. Stimulation of microorganisms to the disadvantage of hydrolysers is a possibility. The rapid reduction of the flooded soil following organic matter decomposition may retard the hydrolysis of parathion as reported earlier for diazinon [7]. These aspects are being investigated.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. S. Y. Padmanabhan, Director, Central Rice Research Institute for helpful suggestions. These investigations were supported, in part, by grants from the International Atomic Energy Agency, Vienna, Austria, and from the Department of Atomic Energy, Government of India.

REFERENCES

[1] ATHWAL, D.S., "IRRI's current research program", Rice, Science and Man, International Rice Res. Inst. (1972) 41-64.

[2] RAJARAM, K.P., SETHUNATHAN, N., Effect of organic sources on the degradation of parathion in flooded alluvial soil, Soil. Sci. 114 (1975) ~~in press~~ 296-300.

[3] SETHUNATHAN, N., Degradation of parathion in flooded acid soils, J. Agric. Food Chem. 21 (1973) 602-04.

[4] SETHUNATHAN, N., Organic matter and parathion degradation in flooded soil, Soil Biol. Biochem. 5 (1973) 641-44.

[5] SIDDARAMAPPA, R., RAJARAM, K.P., SETHUNATHAN, N., Degradation of parathion by bacteria isolated from flooded soil, Appl. Microbiol. 26 (1973) ~~in press~~. 846-849.

[6] SUFFET, I.H., FAUST, S.D., The p-value approach to quantitative liquid-liquid extraction of pesticides from water. 1. Organophosphates, J. Agric. Food Chem. 20 (1972) 52-56.

[7] SETHUNATHAN, N., PATHAK, M.D., Increased biological hydrolysis of diazinon after repeated application to rice paddies, J. Agric. Food Chem. 20 (1972) 586-89.

[8] SETHUNATHAN, N., YOSHIDA, T., A Flavobacterium sp. that degrades diazinon and parathion, Can. J. Microbiol. 19 (1973) 873-75.

[9] SETHUNATHAN, N., Diazinon degradation in submerged soil and rice-paddy water, Advan. Chem. Ser. 111 (1972) 244-55.

[10] GUNDERSEN, K., JENSEN, H.L., Acta Agr. Scand. 6 (1956) 100 - 114. (Cited by ALEXANDER, M., Biodegradation: Problems of molecular recalcitrance and microbial fallibility, Advan. Appl. Microbiol. 7 (1965) 35 - 80.)

[11] JENSEN, H.L., LAUTRUP-LARSEN, G., Microorganisms that decompose nitroaromatic compounds, with special reference to dinitro-ortho-cresol, Acta Agr. Scand. 17 (1967) 115.

[12] RAYMOND, D.G.M., ALEXANDER, M., Microbial metabolism and cometabolism of nitrophenols, Pest. Biochem. Physiol. 1 (1971) 123-30.

[13] O'BRIEN, R.D., Toxic Phosphorus Esters, Academic Press, New York (1960).

[14] ALEXANDER, M., "Microbial degradation and biological effects of pesticides in soil", Soil Biology, Reviews of Research, UNESCO, Paris (1969) 209-40.

[15] SETHUNATHAN, N., YOSHIDA, T., Conversion of parathion to para-nitrophenol by diazinon-degrading Flavobacterium sp., Proc. Inst. Environ. Sci. New York Meeting 18 (1972) 255-57.

[16] SETHUNATHAN, N., Microbial degradation of insecticides in flooded soil and in anaerobic cultures, Residue Revs 47 (1973) 143-65.

Printed by the IAEA in Austria

Residue Reviews (USA), 68, 91-122, 1977.

Parathion: Residues in soil and water

By

N. SETHUNATHAN,* R. SIDDARAMAPPA,* K. P. RAJARAM,*
S. BARIK,* and P. A. WAHID*

Contents

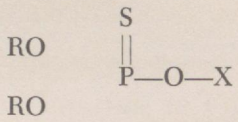
I. Introduction

In recent years, extensive use of toxic organic pesticides, particularly the persistent organochlorines, in agriculture and public health has caused great concern in developed countries from the standpoint of environmental pollution and soil fertility. Organochlorine pesticides, by virtue of their extremely long persistence and instances of several pests developing resistance to them, are now being replaced by the generally less persistent organophosphates and carbamates.

Among the organophosphates, parathion[1] is perhaps one of the most

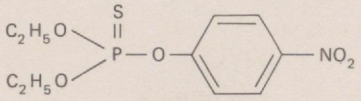
* Central Rice Research Institute, Cuttack-753006, India.
[1] Chemical names of pesticides mentioned in text are given in Table II.

extensively used insecticides in agriculture. Parathion is a member of the phosphoric acid esters group with a general formula of the type:



where R is an alkyl group and X is an organic radical.

Specifically, for parathion, R is an ethyl group and X is a nitrophenyl group as given below:



The corresponding methyl derivative is known as methyl parathion and is also extensively used because of its relatively low mamalian toxicity.

Parathion, like most organophosphates, is relatively less persistent than the organochlorine compounds in soil and aquatic environments. A summary of persistence data of 12 classes of pesticides in several soil types demonstrated the relatively low persistence of organophosphorus insecticides as illustrated in Figure 1 (KEARNEY *et al.* 1969). Parathion appeared to be the least persistent among the organophosphates tested. Interestingly, however, long-term persistence of parathion has also been reported recently (STEWART *et al.* 1971). Although parathion is generally applied to the foliage, frequent applications of this insecticide, necessitated by its relatively low persistence and recent build-up of harmful pests particularly in areas under intensive cultivation (KULSHRESHTHA *et al.* 1974), can eventually lead to its accumulation in the soil and water environments (HARRIS and MILES 1975). Environmental pollution from parathion can also occur from inadvertent spillage of concentrated formulation (WOLFE and DURHAM 1966, WOLFE *et al.* 1973) or from used parathion containers (MUNNECKE and HSIEH 1974).

Organophosphates, in general, are known to be rapidly metabolized in plant and animal tissues, but, until recently, information on their fate in soil and in aquatic environments was limited (ALEXANDER 1969). In more recent years, however, the environmental fate of parathion and its metabolites has received considerable attention, with particular reference to microbial roles in their transformations. This review would highlight the progress in the studies on the (1) persistence of parathion in soil and aquatic environments with emphasis on chemical *versus* microbial aspects of its degradation and (2) metabolism of its predicted metabolites, aminoparathion, *p*-nitrophenol and related nitrophenols in soils, and by microorganisms in pure cultures to provide a more complete picture of parathion degradation to the inorganic end products. The limited studies on the fate of methyl parathion in soil and aquatic environments are also

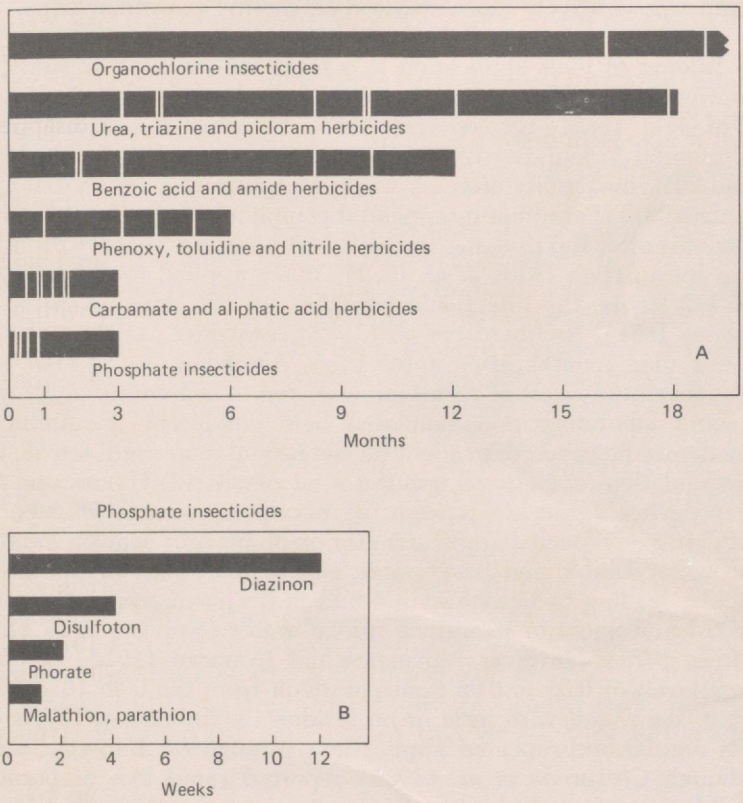


Fig. 1. Persistence of several groups of pesticides (A) and specific organophosphates (B) in soils (KEARNEY *et al.* 1969).

reviewed in this paper in view of similar pathways of degradation for parathion and methyl parathion.

II. Stability in soil

a) Residues

The reported persistence of parathion in soils varies from a few weeks to several years. CARLO *et al.* (1952) reported that no parathion was detected in soil within 16 days of its application at 2.24 kg/ha. LICHTENSTEIN and SCHULZ (1964) reported that in a field study, residues of parathion and methyl parathion dropped to about 0.1 ppm within 90 and 30 days, respectively, after application of these insecticides to

Carrington silt loam soil at 5.60 kg/ha. Also, under laboratory conditions, parathion was relatively more stable than methyl parathion when 30% of the applied parathion and 90% of the methyl parathion disappeared within 12 days of their application to a loam soil. In another study, five and fourteen % of the added parathion remained undegraded over a period of eight weeks (LICHTENSTEIN 1966). Bioassay of field soils treated with a granular formulation of parathion at the rate of 1.12 kg/ha showed less than 20% bioactivity after six weeks of application (BURKHARDT and FAIRCHILD 1967). Parathion disappeared completely from the soils within eight months after top dressing the soils at 3.4 and 6.7 kg/ha with a 20% granular formulation (MOL *et al.* 1972). When applied to the soil at the rate of 112 kg/ha, the insecticide persisted for 325 days (KASTING and WOODWARD 1951). NICHOLSON *et al.* (1962) recovered parathion residues from soils nine months after application. According to SACHER *et al.* (1972), substantial losses of parathion occurred in soil within eight weeks under both laboratory and simulated field conditions. Persistence of parathion was, however, dependent on the formulation used; ten % parathion formulation on charcoal was the most persistent. HARRIS and SANS (1971) reported significant residues of parathion in organic soils, particularly after its repeated application for onion maggot control. Parathion is more water-soluble and hence more mobile than most organochlorine insecticides leading to significant residues in deeper layers of the soil by downward movement or in surface run-off water (STEVENS 1973, HARRIS and MILES 1975). However, VOERMAN and BESEMER (1970) recovered only low levels of 0.01 to 0.06 ppm parathion from the 0 to 10 cm layer of a light, sandy soil with little or no residues in the deeper layers even after its regular and repeated applications throughout a 15-year period.

Although CHISHOLM *et al.* (1955) reported rapid loss of parathion from field plots of sandy loam soil, recent reports by these investigators showed persistence of parathion at low levels in soils over several years. MACPHEE *et al.* (1960) found that parathion persisted in traces for at least five years after its application to the soil. In the field experiments extending over several years at Kentville, Nova Scotia, Canada, the plots received annual applications of 14.1 kg/A of parathion (15.7 ppm soil concentration) from 1949 to 1953. Analysis of soil residues from these plots 16 years after the last application, employing a sensitive flame photometric detector, confirmed that the residue recovered in concentrations of 0.06 ppm from the top six in. was in reality parathion (STEWART *et al.* 1971). Subsequently, CHISHOLM and MACPHEE (1972) observed that 0.2 kg/ha of parathion remained intact even 16 years after the last application in soil samples taken from the plots that received a total of 176 kg/ha of parathion during 1949-1953. IWATA *et al.* (1973) studied the persistence of parathion in six California soils and concluded that long-term, low-level residues could occur in the soils depending on soil type. Such long-term low-level persistence of parathion despite its known instability was attributed to its binding to lipid fraction of organic matter

rendering it unavailable for degradation processes (STEWART *et al.* 1971). Environmental contamination from high concentrates of parathion through waste disposal of containers or air spray operations may also create problems of high residual deposit for extremely long periods. In a study using different formulations and dilutions of parathion WOLFE *et al.* (1973) observed a high level of persistence of parathion over a five-year period in sandy loam soils, particularly following treatment with undiluted commercial grade formulations.

In recent years, the fate and metabolism of pesticides under flooded rice soil conditions have been investigated. Flooded soil environments, generally used for rice cultivation, differ from nonflooded soil in physicochemical and biological characteristics (PONNAMPERUMA 1972). Certain organochlorine insecticides of extremely long persistence in nonflooded soils and other aerobic systems undergo rapid decomposition under predominantly anaerobic flooded soil conditions (SETHUNATHAN 1973 a). Likewise, SETHUNATHAN and YOSHIDA (1973 a), while investigating the persistence of parathion in Philippine rice soils, observed that this insecticide was degraded more rapidly in flooded soils than in nonflooded soils. In all four soils tested, parathion residues reached low levels within 14 days after flooding, whereas in nonflooded soils no appreciable degradation occurred during the same period (Fig. 2). On the other hand, IWATA *et al.* (1973) reported that the effect of flooding on parathion persistence depended on the soil type used. When Madera Sandy Loam soil was fortified with parathion, the insecticide disappeared more rapidly from flooded soils than from moist soils; in flooded soils, the residues dropped from 20 to 0.2 ppm within two weeks after fortification. In

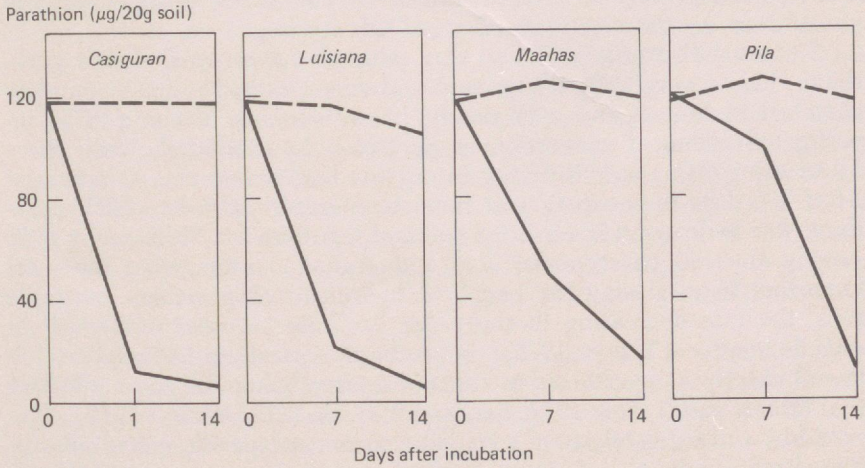


Fig. 2. Persistence of parathion in flooded and upland (nonflooded) soils (SETHUNATHAN and YOSHIDA 1973 a).

contrast, the degradation of parathion in Santa Lucia Silt Loam and Windy Loam was not enhanced by flooding. Rapid degradation of parathion was also noticed in certain Indian soils following flooding (SETHUNATHAN 1973 b).

b) Degradation

Degradation of pesticides in soils is mediated largely by chemical and/or biological means. As for parathion, biological degradation is more important, although limited studies using sterile soil and clay minerals have provided indirect evidence for extremely slow chemical conversion of parathion in soils over long periods of time. Chemical *versus* microbial aspects of parathion decomposition in soils are discussed below.

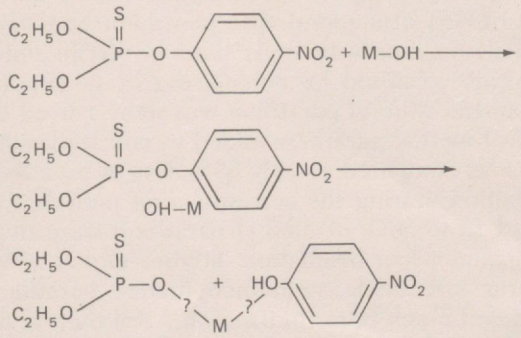
1. Chemical degradation.—The major means of chemical degradation for several organophosphorus pesticides in soil and aquatic ecosystems is *via* hydrolysis at ester linkages. Chemical hydrolysis of organophosphorus insecticides in soils is essentially pH-dependent. For example, malathion can undergo rapid chemical hydrolysis under alkaline conditions (KONRAD *et al.* 1969), ciodrin under acidic conditions (KONRAD and CHESTERS 1969), and diazinon under acidic or alkaline conditions (KONRAD *et al.* 1967). Adsorption-catalysed hydrolysis of these organophosphorus insecticides has also been reported in soils (KONRAD *et al.* 1967 and 1969, KONRAD and CHESTERS 1969).

Among the organophosphorus insecticides, parathion is perhaps one of the most resistant to chemical hydrolysis. Parathion is stable in neutral or acidic pH range, but is hydrolysed rapidly in alkaline conditions yielding *p*-nitrophenol and diethylthiophosphoric acid. The mechanism of hydrolysis essentially involves an attack on a relatively positive site, the phosphorus, by the negatively charged OH^- group.

Studies with sterile soils and clay minerals have revealed that parathion, like several other organophosphorus pesticides, may undergo chemical hydrolysis, but only slowly, in soil systems. YARON (1975) investigated chemical conversion of parathion in irradiated (with three Mrad using ^{60}Co) soils differing in organic matter and clay mineralogy. After 130 days of incubation at room temperature, three to 23% parathion was hydrolysed in air-dried soil and less than ten % in moist soils. Among the soil constituents, clay and organic matter were the most important in catalysing the chemical hydrolysis of parathion in sterile soils, the rate decreasing in the order kaolinite > montmorillonite > organic matter (YARON 1975); interestingly, parathion adsorption followed exactly a reverse order, organic matter being the most effective (SALTZMAN and YARON 1972, SALTZMAN *et al.* 1972, WAHID 1976). Presumably, surface catalysis of parathion occurs on specific active adsorption sites.

The decomposition of parathion on specific kaolinites provided further insight to the understanding of parathion behavior in soils. The kaolinites

catalysed the chemical hydrolysis of parathion, but the hydrolysis was associated with water, temperature, and nature of exchangeable cation. Ca-kaolinite was the most effective in catalysing the hydrolysis followed by Na- and Al-kaolinites (Saltzman *et al.* 1974); about 60% of 500 μg of parathion added was hydrolysed within a week on dry Ca-kaolinite. The presence of water blocked the catalytic effect of kaolinite while a rise in temperature increased it (Yaron and Saltzman 1972). These observations led to the conclusion that parathion molecules react with dissociated water hydroxyls available at the clay surface according to the reaction:



where *M* represents exchangeable cation (Saltzman *et al.* 1974).

Calcium catalysed parathion degradation in sand and soil systems as it did on kaolinites. Addition of calcium salts such as $CaCO_3$ and $CaSO_4$ enhanced the degradation of parathion in almost an inert system, sand, but in soils the effect of calcium salts was not consistent, presumably due to the interaction of certain soil components (Mingelgrin and Yaron 1974). The presence of such free salts in soils, as in several arid zone soils, could, therefore, influence the decomposition of parathion.

According to Adamson and Inch (1973), chemical degradation, rather than microbial, may be of great significance in methyl parathion degradation in solution and soil systems. In solution, methyl parathion was hydrolysed three times faster and was dealkylated 40 times faster than parathion. The decomposition rates observed in natural soil, heat-sterilised soil, and in ion-exchange resin resulted largely from surface catalysis, and microbial degradation played little part in the removal of methyl parathion and parathion from the soil.

2. Biological degradation.—Despite its resistance to chemical degradation, parathion seldom gives rise to significant residues in the environment. Based on several degradation studies using sterile and nonsterile samples and isolated cultures of microorganisms, it has been established convincingly that microbial metabolism is the major means of parathion detoxication in soil and aquatic environments.

Lichtenstein and Schulz (1964) studied the effect of soil micro-

organisms and soil water content on the persistence and metabolism of parathion residues in nonflooded soil system. The insecticide was most persistent in dry soils and least persistent in soils with high water content. Degradation of parathion proceeded either by hydrolysis yielding *p*-nitrophenol and diethylthiophosphoric acid or by nitro-group reduction to its amino analog depending on the populations of the microorganisms; however, parathion degradation appeared to occur principally by nitro group reduction. Yeasts were involved in this transformation, particularly in glucose-amended soils. Addition of sterilising agents such as sodium azide or autoclaving of the soils increased the persistence of parathion indicating microbial participation (LICHTENSTEIN *et al.* 1968). GETZIN and ROSEFIELD (1968) also found that parathion and methyl parathion broke down faster in nonsterile soils than in sterile soils. The rate of degradation in soils sterilised by autoclaving or gamma-irradiation was slow and comparable. Methyl parathion was shorter-lived than parathion; more than 95% of methyl parathion added to nonsterile soils disappeared within one week as compared to only 35% loss of parathion even after a two-week incubation; during the corresponding periods, only 16 to 17% of parathion and 20 to 26% of methyl parathion were degraded in soils sterilised by autoclaving or irradiation. Studies also showed that another organophosphorus insecticide, malathion, unlike parathion and methyl parathion, degraded much faster in irradiated soil than in autoclaved soil, attributed to a heat-labile and alkali-extractable soil fraction capable of degrading malathion, but not other related compounds including parathion. GRIFFITHS and WALKER (1970) reported rapid degradation of parathion by a heat-labile agent, presumably of microbial origin, in soil percolation experiments, but the organisms responsible were not isolated. More recently, SACHER *et al.* (1972) also noticed increased persistence of parathion in soils treated with methyl bromide as a soil sterilant. Moreover, more rapid degradation of parathion occurred in summer months with a half-life of 1.5 weeks than in winter months with a half-life of more than three weeks, presumably due to more intense microbial activity in summer months. LICHTENSTEIN (1966) found that addition of detergents such as ABS (alkyl benzene sulfonate) and LAS (linear alkyl benzene sulfonate), also known for their antimicrobial action, increased the persistence of parathion. Similarly, under flooded conditions, heat treatment of the soils prior to flooding prevented the nitro group reduction (SETHUNATHAN and YOSHIDA 1973 a) and hydrolysis (SETHUNATHAN 1973 b) of parathion. Increased persistence of parathion thus reported in soils sterilised by autoclaving, irradiation or anti-microbial agents presumably resulted from reduced microbial activity (NAUMANN 1970, LICHTENSTEIN *et al.* 1968, GETZIN and ROSEFIELD 1968, SACHER *et al.* 1972, SETHUNATHAN and YOSHIDA 1973 a).

More convincing evidence for microbial involvement in parathion degradation in the soil was obtained by correlating its degradation rate with microbial proliferation and/or by demonstrating its degradation in

isolated cultures of microorganisms. In most studies reported until recently, parathion degradation in soils and in pure cultures proceeded principally by nitro group reduction to aminoparathion. LICHTENSTEIN and SCHULZ (1964) provided experimental evidence by linking the formation of aminoparathion in soils with the proliferation of yeasts. Degradation in Madera sandy soil flooded with water showed an initial lag followed by a logarithmic rate of breakdown indicating the participation of microorganisms, perhaps algae (IWATA *et al.* 1973). NAUMANN (1970) reported that several groups of soil microorganisms can proliferate in soils by utilising parathion and methyl parathion. A soil fungus, *Trichoderma viride*, degraded several insecticides including parathion (MATSUMURA and BOUSH 1968). Cultures of symbiotic nitrogen-fixing bacteria, *Rhizobium japonicum* and *R. meliloti*, produced aminoparathion as the major metabolite and *p*-nitrophenol as a minor metabolite (MICK and DAHM 1970). Recently, RAO and SETHUNATHAN (1974) found that parathion was converted to aminoparathion and certain water-soluble metabolite(s) by a fungus, *Penicillium waksmanii*, isolated from a flooded acid sulfate saline soil. Pure culture studies demonstrated active participation of soil aerobes in the transformation of parathion to aminoparathion, essentially a product of reduction reaction. However, in a predominantly anaerobic system such as flooded soil, facultative or obligate anaerobes may be more involved than aerobes in the reported formation of aminoparathion.

Although nitro group reduction of parathion is a widespread phenomenon in soil and aquatic environments, recent studies have demonstrated that hydrolysis of parathion, considered to be a chemical reaction of minor importance and now largely or solely attributed to microorganisms, is more common and widespread in natural ecosystems than hitherto believed. LICHTENSTEIN and SCHULZ (1964) reported both reduction and hydrolysis of parathion in soil, but experimental evidence for microbial role was established only for the major pathway of nitro group reduction. More recently, KISHK *et al.* (1976) demonstrated enzymatic hydrolysis of methyl parathion with a pH optimum of 7.0 in soils when hydrolysing activity was retarded partially by heat treatment and completely by autoclaving of the soil.

The most convincing report on the biological hydrolysis of parathion as a major means of detoxication in soils was by SETHUNATHAN (1973 b). Parathion was hydrolysed readily by biological action after two to three additions of the insecticide to an alluvial soil flooded with water. Enrichment culture from flooded alluvial soil lost its ability to hydrolyse parathion after autoclaving or Millipore filtration. Moreover, a *Pseudomonas* sp. isolated from this enrichment culture completely hydrolysed parathion, apparently by a cometabolic process, and then metabolised the hydrolysis product, *p*-nitrophenol, liberating nitrite (SIDDARAMAPPA *et al.* 1973) and CO_2 (SUDHAKAR-BARIK *et al.* 1976) as end products. Likewise, another organophosphate, diazinon, reported to hydrolyse in

soil primarily by chemical action (KEARNEY and HELLING 1969), was hydrolysed fairly rapidly by microorganisms developed in flooded rice fields in response to its repeated applications (SETHUNATHAN and PATHAK 1972, SETHUNATHAN 1972). A *Flavobacterium* sp., isolated from diazinon-treated rice fields, readily hydrolysed diazinon and then generated CO_2 after ring cleavage of the pyrimidine moiety (SETHUNATHAN and YOSHIDA 1973 b, SETHUNATHAN 1973 a). The same bacterium hydrolysed parathion to *p*-nitrophenol; the *p*-nitrophenol formed resisted further degradation (SETHUNATHAN and YOSHIDA 1972). Dursban, and not malathion, was also decomposed, but the metabolites were not identified. Interestingly, parathion, diazinon, and Dursban are characterised by a common P-O-C linkage; at least, parathion and diazinon were hydrolysed by cleavage of this bond. The enzyme involved in the hydrolysis of parathion and diazinon by *Flavobacterium* sp. appeared to be constitutive. Contrary to the common belief that hydrolysis of parathion and diazinon in soils is purely chemical, recent work with flooded soils has demonstrated that microorganisms actively participate in the extremely rapid hydrolysis of these organophosphates after their repeated applications to flooded soils.

The conventional chemical detoxication procedures involving alkaline hydrolysis of parathion using strong alkali raises the problems of environmental contamination from the alkali used for hydrolysis and the products of chemical reaction such as *p*-nitrophenol and 2,4-dinitrophenol. Of greater interest is the approach to use adapted mixed microbial cultures as a means of detoxifying high concentrates of parathion arising from used containers, accidental spills, industrial plants, and various other sources (COLEY and STUTZ 1966, HOWE 1969). Mixed cultures often employ a multiplicity of enzyme systems capable of degrading pesticides *via* more than one pathway, if feasible from a molecular point of view, in a sequential reaction process. This allows more effective and/or complete degradation of pesticides in mixed cultures than in pure cultures or chemical reactions.

Monsanto Chemical Company developed an activated sludge pilot plant for processing concentrated parathion wastes from industrial plants (COLEY and STUTZ 1966). Under adequate aeration to supply at least 42.5 m^3/kg (1,500 ft^3/lb) chemical oxygen demand, complete destruction of parathion and *p*-nitrophenol could be accomplished within seven to ten days of treating the blended wastes with biologically activated sludge.

Recently, investigations at the University of California, Davis, revealed the possibility of utilizing adapted mixed cultures for inactivation of high concentrations of parathion. In continuous fermentor or batch culture experiments, HSIEH and MUNNECKE (1972) showed enhanced degradation of concentrated emulsifiable parathion (as high as 1,000 ppm) in adapted microbial cultures at a rate 100 times faster than the rate of hydrolysis in 1 *N* NaOH. Subsequently, a mixed culture of sewage and soil origin was adapted to grow on parathion in a continuous fermentor by gradually increasing parathion concentration in the broth (containing

0.1% glucose) from ten μg/ml to three mg/ml over a 30-day period. At the dilution rate of 0.05 L/hr and under oxygen-sufficient conditions, the adapted mixed culture removed parathion at the rate of 500 ppm/hr and at high concentrations of 5,000 mg/L. *p*-Nitrophenol, generated by enzymatic hydrolysis, produced a lag at concentrations exceeding 0.72 mM. None of the bacterial isolates, mostly *Pseudomanads*, from the mixed culture could utilize parathion as the sole source of energy and carbon while *p*-nitrophenol was metabolised as the sole energy source by eight out of nine isolates (MUNNECKE and HSIEH 1974). The chemical and biological interactions involved in the degradation of parathion in mixed cultures appeared to be more complex with a parathion emulsifiable concentrate (xylene-based) than with technical parathion (MUNNECKE and HSIEH 1975 a). The preferential utilisation of parathion as a carbon source from the mixture of formulation chemicals, was dependent on the select microflora and their growth environment. Slightly alkaline conditions favored the metabolism of parathion over xylene in parathion emulsifiable concentrate as compared to a shift to xylene metabolism under acidic conditions.

Characteristically, rapid degradation of high concentrates of parathion in mixed cultures was accomplished by metabolic pathways involving both oxidation and reduction reactions (MUNNECKE and HSIEH 1976). The primary oxidative pathway involved an initial hydrolysis of parathion to *p*-nitrophenol and diethylthiophosphoric acid. In yet another oxidative pathway, perhaps of secondary importance, parathion was first oxidized to paraoxon followed by the hydrolysis of the latter to *p*-nitrophenol and diethylphosphoric acid. In mixed cultures, paraoxon was hydrolysed faster than parathion. Under low oxygen conditions, parathion was reduced to aminoparathion which was then hydrolysed to aminophenol and diethylthiophosphoric acid. The results indicated that mixed cultures have potential advantages over axenic cultures and chemical processes in preventing the undesirable effects of excessive concentration of parathion wastes in the environment.

Enzymatic hydrolysis of parathion has been demonstrated also in cell-free extracts of adapted mixed or pure cultures of microorganisms. SETHUNATHAN and YOSHIDA (1972 and 1973 b) reported that a cell-free enzyme extract of a *Flavobacterium* sp. showed exceptional ability to hydrolyse parathion and diazinon as did the whole (growing or resting) cells of the same bacterium. With respect to parathion, the reaction ceased at the *p*-nitrophenol stage. The enzyme involved in the hydrolysis was constitutive. More recently, a cell-free preparation of *Pseudomonas* sp. (SIDDARAMAPPA *et al.* 1973) readily hydrolysed parathion but *p*-nitrophenol was not metabolised further (SUDHAKAR-BARIK 1976), despite the exceptional capacity of the whole (growing or resting) cells of the bacterium to degrade parathion beyond *p*-nitrophenol to nitrite (SIDDARAMAPPA *et al.* 1973). Similarly, MUNNECKE and HSIEH (1974) isolated an inducible enzyme, parathion hydrolase, from adapted mixed cultures,

capable of hydrolysing parathion at the rate of 416 nmol/min/mg of protein. The rate of enzymatic hydrolysis of parathion was 2,450 times faster than that of chemical hydrolysis in O. 1*N* sodium hydroxide solution at 40°C. Parathion hydrolase also hydrolysed eight out of ten organophosphorus insecticides at rates ranging from 12 to 1,360 nmol/min/mg of protein (MUNNECKE and HSIEH 1975 b, MUNNECKE 1976 a). Triazophos, paraoxon, diazinon, methyl parathion, Dursban, fenitrothion, and cyanophos, all organophosphates, were hydrolysed by parathion hydrolase at rates 40 to 1,005 times faster than chemical hydrolysis.

Interestingly, mixed cultures metabolised parathion via three different pathways while, in cell-free enzyme systems, primary degradation proceeded by initial hydrolysis of the P-O-C linkage. Also, it is not clear whether *p*-nitrophenol, a product of enzymatic reaction, was metabolised further, as in whole cells, by cell-free enzyme systems from mixed cultures.

The exceptional ability of cell-free enzyme systems, from pure (SETHUNATHAN and YOSHIDA 1972 and 1973 b) and mixed (MUNNECKE 1976 a) cultures, to hydrolyse parathion and several related organophosphates has great implications in the decontamination of high concentrations of aqueous suspensions of these insecticides. As a practical approach of economic importance, MUNNECKE (1976 b) initiated studies to immobilise such active enzyme systems by binding on supports such as cellulose or glass and to use such columns for decontamination of pesticide wastes.

3. Factors.—Factors reported to affect parathion degradation in soils are pH, soil type, soil moisture, and organic matter.

In alkaline soils, degradation of parathion occurs perhaps by chemical hydrolysis (ADAMSON and INCH 1973) while in soils of neutral or near neutral pH, degradation is predominantly microbiological.

According to IWATA *et al.* (1973), persistence of parathion is partially dependent on soil type as illustrated by two types of persistence curves for parathion. Rapid degradation of parathion in four out of six soils resulted from a combination of hydrolysis and strong microbial activity. In the remaining two soils characterised by linear semilogarithmic persistence curves, degradation was extremely slow and was attributed to hydrolysis.

Soil moisture, apart from its effects on the adsorption characteristics and overall biological activity of parathion in soils, may influence its degradation rate, most probably by regulating microbial proliferation. Under nonflooded soil conditions, LICHTENSTEIN and SCHULZ (1964) found that parathion was most persistent in dry soils and least persistent in soils with high moisture content. Of interest is that much faster degradation of parathion occurred in all four Philippine rice soils under flooded conditions than under nonflooded conditions (SETHUNATHAN and YOSHIDA 1973 a). Within two weeks after application, parathion residues declined from 1,000 μg/20 g soil to negligible levels in most soils when flooded with water; during the corresponding period, loss from nonflooded soils

was rather negligible (Fig. 2). Similarly, rapid degradation of parathion was noticed in Indian rice soils under flooded conditions (SETHUNATHAN 1973 b). In contrast, IWATA *et al.* (1973) showed accelerated degradation of parathion, upon flooding, only in one out of three soils tried. Perhaps the effect of soil moisture on the degradation rates of parathion in soils is regulated by the pathways (nitro group reduction/hydrolysis) and means (chemical/microbiological) of its breakdown.

Organic matter, either native or applied, influences the persistence of pesticides in soils. On the one hand, organic matter increases the microbial activity in soils which decreases the persistence of biodegradable pesticides. On the other hand, the adsorption of pesticides onto the organic matter decreases their availability for microbial attack increasing their persistence in the soil. Under flooded conditions organic matter accelerates the reduction of the soil favoring decomposition of certain pesticides susceptible to anaerobic biodegradation (SETHUNATHAN 1973 a). Excess water available under flooded conditions may also successfully compete for adsorption sites on the organic surface displacing pesticides into water.

Organic matter has been implicated as one of the most important factors affecting parathion persistence in soils. According to SETHUNATHAN (1973 b), the persistence of parathion in flooded soils was inversely related to native soil organic matter content, but IWATA *et al.* (1973) found that parathion disappeared at a faster rate from the soils with lower organic matter content under both flooded and nonflooded conditions.

Added organic sources influenced the persistence of parathion in a flooded soil depending on the metabolic pathway involved. In flooded soils inoculated with an enrichment culture which exhibited an exceptional ability to hydrolyse parathion, rice straw amendment inhibited parathion hydrolysis to *p*-nitrophenol and diethylthiophosphoric acid (SETHUNATHAN 1973 c, RAJARAM and SETHUNATHAN 1975); on the other hand, in uninoculated soils, rice straw enhanced the degradation of parathion *via* nitro group reduction. Isotope experiments revealed more rapid conversion of parathion to aminoparathion and an unidentified metabolite in rice straw-amended soil than in unamended soil (RAJARAM and SETHUNATHAN 1975). Decomposition of organic matter in oxygen-depleted flooded soil hastened the drop in reduction-oxidation potential (Eh) of a flooded soil (PONNAMPERUMA 1972). Clearly, such a low potential prevailing in amended soil catalyses the reduction pathway of parathion metabolism. In a subsequent study on the effect of different organic sources, organic amendments particularly high carbon sources, glucose, and rice straw, increased the rate of parathion loss by nitro group reduction under flooded conditions in the order: glucose > rice straw > algal crust > farmyard manure (RAJARAM and SETHUNATHAN 1975). In flooded soils inoculated with parathion-hydrolysing cultures, organic sources inhibited the hydrolysis. The rate of hydrolysis in different amendments followed a reverse order of that for nitro group reduction.

Despite the inhibition of biological hydrolysis by organic amendments, the overall persistence of parathion was not increased because of a shift in the pathway to nitro group reduction which was enhanced by these amendments.

Though not conclusive, studies on the mechanism of inhibition of biological hydrolysis of parathion in flooded soil amended with rice straw showed a water-soluble, heat-resistant factor, capable of inhibiting parathion hydrolysis, accumulated and persisted in rice straw-amended soils incubated under flooded unstirred conditions or under 100% water-holding capacity, but not in amended soils incubated at lower moisture regimes of 25, 50, and 75% water-holding capacity (RAJARAM 1975, RAJARAM and SETHUNATHAN 1976); the toxic factor that developed during incubation in rice straw-amended soil under flooded unstirred conditions was destroyed when the flooded soil system was aerated by shaking or amended with potassium nitrate (RAJARAM 1975). Characterisation of the toxic principle, most probably a product of anaerobic metabolism of rice straw, has not been accomplished yet; it is known, however, that decomposition of rice straw in predominantly anaerobic flooded soils produces a wide array of aliphatic and, perhaps, aromatic acids which accumulate causing phytotoxic problems in rice culture (PONNAMPERUMA 1972).

III. Stability in aquatic environments

a) Residues

Parathion is more water soluble than several organochlorine insecticides and, as a result, its terrestrial application raises the problem of pollution of ponds, streams, and rivers by residues from surface run-off waters. Direct entry of parathion into the aquatic ecosystem also occurs when it is sprayed into the aquatic environment as a larvicide for mosquito control. The persistence of parathion in aquatic systems has, therefore, received attention.

Parathion, like most organophosphorus insecticides, has a short half-life in water and there is little indication of residue build-up. Residues of 0.3 to 70 ppb of parathion have been detected in water (BOWMAN and ORLOSKI 1966, LUDWIG *et al.* 1968). MULLA *et al.* (1966) reported that residues of parathion in the water decreased to 0.01 ppm from an initial level of 0.4 to 0.5 ppm within eight days of its application to a pond at the rate of 1.12 kg/ha. Only traces of the insecticide were recovered after 14 days. Parathion disappeared at a similar rate in a Utah pond water (WARNICK *et al.* 1966), but the insecticide concentration in the bottom muds increased almost ten times the original concentration during seven days. MILLER *et al.* (1967) reported that parathion persisted at concentrations toxic to aquatic organisms for only 96 hr in cranberry bog irrigation water. GRAETZ *et al.* (1970) compared the rate of parathion degradation

in two lake sediments. Sediments from Lake Nagawicka that received waste water effluent from a nearby city appeared to be more active in degrading parathion than less polluted sediments from Lake Tomahawk sediment. In an aqueous/sediment system containing slightly acidic Lake Tomahawk sediment, only 26% of added parathion was degraded in 92 days, while in the system containing 0.5 and 1.5 g of calcareous Lake Nagawicka sediment, the degradation was faster and at the end of 54 days, 28 and 39% of added parathion was degraded. Rapid inactivation of parathion and other organophosphorus insecticides has also been reported in polluted waters in Japan (YASUNO *et al.* 1965). EICHELBERGER and LICHTENBERG (1971) reported complete hydrolysis of parathion within three weeks in raw river water, but not in distilled water, to *p*-nitrophenol and diethylthiophosphoric acid.

In general, concentrations of parathion detected in water are relatively low, but may still pose residue problems because parathion is toxic to *Daphnia* and other aquatic organisms at concentration of 0.4 to 11 ppb and to bluegill fish at 47 ppb (PERRY 1976).

b) Degradation in water and lake sediments

1. Chemical degradation.—Parathion is relatively more stable in water than in soils but is rapidly hydrolysed in alkaline solution. The rate of hydrolysis increased with temperature. RUZUICKA *et al.* (1967) compared the chemical stability of parathion in a buffer solution (pH, 6.0), Thames river water (pH, 8.0), and Irthing river water (pH, 7.5). The half-life of parathion was 43 hr in buffer solution, 65 hr in Thames river water, and 68 hr in Irthing river water. GRAETZ *et al.* (1970) used dry-heat sterilised and nonsterilised lake sediments to ascertain the relative importance of chemical and microbial degradation in the aquatic environments. Microbial degradation rather than chemical was more important although in dry-heat sterilised systems, sediment-adsorbed parathion and parathion in solution were hydrolysed at extremely slow rates to *p*-nitrophenol and diethylthiophosphoric acid. According to COWART *et al.* (1971), hydrolysis of parathion in water followed pseudo-first-order kinetics with a half-life of 25 days.

Chemical oxidation may serve as an effective and economical means of removing high concentrations of organophosphates and carbamates, and not organochlorine compounds, in natural waters. ROBECK *et al.* (1965) used chlorine, potassium permanganate, and ozone for chemical conversion of parathion in natural waters. Chlorine and potassium permanganate at one to five ppm did convert parathion, but to a more toxic compound, undoubtedly paraoxon. Ozone appeared to be very effective in removing parathion [again, conversion to paraoxon].[2] GOMAA and

[2] Editor's note: See GUNTHER, F. A., D. E. OTT, and M. ITTIG: The oxidation of parathion to paraoxon. II. By use of ozone. Bull. Environ. Contam. Toxicol. **5**, 87 (1970).

FAUST (1971 and 1972) studied the kinetics of chemical oxidation of parathion, paraoxon, and *p*-nitrophenol using oxidising agents, chlorine, potassium permanganate, and chlorine dioxide. Several factors *viz.*, temperature, pH, concentration of the oxidant, concentration and type of the pesticide, and contact time, influenced the rate of reactions involving chemical oxidants. For example, the parathion-potassium permanganate reaction produced *p*-nitrophenol at pH 9.0 and a more toxic product, paraoxon, under neutral or acidic conditions. Potassium permanganate was more effective in converting parathion than chlorine and chlorine dioxide. Products of chemical oxidation of parathion detected were paraoxon, *p*-nitrophenol, 2,4-dinitrophenol, 2-hydroxy-5-nitrobenzoic acid, and 2,2-dihydroxy-5,5-dinitrodiphenyl. Paraoxon and 2,4-dinitrophenol are known for their toxic properties. It is, therefore, important to control the environmental factors to enhance the rates of chemical oxidation and, at the same time, prevent the accumulation of any toxic product(s) of chemical reaction.

2. Biological degradation.—The rapid inactivation of fenitrothion and related organophosphates, parathion, and methyl parathion, in polluted waters of Japan was presumably caused by bacteria (YASUNO *et al.* 1965). A bacterium, *Bacillus subtilis*, isolated from these polluted water environments, reduced these insecticides to their amino analogs. The bacterium decomposed methyl parathion past methyl aminoparathion by desmethylation to desmethylaminoparathion (MIYAMOTO *et al.* 1966).

Likewise, Sumithion (fenitrothion) was metabolised by the same bacterium to aminosumithion and desmethylaminosumithion. Both Sumithion and methylparathion contain methoxy groups, in contrast to ethoxy group in parathion, but are related to parathion in possessing common P-O-C, P=S, and nitro groups. Likewise, parathion may undergo successive nitro group reduction and desethylation reactions, but products beyond aminoparathion have not been characterised. Perhaps the unidentified metabolite formed in rice straw-amended soil under flooded conditions (RAJARAM and SETHUNATHAN 1975) is a product of desethylation of aminoparathion.

GRAETZ *et al.* (1970) showed that parathion was readily susceptible to microbial degradation in biologically active lake sediments under both aerobic and anaerobic conditions. In one % peptone solution inoculated with microorganisms from lake sediments, parathion was transformed to aminoparathion. Aminoparathion persisted under anaerobic conditions, but was further degraded under aerobic conditions to a metabolite possessing both phosphoric acid and benzenoid moieties of aminoparathion. In natural lake sediment system supplemented with peptone, aminoparathion, generated as a major metabolite, was in turn degraded to two compounds possessing phosphoric acid and benzenoid moieties of aminoparathion; one of these compounds appeared to be identical to the microbially-produced metabolite in peptone solution.

Degradation of parathion has been demonstrated also in cultures of

algae isolated from water environments. AHMED and CASIDA (1958) recovered only 37% parathion after eight days of its incubation with fresh water alga, *Chlorella pyrenoidosa proteose*. MACKIEWICZ *et al.* (1969) reported that the same alga produced aminoparathion as a major metabolite and an unidentified compound as a minor metabolite. In a subsequent study, the same alga was shown to convert parathion to aminoparathion and three unidentified compounds, two of which were cholinesterase inhibitors (ZUCKERMAN *et al.* 1970). SATO and KUBO (1964) reported that algae accelerated the decomposition of parathion in rice paddies. Algal bloom is a common phenomenon in flood waters of rice fields, but the role of algae in the inactivation of pesticides applied to rice culture is little understood.

In a study on bioaccumulation of pesticides by microbes, cultures of a blue-green alga (*Anacystis nidulans*), a green alga (*Scenedesmus obliqus*), a flagellate (*Euglena gracilis*), and two ciliates (*Paramecium bursaria* and *P. multimicronucleatum*) concentrated parathion 50 to 116 times during a seven-day exposure period (GREGORY *et al.* 1969). The accumulation of parathion by microorganisms and protozoa is probably responsible, at least in part, for the long-term, low-level retention of this insecticide reported in the environment (STEWART *et al.* 1971).

Complete hydrolysis of parathion, rather than nitro group reduction, was noticed in raw river water within three weeks of incubation (EICHELBERGER and LICHTENBERG 1971). This was attributed to biological hydrolysis since little degradation occurred in distilled water during the corresponding period; however, a microbial role in the hydrolysis was not proved conclusively. Recent studies at our laboratory showed that parathion was rapidly hydrolysed in nonsterile samples of water originating from ponds, rivers, and lakes, presumably by a biological action (SUDHAKAR-BARIK 1976). Evidence suggests that biological hydrolysis of parathion is more common and widespread in aquatic ecosystems than hitherto believed.

IV. Stability of breakdown products of parathion

The major breakdown products of parathion detected in soil and aquatic environments are aminoparathion, *p*-nitrophenol, diethylthiophosphoric acid, and paraoxon. Paraoxon, *p*-nitrophenol, and a wide array of products are generated also during chemical oxidation of parathion by chlorine, chlorine dioxide, and potassium permanganate in water-treatment processes (GOMAA and FAUST 1971 and 1972). *p*-Nitrophenol was the major product of potassium permanganate-parathion reaction at pH 9.0 while under neutral or acidic conditions the reaction yielded paraoxon and not *p*-nitrophenol. As far as parathion is concerned, both nitro group reduction and hydrolysis are essentially detoxifying reactions because of the relatively low toxicity of the immediate products of these reactions, viz., aminoparathion, *p*-nitrophenol, and diethylthio-

phosphoric acid. Paraoxon is more toxic, but generally less persistent, than parathion. There are reports, however, that *p*-nitrophenol, and other substituted phenols, often impart off-odor in drinking water, particularly following conventional chlorination treatment in a water plant (BAKER 1963, GOMAA and FAUST 1972 and 1974). From the standpoint of pollution hazard, total mineralisation of parathion beyond paraoxon, *p*-nitrophenol, and aminoparathion to inorganic end products is, therefore, both useful and necessary.

a) Paraoxon

Paraoxon, the oxygen analog of parathion, is potentially more toxic to insects and mammals than the parent compound parathion. Paraoxon was detected during chemical (GOMAA and FAUST 1971 and 1972) and photolytic (FRAWLEY *et al.* 1958, EL-REFAI and HOPKINS 1966, JOINER *et al.* 1971) reactions, and in adapted mixed cultures (MUNNECKE and HSIEH 1976); however, residue problems from paraoxon do not seem to exist in parathion-polluted environments because of its extreme susceptibility to chemical/biological hydrolysis.[3] LICHTENSTEIN and SCHULZ (1964) analysed paraoxon residues in a loamy soil by bioassay and colorimetric techniques after its application at 20 ppm. Complete disappearance of paraoxon occurred within 24 hr accompanied by the formation of *p*-nitrophenol as a major metabolite. Paraoxon, produced in adapted mixed cultures *via* oxidative pathway of secondary importance, was enzymatically hydrolysed to *p*-nitrophenol and diethylphosphoric acid (MUNNECKE and HSIEH 1976). At low substrate concentrations, a crude enzyme preparation from adapted mixed cultures hydrolysed paraoxon at a rate of 11% faster than parathion. Kinetic studies with crude enzyme showed the maximal rate of paraoxon hydrolysis as six μmol/mg of protein/min.

The rate of hydrolysis of organophosphates is determined by the properties of the group attached to the phosphorus (O'BRIEN 1969). The half-lives of some organophosphates at pH 8.0 and at 25°C are: TEPP 73 hr; paraoxon, 22,200 hr; and parathion 203,000 hr (KILGORE 1975). GOMAA and FAUST (1972) studied the kinetics of the chemical hydrolysis and oxidation of parathion and paraoxon in aquatic environments. Both parathion and paraoxon were hydrolysed in alkaline solutions; hydrolysis of paraoxon proceeded at a rate faster than that of parathion at pH 9.0 while the reverse was true under acidic or near neutral conditions. Hydrolytic half-lives at pH 9.0 were 523 hrs for parathion and 70 hrs for paraoxon as compared to the values of 2,594 hrs for parathion and 3,450 hrs for paraoxon at pH 7.4. Furthermore, amendment with potassium

---

[3] Editor's note: However, see GUNTHER, F. A., Y. IWATA, G. E. CARMAN, and C. A. SMITH: The citrus reentry problem: Research on its causes and effects, and approaches to its minimization. Residue Reviews **67**, 1 (1977); these authors review paraoxon residue persistence under certain field conditions.

permanganate accelerated the removal of parathion and its oxygen analog at pH 9.0 and within two hr hydrolysis of both compounds was almost complete.

b) Aminoparathion and p*-aminophenol*

The major metabolite of nitro group reduction of parathion is aminoparathion. Aminoparathion is relatively unstable in soils and in microbial cultures. Most of the aminoparathion applied to a loamy soil at 20 ppm disappeared within 24 hr; no residues of aminoparathion were recovered after three additional days of incubation (LICHTENSTEIN and SCHULZ 1964). *p*-Aminophenol, a predicted hydrolysis product of aminoparathion, was not detected during the decomposition of aminoparathion in soils. Interestingly, no residues of *p*-aminophenol were recovered even from initial soil samples treated with 20 ppm *p*-aminophenol, indicating its extreme instability and consequent loss during extraction and analysis; however, in adapted mixed cultures, aminoparathion produced from parathion under low oxygen tension was hydrolysed to *p*-aminophenol and diethylthiophosphoric acid (MUNNECKE and HSIEH 1976). *p*-Aminophenol was subsequently converted to a brown-colored compound, presumably a polymer that resisted further degradation under aerobic conditions. GRAETZ *et al.* (1970) found that aminoparathion formed in both aerobic and anaerobic media inoculated with microorganisms from a lake sediment was further degraded under aerobic conditions to an unidentified benzene-soluble compound; *p*-aminophenol was not detected. Under anaerobic conditions, aminoparathion resisted further degradation. A cell-free preparation from *Flavobacterium* sp. hydrolysed parathion and not aminoparathion despite a common P=S bond (SETHUNATHAN and YOSHIDA 1972). On the other hand, in a preliminary experiment, parathion hydrolase obtained from adapted mixed cultures appeared to hydrolyse aminoparathion (MUNNECKE and HSIEH 1975 b).

c) Nitrophenols

Phenol derivatives have wide applications in agriculture as herbicides, insecticides, and fungicides. Moreover, phenols are formed as key intermediates in the breakdown of aromatic compounds by soil microorganisms. From the standpoint of pollution, undesirable flavor/odor often result from conventional chlorination of waters containing extremely low concentrations of phenolic compounds. Therefore, *p*-nitrophenol, generated by hydrolysis of parathion and related nitroaromatic compounds, should be degraded further to minimize odor hazard in water-treatment processes. The following discussion deals especially with the metabolism of *p*-nitrophenol and related nitroaromatic compounds by microorganisms.

The introduction of polar groups such as OH^-, NH_2^-, $N\text{-}C^-(O)^-$, COO^-, and NO_2^- to the benzene ring provides a new focal point for

microbial attack in the metabolism of aromatic compounds (HELLING *et al.* 1971, WOODCOCK 1971). The type, position, and number of substituents in the benzene ring influence the rates of degradation of several organic compounds by microorganisms (ALEXANDER and LUSTIGMAN 1966). Biological degradation of nitroaromatic compounds involves two modes of attack, one involving the reduction of a nitro group to an amino group and the other involving the oxidation of a nitro group as nitrite with the concomitant formation of phenol; however, little direct evidence exists with respect to the reduction of the nitro group in nitrophenols. MCCORMICK *et al.* (1976) provided indirect evidence for more rapid reduction of *ortho*-nitro group than for the *para*-nitro group in nitrophenols by comparing the relative rates of hydrogen consumption by cell-free extracts of *Veillonella alkalescens* on 40 mono-, di-, and trinitroaromatic compounds. In yet another instance, both nitro groups in 2,4-dinitrophenol were found to be vulnerable to reduction by a fungus *Fusarium oxysporum* leading to the formation of 2-amino-4- and 4-amino-2-nitrophenol together with an unidentified metabolite (MADHOSINGH 1961). TEWFIK and EVANS (1966) reported successive reduction of both nitro groups in dinitro-*o*-cresol to yield first 3-amino-5-nitro-*o*-cresol and then 3-methyl-5-aminocatechol.

Reports are not lacking to show that the release of nitro group as nitrite is more common than reduction in the microbial metabolism of mono-, di-, and trinitrophenols. ERIKSON (1941) reported that certain strains of *Micromonospora* utilised picric acid (trinitrophenol) and trinitroresorcinol. MOORE (1949) showed that two unspecified proactinomycetes metabolised nitrobenzene as a simultaneous source of carbon and nitrogen.

SIMPSON and EVANS (1953) found that two strains of *Pseudomonas*, isolated from filter beds for detoxication of effluents from a chemical factory, decomposed *o*- and *p*-nitrophenols, respectively, with the formation of nitrite; the strain degrading the *ortho* isomer had no action on the *para* isomer and *vice versa*. Another bacterium formed nitrite from 2,4-dinitrophenol. The mechanism of detoxication, according to the authors, apparently involved an oxidative elimination of the nitro group prior to ring cleavage. The catechol formed by the *o*-nitrophenol-decomposing strain of *Pseudomonas* was then utilised.

A strain of *Corynebacterium simplex*, isolated from soil treated with the herbicide 4-6-dinitro-*o*-cresol (DNOC), utilised DNOC as the sole carbon and nitrogen source liberating more than 70% of the nitrogen in DNOC as nitrite (JENSEN and GUNDERSEN 1955, GUNDERSEN and JENSEN 1956). The decomposition of DNOC proceeded presumably by an initial attack at the *para*-nitro group, followed by attack on the second nitro group. Besides DNOC, *p*-nitrophenol, 2,4-dinitrophenol, and 2,4,6-trinitrophenol (picric acid) were also degraded by the same bacterium with the formation of nitrite. Similarly, several bacterial strains from the soil apparently belonging to *Arthrobacter* or *Pseudomonas* metabolised

DNOC and other aromatic nitro compounds liberating nitrite; none of these strains formed nitrite from dinitrobutyl phenol or *o*- and *m*-nitrophenols (Jensen and Lautrup-Larsen 1967). An unidentified bacterium readily produced nitrite from 2,4-dinitrophenol and DNOC and not from *p*-nitrophenol despite a common *para*-nitro group (Teuteberg 1964).

Raymond and Alexander (1971) reported that a soil bacterium metabolised *p*-nitrophenol as a sole carbon and energy source. Growing cells of this organism produced nitrite from *p*-nitrophenol in stoichiometric amounts while resting cells treated with chloroform generated 4-nitrocatechol from *p*-nitrophenol, but not the end product nitrite. More recently, two strains of *Pseudomonas* (strains 1 and 2) and a *Bacillus* strain exhibiting exceptional capacity to hydrolyse parathion and/or metabolise related nitrophenols have been isolated from flooded soil treated with parathion (Siddaramappa *et al.* 1973, Sudhakar-Barik *et al.* 1976). *Pseudomonas* strain 1 hydrolysed parathion without proliferating and then metabolised *p*-nitrophenol as a sole energy source releasing nitrite (Siddaramappa *et al.* 1973) and carbon dioxide (Sudhakar-Barik *et al.* 1976). *Pseudomonas* strain 2 and *Bacillus* sp. metabolised *p*-nitrophenol while being ineffective in hydrolysing parathion. The *Bacillus* sp. produced nitrite from *p*-nitrophenol (Siddaramappa *et al.* 1973) while with *Pseudomonas* strain 2 the reaction ceased at 4-nitrocatechol (Siddaramappa 1975). Both the *Pseudomonas* strain 1 and the *Bacillus* sp. released nitrite from 4-nitrocatechol indicating the possibility of its formation as an intermediate of *p*-nitrophenol metabolism (Raymond and Alexander 1971) although no accumulation of 4-nitrocatechol occurred during conversion of *p*-nitrophenol to nitrite by these bacteria. Hydrolysis of parathion by *Pseudomonas* strain 1 was accomplished by whole cells (growing and resting) and cell-free preparations of the bacterium; only whole cells, not cell-free preparations, metabolised *p*-nitrophenol (Sudhakar-Barik 1976).

Meta-substituted aromatic compounds are known for their extreme resistance to microbial degradation (Gundersen and Jensen 1956, Kameda *et al.* 1958, Cartwright and Cain 1959, Alexander and Lustigman 1966). In a systematic study, Kameda *et al.* (1958) isolated with ease several strains of *Pseudomonas* capable of utilising *o*- and *p*-methoxybenzoate, *o*- and *p*-aminobenzoate, and *p*-nitrobenzoate, but none of the 34 pseudomonads utilised *m*-methoxy-1, *m*-amino-, and *m*-nitrobenzoates. *m*-Nitrophenol, despite its known recalcitrance (Jensen and Lautrup-Larsen 1967), was shown to undergo decomposition in bacterial cultures. Raymond and Alexander (1971) showed cometabolism of *m*-nitrophenol to nitrohydroquinone by *p*-nitrophenol-grown cells of a bacterium. In a more recent study, Sudhakar-Barik *et al.* (1976) demonstrated more complete transformation of *m*-nitrophenol by a *p*-nitrophenol-degrading *Bacillus* sp. leading to nitrite, presumably formed prior to ring cleavage, and phenol. These results lead to the conclusion that bacteria possessing exceptional capacity to hydrolyse parathion and/or metabolise a variety

Table I. *Microorganisms degrading parathion and its hydrolysis product, p-nitrophenol.*

Compound decomposed	Degrading organism		Decomposition product detected	Related compounds attacked	Reference
	Name	Source			
Parathion					
As sole carbon source	*Pseudomonas* sp.	Flooded soil	*p*-Nitrophenol, nitrite, carbon dioxide	*p*-Nitrophenol, 2,4-dinitrophenol, 4-nitrocatechol	Siddaramappa *et al.* 1973, Sudhakar-Barik *et al.* 1976
	Flavobacterium sp.	Flooded soil	*p*-Nitrophenol	Diazinon, Dursban	Sethunathan & Yoshida 1973 b
	Mixed cultures with at least 9 bacterial isolates (4 *Pseudomonas*; *Brevibacterium* sp., *Azotomonas* sp., *Xanthomonas* sp., and one unidentified)	Soil and sewage	*p*-Nitrophenol, aminoparathion, paraoxon, nitrite, hydroquinone, carbon dioxide	Triazophos, EPN, paraoxon, diazinon, methyl parathion, Dursban, fenitrothion, Cyanophos	Munnecke & Hsieh 1976, Munnecke 1976 a
	Chlorella pyrenoidosa proteose	Water	Aminoparathion and 1-3 unidentified metabolites	——	Ahmed & Casida 1958, Mackiewics *et al.* 1969, Zuckerman *et al.* 1970
In the presence of additional carbon source	*Trichoderma viride*	Soil	——	——	Matsumura & Boush 1968
	Rhizobium japonicum / *Rhizobium meliloti*	Soil	Aminoparathion, *p*-nitrophenol	——	Mick & Dahm 1970
	Penicillium waksmanii	Flooded soil	Aminoparathion	——	Rao & Sethunathan 1974

	Bacillus subtilis	Water	Aminoparathion	Fenitrothion, methyl parathion	Yasuno *et al.* 1965, Miyamoto *et al.* 1966
p-Nitrophenol	*Pseudomonas* sp.	Filter bed	——	——	Simpson & Evans 1953
	Corynebacterium simplex	Soil	Nitrite	Dinitro-*o*-cresol, 2,4-dinitrophenol, 2,4,6-trinitrophenol	Jensen & Gundersen 1955, Gundersen & Jensen 1956
	Pseudomonas sp.	Flooded soil	Nitrite	*m*-Nitrophenol	Raymond & Alexander 1971
	Pseudomonas sp.	Flooded soil	Nitrite	Parathion, 2,4-dinitrophenol, 4-nitrocatechol	Siddaramappa *et al.* 1973, Sudhakar-Barik *et al.* 1976
	Pseudomonas sp.	Flooded soil	4-Nitrocatechol	——	Siddaramappa 1975
	Bacillus sp.	Flooded soil	Nitrite	*m*-Nitrophenol, 4-nitrocatechol	Siddaramappa *et al.* 1973, Sudhakar-Barik *et al.* 1976

of related nitrophenols can develop and proliferate in flooded soils following repeated applications of parathion.

V. Pathways of parathion metabolism

Degradation of parathion in soil, water, and microbial cultures proceeds via nitro group reduction to aminoparathion and/or via hydrolysis at the P-O-C ester bond yielding *p*-nitrophenol and diethylthiophosphoric acid. Chemically, parathion is reduced to aminoparathion involving three consecutive steps as follows:

$$\underset{\text{nitro}}{R\text{–}NO_2} \xrightarrow[-H_2O]{+2H} \underset{\text{nitroso}}{R\text{–}NO} \xrightarrow{+2H} \underset{\text{hydroxylamino}}{R\text{–}NHOH} \xrightarrow[-H_2O]{+2H} \underset{\text{amino}}{R\text{–}NH_2}$$

Enzymatic reduction of parathion to aminoparathion via hydroxylaminoparathion as an intermediate has been demonstrated under anaerobic conditions in plant systems using spinach homogenate (SUZUKI and UCHIYAMA 1975 a) and spinach chloroplasts (SUZUKI and UCHIYAMA 1975 b and c). Similarly, reduction is a major means of parathion metabolism in soil, water, and microbial systems under aerobic and anaerobic conditions, but the sequence in which parathion is reduced to aminoparathion is not known. *p*-Nitrophenol and diethylthiophosphoric acid, products of parathion hydrolysis, are relatively less persistent than the parent molecule and are rapidly metabolised. Evidence suggests that degradation of *p*-nitrophenol by bacteria involves ring hydroxylation with 4-nitrocatechol as key intermediate prior to ring cleavage yielding nitrite and carbon dioxide as end products; however, stepwise reactions in *p*-nitrophenol metabolism leading to the inorganic end products are largely unknown. Also, little information is available on the fate of diethylthiophosphoric acid in aquatic and soil ecosystems. Figure 3 summarises the proposed pathways of parathion metabolism in soil, water, and microbial systems.

VI. Conclusions

Although parathion seldom gives rise to significant residues in soil and aquatic environments, its reported long-term persistence, though at low levels, in some Canadian soils over several years raises problems of environmental contamination. Microorganisms, rather than chemical agents, are almost exclusively involved in the detoxication of parathion in the environment; factors generally associated with microbial activity, *viz.*, temperature, moisture, and organic matter, have been implicated in its degradation. A list of microorganisms capable of degrading parathion and its hydrolysis product, *p*-nitrophenol is presented in Table I. Most recent research has demonstrated that bacteria readily build up in

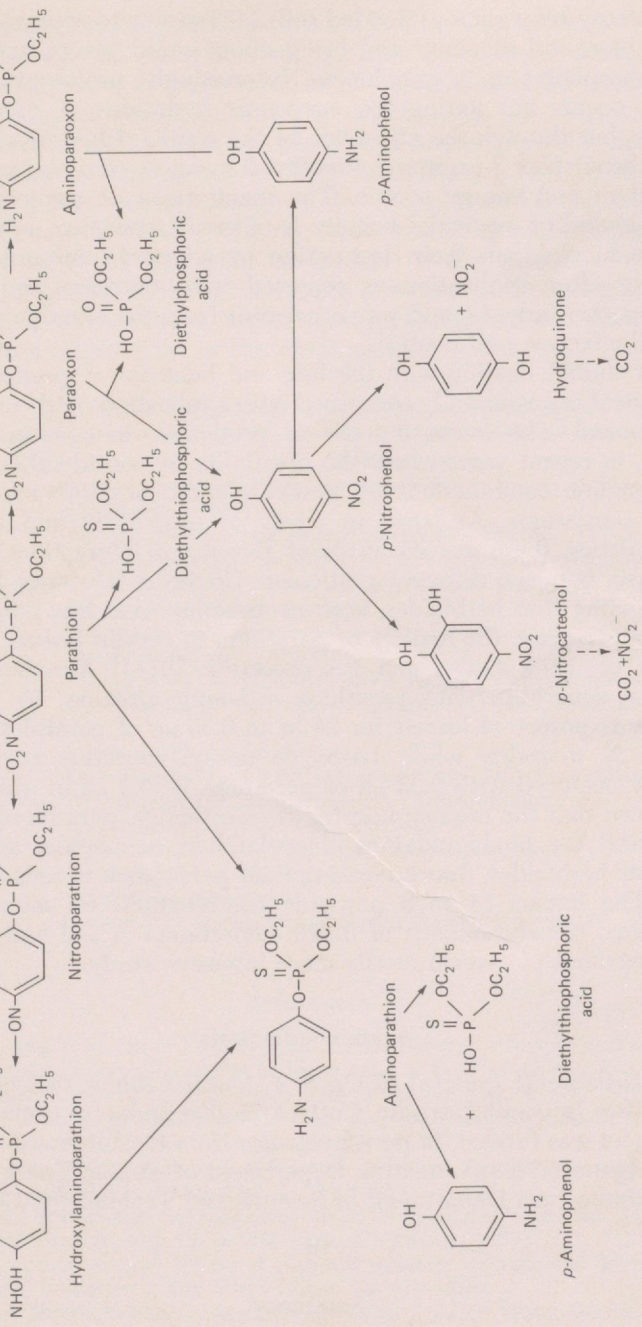


Fig. 3. Proposed pathways of parathion metabolism in microbial, plant, and animal systems.

natural ecosystems such as flooded soils in response to repeated additions of parathion and diazinon and bring about rapid inactivation of these organophosphates upon enrichment. Interestingly, proliferation of these bacteria occurs not during the enzymatic hydrolysis of parathion and diazinon, but through the utilisation of the resultant hydrolysis products, *p*-nitrophenol and 2-isopropyl-4-methyl-6-hydroxy pyrimidine, as exclusive carbon and energy source. The inactivation of pesticides after a desired period of pesticidal activity is of great advantage in minimizing pollution hazard, but their destruction by adapted microorganisms immediately after application as reported with diazinon and parathion following enrichment could prove harmful from the standpoint of economy and effective pest control.

Most studies dealing with the fate and behavior of pesticides in the environment are essentially concerned with applications with an individual test chemical. The increasing use of pesticides, in combination or in rotation, in recent years raises the possibility of considerable impact of such pesticide combinations on pesticide-microflora interactions in the natural ecosystems. At least, in terms of phytotoxic and insecticidal characteristics, there are instances of significant interactions, beneficial or adverse, between different pesticides. Of direct relevance here is the recent finding that herbicides, such as atrazine, simazine, monuron, and 2,4-D, can increase the toxicity of parathion to certain insects (LICHTENSTEIN *et al.* 1973, LIANG and LICHTENSTEIN 1974). Atrazine exhibited maximum synergism with parathion although atrazine, by itself, was nontoxic. Exposure of insects for 24 hr to 0.35 μg of parathion alone led to eight % mortality while, based on dosage mortality curves, 50 % mortality occurred with 0.35 μg of parathion + 6.0 μg of atrazine. It is also known that the organophosphorus insecticides, parathion and diazinon, inhibit the biodegradation of acylanilide, acetamide, and phenylcarbamate herbicides, thus increasing their persistence in soils (KAUFMAN 1972). The impact of such pesticide combinations on microflora and biochemical transformations of major importance in soil and water environments and *vice-versa* merits more intensive study.

Acknowledgments

The authors are grateful to Dr. S. Y. Padmanabhan, former Director, Central Rice Research Institute, Cuttack-753006, India for encouragement. This project was funded, in part, by grants from the International Atomic Energy Agency, Vienna, Austria, Department of Atomic Energy, Government of India, and Department of Science and Technology, Government of India.

Summary

This review describes the fate and behavior of parathion in soil and water environments. Microbial degradation, rather than chemical, is the

best means of detoxication of parathion in the environment. More recently, pathways of parathion metabolism have been demonstrated, though not stepwise, in pure cultures of bacteria isolated from flooded soils or in adapted mixed cultures, in addition to the already existing knowledge on its metabolism by animal and higher plant tissues. Flooding accelerates its degradation in soils via reduction and/or hydrolysis; the effect of added organic matter under flooded conditions is governed by the pathway of its degradation. Most recent demonstration of the increased toxicity of parathion to insects in the presence of certain herbicides emphasizes the need for more intensive study on the impact of such pesticide combinations, rather than an individual test chemical, on soil-microflora interactions in the environment.

Table II. *Pesticides and other chemicals mentioned in text.*

Common name	Chemical name
ABS	alkyl benzenesulfonate
Atrazine	2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine
Ciodrin	2-methylbenzyl-3-(dimethoxyphosphinyloxy) *cis*-crotonate
Cyanophos	*O,O*-dimethyl-*O*-(*p*-cyanophenyl)-thiophosphate
2,4-D	2,4-dichlorophenoxyacetic acid
Diazinon	*O-O*-diethyl-*O*-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate
Dursban	*O,O*-diethyl- *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate
Fenitrothion (Sumithion)	*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate
LAS	linear alkyl benzenesulfonate
Malathion	*O,O*-dimethyl-*S*-[1,2-bis(ethoxycarbonyl) ethyl] phosphorodithioate
Methyl parathion	*O,O*-dimethyl *O*-(*p*-nitrophenyl) phosphorothioate
Monuron	3-(*p*-chlorophenyl)-1,1-dimethylurea
Parathion	*O,O*-diethyl *O*-(*p*-nitrophenyl) phosphorothioate
Simazine	2-chloro-4,6-bis(ethylamino)-*s*-triazine
TEPP	tetraethyl pyrophosphate
Triazophos	1-phenyl-3-(diethoxythiophosphoryloxy)-1,2,4-triazol

References

Adamson, J., and T. D. Inch: Possible relationships between structure and mechanism of degradation of organophosphorus insecticides in the soil environment. Proc. 7th Brit. Insecticide and Fungicide Conference, Brighton, Eng., p. 65 (1973).

Ahmed, M. K., and J. E. Casida: Metabolism of some organophosphorus insecticides by microorganisms. J. Econ. Entomol. **51**, 59 (1958).

Alexander, M.: Biodegradation: Problems of molecular recalcitrance and microbial fallibility. Adv. Applied Microbiol. **7**, 35 (1965).

—— Microbial degradation and biological effects of pesticides in soil. In: Soil biology, reviews of research. Vol. IX, p. 209. Paris: UNESCO (1969).

——, and B. K. Lustigman: Effects of chemical structure on microbial degradation of substituted benzenes. J. Agr. Food Chem. **14**, 410 (1966).

Baker, R. A.: Threshold odors of organic chemicals. J. Amer. Water Works Assoc. **55**, 913 (1963).

Bowman, J. S., and E. J. Orloski: Mosquito News. **26**, 557 (1966), [Cited by Perry, A. S. (1976)].

BURKHARDT, C. C., and M. L. FAIRCHILD: Bioassay of field-treated soils to determine bioactivity and movement of insecticides. J. Econ. Entomol. **60**, 1602 (1967).

CARLO, C. P., D. ASHDOWN, and V. G. HELLER: Persistence of parathion, toxaphene and methoxychlor in soil. Okla. Agr. Exp. Sta. Tech. Bull. T-42, p. 11 (1952).

CARTWRIGHT, N. J., and R. B. CAIN: Bacterial degradation of nitrobenzoic acids. Biochem. J. **71**, 248 (1959).

CHISHOLM, D., and A. W. MACPHEE: Persistence and effects of some pesticides in soil. J. Econ. Entomol. **65**, 1010 (1972).

—— ——, and C. R. MACEACHERN: Effects of repeated applications of pesticides to soil. Can. J. Agr. Sci. **35**, 433 (1955).

COLEY, G., and C. N. STUTZ: Treatment of parathion wastes and other organics. J. Water Pollut. Control Fed. **38**, 1345 (1966).

COWART, R. P., F. L. BONNER, and E. A. EPPS, Jr.: Rate of hydrolysis of seven organophosphate pesticides. Bull. Environ. Contam. Toxicol. **6**, 231 (1971).

EICHELBERGER, J. W., and J. J. LICHTENBERG: Persistence of pesticides in river water. Environ. Sci. Technol. **5**, 541 (1971).

EL-REFAI, A., and T. L. HOPKINS: Parathion absorption, translocation and conversion to paraoxon in bean plants. J. Agr. Food Chem. **14**, 588 (1966).

ERIKSON, D.: Studies on some lake-mud strains of *Micromonospora*. J. Bact. **41**, 277 (1941).

FRAWLEY, J. P., J. W. COOK, J. R. BLAKE, and O. G. FITZHUGH: Effect of light on chemical and biological properties of parathion. J. Agr. Food Chem. **6**, 28 (1958).

GETZIN, L. W., and I. ROSEFIELD: Organophosphorus insecticides degradation by heat-labile substances in soil. J. Agr. Food Chem. **16**, 598 (1968).

GOMAA, H. M., and S. D. FAUST: Thermodynamic stability of selected organic pesticides in aquatic environments. In S. D. FAUST, and J. V. HUNTER (eds.): Organic compounds in aquatic environments, p. 341. New York: Marcel Dekker (1971).

—— —— Chemical hydrolysis and oxidation of parathion and paraoxon in aquatic environments. Adv. Chem. Ser. **111**, 189 (1972).

—— —— Removal of organic pesticides from water to improve quality. In: Pesticides in soil and water, p. 413. Madison, Wis.: Soil Sci. Soc. Amer. (1974).

GRAETZ, D. A., C. CHESTERS, T. C. DANIEL, L. W. NEWLAND, and G. B. LEE: Parathion degradation in lake sediments. J. Water Pollut. Control Fed. **42**, R76 (1970).

GREGORY, W. W. Jr., J. K. REED, and L. E. PRIESTER, Jr.: Accumulation of parathion and DDT by some algae and protozoa. J. Protozool. **16**, 69 (1969).

GRIFFITHS, D. C., and N. WALKER: Microbiological degradation of parathion. Meded. Rijksfac. LandbWet. Gent. **35**, 805 (1970).

GUNDERSEN, K., and H. L. JENSEN: A soil bacterium decomposing organic nitro compounds. Acta Agr. Scand. **6**, 100 (1956).

HARRIS, C. R., and J. R. W. MILES: Pesticide residues in the Great Lakes Region of Canada. Residue Reviews **57**, 27 (1975).

——, and W. W. SANS: Insecticide residues in soils on 16 farms in Southwestern Ontario between 1964-1969. Pest. Monit. J. **5**, 259 (1971).

HELLING, C. S., P. C. KEARNEY, and M. ALEXANDER: Behavior of pesticides in soils. Adv. Agron. **23**, 147 (1971).

HOWE, R. H. L.: Toxic wastes degradation and disposal. Process Biochem. **4**, 25 (1969).

HSIEH, D. P. H., and D. M. MUNNECKE: Accelerated microbial degradation of concentrated parathion. In G. TREVI (ed.): Fermentation technology today, p. 551. Tokyo: Soc. Fermentation Technol. (1972).

IWATA, Y., W. E. WESTLAKE, and F. A. GUNTHER: Persistence of parathion in six California soils under laboratory conditions. Arch. Environ. Contam. Toxicol. **1**, 84 (1973).

JENSEN, H. L., and K. GUNDERSEN: Biological decomposition of aromatic nitrocompounds. Nature **175**, 341 (1955).

——, and G. LAUTRUP-LARSEN: Microorganisms that decompose nitroaromatic com-

pounds, with special reference to dinitro-ortho-cresol. Acta Agr. Scand. **17**, 115 (1967).

JOINER, R. L., H. W. CHAMBERS, and K. P. BAETCKE: Toxicity of parathion and several of its photoalteration products to boll weevils. Bull. Environ. Contam. Toxicol. **6**, 220 (1971).

KAMEDA, Y., E. TOYOURA, and Y. KIMURA: Kanazawa Daigaku yakugakubu kenkyu Nempo. **7**, 37 (1957); through Chem. Abstr. **52**, 4081 (1958).

KASTING, R., and J. C. WOODWARD: Persistence and toxicity of parathion when added to the soil. Sci. Agr. **31**, 133 (1951).

KAUFMAN, D. D.: Degradation of pesticide combinations. In A. S. TAHORI (ed.): Fate of pesticides in environment, p. 175. New York: Gordon and Breach (1972).

KEARNEY, P. C., and C. S. HELLING: Reactions of pesticides in soils. Residue Reviews **25**, 25 (1969).

——, R. G. NASH, and A. R. ISENSEE: Persistence of pesticide residues in soils. In M. W. MILLER and G. G. BERG (eds.): Chemical fallout. Current research on persistent pesticides. Illinois: Thomas (1969).

KILGORE, W. W.: Mechanism responsible for the disappearance of pesticides from the environment. In: Four reviews on important themes. Rome: FAO (1975).

KISHK, F. M., T. EL-ESSAWI, S. ABDEL-GHAFAR, and M. B. ABOU-DONIA: Hydrolysis of methyl parathion in soils. J. Agr. Food Chem. **24**, 305 (1976).

KONRAD, J. C., D. E. ARMSTRONG, and G. CHESTERS: Soil degradation of diazinon, a phosphorothioate insecticide. Agron. J. **59**, 591 (1967).

——, and G. CHESTERS: Degradation in soils of ciodrin, an organophosphate insecticide. J. Agr. Food Chem. **17**, 226 (1969).

—— —— and D. E. ARMSTRONG: Soil degradation of malathion, a phosphorodithioate insecticide. Soil Sci. Soc. Amer. Proc. **33**, 259 (1969).

KULSHRESHTHA, J. P., A. ANJANEYALU, and S. Y. PADMANABHAN: The disastrous brown planthopper attack in Kerala. Indian Farming **24** (9), 5 (1974).

LIANG, T. T., and E. P. LICHTENSTEIN: Synergism of insecticides by herbicides: Effect of environmental factors. Science **186**, 1128 (1974).

LICHTENSTEIN, E. P.: Increase of persistence and toxicity of parathion and diazinon in soils with detergents. J. Econ. Entomol. **59**, 985 (1966).

——, and K. R. SCHULZ: The effects of moisture and microorganisms on the persistence and metabolism of some organophosphorus insecticides in soils, with special emphasis on parathion. J. Econ. Entomol. **57**, 618 (1964).

——, T. W. FUHREMANN, and K. R. SCHULZ: Effect of sterilizing agents on persistence of parathion and diazinon in soils and water. J. Agr. Food Chem. **16**, 870 (1968).

——, T. T. LIANG, and B. N. ANDEREGG: Synergism of insecticides by herbicides. Science **181**, 847 (1973).

LUDWIG, P. D., H. J. DISHBURGER, J. C. MCNEILL, W. O. MILLER, and J. R. RICE: Biological effects and persistence of Dursban insecticide in a salt-marsh habitat. J. Econ. Entomol. **61**, 626 (1968).

MACKIEWICZ, M., K. H. DEUBERT, H. B. GUNNER, and B. M. ZUCKERMAN: Study of parathion biodegradation using gnotobiotic techniques. J. Agr. Food Chem. **17**, 129 (1969).

MACPHEE, A. W., D. CHISHOLM, and C. R. MACEACHERN: The persistence of certain pesticides in the soil and their effect on crop yields. Can. J. Soil Sci. **40**, 59 (1960).

MADHOSINGH, C.: The metabolic detoxication of 2,4-dinitrophenol by *Fusarium oxysporum*. Can. J. Microbiol. **7**, 553 (1961).

MATSUMURA, F., and G. M. BOUSH: Degradation of insecticides by a soil fungus, *Trichoderma viride*. J. Econ. Entomol. **61**, 610 (1968).

MCCORMICK, N. G., F. E. FEEHERRY, and H. S. LEVINSON: Microbial transformation of 2,4,6-trinitrotoluene and other nitroaromatic compounds. Applied Environ. Microbiol. **31**, 949 (1976).

MICK, D. L., and P. A. DAHM: Metabolism of parathion by two species of *Rhizobium*. J. Econ. Entomol. **63**, 1155 (1970).

MILLER, C. W., N. E. TOMLINSON, and R. L. NORGEREN: Persistence and movement of parathion in irrigation waters. Pest. Monit. J. **1**, 47 (1967).

MINGELGRIN, U., and B. YARON: The effect of calcium salts on the degradation of parathion in sand and soil. Soil Sci. Soc. Amer. Proc. **38**, 914 (1974).

MIYAMOTO, J., K. KITAGAWA, and Y. SATO: Metabolism of organophosphorus insecticides by *Bacillus subtilis*, with special emphasis on Sumithion. Japan J. Exp. Med. **36**, 211 (1966).

MOL, J. C. M., D. L. HARRISON, and R. H. TELFER: Parathion toxicity to sheep and persistence on pasture and in soil. New Zealand J. Agr. Res. **15**, 306 (1972).

MOORE, F. W.: The utilization of pyridine by microorganisms. J. Gen. Microbiol. **3**, 143 (1949).

MULLA, M. S., J. O. KEITH, and F. A. GUNTHER: Persistence and biological effects of parathion residues in water fowl habitats. J. Econ. Entomol. **59**, 1085 (1966).

MUNNECKE, D. M.: Enzymatic hydrolysis of organophosphate insecticides, a possible pesticide disposal method. Applied Environ. Microbiol. **32**, 7 (1976 a).

—— An immobilized pesticide degrading enzyme. 5th Internat. Fermentation Symp., Berlin (mimeographed) (1976 b).

——, and D. P. H. HSIEH: Microbial decontamination of parathion and *p*-nitrophenol in aqueous media. Applied Microbiol. **28**, 212 (1974).

—— —— Microbial metabolism of parathion + xylene pesticide formulation. Applied Microbiol. **30**, 575 (1975 a).

—— —— Development of microbial systems for the disposal of concentrated pesticide suspensions. In: 27th Internat. Symp. Crop Protection, p. 1237, Rijksuniversiteit Gent, Belgium (1975 b).

—— —— Pathways of microbial metabolism of parathion. Applied Environ. Microbiol. **31**, 63 (1976).

NAUMANN, K.: Zur Dynamik der Bodenmikroflora nach Anwerdung von Pflanzenzenzchutzmitteln. V. Untersuchungen über die moglichen Ursachen der stimulierenden Wirkung von Parathion-methyl auf die Bodenbakterien. Phytopathol. Z. **68**, 9 (1970).

NICHOLSON, H. P., H. J. WEBB, G. J. LAUER, R. E. O'BRIEN, A. R. GRZENDA, and D. W. SHANKLIN: Insecticide contamination in farm ponds. I. Origin and duration. Trans. Amer. Fish. Soc. **91**, 213 (1962).

O'BRIEN, R. D.: Insecticides: Action and metabolism. New York: Academic Press (1969).

PERRY, A. S.: Impact of agricultural and forestry uses of pesticides on aquatic resources. In: Impact monitoring of agricultural pesticides, p. 86. Rome: FAO (1976).

PONNAMPERUMA, F. N.: The chemistry of submerged soils. Adv. Agron. **24**, 29 (1972).

RAJARAM, K. P.: Studies on metabolism of pesticides in rice soils. Ph.D. thesis, Orissa Univ. Agr. Technol. Bhubaneswar, India (1975).

——, and N. SETHUNATHAN: Effect of organic sources on the degradation of parathion in flooded alluvial soil. Soil Sci. **119**, 296 (1975).

—— —— A factor inhibiting parathion hydrolysis in organic matter-amended soil under flooded conditions. Plant Soil **44**, 683 (1976).

RAO, A. V., and N. SETHUNATHAN: Degradation of parathion by *Penicillium waksmani* Zaleski isolated from flooded acid sulphate soil. Arch. Microbiol. **97**, 203 (1974).

RAYMOND, D. G. M., and M. ALEXANDER: Microbial metabolism and cometabolism of nitrophenols. Pest. Biochem. Physiol. **1**, 123 (1971).

ROBECK, G. G., K. A. POSTAL, J. M. COHEN, and J. F. KRIEISSL: Effectiveness of water treatment processes in pesticide removal. J. Amer. Water Works Assoc. **57**, 181 (1965).

RUZUICKA, J. H., J. THOMPSON, and B. B. WHEALS: The gas chromatographic determination of organophosphorus pesticides. Part II. A comparative study of hydrolysis rates. J. Chromatog. **31**, 37 (1967).

Sacher, R. M., G. F. Ludwik, and J. M. Deming: Bioactivity and persistence of some parathion formulations in soil. J. Econ. Entomol. **65**, 329 (1972).
Saltzman, S., L. Kliger, and B. Yaron: Adsorption-desorption of parathion as affected by soil organic matter. J. Agr. Food Chem. **20**, 1224 (1972).
——, and B. Yaron: Parathion adsorption from aqueous solutions as influenced by soil components. In A. S. Tahori (ed.): Fate of pesticides in the environment, p. 88. New York: Gordon and Breach (1972).
——, and U. Mingelgrin: The surface catalyzed hydrolysis of parathion on kaolinite. Soil Sci. Soc. Amer. Proc. **38**, 231 (1974).
Sato, R., and K. Kubo: The water pollution caused by organophosphorus insecticides in Japan. Adv. Water Pollut. Res. **1**, 95 (1964).
Sethunathan, N.: Diazinon degradation in submerged soil and rice-paddy water. Adv. Chem. Series **111**, 244 (1972).
—— Microbial degradation of insecticides in flooded soil and in anaerobic cultures. Residue Reviews **47**, 143 (1973 a).
—— Degradation of parathion in flooded acid soils. J. Agr. Food Chem. **21**, 602 (1973 b).
—— Organic matter and parathion degradation in flooded soil. Soil Biol. Biochem. **5**, 641 (1973 c).
——, and M. D. Pathak: Increased biological hydrolysis of diazinon after repeated application in rice paddies. J. Agr. Food Chem. **20**, 586 (1972).
——, and T. Yoshida: Conversion of parathion to *p*-nitrophenol by diazinon-degrading *Flavobacterium* sp. Proc. Ann. Meeting Inst. Environ. Sci. **18**, 255 (1972).
—— —— Parathion degradation in submerged rice soils in the Philippines. J. Agr. Food Chem. **21**, 504 (1973 a).
—— —— A *Flavobacterium* sp. that degrades diazinon and parathion. Can. J. Microbiol. **19**, 873 (1973 b).
Siddaramappa, R.: Biodegradation of insecticides in rice soils. Ph.D. thesis, Orissa Univ. Agr. Technol. Bhubaneswar, India (1975).
——, K. P. Rajaram, and N. Sethunathan: Degradation of parathion by bacteria isolated from flooded soil. Applied Microbiol. **26**, 846 (1973).
Simpson, J. L., and W. C. Evans: The metabolism of nitrophenols by certain bacteria. Biochem. J. **52**, XXIV (1953).
Stevens, T. (1973): Quoted by Harris, C. R., and J. R. W. Miles: Residue Reviews **57**, 27 (1975).
Stewart, D. K. R., D. Chisholm, and M. T. H. Ragab: Long term persistence of parathion in soil. Nature **229**, 47 (1971).
Sudhakar-Barik: Unpublished data (1976).
——, R. Siddaramappa, and N. Sethunathan: Metabolism of nitrophenols by bacteria isolated from parathion-amended flooded soil. Antonie van Leeuwenhoek **42**, In press (1976).
Suzuki, T., and M. Uchiyama: Pathways of nitro reduction of parathion by spinach homogenate. J. Agr. Food Chem. **23**, 281 (1975 a).
—— —— Photoreduction of parathion by spinach chloroplasts. Chem. Pharm. Bull. **23**, 2175 (1975 b).
—— —— Photoreduction of parathion by spinach chloroplasts. **II.** Ferrodoxin-independent photoreduction of parathion by heated chloroplasts with an artificial electron donor system. Chem. Pharm. Bull. **23**, 2290 (1975 c).
Teuteberg, A.: Untersuchungen über den Abbau von Halogenitrobenzolen durch Bodenbalterien. Arch. Microbiol. **48**, 21 (1964).
Tewfik, M. S., and W. C. Evans: The metabolism of 3,5-dinitro-*o*-cresol (DNOC) by soil micro-organisms. Biochem. J. **99**, 31P (1966).
Voerman, S., and F. H. Besemer: Residues of dieldrin, lindane, DDT, and parathion in a light sandy soil after repeated application throughout a period of 15 years. J. Agr. Food Chem. **18**, 717 (1970).
Wahid, P. A.: Unpublished data (1976).

WARNICK, S. L., R. F. CAUFIN, and A. R. CAUFIN: Concentrations and effects of pesticides in aquatic environments. J. Amer. Water Works Assoc. **58**, 601 (1966).

WOLFE, H. R., and W. F. DURHAM: Spillage of pesticides and residues in soil. Proc. 62nd Meet. Wash. St. Hort. Assoc., p. 91 (1966).

——, D. C. STAIFF, J. P. ARMSTRONG, and S. W. COMER: Persistence of parathion in soil. Bull. Environ. Contam. Toxicol. **10**, 1 (1973).

WOODCOCK, D.: Metabolism of fungicides and nematicides in soils. In A. D. MCLAREN, and J. SKUJINS (Eds.): Soil Biochemistry, vol. 2, p. 337. New York: Marcel Dekker (1971).

YARON, B.: Chemical conversion of parathion on soil surfaces. Soil Sci. Soc. Amer. Proc. **39**, 639 (1975).

——, and S. SALTZMAN: Influence of water and temperature on adsorption of parathion in soils. Soil Sci. Soc. Amer. Proc. **36**, 583 (1972).

YASUNO, M., S. HIRAKOSO, M. SASA, and M. UCHIDA: Inactivation of some organophosphorus insecticides by bacteria in polluted water. Japan J. Exp. Med. **35**, 545 (1965).

ZUCKERMAN, B. M., K. DEUBERT, M. MACKIEWICZ, and H. GUNNER: Studies on the biodegradation of parathion. Plant Soil **33**, 273 (1970).

Manuscript received December 7, 1976; accepted December 30, 1976.