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Impact of pesticides on soil and water microflora and fauna in wetland ricefields

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The impact of pesticides on the microorganisms and invertebrates that contribute to the fertility of rice soils are assessed from bibliographic data and field experiments. The paper summarizes the major characteristics of pesticide behavior in wetland soils, analyzes experimental data from an extensive bibliographic survey, and summarizes the results of field studies and surveys. Pesticides often degrade faster in tropical ricefields than in temperate upland soils because of reducing conditions, temperatures, and pH. Floodwater also may favor pesticide dilution. These factors may explain the fact that pesticides had no microbiological effect in 73% of the tests performed in situ. The data from the literature suffer from several biases in terms of pesticides, environments, populations, and activities tested, but they do show some trends: pesticides exhibit more marked impacts in laboratory experiments than in situ, herbicides have greater microbiological and algological impact than insecticides, insecticides have greater impact on water and soil invertebrates than herbicides, bacteria exhibit a higher sensitivitiy to pesticides than other microorganisms, microbial activities exhibit a higher sensitivity to pesticides than population densities, and biological N$_2$ fixation sensitivity to pesticides is higher than average. When significant effects were observed in situ at concentrations corresponding to the recommended level for field application, they most often did not last long. Field experiments showed that N fertilizer had more impact on algal and invertebrate populations in floodwater than pesticides. Pesticides had significant effects on photosynthetic activity in the floodwater and on populations of aquatic oligochaetes in the soil. Field surveys did not show significant long-term effects of pesticides. Available data partly confirm the common belief that pesticides applied at recommended levels and intervals seldom markedly affect soil microorganisms and their activities. However, there is evidence of significant effects on nontarget microorganisms and aquatic and soil invertebrates important for soil fertility. Because few studies have collected field data for the duration of a crop, there are no long-term experiments, and it is premature to draw conclusions on the possible long-term impacts of pesticides on ricefield fertility.

At the levels of inorganic fertilizer usually applied in ricefields, most N absorbed by the plant originates from soil. Available soil N is released by the turnover of a microbial biomass that represents only a few percent of total soil N (Watanabe et al 1988). Crop residues, rhizosphere exudates, algae, and aquatic plants contribute nutrients that replenish the microbial biomass. Nutrients that accumulate in algae and aquatic plants (including biologically fixed N_2) and in the detritus layer at the soil-water interface are recycled by the zooplankton and reincorporated into the soil by oligochaetes (Roger and Kurihara 1988).

A 60% increase in rice production is needed in the next 30 yr (IRRI 1990). Therefore, it is important to understand and predict how factors associated with crop intensification, especially pesticide use, affect soil fertility through their effects on nontarget organisms.

Pesticide behavior in wetland soils

Pesticide use and concentration in farmers' fields. A wide range of pesticides is used in wetland ricefields. About 150 chemicals have been tested for their impact on ricefield microflora. Recommended levels of pesticides for field application range from a few hundred grams to a few kg ai/ha, with a median of about 2 kg. The median is higher for herbicides (2.5 kg ai/ha) than for fungicides (1.7 kg ai/ha) and insecticides (1.1 kg ai/ha).

However, farmers often apply less than the recommended amount of pesticides. A survey conducted in 1989 in 32 farms in the Laguna area of the Philippines indicated that the amount of pesticide applied during a cropping season per field ranged from 0.5 to 2.5 kg ai/ha. The quantities of individual pesticides used per cropping season averaged 0.3 kg ai/ha and did not exceed 1 kg ai/ha (Roger et al 1990).

In this paper, unless otherwise indicated, results obtained at concentrations corresponding to the recommended level for field application (RLFA) are considered. Concentrations of 10-500 ppm, often tested in vitro, are used more to estimate a lethal level than to reflect the field situation.

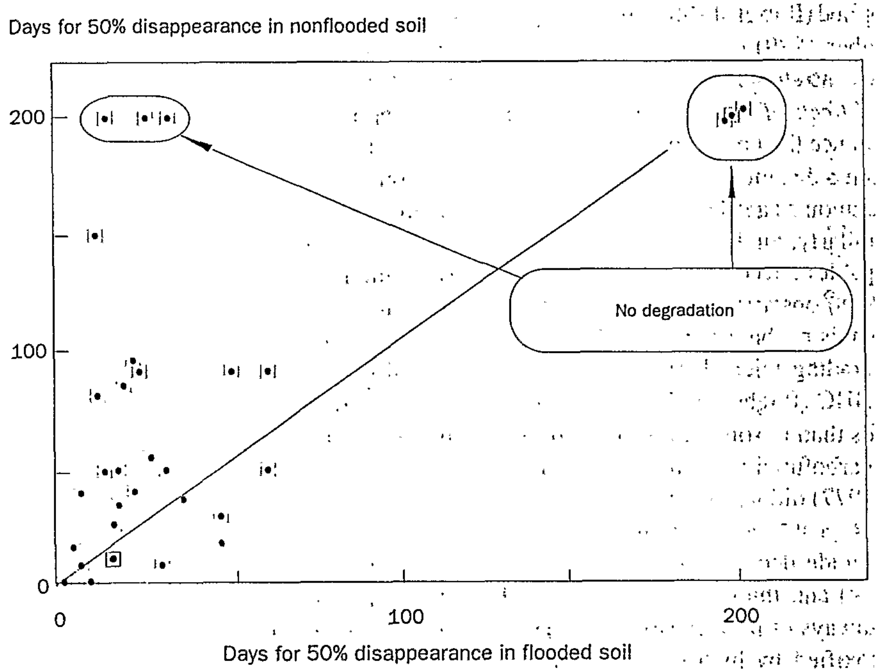
Characteristics of metabolism and behavior of pesticides in wetland soils. The metabolism of pesticides and their microbiological effects in ricefields depend on soil properties, climatic factors, pesticide application method, and synergistic or antagonistic effects among pesticides and between pesticides and fertilizers. Pesticide behavior in ricefields shows characteristics specific to wetland conditions. Pesticide degradation is favored by temperatures and pH, which usually stabilize in a range that favors microbial activity, and by reducing conditions caused by submersion and further accelerated by the incorporation of organic matter. These factors result in rapid detoxification of certain pesticides known to persist in aerobic systems (Sethunathan and Siddaramapa 1978).

Relative importance of biological and chemical decomposition. Pesticides usually degrade much faster in nonsterile soils than in sterilized soils, which demonstrates the importance of microbial degradation (Raghu and MacRae 1966, MacRae et al 1967, Sethunathan and MacRae 1969a, Gowda and Sethunathan 1976, Nakamura et al 1977, Adhya et al 1981, Funayama et al 1986). In uplands, bacteria and fungi are mainly responsible for pesticide transformations. In wetlands, fungi are probably less important; whereas, the role of algae may be significant for parathion (Sato and Kubo 1964). Rhizospheric bacteria may also play a significant role. In an unplanted flooded soil, less than 5% of the ^{14}C of labeled parathion was evolved as CO_2 in 15 d; whereas, 23% was evolved in a planted soil (Reddy and Sethunathan 1983).

Pesticide degradation also occurs in the absence of microflora (Sethunathan and MacRae 1969a, Sudhakar-Barik and Sethunathan 1978) which suggests that chemical transformations catalyzed by redox reactions may be common. The relative importance of nonbiological degradation varies with pesticides and environmental conditions. Nonbiological degradation of insecticides ranges from 30 to 90% of the degradation in soil (Agnihotri 1978). Carbofuran in water degrades mainly by nonbiological process(es) related to the initial pH, but in soil, degradation is associated with microbial activities (Siddaramappa and Seiber 1979).

Pesticides can also disappear from ricefields because of volatilization (Soderquist et al 1977). Gaseous exchanges that take place between the soil and the atmosphere through the rice plant may favor losses of pesticides by volatilization, as observed for carbofuran (Siddaramappa and Watanabe 1979).

Effect of anaerobiosis. Comparisons of pesticide stability often show a longer persistence in nonflooded soils than in flooded soils (Fig. 1). A negative redox potential favors degradation of γ-BHC, DDT, endrin, and toxaphene (Willis et al 1974, Sethunathan et al 1976, 1980). Pesticide degradation can be very rapid in prereduced soils; 48-86% of the parathion disappeared when it was shaken for 5 s with reduced soil (Wahid et al 1980).



1. Relative stability of 30 pesticides in flooded and nonflooded soil (drawn from data by Sethunathan and Siddaramappa 1978).

Incorporation of organic matter, which hastens the drop in redox potential in flooded soils and increases microbial activity, favors pesticide degradation and has been observed with straw (Gowda and Sethunathan 1976, Venkateswarlu and Sethunathan 1979, Adhya et al 1981), compost (Chopra and Magu 1986), and green manure (Ferreria and Raghu 1981). However, some pesticides (e.g., γ-BHC) may reduce the drop in redox potential of rice soils (Pal et al 1980).

Some reports indicate no difference in degradation in upland and flooded conditions (Castro and Yoshida 1971) or a longer persistence, as for molinate (Deuel et al 1978), thiobencarb (Nakamura et al 1977), and phorate (Walter-Echols and Lichtenstein 1978). Pesticide losses by volatilization can also be retarded in flooded environments, as shown for trifluralin (Parr and Smith 1973).

Water management and method of pesticide application. In uplands, pesticides remain at the soil surface until they are incorporated into the soil by cultivation or watering. In wetlands, a faster dilution can be expected, and variations depend on solubility and surfactants. Water management and the method of pesticide application may affect pesticide toxicity, but no information is available.

Different application methods can induce significant differences in pesticide behavior. Pentachlorophenol (PCP) that was incorporated into the soil with lime stimulated N_2-fixing blue-green algae (BGA), but when it was surface-applied, even at low levels, it was depressive and had a long residual effect (Ishizawa and Matsuguchi 1966). When soil-incorporated, γ-BHC inhibited rhizospheric biological N_2 fixation (BNF), but γ-BHC stimulated rhizospheric BNF throughout crop growth when water-applied (Rao et al 1983). Incorporation of carbofuran at about 3 cm (Siddaramappa and Seiber 1979) or placement in the root zone (Siddaramappa et al 1979) reduced its concentration in water and increased its persistence in soil.

Effects of repeated application. Repeated applications of the same pesticide may enhance the growth of specific degrading microflora. A *Flavobacterium* sp., isolated from a diazinon-treated ricefield, had an exceptionally high capability to metabolize diazinon as a sole carbon source (Sethunathan 1972, Sethunathan and Pathak 1972). Similarly, Sudhakar-Barik et al (1976) isolated a *Pseudomonas* sp. and a *Bacillus* sp. capable of decomposing nitrophenols from parathion-amended flooded soil. Watanabe (1978) observed a 1000-fold difference in the number of PCP-decomposing microorganisms between treated and untreated soil. The enhancement of specialized degrading microflora caused a faster inactivation of diazinon (Sethunathan 1972), γ-BHC (Raghu and MacRae 1966), and aldicarb (Read 1987) in previously treated soils than in soils never exposed to those pesticides. However, repeated applications of carbofuran (Venkateswarlu and Sethunathan 1978) and thiobencarb (Nakamura et al 1977) did not increase degrading microflora.

Repeated applications of pesticides may also change the metabolic pattern of pesticide decomposition. This was observed with parathion (Sudhakar-Barik et al 1979) and thiobencarb (Moon and Kuwatsuka 1984). Changes in the degradation pathways of thiobencarb led to environmental problems. This herbicide is generally detoxified by hydrolysis, but its repeated application to flooded soil favored the multiplication of anaerobic bacteria that decomposed it by reductive dechlorination and produced a phytotoxic compound (Moon and Kuwatsuka 1984).

Literature analysis

Establishment of data base

Five hundred and forty-seven references were collected according to the following criteria:

(1) All papers dealing with the effects of pesticides on microorganisms (including microalgae) and microbial activities in ricefields, with ricefield soil, or with microbial or algal strains isolated from ricefields or known to be present in ricefields;

(2) A few papers dealing with methodological aspects or presenting data useful for comparison; and

(3) Bibliographic reviews that included references on wetland soils.

Bias and limitations of the data base. Only half of the papers present quantitative estimates of the effects of pesticides on microbial populations or their activities (Table 1). In addition, the information is biased. The literature on microalgae is most abundant and deals mostly with herbicides (62% of the records) and BGA. The literature on other microorganisms deals mostly with insecticides (80% of the records). Moreover, most studies are small-scale laboratory toxicity tests with algal cultures or test tube or flask experiments with a few grams of soil (Table 2).

Table 1. Topics in bibliographic data base regarding microbiological impacts of pesticides in ricefields.

Topic		Number of references
Methodological aspects including bioassays		13
Decomposition and persistence of pesticides in rice soils		140
Effects on heterotrophic microbial populations and activities		91
Effects on algae		272
Algicides and algal weeds	38	
Effects on nontarget algae: quantified effects on growth and activities	149	
Effects on nontarget algae: qualitative effects	29	
Bioconcentration in algae	13	
Effects on algal grazers	11	
Effects on symbiotic BGA (azolla)	5	
Adaptation and resistance of algae to pesticides	27	
Miscellaneous		26
Reviews including references to wetland soils		18
Total		547

Table 2. Methods used to quantify microbiological impacts of pesticides in ricefields.

Type of experimental design	Algological studies (No. of reports)	Bacteriological studies (No. of reports)
Cultures of microorganisms	130	2
Cultures of microorganisms with soil	6	0
Soil in test tubes or beakers	0	24
Pot experiments	3	21
Field experiments	10	14
Method not available	0	10
Total	149	71

Experiments with cultures of microorganisms give an index of strain sensitivity to pesticides. However, it is difficult to compare the results of different studies and to accurately assess the relative toxicity of pesticides because the methods used to assess their effects are variable and toxicity in vitro depends on culture conditions (Kar and Singh 1979b), nutrient concentration (Kar and Singh 1979c), and initial size of the inoculum (Das 1977). It is also difficult to draw general conclusions because microorganisms of the same taxon may show different responses to the same pesticide (Hutber et al 1979, Chen 1986). In addition, results obtained in vitro cannot be extrapolated to field conditions because:

(1) Toxicity is likely to be higher in cultures than in situ where pesticide degradation is enhanced by soil microflora, nonbiological decomposition, leaching, volatilization, and soil adsorption. For example, 5 ppm propanil prevented the growth of BGA in culture, but not in the presence of unsterilized or sterilized soil or in situ (Ibrahim 1972, Wright et al 1977).

(2) Toxicity depends on the initial microbial population, its nutrient status, and the method of pesticide application. These conditions are likely to differ markedly in vitro and in situ.

(3) In vitro experiments often test pure ingredients; whereas, in situ toxicity depends on the formulation. Some additives used as surfactants in commercial formulations are detrimental to algae or enhance the effect of the pesticide (Arvik et al 1971).

(4) In situ toxicity also depends on degradation products. Some can be more inhibitory than the parent compound, as shown for atrazine (Wright et al 1977, Stratton 1984).

(5) Many studies were conducted with pesticide concentrations higher than those resulting from the RLFA. These studies are of little value for drawing conclusions, unless no significant effect is recorded.

Because of the possible combinations to be tested (nature of pesticide × pesticide concentration × environmental conditions × microorganisms or microbial activities) and the methodological limitations of the studies, the literature on microbiological impacts of pesticides in ricefields is very fragmentary.

Effects on microalgae

Two major effects of pesticides on ricefield algae have been recorded: selective toxicity that affects the composition of the algal population, and growth promotion because of a decrease in the invertebrate populations that graze on algae.

General trends. The data base tabulates 1045 records of effects on algae. However, the majority of the tests were performed at concentrations higher than the RLFA probably because most studies were conducted in vitro (96%) and aimed at establishing the lethal concentration (LC_{50}) for the strains rather than testing the possible effects in situ. Four hundred and seven records of pesticide effects obtained at concentrations corresponding to the RLFA were analyzed (Table 3).

No pesticide effect was reported in 39% of the records. But, in 62% of the records obtained in situ or in the presence of soil, there was no effect. This observation confirms that pesticide effects are more marked in vitro than in situ. However, most data were obtained in vitro and this bias must be kept in mind.

Effects of algicides and fungicides. Many of the fungicides were tested primarily as algicides and are therefore considered with algicides. Algicides are usually applied in ricefields to control macrophytic (*Chara* spp., *Nitella* spp.) or mat-forming algae (*Spirogyra* spp., *Hydrodyction* spp.). Several reports indicate a preferential inhibitory effect of algicides on green algae that promotes BGA growth. This was observed with simetryn (Yamagishi and Hashizume 1974) and algaedyn (Almazan and Robles 1956). This may explain why total inhibition by algicides and fungicides was recorded in only 30-40% of the observations.

Effects of insecticides. Insecticides had a low impact on the tested algae (mostly BGA). A high percentage of the records indicated no inhibition (Table 3).

Several reports indicate a preferential inhibitory effect of insecticides on green algae that promotes BGA growth. This was observed with γ-BHC (Ishizawa and

Table 3. Summary of data on effect of 109 pesticides on ricefield microalgae at concentrations corresponding to RLFA.

Nature of data	Number of observations	% of the observations corresponding to each inhibition level				
		None	<50	50	>50	100
All data	407	39	19	26	2	4
All data in situ or with soil	39	62	8	3	3	6
Algicides (3 tested)	33	3	0	67	0	30
Fungicides (22 tested)[a]	30	40	10	7	0	43
Herbicides (57 tested)	252	33	25	28	2	12
Herbicides, in situ or with soil	24	58	8	4	4	25
Insecticides (28 tested)	97	67	11	14	3	4
Insecticides, in situ or with soil	10	90	10	0	0	0

[a] Several fungicides also act as algicides.

Matsuguchi 1966, Raghu and McRae 1967) and PCP, a pesticide that was used as an insecticide and an herbicide (Watanabe 1977). Simultaneously, insecticides inhibited invertebrates that feed on algae (grazers) and promoted BGA and photodependent BNF. This was observed with parathion (Hirano et al 1955), phorate (Srinivasan and Emayavaramban 1977), and carbofuran (Tirol et al 1981).

However, insecticide application did not invariably increase photodependent BNF. Some inhibitory effect was reported for PCP in situ (Ishizawa and Matsuguchi 1966). In the long term, insecticide use might also be detrimental to BGA if it decreased the diversity of aquatic invertebrates and caused a proliferation of algal grazers.

The relative acute lethal toxicity of carbofuran to the ostracod *Heterocypris luzonensis* was 2.4 mg/ml and that of lindane was 56.0 mg/ml (Grant et al 1983). Resistance to conventional pesticides allows large densities of ostracods to develop after pesticide application (5000-15000/m^2), particularly because natural predators succumb first. Ostracod populations can remove algal blooms in a few days. Takamura and Yasuno (1986) reported the proliferation of chironomids and ostracods in herbicide- and insecticide-treated fields, while the number of their natural predators decreased. Microalgae decreased in herbicide-treated plots and did not increase in insecticide-treated plots probably because of grazing.

Effects of herbicides. Herbicides are most detrimental to algae. Partial or total inhibition was reported in 67% of the in vitro tests and in 42% of the tests performed in situ or in the presence of soil (Table 3). Herbicides can inhibit BGA and photodependent BNF, as shown with PCP (Ishizawa and Matsuguchi 1966) and several formulations used in ricefields (Srinivasan and Ponnuswami 1978). Some herbicides, specifically affect the N_2-fixing ability of BGA because inhibition was observed in N-free medium but not in the presence of inorganic N. This was observed with dichlone (fungicide/algicide) (Kashyap and Gupta 1981) and butachlor (Kashyap and Pandey 1982).

Few trials have tested the interaction between herbicides and algal inoculation. Kerni et al (1984) concluded that butachlor at 5-30 kg/ha had no effect in inoculated plots. El-Sawy et al (1984) reported, from a pot experiment, that when algal inoculation was effective, herbicide application had no effect or a positive effect (14 of 16 cases). Negative effects (2 of 16 cases) were observed with propanil.

Holst et al (1982) tested 15 pesticides on *Azolla mexicana*. Bipyridilium and phenolic herbicides were the most detrimental and caused up to a 75% reduction in BNF at 0.1 ppm. Chloramben and benomyl (a fungicide) at 10 ppm caused an 84-99% reduction in BNF without affecting growth. Growth and BNF were reduced by other benzoic, triazine, dinitroanaline, and urea herbicides tested at 0.1-10 ppm. Naptalam was the only herbicide tested that had no effect on growth or BNF at 10 ppm.

Effects on nonphotosynthetic microorganisms and their activities

General trends. Contrary to experiments with microalgae, most tests on microflora and their activities were performed in the presence of soil, either in small-scale experiments (51% of the data) or in situ (47% of the data) (Table 4). Most experiments were also at concentrations corresponding to the RLFA. The data base tabulates 606 records.

Table 4. Summary of in situ and in vitro data on microbiological effects of pesticides in ricefields at concentrations corresponding to the RLFA (methodological aspects).

Group	Number of records	Data for each effect (%)				
		All negative	Negative trend	No effect	Positive trend	All positive
All data	606 (100%)	8	12	60	11	9
Summary by experimental design (606 records)						
Field experiments	309 (51%)	5	17	73	4	2
Pot and flask expts.	283 (47%)	10	8	46	18	19
Summary by environment (590 records)						
Soil	347 (59%)	7	12	52	16	14
Rhizosphere	243 (41%)	8	13	70	5	5
Summary by pesticide group (600 records)						
Fungicides	58 (10%)	5	0	50	24	21
Herbicides	102 (17%)	13	23	30	21	14
Insecticides	440 (73%)	6	11	68	7	8

About 60% of the records deal with populations or activities in the bulk of the soil and 40% deal with the rhizosphere. Data suffer from a strong bias because 73% of the records deal with insecticides.

On average, 20% of the trials reported a negative effect of pesticide application. No significant effect was observed in 60% of the cases; positive effects were recorded in 20% of the cases.

Experiments in situ showed a higher percentage of no effect (73%) than small-scale experiments (46%), which confirms that the small-scale experiments may overestimate pesticide effects. Extreme effects (all negative or all positive) were also more frequent in small-scale trials.

Pesticide effects are more marked in the bulk of soil (52% no effect) than in the rhizosphere (70% no effect), which is a more active and probably more resilient microenvironment than nonrhizospheric soil. Herbicides affected the microflora or its activities more often (30% no effect) than fungicides (50% no effect) and insecticides (68% no effect).

On average, populations of microorganisms were less affected by pesticides (58% no effect) than microbial activities (46% no effect) (Table 5).

Within microbial populations, fungi (81% no effect) and actinomycetes (62% no effect) were less sensitive to pesticides than bacteria (52% no effect). Because the relative abundance of actinomycetes and fungi is much lower in wetland soils than in upland soils, the imbalance of the data with regard to their distribution among microbial groups (70% of data on bacteria) reflects the field situation.

Table 5. Summary of in situ and in vitro data on microbiological effects of pesticides in ricefields at concentrations corresponding to the RLFA (organisms).

Group	Number of records	Data for each effect (%)				
		All negative	Negative trend	No effect	Positive trend	All positive
Summary for microbial counts (249 records, 51% of data for each effect)						
All microbial counts	249 (100%)	10	10	58	13	9
Actinomycetes	37 (15%)	3	19	62	8	8
Bacteria	175 (70%)	13	9	52	15	11
Fungi	37 (15%)	5	5	81	8	0
Summary for measurements other than microbial counts (357 records, 47% of all data)						
All measurements	357 (100%)	6	14	61	10	10
Microbial activities	225 (63%)	8	18	46	13	15
Enzymatic activities	123 (34%)	0	7	93	1	0
Others	9 (3%)					

Table 6. Summary of in situ and in vitro data on microbiological effects of pesticides in ricefields at concentrations corresponding to the RLFA (N cycle).

Group	Number of records	Data for each effect (%)				
		All negative	Negative trend	No effect	Positive trend	All positive
All data	606 (100%)	8	12	60	11	9
Data N cycle	302 (50% of all data)	8	15	48	16	13
Summary for BNF (176 records, 29% of all data, 58% of data on N cycle)						
All data on BNF	176 (100%)	2	23	31	26	19
Bacterial counts	69 (39%)	4	3	52	23	17
BNF measurements	107 (61%)	0	36	18	27	20
In bulk of soil	95 (54%)	1	12	25	37	25
In rhizosphere	81 (46%)	2	36	38	12	11
Fungicides	25 (14%)	0	0	20	52	28
Herbicides	26 (15%)	0	23	23	35	19
Insecticides	125 (71%)	2	27	35	18	17
Summary for other aspects of N cycle (126 records, 21% of all data, 42% of data on N cycle)						
All other aspects	126 (100%)	16	6	71	3	5
Nitrification	54 (43%)	30	4	61	0	6
Denitrification	47 (37%)	6	4	87	2	0
Others	25 (20%)	4	12	60	12	12

Microbial activities were more affected than enzymatic activities. Among 123 tests on 10 soil enzymes, 93% showed no effect of pesticide application. Only β-glucosidase reacted negatively to pesticide application.

Effects on nitrogen cycle. Half of the records in the data base deal with the N cycle. About 60% of the data on N cycle concern BNF and 30% concern nitrification and denitrification (Table 6).

N_2-fixing microflora and BNF were more affected by pesticides (31% no effect) more than other populations and activities of the N cycle (71% no effect). The low percentage of nonsignificant effects on BNF was mostly due to the higher number of positive effects (45%) that were observed indiscriminately with fungicides, herbicides, and insecticides. Compared with the data from the whole data base, data on BNF confirm a higher sensitivity of the nonrhizospheric microflora to pesticides and a more marked impact of fungicides and herbicides than of insecticides. A noticeable difference, as compared with the whole data base, is that populations were much less affected (52% no effect) than activities (18% no effect).

With 25% of negative effects and 45% of positive effects, BNF seems to be quite versatile in its response to pesticides applied at concentrations corresponding to the RLFA. Nayak and Rajaramamohan Rao (1980) using benomyl, carbofuran, and γ-BHC applied at the RLFA (5 ppm) in five soils and in ^{15}N tracer techniques under laboratory conditions (5-g soil samples) found both positive and negative effects on N_2 fixation. Most often, a positive effect was observed, but a single pesticide could exhibit a negative or positive effect depending on the soil type. Also Rao et al (1983) reported variable effects of the same pesticide that depended on the method of application.

Nitrification was not affected by pesticides in about 60% of the cases. This value is similar to the average of the data base. However, negative effects were much more frequent (34% of the cases) than positive effects (6% of the cases). This inhibition cannot be considered detrimental because it reduces losses from N fertilizer. In fact, the identification of efficient and economically feasible nitrification inhibitors is an important objective of research on microbial management of ricefields (Roger et al 1992).

Denitrification was not affected by pesticides in 87% of the cases. This is probably because the denitrifying microflora, being complex and very versatile, are able to metabolize or resist a wide range of substrates. As a result, high pesticide levels are needed to inhibit denitrification. Mitsui et al (1964) tested the effect of eight dithiocarbamate pesticides in a rice soil and found that 20 ppm metham-sodium or 100 ppm of the other pesticides was required to significantly decrease denitrification 2-5 d after pesticide application. These concentrations are higher than the RLFA.

Significant effects of pesticides were less often recorded in situ than in vitro and they were more often negative than positive. However, the same percentage of positive and negative effects (20%) was recorded with the whole data set (Table 7).

Most trends observed with the whole data set were also observed in situ: greater impact of herbicides than of insecticides; higher sensitivitiy of bacteria to pesticides than fungi, actinomycetes, and algae; higher sensitivity of microbial activities to pesticides than population densities (especially with BNF); and a higher sensitivity of

Table 7. Summary of in situ data on microbiological and algological effects of pesticides in ricefields at concentrations corresponding to the RLFA.

Group	Number of records	Data for each effect (%)				
		All negative	Negative trend	No effect	Positive trend	All positive
Data in situ and in vitro	606	8	12	60	11	9
Data in situ	351	5	16	73	5	2
Herbicides	50	8	18	64	10	0
Insecticides	297	4	14	75	4	3
Algae	42	7	10	71	10	2
Actinomycetes	29	0	24	76	0	0
Bacteria	84	17	13	57	6	7
Fungi	29	0	7	86	7	0
All counts of microorganisms	184	9	13	68	6	4
Microbial activities	65	0	45	46	8	2
Soil enzymes	102	0	2	98	0	0
BNF (algae not included)						
BNF (all data)	93	2	32	39	15	12
BNF populations	35	6	6	63	3	23
BNF activity	58	0	48	24	22	5

BNF to pesticides (39% no effect) than the average sensitivity observed with all the data in situ (73% no effect).

Most field studies dealing with microflora present no statistical analyses of the data, but results of microbial enumerations after pesticide application usually indicate either an absence of effect or a transitory change of population densities followed by a recovery within 2 or 3 wk. Two studies of rhizospheric BNF indicate long-lasting stimulatory (Mahapatra and Rao 1981) or inconsistent (Rao et al 1983) effects. This probably reflects the long-term effects of pesticides on the rice plant rather than a direct effect on the microflora.

The only field study that was conducted over several crop cycles (Nishio and Kusano 1978) showed that nitrification and total bacterial populations were not specifically different in soils that received insecticide for four consecutive years. However, counts of bacteria tolerant of organophosphate insecticides were two to four times higher in treated soils.

Effects on invertebrate populations

Insecticides are the most active pesticides on floodwater invertebrates. They cause a decrease in populations followed by a proliferation of primary consumers such as ostracods, chironomid and mosquito larvae, and molluscs (Ishibashi and Ito 1981, Roger and Kurihara 1988) and a decrease in populations of predators such as odonate larvae (Takamura and Yasuno 1986). Ostracods recover rapidly after pesticide application because of their resistance to pesticides and the large number of eggs that are produced parthenologically (Lim and Wong 1986).

In situ experiments and surveys

Impacts of pesticides in relation to N fertilization

The combined effects of N fertilizer, the insecticide carbofuran, and the herbicide butachlor were studied on major populations of aquatic and soil invertebrates and on floodwater primary production.

There was a negative correlation between BGA growth and the level of fertilizer applied. Deep placement of fertilizers markedly decreased the inhibitory effect of N fertilizer on BGA growth (Table 8). There was some stimulating effect of pesticide application on BGA abundance (when N fertilizer was not applied or was deep-placed), and on photosynthetic activity in the floodwater.

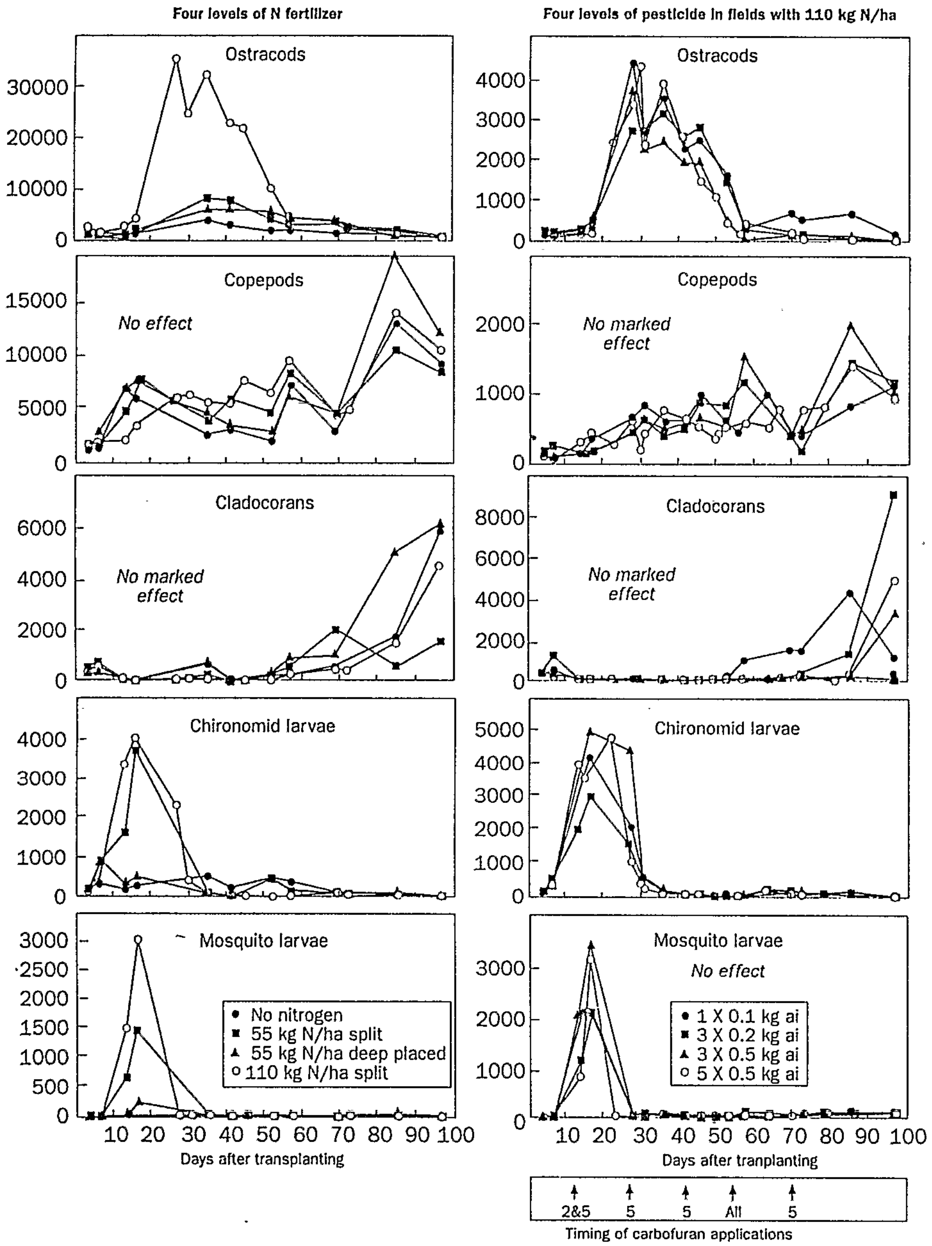
The dynamics of invertebrate populations followed a similar pattern in most plots (Fig. 2). There was a peak of chironomid and mosquito larvae at 12 d after transplanting (DT) and a peak of ostracods, the most abundant organisms, at 40 DT. Copepods established early in the crop cycle and increased in number during the second half of the cycle. Cladocerans started to multiply only during the last third of the crop cycle. Populations of ostracods and chironomid and mosquito larvae were much more abundant in the plots that received the largest quantity of agrochemicals than in the fallow plots.

There was a marked positive effect of N fertilizer on populations of algivorous aquatic arthropods (ostracods and chironomid and mosquito larvae) but not copepods and cladocerans which developed late in the crop cycle (Fig. 2). Pesticides had no marked effect on zooplankton. Pesticides partially inhibited the development of aquatic oligochaete populations (Table 8).

Table 8. Effect of rice, urea, and pesticides on aquatic invertebrates and N_2-fixing BGA[a], IRRI, 1990 dry season.

Organism	Rice[b]	N fertilizer		Pesticides	
		Deep placement	Increasing level	All N treatments (2 pesticide levels)	At 110 Kg N/ha (4 pesticide levels)
Ostracods	-	-	+++	– (3/11)	– (5/45)
Copepods	0	0	0	– (1/11)	– (4/45)
Cladocerans	-	+	0	– (3/11)	– (5/45)
Chironomid larvae	-	-	+++	0	0
Mosquito larvae	+	-	+++	0	0
Aquatic oligochaetes	nd	nd	++	–[c]	-
Snails (11/12)	nd	nd	0	nd	0
N_2-fixing BGA	-	+++	-	+++	+
Dissolved O_2 (0-20 d)	-	-	+++	+ (6/32)	0

[a] Legend: 0 = no effect; +++ or - = clear positive or negative effect; + or - = not very marked, possibly incidental, positive or negative effect (values in parentheses are the number of significant differences over the total number of records); nd = no data. [b] Effect observed during the dry season in 1990 but not in 1991. [c] Comparison between planted and fallow plots with no N fertilizer applied.



2. Effect of N fertilizer and pesticide application on the dynamics of zooplankton during a dry-season crop cycle in the IRRI farm. Each value is the average of measurements in four replicated plots.

This experiment, conducted at current rates of agrochemical use in farmers' fields, showed a clear stimulatory effect of N fertilizer on algivorous aquatic arthropods that was associated with an increase in the primary productivity of the floodwater. Pesticides had no marked effect on these organisms when considered at the crop cycle level. Among tested invertebrates, only aquatic oligochaetes exhibited a significant negative response to pesticide application; however, this effect could not be reproduced over two successive dry season crops.

Nitrogen broadcasting inhibited the growth of N_2-fixing BGA and favored the growth of eukaryotic algae. But, agrochemical use did not markedly reduce primary production in floodwaters. By favoring algivorous aquatic arthropods, pesticides also favored nutrient recycling. The negative effect of pesticides on aquatic oligochaetes indicates that pesticide use might reduce the translocation of recycled nutrients, accumulating at the soil-water interface, to deeper soil. This would reduce their availability to the rice plant.

Impact of pesticides in rice-fish culture

An experiment was conducted to study some of the biological effects of pesticides on fish and to quantify major components of the agroecosystem to develop a static model of N cycling in fields with and without fish (Lightfoot et al 1990) using the Ecopath program (Christensen 1990).

Pesticide application caused a significant increase in the photosynthetic activity of floodwater and reduced populations of aquatic oligochaetes (Table 9). There was no significant effect on surface-soil N, microbial biomass, available N, populations of N_2-fixing BGA, and fish and rice yields.

Surveys in farmers' fields

For two consecutive seasons, available N, microbial biomass, N_2-fixing BGA, and aquatic oligochaetes were quantified in 32 farms in Laguna and 30 farms in Lucban

Table 9. Summary of effects of pesticide treatment on rice-fish experiment, IRRI, 1990 wet season.

Variable	No pesticide	With pesticide	p
Dissolved O_2 (av during first two weeks) (ppm)	8.2	16.0	0.01
Dissolved O_2 (av during the crop cycle) (ppm)	6.8	8.6	0.01
Av[a] bulk density of surface soil (g/cm^3)	0.70	0.71	0.73
Av change in surface soil N%[b]	0.015	0.022	0.12
Av available N (0-10 cm)(kg/ha)	18.3	16.3	0.16
Av extractable N after fumigation (0-10 cm) (kg/ha)	64.2	62.3	0.62
Av flush N (0-10 cm) (kg/ha)	45.9	46.0	0.97
Av number of N_2-fixing BGA (colony-forming units/cm^2)	6.7x10^4	5.4x10^4	0.35
Av number of oligochaetes (no./m^2)	1760	183	0.01
Fish yield (kg/ha)	199	179	0.36
Rice yield (t/ha)	4.4	4.8	0.21

[a] Average values are the mean of four measurements during the crop (4 samplings). [b] Difference between the first 2 cm of soil and the deeper (2-10 cm) layer of soil.

Table 10. Comparison of average soil and biological properties in Laguna and Lucban.[a]

	Laguna		Lucban		p
	Mean	Range	Mean	Range	
Pesticides (kg ai/ha per crop)	1.5	0.5-2.5	0.0	0.0-0.0	**
Fertilizer N DS (kg/ha per crop)	103	33-184	35	8-92	**
Fertilizer N WS (kg/ha per crop)	67	0-134	22	7-60	**
pH	8.0	5.7-7.5	5.6	4.7-6.1	**
C (%)	2.23	1.48-3.35	2.55	1.99-3.17	**
N (%)	0.22	0.15-0.31	0.28	0.19-0.60	**
C/N	10.0	7.5-11.7	9.3	5.3-11.2	**
Available P (Olsen) ppm	31.3	2.7-81	8.2	2.2-71	**
Active Fe	1.13	0.34-2.36	3.59	1.61-4.57	**
Bulk density, 0-2 cm (g dw/cm^3)	0.59	0.45-0.75	0.43	0.28-0.61	**
Bulk density, 0-10 cm (g dw/cm^3)	0.67	0.50-0.89	0.54	0.33-0.56	**
Available N, 0-2 cm (kg/ha)	8.0	4.4-27.7	5.6	3.2-8.3	**
Available N, 0-10 cm (kg/ha)	27.2	16-89	19.3	10- 33	**
Flush N, 0-2 cm (kg/ha)	18.7	9.8-33.7	8.4	5.1-14.0	**
Flush N, 0-10 cm (kg/ha)	84.7	51-134	44.0	22-72	**
Blue-green algae (1000/cm^2)	87	30-300	17	0.7-94	**
Aquatic oligochaetes (1000/m^2)	6.2	0.1-18.3	1.2	0-16.8	**

[a]Values are the average of 4 measurements at the sequencing and at the end of the WS and DS.

(Philippines). Sampling was performed at the beginning of the crop cycle, before pesticide application, and at the end of the crop cycle before the soil was drained to identify possible long-term effects of pesticide use.

Only rodenticides were used in the farms in Lucban. In Laguna, a wide range of formulations and quantities of pesticides was applied. The quantities of individual pesticides used per cropping season averaged 0.3 kg ai/ha per crop and never exceeded 1 kg ai/ha per crop. The quantities of active ingredients used during a cropping season ranged from 0.5 to 2.5 kg/ha. Most values were between 1 and 2 kg/ha.

There were significant differences in the mean values of all the measurements taken in both areas (Table 10). Flush N and densities of BGA and aquatic oligochaetes were significantly lower in Lucban where no pesticides were used. These differences were probably due to soil properties and environmental conditions. In particular, soil pH and available N and P contents were much lower in Lucban. In Laguna, there was an inhibitory effect ($p = 0.05$) of herbicides + molluscicides and a positive effect of insecticides on BGA at the end of the dry season.

Summary and conclusion

As in any cultivated soil, the impacts of pesticide microflora and fauna in wetland soils depend on their persistence, the concentrations attained in the environment, and synergistic or antagonistic effects among pesticides and between pesticides and fertilizers. Pesticide degradation in flooded soils results from biological activities (mostly by bacteria and algae), chemical transformations catalyzed by redox reactions such as the iron redox system, and volatilization, including gaseous exchanges between the soil and the atmosphere through the rice plant. Pesticide degradation is often faster in flooded than in nonflooded soils.

Two major effects of pesticides on ricefield algae have been recorded: 1) a selective toxicity of all types of pesticides, which affects the composition of the algal population and often favors BGA, and 2) a short-term growth-promoting effect of insecticides on microalgae because of a temporary decrease in invertebrate populations that graze on algae. However, in the long term, insecticides might become indirectly detrimental to algae if they decrease the diversity of aquatic invertebrates and cause the proliferation of algal grazers that are resistant to insecticides. Grazing pressure may partly explain the dominance in many ricefields of strains of BGA that form mucilaginous macrocolonies (such as *Nostoc* spp.) and are more resistant to grazing than unicellular strains or strains that form individual filaments.

Field studies on algae mostly report that algal growth is enhanced by insecticide application. Several of these studies dealt with the promotion of photodependent BNF by the control of grazers with chemical pesticides or pesticides of plant origin. Microalgae can adapt themselves or develop mutants resistant to pesticides.

Pesticide effects were more marked in the bulk of soil than in the rhizosphere, which is a more active and probably more resilient microenvironment than the nonrhizospheric soil. Pesticide volatilization through the rice plant may also explain this difference.

Herbicides had more significant effects than insecticides. Microbial activities were more sensitive to pesticides than to population densities. This trend was especially obvious with data on BNF. Within microbial populations, fungi and actinomycetes were less sensitive to pesticides than bacteria.

Nitrification was either not affected, or negatively affected, by pesticides. This is beneficial because losses from N fertilizer are reduced. There was little effect on denitrification.

Insecticide application may favor the proliferation of primary consumers that are resistant to pesticides because of a decrease in the populations of predators. This suggests, but does not demonstrate, a decrease in the biodiversity of the predator population in ricefields where pesticides are used.

Floodwater biology was more affected by N fertilizer than by pesticides. The effects of pesticides were more marked on soil-aquatic oligochaetes than on zooplankton.

Pesticides applied on soil at recommended levels rarely had a detrimental effect on microbial populations or their activities. When significant changes were observed during tests that last for several weeks, populations or activities usually recovered after 1-3 wk. Pesticides applied at recommended levels and intervals are seldom deleterious to the beneficial microorganisms and their activities. However, invertebrate populations seem to be more sensitive to pesticides than microflora.

There are reports of significant effects of pesticides on nontarget microorganisms that are important to soil fertility. Pesticides may only have temporary effects, but when applied repeatedly, they could lead to the disappearance or depression of components of the microbial community. This could lead to a new equilibrium and to detrimental changes in the pattern of microbial decomposition.

Ricefield algae can significantly contribute to the bioconcentration of pesticides. This is important when the ricefield ecosystem is considered as a possible environment for aquaculture (rice-fish, rice-shrimp).

A major concern is that knowledge about the impact of pesticides in wetland soils is too fragmentary to draw anything but general conclusions. It is important to emphasize that impacts of pesticides on the soil-floodwater ecosystem can be significant without being detrimental. For example, a shift in the community structure of the algae may not affect soil fertility if aquatic primary production remains unchanged. Therefore, caution is required when qualifying the nature of the impact, which should be considered in the context of ecosystem equilibrium, not in isolation.

It would be as unwise to under- or overestimate the significance of pesticide impact in wetland soil. Underestimation could cause ecological damage. Overestimation could restrict the judicious use of pesticides. Studies of microbial degradation of pesticides and their influence on microflora and microbial activities in flooded rice soils have mostly been restricted to short-term laboratory experiments. These studies must be performed under more realistic field conditions and cultural practices on a long-term basis.

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