pg 32
p. 5

BIBLIOGRAPHIC AND EXPERIMENTAL ASSESSMENT OF THE IMPACTS OF PESTICIDES ON SOIL AND WATER MICROFLORA AND FAUNA IN WETLAND RICEFIELDS

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SUMMARY

The impacts of pesticides on microorganisms and invertebrate that contribute to maintain rice soils fertility are assessed from the analysis of bibliographic data and from field experiments conducted by the authors. The first part of the paper summarizes the major characteristics of pesticide behavior in wetland soils. The second part analyzes experimental data from an extensive bibliographic survey (547 references) that has lead to the establishment of a database on microbiological impacts of pesticides in ricefields. The third part summarizes the results of field studies and surveys.

Pesticide degradation in tropical ricefields is often faster than in temperate upland soils because of reducing conditions, and temperatures and pH which usually stabilizes in a range favoring microbial activity. Floodwater also may favor pesticide dilution and reduce their microbiological effects as compared with upland conditions. This may explain an absence of microbiological effect of pesticides in 73 % of the tests performed *in situ.*

The dataset established from the litterature suffers from several bias in terms of pesticides, environments, populations, and activities tested, but shows some general trends, namely:

- pesticides exhibit more marked impacts in laboratory experiments than *in situ,*
- herbicides have more microbiological and algological impacts than insecticides,
- insecticides have more impacts 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
- BNF sensitivity to pesticides is higher than the average value of the database.

When significant effects were observed *in situ* at concentrations corresponding to the recommended level for field application they were most often not lasting.

Field experiments by the authors showed that nitrogen fertilizer had more impact on algal and invertebrate populations in floodwater than pesticides. Pesticides had significant effects on photosynthetic activity in floodwater and on populations of aquatic oligochaetes in soil. Field surveys did not show significant effects of pesticides on long term basis, but a bias in the distribution of the soil properties of the forms refrains definite conclusions.

Available data seems to partly confirm the common belief that pesticides applied at recommended level and intervals seldom markedly affect soil microorganisms and their activities. However there is also evidence of significant effects on non target microorganisms, and aquatic and soil invertebrates of importance to soil fertility. With regard to the low number of field data measured for the whole duration of a crop and the absence of long term experiments, it would be premature to draw conclusions on the possible long term impacts of pesticide use on ricefields fertility.

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CONTENTS

INTRODUCTION

Research on rice nutrition has shown that, at the levels of inorganic fertilizer usually applied in ricefields, most N absorbed by the plant originates from soil. Available soil nitrogen is released by the turnover of a microbial biomass which represents only a few percent of total soil N (Watanabe et al. 1988). Crop residues, rhizosphere exudates, algae and aquatic plants contribute nutrients that allow the replenishment of microbial biomass. Nutrients accumulating 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 & Kurihara 1988).

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

This paper considers the impacts of pesticides on (1) soil microflora and microalgae in water, which are responsible for the maintenance of soil fertility, and (2) the invertebrate populations, which contribute to recycle and translocate nutrients in floodwater and soil. The first part summarizes the major characteristics of pesticide behavior in wetland soils. The second part analyzes experimental data from an extensive bibliographic survey (547 references) that has lead to the establishment of a database on microbiological impacts of pesticides in ricefields. The third part summarizes the results of field studies and surveys conducted by the authors.

PESTICIDE BEHAVIOR IN WETLAND SOILS

Pesticide use and concentration in farmer fields

A wide range of pesticides is used in wetland ricefields. Currently, about 150 chemicals have been tested for their impacts on ricefield microflora. Recommended level for field application (RFLA) of traditional pesticides range from a few hundred grams to a few kg active ingredient per hectare (a.i. ha^{-1}); with a median of about 2 kg (Fig. 1). The median is higher for herbicides (2.5 kg a.i. ha^{-1}) than for fungicides (1.7 kg a.i. ha^{-1}) and insecticides (1.1 kg a.i. ha^{-1}).

However, farmers often apply reduced doses of pesticides. A survey conducted in 1989 by IRRI in 32 farms of the Laguna area in the Philippines indicated that the total quantity of pesticide used during a cropping season in a field ranged from 0.5 to 2.5 kg a.i. ha^{-1}. The quantities of individual pesticides used per cropping season averaged 0.3 kg a.i. ha^{-1} and did not exceed 1 kg a.i. ha^{-1} (Roger et al. 1990).

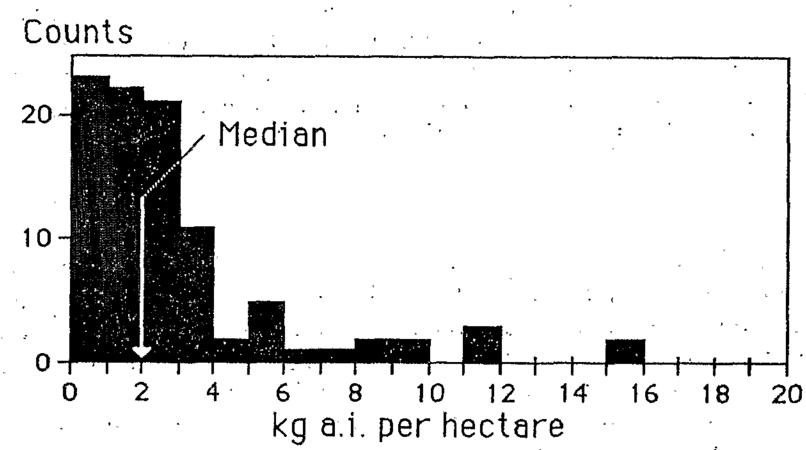

Fig. 1. Histogram of the average recommended doses of 94 pesticides tested for their microbiological effects in ricefields

When interpreting experimental results, one should keep in mind the range of pesticide concentrations that can be expected in farmer fields after application. The upper limit can be calculated by assuming that pesticide is applied on a nonflooded soil and remains in its two first centimeters. In this case, 1 kg a.i. ha^{-1} corresponds to 10 ppm on dry soil basis (bulk density 0.5). A lower limit can be calculated by assuming an even distribution in 10 cm of water and 10 cm of puddled soil of a water-soluble pesticide. In this case, 1 kg a.i. ha^{-1} corresponds to 0.4 ppm. Field situation is probably closer to the lower value.

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

Characteristics of metabolism and behavior of pesticides in wetland soils

As in any cultivated soil, the metabolism of pesticides and their microbiological effects in ricefields depends on soil properties, climatic factors, the method of pesticide application, and synergistic/antagonistic effects among pesticides and between pesticides and fertilizers. However, pesticide behavior in ricefields presents characteristics specific to wetland conditions. In particular, their degradation in tropical ricefields is favored by (1) temperatures and pH which usually stabilizes in a range favoring microbial activity, and (2) reducing conditions caused by submersion and further accelerated by organic matter incorporation. This results in rapid detoxication of certain pesticides known to persist in aerobic systems (Sethunathan & Siddaramapa 1978).

Relative importance of biological and chemical decomposition

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

Pesticide degradation was also observed in the absence of microflora (Sethunathan & MacRae 1969a; Sudhakar-Barik & Sethunathan 1978) showing that chemical transformations catalyzed by redox reactions such as the iron redox system may also be common. The relative importance of non biological degradation varies with pesticides and environmental conditions. For various insecticides, it ranged from 30 to 90% of the degradation in soil estimated in the presence of microflora (Agnihotri 1978). Degradation of Carbofuran in water was mainly by non-biological process(es) and was related to the initial pH; but in soil, it was associated with microbial activities (Siddaramappa & Seiber 1979).

Beside microbiological and chemical degradation/transformation, pesticides can disappear from the ricefield through volatilization (Soderquist et al. 1977). Gaseous exchanges that take place between the soil and the atmosphere through the rice may favor losses of pesticides by volatilization, as observed for Carbofuran (Siddaramappa & Watanabe 1979).

Effect of anaerobiosis

The comparison of pesticide stability in wetland and upland conditions (Fig. 2) often shows a longer persistence in nonflooded soils than in flooded soils. A negative redox potential favored the degradation of HCH, DDT, Endrin, and Toxaphene (Willis et al. 1974; Sethunathan et al. 1976, 1980). Pesticide degradation can be very rapid in prereduced soils as shown with Parathion which exhibited 48-86% disappearance when shaken for 5 seconds with reduced soil (Wahid et al. 1980).

Organic matter incorporation, which hastens the drop in redox potential in flooded soils and increases microbial activity, favors pesticide degradation, as observed with straw (Adhya et al. 1981; Gowda & Sethunathan 1976; Venkateswarlu & Sethunathan 1979), compost (Chopra & Magu 1986), and green manure (Ferreria & Raghu 1981). On the other hand, some pesticides may reduced the drop in redox potential of rice soils as observed with HCH by Pal et al. (1980).

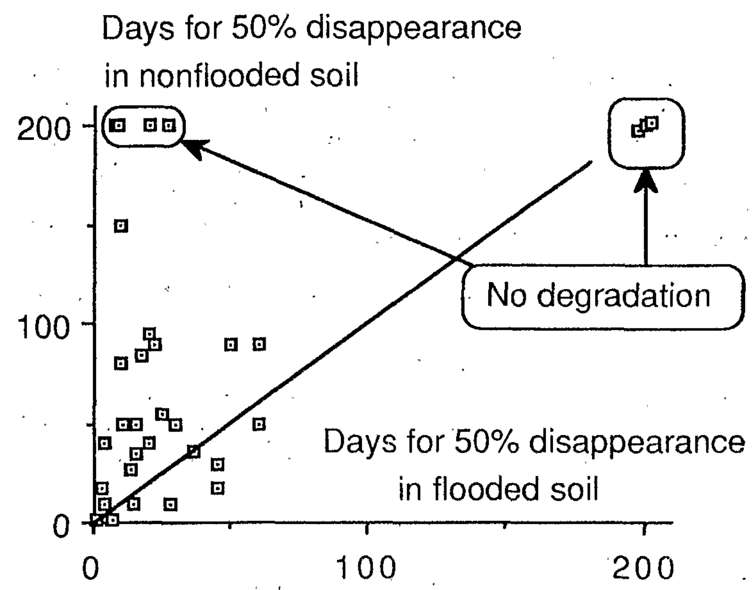

Fig.2 : Relative stability of 30 pesticides in flooded and nonflooded soil
(drawn from data by Sethunathan & Siddaramappa, 1978)

Some reports indicate no difference in degradation in upland and flooded conditions (Castro & Yoshida 1975) or a longer persistence, as for Molinate (Deuel et al. 1978), Benthiocarb (Nakamura et al. 1977), and Phorate (Walter-Echols & Lichtenstein 1978). Also, pesticide losses by volatilization can be retarded in flooded environments, as shown for Trifluralin (Parr & Smith 1973).

Water management and method of pesticide application

In uplands, pesticide remains at the soil surface until cultivation or watering incorporates them into the soil. In wetlands, a faster dilution can be expected, with variations depending upon solubility and surfactants used. Water management and the method of pesticide application might affect pesticide toxicity with regard to dilution and movements in soil, but no information is available on this aspect.

In ricefields, pesticides can be sprayed, applied in the floodwater, incorporated into the soil, or used for dipping rice seedling at transplanting. The different methods can induce significant differences in pesticide behavior. Pentachlorophenol incorporated in soil with lime stimulated N_2-fixing BGA; but if surface-applied, even at low levels, it was depressive with a long residual effect (Ishizawa & Matsuguchi 1966). When soil-incorporated, HCH inhibited rhizospheric BNF, while it was stimulating throughout the crop when water-applied (Rao et al. 1983). Incorporation of Carbofuran at about 3 cm (Siddaramappa & Seiber 1979) or placement in the root zone (Siddaramappa et al. 1979) reduced its concentrations in water and increased its persistence in soil .

Effects of repeated application

Repeated application of the same pesticide has been reported to enhance the growth of the specific degrading microflora. A *Flavobacterium* sp., isolated from a diazinon treated ricefield, had an exceptionally high capability to metabolize diazinon as sole carbon source (Sethunathan 1972; Sethunathan & Pathak 1972). Similarly, Sudhakar-Barik et al. (1976) isolated a *Pseudomonas* and a *Bacillus* able to decompose 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 the specialized degrading microflora caused a faster inactivation of Diazinon (Sethunathan 1972), gamma-BHC (Raghu & MacRae 1966), and Aldicarb (Read 1987) in soils previously treated than in soils never exposed to those pesticides.

However, repeated application of Carbofuran (Venkateswarlu & Sethunathan 1978) and Benthiocarb (Nakamura et al. 1977) did not lead to the build-up of the degrading microflora.

Repeated application of pesticide was also reported to change the metabolic pattern of pesticide decomposition. This was observed with Parathion (Sudhakar-Barik et al. 1979) and Benthiocarb (Moon & Kuwatsuka,1984). Changes in degradation pathways of Benthiocarb lead to environmental problems. This herbicide is generally detoxified by hydrolysis, but its repeated application to flooded soil favoured the multiplication of anaerobic bacteria that decompose it by reductive dechlorination, resulting in the formation of a very phytotoxic compound (Moon & Kuwatsuka 1984).

ANALYSIS OF EXPERIMENTAL DATA FROM THE LITERATURE

Most of the earlier information on pesticide effects on non-target soil microorganisms comes from observations in upland temperate soils. Anderson's review (1978) lists almost 500 references and tabulate 1016 records on microbiological effects of pesticides among which only 11 deal with rice soils. During the last two decades, more information on tropical wetlands has become available.

Database establishment

We have collected bibliographic references according to the following criteria:
(1) all papers dealing with the effects of pesticides on microorganisms -- including microalgae-- and microbial activities reporting studies conducted in ricefields, with ricefield soil, or with microbial or algal strains isolated from ricefields or known to be present in ricefields..
(2) few papers of interest for methodological aspects or presenting data useful for comparison.
(3) bibliographic reviews including references on wetland soils.

This has lead to the collection of 547 references which were entered in a specially designed program written in Hypertalk language.

Bias and limitations of the database

With 547 references entered in the bibliographic database, the literature on microbiological aspects of pesticide use in ricefields seems quite abundant. However, only half of the papers presents quantitative estimates of the effects of pesticides on microbial populations or their activities (Table 1).

Table 1.Main topics of the bibliographic database on microbiological impacts of pesticides in ricefields

Topics		Number of references
Methodological aspects including bioassays		13
Decomposition and persistance 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**

In addition, this information is markedly biased. The literature on microalgae is more abundant than that on other microorganisms and deals mostly with herbicides (62% of the records) and blue-green algae (BGA). The literature on other microorganisms deals mostly with insecticides (80% of the records). Moreover, most studies are small scale laboratory experiments consisting in toxicity tests with algal cultures or test tube or flask experiments with a few grams of soil (Table 2).

Experiments with cultures of microorganisms give an index of strains 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 very variable and the toxicity *in vitro* depends on the culture conditions (Kar & Singh 1979b), the nutrient concentration (Kar & Singh 1979c), and the initial size of the inoculum (Das 1977). It is also difficult to draw general conclusions because microorganisms of a same taxon may show very different responses to the same pesticide (Chen PeiChung 1986; Hutber et al. 1979). In addition, results obtained *in vitro* can hardly be extrapolated to field conditions because:

- 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 and *in situ* (Ibrahim 1972; Wright et al. 1977).
- Toxicity depends on the initial microbial population, its nutrient status, and the method of pesticide application. This conditions are likely to markedly differ *in vitro* and *in situ*..
- *In vitro* experiments often test pure ingredients, while *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).
- *In situ* toxicity also depends on degradation products. Some can be more inhibitory than the parent compound, as shown for Atrazine (Stratton 1984; Wright et al. 1977).
- Many studies were conducted with pesticide concentrations far or higher than those resulting from the recommended level for field application (RLFA). Such studies are of little value for drawing conclusions, except when no significant effect was recorded.

Considering the numerous possible combinations to be tested (nature of pesticide x pesticide concentration x environmental conditions x microorganisms or microbial activities), and the methodological limitations of the studies currently performed, the literature on microbiological impacts of pesticides in ricefields appears to be very fragmentary.

Carbofuran, Furadan

Find

Carbofuran, Furadan (insecticide, nematicide, molluscicide) (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate): Systemic acaride, insecticide and nematicide, applied to foliage at 0.25-1.0 kg a.i. ha-1 and to seed furrow at 0.5-4.0 kg ha-1.

- Adhikari (1989) Carbofuran had no significant effect on Westiellopsis prolifica growth measured in vitro 15 d after inoculation until 3 ppm, 50% inhibition was at 7.5 ppm, total inhibition was at 30ppm.
- Ghost & Saha (1988),
- Jena & Rajaramamohan Rao (1987)
- Kar & Singh (1978) lin vitro, 0.75 ppm enhanced growth and N2-fixation by Nostoc muscorum, 1.5-3 ppm was not or slightly inhibitory, 36 ppm was algicidal.

kg/ha or ppm	≤ 0.5	0.6	0.7	0.8	0.9	1	2	3	4	5	6	7	8	9	10	20	30	40	50	60	70	100	>100
Recommanded level (kg/ha)	●●	●●	●●●	●●	●●●	●●●	●●	●															
inhibition: none	A		1				S	1			S				7	5	1						
inhibition: <50%							1			S						1							
inhibition: 50%												2			1				4			1	
inhibition: >50%																	1						1
inhibition: 100%										1							1	1					

Fig. 3. Example of a record on pesticide effects on microalgae in the Hypercard stack .

Card are connected to the bibliographic database. The upper field provides general information on the pesticide and a summarization of the effects recorded by the authors listed in the bibliographic database. The lower field shows the recommanded level for application (black dots) and the recorded effects. Numbers in boxes are the number of algal strains presenting a given inhibition at a given concentration of pesticide. "S" refers to a field or pot experiment (soil). "A" refers to symbiotic BGA (Azolla). The vertical bold line indicates an estimate of the upper limit of field concentration calculated on the basis of the RLFA and 1 kg a.i. ha^{-1} = 2ppm

Tabulation of the results

Algological data collected in the database are primarily percentages of inhibition estimated by various measurements on cultures (dry weight, fresh weight, total nitrogen, chlorophyll content, etc.) They were tabulated for each pesticide, using a grid combining a geometric scale of pesticide concentration and four levels of inhibition: none, < 50%, 50%, > 50%, and total inhibition (Fig 3). This tabulation allows to identify results obtained at concentrations higher than the RLFA. Data were then entered in a spread sheet and summarized (Table 3).

Data for other microorganisms are bacterial counts and activity measurements, often performed at several time intervals after pesticide application. Inhibition, no effect, and enhancement were reported. For tabulating such data, each experiment was attributed a score of 1 within a 5 cases scale:

- all negative: for all measurements the treatment was statistically lower than the control,
- negative trend: various effects were recorded, the balance was negative,
- no effect: no statistically significant difference between treatment and control,
- positive trend: various effects were recorded, the balance is positive, and
- all positive: for all measurements the treatment was statistically higher than the control.

Table 3. Summarization of the data on the effect of 109 pesticides on ricefield microalgae at concentrations corresponding to the recommanded level for field application .

Nature of the data	number of data	% of data corresponding to each of the above five levels of inhibition none	< 50%	50 %	> 50%	100%
All data	407	39	19	26	2	14
All data *in situ* or with soil	39	62	8	3	3	26
Algicides (3 tested)	33	3	0	67	0	30
Fungicides (22 tested)*	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

* several fungicides act also as algicides

Results were then tabulated in a spread sheet containing for each record: the name of the pesticide, its nature (herbicide, insecticide ....), the range of the RLFA, the dose(s)/concentration(s) used for the experiment, the type of experimental design (*in situ*, pot experiment, flask experiment ...), the population/activity measured, the environment (soil, rhizosphere ...), the duration of the experiment, the dates of the observations in days after pesticide application, and the effect at the RLFA (5 columns) and at concentrations higher than the RLFA (5 columns).

When no statistical analysis was performed, which was most often observed with bacterial enumerations, we have assumed that data were log-normally distribution and considered as significantlly different results at least three times higher or lower than the control (Roger et al. 1991).

Effects on microalgae

General trends

The database tabulates 1045 records of effects on algae. However 638 tests were performed at concentrations higher than that corresponding to the RLFA, probably because most studies were conducted *in vitro* (96 %) and aimed at establishing LC_{50} or the lethal concentration for the strains rather than testing the possible effects *in situ*. In this section, we analyse the 407 records of pesticide effects obtained at concentrations corresponding to the RLFA (Table 3).

An absence of effect of pesticides was reported in 39 % of the total number of records but only in 62 % of the records obtained *in situ* or in the presence of soil. This 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 in the following discussion.

Two major effects of pesticides on ricefield algae have been recorded: (1) a selective toxicity which affects the composition of the algal population, and (2) a growth promoting effect of insecticides due to the decrease of invertebrate populations that graze on algae.

Effects of algicides and fungicides

Many fungicides for use in ricefield were tested primarily as algicides and are therefore considered together with algicides. Algicides are usually applied in ricefields to control macrophytic (*Chara* spp., *Nitella* spp.) or mat forming algae (*Spirogyra* spp., *Hydrodyction* spp.). Microalgae are usually not considered as weeds. Several reports indicates a preferential inhibitory effect of algicides on green algae which results in the promotion of BGA growth. This was observed with Symetryne (Yamagishi & Hashizume 1974) and Algaedyn (Almazan & Robles 1956). This may explain why only 30-40 % of total inhibition were recorded with algicides and fungicides.

Effects of insecticides

Insecticides had a low impact on tested algae (mostly BGA), as shown by the high percentages of record indicating no inhibition in the whole database (67 %) and *in situ* (90 %) (Table 3).

Several reports indicates a preferential inhibitory effect of insecticides on green algae which resulted in the promotion of BGA growth. This was observed with BHC (Ishizawa & Matsuguchi 1966, Raghu & McRae 1967), and PCP (Watanabe 1977). Simultaneously, insecticides inhibited invertebrates that feed on algae (grazers), thus promoting furthermore BGA and photodependant biological N_2 fixation (BNF). This was observed with Parathion applied in the floodwater (Hirano et al. 1955), Phorate (Srinivasan & Emayavaramban 1977) and Carbofuran (Tirol et al. 1981).

However, insecticide application did not invariably increase photodependant BNF. Some inhibitory effect was reported for PCP *in situ* (Ishizawa & Matsuguchi 1966). Also, on a long term, insecticide use might become detrimental to BGA by decreasing the diversity of aquatic invertebrates and causing proliferation of algal grazers. The relative acute lethal toxicity of Carbofuran to the ostracod *Heterocypris luzonensis* was 2.4 $\mu g\ ml^{-1}$ and that of Lindane was 56.0 $\mu g\ ml^{-1}$ (Grant et al. 1983). Such resistance to conventional pesticides allows large densities of ostracods to develop after pesticide application (5,000 -15,000 m^{-2}), particularly as the natural predators succumb first. Ostracod populations may cause the disappearance of 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

Among pesticides not aiming at algal control, herbicides seems to be most detrimental to algae, causing partial or total inhibition 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 photodependant BNF, as shown with PCP--a pesticide that is used as insecticide and herbicide-- (Ishizawa and Matsuguchi 1966) and several formulations used in ricefields (Srinivasan & Ponnuswami 1978). Some herbicides seem to affect specifically the N_2-fixing ability of BGA as indicated by an inhibition observed in N-free medium but not in the presence of inorganic N. This was observed with Dichlone (fungicide/algicide) (Kashyap & Gupta 1981) and Machete (Kashyap & Pandey 1982).

In the numerous experiments on algal inoculation of rice fields (Roger 1990) very few trials have tested the interaction between herbicides and algal inoculation. Kerni et al. (1984) concluded to the absence of effect of Butachlor at 5-30 kg ha^{-1} in inoculated plots. El-Sawy et al (1984) reported, from a pot experiment, that when algal inoculation was effective, herbicide application had most often no effect or a positive effect over the inoculated control (14 of 16 cases). Negative effects (2 of 16 cases) were observed with Propanyl.

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

Table 4. Summary of *in situ* and *in vitro* data on microbiological effects of pesticides in ricefields at concentrations corresponding to the recommended level for field application: methodological aspects

Groups	number of data	% of 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 data)						
Field experiments	309 (51 %)	5	17	73	4	2
Pot and flask expts.	283 (47 %)	10	8	46	18	19
Summary by environment (590 data)						
Soil	347 (59 %)	7	12	52	16	14
Rhizosphere	243 (41 %)	8	13	70	5	5
Summary by pesticide group (600 data)						
Fungicides	58 (10 %)	5	0	50	24	21
Herbicides	102 (17 %)	13	23	30	21	14
Insecticides	440 (73 %)	6	11	68	7	8

* see in the text for the definition of the effects

Effects on nonphotosynthetic microorganisms and their activities

General trends

Contrary to experiments with microalgae, most tests on microflora and its 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). Also, most experiments were at concentrations corresponding to the RLFA. The database tabulates 606 records obtained at such concentrations. About 60 % of the records deal with populations or activities in the bulk of soil and 40 % deal with the rhizosphere. Data suffer from a strong bias in the nature of pesticides tested, 73 % of the records being on insecticides (Table 4).

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

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

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

The summarization of effects according to counts of microbial populations and other types of measurements (Table 5) shows that, on an average, populations of microorganisms were less affected by pesticides (58 % of no effects) than microbial activities (46 % of no effects).

Within microbial populations, fungi (80 % of no effect) and actinomycetes (62 % of no effect) were less sensitive to pesticides than bacteria (52 % of no effect). As 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 distibution among microbial groups (70 % of data on bacteria) reflects the field situation.

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

Table 5. Summary of *in situ* and *in vitro* data on microbiological effects of pesticides in ricefields at concentrations corresponding to the recommended level for field application: organismal aspects

Groups	number of data	% of data for each effect: all negative	negative trend	no effect	positive trend	all positive
All data	606 (100 %)	8	12	**60**	11	9
Summary for microbial counts (249 data, 51 % of all data)						
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 data, 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 %)					

Effects on nitrogen cycle

Half of the records of the database deal with N cycle. About 60% of the data on N cycle concern BNF and 30 % concern nitrification and denitrification. Data on other aspects are not numerous enough to allow conclusions (Table 6).

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

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 gamma-BHC applied at the RLFA (5ppm) in five soils and ^{15}N tracer techniques under laboratory conditions (5g 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 negative or positive effect depending on the soil type. Also Rao et al. (1983) reported variables effects of the same pesticide depending 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 database. 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 nitrogen fertilizer. In fact the identification of efficient and economically feasible nitrification inhibitors has been an important objective of the research on the 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, is able to metabolize or to resist a wide range of substrates. As a result, high pesticide levels are needed to inhibit denitrification. Mitsui et al. (1964), testing the effect of 8 dithiocarbamate pesticides in a rice soil, found that 20 ppm Vapam or 100 ppm of the other pesticides was required to significantly decrease denitrification at 2 and 5 d after pesticide application. Such concentrations are higher than the RLFA.

Table 6. Summary of *in situ* and *in vitro* data on microbiological effects of pesticides in ricefields at concentrations corresponding to the recommended level for field application: nitrogen cycle

Groups	number of data	% of 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 data, 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 other aspects of N cycle (126 data, 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

Table 7. Summary of *in situ* data on microbiological and algological effects of pesticides in ricefields at concentrations corresponding to the recommended level for field application.

Groups	number of data	% of 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

Considering only results from *in situ* experiments (Table 7) provides information measured under realistic conditions, but markedly reduces the size of the dataset and the number of conclusions that can be drawn.

Significant effects of pesticides were less often recorded *in situ*than *in vitro* and they were more often negative than positive whereas the same percentage of positive and negative effects (20 %) was recorded with the whole dataset.

However most trends observed with the whole dataset were also obseved *in situ*, namely:

- more impacts of herbicides than of insecticides,
- a higher sensitivitiy of bacteria to pesticides than that of fungi, actinomycetes, and algae,
- a higher sensitivity of microbial activities to pesticides than that of population densities. This last trend was especially obvious with data on BNF (Table 7), and
- a higher sensitivity of BNF to pesticides (39 % of no significant effects) than the average sensitivity observed with the whole set of data *in situ* (73 % of no significant effects).

Most fields 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 two or three weeks. Two studies of rhizospheric BNF indicate long lasting stimulatory (Mahapatra & Rao 1981) or inconsistant (Rao et al. 1983) effects. This probably reflects more the long term effects of pesticides on the rice plant than a direct effect on the microflora.

The only field study conducted over several crop cycles (Nishio & Kusano 1978) showed that nitrification and total bacterial populations in soils having received insecticide for 4 consecutive years were not significantly different from those in the control. However, counts of bacteria tolerant to organophosphate insecticides were 2 to 4 times higher in treated soils.

Effects on invertebrate populations

Insecticides are the most active pesticides on floodwater invertebrates. Their application caused a decrease in populations followed by the proliferation of primary consumers : Ostracods, Chironomid and mosquito larvae, and molluscs (Ishibashi & Itoh 1981, Roger & Kurihara 1988) while populations of predators such as Odonate larvae decreased (Takamura & Yasuno 1986). Ostracods recover rapidly after pesticide application because of their resistance to pesticides and the large number of eggs produced parthenologically (Lim & Wong 1986).

It is generally admitted that crop intensification and agrochemical use decrease biodiversity. Few data on species diversity are available for aquatic invertebrates (Table 8) . These data were obtained by different methods of sampling and the time frame of the samplings were different. They show a marked decrease of the values recorded since 1975 that might probably be taken as a rough indication of a decrease in total number of species, but, indeed, does not demonstrate the generally accepted concept that crop intensification has decreased biodiversity in ricefields (Roger et al. 1991).

Table 8. Number of species/taxa of aquatic invertebrates (protozoas excluded) recorded in ricefields by different authors

Reference and characteristics of the record	Number of species/taxa
• Heckman (1979) (species), one traditional field, one-year study 1975 (Thailand)	**183**
• Lim (1980) (taxa), two-year study of pesticide application (Malaysia)	**39**
• Takahashi et al. (1982) (taxa) 4 fields, single samplings (California)	**10 - 21**
• IRRI 1985 and Roger *et al.* 1985 (species) single samplings in 18 fields where pesticide was applied (Philippines and India)	**2 - 26**

SUMMARY OF IN SITU EXPERIMENTS AND SURVEYS BY THE AUTHORS

We have conducted field experiments and surveys in farmer fields over several cropping seasons. The effect of pesticides was assessed by monitoring, with standardized methods, the soil microbial biomass, the photosynthetic activity in the floodwater, N_2-fixing BGA, zooplankton, and soil invertebrates.

Methods of measurement

Soil microbial biomass was estimated as the difference in N mineralized after 4 weeks of incubation in anaerobiosis between a soil sample in which most of the microflora was killed by a treatment with chloroform and an untreated control. This value (flush N) is only an index of the microbial biomass. To obtain an exact estimate it has to be multiplied by a correction factor which depends on the C/N of the major components of the microflora. The use of an average correction factor is restricted to soils that do not contain large amounts of undecompsoed organic material. Therefore, we used non corrected flush values. The method is only semi-quantitative, but it is faster, and less tedious than direct or indirect counts. In addition, the measurement in the nonfumigated control also provides estimates of soil available nitrogen and soil bulk density.

The impact of pesticides on photosynthetic activity in floodwater was estimated by measuring dissolved O_2 around 1-2 pm and enumerating N_2-fixing BGA, using the plating method and composite soil samples comprising 10 core subsamples of the top 0.5 centimeter of fresh soil. BGA were chosen to assess the impacts of pesticides on the photosynthetic aquatic biomass because of (1) their recognized role in maintaining the N fertility of traditional ricefields, and (2) the existence of an important set of data on BGA in ricefields which allows comparisons (Roger 1989).

Zooplankton was studied by enumerating populations of Ostracods, Copepods, Cladocerans, and Chironomid and mosquito larvae in core samples comprising the floodwater and the surface soil.

Aquatic oligochaetes were chosen to characterize the possible impacts on soil fauna because a previous study and a literature survey (Roger & Kurihara 1988) showed that they are a major component of the submerged soil fauna, stimulating organic matter decomposition and translocating nutrients in the soil profile. They were shown to affect weed growth, soil properties, and the nutritional status of the floodwater. Aquatic oligochaetes were enumerated from composite samples of several soil cores 27 mm in diameter collected along a transect in the fields or the plots.

Impacts of pesticides in relation with N fertilization.

An experimental design of 65 plots (16 m^2 each, 5 replicates) was used to study the combined effects of N fertilizer, the insecticide Carbofuran, and the herbicide Butachlor on major populations of aquatic and soil invertebrates, and on floodwater primary production. We used one unplanted unfertilized control and 12 selected combinations of five N treatments (no N, 55 and 110 kg N ha^{-1} broadcast split, 55 kg N ha^{-1} deep-placed, and Azolla incorporated before transplanting) and four levels of pesticides (one application of carbofuran at 0.1 kg a.i. ha^{-1}, two applications of 0.3 kg each, three applications of 0.5 kg each, and five applications of 0.5 kg each). The three treatments with 2 and 5 applications of carbofuran also received one application of 0.375 kg a.i. ha^{-1} of Butachlor.

A very clear negative correlation between BGA growth and the level of fertilizer applied was observed. Deep-placement markedly decreased the inhibitory effect of N fertilizer on BGA growth (Table 9). Some stimulating effect of pesticide application was observed on BGA abundance when N fertilizer was not applied or deep-placed, and on photosynthetic activity in the floodwater .

The dynamics of invertebrate populations followed a similar pattern in most plots (Fig. 4) with a peak of Chironomid and mosquito larvae at 12 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 crop 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 receiving the largest quantity of agrochemicals than in fallow plots.

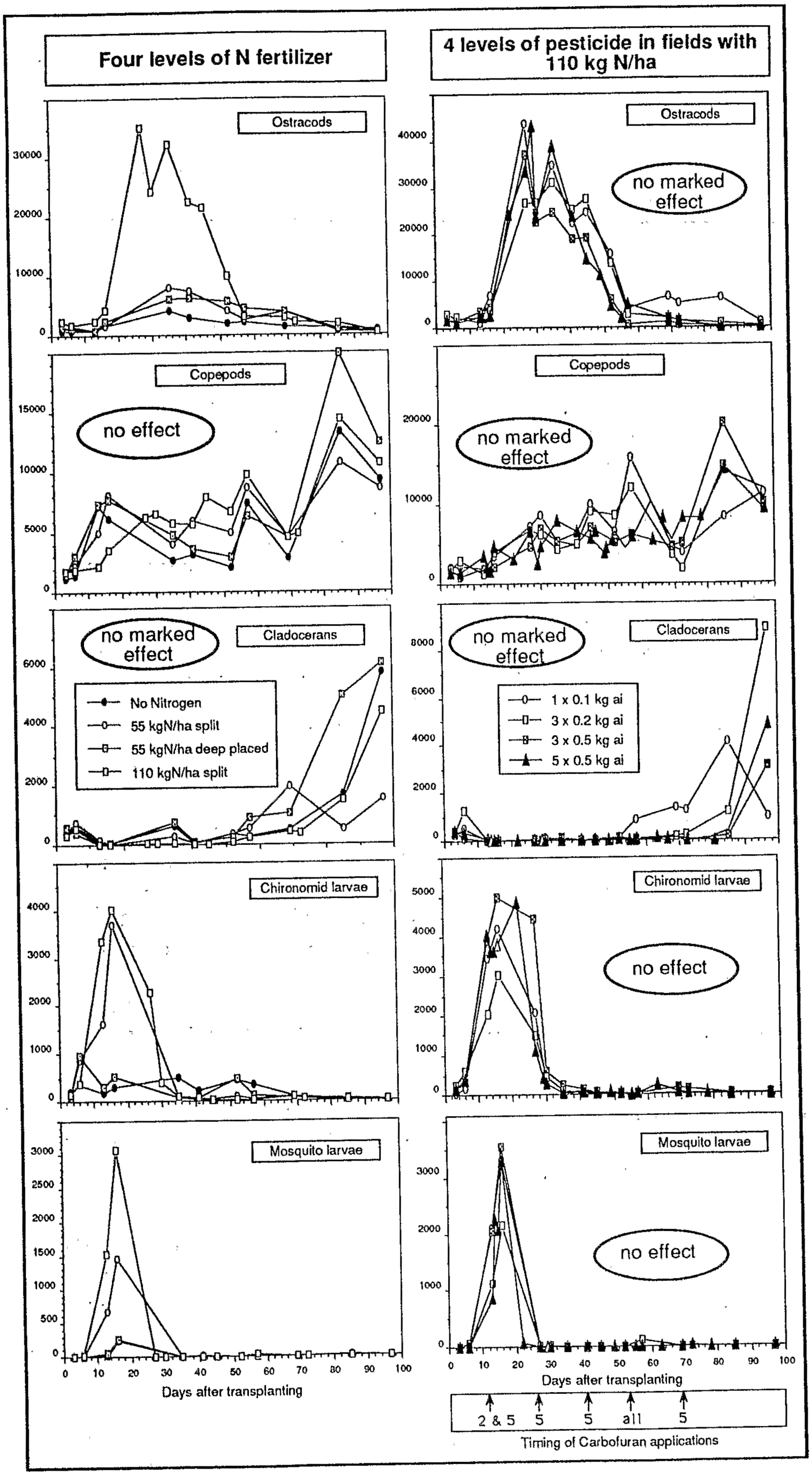

Figure 4. Effect of N fertilizer and pesticide application on the dynamics of zooplankton during a dry season crop cycle in the IRRI farm. Each value is the average of measurements in four replicated plots

Table 9. Effect of rice, urea and pesticides on aquatic invertebrates and N_2-fixing BGA [a]. IRRI DS 1990

Organisms	Rice[b]	N fertilizer Deep Placement	N fertilizer Increasing Level	Pesticides all N treatments 2 pesticide levels	Pesticides at 110 N 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]	---
(11/12)Snails	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 parenthesis are the number of significant differences over the total number of records); nd: no data. [b] *Effect observed during DS 1990 but not in 1991.* [c] Comparison between planted and fallow plots with no N fertilizer applied.

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 4). Pesticides had no marked effect on zooplankton. Few significant negative effects were found for Ostracods, Copepods, and Cladocerans by statistical analysis of daily measurements, but they represented a very low percentage of the total number of measurements (Fig 4). On the contrary, pesticide was found to partially inhibit the development of aquatic Oligochaete populations (Table 9).

This experiment, conducted at rates of agrochemicals currently used in farmer fields showed a clear stimulatory effect of N fertilizer on algivorous aquatic Arthropods associated with an increased floodwater primary productivity. 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 was not reproducible 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 floodwater. By favoring algivorous aquatic Arthropods, it also favored nutrients recycling. On the other hand, the negative effect of pesticides on aquatic oligochaetes indicates that pesticide use might reduce the translocation into deeper soil of recycled nutrients accumulating at the soil/water interface, and thus reduce their availability to rice plant.

3.3. Impact of pesticides in rice-fish culture

We studied in collaboration with scientists of ICLARM and CLSU some of the biological effects of fish stocking and pesticide use in an experimental design of 12 experimental plots with four replicates and two treatments (with and without fish x with and without pesticide) at CLSU during the wet season of 1990. The study also aimed at quantifying major components of the agroecosystem in order to develop a static model of N cycling in fields with and without fish (Lightfoot et al. 1990) using the Ecopath program (Christensen & Pauli 1990). Fertilizer was applied in all plots at 5 and 30DT as urea and ammonium phosphate at 106 kg N ha^{-1} and 20 kg P ha^{-1}.

Table 10. Summarization of the effects of pesticide treatment in a rice-fish experiment. WS 1990.

Variable	no pesticide	with pesticide	p
Dissolved O_2 (average during two first weeks) (ppm)	**8.2**	**16.0**	**0.01**
Dissolved O_2 (average during the crop cycle) (ppm)	**6.8**	**8.6**	**0.01**
Average[a] bulk density of surface soil (g cm^3)	0.70	0.71	0.73
Average change in surface soil N% [b]	**0.015**	**0.022**	**0.12**
Average available N (0-10 cm)(kg ha^{-1})	18.3	16.3	0.16
Average extractable N after fumigation (0-10 cm) (kg ha^{-1})	64.2	62.3	0.62
Average flush N (0-10 cm) (kg ha^{-1})	45.9	46.0	0.97
Average number of N^2-fixing BGA (CFU cm^{-2})	6.7 10^4	5.4 10^4	0.35
Average number of oligochaetes (nb. m^{-2})	**1760**	**183**	**0.01**
Fish yield (kg ha^{-1})	199	179	0.36
Rice yield (t ha^{-1})	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 two centimeter of soil and the deeper (2-10 cm) layer of soil

Pesticide treated plots received 16.7 kg ha^{-1} Furadan 3G (granular insecticide with 3% a.i. carbofuran) at 1 DT, 5 kg ha^{-1} Machete 5G (granular herbicide with 5% a.i. Butachlor) at 3 DT, and Telustan (molluscicide with 60 % a.i. triphenyltin hydroxide) applied as a solution (6 tbsp per liter per 1,200 m^2) at 1DT. *Tilapia* fingerlings, (initial weight of 7.22 g $animal^{-1}$), were stocked at 11 DT, at a density of 12,000 ha^{-1}.

Results (Table 10) show that pesticide application caused a statistically significant increase of the photosynthetic activity in floodwater and reduced populations of aquatic oligochaetes. There was no significant effect on surface soil nitrogen, microbial biomass, available N, populations of N_2-fixing BGA, and fish and rice yields.

3.4. Surveys in farmer fields

For two consecutive seasons, available N, microbial biomass, N_2-fixing blue-green algae, and aquatic Oligochaetes were quantified in 32 farms of the Laguna area and 30 farms of the Lucban area (Philippines) where the Social Sciences Division of IRRI had recorded agrochemical use and rice yields for several years.

Sampling was performed at the beginning of the crop cycle, before pesticide application, and at the end of the crop cycle, as far as possible from the last pesticide application, but before the soil was drained. This sampling schedule was chosen in order to try to identify possible long term effects of pesticide use by studying the correlation between biological data with data on pesticide use when the short term effect of pesticide application was not effective anymore.

No pesticide other than rodenticides were used in the farms of the Lucban area. In the farms of Laguna a wide range of pesticides in terms of formulation (21) and quantity (Fig.) was applied. The quantities of individual pesticides used per cropping season averaged 0.3 kg a.i. ha^{-1} $crop^{-1}$ and never exceeded 1 kg a.i. ha^{-1} $crop^{-1}$. The total quantities of active ingredients used during a cropping season in a field ranged from 0.5 to 2.5 kg ha^{-1}. Most values were between 1 and 2 kg ha^{-1}.

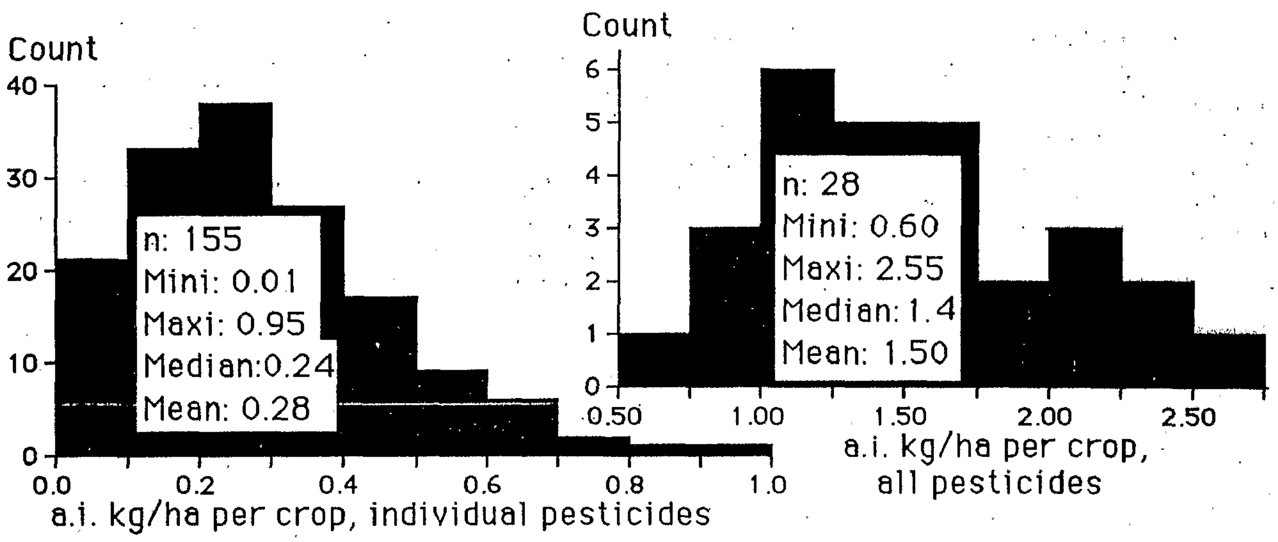

Fig. 9. Histogram and statistics of the quantities of active ingredient of individual pesticides and total of pesticides used per cropping season (kg a.i. ha^{-1} crop $^{-1}$) by farmers in the Laguna area in 1998.

Table 11. Comparison of average soil and biological properties in Laguna and Lucban areas

	Laguna		Lucban		p
	mean	range	mean	range	
Pesticides (kg a.i. ha^{-1} $crop^{-1}$)	1.5	(0.5 - 2.5)	0.0	(0.0 - 0.0)	**
Fertilizer N DS (kg ha^{-1} $crop^{-1}$)	103	(33 - 184	35	(8 - 92)	**
Fertilizer N WS (kg ha^{-1} $crop^{-1}$)	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^{-1})	8.0	(4.4 - 27.7)	5.6	(3.2 - 8.3)	**
Available N, 0 - 10 cm (kg ha-1)	27.2	(16-89)	19.3	(10- 33)	**
Flush N, 0 - 2 cm (kg ha^{-1})	18.7	(9.8 - 33.7)	8.4	(5.1 - 14.0)	**
Flush N, 0 - 10 cm (kg ha-1)	84.7	(51 - 134)	44.0	(22 - 72)	**
Blue-green algae (1000 x cm^{-2})	87	(30 - 300)	17	(0.7 - 94)	**
Aquatic Oligochaetes (1000 x m-2)	6.2	(0.1 - 18.3)	1.2	(0 - 16.8)	**

* values are the average of 4 measurement at the sequencing and the end of the WS and DS

Results of analyses showed significant differences in the mean values of all the measurements performed in both areas, including N fertilizer application and soil properties (Table 11). Flush N and densities of BGA and aquatic Oligochaetes where significantly lower in the Lucban area where no pesticide was used that in the Laguna area. These differences were most probably due to soil properties and environmental conditions. In particular, soil pH and available N and P contents were much lower in the Lucban area than in the Laguna area. This showed that Lucban area could not be used as a no pesticide control for measurements performed in Laguna area where pesticide was used. Therefore, the analysis of the possible long term pesticide impacts was restricted to the farm of Laguna, trying to relate variations of the biological variables with variations in quantities of pesticide used.

In a first approach, we calculated linear correlation between the biological variables determined at each sampling times and the quantities of active ingredient of insecticide, herbicide, molluscicide, and the sum of herbicide and molluscicides applied during the dry and the wet season. Among the 160 coefficients of correlation calculated, only two were significant. They indicated an inhibitory effect of herbicides + molluscicides and a positive effect of insecticides on BGA at the end of the dry season. The level of the correlation ($p = 0.05$) indicated a weak relationship.

A second approach was to study the level of significance of the difference between average biological values in farms where selected pesticides were or were not used. The t test of Pearson applied on normalized data was utilized. Only pesticide used or not used by more than 5 farmers were tested. None of the 60 tests performed indicated a significant difference.

4. SUMMARY AND CONCLUSIONS

As in any cultivated soil, the impacts of pesticide microflora and fauna in wetland soils depends upon their persistence, the concentrations attained in the environment, and synergistic/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 in tropical ricefields is favored by (1) temperatures and pH which usually stabilize in a range favoring high microbial activity, and (2) reducing conditions caused by submersion and further accelerated by organic matter incorporation. As a result, pesticide degradation is often faster in flooded than in nonflooded soils.

In ricefields, pesticides can be sprayed, applied in the floodwater, incorporated into the soil, or used for dipping rice seedling at transplanting. The different methods can induce significant differences in pesticide behavior. However because of the presence of floodwater and puddled soil, a faster dilution can be expected as compared with uplands where pesticide remains at the soil surface until cultivation or watering incorporates them into the soil.

As a result of a shorter persistence and faster dilution, pesticide should have less impact on soil microflora in wetland ricefields than in upland soils.

In his review on the effects of pesticides on nontarget microorganisms, Anderson (1978) used the notion of "ratios of effects" to analyse 1016 records on microbiological effects of pesticides in soils. Using sets of experimental data corresponding to various combinations between a group of pesticides and a population or activity, all stimulatory effects, and instances where there was no effect, were counted as positive. All inhibitions where counted as negative. The ratio of positive to negative counts was called the "ratio of effects". The average of 27 ratios calculated by Arvik for herbicides, fungicides, and insecticides in experiments or observations dealing almost exclusively with upland soil is 1.39, which corresponds to 42 % of negative effects. The 606 data on wetland soils recorded in our database indicate only 20% of negative effects.

About half of 547 papers dealing with the impacts of pesticides on ricefield microflora and aquatic invertebrates present quantitative data. However, less than 8% of the quantitative studies were conducted *in situ* and a high percentage of the laboratory experiments was conducted at concentrations higher than the RLFA. We selected for analysis only data from experiments conducted at pesticide levels corresponding to the RLFA but data are also biased in terms of organisms and pesticides tested. Therefore their analysis allowed only the identification of general trends.

An absence of effect of pesticides on algae was reported in 39 % of the total number of records but only in 62 % of the records obtained *in situ* or in the presence of soil. This confirms that pesticide effects are more marked *in vitro* than *in situ.* Among pesticides not aiming at algal control, herbicides were most detrimental to algae, causing partial or total inhibition in 67% of the *in vitro* tests and in 42% of the tests performed *in situ* or in the presence of soil.

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 favor BGA, and (2) a short term growth promoting effect of insecticides on microalgae due to a temporary decrease of invertebrate populations that graze on algae. However, on the long term, insecticide use might become indirectly detrimental to algae by decreasing the diversity of aquatic invertebrates and causing the proliferation of algal grazers resistant to insecticides such as Ostracods. Grazing pressure may partly explain the dominance in many ricefields of strains of BGA forming mucilaginous macrocolonies, such as *Nostoc* spp., which are more resistant to grazing than unicellular strains or strains forming individual filaments.

Field studies on algae mostly report an enhancement of algal growth due to insecticide application. Several of these studies were in fact dealing with the promotion of photodependant BNF by controlling grazers with chemical pesticides or pesticides of plant origin.

No bibliographic data are available on long term effects of pesticides on algae, but studies (Table 1), mostly on BGA, indicate that microalgae can adapt themselves or develop mutants resistant to pesticides.

In 606 tests of the effect of pesticides applied at the recommended dose on a microbial population or activity in wetland soils or in rice rhizosphere, an inhibitory effect was recorded in about 20 % of the cases, no effect was recorded in about 60 % of the cases, and a promoting effect was recorded in 20 % of the cases. Data were biased by the high percentage (73 %) of records on insecticides. Effects were more frequently observed in laboratory experiments (54 % of the cases) than *in situ* (27 %), confirming that small scale and *in vitro* experiments overestimate pesticide effects. In addition, significant effects were more often negative than positive *in situ* whereas the same percentage of positive and negative effects (20 %) was calculated from the whole dataset.

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. Also pesticide volatilization through the rice plant may also explain this difference.

Herbicides had more often significant effects (70%) than insecticides (32%). Microbial activities were more sensitive to pesticides than population densities. This trend was especially obvious with data on BNF. Within microbial populations, fungi and actinomycetes were less sensitive to pesticides than bacteria.

Data on N cycle show that nitrification was either not affected or negatively affected (34 % of the cases) which is rather beneficial as it reduces losses from N fertilizer. Denitrification was little affected (13 % of the cases). With 25 % of negative effects and 45 % of positive effects, N_2-fixing microflora and BNF were more affected by pesticides than other populations and activities of the N cycle (no effect: 71 %) but seemed quite versatile in their response to pesticides. A higher number of positive effects was observed indiscriminately with fungicides, herbicides, and insecticides. N_2-fixing populations were much less affected (no effect: 52 %) than their activitiy (no effect: 18 %).

Data on other aspects of N cycle were not numerous enough to allow conclusions .

A few data from the literature indicate that insecticide application may favor the proliferation of primary consumers resistant to pesticides by decreasing populations of predators and suggest, but do not demonstrate, a decrease in their biodiversity in ricefields where pesticide use was introduced.

Field studies by the authors showed floodwater biology was more affected by N fertilizer more than by pesticides. The effects of pesticides were more marked on soil aquatic Oligochaetes than on zooplankton. Aquatic Oligochaetes may be an indicator of pesticide use in wetland soils.

Field and laboratory studies with soil usually showed that 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 lasting for several weeks, a recovery of populations or activities was usually observed after 1 to 3 weeks.

The analysis of the literature and our own observations seems to partly confirm the common belief that pesticides applied at recommended levels and intervals are seldom deleterious to the beneficial microorganisms and their activities. On the other hand, invertebrate populations seems to be more sensitive to pesticides than microflora.

However, available information raise several concerns. These are reports of significant effects of pesticides on non-target microorganisms of importance to soil fertility. Pesticides might have only temporary effects but, when applied repeatedly, could lead to the disappearance or depression of components of the microbial community, thus leading to a new equilibrium and changes in the pattern of their microbial decomposition that might be detrimental.

Several references confirm that ricefield algae, as many microalgae in freshwater environments can significantly contribute to the bioconcentration of pesticides (Table 1). This aspect is important when considering the ricefield ecosystem as a possible environment for aquaculture (rice-fish, rice-shrimp).

The major concern is that the current stage of the knowledge on impacts of pesticides in wetland soils is too fragmentary to draw conclusions other than general trends. 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 algal community structure may not affect soil fertility, providing that aquatic primary production is unchanged. Therefore one should be cautious when qualifying the nature of impacts which should be considered in the contest of the ecosystem equilibrium and not in isolation.

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

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