10 THE IMPACT OF PESTICIDES ON RICEFIELD MICROFLORA: AN ANALYTICAL REVIEW OF THE LITERATURE

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10.1. Introduction

The 60 percent increase in rice production needed to meet the requirements of a fast-growing human population in the next thirty years (IRRI, 1990) should be obtained with practices that maintain or enhance the quality of the environment and conserve or enhance natural resources. Alterations in soils caused by crop production techniques that use high inputs of agrochemicals are not necessarily undesirable, especially as crop intensification produces more food on less land. But there is no assurance that, in the long term, crop intensification will not affect soil fertility.

At the levels of inorganic fertilizer usually applied in ricefields, most N absorbed by the plant originates from soil where it is released by the turnover of a microbial biomass that represents only a few percent of total soil N (Watanabe, De Datta, and Roger, 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 zooplankton and reincorporated into the soil by oligochaetes, which are therefore key components of the ricefield fertility (Roger and Kurihara, 1988).

Therefore it is important to understand and predict how factors associated with crop intensification, especially pesticide use, may affect the soil microbial biomass directly through toxic effects, or indirectly by decreasing the productivity of the photosynthetic aquatic biomass and inhibiting invertebrate populations responsible for nutrient recycling and translocation. There is also a concern about the enhanced use of pesticides that might lead to a reduced pesticide efficiency because of shifts in microbial populations toward organisms more efficient in pesticide degradation.

Aspects dealing with pesticide impacts on soil and water micro and mesofauna are reviewed by Simpson and Roger (Chapter 9). Those dealing with pesticide degradation by microorganisms are reviewed by Roger and Bhuiyan (Chapter 5). This chapter focuses on the impacts on phototrophic and heterotrophic microorganisms in ricefield floodwater and soil.

Most of the earlier information on pesticide effects on nontarget soil microorganisms comes from observations in upland temperate soils (Wainright, 1978; Anderson, 1978). Anderson's review (1978) lists almost 500 references and tabulates 1,016 records on microbiological effects of pesticides, among which only eleven deal with rice soils. During the last two decades, more information on tropical wetlands has become available but emphasis has been on phototrophic microorganisms (Table 10.1).

The effects of pesticides on nitrogen transformations in soils were reviewed by Goring and Laskowski (1982) and Lal and Lal (1988); effects on nitrogen transformations in wetland soils were reviewed by Ray and Sethunathan (1988).

The literature dealing with the interactions between microalgae and pesticides published from 1946 to 1975 was reviewed by Butler (1977). The work published during this thirty-year period includes pesticide degradation, bioaccumulation, and bioassay; but the majority of the papers deal with pesticide toxicity tested *in vitro*. In their review on the influence of pesticides on soil algae McCann and Cullimore (1979) came to the similar conclusion that most information was from *in vitro* experiments mostly with herbicides. They pointed out that *in situ* studies were very scarce and results obtained by different workers for the same pesticide frequently conflicted. The literature dealing with the effects of pesticides on cyanobacteria (Padhy, 1985; Kumar, 1988) presents the same characteristics and most of the work concerns *in vitro* determination of lethal doses of pesticides on different strains (Padhy, 1985).

The first part of this review summarizes (1) the methodological aspects of the study of pesticide impacts on wetland soil microflora and places emphasis on the limitations of *in vitro* and small-scale experiments that have been the most frequent approach for such studies and (2) the factors that affect pesticide impact on ricefield microflora. The second part presents the analysis of quantitative data collected in 210 references originating from a larger database on microbiological

Table 10.1. Main Topics of the Bibliographic Data Base on Microbiological Impacts of Pesticides in Ricefields

Topics		*Number of References*
Methodological aspects including bioassays		13
Decomposition and persistence of pesticides in rice soils		140
Effects on heterotrophic microbial populations and activities		71
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 cyanobacteria (Azolla)	5	
Adaptation and resistance of algae to pesticides	27	
Miscellaneous		26
Reviews including references to wetland soils		18
Total		527

aspects of pesticide use in ricefields (Table 10.1). In the summary of recorded effects, emphasis is placed on field experiments.

Two annotated bibliographies on pesticide impacts on (1) ricefield algae and cyanobacteria and (2) nonphototrophic microorganisms of ricefield, are annexed to this review (Appendices D and E, respectively).

10.2. Methodological Aspects of the Study of Pesticide Impacts on Soil and Water Microflora

Methods used to study pesticide impacts on ricefield microflora include tests on cultures of microorganisms isolated from rice soils, small-scale experiments on soil in test tubes and beakers, and pot and field experiments.

10.2.1. In Vitro Experiments

Many studies of the effects of pesticides on soil microflora are laboratory experiments conducted with cultures of microorganism. This is especially characteristic

Table 10.2. Methods Used to Quantify Microbiological Impacts of Pesticides in Ricefields

Type of Experimental Design	*Algological Studies n° of Reports*	*Bacteriological Studies n° 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

of studies of soil algae and cyanobacteria for which most experiments have been performed in flasks with axenic or unialgal cultures of single species (Table 10.2).

Experiments with cultures of microorganisms can give an index of the sensitivity of the strains to pesticides, but it is difficult to draw general conclusions from such data because microorganisms of a same taxon often show different responses to the same pesticide (Hutber, Rogers, and Smith, 1979) and toxicity *in vitro* depends on the culture conditions (Kar and Singh, 1979a), the nutrient concentration (Kar and Singh, 1979b), and the initial size of the inoculum (Das, 1977). This is especially well demonstrated with cyanobacteria.

Marked differences in response of *Nostocaceae* (cyanobacteria) to pesticide *in vitro* were reported for Bavistin (carbendazin) for which tolerated concentrations were 300 ppm for *Westiellopsis* sp., 100 ppm for *Aulosira* sp., 100 ppm for *Nostoc* sp., 50 ppm for *Tolypothrix* sp. while 1 ppm was algicidal for *Calothrix* sp. (Gangawane, 1980). *Anabaena cylindrica* could tolerate concentrations of dichlone ten times higher than *Nostoc calcicola* and *Anacystis nidulans* (Kashyap and Gupta, 1981). Gadkari (1987) reported a two to ten times higher resistance of *Nostoc muscorum* to triazine herbicides as compared with *Anabaena cylindrica*. Even cyanobacteria of a same genus may show very different responses to the same pesticide (Venkataraman and Rajyalakshmi, 1971, 1972). Hutber, Rogers, and Smith (1979) found that 100 ppm of glyphosate was needed to reduce the growth of *Aphanocapsa* 6,714 by 50 percent while 2 ppm could achieve the same with *Aphanocapsa* 6,308. Chen (1986) found significant difference in the effects of various herbicides on N_2 fixation and photosynthetic activity by two strains of *Anabaena*.

The effect of culture conditions was demonstrated with carbofuran, which was more toxic to *Nostoc muscorum* under conditions reflecting or causing a low growth of the cyanobacteria (low inoculum, low light intensity, acidic pH) than

under conditions favorable for its growth (high inoculum, sufficient light, and acaline pH) (Kar and Singh, 1979a). Carbofuran toxicity decreased with the level of nutrients in the medium (Kar and Singh, 1979b). The initial size of the inoculum had a significant role on the tolerance of *Anabaenopsis raciborskii, Anabaena aphanizomonoides, A. spiroides,* and *Microcystis flos-aquae to* 2,4-D and BHC (Das, 1977).

As pointed out by Hutber, Rogers, and Smith (1979), it is difficult to compare the results of different *in vitro* studies and to accurately assess the relative toxicity of pesticides on microorganisms because of (1) variations in the methods used to assess effects on growth, (2) the use in some cases of slow-growing organisms cultured under suboptimal conditions, and (3) the comparison of growth from a single sampling of cultures.

In addition toxicity tests were often performed in a way that hardly permits comparisons and extrapolations. Most *in vitro* experiments only indicate the concentration of pesticide used in the culture medium and not the recommended level for field application (RLFA). Assuming a floodwater depth of 5 to 10 cm and a homogeneous dissolution of pesticides—which is indeed quite far from the reality—the application of 1 kg of active ingredient (ai) per hectare would correspond to 1 to 2 ppm ai in water. Assuming a puddled layer of 15 cm with a bulk density of 0.5, the application of 1 kg ai/ha would correspond to about 1.33 ppm on soil dry weight basis. As in the field most pesticides are usually applied at dosages lower than 3 kg ai/ha, concentrations of 10 to several hundred ppm often tested in flask cultures appears to be used more to estimate a lethal level than to reflect field situation. They are of little value for drawing conclusions on impacts, except when no significant effect was recorded.

Results of *in vitro* trials can hardly be extrapolated to field conditions for several reasons summarized thereafter:

- Toxicity is likely to be higher in flask cultures than in the field. In soil, many factors interact with pesticides and modify their effect as compared with flask culture of a single organism and enhance pesticide degradation. These factors include biological degradation of the pesticide by the soil microflora, nonbiological degradation, photodecomposition, leaching, volatilization, and adsorption to the soil particles. For example, 5 ppm propanil prevented the growth of several cyanobacteria in flask cultures, but the same concentration did not produce any inhibition in the presence of unsterilized or sterilized soil and *in situ* (Ibrahim, 1972; Wright, Stainhope, and Downs, 1977).
- Toxicity depends on the initial microbial population and its nutrient status. These conditions are likely to markedly differ *in vitro* and *in situ.*
- In the field, toxicity depends on the method of pesticide application and water management.

- Toxicity depends on the formulation of the pesticide. *In vitro* experiments frequently test pure ingredients while, in the field, toxicity depends on the formulation. In particular some additives used in commercial formulations were shown to be detrimental to algae. The surfactant Renex 36 at a final concentration of 1.6 ppm in addition to the herbicide HOE-23408 at 2 ppm caused a much greater decrease of the soil algal population than when the herbicide was used alone (Linka, 1978). Similarly, the surfactant used in a commercial preparation of Picloram-D affected algal growth while the pesticide did not (Arvik, Willson, and Darlington, 1971).

  Stratton, Burrell, Kurp, and Corke (1980) pointed out that acetone used to dissolve pesticides in *in vitro* tests may be responsible for a very significant part of the toxicities observed not only as direct effect but as synergistic effect between the solvent and the pesticide. The authors concluded that when 1 percent acetone was used to dissolve permethrin, the toxicity of the pesticide on *Anabaena cylindrica* was overestimated by twenty times.
- In the field toxicity depends on both the pesticide and the degradation products. A pesticide considered harmless in the laboratory may be dangerous when applied in the field due to the production of product(s) having different toxicity than the parent compound. The degradation product of propanil, 2,4-dichloroaniline, was less toxic than propanil towards the growth of *Gloeocapsa alpicola* whereas the degradation products of atrazine were more toxic than the parent compound toward *Anabaena inaequalis* (Stratton, 1984; Wright, Stainhope, and Downs, 1977). Metabolic products of aldrin, dieldrin, and endrin can be as inhibitory to algal growth than the parent compound (Batterton, Bousch, and Matsumura, 1971). 3,4-dichloroaniline, the primary product of propanil degradation, is less inhibitory than propanil, but at the concentration of propanil used in the field (12 ppm), the degradation product can still be inhibitory for some cyanobacteria (Wright, Stainhope, and Downs, 1977).

In vitro experiments are of limited practical value and should be limited to toxicity tests under standardized conditions in order to allow comparisons. A possible standardization could be the determination of the concentrations that would reduce by 50 percent the growth of reference organisms in exponential phase and the concentration that would totally inhibit their growth (Hutber, Rogers, and Smith, 1979).

10.2.2. Studies with Soil

Methods that can be used to determine the effects of herbicides on soil microorganisms were summarized by Greaves et al. (1978) in a technical report of the

Weed Research Association. These methods are basically classical microbiological techniques, which include various soil analysis, the measurement of respiration and enzymatic activities (nitrogenase, phosphatase, dehydrogenase, urease, and cellulose decomposition), and bacterial counts. These methods can be used for any type of pesticide.

Most of the studies of the effect of pesticides on soil microflora have been performed as small-scale experiments in test tubes, beakers, and pots (Table 10.1). Such conditions might overestimate pesticide impact as compared with field situation because of (1) a longer persistence of pesticides due to the absence of rice plant and little variations of environmental conditions (temperature, redox, light, wind) and (2) a more even distribution pesticides and a better contact with microorganisms than *in situ*. The high pesticide concentrations sometimes used in such experiments may also overestimate pesticide efficiency because high concentrations seems to slow down pesticide degradation as shown with molinate (Deuel, Turner, Brown, and Price, 1978) and trifluralin, which degraded very slowly at 200 ppm and rapidly at 1.0 and 0.1 ppm (Parr and Smith, 1973).

Field trials have been mostly used for monitoring the persistence of pesticides and more rarely for record of bacterial populations and microbial activities. No long-term experiment monitoring the effect of pesticides on wetland soils microbiology has yet been reported in the literature.

Very few studies have compared *in situ* and *in vitro* effects of pesticides. In a study of 2,4-D and picloram, used in mixture in commercial herbicides, Arvik, Willson, and Darlington (1971) found no long-term effect of applications at the RLFA (0.3 to 1.1 kg ai/ha) on the composition of algal population *in situ*. Decrease in growth, determined *in vitro*, began at concentrations corresponding to the application of about 560 kg/ha of the herbicides.

10.2.3. Microcosms and Models

Several authors have develop small-scale models (microcosms) of ricefields or aquatic ecosystems to study or predict the bioaccumulation and dissipation of various pesticides applied to flooded ricefields (Chapter 5). Such methods offers an interesting alternative for detailed pesticide studies under controlled conditions, but they have not yet involved the study of the microbial components.

10.2.4. Establishment of the Database Used in This Study

Bibliographic references were collected according to the following criteria:

- All papers dealing with the effects of pesticides on microorganisms and microbial activities, and reporting studies in ricefields, with ricefield soil,

or with microbial, cyanobacteria or microalgae strains isolated from ricefields or known to be present in ricefields;
- Few papers of interest for methodological aspects or presenting data useful for comparison; and
- Bibliographic reviews including references on wetland soils.

Quantitative data on pesticide impacts were collected from this database, tabulated, and analyzed.

10.2.4.1. Bias and Limitation of the Database. Half of the papers of the database presents quantitative estimates of the effects of pesticides on microbial populations or their activities (Table 10.1). The literature on microalgae is more abundant (149 references) than that on other microorganisms (71 references) (Table 10.2) and deals mostly with herbicides (62 percent of the records) (Table 10.3) and cyanobacteria. The literature on other microorganisms deals mostly with insecticides (80 percent of the records). Moreover, most studies are small-scale laboratory experiments consisting of toxicity tests with algal cultures or test tube or flask experiments with a few grams of soil (Table 10.2).

Considering the numerous possible combinations to be tested (nature of pesticide × pesticide concentration × environmental conditions × 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.

10.2.4.2. Tabulation of the Results. Data on cyanobacteria and microalgae collected in the database were 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 in a especially designed Hypercard stack, using a grid combining a geometric scale of pesticide concentration and four levels of inhibition: none, <50 percent, 50 percent, >50 percent, and total inhibition (Figure 10.1). This tabulation allowed to identify results obtained at concentrations higher than the RLFA. Data obtained at concentrations corresponding to the RLFA were then entered in a spread sheet and summarized (Table 10.3).

Data for other microorganisms were 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 one within a five-case scale:

- All negative: the treatment was statistically lower than the control for all measurements;
- Negative trend: various effects were recorded, the balance was negative;

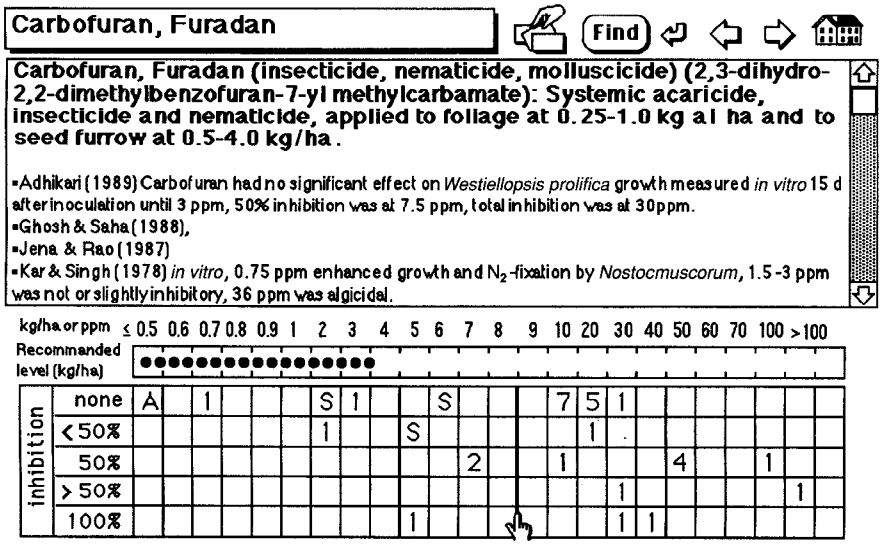

Figure 10.1. Example of a Record on Pesticide Effects on Microalgae in the Hypercard Stack

Cards are connected to the bibliographic database. The upper field provides general information on the pesticide and a summary of the effects recorded by the authors listed in the bibliographic database. The lower field shows the recommended 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 cyanobacteria (*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 ai/ha^{-1} = 2 ppm

- No effect: no statistically significant difference between treatment and control;
- Positive trend: various effects were recorded, the balance was positive; and
- All positive: for all measurements the treatment was statistically higher than the control.

Results were then tabulated in a spreadsheet 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 (five columns) and at concentrations higher than the RLFA (five columns).

Table 10.3. Summary of Data on the Effect of 109 Pesticides on Ricefield Microalgae at Concentrations Corresponding to the Recommended Level for Field Application

Nature of the Data	*Number of Data*	*Percent 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)[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

Note: Data on microalgae and cyanobacteria presented in this table and the following ones are from: Adhikari (1989), Ahmad and Venkataraman (1973), Arvik, Willson, and Darlington (1971), Arvik, Hyzak, and Zimdahl (1973), Battino-Viterbo, Minervini-Ferrante, and Bisiach (1973), Bisiach (1971, 1972), Bongale (1985), Cameron and Julian (1984), Chen Pei Chung (1986), Das and Singh (1977a, b), Das and Singh (1978), Das and Singh (1979), DaSilva, Henriksson, and Henriksson (1975), Dunigan and Hill (1978), Dunigan, Hutchinson, and Hill (1979), El-Haddad (1984), El-Sawy et al. (1984), Fritz-Sheridan (1982), Gangawane (1979, 1980), Gangawane and Kulkarni (1979), Gangawane and Saler (1979), Gangawane, Chaporkar, and Khalil (1982), Ghosh and Saha (1988), Gibson (1972), Gupta and Saxena (1974), Hamdi, El Nawawy, and Tewfik (1970), Holst, Yopp, and Kapusta (1982), Hutber, Rogers, and Smith (1979), Ibrahim (1972), Inger (1970), Ishizawa and Matsuguchi (1966), Kar and Singh (1978, 1979a, 1979b, 1979c), Kashyap and Gupta (1981), Kashyap and Pandey (1982), Kaushik and Venkataraman (1983), Khalil, Chaporkar, and Gangawane (1980), Kumar and Singh (1981), Singh, Tiwari, and Singh (1986), Mallison and Cannon (1984), Maulc and Wright (1984), Megharaj, Venkateswarlu, and Rao (1988a, 1988b), Megharaj, Venkateswarlu, and Rao (1989a, 1989b), Mehta and Hawxby (1979), Minervini-Ferrante, Battino-Viterbo, and Bisiach (1974), Mukherji and Laha (1979), Mukherji and Ray (1966), Mukherji and Sengupta (1964), Singh (1973, 1974), Pande, Rekha Sarkar, and Krishnam-Oorthi (1981), Pandey (1985), Pandey and Kashyap (1986), Pandey and Tiwari (1986), Patnaik and Ramachandran (1976), Pillay and Tchan (1972), Singh, Singh, and Singh (1983), Singh, Rana, and Carg (1986), Raghu and MacRae (1967), Saha, Mandal, Sannigarahi, and Brandhopadhya (1982), Saha, Sannigarahi, Brandhopadhya, and Mandal (1984), Sardespande and Goyal (1982), Satapathy and Singh (1987), Schauberger and Wildman (1977), Sharma and Gaur (1980), Shivaram and Shetty (1988), Singh and Singh (1988), V.P. Singh et al. (1978), Sinha, Pal, and Trial (1986), Smith, Flinchum, and Seaman (1977), Srinivasan (1981), Srinivasan and Ponnuswami (1978), Stratton and Corke (1981), Subramanian (1982), Subramanian and Shanmugasundaram (1986), Theivendirajah and Jeyaseelan (1981), Tiwari, Pandey, and Mishra (1981), Tiwari, Pandey, Mishra, and Srivastava (1982), Tiwari, Pandey, and Misra (1984), Tubea Hawxby, and Mehta (1981), Vaishampayan (1985), Vaishampayan and Prasad (1982), Vaishampayan, Singh, and Singh (1978), Vance and Drummond (1969), Venkataraman and Rajylakswami (1971), Wegener, Aldag, and Meyer (1985), Wright and Maule (1982), Zargar and Dar (1990).

a. Several fungicides act also as algicides.

When no statistical analysis was performed by the authors, which was most often observed with bacterial enumerations, we have assumed that data were of lognormal distribution and considered as significantly different the results at least three times higher or lower than the control (Roger, Jimenez, and Santiago-Ardales 1991).

10.3. Factors Affecting Pesticide Toxicity on Soil Microorganisms

10.3.1. Soil Properties

Little information is available on soil properties that affect pesticide impacts on ricefield microorganisms. The few studies conducted with several soils report some differences in pesticide impacts. In particular the response of N_2-fixing organisms to benomyl, carbofuran and gamma-BHC varied with the soil type (Rajaramamohan Rao, 1980). Mephosfolan had positive or negative effects on the populations of bacteria and actinomycetes depending on the soil studied (Sivaraj and Venugopal, 1979). However, data are too scarce to draw general conclusions. Observations with cultures of cyanobacteria allow some hypothesis. As a general trend, it is usually found that pesticide toxicity to cyanobacteria increases when the growth medium is adjusted to acidic range. It is probable that cyanobacteria, which prefer alkaline environments, are less tolerant to pesticides in acidic than in neutral or alkaline soils. This has been observed in liquid cultures (Das, 1977) but not confirmed by studies in soil. *Anacystis nidulans* was found to tolerate 1 percent ClNa and DDT at 0.8 ppm separately, but growth was inhibited in the presence of both compounds (Batterton, Bousch, and Matsumura, 1972). This aspect might have implications in brackish rice soils.

10.3.2. Water Management and Method of Pesticide Application

A faster pesticide dilution in wetland than in upland soils might be expected, with variations depending upon solubility and the surfactants used. Megharaj, Venkateswarlu, and Rao (1988b) in laboratory experiments observed that carbofuran applied at 5 kg/ha had no effect in flooded soil but caused a transitory decrease in total algal population at ten days in nonflooded soil that disappeared at twenty days. The relations between floodwater management and methods of pesticide application might affect the toxicity of the pesticides with regard to dilution and movements in soil. Water depth is taken into account for algicide

application (Mukherji, 1968) but little information is available on this aspect for other pesticides.

Rao, Pasalu, and Rao (1983) found significant differences in the effect of the same pesticide when applied in the floodwater, incorporated into the soil, or used for dipping rice seedling. In particular HCH incorporated in the soil caused an initial inhibition up to seventy days of rhizosphere soil nitrogenase, while it was stimulating throughout the rice growing period when applied in floodwater. Pentachlorophenol incorporated in soil with lime stimulated N_2-fixing cyanobacteria; but if broadcast in floodwater, even at low levels, it was depressive with a long residual effect (Ishizawa and Matsuguchi, 1966).

10.3.3. Synergistic Effects and Interactions with Other Agrochemicals

Pesticides in combination may interact with each other and alter their respective toxicity on microorganisms (Arvik, Willson, and Darlington, 1971; Chinnaswamy and Patel, 1983; Stratton, 1984). Synergistic stimulatory effects of pesticides on N_2 fixation were reported for combinations of carbofuran with benomyl, nitrofen, and gamma-HCH. On the contrary, diazinon slightly retarded the stimulatory effect of benomyl and carbofuran (Nayak and Rajaramamohan Rao, 1982). Ray, Ramakrishna, and Sethunathan (1980) reported a synergistic increase in the inhibition of nitrification by a combined application of HCH and carbofuran.

Repeated application of the same pesticide has been reported to enhance the growth of the related specific decomposing microorganisms and cause the rapid inactivation of the pesticide. This aspect is summarized by Roger and Bhuiyan (Chapter 5).

Nitrogen fertilizer is known to inhibit N_2-fixation at different levels. Concomitant use of N fertilizer and pesticides in ricefields may affect pesticide toxicity to N_2-fixing organisms. In the case of cyanobacteria, carbofuran toxicity was higher under N_2 fixing conditions than under heterotrophic growth on nitrate (Kashyap and Gupta, 1981; Kashyap and Pandey, 1982; Pandey, Srivastava, and Tiwari, 1984).

10.4. Impacts on Photosynthetic Microorganisms

10.4.1. Effects on Populations of Microalgae and Cyanobacteria

Pesticides have three major effects on ricefield algae and cyanobacteria: (1) a selective toxicity that affects preferentially green algae and thus promote

cyanobacteria growth, (2) a short-term promoting effect of insecticides on microalgae, due to a temporary decrease of invertebrates that graze on algae, (3) a selective effect of insecticides on cyanobacteria by causing a recruitment of algal grazers, which results in the dominance of strains forming mucilaginous macrocolonies resistant to grazing. There are also reports indicating no significant effects of pesticides applied at RLFA on algal flora in the presence of soil (Megharaj, Venkateswarlu, and Rao, 1988a, 1988b).

The database tabulates 1,045 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 percent) 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 analyze the 407 records of pesticide effects obtained at concentrations corresponding to the RLFA (Table 10.3).

An absence of effect of pesticides was reported in 39 percent of the total number of records but only in 62 percent 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.

10.4.1.1. 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 cyanobacteria growth. This was observed with symetryne (Yamagishi and Hashizume, 1974) and algaedyn (Almazan and Robles, 1956). This may explain why only 30 to 40 percent of total inhibition were recorded with algicides and fungicides.

10.4.1.2. Effects of Insecticides. Insecticides had a low impact on tested cyanobacteria and algae, as shown by the high percentage of records indicating no inhibition in the whole database (67 percent) and *in situ* (90 percent) (Table 10.3).

A preferential inhibitory effect of insecticides on green algae, which resulted in the promotion of cyanobacteria growth, was observed with BHC (Ishizawa and Matsuguchi, 1966; Raghu and MacRae, 1967a, 1967b) and PCP (Watanabe, 1977). Simultaneously, insecticides inhibited invertebrates that feed on algae (grazers), thus promoting furthermore BGA and photodependant biological N_2 fixation. This was observed with parathion applied at 1 to 5 ppm in the floodwater (Hirano, Shiraishi, and Nakano, 1955), ethyl parathion applied at 0.2 ppm (Osa-Afiana and Alexander, 1981) phorate (Srinivasan and Emayavaramban,

1977), and carbofuran (Tirol, Santiago, and Watanabe, 1981). Similarly parathion controlled grazers in a lake in the United States, and favored *Anabaena* growth (Cook and Gonner, 1963).

However, insecticide application did not invariably increase photodependant BNF. Some inhibitory effect was reported for PCP *in situ* (Ishizawa and Matsuguchi, 1966).

Also, in the long term, insecticide use might become detrimental to N_2-fixing cyanobacteria 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 µg/ml and that of lindane was 56.0 µg/ml (Grant, Eagan, and Alexander, 1983). Such resistance to conventional pesticides allows large densities of ostracods to develop after pesticide application (5,000 to 15,000/sq m), 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.

10.4.1.3. Effects of Herbicides. Algae, as photosynthetic organisms, should be expected to be more sensitive than other microorganisms to herbicides, especially the photosynthetic inhibitors. Among pesticides not aiming at algal control, herbicides appears to be most detrimental to algae, causing partial or total inhibition in 67 percent of the *in vitro* tests and in 42 percent of the tests performed *in situ* or in the presence of soil (Table 10.3). Several unicellular eukaryotic algae most common in ricefields (*Chlorella, Chlamydomonas, Euglena*) have been shown to be sensitive to photosynthetic inhibitors herbicides (Arvik, Hyzak, and Zimdahl, 1973). Herbicides can inhibit cyanobacteria 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 and Ponnuswami, 1978). Some herbicides seem to affect specifically the N_2-fixing ability of cyanobacteria 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 and Gupta, 1981) and machete (butachlor) (Kashyap and Pandey, 1982).

10.4.2. Effects on Photodependant Biological N_2 Fixation

Raghu and MacRae (1967a, 1967b) were probably the first to report marked stimulation of growth of indigenous cyanobacteria and nitrogen fixation on the

application of gamma-HCH in submerged paddy soils even at 5 kg/ha. This stimulation was attributed to the toxic action of gamma-HCH on algal grazers. Similarly, increased nitrogenase activity in paddy water treated with carbofuran (6 kg ai/ha) was attributed to inhibition of micro-crustaceans and consequent build-up of N_2-fixing cyanobacteria (Tirol, Santiago, and Watanabe, 1981).

However, pesticide application do not invariably increases BNF by cyanobacteria. Insecticide HCH at 50 μg/g (Ishizawa and Matsuguchi, 1966), and the herbicides CNP (2,4,6-trichlorophenyl 4-nitro-phenyl ether) (Matsuguchi, 1979) and propanil (Habte and Alexander, 1980) inhibited the nitrogenase activity of cyanobacteria in a flooded soil. Some pesticides seem to affect specifically the N_2-fixing ability of cyanobacteria as indicated by the observation that the inhibitory effect of dichlone (Kashyap and Gupta, 1981) and butachlor (Kashyap and Pandey, 1982) on N_2-fixing strains growing in N-free medium was markedly decreased or reversed by inorganic N sources. Whereas herbicides seem to be the most detrimental pesticides for photodependant biological N_2 fixation, several species of cyanobacteria tolerated levels (100 to 500 ppm) of 2,4-D much higher than RLFA suggesting that this herbicide might be compatible with cultural practices aiming at promoting cyanobacteria growth as biofertilizer (Venkataraman and Rajyalakshmi, 1971, 1972).

In the numerous experiments dealing with inoculation of ricefields with N_2-fixing strains of cyanobacteria (Roger, 1991) almost no field trials have tested the interaction between pesticides and algal inoculation. Kerni, Shant, Singh, and Gupta (1983) and Kerni, Shant, Gupta, and Singh (1984) concluded to the absence of effect of butachlor applied at 5–30 kg/ha in inoculated plots. El-Sawy et al. (1984) in a pot experiment tested the interaction between cyanobacteria inoculation and four herbicides by measuring plant characteristics and soil nitrogen at 40 days after transplanting (DAT). They found 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 (two of 16 cases) were observed with propanil.

Information on the effects of pesticides on BNF by N_2-fixing cyanobacteria symbiotic of *Azolla* is limited. Insecticides, by decreasing pest incidence, usually favor *Azolla* growth (Satapathy and Singh, 1987; Singh and Singh, 1988). Herbicides have more often detrimental effect. Holst, Yopp, and Kapusta (1982) tested *in vitro* the effect of fifteen herbicides on growth and N_2 fixation of *Azolla mexicana*. Bipyridilium and phenolic herbicides were the most detrimental, causing up to a 75 percent reduction in N_2 fixation and nitrate reduction at 0.1 ppm. Chloramben and benomyl at 10 ppm caused an 84 to 99 percent reduction in N_2 fixation without affecting nitrate reduction or growth. Simazine at 10 ppm stimulated nitrate reduction 20 fold, causing a 99 percent reduction in N_2 fixation. Growth and N_2 fixation were reduced by other benzoic, triazine, dinitroanaline,

and urea herbicides tested at concentrations between 0.1 and 10 ppm. Naptalam was the only pesticide tested that had no effect on growth or N_2 fixation at 10 ppm. *In situ*, preemergence herbicide applied about one week before *Azolla* inoculation had only limited effects on *Azolla*, while postemergence herbicides were more detrimental (Singh and Singh, 1988).

10.4.3. Bioconcentration of Pesticides in Microalgae and Cyanobacteria

Microalgae and cyanobacteria, the base constituents of the aquatic food web in wetland ricefields, have a high surface area/volume ratio, which give them a significant potential for sorption of, and reaction with pesticides (Wright, 1978). They can concentrate pesticide manyfold and, in general, are more resistant to their toxic effects than the food web's high members (Vance and Drummond, 1969). Little field data is available on bioconcentration of pesticide by phototrophic microorganisms in ricefields, but data from laboratory experiments and studies in microcosms and freshwater ecosystems, demonstrate that microalgae and cyanobacteria in ricefields may play an important role in accumulating pesticides that become available to bioconcentration through the food web.

For example, cyanobacteria concentrated 100 to 250 times chlorinated pesticides introduced at a level of 1 mg/L in the culture medium (Vance and Drummond, 1969). Maximum bioaccumulation ratio of fenitrothion ranged from 44 to 105 in living cells and from 100 to 1,810 in dead cells of *Chlorella vulgaris, Nitschia closterium*, and *Anabaena flos-aquae*. Corresponding values for DDT were 420 to 82,000 and 1,000 to 210,000 (Kikuchi et al., 1984).

This aspect is important when considering the ricefield ecosystem as a possible environment for aquaculture (rice-fish, rice-shrimp).

10.4.4. Results from Field Experiments

Most field studies on microalgae or cyanobacteria conducted in the field deal on either algicides used to control algal blooms of microalgae (Dunigan and Hill, 1978; Dunigan, Hutchinson, and Hill, 1979) or the application of synthetic insecticides (Srinivasan, 1981; Grant, Eagan, and Alexander, 1983, Grant, Roger, and Watanabe, 1986) or insecticides of plant origin (Watanabe et al., 1981; Grant, Eagan, and Alexander, 1983; Grant, Seegers, and Watanabe, 1984) to promote photodependant biological N_2-fixation by controlling cyanobacteria grazers.

Experiments to assess the effect on nontarget algae and cyanobacteria deal mostly with herbicide application. In tropical countries little impact of herbicide was reported. Arvik, Willson, and Darlington (1971) found no change in the

composition of the algal flora over an 18-month period after the application of a 1:4 commercial mixture of 4-amino- 3,5,6-trichloropicolinic acid (picloram) and (2,4-dichlorophenoxy) acetic acid (2,4-D) at RLFA. Srinivasan and Ponnuswami (1978) found either no significant effect or a slight inhibitory effect on cyanobacteria of seven herbicides applied at RLFA. Singh, Rana, and Carg (1986) studying the effect of butachlor, thiobencarb, and 2,4-D on N_2-fixing cyanobacteria found that the recommended rates of herbicide did not result in major changes in the composition of the algal population. All herbicides increased the proportion of *Nostoc*, while propanil reduced the proportion of *Anabaena*.

In temperate countries, some inhibitory effect of herbicides was reported. In Japanese ricefields, benthic algae decreased with applications of herbicide but did not increase markedly in fields treated with insecticide (Takamura and Yasuno, 1986). In Italian ricefields, heterocystous and nonheterocystous cyanobacteria, and microalgae were differently affected by the repeated use of simazine (4 kg/ha), the first being more severely affected. The herbicide produced a reduction in the species diversity, which was very evident in the case of heterocystous cyanobacteria (Tomaselli, Giovannetti, and Materassi, 1987).

Resistance to biocides is a common phenomenon. Cyanobacteria can be adapted to increased concentrations of pesticides (Sharma and Gaur, 1981). When several strains of algae are tested for sensitivity to pesticides, strains resistant to field levels are usually identified (Gadkari, 1987). Spontaneous mutants resistant to pesticides (monuron, blitox) have been isolated (Vaishampayan, 1984, 1985; Vaishampayan and Prasad, 1982). The study of various classes of herbicides by Hawxby, Tubea, Ownby, and Basler (1977) showed that s-triazines and substituted ureas could alter phytoplankton composition by selective inhibition of certain species. As sensitivity to a given pesticide may vary between quite large limits among algal strains, pesticide application might cause shifts in dominant strains within the algal/cyanobacterial community rather than a decrease of the whole algal biomass.

10.5. Impacts on Nonphotosynthetic Microorganisms and Their Activities

10.5.1. General Trends

Contrary to experiments with microalgae and cyanobacteria, most tests on nonphotosynthetic microflora and its activities were performed in the presence of soil, either in small-scale experiments (51 percent of the data) or *in situ* (47 percent of the data) (Table 10.4). Also, most experiments were performed at concentrations corresponding to the RLFA. The database tabulates 606 records obtained at such concentrations. About 60 percent of the records deal with

Table 10.4. 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	Percent of Data for Each Effect[a]				
		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

Note: Data on nonphotosynthetic microorganisms presented in this table and the following ones are from: Akhtar, Solangi, and Baig (1986), Azad and Khan (1968), Baruah and Mishra, (1986), Chen Ching Chao (1983), Charyulu, Ramakrishna, and Rao (1980), Chen (1980), Chendrayan and Sethunathan (1980), De and Mukhopadhyay (1971), Endo, Kusaka, Tan, and Sakai (1982), Furusaka (1978), Gowda, Rao, and Sethunathan (1977), Jayachandran and Chandramohan (1977), Jena and Rajaramamohan Rao (1987), Kandasamy et al. (1975), MacRae, Raghu, and Castro (1967), Mahapatra and Rao (1981), Mandal, Bandyopadhyay, Bandyopadhyay, and Maity (1987), Mitsui, Watanabe, Honma, and Honda (1964), Nair, Ramakrishnan, and Sithanatham (1974), Nayak and Rajaramamohan Rao (1980, 1982), Nishio and Kusano (1978), Palaniappan and Balasubramanian (1985), Patnaik, Panda, and Dash (1986), Purushothman, Venkataraman, and Kasirajan (1976), Raghu and MacRae (1967a, 1967b), Ramakrishna, Rao, and Sethunathan (1978), Ramakrishna, Gowda, and Sethunathan (1979), Ramakrishna and Sethunathan (1982), Rao, Pasalu, and Rajaramamohan Rao (1983), Rao Prasad, and Rajaramamohan Rao (1984), Ray, Ramakrishna, and Sethunathan (1980), Ray and Sethunathan (1980), Roy, Sinha, and Mukherjee (1975), Russo (1970), Sathasivan, Palaniappan, and Balasubramaniyan (1982), Sato (1987), Sethunathan and MacRae (1969), Singh, Tiwari, and Singh (1986), Sivaraj and Venugopal (1979), Sivasithamparam (1970), Tirol, Santiago, and Watanabe (1981), Turner (1979), Yeomans and Bremner (1985).

a. See text for definition of effects.

populations or activities in the bulk of soil and 40 percent deal with the rhizosphere. Data suffer from a strong bias in the nature of pesticides tested, 73 percent of the records being on insecticides (Table 10.4).

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

Table 10.5. 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	Percent of Data for Each Effect[a]				
		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%)					

a. See text for definition of effects.

Experiments *in situ* showed a higher percentage of no significant effects (73 percent) than small-scale experiments (46 percent), 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 percent) than in the rhizosphere (no effect: 70 percent), 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 percent) than fungicides (no effect: 50 percent) and insecticides (no effect: 68 percent).

The summary of effects according to counts of microbial populations and other types of measurements (Table 10.5) shows that, on an average, populations of microorganisms were less affected by pesticides (58 percent of no effects) than microbial activities (46 percent of no effects).

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

Microbial activities were more affected than enzymatic activities. Among

Table 10.6. 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

		Percent of Data for Each Effect[a]				
Groups	*Number of Data*	*All Negative*	*Negative Trend*	*No Effect*	*Positive Trend*	*All Positive*
All data	606 (100%)	8	12	60	11	9
Data on 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 of 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

a. See text for definition of effects.

123 tests on ten soil enzymes, 93 percent showed no effect of pesticide application. Only ß-glucosidase reacted negatively to pesticide application (Purushothaman, Venkataraman, and Kasirajan, 1976).

Half of the records of the database deal with N cycle. About 60 percent of the data on N cycle concern BNF and 30 percent concern nitrification and denitrification. Data on other aspects are not numerous enough to allow general conclusions (Table 10.6).

10.5.2. Effects on Heterotrophic Biological N_2 Fixation

N_2-fixing microflora and BNF were more affected by pesticides (no effect: 31 percent) than other populations and activities of the N cycle (no effect: 71 percent). The low percentage of nonsignificant effects on BNF was mostly due to a higher number of positive effects (45 percent), 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 percent) than the activities (no effect: 18 percent).

With 25 percent of negative effects and 45 percent 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 (5 ppm) 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, Pasalu, and Rajaramamohan Rao (1983) reported variables effects of the same pesticide depending on the method of application.

10.5.3. Effects on Nitrification-Denitrification

Nitrification was not affected by pesticides in about 60 percent of the cases. This value is similar to the average of the database. However, negative effects were much more frequent (34 percent of the cases) than positive effects (6 percent of the cases). Nitrification 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, Zimmerman and Lumpkin, 1993).

Denitrification was not affected by pesticides in 87 percent 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, Watanabe, Honma, and Honda (1964), testing the effect of eight dithiocarbamate pesticides in a rice soil, found that 20 ppm Vapam (metham) or 100 ppm of the other pesticides was required to significantly decrease denitrification at two and five days after pesticide application. Such concentrations are higher than the RLFA.

10.5.4. Results from Field Experiments

Most field studies dealing with microflora present no statistical analyses of the data, but results of microbial enumerations after pesticide application often

indicate either an absence of effect or minor effects (Patnaik, Panda, and Dash, 1986).

Both positive and negative effects of pesticides have been observed. A positive effect of insecticide gamma-BHC on N_2 fixation and populations of anaerobic, phosphate-dissolving bacteria was reported by Raghu and MacRae (1967a, 1967b). A decrease in microbial population was reported after the application of insecticides diazinon, cytrolane, carbofuran, carbaryl + lindane, quinalphos, and Dursban at RLFA whereas fungal populations were not affected (Purushothaman, Venkataraman, and Kasirajan, 1976). Total count of benthic bacteria decreased from the other of 10^{10} cells/ml to the order of 10^9 cells/ml after the simultaneous application of insecticide and herbicide (Takamura and Yasuno, 1986).

However changes in bacterial populations were usually followed by a recovery within two or three weeks. Herbicide butachlor had no significant effect on populations of fungi and actinomycetes but possibly increased total populations of bacteria for about two weeks (Chen, 1980). Insecticides sevidol, endrin and gamma-BHC applied at 0.45 to 2.25 kg ai/ha had no adverse effect on the bacterial population and available N, P, and K of the soil observed at harvest (Nair, Ramakrishnan, and Sithanantham, 1974).

A transitory effect of pesticide application at RLFA on microbial populations was also reported for herbicide benthiocarb (Sato, 1987). Inhibitory effect of diazinon, carbofuran, and endosulfan at recommended dose were observed on microbial populations at three and nine days after application, but they recovered at twenty days (Roy, Sinha, and Mukheijee, 1975).

One study reported a decrease in rhizospheric BNF measured at sixty and seventy-five days after transplanting in a field where pesticide were sprayed at fifty, sixty, and seventy-five days after transplanting (Nayak and Rao, 1980). On the other hand, two studies of rhizospheric biological N_2 fixation reported a long lasting stimulatory (Mahapatra and Rao, 1981) or inconsistent (Rao, Pasalu, and Rao, 1983) effects of insecticides. This probably reflects more the long-term effects of pesticides on the rice plant than a direct effect on the microflora. The stimulatory effect of gamma-BHC on rhizospheric populations of *Azospirillum* sp. and *Azotobacter* was associated with a reduction of the drop in redox potential of the field soil up to eighty days after transplantation under submerged conditions (Mahapatra and Rao, 1981).

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

Considering only results from *in situ* experiments (Table 10.7) provides

Table 10.7. 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	Percent of Data for Each Effect[a]				
		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 (cyanobacteria 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

a. See text for definition of effects.

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 percent) was recorded with the whole dataset.

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

- More impacts of herbicides than of insecticides;
- A higher sensitivity 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 10.7); and

- A higher sensitivity of BNF to pesticides (39 percent of no significant effects) than the average sensitivity observed with the whole set of data *in situ* (73 percent of no significant effects).

10.6. Conclusions

The impacts of pesticide microflora in wetland soils depends on their persistence, the concentrations attained in the environment, and synergistic/antagonistic effects among pesticides and between pesticides and fertilizers. 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. 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. Therefore, pesticide degradation is often faster in flooded than in nonflooded soils and other aerobic systems. As a result of a shorter persistence and faster dilution, pesticides 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 "ratio of effects" to analyze 1,016 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 were counted as negative. The ratio of positive to negative counts was called the "ratio of effects." The average of twenty-seven ratios calculated by Anderson (1978) for herbicides, fungicides, and insecticides in experiments or observations dealing almost exclusively with upland soil is 1.39, which corresponds to 42 percent of negative effects. The 606 data on wetland soils recorded in our database indicate only 20 percent of negative effects.

About half of 547 papers dealing with the impacts of pesticides on ricefield microflora present quantitative data. However, less than 8 percent 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 were 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 percent of the total number of records but only in 62 percent 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 percent of the *in vitro* tests and in 42 percent of the tests performed *in situ* or in the presence of soil. Recorded effects of pesticides on ricefield algae are: (1) a selective toxicity of all types of pesticides, which affects the composition of the algal population and often favor cyanobacteria, 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, in the long term, insecticide use might cause the proliferation of algal grazers resistant to insecticides such as Ostracods. Grazing pressure may partly explain the dominance in many ricefields of strains of cyanobacteria 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 mostly on cyanobacteria 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 percent of the cases, no effect was recorded in about 60 percent of the cases, and a promoting effect was recorded in 20 percent of the cases. Data were biased by the high percentage (73 percent) of records on insecticides. Effects were more frequently observed in laboratory experiments (54 percent of the cases) than *in situ* (27 percent), 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 percent) 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. Pesticide volatilization through the rice plant may also explain this difference.

Herbicides had more often significant effects (70 percent) than insecticides (32 percent). Microbial activities were more sensitive to pesticides than population densities. This trend was especially obvious with data on BNF. This observation might indicate a short-term effect of pesticides, sufficient to affect metabolic activities but not the viability of the microorganisms.

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 percent of the cases), which is rather beneficial as it reduces losses from N fertilizer. Denitrification was little affected (13 percent of the cases). With 25 percent of negative effects and 45 percent of positive effects, N_2-fixing microflora and biological N_2- fixation were more affected by pesticides than other populations and activities of the N cycle (no effect: 71 percent) 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 percent) than their activity (no effect: 18 percent).

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

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 one to three weeks.

The analysis of the literature 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. Wainright (1978) already concluded that pesticides, with the exception of fumigants and some broad spectrum fungicides, have little deleterious influence on soil processes when applied at field rates. Invertebrate populations seems to be more sensitive to pesticides than microflora (Chapter 9).

However, available information raise several concerns. These are reports of significant effects of pesticides on nontarget 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 10.1). 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 that 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 of 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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