MICROBIOLOGICAL ASPECTS OF PESTICIDE USE IN WETLAND RICEFIELDS

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CONTENTS

1. INTRODUCTION

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. However there is a concern about the enhanced use of pesticides that might alter the microflora responsible for the maintenance of soil fertility and also lead to a reduced pesticide efficiency because of shifts in microbial populations toward organisms more efficient in pesticide degradation.

Most of the earlier information on the effects of pesticides on non-target soil microorganisms comes from observations in upland temperate soils (Anderson 1978). However, during the last two decades, information on tropical wetland soils-- where most of world rice is produced--has become available.

This review consider three major aspects of the microbiology of pesticide utilization in soils used for wetland rice cultivation:
(1) the methods that are used or could be used to study the relations between pesticides and microflora,
(2) the role of microflora in degrading pesticides applied in ricefields, and
(3) the effects of this pesticides on the non target microorganisms.

Several reviews are available that covers specific parts of these topics. They include the degradation of pesticides in tropical soils (Sethunathan & Siddaramapa 1978; Sethunathan et al. 1982) the effects of pesticides on nitrogen transformations in flooded soils (Ray & Sethunathan, 1988), and the effects of pesticides on N_2-fixing blue-green algae (BGA) (Chinnaswamy & Patel 1984; Padhy 1985; Kumar 1988).

The literature on microbiological aspects of pesticide use in ricefields is quite abundant as shown by the non exhaustive list of references quoted in this review. However, considering (1) the numerous possible combinations to be tested (nature of pesticide x pesticide concentration x environmental conditions x microorganisms or microbial activities), and (2) the methodological limitations of the studies currently performed, this literature is still extremely fragmentary. In particular the available information is markedly biased because (1) most of the information comes from *in vitro* experiments, (2) the literature on microalgae is quantitatively as abundant as that on all other microorganisms and deals almost only with N_2-fixing blue-green algae (BGA), and (3) the literature on non photosynthetic microorganisms deals mostly with insecticides. Anderson (1978) in the introduction of his review on pesticide effects on non-target soil microorganisms stated: " *It is not intended in this paper to condemn, condone, or propound arguments regarding particular pesticides* (or groups of pesticides), *since the available evidence will not permit it. At the most, generalizations can be made and trends identified"* . Obviously, Anderson's statement also applied to this review. To avoid the enumeration of fragmentary experimental results classified according to groups of pesticides or micro organisms, data have been tabulated (Appendix 1) and emphasis is placed on general trends.

Table 1. Methods used for studying pesticide transformations and/or their effects on microbial populations and microbial activities in wetland soils*.

Type of experimental design	n° of reports*
Algological studies.	
Flask culture of microorganisms	102
Pot experiments	6
Field experiments	12
Total	120
Bacteriological studies	
Flask cultures of microorganisms	6
Soil in test tubes or beakers	52
Pot experiments	13
Microcosm experiments	10
Field studies on degradation and residues	12
Field studies on populations and activities	8
Total	101

* data are from a literature data base that can be obtained from the author on request.

2. METHODOLOGICAL ASPECTS

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 of studies of soil algae for which 85% of experiments have been performed in flasks with axenic or unialgal cultures of single species (Table 1).

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 as microorganisms of a same taxon may show different responses to the same pesticide (Chen PeiChung 1986; Hutber et al 1979) and toxicity *in vitro* depends on the culture conditions (Kar and Singh 1979b), the nutrient concentration (Kar and Singh 1979c) and the initial size of the inoculum (Das 1977) .

As pointed out by Hutbert et al. (1979), it is difficult to compare the results of different studies and to accurately assess the relative toxicity of pesticides on microorganisms because of variations in the methods used to assess effects on growth, the use in some cases of slow-growing organisms cultured under suboptimal conditions, and 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 dose in the field. Assuming a floodwater depth of 5-10 cm and an homogeneous dissolution of pesticides -- which is indeed quite far from the reality --, one kg of active ingredient (a.i.) applied per hectare would correspond to 1-2 ppm a.i. in water. Assuming a puddled layer of 15 cm with a bulk density of 0.5, the application of 1 kg a.i. ha^{-1} would correspond to about 1.33 ppm on soil dry weight basis. As in the field pesticides are usually applied at dosages lower than a few hundred grams of a.i. ha^{-1}, 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.

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 (1) biological degradation of the pesticide by the soil microflora, (2) non biological degradation, and (3) leaching, volatilization, and/or adsorption to the soil particles. For example, 5 ppm propanil prevented the growth of several BGA 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 et al., l977).

• Toxicity depends on the initial microbial population and the nutrient status, and this conditions are likely to markedly differ *in vitro* and *in situ*.

• In the field, toxicity depends on the method of pesticide application (see section 4.3)

• In the field, 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 et al. 1971).

• 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 (DCA), was less toxic than propanil towards the growth of *Gloeocapsa alpicola* whereas the degradation products of atrazine were more toxic than the parent compound towards *Anabaena inaequalis*.(Stratton 1984; Wright, Stainthorpe and Downs 1977).

Metabolic products of Aldrin, Dieldrin, and Endrin can be as inhibitory to algal growth than the parent compound (Batterton et al., 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 BGA (Wright et al., l977).

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% the growth of reference organisms in exponential phase and the concentration that would totally inhibit their growth (Hutbert et al. 1979).

2.2. Studies with soil

Methods that can be used to determine the effects of herbicides on soil microorganisms were summarized by Graves 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, cellulose decomposition) and bacterial counts. These method can be used for any type of pesticide. Sethunathan et al. (1980) suggested that redox potential, reducing capacity and certain reduction reactions, assayed for example in terms of nitrate disappeared or reduced iron formed, could be used as simple and suitable indicators of the pesticide-degrading capacity of anaerobic systems. Sethunathan et al. (1980) also suggested that dehydrogenase activity, which is very active in predominantly anaerobic flooded soil may have the obvious advantage over the $^{14}CO_2$ evolution technique as an assay for the pesticide-degrading capacity of anaerobic systems.

Most of the studies of the effect of pesticides on soil microflora have been performed in small scale experiments in test tubes and beakers (Table 1). However the high pesticide concentrations sometimes used in such experiments may overestimate pesticide efficiency because high concentrations seems to slow down pesticide degradation as shown with Trifluralin which degraded very slowly at 200 ppm and rapidly at 1.0 and 0.1 ppm (Parr & 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 experiments monitoring the effect of pesticides on wetland soils microbiology has yet been reported in the literature.

2.3. Microcosms and models

Several authors have tried to develop small scale models (microcosms) of ricefields or aquatic ecosystems to study the fate of pesticides. Chen et al. (1982) used a microcosm including rice plants and 10 organisms (grasshopper, brown plant hopper, mosquito larva, wolf spider, water flea, dragonfly naiad, giant duckweed, mosquito fish algae, and snails) to study the fate of ^{14}C labelled thiobencarb. Tomizawa (1980) used a similar approach to study the persistence and bioaccumulation of several pesticides in aquatic ecosystems. Microcosms were designed by Higiashi (1987), Seiber et al. (1986), and Isensee et al. (1982) to study and/or predict the dissipation of various pesticides applied to flooded ricefields.

Such methods offers an interesting alternative for detailed pesticide studies under controlled conditions but they have not yet involved the study of the microbiological components.

2.4. Bioassays using microorganisms

Microbial bioassays are relative methods in which the growth responses of an organism to a water or a soil, is used to obtain information on the quality of the environment. Bioassays are popular in limnology because they are suitable to asses the environmental impact of pollutants that cannot be adequately determined on the basis of chemical or physical parameters alone. Algal bioassays have also been used to study specific chemical by comparing observed effects (growth or motility) with a standard curve obtained with

preparations of known composition and concentration. Algal bioassay are particularly attractive in aquatic ecosystems because algae are the first link in the food chain.

Several authors have used soil algae for bioassay of pesticides in soils. In particular, the sensitivity of *Chlorella* to photosynthetic inhibitors, and its ease of culture have permitted its use as a bioassay organism for pesticides, especially photosynthetic inhibitors herbicides (Butler 1977). The blue-green alga *Phormidium* was used by Noll and Bauer (1973) to detect a range of 22 herbicides. Trichome migration was markedly affected by most herbicides, and concentrations from 0.3 to 15.0 ppm could be detected in as little as 3 hours. Mallison and Cannon (1984), from spot tests, disk assays, growth curves, and one-step growth experiments concluded that *Plectonema boryanum* (BGA) may be a useful indicator for phototoxic agents in bioassay procedures. Bongale (1985) suggested the possibility of utilizing the germination of spores of BGA as a bioassay for estimating pesticide residues in soils.

With regard to their ecological importance in wetland soils, BGA could be a well adapted material for bioassays in ricefields but such methods have not yet been adopted.

3. PESTICIDE METABOLISM IN WETLAND SOILS

3.1 Degradation/transformation

Flooded rice soil is an ideal environment for rapid detoxication of certain pesticides known to persist in non flooded soils and other aerobic systems (Sethunathan and Siddaramapa, 1978). Pesticide degradation is favored by temperature and reducing conditions caused by submersion and further accelerated by organic matter incorporation, as well as a pH which usually stabilizes in a range favoring microbial activity (6.7 to 7.2) (Ponnamperuma 1972). Pesticide degradation can be extremely rapid in prereduced soils as shown with Parathion which exhibited instantaneous degradation with 48-86% disappearance when shake for 5 seconds with reduced non sterile soil (Wahid et al. 1980).

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

3.1.1. Factors responsible for pesticide degradation/transformation

Anaerobic microorganisms are particularly implicated in pesticide transformation in wetland soils, but chemical transformations catalyzed by redox reactions such as the iron redox system may also be common. The importance of microbial degradation was shown by several experiments comparing the fate of pesticides in normal soil with that in a sterilized control. Usually, a much faster degradation was observed when an active microflora was present (Adhya et al. 1981; Funayama et al. 1986; Gowda and Sethunathan 1976; MacRae et al. 1967; Nakamura et al. 1977; Raghu & MacRae 1966; Sethunathan and MacRae 1969), but degradation was also observed in the absence of microflora (Sethunathan & MacRae 1969; Sudhakar-Barik & Sethunathan 1978).

The relative importance of non biological degradation varies with pesticides. 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).

3.1.2. Microflora

In upland conditions, bacteria and fungi are considered to be mainly responsible for pesticide transformations in soils. In wetland soils, fungi are probably less important whereas microalgae might have a significant role. Sato and Kubo (1964) found that parathion was degraded within a few days in rice fields and that the presence of algae greatly accelerated the degradation rate of *in vitro*..

Experiments in flask have shown that the toxic effect of BHC, HCH, and carbofuran on axenic cultures of BGA was reduced by repeated inoculation and removal of the algae. (Das and Singh 1977; Kar and Singh 1979). But the mechanism responsible for the decrease of toxicity of the was not elucidated, it could have been pesticide degradation by the alga, or accumulation and removal with the harvested algae, or spontaneous degradation of the pesticide.

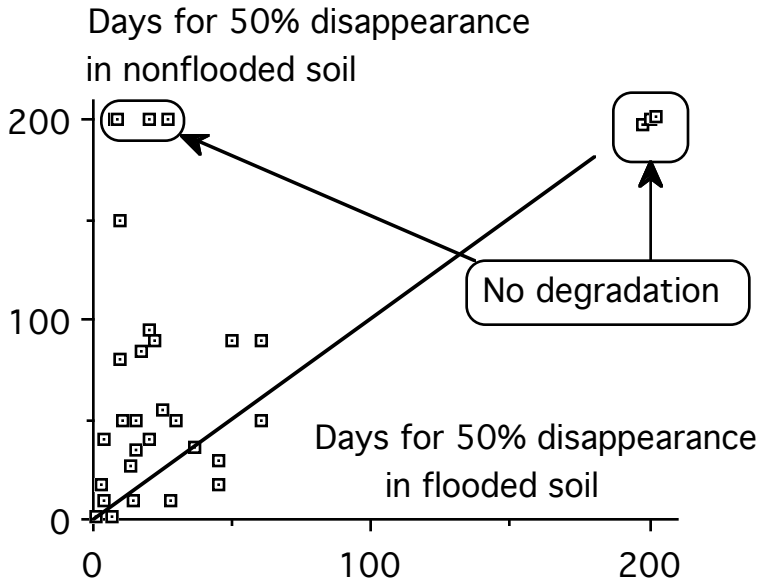
Rhizospheric bacteria may play a significant role in pesticide degradation. In an unplanted flooded soil, less than 5.5% of the ^{14}C of labelled parathion was evolved as $^{14}CO_2$ in two weeks whereas 22.6% was evolved in planted soil (Reddy & Sethunathan 1983a).

3.1.3. Effect of anaerobiosis (wetland vs dryland)

The comparison of the relative stability of commonly used pesticides in wetland and upland conditions (Fig. 1) shows that most of the pesticides have a much longer persistence in non flooded soils than in flooded soils.

There is a relation between soil redox and pesticide degradation. Gamma and beta isomers of HCH decomposed rapidly in non sterile soils capable of attaining redox potentials of -40 to -100 mV within 20 days after flooding.

Fig. 1 : Relative stability of 30 pesticides in flooded and non flooded soils*



*drawn from data by Sethunathan & Siddaramappa, 1978.

Degradation was slow in soils low in organic matter and in soils with very low pH and positive Eh after several weeks of flooding (Sethunathan et al. 1976). A negative redox

potential also favored the degradation of DDT, Endrin, and Toxaphene, while Trifluralin degradation was favored by a redox between + 50 and +150 (Willis et. al. 1974; Sethunathan et al. 1980). Sethunathan and Siddaramapa (1978) suggested that the alternate oxydation and reduction processes in non continuously flooded soils may favor degradation of pesticides. Brahmaprakash et al. (1985) observed that the degradation of anaerobically unstable HCH isomers in the rice rhizosphere was not retarded by the possible aeration of a flooded soil by roots.

However, they are also reports indicating no difference in degradation under upland and flooded conditions (Castro & Yoshida,1975) or even a longer persistence of pesticides under flooded conditions. Molinate dissipation rate significantly decreased at Eh values lower than 70 mV (Deuel et al. 1978),^{14}C-Benthiocarb was rapidly degraded under oxidative conditions, but slowly under reductive conditions (Nakamura et al. 1977), and Phorate was much more persistent under flooded than under non flooded conditions (Walter-Echols & Lichtenstein 1978).

Also, pesticide losses by volatilization might be retarded in the flooded anaerobic environment as shown by Parr & Smith (1973) for Trifluralin.

The mechanisms of degradation/transformation differ in wetland and upland conditions and a same compound my be degraded according to different pathways depending upon the environmental conditions. For example, Trifluralin degrades by a pathway involving sequential dealkylation of propyl groups in aerobic conditions, and by a pathway involving initial reduction of the nitro groups in anaerobic conditions (Parr & Smith 1973).

In flooded conditions, reductive dechlorination (DDT), hydrolysis (diazinon and parathion), and nitro-group reduction (parathion) have been demonstrated. Dehydrochlorination (DDT), ring cleavage (IMHP), and epoxidation (aldrin and heptachlor) are apparently blocked or less favored in oxygen-depleted flooded soil (Sethunathan 1972, Hill 1978).

3.1.4. Effect of organic matter

Organochlorine insecticides were found to degrade faster in soils with high organic matter content (Castro and Yoshida 1971). Several reports shows that organic matter incorporation, which increases microbial activity and hastens the drop in redox potential in flooded soils, favors pesticide degradation. This was observed for straw incorporation (Adhya et al. 1981; Chopra and Magu 1986; Gowda and Sethunathan 1976; Venkateswarlu & Sethunathan N 1979) as well as for green manure incorporation (Ferreria and Raghu 1981). However, Castro and Yoshida (1971) observed this effect only when soil organic matter was low.

3.1.5. Effect of soil properties

The degradation of some pesticides is affected by soil pH. Sethunathan et al. (1982) presented several example indicating that both alkaline and acidic conditions could enhance the decomposition of specific pesticides or groups of pesticides. They indicated that organophosphates and carbamates are more affected by soil pH than organochlorine insecticides. Microbial degradation of carbofuran in three soils was slowest in soil with the lowest pH (Siddaramappa et al. 1979).

Carbaryl was more persistent in an acid sulfate soil (pH 3.7) than in other acid soils (pH 4.2-4.8) which was attributed to a low bacterial activity (Gill and Yeoh 1980). On the

other hand Wahid & Sethunathan (1979) observed that hydrogen sulphide, the end product of sulfate reduction which is frequent in such soils, was involved in the degradation of parathion.

3.1.6. Long term effects of pesticide application

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. A second application of gamma-BHC to a soil, 55 days after the first one, showed a faster degradation of the pesticide (Raghu & MacRae 1966). Diazinon persisted for about 15 days in a flooded soil (pH 6.6) that had been treated previously with this insecticide; but, it persisted for about 60 days in a soil that had never been exposed to diazinon (Sethunathan 1972). Two successive annual applications of aldicarb, as subsurface band-in-row treatments at 4 kg a.i. ha^{-1} in the same field, resulted in the development of microorganisms that rapidly broke down aldicarb and accelerated degradation of aldicarb in soil and water (Read 1987).

Several bacteria having the ability to degrade a given pesticide were isolated from soils or water of fields previously treated with this pesticide. A Flavobacterium sp., isolated from water of a diazinon treated rice field, had exceptionally high capability to metabolize diazinon as sole carbon source (Sethunathan 1972; ; Sethunathan & Pathak 1972). Similarly Sudhakar-Barik et al. (1976) isolated a *Pseudomonas* sp. and *Bacillus* sp. able to decompose nitrophenols from parathion-amended flooded soil. A substantial portion (23% for *Pseudomonas* sp. and 80% for *Bacillus* sp.) of radioactivity applied as p-nitrophenol was accounted for as CO_2 at the end of a 72-h period. Watanabe (1973, 1977) isolated

pentachlorophenol-decomposing and PCP-tolerant bacteria from soils and observed a 1000-fold difference in the number of PCP-decomposing microorganisms between treated and untreated soil (Watanabe 1978).

However, there are reports indicating that repetitive application of pesticides did not lead to the build-up of the degrading microflora. This was observed in laboratory experiments with carbofuran (Venkateswarlu & Sethunathan 1978) and benthiocarb (Nakamura et al. 1977). Also Ohisa & Yamaguchi (1978) observed that the enrichment of soil populations of *Clostridium rectum*, a bacterium isolated from ricefield soil that degrades BHC by a cometabolic process, was not caused by BHC application but by the addition of peptone.

Repeated application of pesticide has also been reported to cause changes in the metabolic pattern of pesticide decomposition (Sudhakar-Barik et al. 1979). The principal metabolic pathway for the insecticide parathion shifted from nitro group reduction to hydrolysis upon the repeated additions of parathion or its hydrolysis product, p-nitrophenol, to a flooded soil. Both nitro group reduction and hydrolysis are cometabolic reactions; the build-up of parathion-hydrolysing micro-organisms after parathion additions was at the expense of the product of the primary cometabolic hydrolysis, p-nitrophenol (Sethunathan et al. 1980).

Changes in degradation pathways may lead to environmental problems. For example, Benthiocarb is generally detoxified by hydrolysis, but its repeated application to

flooded soil favoured the multiplication of anaerobic bacteria that decompose Benthiocarb by reductive dechlorination, resulting in the formation of a very phytotoxic compound (Moon and Kuwatsuka,1984).

3.2. Bioconcentration in microorganisms

Whereas bioconcentration of pesticides in the various elements of the food chain is a major topic of interest in pesticide studies, little attention has been paid to this aspect in microbial studies. The few available data refer to pesticide accumulation in vitro by BGA common in ricefields (Fitzgerald 1975; Das and Singh 1977; Kar and Singh 1979). However data from freshwater environments have demonstrated the ability of microalgae to accumulate pesticides (Wright 1978).

4. FACTORS AFFECTING PESTICIDE TOXICITY ON MICROORGANISMS

4.1.Soil properties

As a general trend, it is usually found that pesticide toxicity to BGA increases when the growth medium is adjusted to acidic range. It is probable that the 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% ClNa and DDT at 0.8 ppm separately, but growth was inhibited in the presence of both compounds (Batterton et al. 1972). This aspect might have implications in saline rice soils.

4.2. Water management

In upland soils pesticide remains mostly at the soil surface -- the area of maximum algal activity-- until cultivation or watering incorporates into the soil. In wetlands a faster dilution of pesticides might be expected with, indeed, variations depending upon solubility and surfactants used. The relations between floodwater management and pesticide application might affect the toxicity of the pesticides with regard to dilution and movements in soil but no information is available on this aspect.

4. 3. Method of pesticide application

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 and Matsuguchi,1966).

Rao et al. (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 70 days of rhizosphere soil nitrogenase, while it was stimulating throughout the rice growing period when applied in floodwater.

Application carbofuran to a depth of about 3 cm from the surface of flooded soil reduced the concentrations in standing water but increased the persistence in soil significantly (Siddaramappa & Seiber 1979). Placement in the root zone of rice also resulted in a significant increase in persistence in soil as compared with water application (Siddaramappa et al. 1979).

4. 4. Synergistic effects between pesticides

Pesticides in combination may interact with each other and alter their respective toxicity (Chinnaswamy and Patel 1983; Torres and O'Flaherty 1976; Arvik et al 1971; Stratton 1983; Metting and Rayburn 1979). The fungicide benomyl significantly increased the persistence of generally short-lived parathion in flooded soil by inhibiting nitro group reduction and hydrolysis (Sudhakar-Barik & Sethunathan 1979; Sethunathan et al. 1980). Benzene hexachloride was more inhibitory on *Anabaena flos-aquae* in combination with fernoxone than alone (Chinnaswamy and Patel 1983). Synergistic stimulatory effects of pesticides on N_2 fixation were reported for combinations of carbofuran with benomyl, nitrofen and -HCH. On the contrary, diazinon slightly retarded the stimulatory effect of benomyl and carbofuran (Nayak & Rajaramamohan Rao 1982). Ray et al. (1980) reported a synergistic increase in the inhibition of nitrification by a combined application of HCH and carbofuran.

4.5. Interaction with other agrochemicals

Nitrogen fertilizer is known to inhibit at different levels N_2-fixation. The concomitant use of pesticides and fertilizer in rice fields may further augment the pesticide toxicity to N_2-fixing BGA.

The addition of various nitrogenous fertilizers such as ammonium sulfate or urea along with carbofuran influenced the duration of the insecticide's activity in soil and water (Siddaramappa & Seiber 1979).

5. EFFECTS ON MICROBIAL POPULATIONS

5.1. Effects on microalgae

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.

Several reports indicates a preferential inhibitory effect of pesticides on green algae which results in the promotion of BGA growth. This was observed with BHC (Ishizawa & Matsuguchi 1966, Raghu & McRae 1967), PCP (Watanabe 1977), Symetryne (Yamagishi and Hashizume 1974), and algaedyn (Almazan & Robles 1956).

Several insecticides have been reported to be harmless to BGA while they killed algal grazers, thus promoting BGA growth. This was observed in ricefields for parathion applied at 1 to 5 ppm in the irrigation water (Hirang et al. 1955), methyl parathion (Folidol) applied at a 25 ppm (Anonymous 1977), and Phorate (Srinivasan & Emayavaramban 1977). Similarly parathion controlled grazers in a lake in the USA, and favored *Anabaena* growth (Cook & Conners 1963).

However, on a long term basis, insecticide application might be detrimental to BGA by decreasing species diversity and causing a rapid recruitment of ostracods. The relative acute lethal toxicity of carbofuran to the ostracod *Heterocypris luzonensis* was 2.4 μg ml^{-1} and that of Lindane was 56.0 μg ml^{-1} (Grant et al. 1983a). 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. Such populations may cause the disappearance of algal blooms in a few days. A field study by Takamura and

Yasuno (1986) reports the development of large populations of chironomids and ostracods in herbicide and insecticide treated fields. Simultaneously, the number of natural predators of chironomids and ostracods decreased. Benthic algae decreased in herbicide treated plots and did not increase in insecticide treated plots probably because of grazing by ostracods.

There are also several reports indicating no significant effects of pesticides applied at recommended level on algal flora in the presence of soil (Megharaj et al. 1988).

Table 2. Summarization of the data from published reports on the effects of pesticides on microflora and microbial activities in wetland ricefields. Summary per microbiological groups and microbial activities*

Population/ Activity	Pesticide tested*			Effect **		
	F	H	I	-	=	+
MICROBIAL POPULATIONS,						
Actinomycetes	**0**	**5**	**26**	**4**	**19**	**7**
Fungi	**0**	**1**	**25**	**7**	**18**	**1**
Bacteria						
Total bacteria in soil	0	5	15	4	13	3
Total bacteria in phyllosphere	0	0	7	0	7	0
Total bacteria in rhizosphere	0	0	7	0	7	0
N cycle other than BNF	0	9	5	6	3	5
N_2-fixing bacteria	0	1	20	1	15	6
Various physiological groups	0	5	5	1	2	7
Miscellaneous groups	0	2	4	3	3	1
Total of bacterial counts	**0**	**22**	**63**	**15**	**50**	**22**
SOIL PROPERTIES (N, P, K availability)	**3**	**7**	**0**	**1**	**0**	**8**
SPECIFIC ENZYMATIC ACTIVITIES						
Amylase	0	1	8	0	8	1
Cellulase	0	0	8	0	8	0
Dehydrogenase	1	4	3	0	6	3
Dextranase	0	0	7	0	7	0
Invertase	0	1	14	0	15	0
Phosphatase	0	0	13	0	13	0
Urease	0	4	1	0	4	1
ß-glucosidase	0	0	13	4	9	0
Others	0	0	2	0	2	0
Total of enzymatic activities	**1**	**10**	**69**	**4**	**72**	**5**
MICROBIOLOGICAL ACTIVITIES						
O_2 uptake or CO_2 production	0	5	2	2	1	3
OM decomposition/mineralization	1	2	1	0	4	0
Nitrification	2	6	15	14	6	2
Denitrification	9	9	12	4	16	0
N_2-fixation (soil)	8	9	24	1	3	28
N_2fixation in rhizosphere	0	0	32	13	10	9
Total of microbiological activities	**20**	**31**	**86**	**34**	**40**	**42**
GRAND TOTAL	**24**	**76**	**269**	**65**	**199**	**85**

Values are nº of reports of the effect of one pesticide on a single microbial group or a microbial activity
I: insecticide; H: herbicide; F: fungicide; - : inhibition; =: no effect; +: enhancement
The origin of the data is the same as in table 3. Extensive data are in Annex 1.

Table 3. Summarization of the data from published reports on the effects of pesticides on microflora and microbial activities in wetland ricefields. Summary per type of pesticide*

	inhibition	no effect	enhancement	Total
Fungicide	2	8	6	16
Herbicides	17	11	21	49
Insecticides	39	172	46	257
Mixtures	0	0	10	10

*Values are nº of reports of the effect of one pesticide on a single microbial group or a microbial activity Data from: Azad & Khan; Baruah & Mishra, 1986; Charyulu et al., 1980; Chendrayan & Sethunathan, 1980; Chopra & Magu, 1986; Endo et al., 1982b; Jayachandran & Chandramohan, 1977; Jena & Rajaramamohan Rao, 1986; Kandaswamy et al., 1975; Mac Rae & Castro, 1967; Mahapatra & Rao, 1981; Mandal et al., 1987; Mitsui et al.,1964; Nair et al., 1974; Nayak & Rajaramamohan Rao, 1980; Nayak et al., 1980; Nishio & Kusano, 1978; Palaniappan & Balasubramanian, 1985; Purushothman et al., 1976; Raghu & Mac Rae, 1967; Ramakrishna & Sethunathan,1982; Rao et al., 1983; Ray & Sethunathan, 1980; Roy et al., 1975; Russo, 1970; Sathasivan et al., 1982; Sato, 1987; Sethunathan & MacRae, 1969; Singh et al., 1986; Sivaraj & Venugopal, 1979; Sivasithamparam, 1970; Tirol et al., 1981; Turner, 1979; Yeomans & Bremner, 1985. Extensive data are in Annex 1.

5.2. Effects on populations of non photosynthetic microorganisms.

A non exhaustive compilation of the recorded effect of pesticides on (1) populations of microorganisms other than algae and (2) microbial activities in wetland rice soils is annexed (Annex 1). Tables 2 and 3 present a summarized analysis of the results of this compilation. As 82 % of the data refer to insecticides (Table 2), the significance of the analysis is obviously limited by the imbalance of the nature of the pesticides tested.

On an average 17% of the trials report a decrease in bacterial population after a pesticide application, in 58% of the cases no significant change was observed, and in 25% of the cases an increase was recorded. Actinomycetes show a trend similar to that of bacterial counts. Populations of fungi seems to be most sensitive to pesticides. However it has to be kept in mind that the relative abundance of actinomycetes and fungi is much lower in wetland soils than in upland soils.

Among the different groups of bacteria, N_2-fixers seems to be the less negatively affected, while other bacteria of the N cycle are relatively the most frequently inhibited. When considering original data (see Annex 1) no trend is obvious within this last group as negative effects were recorded for ammonium oxidizers, denitrifiers, and nitrite oxydizers. The values presented for N_2-fixing bacteria are biased by a large number of tests with phyllospheric Azotobacter which have little agroecological implication. The absence of inhibitory effect on populations of nonrhizosperic N_2-fixers is in agreement with a very low number of records of negative effects of pesticide application on nitrogen fixation in soil but the absence of effect on populations of rhizosperic N_2-fixers do not with the relatively high frequency of inhibition of BNF in rhizosphere (Table 2).

6. EFFECTS ON MICROBIAL ACTIVITIES

6.1. Effect on enzymatic activities

Among 81 tests on 10 soil enzymes 89% show no effect of pesticide application on soil enzymatic activities. Only ß-glucosidase reacted negatively to pesticide application (Table 2).

6.2. Effects on biological nitrogen fixation

6.2.1. Heterotrophic BNF

Results regarding heterotrophic BNF show a very frequent enhancement of soil BNF while rhizospheric BNF was inhibited at least transitorily in 49% of the cases (Table 2).

Nayak & Rajaramamohan Rao (1980) using benomyl, carbofuran and gamma-BHC applied at the recommended field level (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. In most cases a positive effect was observed but a single pesticide could exhibit nmegative 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.

6.2.2. Photodependant BNF

Raghu and MacRae (1967) were probably the first to report marked stimulation of growth of indigenous blue-green algae and nitrogen fixation on the application of -HCH in submerged paddy soils even at 5 kg/ha. This stimulation was attributed to the toxic action of -HCH on algal grazers. Similarly, increased nitrogenase activity in paddy water treated with carbofuran (6 kg a.i./ha) was attributed to inhibition of micro-crustaceans and consequent build-up of nitrogen-fixing blue-green algae (Tirol, Santiago and Watanabe 1981).

However, pesticide application do not invariably increases BNF by BGA. The application of 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 BGA in flooded soil. Some pesticides seems to affect specifically the nitrogen fixing ability of BGA as indicated by the observation that the inhibitory effect of dichlone (Kashyap & Gupta 1981) and Machete (Kashyap & Pandey 1982) observed on N_2-fixing strains growing in N-free medium was markedly decreased or reversed by inorganic N sources.

In the numerous experiments dealing with algal inoculation of rice fields (Roger 1990) almost no field trials have tested the interaction between pesticides and algal inoculation. Kerni et al. (1983, 1984) concluded to the absence of effect of Butachlor applied at 5-30 kg ha^{-1} in inoculated plots. El-Sawy et al (1984) in a pot experiment tested the interaction between BGA inoculation and four herbicides by measuring plant characteristics and soil nitrogen at 40 DT. 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 (2 of 16 cases) were observed with propanyl.

Information on the effects of pesticides on BNF by Azolla is limited. Holst et al. (1982) tested the effect of 15 pesticides on growth and nitrogen assimilation of *Azolla mexicana*. Bipyridilium and phenolic herbicides were the most detrimental, causing up to a 75% reduction in nitrogen fixation and nitrate reduction at 0.1 ppm. Chloramben and benomyl at 10 ppm caused an 84 to 99% reduction in nitrogen fixation without affecting nitrate

reduction or growth. Simazine at 10 ppm stimulated nitrate reduction 20 fold, causing a 99% reduction in nitrogen fixation. Growth and nitrogen assimilation 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 nitrogen assimilation at 10 ppm.

6.2.2. Nitrification-denitrification

Among microbial activities, nitrification was most frequently recorded as sensitive to pesticide application. Denitrification was much less affected (Table 2). Mitsui et al. (1964) conducted extensive pot and laboratory experiments on the effect of 8 dithiocarbamate pesticides on denitrification in a rice soil. Vapama and Dithane (Zineb) depressed nitrification of urea under upland conditions, while none of the pesticides tested had a depressive effect on denitrification of nitrate applied under water-logged conditions. Among dithiocarbamate compounds, Vapam applied at 20 ppm decreased denitrification by approximately by 40%. Other pesticides required a concentration of 100 ppm to decrease significantly denitrification at 2 and 5 days after pesticide application. Inhibition rates ranged from 0 to 56%. It was concluded that soil denitrification is less sensitive to pesticides than nitrification. However, the high concentration of pesticide used should be kept in mind when interpreting this data.

Effects on other microbial activities concern only restricted number of cases and do not allow conclusions (Table 2).

When considering the data summarized per main type of pesticide (Table 3), herbicides seems to exhibit relatively more often detrimental effects on soil microflora than insecticides.

9. CONCLUSION

Flooded rice soil is an ideal environment for rapid detoxication of many pesticides. Degradation is usually faster in flooded soils than in non flooded soils and other aerobic systems.

Studies conducted in the presence of soil tend to show that pesticides at normally recommended field rates and intervals are seldom deleterious to the beneficial organisms and their activities. In 349 tests of the effect of a pesticide (sometimes applied at a rate higher than the recommended dose) on a microbial population or activity in wetland soils or in rice rhizosphere, an inhibitory effect was recorded in about 19 % of the cases, no effect was recorded in about 57 % of the cases, and a promoting effect was recorded in 24 % of the cases. When tests lasted for several week, many of the inhibitory effects were observed to be transitory as a recovery of populations or activities was observed after 1 to 3 weeks. Most of these data were obtained under laboratory conditions which might exaggerate the effect of pesticides.

Only 12 studies on algae and 8 studies on non photosynthetic bacteria were conducted in the field. Field studies on algae mostly reported an enhancement of algal growth due to insecticide application -- some of these studies were in fact dealing with the promotion of photodependant BNF controlling grazers with chemical pesticides or pesticides of plant origin--. Five of the eight fields studies dealing with non photodependant microorganisms reported no effect of pesticide application on microbial

populations or activities, two showed a transitory drop of populations after pesticide application followed by a recovery within two to three weeks. One study indicated a decrease in N_2-fixing activity associated with rice measured at 60 and 75 days after transplanting in a field where pesticide were sprayed at 50, 60, and 75 days after transplanting (Nayak et al. 1980)

These observations seems to partly confirm the common belief that pesticides applied at recommended level and intervals seldom markedly affect the soil microorganisms and their activities. However, they are reports of significant effects of pesticides on non target microorganisms of importance to soil fertility.

The current stage of the knowledge of wetland soils is too fragmentary to draw conclusions.

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. Pesticides might have only temporary effects but, when applied repetitively, 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.

The maintenance of soil fertility in wetland ricefields is a complex process involving the replenishment of soil nutrients through crop residues and the photosynthetic aquatic biomass in floodwater (Roger and Kurihara, 1988). Therefore, attention has to be paid to long term effects of pesticide application on the ecology of the photic zone (floodwater and surface soil) in relation with N cycling and the effects of soil microbial biomass in relation with N availability. With regard to the major role of the microfauna in recycling N in the rice field ecosystem, attention should also be paid to the effect of pesticides on invertebrates populations. In such studies, emphasis has to be placed on long-term field experiments.

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