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Impact of long term pesticide usage on soil properties using radiotracer techniques

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FOREWORD

An important activity of the United Nations Food and Agriculture Organization (FAO) is to assist Member States to ensure that, as far as possible, pesticides are used effectively and safely. To this end, FAO has published *Guidelines for the Registration and Control of Pesticides,* which forms the basis of most national pesticide registration schemes. Among the recommendations is that data should be provided to show that a candidate pesticide has no unacceptable effects on non-target organisms. Soil micro-flora and fauna, because of their central role in maintaining soil fertility, are highly ranked in this context.

Concern has been expressed that the data normally presented may not be adequate to predict the effects on soil micro-organisms of repeated, heavy, multiple applications of pesticides that are common in monocultures of crops, such as cotton, maize and rice. Evaluation of the effects of such pesticide regimes requires studies of a range of soil microbial activities, some of which require the use of ^{14}C-labelled pesticides. Therefore, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture organised a Co-ordinated Research project to assess whether or not there is a need to extend the data requirements for pesticide registration to cover such extreme agricultural practices. This TECDOC summarises the outcome of this programme and includes papers presented at the final Research Co-ordination meeting held in Hangzhou, China, 24–28 May 1999.

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SUMMARY OF THE CO-ORDINATED RESEARCH PROGRAMME

1. Introduction

Crop losses to pests, pathogens and weed competition exceeded 40% worldwide in 1989–90 [1], ranging from 28% in Europe to almost 49% in Africa. In financial terms this is of the order of $250 billion. To try to contain these losses, farmers and growers use chemical pesticides. Although pesticides are credited with success in increasing food production and helping to protect man and animals against disease vectors, there are concerns that they have the potential to harm human and environmental health and even compromise the sustainability of agricultural systems.

Part of a pesticide application usually reaches the soil, even if sprayed on the growing crop, and so may have an effect on organisms living in the soil. Therefore, it is important to study the possible effects of specific practices on soil properties. Such possibilities are of particular concern where pesticides are applied at high rates, repeatedly, over many years, as occurs in crops such as maize and cotton which often receive multiple applications of several pesticides during a single growing season.

Soil is a dynamic living system with a variety of micro-and macro-flora and fauna including bacteria, actinomycetes, fungi, nematodes, arthropods, crustaceans and earthworms. They play a primary role in the degradation of plant and animal residues and other organic matter in the environment as well as in nitrogen fixation, nitrification and the release of nutrients from soil minerals [2]. Anything that affects their activities might affect the function of soils not only in crop production, but also in the global carbon and nitrogen cycles and in the removal of a range of environmental pollutants. The consequences could thus be serious. Hence the *FAO Guidelines for the Registration and Control of Pesticides* [3], which are followed by national registration schemes, require studies of the effects of candidate compounds on soil activities.

This requirement has lead to considerable research on the impact of pesticides on soil and their fate and degradation following single applications for short periods. The observed effects have been minor and short lived [4] even for old compounds that were developed before the introduction of modern, rigorous registration procedures. However, little work has been reported on the effects of heavy, repeated and long term applications of pesticides on soil other than a UK study with four herbicides applied individually for 16 years which found no effects on soil properties and crop yield [5]. There seem to have been no relevant studies involving sequences of several compounds. Recognizing the need for research in this area, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture initiated this 5-year Co-ordinated Research project (CRP)

2. Objectives of the CRP

To promote the safe and efficient use of pesticides and fair practice in their sale and distribution, the FAO has developed the *International Code of Conduct on the Distribution and Use of Pesticides*. 'Safe and efficient use' includes the requirement for practices which minimize possible adverse effects on humans and the environment. The guidelines [3] for data requirements for pesticide registration relating to environmental considerations include assessment of degradation, adsorption/desorption, leaching and effects on soil microorganisms. The main objective of the CRP was to support this FAO initiative with co-ordinated studies of the effects on soil activities of heavy, repeated applications of pesticides, as commonly used in maize and cotton production. As discussed above, studies of this sort are not normally done so

the results were expected to show whether or not registration requirements need to be extended to include them.

The second objective of the CRP was to encourage interdisciplinary co-operation within the participating countries as the programme required expertise in agronomy, chemistry, microbiology and soil science.

Studies with radiolabelled pesticides were essential to the programme.

3. Methods and materials

3.1. Experimental outline

The programme involved assessments of populations of soil microorganisms and of biochemical processes in order to:

(a) evaluate the effects of repeated long term (up to four years applications of pesticides, commonly used in cotton, corn or rice agrosystems, on specific soil parameters such as: bioactivity and physical and chemical characteristics.
(b) study, using radio tracer techniques, binding phenomena within the framework of repeated long term treatments (to determine if there is a cumulative effect).
(c) assess the soil capacity to release the bound residues and whether this capacity is being affected by heavy pesticide applications.

Items b & c can only be studied using isotope-labeled pesticides.

Experiments were performed for four years (1995–1998) in corn and cotton field plots and in the laboratory. Soil plots were laid out according to Test Protocol 1 (Appendix I). Soil samples were collected from different types of plot: (a) control soil plots, which received no pesticide applications; (b) pesticide treated experimental plots, which received pesticide applications similar to farmer's plots; (c) where possible, plots in nearby farmer's fields with a history of heavy pesticide use.

The programme was planned to allow studies in cotton, maize and rice but in practice cotton was chosen by all participants except those in Canada, where maize was grown. The pesticides were those which are commonly used on these crops in the participating countries and so differed from country to country. The least intensive applications were in China and Canada (3 applications of methamidophos and atrazine respectively), Egypt (one application of each of 3 compounds per year) and the Philippines (5 applications of prophenofos per year). Elsewhere annual pesticide use was much higher: India, 5–6 compounds; Pakistan, 8–10 applications of mixtures; Brazil 10–12 applications including some mixtures; Thailand 11–16 applications including some mixtures. This broad approach meant that inter-country comparisons could not be made with respect to particular compounds but it did ensure the inclusion of some of the most extreme pesticide use practices.

Soil samples were collected before and after pesticide applications and brought to the laboratory for assessments of: microbial populations (largely bacteria and fungi); basal and substrate-induced respiration; iron reduction; nitrification; activity of the enzymes dehydrogenase, aryl sulfatase and arginine deaminase; soil capacity to bind, release and mineralize ^{14}C-ring-labeled pesticides. Study protocols are shown in Appendix-I.

For many participants, this CRP was an adjunct to ongoing programmes, so some did not carry out all the assessments for which there were protocols and some reported additional experiments.

3.2. Assessment procedure

The interpretation of observed effects on soil activities is difficult. Quantitative and qualitative changes in species numbers and, in consequence, in biochemical activity are subject to a number of feedback processes which work towards re-establishing the former steady state.

Apparent stimulation may follow an initial biocidal action and, indeed, this is exploited in cultural systems that involve soil 'sterilisation' by heat (eg steam or solarization) or chemical means. Microbial populations are influenced by vegetation, not least because most active organisms live in the rhizosphere. Thus soil under a weedy crop is likely to have a different population from one that is weed free regardless of whether weeds are controlled mechanically or chemically.

A further complication is that depressions of microbial populations by 50–90% happen naturally in response to drought, flooding or temperature change. Generally recovery occurs in about 30d so it has been proposed [6] that effects lasting less than 30d can be ignored and only those lasting over 60d give cause for concern.

This criterion could not be applied directly in this CRP because in most cases there was less than 60d between successive pesticide applications. However, data from the untreated control plots gave an indication of the magnitude of natural fluctuations at each site with which to compare the effects of pesticide treatment. Thus any change in a property that was no greater than the annual fluctuation in values from the control, is not considered to be biologically important even if there was a statistically significant difference between treated and control values at any particular time.

Many of the participants' reports discuss results in terms of changes in soil properties following each pesticide application. It is important to remember, however, that the effect of any particular pesticide in a sequence is likely to be influenced by earlier applications of other compounds so it will not be valid to construct a list ascribing specific effects to individual pesticide or mixtures. The aim is to assess the effects of overall pesticide treatment systems.

4. Results

4.1. The effect of pesticides on soil microbial populations

Microbial populations may be affected by many factors including environmental changes and pollution with xenobiotic chemicals. It has been known for over 25 years [7] that, in short term experiments, pesticides may stimulate, inhibit, or have no effect on microbial numbers. The results of this programme with high rates of application over 3–4 years are consistent with this pattern.

In Brazil, microbial numbers were recorded only in the 3rd (and last) year. There were fewer bacteria in experimental than control plots at the beginning of the year before the final crop was sown but their number rose to over 3 times the control figure in one sampling but was lower subsequently. By the end of the season the difference in numbers was small, 0.4×10^6 colony forming units (CFU)/g in the control and 0.28×10^6/g in the treated plots. It is noteworthy that number of bacteria in the control plots was 3.4×10^6/g at the intercrop sampling so the difference at the end of the experiment seems trivial compared with the

natural fluctuation. The situation with fungi was similar though the number of CFUs in the controls fell from 36 to 3.0×10^3/g over the season while the corresponding numbers for the treated plots were 4.0 and 2.3×10^3/g.

The results with atrazine in Canada showed some reduction in both bacterial and fungal numbers during the first 2 years but there were no differences at the end of the 3rd year. Plots treated with methamidophos 3 times a year in China contained slightly fewer bacteria than the controls at most sampling dates but the numbers were well within the range of natural fluctuation ($0.52 – 11.9 \times 10^6$ /g).

Numbers of fungi were sometimes higher and sometimes lower in the treated plots but always within the range of natural fluctuation ($1.1 - 17.46 \times 10^4$/g).

Numbers of bacteria and actinomycetes in the Egyptian study were always lower in treated than control plots following each application in the sequence of monocrotophos, methomyl and carbaryl in all 4 years. Fungi were generally, but not always, also lower in the treated plots. Populations of all 3 groups showed some powers of recovery but the numbers recorded in treated plots at the pre-spray sampling in June 1998 were below the ranges that occurred in the controls.

There were transient differences in numbers of bacteria, fungi, actinomycetes and *Azotobacter* between treated and control plots in India but there were no differences in the final sampling in 1998. The picture in Pakistan was similar with positive and negative differences at various times and at the end of the experiment there were no biologically significant differences in numbers of bacteria or fungi.

Five annual applications of profenofos seemed to have had no real affect on numbers of bacteria, bacilli or fungi in the Philippines. There was an initial temporary reduction in bacteria in the farm plots which had previously received cypermethrin and isoprocarb but at the end of 4 years there were no significant differences.

Only the pesticide application regime used in Egypt produced a lasting effect on numbers of bacteria, fungi and actinomycetes in the soil. Elsewhere even the transient effects were inconsistent. For example numbers of bacteria increased following dimethoate application in Pakistan in 1994 but reduced in 1998.

The conclusion must be that any effect on numbers of micro-organisms produced by the pesticides used in this programme is likely to be insignificant compared with the other factors involved.

4.2. The effect of pesticides on soil respiration and biomass

Soil respiration, as indicated by oxygen consumption and CO_2 evolution, is considered as an indicator of microbiological activity although it must be interpreted with caution [6]. The rate of soil respiration depends on the physiological condition of the organisms as well as edaphic conditions such as temperature and soil moisture

In this programme, both Basal Respiration and Substrate Induced Respiration (respiration following amendment of the soil with glucose) were measured. The ratio of Basal to Induced Respiration gives the Respiration (or Metabolic) Quotient from which an estimate of biomass can be calculated. The participants varied as to which of these data they reported.

Values for metabolic quotient and biomass in Brazil varied without an obvious pattern. For example, they were lower than control values following monocrotophos application in the first year but were higher in the second year and were lower than controls following treatment with methyl parathion and deltamethrin, alone but not after treatment with a mixture of the two. Values were higher in treated than control plots between the 2nd and 3rd crops but there were no differences following the final harvest.

In Canada, where only atrazine was applied, basal respiration was lower than control in the treated experimental plots in the second year but was higher in the farm plots. After some fluctuations, biomass was the same in all plots in the final samples taken at the end of the 3rd year.

In China there were no clear differences in basal respiration between controls and plots treated with methamidophos but, curiously, respiration rates in the 4th year were on average only about 20% of the average for the first year.

There is a hint of a reduction in biomass caused by pesticides in the Egyptian results where on 6 of 12 pre spray sampling occasions it was substantially lower than in the controls. However, it was only once lower than the lowest control value recorded in the 4 years. Further, pre-spray figures were never significantly lower than those found 48h after the previous spray application so it is likely that any damage will not be permanent.

Over the 4 year experimental period in India there was one occasion for basal respiration and 2 for substrate induced respiration when the values for treated plots were lower than those than in the controls. There were no differences in the final samples.

Various increases and reductions shortly after pesticide application were recorded in Pakistan but at harvest in the final year basal and induced respiration and biomass were higher in the experimental and farm plots than in the controls. However, the values were all within the range of control values found over the 4 years.

Values for basal respiration from control plots in the Philippines fluctuated in the same way as in those treated with profenofos while the controls for substrate induced respiration fluctuated so widely (119.5 – 874 μg CO_2/g soil^{-1}h) that all but one value (which was higher) from the treated plots was within the range. There were fluctuations in basal respiration in the experiment in Thailand but in the samples taken after harvest there were no real differences between control and treated plots although at the end of the second year respiration in the treated plots was higher statistically.

Heat production in the soil is an alternative indicator of biological activity which was used in Germany. Bromoxynil applied at 0.615 mg a.i./kg dry soil (equivalent to a normal agricultural rate) in the laboratory did not affect basic or substrate-induced heat production or metabolic heat quotient in the soil. Only at a concentration 1000 times higher were there reductions both in basal and substrate-induced heat production. The experiment lasted only 27d so the long term significance of this observation cannot be judged.

Thus no participant reported a conclusive effect on respiration or figures derived from it although the data from Egypt, taken together with those for numbers of micro-organisms, are not entirely reassuring.

4.4. The effect of pesticides on nitrification

Nitrification is a biological oxidation process by which ammonium released from soil organic matter (ammonification) is oxidized to nitrite by *Nitromonas* spp. and then to nitrate by *Nitrobacter* spp. Nitrate is the principal nitrogen source for plants so inhibition of these organisms could reduce soil fertility

There were indications of inhibition of nitrification in several countries although not in Brazil where on only two occasions were there statistically significant differences between control and treated plots.

The atrazine treatments used in Canada reduced nitrification in both experimental and farm plots although the effect was not great in the latter. Values recovered almost to the level in control plots by the beginning of the 2nd and 3rd crop seasons so the long term significance may not be serious. However, the lowest level recorded in the experimental plots in the second growing season was less than half that of the control which might be significant where ammonium salts are used as nitrogen fertilizers.

Nitrification was lower in treated than control plot following each pesticide application in Egypt by 10% to over 50%. Only twice, before the 2nd spray date in 1995 and before the first application in 1998 had nitrification recovered in the treated plots to the level in the controls so, as with the system in Canada, the effect may have biological significance.

The results from the experiment in Pakistan are confusing not least because of large differences in the values from control soils taken before the treated plots were sprayed and two days later. In one case the difference was more than six fold. In 1997 in samples taken after application of profenofos +cypermethrin and after profenofos alone, nitrification in the farm plots was higher than in controls while in the experimental plots it was lower. Despite this, the pre-spray values in the experimental plots were lower than the post-spray values in both cases, making interpretation impossible. The same situation arose in 1998 in the experimental plots following application of profenofos + alphamethrin. The data show clearly the effect of the weather, as most of the figures for 1998 are ten or more times greater than those for 1997.

In Thailand where a range of compounds was applied 11–16 times per year samples were taken pre- and post- spray on 4 occasions per year. In the first year there were no differences but in the 2nd and 3rd years nitrification was usually lower in treated plots post spray but always recovered before the next application with values higher than those of the controls. Samples taken 4 and 6 weeks after the final harvest showed no differences.

The study in China was done in the laboratory and methamidophos or dimethoate was applied three times at intervals of 36 days. There was an initial inhibition at all rates of treatment (up to 10 μg/g but levels were back to those of the controls in 36 days or less. There was also a laboratory experiment in Germany with bromoxynil. Only a rate equivalent to 1000 times the normal agricultural rate caused substantial inhibition although a 100 fold rate caused some inhibition for 2 weeks.

The results from Canada, Egypt and, to a lesser extent, from Thailand show that nitrification may be vulnerable to some pesticide practices. The agricultural significance, if any, is unclear. If nitrate availability to the growing crop is reduced sufficiently to affect yield the problem might be overcome my modifying fertilizer practice if the need for pest control is

overwhelming. In cropping systems which may cause nitrate pollution of water, inhibition of nitrification may be a benefit. Indeed a nitrification inhibitor, nitrapyrin, has been used commercially in this context.

4.5. The effect of pesticides on iron reduction in the soil

The reduction of the ferric ion to the ferrous ion, whose salts are more soluble and hence more available to plants, is an indicator of the activity of anaerobic microorganisms in the soil. It has been proposed as a test for the effect of pesticides on microbial activities [8, 9]. Whilst soils are largely aerobic unless flooded, as in the case of paddy soils, there are usually anaerobic microsites in otherwise aerobic soils [10]. Therefore anaerobic activities are of more than academic interest.

The results are broadly similar to those for nitrification, with the greatest effects occurring in Egypt with smaller differences in Canada and Thailand. In the first 2 years in Egypt rates of iron reduction were lower in treated than control plots after each pesticide application but recovered by the time of the subsequent spray. In the 3rd and 4th years levels were always lower in the treated plots and the figure for the samples taken before the final application in 1998 were 0.78 μg Fe^{2+}/g compared with 2.85 μg/g in the controls. The lowest control value in the 4 years was 1.54 μg/g so this can be regarded as a significant difference.

In Canada values in experimental plots were lower than in the controls with some, but not complete, recovery between seasons. The figures for the farm plots were always lower then the controls but they increased over the 3 years so if atrazine was having an effect, it was masked by other factors.

Figures for the first year in Thailand were generally lower in the treated plots but with an indication of post-harvest recovery. The results were similar in the 2nd year but without a post-harvest recovery and on two occasions the values from treated plots were below the lowest control value. Differences were greater in the 3rd year and on 5 occasions treated plot figures were below the lowest control value. Four
weeks after harvest the figure from the treated plots was less than 50% of that of the controls. As the difference was only about 10% after 8 weeks, statistically significant but within the range of control values, recovery in the off-season seems possible.

In Brazil, India and Pakistan there were statistically significant differences, both positive and negative between treated and control plots but within the seasonal fluctuation of the controls and there were differences in the final samples only in Pakistan where the figures from experimental and farm plots were higher than the controls.

Overall, only the pesticide regime used in Egypt produced results for iron reduction that would justify further investigation.

4.6. The effect of pesticides on soil dehydrogenase activity

Dehydrogenases are oxidoreductases and these enzymes are responsible for oxidation of organic matter. Increase in dehydrogenase activity caused by pesticides has been reported [11,12,13]. The field experiments reported in this programme did not show any clear differences between treatment and control values. There were some divergences but they were within the range of fluctuations of control values. In the laboratory experiment in China each application of dimethoate or methamidophos to the soil at the recommended application rate

inhibited dehydrogenase activity, but this effect was temporary and the activity recovered within 36 days on each occasion. In laboratory tests in Germany bromoxynil at a concentration equivalent to 100-fold the field application rate had reversible inhibitory effect on dehydrogenase activity but a 1000 fold concentration stopped activity completely and irreversibly within the 4 weeks the study lasted.

4.7. The effect of pesticides on soil arginine deaminase activity

Ammonification of arginine is brought about by arginine deaminase activity which is closely related to soil respiration and so is an indicator of microbial activity.

Again, the strongest evidence for a deleterious effect was seen in the Egyptian results. There were substantial reductions (by 70–90% of control figures) in activity following each pesticide application over the 3 years. The level had just about recovered by the beginning of the 2nd year and again at the beginning of the 3rd but there was not a corresponding figure to test if there was a similar recovery after the 3rd crop season. Assuming it would have done, there is perhaps no long term concern but the reduction in activity during the cropping period suggests more detailed work on plant response is desirable.

The results from Brazil show extremely large variations in values for control soils (0.011 – 0.376 μg $N/h/g^{-1}$ soil) and on 2 occasions the value for treated plots was much higher than the highest control value. There were, however, several occasions when control values were statistically higher and, given the variability of the data, it would be unsafe to conclude that the pesticide regime stimulated arginine deaminase.

In Canada, India and Pakistan there were occasions in which the values for treated plots were higher or lower than the range of control figures but at the end of the experiment there were no real differences although in Canada the farm plot figures and in India the experimental plot figures were statistically higher than the controls.

4.8. The effect of pesticide applications on the activity of aryl sulfatase in the soil

There was an indication in Canada of slight stimulation of aryl sulfatase activity in plots treated with atrazine. Elsewhere there was no significant effect on the activity of the enzyme. For this reason this study was discontinued after the third year.

4.9. Relationships between the various microbiological properties measured

It was concluded over 30 years ago [14] that, with the techniques then available, enzyme activity rarely correlates with microbial activity. Table 1 shows the correlation coefficients between the microbiological activities measured, although not all participants reported data that could be used in this way. It is clear from the table that the conclusion is still valid as only 2 values were above 0.7. This reaffirms that a range of assessments of the effects of pesticides on soil micro-organisms is necessary.

4.10. The effect of pesticides on binding and persistence of residues in the soil

Studies with ^{14}C labeled compounds have shown that frequently the extraction procedures used in pesticide residue analysis do not remove all of the applied radioactivity. Such unextractable materials are commonly known as ‘bound residues’ although they include ^{14}C that has been incorporated into other compounds following transformation of the compound

originally applied. The biologically available fraction of the radioactivity seems to be largely associated with such transformation products with only a small component coming from unextracted parent compound [15, 16]. However, these conclusions are drawn from studies using single compounds albeit sometimes involving repeated annual applications. This programme was concerned to check that similar conclusions apply when mixtures and sequences of pesticides are applied often several times a season and at high rates.

Pesticides are removed from soil by the processes of volatilization, degradation (microbiological and abiotic) and leaching. The overall kinetics of dissipation thus depends on the rates of several processes so does not usually fit a specific rate law. Curve fitting procedures are frequently used to obtain a mathematical description but often the rate approximates to first order. As it is the resultant of several processes it should be referred to as 'pseudo first order' and the term DT_{50} (time to 50% loss) used instead of 'half-life' ($t_{0.5}$).

Differences in levels of unextracted ^{14}C between previously treated and untreated plots were small. In Brazil (methyl parathion), Egypt (monocrotofos, carbaryl, 2,4-D and pirimiphos-methyl), Pakistan (methamidophos) unextracted ^{14}C tended to be higher in control than treated plots with the converse situation in Canada (atrazine), Philippines (2.4-D) and Thailand (monocrotophos) but differences were not always statistically significant. In China (methamidophos) differences were not consistent between sampling dates.

Two participants reported additional laboratory experiments. In Canada ^{14}C from radiolabelled 2,4-D was less extractable from soil previously treated with atrazine and carbofuran. Similarly in Egypt soil from the field-treated plots retained slightly less ^{14}C from 2,4-D, carbofuran and pirimiphos-methyl than control soil, with binding greater under anaerobic than aerobic conditions. Again the differences were small.

With regard to rates of dissipation, there were in general no differences between treated and control plots except in Egypt where DT_{50} values for carbaryl and monocrotophos were lower in treated plots, suggesting the possibility of the induction of a specialized microbial population adapted to degrade these compounds [17]. This might be anticipated following prolonged heavy pesticide applications so it is a little surprising that there were no similar indications elsewhere. It is more surprising that in Thailand the DT_{50} value for monocrotophos was lower in the control soil.

Participants who compared leaching of labeled compounds in control and treated plots (China, Egypt and Pakistan) reported no real differences, although in Egypt monocrotophos and carbaryl concentrations were a little higher in the lower horizons in the control soil, probably because of lower breakdown rates as when expressed as percentages of total residues they were similar.

4.11. The effect of pesticides on the mineralization of ^{14}C-labelled pesticides in the soil

Complete degradation of pesticides produces simple inorganic substances, including CO_2 and water, hence the process is known as mineralization. Any inhibition of soil microbial populations due to the use of pesticides will be expected to reduce mineralization of the applied pesticides. Measurement of $^{14}CO_2$ derived from ^{14}C-labelled pesticides is the most practical way of assessing mineralization even for compounds containing Cl, N and P where other isotopic techniques could also be used.

Table 1. Correlations between microbiological properties

		Biomass	DeH_2ase	Nitrification	Arginine deaminase	ATP	Arylsulfatase	Bacterial numbers	Fungal numbers	Fe III reduction
Biomass	Brazil		0.181	-0.183	-0.149		-0.316	0.357	-0.130	0.088
	Canada		-0.121	0.224	0.249	0.640	0.052	-0.126	0.059	0.495
	Egypt		0.329	0.436				0.284	0.022	
	Pakistan		0.131	0.034	0.134			0.036	-0.102	0.064
DeH_2ase	Brazil			0.005	0.222		0.636	0.432	0.435	0.411
	Canada			-0.676	-0.470	0.267	0.480	-0.410	0.278	-0.229
	Egypt			0.435				0.543	0.528	
	India				0.112			0.137	-0.346	-0.361
	Pakistan			-0.792	0.159			-0.162	0.397	-0.046
Nitrification	Brazil				0.057		0.096	0.044	0.294	0.133
	Canada				0.384	0.197	0.059	0.264	--0.413	0.256
	Egypt							0.624	0.475	
	Pakistan				0.102			0.190	-0.299	-0.0005
Arginine	Brazil						-0.032	-0.285	0.118	0.073
deaminase	Canada					0.521	-0.061	0.120	0.052	0.344
	India							-0.110	0.011	-0.306
	Pakistan							0.107	0.012	0.063
ATP	Canada						-0.079	-0.031	-0.013	0.176
Aryl-	Brazil									0.039
sulfatase	Canada							-0.105	0.336	-0.009
Bacteria	Brazil								0.565	-0.240
	Canada								-0.395	0.017
	Egypt								0.751	
	India								-0.042	-0.243
	Pakistan								-0.343	-0.064
Fungi	Brazil									-0.355
	Canada									0.107
	India									0.116
	Pakistan									-0.132

Unfortunately there seems to have been some misunderstanding concerning this final assessment as some participants made measurements of pesticide mineralization under various conditions but did not compare soil with a history of heavy pesticide use with control soil.

In Brazil the percentage of ^{14}C applied in 2,4-D evolved as $^{14}CO_2$ after 42d incubation ranged from less than 5% to about 45% in control soil and from about 10% to just under 39% in treated soil. At various sampling times control values were higher than those from treated soil and *vice versa*. Control values were higher than treated soil values in samples taken between the 1994 and 1995 crops and in those taken after the final harvest. The relationship was reversed in samples taken between the 1996 and 1997 crops.

In Egypt not only 2,4-D but also carbofuran and pirimiphos-methyl were studied and incubations were made under both aerobic and anaerobic condition. Differences in mineralization rates between control and treated soils were small for all 3 compounds although in 43 of the 44 pairs of samples the rate was higher in the control samples. Rates were always higher in aerobic than anaerobic conditions. Mineralization of 2,4-D (about 30% applied ^{14}C released as $^{14}CO_2$ in 90d) was faster than that of the other compounds (which released 10 – 14% of the applied radioactivity).

The assessment in Pakistan was made on only one set of samples in 1997 and again in 1998. In both years mineralization of 2,4-D was fastest in control soils with about 18% applied ^{14}C evolved as CO_2 after 42d in 1997 and 4.4% in 1998 compared with corresponding figures of about 15% and 3.8% for soil from experimental plots and 12% and 2% for soil from farm plots.

Mineralization of 2,4-D was even slower in the Philippines with only about 1.5% of the applied ^{14}C evolved in 56d in the only study conducted (1998). Not surprisingly with such a low figure, the difference between treated and control soil was not statistically significant. These figures and the values for 1998 in Pakistan are surprisingly low as 2,4-D is not regarded as a very persistent molecule with DT_{50} times usually in the range of 15–30d [18].

Thus the pesticide regimes used in Brazil, Egypt, Pakistan and the Philippines did not have a major effect on the ability of soil to mineralize 2,4-D though there were indications of slight inhibition.

5. Conclusions and recommendations

The general conclusion must be that even the heavy and frequent rates of pesticide application used in cotton cultivation do not have drastic long term effects on the soil properties measured in this programme. Cotton is widely regarded as the crop which receives the heaviest pesticide treatment so this conclusion is reassuring. Other crops receive lower rates of application and newer pesticides are more specific and environmentally benign than those used here, so this programme does not provide evidence that registration requirements for data on effects of individual compounds on soil micro-organisms should be made more stringent. However, the lack of correlation between the various microbial activities confirms the importance of not relying on the assessment of a single process.

The case for careful monitoring of pest control systems is stronger. The sequence of monocrotophos, methomyl and carbaryl used in Egypt caused quite substantial changes in several activities in the short term and lesser effects were caused by systems used in other countries. This programme did not include assessments of crop yield so it would be

worthwhile to review trends in crop yields in cotton monoculture systems to obtain a crude assessment of levels of soil fertility.

The studies with radiolabelled compounds showed that repeated pesticide use did not affect the production of unextractable ^{14}C residues ('bound residues') of another compound or the ability of a soil to mineralize another compound. Hence there is no clear need for further work in this area.

The results give further support to the idea that more research is needed to understand the factors controlling the activities of soil micro-organisms. Until this has been done, the influence of a pesticide programme on soil fertility can really only be assessed through measurements of crop yield.

REFERENCES

[1] OERKE, E-C., et al., "Conclusions and perspectives", Crop Production and Crop Protection, (OERKE, O-C., et al., Eds), Elsevier, Amsterdam, Netherlands (1994) 742–770.

[2] DOETSCH, R.N., COOK, T.M., Eds, Introduction to Bacteria and Their Ecobiology. Univ. Park Press, Baltimore, Maryland, USA (1974).

[3] FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, Guidelines for the Registration and Control of Pesticides, FAO, Rome (1985).

[4] GREAVES, M.P., "Side-effect testing: an alternative approach", Pesticide Effects on Soil Microflora. (SOMERVILLE, L., GREAVES, M.P. Eds.), Taylor and Francis, London (1987) 183.

[5] FRYER, J.D. et al., Field experiments to investigate long term effectsof repeated applications of MCPA, triallate, simazine and linuron. II Crop performance and residues 1969–1978, Weed Res **20** (1980) 103–110.

[6] GREAVES, M.P., MALKOMES, H.P., "Effects on soil microflora", Interactions Between Herbicides and the Soil (HANCE, R.J., Ed), Academic Press, London (1980)223–253.

[7] GROSSBARD, E. "Effects on the soil microflora", Herbicides: Physiology; Biochemistry; Ecology, Vol 2, (AUDUS, L.J., Ed), Academic Press, London (1976) 99–148.

[8] WELP, G., BREMNER, G., Der Fe(III)-Reductionstest, ein einfaches Verfahren zur Abschatzung der Wirkung von Umweltchemikalein auf die mikrobielle Aktivitat in Boden, Z. pflanzenernahr, Bedenk. **148** (1985) 10.

[9] PAL, S.S., et al., Effect of benomyl on iron and manganese reduction and redox potential in flooded soil, J. Soil Sci., **30** (1979) 155.

[10] HAIDER, K., "Anaerobic microsite in soils and their possible effects on pesticide degradation" Pesticide Chemistry, Human Welfare and the Environment, Vol 3, (MYAMOTO, J., KEARNEY, P.C., Eds), Pergamon, Oxford (1983) 351–356.

[11] TU, C.M., Effect of five insecticides on microbial and enzymatic activities in sandy soil, J. Environ. Sci. Health, **B 30** (1995) 289.

[12] JENKINSON, D.S., POWLSON, D.S., The effect of biocidal treatments on metabolism in soil, Soil Biol. Biochem. **8** (1976) 209.

[13] NAUMANN, K., Die Wirkung einiger Umweltfactoren auf die Reaction der Bodenmicrofloragegenuber Pflanzenschutzmitteln, Zbl. Bakt. Abt. II **127** (1972) 379.

[14] SKUJINS, J.J., "Enzymes in soil", Soil Biochemistry, (McLAREN, A.D., PETERSON, G.H., Eds), Edward Arnold, London,(1967) 371–414.

[15] HANCE, R.J. et al., "Biofügbarkeit von Rückständen", Okotoxikologie von Pflanzenschutzmitteln: Sachstandbericht/Deutsche Forschungsgemeinschaft, VCH, Weinham (1994) 64–81.

[16] BURAEL, P., et al., “Soil-bound residues”, The Lysimeter Concept, (FÜHR, F., et al., Eds), American Chemical Society, Washington, D.C. (1998) 177–188.
[17] TORSTENSSON, N.T.L. “Microbial decomposition of herbicides in soil”, Progress in Pesticide Biochemistry and Toxicology, Vol 6 Herbicides, (HUTSON, D.H., ROBERTS, T.R. Eds) John Wiley & Sons (1987) 249–270.
[18] NASH, R.G., “Dissipation from soil” Environmental Chemistry of Herbicides, Vol 1 (GROVER, R.J. Ed) CRC Press, Boca Raton (1988) 131–170.

Impact of long term applications of cotton pesticides on soil biological properties, dissipation of [^{14}C]-methyl parathion and persistence of multi-pesticide residues*

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Abstract. Biological parameters were followed in soils from a cotton farm (Tatui) where the recommended pesticides have been used for years, and from an experimental field (São Paulo) which was subdivided in two areas: one received the recommended pesticides and the other was maintained untreated. The soil bioactivities monitored from 1995 to 1998, after different pesticide applications, were: basal and glucose-induced respiration; anaerobic activity; nitrification rate; activity of the enzymes: dehydrogenase, aryl sulfatase and arginine deaminase; the soil capacity to mineralize an aromatic pesticide molecule ([^{14}C]-2,4-D), fungal and bacterial contributions for soil respiration until the beginning of 1998, and fungal and bacterial numbers from the beginning of 1998. The dissipation of [^{14}C]-methyl parathion - one of the recommended pesticides - was followed by radiometric techniques only in São Paulo, but persistence of multi-residues was determined in both soils by gas-liquid chromatography. All the biological parameters varied each sampling time and values also varied among soil samples, being inhibited or stimulated by the different pesticide applications, but they mostly recovered the initially detected activity. Dissipation of methyl parathion was fast and not affected by the other pesticide applications. Pesticide residues varied between the two soils but were mostly low after all applications, which indicates their dissipation.

1. Introduction

The use of pesticides has proved to be the only means to protect crops on a large scale. However, the effects of pesticide usage must be seen also in the context of soil pollution and sustainability of the agroecosystem.

Some crops, such as cotton, need heavy repeated applications of different pesticides, including organophosphates, carbamates, pyrethroids, and organochlorines [1] which reach the soil. Most of the studies of the behaviour of pesticides in the soil have focused mainly the behaviour of only one pesticide, but, although the results are very useful, the soil conditions from real situations are not being fully investigated.

The soil is a heterogeneous environment in which the microbial population is involved in important cycles of the essential elements [2,3]. Pesticides reaching the soil may affect non-target organisms, disturbing the local metabolism [3,4,5] which is essential for soil fertility, and also for pesticide degradation processes [6].

Although there is a series of tests used to measure biodegradation of pesticides, the individual resultsof each test have limited value [7]. The combination of results from various tests provides information for a more realistic evaluation on the pesticide impact on the soil microbial population [6], and on the complexity of the soil population dynamics [8].

This work evaluated some biological and chemical parameters in soils treated with different pesticides, as recommended for cotton crops. At the same time, the dissipation of one of the recommended

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pesticides — ^{14}C-methyl parathion — under the influence of the other pesticide applications, was also studied using nuclear techniques. The remaining residues of the applied pesticides were also determined by solvent extraction and gas liquid-chromatography of the soil samples during the crop and between crop seasons.

2. Material and methods

Soil from a cotton experimental station (Tatui, São Paulo State) was collected after different pesticide applications (Fig. 1), and in the interval between crop seasons. The same application schedule used in Tatui was followed in half of an experimental field area in São Paulo city. The other half did not receive pesticides.

The rate and order of all pesticide applications (Fig. 1) were (per hectare) in the 1995-1996 season: monocrotophos (1.0 L); dimethoate (0.5 L); dimethoate again (0.5 L); endosulfan (1.2 L); deltamethrin (0.5 L); endosulfan (2.0 L); deltamethrin (300 mL); methyl parathion (1.0 L); endosulfan (2.0 L), and carbaryl (2.5 kg). Trifluralin (2.0 L/ha) was applied between the two crop seasons. In 1996-1997 the order was: monocrotophos (1.0 L); monocrotophos again (1.3 L); endosulfan (1.25 L); methylparathion (1.2 L); endosulfan (1.2 L); endosulfan (1.0 L) plus methyl parathion (1.0 L); endosulfan (1.5L) plus methyl parathion (1.5 L); endosulfan (2.0 L) plus methyl parathion (2.0 L); deltamethrin (1.0L); again deltamethrin (250 mL); endosulfan (1.2 L), and after the cropping, deltamethrin (250 mL) plus methyl parathion (1.25 L). Again trifluralin was applied between crop seasons In 1997 – 1998 the order was: diuron (4 kg); methyl parathion (1.2 L); endosulfan (1L); endosulfan again (1 L); methyl parathion (1 L) plus endosulfan (1 L); deltamethrin (350 mL); methyl parathion (1 L); deltamethrin (1L); and methyl parathion (2.5 L) plus deltamethrin (1.25 L).

Soil samples were taken before the first pesticide application (Sampling 0), and after the first treatments with: monocrotophos (S. 1); methyl parathion (S. 2) and carbaryl (S. 3), and after the first harvest (S. 4). In the second year of the study the samples were taken after the two applications of monocrotophos (S. 5); the first application of methyl parathion (S. 6); the first application of deltamethrin (S. 7), and after the mixture of methyl parathion and deltamethrin (S. 8). Another sampling between the crop seasons (S. 9), and then, after the first application of endosulfan (S. 10); deltamethrin (S. II); the mixture of methyl parathion plus deltamethrin (S. 12), and after the crop season (S.13) as shown in Fig. 1.

The following parameters were studied in the soils: basal and glucose induced respiration [9]; Fe-III reduction [8]; total N and nitrification [10]; capacity to mineralize ^{14}C-2,4-D as model aromatic molecule [11]; and the activity of the enzymes: dehydrogenase [12]; aryl sulfatase [13] and arginine deaminase [14]. The fungal and bacterial contributions for soil respiration were also evaluated until S. 8 using selective inhibitors [15, 16]. Nine (10 g) replicates of each area (i. e, Tatui, São Paulo treated sub-area, and São Paulo untreated sub-area) from the 0-15 cm soil layer were placed in biometer flasks [18] and the water content was adjusted to 55% 0f maximum water holding capacity.

The behaviour and fate of ^{14}C-methyl parathion was followed in soil columns in hard PVC tubes (5 cm i.d. × 50 cm long) which were driven in the Sao Paulo experimental field. Ring [^{14}C]-methyl parathion, 95% pure, with specific activity of 1.073 GBq mmol^{-1} purchased by IAEA was applied on the soil surface of each tube in 5 mL of a hexane solution containing 148 kBq of the radiolabelled compound dissolved in 0.6 mg of methyl parathion, in a rate equivalent to 2 μg g^{-1} in the top 15 cm of the soil columns. This application was made at the same time as the first recommended application of methyl parathion. The area of the experimental field was subdivided in two sub-areas: one received all pesticide treatments and the other received only the ^{14}C-methyl parathion into the tubes. At sampling times (0,3,6,9 and 12 months after the application) three tubes from each sub-area were collected, divided in two sections (0-15cm; 15-50 cm) from which the soil was taken and thoroughly mixed for analysis[17].

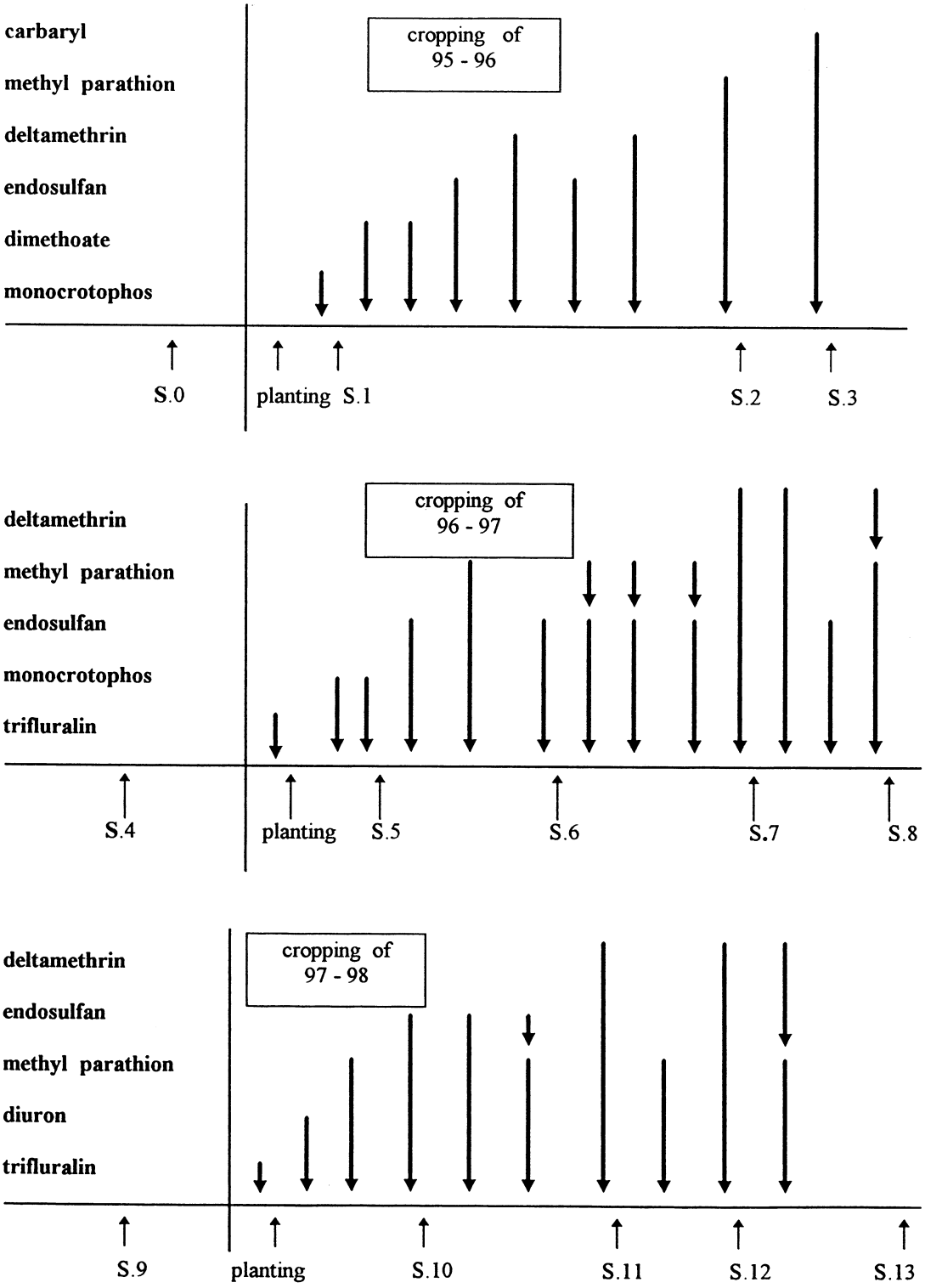

FIG. 1. Schedule of pesticide applications and samplings (S) during three crop seasons

Three replicates were treated with 75 mg of streptomycin in 50 mg of talcum; three replicates were treated with 150 mg of cycloheximide in 50 mg of talcum, and the three control replicates were treated only with 50 mg of talcum [19]. All replicates received 4 mg of glucose g^{-1} soil and were kept at 28°C during the week of the test. The KOH of the side arm of the flasks was changed after 6h and 1, 2, 3 and 4 d. The CO_2 produced was determined by titration with HCI, as for the basal and substrate induced respiration test. All these parameters were studied until S.8 in the 0–15 cm and 15–30 cm soil layers, and then, only in the surface layer. From S.8 to S.13 the total bacterial and fungal populations

were also examined by serial-dilution techniques and the pour-plate method, according to Johnson *et al*. [20].

Multi-pesticide residues were monitored in the surface (0-15 cm) of treated soils. Triplicate soil samples (50 g) were Soxhlet extracted with 150 mL of methanol during 8 hours. Aliquots of 100 mL of the organic extracts were concentrated to dryness in rotary evaporator (Büchi 461) at 35°C. Residues were quantitatively suspended in 2 mL of methanol (pesticide grade) which was totally transferred to Bond Elut C_{18} cartridges (500 mg octadecyl) previously conditioned with 2 x 3 mL MilliQ water and 2 x 4 mL methanol. The cartridges were extracted with (2 x) 5 mL of methanol which was concentrated to dryness and re-suspended in 2 mL of methanol. These 2 mL extracts were analyzed by gas-chromatography with an electron capture detector (GC-ECD). Tatui soil samples from S.0 to S.10 were analyzed in a Hewlett-Packard 5890 II gas chromatograph equipped with a split/splitless injector, ^{63}Ni electron capture detector and HP Chemystation terminal to integrate peak areas. An Ultra-2 fused silica capillary column (cross-linked 5% phenylmethyl polysiloxane, 25 m x 0.2 mm i.d.) was used with nitrogen as carrier gas at 30 mL min^{-1} Injector and detector temperatures were, respectively, 280 and 320°C, Injections (1 μL) were made in the splitless mode under a temperature programme starting at 200°C, increasing at 5°C min^{-1} to 280°C (10 min.). All other samples were analyzed in a Varian 3400 instrument equipped with split/splitless injector, ^{63}Ni electron capture detector, and a Varian Star Chromatography Workstation terminal. The column was a megabore DB 17 with 10 μm film, 30 m x 0.53 mm i.d., with nitrogen as carrier gas at 1.5 mL min^{-1}. Injector and detector temperatures were 250 and 300°C, respectively. The splitless mode was also used under a temperature programme starting at 170°C and increasing at 10°C min^{-1} to 270°C (15 min.) Peak areas were quantified from standard curves of pure pesticides and analyzed by the Meier-Zund method [21].

Some soil conditions (pH, moisture and temperature) were measured in the fields at the sampling times by a Soil pH and Humidity Tester (Takemura DM-5) and thermometer. Other physico-chemical characteristics were measured by the Soil Science Dept. of the Superior Agriculture School of the São Paulo University (ESALQ/USP).

3. Results and discussion

Soil and weather conditions at sampling times are presented in Figs. 2 and 3. Soil physico-chemical characteristics almost did not differ during the experiment. Soil pH varied from 4.9 to 5.7 in Tatui and from 4.4 to 5.3 in São Paulo; organic matter was mostly 20 g dm^3 in both depths of Tatui; from 25 to 36 g dm^3 in the 0-15 cm layer and from 26 to 33 g dm^3 in the 15-30 cm layer of São Paulo. Among the main elements studied, only the amounts of phosphorus varied during the experiment. It varied from 33 to 91 mg dm^3 and from 22 to 99 mg dm^3 in Tatui soil in the of 0-15 and 15-30 cm layers, respectively (Fig. 4). The mixture methyl parathion plus deltamethrin (S.8) caused a decline in the phosphorus content of Tatui at 0-15 cm, but the range recovered afterwards. At 15-30 cm, the application of deltamethrin alone (S.7) caused an increase of phosphorus. In Sao Paulo, the amounts of phosphorus in the treated area varied from 91 and 207 and from 53 to 128 mg dm^3 at 0-15 and 15-30 cm,respectively. In São Paulo in the untreated area it varied, respectively, from 109 to 266 and from 41 to 231 mg dm^3. As a decrease of phosphorus content was detected only in the untreated area (both depths), after S.6 followed by an increase after S.7, the applications of endosulfan and deltamethrin seemed to maintain the phosphorus contents more uniformly in the treated area (Fig. 4).

Results on enzyme activities, total N and nitrification, Fe-III reduction, metabolic quotient, microbial biomass and colony forming units are presented in Tables 1, 2 and 3, respectively for the soil samples from Tatui and São Paulo with pesticide applications and São Paulo without applications.

The dehydrogenase activity in Tatui varied from about 23 (S. 0) to 151 μg (S. 9) of Formazan formed per gram of dry soil, being always a little higher in the 0-15 cm layer (Table 1). Values decreased after S. 9, but at the end (S. 13) they were near to those at the beginning of the experiments.

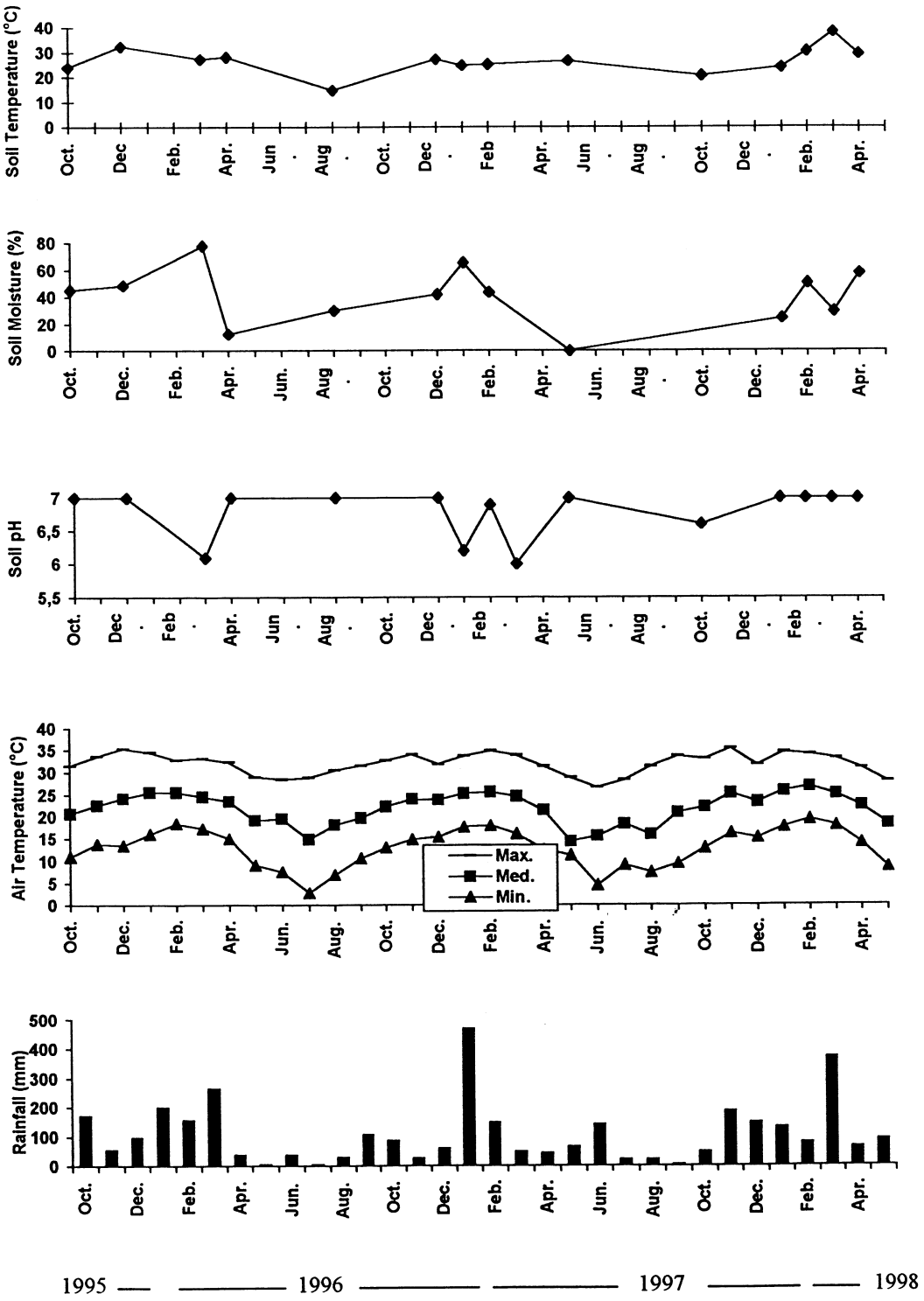

FIG. 2. Climatic and soil conditions of Tatuí during the experimental time

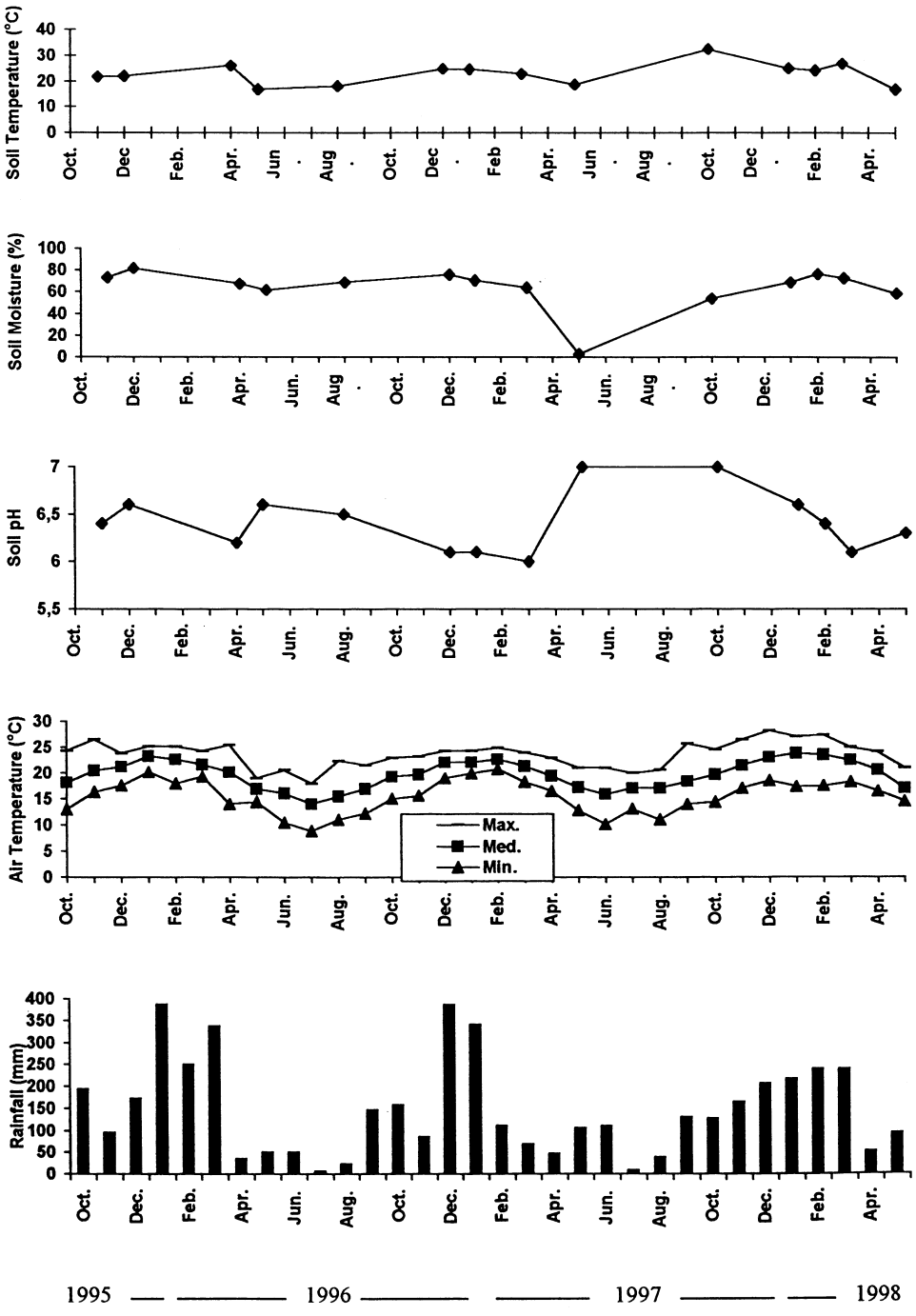

FIG. 3. Climatic and soil conditions of São Paulo during the experimental time

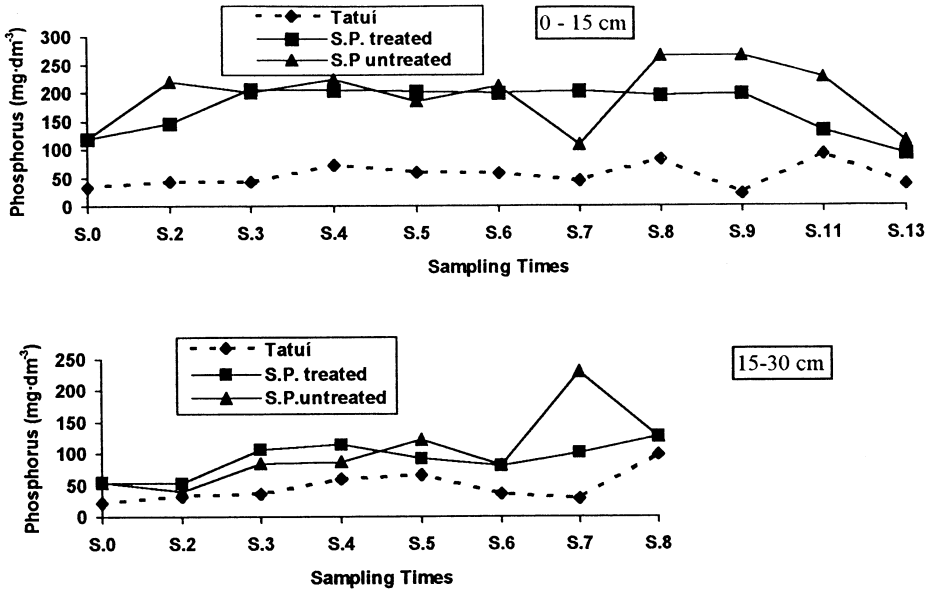

FIG. 4. Phosphorus contents of Tatuí and São Paulo treated and untreated areas after different sampling times

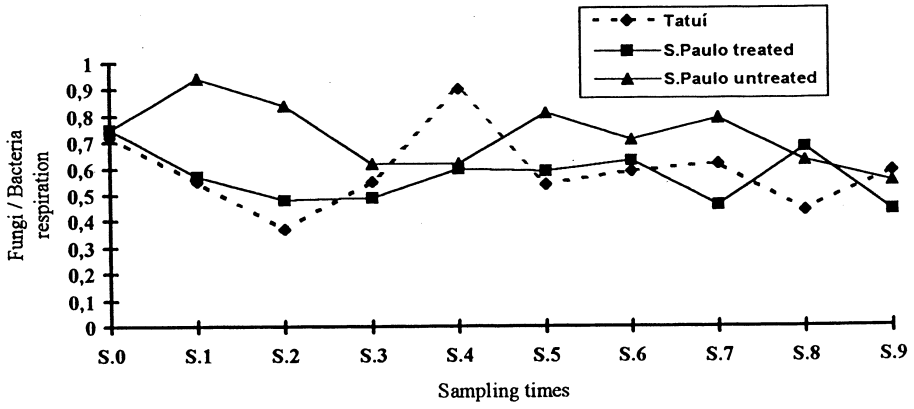

FIG. 5. Contribution of microbial individual groups for soil respiration (0 - 15 cm)

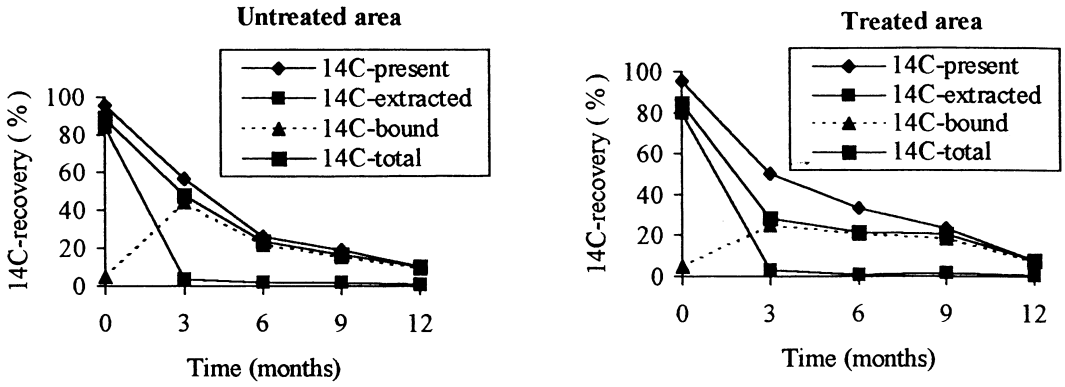

FIG. 6. ^{14}C-recovery from soil of São Paulo under cotton crop treated with [^{14}C]-methyl parathion which received or not other pesticide applications

In São Paulo treated area (Table 2) the variation was from 31 (S. 2, 15-30 cm layer) to more than 250 µg (S.1, 0-15 cm layer) of Formazan g^{-1} soil; and, in the untreated area (Table 3) the variation was from about 26 (S.2, 15-30 cm) to 240 µg (S.9, 0- 15 cm) and, again decreased to the beginning values. Thus, the dehydrogenase activity was always much higher in the heavy clay soil of São Paulo than in the clay soil of Tatui. Although not very different, the values in São Paulo were mostly a little higher in the treated area, indicating that the pesticide applications stimulated the activity of this enzyme.

The range of the enzyme aryl sulfatase activity was also higher in São Paulo soil (from 0.75 to 11 µg of p-nitrophenol formed) than in Tatui soil (from 0 to 3.8 µg), and the highest detected values were again found in the treated area of São Paulo. There was a high increase in the values detected after the first pesticide treatments of the second crop season (S.5, Tables 1,2 and 3), but, as the same occurred in São Paulo untreated area, it was probably caused by changes in climate. As results indicated no defined effect on the activity of this enzyme, the test was interrupted after S.8.

The amount of nitrogen released by the action of arginine deaminase increased in both soil layers until S.2 in Tatui and until S.4 in São Paulo treated area; but in Sao Paulo untreated area it was very low and near the same until S.3 (Tables 2,3 and 4). At S.6, the amounts of released nitrogen decreased to near zero in almost all soil samples. Anyway, the highest detected value in Tatui and in São Paulo treated area (0.55 and 0.63 µg N h^{-1} respectively) was found at S.10, right after endosulfan application. Excepting S.5 the values for the N-released by arginine deaminase were always higher in the São Paulo treated area than in the untreated (Tab. 2 and 3).

Amounts of N-total, N-ammonium and N-nitrate were small and always smaller in Tatui than in São Paulo areas, indicating the influence of soil characteristics. The ranges of N-total and N-ammonium were very close during the experimental time in all soil samples, but the N-nitrate seemed to be stimulated in Tatui and São Paulo treated areas after the first application of monocrotophos (S.1).

In Tatui, after the application of endosulfan the nitrate was a little stimulated (S. 6, Table.1), but in São Paulo it was inhibited (Tables 2 and 3), which shows again the influence of soil characteristics.

Anaerobic metabolism, as determined by Fe^{3+} reduction, varied among sampling times and soil depths (Tables 1,2 and 3), but in the opposite way to the phosphorus content and enzyme activities i.e, the highest values were mostly found in the reddish soil from Tatui (Table 2). Between crop seasons in 96-97 and 97-98 (S. 9) all the three soil samples had the Fe^{3+} reduction inhibited, but the activity recovered after planting and application of endosulfan (S. 10), which could be related to the presence of plants.

Table 1. Biological indicators of Tatui soil activity under a cotton crop, before and after different pesticide applications (per gram of dry soil)

Sampling Depth (cm)	desidrog.[a] µg Formazan	aryl sulfatase µg p-nitrophenol	arginine deaminase µg N/ hour	N total µg N	NH_4^+ µg NH_4	NO_3^- µg NO_3^-	Fe µg Fe^{2+}	M.Q (resp.[b]) Basal / Induc.	Biom.[c] µg C	C.F.U.[d] Bacteria (x10^5)	C.F.U.[d] Fungi (x10^3)
S.0											
0-15	33.23 ±7.17	1.93 ±1.17	0.033 ±0.022	0.063 ±0.029	0.040 ±0.015	0.037 ±0.006	60.94 ±1.93	0.089	0.040	-	-
15-30	23.09 ±5.65	3.38 ±1.05	0.024 ±0.004	0.071 ±0.032	0.045 ±0.009	0.027 ±0.032	71.60 ±21.66	0.129	0.058	-	-
S. 1											
0-15	80.05 ±20.14	2.47 ±0.64	0.028 ±0	0.092 ±0.028	0.037 ±0.005	0.055 ±0.023	19.38 ±9.30	0.250	0.113	-	-
15-30	82.31 ±11.41	1.23 ±0.37	0.020 ±0.005	0.125 ±0.058	0.028 ±0.031	0.097 ±0.031	0.943 ±0.45	0.167	0.075	-	-
S.2											
0-15	63.71 ±22.38	3,81 ±3.00	0.367 ±0.022	0.037 ±0.010	0.024 ±0.017	0.013 ±0.012	38.41 ±5.22	0.395	0.178	-	-
15- 30	45.63 ±6.14	3.06 ±0.18	0.366 ±0.017	0.052 ±0.021	0.041 ±0.019	0.011 ±0.002	54.93 ±10.81	0.023	0.010	-	-
S.3											
0-15	52.81 ±1.34	0.50 ±0.44	0.116 ±0.021	0.070 ±0.041	0.037 ±0.022	0.032 ±0.020	30.66 ±4.59	0.067	0.030	-	-
15- 30	45.96 ±5.84	0.85 ±0.45	0.228 ±0.034	0.061 ±0.036	0.030 ±0.0 10	0.030 ±0.026	30.14 ±13.23	0065	0.029	-	-

able 1. cont.

15	69.85 ±1.96	0	0.081 ±0.013	0.071 ±0.034	0.022 ±0.017	0.048 ±0.017	22.90 ±2.12	0.116	0.052	-	-
15- 30	51.39 ±6.83	0	0.122 ±0.026	0.099 ±0.025	0.032 ±0.008	0.067 ±0.028	27.97 ±11.45	0.050	0.023	-	-
S.5											
0 - 15	37.76 ±1.93	2.98 ±0.21	0.178 ±0.163	0.087 ±0.029	0.040 ±0.007	0.047 ±0.022	*75.30* ±6.30	0.042	0.019	-	-
15-30	33.58 ±11.76	3.50 ±0.36	0.070 ±0.032	0.095 ±0.026	0.040 ±0.012	0.069 ±0.004	38.50 ±2.34	0.047	0.021	-	-
S.6											
0-15	74.41 ±5.00	2.20 ±0.10	0.05 ±0.051	1 0.116 ±0.024	0.040 ±0.009	0.076 ±0.016	16.50 ±11.00	0.075	0.034	-	-
15- 30	68.87 ±30.83	0.81 ±0.10	0	0.102 ±0.007	0.039 ±0	0.063 ±0.007	37.33 ±3.37	0.138	0.062	-	-
S.7											
0-15	66.98 ±1.88	0.56 ±0.07	0.015 ±0.009	0.100 ±0.020	0.050 ±0.014	0.056 ±0.023	52.20 ±7.20	0.109	0.049	-	-
15-30	35.37 ±1.02	0.73 ±0.11	0.026 ±0.004	0.103 ±0.010	0.049 ±0.011	0.054 ±0.006	161.90 ±3.70	0.157	0.071	-	-
S.8											
0-15	103.77 ±3.82	0.49 ±0.30	0.140 ±0.0 10	0.073 ±0.024	0.038 ±0.008	0.035 ±0.0 16	16.80 ±8.00	0.083	0.037	3.1	2.7
15-30	67.45 ±6.99	0.76 ±0.35	0.131 ±0.026	0.040 ±0.005	0.028 ±0.008	0.011 ±0.004	3.90 ±0.60	0.093	0.042	-	-

Table 1. cont.

S.9											
0-15	151.07 ±21.35	-	0.014 ±0.008	0.032 ±0.009	0.020 ±0.008	0.012 ±0.009	17.0 ±7.10	0.460	0.207	5.7	2.3
S. 10											
0-15	73.42 ±18.80	-	0.551 ±0.077	0.073 ±0.012	0.028 ±0.007	0.045 ±0.005	64.8 ±16.60	0.038	0.017	0.3	2.0
S. 11											
0-15	63.65 ±5.80	-	0.247 ±0.174	0.050 ±0.010	0.030 ±0.006	0.02 1 ±0.012	18.9 ±5.97	0.818	0.368	1.4	2.7
S.12											
0-15	56.75 ±2.64	-	0.092 ±0.074	0.071 ±0.030	0.025 ±0.011	0.047 ±0.020	52.9 ±16.90	0.014	0.006	5.1	3.0
S. 13											
0-15	64.50 ±14.81	-	0.188 ±0.015	0.109 ±0.012	0.065 ±0.006	0.044 ±0.006	60.47 ±11.47	0.126	0.057	0.3	0.7

[a]Desidrogenase; [b]Metabolic Quotient =Basal Respiration / Induced Respiration

[c]Biomass = Q.M. × 0.45; [d]C.F.U.= colony forming units

Table 2. Biological indicators of São Paulo soil activity under a cotton crop, before and after different pesticide applications (per gram of dry soil)

Sampling Depth (cm)	rog.[a] µg Formazan	aryl sulfatase µg p-nitrophenol	arginine deaminase µg N/ hour	N total µg N	NH_4^+ µg NH_4	NO_3^- µg NO_3^-	Fe µg Fe^{2+}	M.Q (resp.[b]). Basal / Induc	Biom.[c] µg C	C.F.U.[d] Bacteria ($x10^5$)	C.F.U.[d] Fungi ($x10^3$)
S.0										-	-
0-15	134.18 ±25.41	7.35 ±1.41	0.030 ±0.001	0.125 ±0.016	0.054 ±0.004	0.072 ±0.002	21.15 3.06	0.108	0.049		
										-	-
15-30	73.12 ±12.19	3.91 ±0.44	0.025 ±0.002	0.126 ±0.032	0.046 ±0.009	0.080 ±0.025	4.88 ±2.11	0.014	0.006		
S.1										-	-
0-15	258.77 ±16.86	11.61 ±0.67	0.123 ±0.068	0.184 ±0.00 1	0.097 ±0.029	0.087 ±0.028	13.97 ±3.67	0.066	0.030		
										-	-
15-30	137.72 ±10.98	7.26 ±1.44	0.048 ±0.002	0.142 ±0.039	0.085 ±0.009	0.057 ±0.035	30.57 ±11.46	0.027	0.012		
S.2										-	-
0-15	167.96 ±11.16	4.80 ±1.64	0.28 1 ±0.012	0.077 ±0.016	0.048 ±0.010	0.030 ±0.009	28.83 ±5.06	0.083	0.037	,	
										-	-
15-30	31.48 ±2.97	3.04 ±2.35	0.259 ±0.003	0.057 ±0.037	0.025 ±0.010	0.032 ±0.028	20.30 ±3.02	0.081	0.036		
S.3										-	-
0-15	87.73 ±10.25	5.08 ±0.91	0.376 ±0.049	0.096 ±0.003	0.068 ±0.007	±0.005	52.41 ±10.99	0.054	0.024		
										-	-
15-30	119.04 ±18.64	2.80 ±1.56	0.284 ±0.120	0.070 ±0.007	0.036 ±0.004	0.033 ±0.003	42.26 ±4.04	0.150	0.068		

Table 2. cont.

S.4											
0 - 15	178.66 ±6.06	4.87 ±0.15	0.584 ±0.156	0.154 ±0.048	0.073 ±0.009	0.081 ±0.046	56.67 ±2.46	0.083	0.037	-	-
15 -30	107.76 ±5.25	4.78 ±0.46	0.298 ±0.133	0.137 ±0.040	0.045 ±0.010	0.092 ±0.040	26.27 ±4.31	0.112	0.050	-	-
S.5											
0-15	118.87 ±11.98	5.51 ±1.84	0.053 ±0.051	0.120 ±0.009	0.063 ±0.008	0.057 ±0.014	50.77 ±2.41	0.100	0.045	-	-
15 -30	83.70 ±9.70	3.30 ±0.49	0.040 ±0.030	0.13 1 ±0.002	0.056 ±0.003	0.074 ±0.005	36.96 ±5.70	0	0	-	
S.6											
0 - 15	192.99 ±20.93	9.26 ±0.82	0.011 ±0.005	0.089 ±0.033	0.050 ±0.013	0.039 ±0.021	53.41 ±15.85	0.050	0.023	--	-
15 -30	62.85 ±18.14	6.10 ±0.31	0.001 ±0	0.115 ±0.015	0.057 ±0.025	0.058 ±0.014	38.83 ±7.68	0.032	0.014	-	--
S.7											
0-15	105.13 ±12.59	3.77 ±0.34	0.044 ±0.022	0.117 ±0.019	0.061 ±0.008	0.056 ±0.012	17.60 ±4.90	0.088	0.040	--	-
15-30	31.48 ±13.58	2.64 ±0.17	0.059 ±0.057	0.105 ±0.016	0.054 ±0.011	0.058 ±0.013	74.10 ±5.90	0.117	0.053	-	-
S.8											
0-15	59.62 ±8.50	5.28 ±0.44	0.118 ±0.048	0.072 ±0.016	0.044 ±0.012	0.028 ±0.009	19.30 ±1.70	0.083	0.037	0.4	4.8
15-30	69.79 ±7.59	2.98 ±0.48	0.164 ±0.0 15	0.101 ±0.032	0.040 ±0.002	0.057 ±0.027	66.50 ±13.0	0.128	0.058	-	-

able 2 cont.

15	200.11 ±38.14	-	0.020 ±0.004	0.094 ±0.006	0.070 ±0.004	0.024 ±0.009	19.03 ±3.23	0.270	0.122	19.0	4.0
S.10 0-13	118.47 ±3.00		0.626 ±0.094	0.073 ±0.005	0.038 ±0.005	0.035 ±0	52.49 ±14.64	0.008	0.004	17.3	24.0
S.11 0-15	271.59 ±15.31		0.471 ±0.125	0.059 ±0.002	0.030 ±0.007	0.030 ±0.005	49.49 ±11.15	0.116	0.052	2.8	17.0
S.12 0- 15	143.01 ±5.51		0.150 ±0.022	0.070 ±0.007	0.070 ±0.007	0	29.36 ±7.22	0.038	0.017	16.0	0.7
S. 13 0-15	91.06 ±7.57		0.293 ±0.024	0.148 ±0.009	0.080 ±0.003	0.067 ±0.003	50.23 ±2.02	0.080	0.036	2.8	2.3

[a]Dehydrogenase; [b] Metabolic Quotient =Basal Respiration / Induced Respiration; [c]Biomass = Q.M. × 0.45; [d]C.F.U =colony forming units

Table 3. Biological indicators of São Paulo soil activity under a cotton crop, without pesticide applications (per gram of dry soil)

Sampling Depth (cm)	desidrog.[a] µg Formazan	arylsulfatase µg p-nitrophenol	arginine deaminase µg N/ hour	N total µg N	NH_4^+ µg NH_4	$N0_3^-$ µg $N0_3$	Fe µg Fe^2	M.Q. (resp).[b] Basal / Induc	Biom.[c] µg C	C.F.U Bacteria ($x10^5$)	C.F.U Fungi ($x10^{3)}$
S.0											
0-15	134.18 ±25.41	7.35 ±1.41	0.050 ±0.001	0.125 ±0.016	0.054 ±0.004	0.072 ±0.002	21.15 ±3.06	0.108	0.049	-	-
15-30	73.12 ±12.19	3.91 ±0.44	0.025 ±0.002	0.126 ±0.032	0.046 ±0.009	0.080 ±0.025	4.88 ±2.11	0.014	0.006	-	-
S. 1											
0-15	218.67 ±19.45	3.40 ±2.68	0.061 ±0.044	0.122 ±0.023	0.063 ±0.012	0.059 ±0.012	1.94 ±1.86	0.108	0.049	-	-
15-30	100.84 ±3.02	4.87 ±2.01	0.049 ±0.033	0.053 ±0.026	0.041 ±0.020	0.012 ±0.007	18.51 ±0.70	0.045	0.020	-	-
S.2											
0-15	107.45 ±4.74	3.78 ±1.27	0.037 ±0.021	0.094 ±0.018	0.050 ±0.008	0.044 ±0.011	21.82 ±0.59	0.167	0.075	-	-
15-30	25.76 ±2.57	1.01 ±0.31	0.038 ±0.020	0.054 ±0.012	0.035 ±0.005	0.018 ±0.012	29.76 ±4.63	0.181	0.082	-	-
S.3											
0-15	110.09 ±5.54	0.75 ±0.07	0.065 ±0.034	0.096 ±0.045	0.059 ±0.02 1	0.037 ±0.030	25.15 ±8.03	0.080	0.036	-	-
15-30	45.34 ±7.68	2.86 ±0.28	0.047 ±0.046	0.088 ±0.048	0.051 ±0.035	0.041 ±0.012	62.70 ±14.48	0.118	0.053	-	-

able 3. cont.

0-15	147.65 ±26.65	2.37 ±0.90	0.284 ±0.085	0.161 ±0.046	0.048 ±0.010	0.113 ±0.038	32.87 ±3.47	0.083	0.037	-	-
15-30	51.13 ±7.62	0.73 ±0.07	0.393 ±0.069	0.153 ±0.041	0.061 ±0.017	0.092 ±0.025	24.46 ±8.38	0.053	0.024	-	-
S.5											
0-15	144.22 ±21.64	9.76 ±2.88	0.212 ±0.055	0.115 ±0.015	0.048 ±0.004	0.067 ±0.011	70.07 ±5.06	0.077	0.035	-	-
15-30	102.49 ±12.24	4.38 ±0.42	0.232 ±0.138	0.136 ±0.031	0.054 ±0.014	0.082 ±0.024	31.05 ±0.96	0.077	0.035	-	-
S.6											
0-15	94.59 ±6.83	3.66 ±0.65	0.007 ±0.006	0.112 ±0.037	0.050 ±0.022	0.062 ±0.015	56.80 ±8.28	0.076	0.034	-	-
15-30	43.87 ±2.85	1.99 ±0.32	0.009 ±0.002	0.124 ±0.008	0.052 ±0.012	0.072 ±0.017	79.83 ±27.32	0.084	0.038	-	-
S.7											
0-15	90.96 ±7.61	3.55 ±0.15	0.052 ±0.050	0.114 ±0.013	0.053 ±0.011	0.058 ±0.007	9.04 ±2.30	0.128	0.058	-	-
15-30	53.69 ±11.02	2.60 ±0.05	0.034 ±0.006	0.109 ±0.011	0.049 ±0.004	0.055 ±0.0 15	13.90 ±0.60	0.103	0.464	-	-
S.8											
0-15	115.73 ±16.39	4.55 ±0.13	0.197 ±0.017	0.062 ±0.011	0.054 ±0.011	0.008 ±0	14.90 ±4.30	0074	0.033	0.9	5.3
15-30	100.71 ±4.66	3.10 ±0.06	0.039 ±0.016	0.080 ±0.021	0.044 ±0.006	0.035 ±0.015	12.10 ±0.46	0.083	0.037	-	-

(cont.

Table 3. cont.

S.9											
0-15	240.20 ±24.02	-	0.027 ±0.006	0.138 ±0.027	0.068 ±0.010	0.069 ±0.031	3.93 ±1.34	0.150	0.068	34.0	36.0
S.10											
0-15	137.52 ±37.10	-	0.140 ±0.030	0.071 ±0.009	0.028 ±0.007	0.043 ±0.009	13.3 ±7.85	0.011	0.005	5.6	22.0
S. 11											
0-15	235.79 ±21.13	-	0.376 ±0.170	0.066 ±0.016	0.028 ±0.007	0.038 ±0.010	0.90[e]	0.160	0.072	10.0	6.1
S.12											
0-15	105.94 ±5.79	-	0.108 ±0.036	0.091 ±0.012	0.052 ±0.004	0.039 ±0.016	55.9 ±2.05	0.161	0.072	15.0	9.0
S. 13											
0-15	54.69 ±8.69	-	0.205 ±0.006	0.134 ±0.019	0.068 ±0.007	0.066 ±0.021	53.07 ±13.78	0.080	0.036	4.0	3.0

[a]Dehydrogenase; [b] Metabolic Quotient = Basal Respiration / Induced Respiration; [c]Biomass = M.Q. x 0.045; [d]C.F.U = colony forming units
[e] Result from only one sample

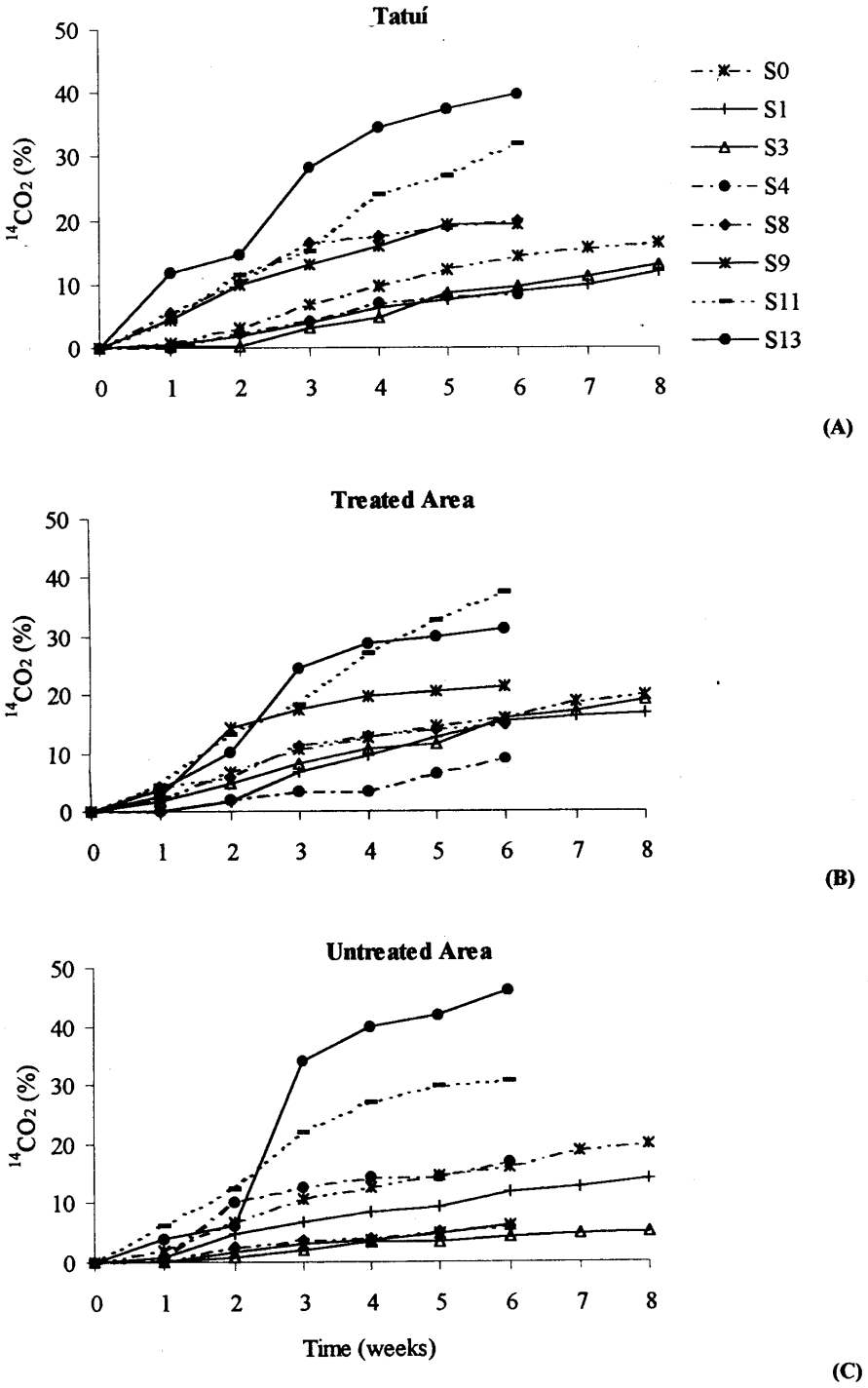

FIG. 7. Mineralization of ^{14}C-2,4-D in soils under cotton crop of Tatuí (A) and São Paulo treated (B) or untreated with other pesticides (C)

As could be expected from the soil layer with known higher microbial activities, basal and induced respiration were higher in the 0 - 15 cm soil layers. The amount of CO_2 produced by glucose-induced respiration varied from about 3 (S. 9) to 50 (S. 0) μg CO_2 h^{-1} g^{-1} soil of Tatui; from 8 (S.1, S.8) to 143 (S. 10) μg CO_2 h^{-1} g^{-1} treated soil of São Paulo, and from 9 (S.1) to 84 (S.10) μg CO_2 h^{-1} g^{-1} untreated soil of São Paulo, which indicates a higher respiration activity by the soil microbial population of São Paulo after treatments. Values of the Metabolic Quotient (M.Q.) and Biomass varied among samplings, but a clear stimulation was observed between crop seasons of 1997 and 1998 (S. 9). Nevertheless, as after S. 9 an inhibition was observed in all (treated and untreated) soils, the climatic conditions appear to be the most influential factor (Tables 2,3 and 4). After application of deltamethrin (S. 12) only the Tatui and São Paulo treated soils showed an inhibition effect.

When selective inhibitors were used, the quotients streptomycin / cycloheximide in the respiration curve points of higher CO_2 production (mostly at the second day of the test) were always smaller than one (Fig. 5), indicating the prevalence of bacterial respiration. The range of the fungi / bacteria respiration quotients was higher in soil of the untreated area of São Paulo (from 0.6 to 0.9), followed by the soil of Tatui (0.4 - 0.9). But, it varied very little in soil of the treated area of São Paulo (0.5 - 0.7), although it was stimulated by deltamethrin followed by an inhibition caused by the mixture deltamethrin-methyl parathion (respectively S. 7 and S. 8, Fig. 5). As in other tests, there was no indication of a long lasting influence from any particular pesticide application.

Dissipation of ^{14}C-methyl parathion in São Paulo was very fast, independent of the other pesticide applications (Fig. 6). In only three months, the ^{14}C-extractable recoveries decreased from about 80% to 3-4% in both areas, while the ^{14}C-bound increased from 5% to 44% in the untreated area and to 25% in the treated area. Afterwards, even the bound residue amounts decreased and the ^{14}C-total recoveries were 10.0% and 7.6% after one year, respectively in the untreated and treated areas. As ^{14}C-extractable and ^{14}C-bound recovered after one year were respectively, 0.8% and 9.4% in the untreated area, and 0.5% and 7.2% in the treated area, the main losses can be assigned to volatilization and/or mineralization of the pesticide to the atmosphere. Even the half-lives were similar in both treated and untreated areas, i.e, 3.6 and 3.7 months, respectively.

Rates of mineralization of ^{14}C-2,4-D in Tatui (Fig. 7A) were in the following descending order: after all the treatments, between crop seasons (S. 13) > after treatment with deltamethrin (S. 11) > treatment with the mixture of deltamethrin + methyl parathion (S. 8) ≈ between crop seasons of 96 and 97 (S. 9). Treatments with monocrotophos (S. 1) and methyl parathion alone (S. 3), and between crop seasons of 95 and 96 (S. 4) resulted in inhibition. In São Paulo (Fig. 7-B and C) the stimulation was again very clear after application of deltamethrin (S. 11) > after all the treatments (S. 13) ≥ between crop seasons of 96 and 97 (S. 9). But the results showing stimulation differed from the untreated area only between crop seasons of 96 and 97 (S. 9) and after the mixture deltamethrin + methyl parathion (S. 8). Between the crop seasons of 95 and 94 (S. 4) there was a clear inhibition of the ^{14}C-2,4-D mineralization as compared with the untreated area. Although all the treatments (S. 13) stimulated $^{14}CO_2$ production from ^{14}C-2,4-D in the treated area, the stimulus was much higher in the untreated area, indicating the climatic influence on this parameter.

Results for the persistence of the different applied pesticides in soils are presented in Tables 4and 5. Amounts of trifluralin varied in Tatui being higher some while after its application, but decreasing onwards (Table 4). Methyl parathion was only detectable in samplings near or right after its application, but endosulfàn was always detected, although in decreasing amounts as time passed. Its metabolite endosulfàn sulphate was detected some time after the application of endosulfan, indicating a time interval for its formation. In São Paulo (Table 5), trifluralin, dimethoate and methyl parathion were detected only in the samplings soon after their applications. As in Tatui, endosulfan and its metabolite endosulfan sulphate were the most detected but in higher amounts than in Tatui, indicating their higher persistence in the soil of São Paulo.

In Tatui, higher amounts and more types of pesticide residues were found at S.3, S.5, S.8 and S.11 (Table 4) which seems to have influenced Metabolic Quotient and Microbial Biomass because they were clearly inhibited at S.3, S.5 and S.8, but definitely stimulated at S.11 (Table 1). In the same way, the highest amounts of pesticide residues at S.11 and also the climatic conditions in São Paulo treated area (Table 5) seem to be related mainly with Metabolic Quotient and Microbial Biomass (Table 2), which increased as had also occurred in São Paulo untreated area (Table 3), but rapidly decreased after S 11. Soil numbers of bacteria were also influenced by the pesticide residues at S. 11 because while bacterial counts were stimulated in Tatui and São Paulo untreated area, the presence of residues deeply inhibited the bacterial numbers in the treated area. At S.11 and S. 12, soil fungi was clearly influenced only in the gley humic of São Paulo, because residues inhibited them more pronouncedly as compared with the untreated area (Tables 2 and 3). Residues present in soils between crop seasons of 96 and 97 (S.9) and after all the treatments (S.13) influenced very clearly the mineralization of ^{14}C-2,4-D in both treated soils.

None of the results showed large and/or lasting effects of pesticide applications on the various studied soil parameters, but the real and long term meaning of the temporary changes remain to be completely understood.

Table 4. Pesticide residues in soil of Tatui (mg kg^{-1})

Sampling	Pesticide				
	Trifluralin	Methyl Parathion	Endosulfan (1)	Endosulfan (2) **	Endosulfan Sulphate***
0	0.174 ±0.024	- -	0.115 ±0.007	- -	+
2	0.175 ±0.010	- -	0.095 ±0.002	- -	- -
3	0.368 ±0.038	0.200 ±0.025	0.398 ±0.035	- -	+
5	0.383 ±0.027	0.373 ±0.017	0.102 ±0.001	- -	- -
6	0.162 ±0.005	- -	0.114 ±0.019	- -	- -
8	0.093 ±0.093	2.090 ±0.216	0.104 ±0.001	- -	+
9	0.018 ±0.004	- -	0.079 ±0	- -	- -
10	0.210 ±0.019	- -	0.105 ±0.007	- -	- -
11	0.129 ±0.014	- -	0.035 ±0.001	0.373 ±0.026	0.104 ±0.011
13	0.099 ±0.009	- -	0.011 ±0	0.223 ±0.007	0.119 ±0.007

* - - not detected; ** analysis of samplings 11 and 13 distinguished 2 peaks;
*** only qualitatively detectable until sampling 10 and quantitatively detectable afterwards

Table 5. Pesticide residues in soil of São Paulo treated area

Sampling	Pesticide (mg kg^{-1})					
	Trifluralin	Dimethoate	Methyl Parathion	Endosulfan (1)	Endosulfan (2)	Endosulfan Sulphate
0	- -	- -	- -	- -	- -	- -
2	- -	0.203 ±0.011	- -	--	0.241 ±0.038	0.190 ±0.020
3	- -	- -	0.177 ±0.023	- -	- -	- -
5	0.069 ±0.018	- -	0.143 ±0.030	- -	- -	0.082 ±0.014
6	- -	- -	0.116 ±0.038	0.165 ±0.0 17	0.367 ±0.039	0.192 ±0.020
8	- -	- -	0.393 ±0.011	0034 ±0.007	0.387 ±0.023	0.263 ±0.016
9	- -	- -	- -	- -	0.175 ±0.031	0.216 ±0.103
10	- -	- -	- -	0.166 ±0.006	0.439 ±0.012	0.286 ±0.009
11	- -	- -	- -	0.398 ±0.023	1.483 ±0.028	0.610 ±0.037
13	- .	- - -	- -	0.041 ±0.023	0.318 ±0.039	0.365 ±0.028

*- - not detected

OTHER OUTPUTS

Parts of the project have generated data for two Master Theses, 8 presentations in national scientific meetings and 1 international meeting.

REFERENCES

[1] PAPP, I.L.G., et al., Manual do Produtor do Algodão, Bolsa de Mercadorias & Futuros, São Paulo (1992) 158

[2] NANNIPIERI, P. et al., "Ecological significance of the biological activity in soil", Soil Biochemistry, Vol. 6 (BOLLAG, J.-M., STOTZKY, O., Eds.), Marcel Dekker Inc., New York (1990) 293–355.

[3] TOPP, E., et al, "Pesticides: microbial degradation and effects on microorganisms", Modem Soil Microbiology (van ELSAS, J.A., et al., Eds.), Marcel Dekker Inc., New York (1997) 547–573.

[4] JENKINSON, D.S., POWLSON, D.S., The effects of biocidal treatment on metabolism in soil. I. Fumigation with chloroform, Soil Biol. Biochem. **8** (1976) 167–177.

[5] KALE, S.P., RAGHU, K., Relationship between microbial parameters and other microbial indices in soil, Bull. Environ. Con. Toxicol. **43** (1989) 941–945.

[6] KEARNEY, P.C., KELLOGG, S., Microbial adaptation to pesticides, Pure & Appl. Chem. 57 (1985) 389–403.

[7] JOHNEN, B.G., DREW, E.A., Ecological effects of pesticides on soil microorganisms, Soil Sci. **123** (1977) 3 19–324.

[8] ZELLES, L., et al., Comparison of methods to test chemicals for side effects on soil microorganisms, Ecotox. Environ. Safety **12** (1986) 53–69.

[9] ANDERSON, J.P.E., DOMSCH, K.H., A physiological method for quantitative measurements of microbial biomass in soil, Soil Biol. Biochem **10** (1978) 215–221.
[10] CAMARGO, O.A., et al., Métodos de Análise Quimica, Mineralógica e Fisica de Solos do Instituto Agronômico de Campinas, Boletin Técnico n° 106, Instituto Agronômico, Campinas (1986) 94.
[11] KLOSKOWSKI, R., et al., Laboratory screening of distribution, conversion and mineralization of chemicals in the soil-plant-system and comparison to outdoor experimental data, Chemosphere **10** (1981) 1089–1100.
[12] SCHUSTER, E., SCHRODER, D., Side-effects of sequentially-applied pesticides on non-target soil microorganisms: field experiment, Soil Biol. Biochem. **22** (1990) 367–373.
[13] TABATABA!, M.A., BREMNER, J.M., Arylsulfatase activity of soils, Soil Sci. Soc. Am. Proc. **34** (1970) 225–229.
[14] ALEF, K., KLEINER, D., Arginine ammonification, a simple method to estimate microbial activity potentials in soil, Soil Biol. Biochem. **18** (1986) 233–235.
[15] ANDERSON, J.P.E., DOMSCH, K.H., Use of selective inhibitors in the study of respiratory activities and shifts in bacterial and fungal population in soil, Ann. Micr. **24** (1974) 189–194.
[16] ANDERSON, J.P.E., DOMSCH, K.H., Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils, Can. J. Microbiol. **21** (1975) 3 14–322.
[17] ANDREA, M.M., et a!., Dissipation and degradation of DDT, DDE and parathion in Brazilian soils, J. Environ. Sci. Health **B29** 1 (1994) 121–132.
[18] BARTHA, R., PRAMER, D., Features of a flask and method for measuring the persistence and biological effects of pesticides in soil, Soil Sci. **100** (1965) 68–70.
[19] SONG, H.-G., et al., Hydrocarbon mineralization in soil: relative bacterial and fungal contributions, Ecotox. Environ. Safety **18** (1986) 109–111.
[20] JOHNSON, L.S., et al., Methods for studying soil microflora-plant disease relationships, Burgess Publishing Company, U.S.A. (1960) 178.
[21] MEIER, P.C., ZOND, R.E., Statistical methods in analytical chemistry, John Wiley & Sons, Inc., New York (1993) 321.

Impact of repeated long term application of atrazine on soil properties and bound residues formation

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Abstract. The effect of repeated long term application of the herbicide atrazine on the activities of microorganisms, enzymes, as well as on the bound residues formation, was investigated. Bacteria, fungi and soil respiration were in general inhibited in the first year of application. However, in the second and third year no such trend was observed. Similarly, a decreasing trend in the Fe(III)-reduction, nitrification and arginine deamination was observed in the first year whereas in the subsequent two years no such trend• was prominent. The dehydrogenase and arylsulfatase activities showed an increasing trend after the application of the herbicide. Column studies showed that extractable residues of atrazine and carbofuran gradually decreased after the application of the pesticides. Amendments of the soil containing ^{14}C-bound residues did not increase $^{14}CO_2$ evolution. Unextractable ^{14}C was higher and mineralization of ^{14}C-2,4-D was lower in previously untreated soil than in soils with histories of atrazine and carbofuran application.

1. Introduction

Repeated long term application of pesticides to the soil may cause the chemical to accumulate to the point that it may have deleterious effects on soil microbiological and biochemical activities thereby creating an unhealthy soil having a lasting impact on soil fertility. At present, little is known of such effects and those recorded are restricted to few pesticides and involves changes in single microbial activities or populations, which are regarded as having no importance in terms of overall activity. Recently, the impact of pesticides on microbial populations and their activities as well as on other parameters such as respiration, soil enzymes and other soil biochemical processes, has been studied by several workers. However, most of these studies were undertaken following single applications of pesticides for a short period, which does not represent a real situation in the field. Investigations of the effects of long term use, sequence and combinations of pesticides would help to understand what might happen in practical agricultural situations.

The present study is a part of the joint research coordination program initiated by FAO/IAEA on the impact of long term pesticide usage on soil biochemical properties. The study was conducted under the laboratory and field conditions to evaluate the effect of atrazine on activities of microorganisms and enzymes as well as on the bound residues formation in a sandy loam and loam soil. The field trial involved the application of atrazine and carbofuran in combination as used by the farmers in the corn growing areas. Experimental procedures described in the Protocols established by FAO/AEA were followed to measure soil bioactivity and other parameters.

2. Materials and methods

2.1. Soil treatment

Field plots (approx. 600 m^2) were established in triplicate on a sandy loam soil in eastern Ontario near the Experimental Farm, Ottawa (Canada). The soil had not received any pesticide application for the past 10 years and has been left fallow or used to grow grass and alfalfa. Three sets of plots were established. Plot A received one fertilizer treatment (NPK) but no pesticide application. Plot B received one fertilizer treatment and two atrazine applications at 2.93 kg/ha. Corn was grown in both

plots A and B during the 4 months period. Plot C constituted a farmer's field (loam soil) with corn plot which had received atrazine and carbofuran (1-3 kg/ha) every year (2–3) times and NPK fertilizer. The three plots received treatments for two years.

2.1.1 Sampling

Soil samples were collected only from the ploughed layer (0-15 cm) at different time intervals for the two growing season as outlined below. A minimum of 20 cores were collected (id 2.5 cm) per plot, pooled, mixed thoroughly and stored. Further processing of the sample was done as outlined in the protocol. Each year the duration of the experiment was about 4.5 months. Soil samples from plots A and B were collected as follows: 1st sample - zero time before the application of any pesticide or fertilizer; 2nd sample - 2 days after the first application of atrazine; 3rd sample - 2 months after zero time (before second application of atrazine); 4th sample - 2 days after second herbicide application; and 5th sample - 4.5 months after zero time (harvest time). In the third year soil samples were taken at the start and end of growing season without the application of the herbicide or fertilizer.

Soil samples from plot C were collected each year before the application of the chemicals (1st sample); 2.5 months after the first sampling (2nd sample); and harvest time 3rd sampling). In the third year samples were collected only at the start and end of the growing season.

2.1.2. Analysis

All the analyses were undertaken following the procedures outlined in the protocols. All measurements were made on triplicate samples and the results are reported on an oven dry basis.

2.3. Binding and release of the chemicals under field conditions

Plot A was used for this experiment and the experimental protocol 9 was followed as closely as possible. Hard PVC cylinders were used as containers for experimental plots. Cylinders were inserted into the field plots and treated as follows: there was one ^{14}C-atrazine application (2.93 kg/ha) and one fertilizer treatment in August of the first year and second years but in the third year no treatment was applied.

2.3.1 Sampling and analysis

The procedure outlined in the protocol was followed. In the first year soil samples were collected from plot A in August (before application, 1st sample); August (immediately after application, 2nd sample) and then in October (3rd sample). In the second year the samples were collected in August (before application, 1st sample), August (after pesticide application, 2nd sample) and in October (3rd sample). All measurements were made on triplicate samples and the results are reported on an oven dry basis.

2.4. Release of bound ^{14}C from plot A soil after amendment

Soil samples (25 g) from the cylinder of plot A (3rd sample of each year) containing bound ^{14}C-atrazine (after solvent extraction) were treated with glucose (3 mg/g). Unamended soil containing bound ^{14}C-atrazine was used as a control. The samples were incubated for 30 days at 22°C. Analysis of the KOH trap for $^{14}CO_2$ as well as the extractable and bound ^{14}C was carried out as outlined in the protocol.

3. Results and discussion

The characteristics of the two soils used in this study are summarized in Table 1.

Table 1. Physical and chemical properties of the soil

Soil type	pH*	CEC**	% Organic C	% Sand	% Silt	% Clay
Sandy loam	6.3	71	1.16	72.2	20.5	7.3
Loam	5.0	87	1.90	37.5	48.8	13.7

*1:1 soil to water mixture; * * in Meq/kg

The population of bacteria and fungi showed initial inhibition after the application of atrazine in the first two years. The population of fungi increased in the first year after the application of the pesticides but followed a trend of inhibition in the second year. A similar effect was observed in the farmer's field which received atrazine and carbofuran applications. However, in the third year when no chemical treatment was applied, the population nearly remained the same as the control plot (Table 2).

Respiration is considered as an indicator of biological activity in soil. Application of pesticides appears to have had an inhibitory effect on soil respiration. However, a stimulatory effect was observed in Plot C soil. It was also observed that the microbial mass of pesticide treated soil was in general less than the untreated soil (Table 3).

Table 2. Microbial population in soil samples collected from the three plots

Microflora		Plot A Time of sampling					Plot B Time of sampling					Plot C Time of sampling		
		1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th	1st	2nd	3rd
Bacteria	(y-l)	203	207	220	201	195	196	164	210	189	198	267	101	123
10^5/g of	(y-2)	210	212	227	231	200	215	182	173	195	203	304	210	254
soil	(y-3)	198				202	211				210	244		228
Fungi	(y-l)	20	17	21	23	20	22	31	28	34	25	15	29	22
(10^3/g of	(y-2)	27	28	23	20	18	31	22	23	24	24	21	18	16
soil)	(y-3)	21				24	26				21	24		21

(y-1): First year; (y-2): Second year; (y-3): Third year

The Fe(III)-reduction test could be taken as a measure of the potential activity of soil microorganisms. There was obvious effect of pesticides on this parameter although a decreasing trend was observed following the pesticide application (Table 4). Nitrification in soil is mainly carried out by *Nitrosomonas* spp. and *Nitrobacter* spp. Plot B Soil exhibited an inhibitory effect whereas Plot C soil receiving atrazine and carbofuran showed a slight stimulating effect following the pesticide application.

Table 3. Respiration and biomass in soil samples

Respiration		Soil: Plot A Time of sampling 1st	2nd	3rd	4th	5th	Plot B Time of sampling 1st	2nd	3rd	4th	5th	Plot C Time of sampling 1s	2nd	3rd
Basal	(y-l)	0.94	0.87	0.92	0.82	0.80	0.97	0.72	0.95	0.61	0.90	1.21	0.99	1.35
(96h)	(y-2)	0.90	0.93	0.88	0.84	0.88	0.91	0.74	0.68	0.55	0.59	1.33	1.53	1.58
	(y-3)	0.97				0.99	0.96				0.90	1.11		1.18
Substrate	(y-1)	0.93	0.89	0.91	0.93	0.91	0.99	0.99	0.90	0.88	1.01	2.70	1.12	2.10
Induced	(y-2)	1.11	1.23	1.10	1.09	1.28	1.32	1.25	0.98	0.81	1.11	2.44	2.45	2.00
(4h)	(y-3)	1.22				1.31	1.28				1.19		1.80	1.60
Quotient	(y-1)	1.01	0.98	1.01	0.88	0.88	0.99	0.72	1.05	0.69	0.89	0.45	0.88	0.64
	(y-2)	0.81	0.76	0.80	0.77	0.69	0.69	0.59	0.69	0.68	0.53	0.55	0.62	0.79
	(y-3)	0.79				0.76	0.75				0.76	0.61		0.73
Biomass	(y-1)	0.45	0.44	0.45	0.34	0.34	0.44	0.32	0.45	0.31	0.40	0.20	0.38	0.23
	(y-2)	0.36	0.34	0.36	0.35	0.31	0.31	0.27	0.31	0.31	0.24	0.25	0.28	0.36
	(y-3)	0.36				0.34	0.33				0.34	0.27		0.33

Table 4. Fe(III) reduction and nitrification in soil samples

		Soil: Plot A Time of sampling 1st	2nd	3rd	4th	5th	Plot B Time of sampling 1st	2nd	3rd	4th	5th	Plot C Time of sampling 1st	2nd	3rd
Fe(III) redn.	(y-I)	1.05	1.30	1.10	1.01	0.92	0.98	0.91	0.95	0.94	0.52	0.47	0.38	0.52
(mg Fe^{2+}/g	(y-2)	1.22	1.27	1.02	1.09	1.01	0.93	0.90	0.92	0.82	0.71	0.51	0.50	0.64
of soil)	(y-3)	1.10				0.90	0.85				0.67		0.66	0.69
Nitrification	(y-1)	8.2	8.9	8.0	8.2	8.1	9.0	4.3	7.4	3.1	5.0	7.8	9.5	4.6
µg (NO_2+NO_3)	(y-2)	8.8	9.4	9.2	9.0	9.3	8.5	5.1	5.9	5.0	4.3	8.6	8.0	7.9
-N/g of soil	(y-3)	8.4				9.9	7.2				7.0	7.7		6.3

Dehydrogenase activity is a measure of the oxidative microbial activity in soils. This system consists of different enzymes which play a role in the initial stages of soil organic matter production. Pesticide application appeared to increase the dehydrogenase activities in both Plots B and C (Table 5). The arylsulfatase hydrolyses sulfate esters with an aromatic moiety and have received more attention than the other group of sulfatases. Table 5 shows the effects of pesticides treatments on soil arysulfatase activity. The activity of this enzyme appeared to increase after the pesticide application. Arginine deamination indicates the ammonification ability of microorganisms which in turn may reflect on the microbial population in the soil samples. This activity was somewhat inhibited by the application of the pesticide. The ATP content diminished after atrazine application but recovered to almost the initial value during the period of the experiment. However, in Plot C soil no such decrease was observed.

Table 5. Enzyme activities in soil samples

		Soil												
		Plot A Time of sampling					Plot B Time of sampling					Plot C Time of sampling		
		1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th	1st	2nd	3rd
Dehydrogenase	(y-1)	34.9	30.1	25.3	27.9	30.0	26.0	44.8	33.1	54.3	52.0	16.7	38.0	48.3
(μg Formazan	(y-2)	36.3	33.9	35.8	36.0	36.7	25.8	38.9	36.6	48.8	50.9	26.1	42.0	48.8
/10 g soil)	(y-3)	33.3				32.8	34.1				36.5	30.0		36.2
Arylsulfatase	(y-1)	21.1	13.4	34.5	20.1	25.5	20.8	33.0	24.7	31.8	56.8	10.5	13.4	25.6
μg p-nitrophenol	(y-2)	23.4	24.5	27.1	24.3	27.0	21.9	29.9	28.4	30.0	37.1	26.1	21.0	24.7
released	/(y-3)	19.9				23.5	22.7					27.3	21.1	28.9
g soil h^{-1}														
Arginine de-	(y-l)	2.0	2.1	1.7	2.5	1.8	2.7	1.1	2.5	1.0	2.1	1.9	1.3	1.6
aminase	(y-2)	2.4	2.7	2.0	2.1	2.0	3.1	2.2	2.1	1.3	1.5	1.4	1.3	1.4
μg NH_4-N/	(y-3)	2.0				2.4	3.3				3.2	1.9		2.0
g soil h^{-1}														
ATP	(y-1)	3.2	3.1	3.0	2.9	2.9	3.4	2.1	3.1	2.0	3.0	2.1	2.9	1.7
(μg/g of soil)	(y-2)	2.5	2.2	2.1	2.6	2.3	2.8	1.9	2.1	2.3	2.7	1.8	2.5	2.4
	(y-3)	2.8				2.6	3.6				3.2	2.9		2.3

An estimate of binding of ^{14}C-atrazine when applied to soils is shown in Table 6. In Plot A soil the extractable ^{14}C was about 30% of the originally applied at the time of the 3rd sampling in both years. However, the bound residues formation was about 50% of the material originally applied.

Bound residues of atrazine increased with time, two months after the herbicide application 45% of the applied atrazine was bound to the soil in the first year and 53% the following year (Table6). Amendment of the soil containing atrazine residues with glucose did not increase the release of $^{14}CO_2$ or the formation of unextractable residues from the soil (Table7). Binding of 2,4-D to the untreated soil (Plot A) was greater than the soil in Plot B, which had been treated with atrazine only and Plot C, which had received three applications each of atrazine and carbofuran (Table 8). Thus applications of atrazine, plus or minus carbofuran, reduced the capacity of the soil to retain 2,4-D residues

Table 6. Dissipation of ^{14}C labelled atrazine in field plot previously treated with atrazine (% of applied)

	Extractable Time of sampling			Nonextractable Time of sampling		
	1st	2nd	3rd	1st	2nd	3rd
Year 1	0	98	28	0	2	45
Year 2	6	96	31	11	15	53

Table 7. Effect of glucose amendments on the distribution of radioactivity in soil containing ^{14}C bound residues.

Soil (3rd sample)	% of total ^{14}C at day 30		
	$^{14}CO_2$	Extractable ^{14}C	Bound ^{14}C
Year-l, no glucose	2	7	91
Year-l, with glucose	2	9	87
Year-2, no glucose	2	5	93
Year-2, with glucose	1	11	88

Table 8. Mineralization of ^{14}C-labelled 2,4-D in soil treated with atrazine

Soil	Cumulative ^{14}C (% of applied)			
	Solvent	CO_2	Extracted	Bound
Plot A (5^{th} sample)	7.9	2.6	44.7	37.6
PlotB (5th sample)	3.8	11.2	54.2	27.5
Plot C (3^{rd} sample)	14.7	25.3	29.8	26.5

Table 9. Recovery of atrazine and carbofuran applied to a field soil.

Time of Sampling	Extractable residues (% of applied)	
	Atrazine	Carbofuran
August,1995	100	100
October, 1995	37	32
June, 1996 (before application)	6	0
August, 1996 (before application)	48	26
October, 1996	43	12

The soil in PVC cylinders in the field was treated with one application each of atrazine and carbofuran in August 1995, June, 1996 and August 1996. Analysis of soil samples at different times showed two months after the first application 37% of the applied atrazine and 32% carbofuran was present in the soil as extractable residues (Table 9). Prior to treatment in June 1996 only 6% of the atrazine and none of the carbofuran was extractable. After 14 months 43% of the applied atrazine was extractable residues and only 12% of the carbofuran. These data indicate faster dissipation of carbofuran than atrazine in the soil which had previously been treated with the same pesticides.

Impact of repeated insecticide application on soil microbial activity*

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Abstract. The effects of repeated insecticide application on soil microbial activity were studied both in a cotton field and in the laboratory. The results of experiment show that there are some effects on soil microbial activities, such as the population of soil microorganisms, soil respiration, dehydrogenase activity and nitrogen fixation. The degree of effects depends on the chemical dosage. Within the range of 0.5–10.0 μg/g air-dry-soil, the higher the concentration, the stronger effect. In this experiment, the effect disappeared within 4, 8 or 16 days after treatment, depending on the dose applied. In field conditions, the situation is more complex and the data of field experiment show greater fluctuation.

1. Introduction

Agrochemicals have a very important role in agricultural production. To reduce damage to crops, many kinds of pesticides are applied for insect, diseases and weed control in China. Scientists have done much work on the fate and metabolism of pesticides, and obtained much useful data [1, 2]. Yet most of the studies were focused on a single application for a short period, and knowledge of pesticide effects on soil properties, especially repeated long term applications, has been limited. Microorganisms are an important component of the soil ecosystem. The effects of agrochemicals on soil microbial activity are of great concern to biologists [3, 4]. Methamidophos and dimethoate are two of the most intensively used insecticides for cotton insect control in China. This study tries to investigate impact of insecticide application on cotton field soil ecosystems, mainly on microorganisms.

2. Materials and methods

2.1. Field experiment — effects of repeated applications of methamidophos on the activity of soil micro-organisms

Soil samples from the cotton field were taken 48h after each insecticide application, and soil microbial activities were analyzed. The soil was a Fluvio Marine Yellow Loam with the composition; Silt 39%, Sand 43%, Clay 18%, P_2O_5 50%, K_2O 1.87%; O. M.,2.12%; NH_4^+ N: 20 μg/g, NO_3^- N: 13 μg/g.

2.2. Laboratory experiments

2.2.1. Effects of repeated application of methamidophos on the activity of soil microorganisms

The soil was treated 3 times with methamidophos at intervals of 36 days. The doses rates of methamidophos were 0 (as control), 0.5, 2.5, 5.0, 10.0 μg/g air-dry-soil. Sampling was done on the 1st,

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4th, 8th, 16th, 24th, 36th days after the soil treatment. The activities of phosphatase, dehydrogenase, respiration and nitrogen fixation were determined at each sampling time.

2.2.2. Effects of Dimethoate on the activity of soil microorganisms

The soil was treated once with dimethoate at dose rates of 0 (as control), 0.5, 2.5, 5.0, 10.0 μg/g air-dry-soil. The treated soils were placed in glass bottles capped by holed caps; the moisture content was adjusted to 60% of the maximum field capacity. Sampling was done 1, 4, 8, 16, 24 days after treatment. The activity of phosphatase, dehydrogenase, soil respiration, nitrogen fixation was determined.

3. Results and discussion

3.1. Field experiment — effects of repeated applications of methamidophos on the activity of soil micro-organisms

Cotton field soil samples were taken during 1995-1998. Populations of bacteria and fungi, soil respiration and dehydrogenase activity are shown in Tables 1-3. All the experimental results show fluctuation. Some figures show an increase, some show a decrease. In field conditions, the application rate was 375 g of pure methamidophos per hectare. The estimated concentration of methamidophos in the top 15cm layer of soil is about 0.2-0.3 μg/g air-dried soil. In laboratory experiments [5], soil microbial activity was slightly affected by a single application at the dose of 0.5 μg/g air-dried soil and it recovered very quickly, especially at the low dose level. In field conditions, the situation is more complex, so the fluctuation in the data of the field experiment is understandable. However, the general tendency is similar.

3.2. Laboratory experiments

3.2.1. Effects of repeated application of methamidophos on the activity of soil microorganisms

Fig. 1, Fig. 2 and Fig. 3 show the effects of repeated application of methamidophos on the activities of dihydrogenase, respiration and nitrogen fixation carried out in lab experiment.

From Fig. 1, it can be seen that until the 8th day after the first application, dehydrogenase activity was inhibited by all treatments. After 24 days, treatments of 5.0 μg/g and 10 μg/g show stimulation. After the second application, treatments of 5.0 μg/g and 10 μg/g show serious inhibition, especially at the dose of 10 μg/g. With the third application, initially all the treatments show inhibition which had just disappeared at the end of the experiment, except the treatment of 10 μg/g. It may be concluded that the lower concentration of methamidophos, the weaker the inhibiting effects on dehydrogenase activity.

Fig. 2 shows the effect of repeated application of methamidophos on soil respiration. Generally, the effects were weak. Most of samples show a very slight stimulation.

Fig. 3 shows the effects of repeated application of methamidophos on nitrogen fixation. It is a little complex. After the first application, inhibition is dominant. Thirty six days after the treatment it recovers. The higher concentration, the stronger effect. After-the second application the pattern is inhibition-stimulation-recovery; only the 0.5 μg/g treatment does not show stimulation. Following the third application, all the treatments show inhibition and recovery.

Table 1. Effect of methamidophos on microbial populations in the 0-15 cm layer of field soil

Sampling date	950730	950818	950912	951121*
	Bacteria, $\times 10^6$/g soil (% of check)			
Check	9.12(100.0)	4.87(100.0)	4.14(100.0)	1.32 (100.0)
Treated	8.53(93.5)	4.53(93.0)	3.2 1(77.5)	1.06(80.3)
	Fungi, $\times 10^4$/g soil (% of check)			
Check	2.3(100.0)	1.6(100.0)	1.3(100.0)	1.1(100.0)
Treated	2.8(121.7)	1.7(106.3)	1.7(130.8)	1.4(127.3)
Sampling date	960719	960803	960818	961128*
	Bacteria, $\times 10^6$/g soil (% of check)			
Check	11.9(100.0)	8.72(100.0)	8.6(100.0)	0.52(100.0)
Treated	8.35(70.2)	6.68 (76.6)	7.17(83.3)	0.43(82.7)
	Fungi, $\times 10^4$/g soil (% of check)			
Check	6.99(100.0)	5.44(100.0)	7.46(100.0)	1.78(100.0)
Treated	7.34(105.0)	7.36(135.3)	5.11(68.5)	1.68(94.4)
Sampling date	970701	970719	970809	971110*
	Bacteria, $\times 10^6$/g soil (% of check)			
Check	6.74 (100)	5.17 (100)	5.82 (100)	3.3 (100)
Treated	5.96 (84.4)	5.23(101.2)	4.84(83.2)	2.31(70.0)
	Fungi, $\times 10^4$/g soil (% of check)			
Check	2.56(100.0)	1.67(100.0)	2.38(100.0)	1.51(100.0)
Treated.	2.62(102.3)	2.58(154.4)	2.37(99.6)	1.84(121.8)
Sampling date	980806	980815	980829	981129*
	Bacteria, $\times 10^6$/g soil (% of check)			
Check	6.64(100.0)	5.85(100.0)	7.78(100.0)	8.27(100.0)
Treated.	5.57(83.9)	5.64(96.4)	5.77(74.2)	7.29(88.1)
	Fungi, $\times 10^4$/g soil (% of check)			
Check	1.39(100.0)	1.65(100.0)	1.45(100.0)	1.64(100.0)
Treated	1.58(113.7)	1.81(109.7)	1.56(107.6)	1.19(72.6)

Table 2. Effect of methamidophos on soil respiration in field conditions
(CO_2 released: nmol/g air-dried soil in 24h)

Sampling date	Check Plot	Treated Plot
950730	48.9	45.9
950818	37.3	40.1
950912	42.1	43.1
951121	38.6	40.9
960719	28.2	27.5
960802	34.1	41.8
960818	38.5	35.6
961128*	16.7	14.3
970701	8.8	11.1
970719	10.9	13.1
970809	17.2	10.6
971110*	19.5	27.2
980806	10.2	9.21
980815	9.4	7.6
980829	8	5.4
981129*	8.8	6.6

Table 3. Effect of methamidophos on the activity of dehydrogenase
(μg phenol /g air-dried soil)

Sampling date	Check Plot	Treated Plot
950730	15	16.1
950818	15.6	10.9
950912	30	20.2
951121*	12.2	1.8
980806	7.2	5.1
980815	7.9	2.9
980829	6.3	3.3
981129	5.1	3.3

*Sampling was done after the harvest of cotton plants

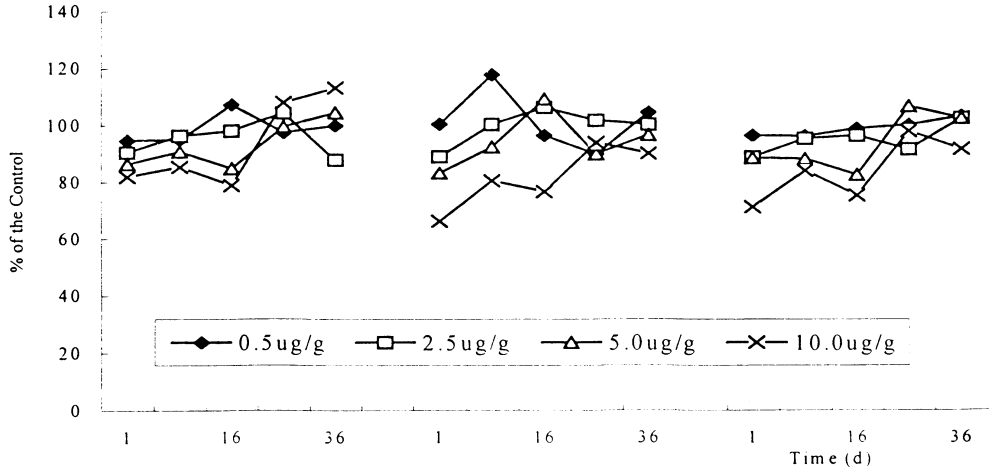

Fig.1 Relation Between concentration of Methamidophos and Dehydrogenase Activity

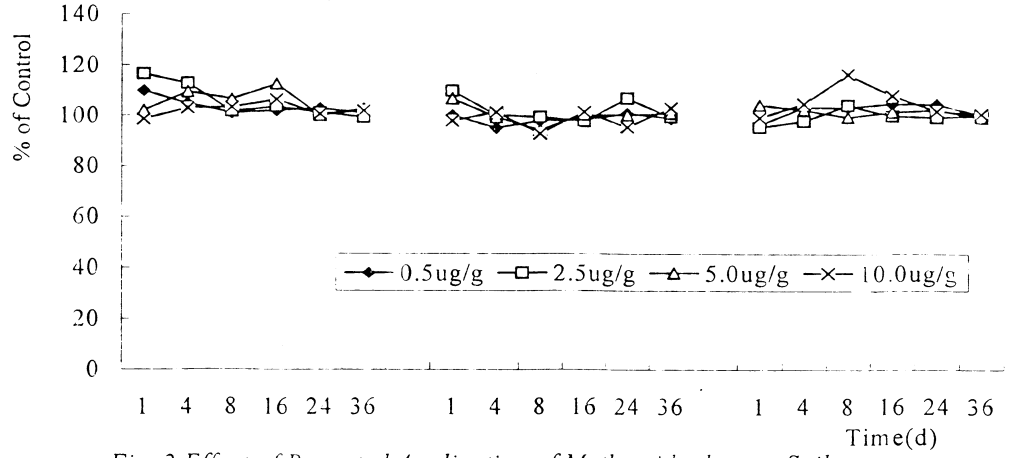

Fig. 2 Effect of Repeated Application of Methamidophos on Soil Respiration

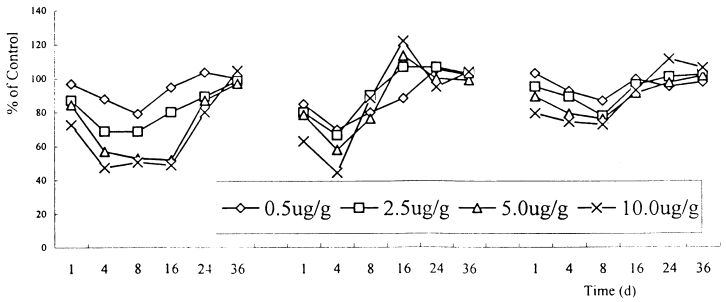

Fig. 3 Effect of Repeated Application of Methamidophos on Nitrogen Fixation

3.2.2. Effects of dimethoate on the activity of soil microorganisms

Table 4. shows the effect of dimethoate on soil dehydrogenase activity. Inhibition is the main effect, with earlier recovery at the lower concentration. At dose levels of 0.5 and 2.5 µg/g air-dried soil, it recovers from inhibition on the 8th day. At the 5.0 and 10.0 µg/g air-dried soil levels, it recovers on the 16th day after treatment.

Table 4. Effect of dimethoate on activity of dehydrogenase in soil (% of control**)**

Time (in days)	Concentration of Dimethoate µg/g				
	0	0.5	2.5	5	10
1	100(9.83)*	77.92	75.48	69.99	65.82
4	100(7.83)	82.76	81.35	78.29	73.05
8	100(6.11)	100	101.47	95.58	87.27
16	100(6.04)	100.66	95.58	100.99	102.32
24	100(7.64)	100.7	87.27	99.27	99.62

*Figures in parentheses are nmol phenol/g-air dried soil

Table 5 lists the data for the effect of dimethoate on soil respiration. At the dose level of 0.5 µg/g air-dried soil it shows a weak stimulation. At 2.5 and 5 µg/g air-dried soil there is inhibition followed by recovery by the 8th and 16th days after treatment respectively.

Table 5. Effect of dimethoate on soil respiration (% of control)

	Concentration of Dimethoate (µg/g)				
Time (in days)	0	0.5	2.5	5	10
1	100(333.64)*	104.9	96.8	96.1	100.9
4	100(387.55)	103.7	95.5	91.3	97.3
8	100(262.34)	99.6	101.6	99.3	101
16	100(293.28)	100.9	101.6	103.2	102.1
24	100(308.42)	97.4	99.7	96.2	97.4

* Figures in parentheses are nmol CO_2/g-air dried soil

Table 6 shows the effect of dimethoate on nitrogen fixation. Inhibition becomes greater and lasts longer with increase in dose. However, even at the highest dose rates there was recovery on the 24th day after treatment.

Table 6. Effect of dimethoate on nitrogen fixation (% of control)

	Concentration of Dimethoate (µg/g)				
Time (in days)	0	0.5	2.5	S	10
1	100(88.41)*	100	100.1	90.4	88.7
4	100(136.02)	95.6	95.5	85	86.4
8	100(100.16)	92	84.2	81.5	67.8
16	100(89.68)	101.6	94.5	96.9	94.5
24	100(74.81)	100	98.4	101.8	100

* Figures in parentheses are nmol C_2H_2/g-air dried soil

4. Conclusions

Soil microbial activities can be influenced by the application of agrochemicals, including the population of soil microorganisms, the dehydrogenase activity, the soil respiration, nitrogen fixation and others. The degree of effect depends on the dosage applied. The effects of repeated application in some degree are different from those of a single application. In field conditions, the amounts of agrochemicals entering the soil depend on the dosage, the application method and properties of chemical. In this experiment, the chemical used was methamidophos. The amount in the soil is less than 0.3 μg/g air-dried soil, and also the half-life of the chemical is rather short in soil, so the effect is not significant. If there are any effects, they would disappear quickly. And would not cause an ecological problem from the microbial point of view.

REFERENCES

[1] WAIWRIGHT, M.. A review of the effect of pesticides on microbial activity in soil, J. Soil. Sci. **29** (1978), 287–298.

[2] MOORMAN, J. B., A review of pesticide effects on microorganisms and microbial processes related to soil fertility, J. Prod. Agric. **21** (1989), 14–23.

[3] Hang MING, Yuhua ZHAO, Yiton LU, Effect of trifluralin on soil microbes and earthworms, J. Rural Eco-Environ. **3** (1993) 40–43 (in Chinese).

[4] TU C.M. Effect of four experimental insecticides on enzyme activities and level of adenosine triphosphate in mineral and organic soils, J. Health Environ. Sci. **B25** (6) 1990, 787–800.

[5] Bujin XU, Yongxi ZHANG et al. “Effects of methamidophos on soil Microbial Activity”, Environmental Behaviour of Crop Protection Chemicals, IAEA-SM-343/l 9, 1997.

A study of the fate of methamidophos in soil*

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Abstract. The persistence, degradation and mineralization of methamidophos in a cotton field soil was studied in laboratory experiments. Results show that it is easily degraded, with a half-life of only a few days. At 30°C, within 3 days. less than 25% of applied radioactivity was extracted in ethyl acetate:methanol (8:2 v/v). Thin layer chromatography showed that by the 15th day all the ^{14}C-methamidophos had disappeared although nearly 16% of the radioactivity remained in the sample. In a study of mineralization, less than 9% of the radioactivity appeared as $^{14}CO_2$ within 24 days.

1. Introduction

After field application of a pesticide, some will eventually get into the soil. Therefore there is interest in the behaviour of pesticides in soil [1,2,3,4]. Methamidophos is one of the intensively used pesticides for cotton pest control. Much work has been done on methamidophos residues in crops in China, but little related to its behavior in soil. This study focused on its fate in soil using ^{14}C-methamidophos.

2. Materials and methods

2.1. Soil

The soil was a Fluvio Marine Yellow Loam with the composition; Silt 39%, Sand 43%, Clay 18%, P_2O_5 50%, K_2O 1.87%; O. M.; 12%; NH_4^+ N: 20 μg/g and NO_3^- N: 13 μg/g**.**

2.2. Experiments

2.2.1. The persistence of ^{14}C-Methamidophos in soil

2.2.1.1. Treatment

This was a soil column experiment with two treatments.
Treatment A: dimethoate was applied to the soil surface one week before applying ^{14}C methamidophos.
Treatment B: ^{14}C-methamidoplios alone was applied.

Samples were taken 0, 3, 6, 9, 12, 18, 24 and 30 months after treatment.

2.2.1.2. Sample preparation

(1) A 20.0 g soil sample was placed in a 250 mL flask, and 50 mL extraction solvent (ethyl acetate: methanol 8:2, v/v was added and the flask allowed to stand for for 3h;
(2) The flask was shaken for 30 min., then 7.5 g $(NH_4)_2SO_4$ was added, the flask allowed to stand for 3h, then shaken for a further 30 min;
(3) The slurry was filtered, and the residue washed with 3 × 10 mL portions of the extraction solvent;

* The project was supported by IAEA under the research contract 8079/RB and by the provincial Science and Technology Committee of Zhejiang, China.

(4) The filtrate was dried by passage through a column filled with 20 g anhydrous Na2SO4;
(5) The column was washed with 3 × 10 mL ethyl-acetate: methanol 9:l (v/v);
(6) The solution was concentrated to 10.0 mL in a rotary evaporator.

2.2.1.3. Total radioactivity determination

A 1.00 g air-dried soil sample was oxidized in OX-600 Biological Oxidiser and the released $^{14}CO_2$ was absorbed by an ethanolamine based liquid scintillation solution. The radioactivity in the sample was determined in a Packard 1900 TR Liquid Scintillation Counter.

2.2.2. The Degradation of ^{14}C-Methamidophos in soil

2.2.2.1 Treatment

Air dried soil samples (20 g) were put into flasks and 1.48×10^4 Bq ^{14}C-methamidophos and 0.1 mg carrier (cold methamidophos) was added to each followed by 8 mL water to bring the soil moisture content to 25% which was maintained during experiment. The flasks were incubated at 30°C and samples taken after 0, 1, 3, 7, 14, 30d.

2.2.2.2. Sample extraction

Extraction solvent (ethyl acetate:methanol 8:2 v/v) was added to each sample flask. The flasks were left to stand overnight then shaken at 250 rpm for 1h. Then 7.5 g of $(NH_4)_2SO_4$ was added to each flask which was then shaken for a few minutes, left to stand for 4h, then shaken again for 1h before filtration. The filter was washed with extraction solvent (3 x 10mL). Ten g Na_2SO_4 was added to the filtered extracts; which were then passed through a column of Celite and active charcoal. The purified extracts were concentrated to 2 mL in a rotary evaporator

2.2.2.3. Radioactivity measurement

Radioactivity was measured by LSC. The degradation products were separated by TLC using an eluant of trichloromethane: methanol 85:15, (v/v) and measured by a beta-gamma scanner.

2.2.3. The mineralization of ^{14}C-methamidophos in soil

2.2.3.1. Experimental procedure

Air dried soil (50 g) was put into a 500 mL flask, and 3.67×10^4 Bq of ^{14}C-methamidophos plus 10 µg /g soil of cold methamidophos were added. The soil was mixed with the chemicals thoroughly and 10 mL water was added. The flask was stoppered with a rubber bung fitted with inlet and outlet tubes and incubated at 24^{o}C. Samples from the CO_2 trap were taken every 24h.

3. Results

3.1 Persistence of ^{14}C methamidophos

Results are shown in Table 1.

Table 1 shows that within 3 months more than 80% of the applied radioactivity had disappeared. There is no significant difference between the two treatments in respect of radioactivity disappearance. The chemical moved down slowly to lower layers. There was no radioactivity in the samples below 30 cm until 18 months. In the last sample, radioactivity below 30 cm was only 0.38%, and the total radioactive residue was about 9%.

Table 1. ^{14}C-activity in soil at each sampling time

Sampling time (month)		Soil layer (cm)	Extractable (dpm/g soil)	% of applied	Combustion (dpm/g soil)	% of applied
0	A	0-15	18360	75	23740	97
		15-30	0	0	0	0
	B	0-15	18480	75	23900	97
		15-30	0	0	0	0
3	A	0-15	686	2.48	4800	16.9
		15-30	117	0.44	44	0.17
	B	0-15	596	2.59	5050	17.7
		15-30	107	0.4	32	0.12
6	A	0-15	300	1.25	4400	16.45
		15-30	20	0.07	50	0.2
	B	0-15	236	0.98	4070	16.3
		15-30	20	0.09	30	0.13
9	A	0-15	290	1.25	4440	17.8
		15-30	10	0.05	30 0	0.12
	B	0-15	270	1.08	4300	18
		15-30	14	0.06	35	0.14
12	A	0-15	145	0.56	4190	16.3
		15-30	50	0.2	50	0.2
		Under 30	0	0	80	0.36
	B	0-15	140	0.56	3680	14.2
		15-30	40	0.16	180	0.7
		Under 30	0	0	70	0.22
18	A	0-15	113	0.45	3611	14
		15-30	30	0.12	392	1.55
		Under 30	54	0.18	42	0.14
	B	0-15	107	0.4	3663	13.5
		15-30	53	0.22	410	1.69
		Under 30	44	0.13	38	0.11
24	A	0-15	128	0.41	3944	12.99
		15-30	29	0.11	132	0.51
		Under 30	25	0.1	80	0.31
	B	0-15	159	0.57	3090	11.4
		15-30	38	0.14	78	0.29
		Under 30	13	0.04	58	0.19
30	A	0-15	167	0.6	1824	6.73
		15-30	26	0.1	71	0.87
		Under 30	32	0.11	103	0.15
	B	0-15	220	0.79	1662	7.32
		15-30	72	0.27	88	0.33
		Under 30	39	0.13	112	0.38

3.2 Degradation of methamidophos

Results are shown in Tables 2 & 3. Thus methamidophos is rapidly degraded in soil. Under the experimental conditions, the extractable residue was less than 2% of that applied by the 7th day. On the 15th day, the total radioactive residue determined by oxidation was 15.8% and ethyl acetate:methanol extractable was 1.2% and there was no methamidophos peak in the TLC plate, which means almost all of ^{14}C-methamodophos was degraded.

Table 2. The residue of ^{14}C-methamidophos in ethyl-acetate:methanol extracts

Sampling time (d)	Total activity recovered (Bq)	% of zero time
0	8569	100
1	7138	83.3
3	2121	24.8
7	261	3.0
15	107	1.2
30	52	0.6

Table 3. TLC of ^{14}C-methamidophos in soil

Sampling time (d)	Total activity	Activity of Peak	% of total in peak
0	4102	3819	93.1
1	4943	4643	92
3	1766	1451	82.2
7	784	488	62.2
15	217	no peak	

3.3. Mineralization of ^{14}C methamidophos in soil

Table 4 shows that methamidophos is easily mineralized in soil. Within 24 days about 9% of the ^{14}C activity was released as $^{14}CO_2$

Table 4. Mineralization of methamidophos in soil

Sampling time (d)	$^{14}CO_2$ recovered (dpm)	Accumulated recovery (%)
0	0	0
1	79000	3.36
2	40830	5.09
3	45450	7.02
4	16190	7.71
5	6920	8.01
6	3750	8.16
7	2730	8.28
12	1350	8.64
17	920	8.84
24	530	8.98

4. Conclusions

(a) The half-life of methamidophos in soil is rather short, only a few days. It is easily degraded. Within 3 days at 30°C, the radioactivity in extracts was less than 25% of that applied.
(b) Thin layer chromatography showed that after 15d none of the residual radioactivity corresponded with the methamidophos peak which means all the ^{14}C-methamidophos had disappeared
(c) Less than 9% of the applied radioactivity was released as $^{14}CO_2$ in 24d. The remainder could be fixed in the soil or released from soil as other simpler compounds.

REFERENCES

[1] HENDRY, K. M, RICHARDSON, C. J., Soil biodegradation of carbofuran and furathiocarb following Soil Pretreatment with these pesticides, Environ. Toxicol. Chem. **7** (1988) 763–774.

[2] ENTRY, J. A. et al., Microbial mineralization of atrazine and 2, 4-dichlorophenoxyacetic acid in riparian pasture and forest soil, Biol. Fertility Soils, **18** (1994) 89–94.

[3] JAMES, T. K. et al, Degradation of primisulfuron-methyl and metsulfuron-methyl in soil, Weed Research, **35** (1995) 113–420.

[4] HILL, L.R. and WRIGHT, S. J. L., "Pesticide microbiology", Microbiological Aspects of Pesticide Behaviour in the Environment, Academic Press Inc (London) Ltd. (1978).

Mineralization of ^{14}C-labeled agrochemicals in soil*

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Abstract. ^{14}C-labeled compounds were used to study the mineralization of methamidophos, 2,4-D and metsulfuron in soil. Mineralization rate was influenced by the type of soil, concentration of chemical in the soil, the initial soil microbial population and the nature of the chemical.

1. Introduction

Agrochemicals have played a very important rôle in agricultural production. Many kinds of agrochemicals have been used in China for insect, diseases and weed control [1]. Methamidophos is a widely used insecticide in cotton; 2,4-D has been used as a plant growth regulator and herbicide for a long period, and metsulfuron is a newly introduced herbicide. Soil micro-organisms are actively engaged in mineralization and co-metabolism processes [2,3,4]. Much work related to agrochemical mineralization has been done since the 1960's [5,6,7]. This study is focused on the mineralization of methamidophos, 2,4-D and metsulfuron using ^{14}C-labeled compounds to obtain a clearer picture of the fate of these chemicals in the soil. $^{14}CO_2$ released from soil treated with ^{14}C-labeled compounds is used as a measure of mineralization [8].

2. Materials and methods

2.1. Soils

(a) Fluvio Marine Yellow Loamy Soil: organic matter 2.12%; pH value 6.8; maximum water holding capacity 32.11%
(b) Quaternary Red Clay (Red Earth): organic matter 2.26%; pH value 5.1; maximum water holding capacity 41.27%

2.2. Chemicals

^{14}C-methamidophos and ^{14}C-2,4-D were provided by the IAEA. ^{14}C-metsulfuron was provided by the Institute for Application of Atomic Energy in Agriculture, Chinese Academy of Agricultural Sciences.

2.3. General experimental procedure

Air dried soil (50g) was put into a 500 mL flask, and the radioactive chemical mixed with unlabelled compound were added. The soil was mixed with the chemicals thoroughly and 10 mL water was added. The flask was stoppered with a rubber bung fitted with inlet and outlet tubes and incubated at 24^{o}C Samples of the ethanolamine based liquid scintillation solution from the CO_2 trap were taken at intervals. Samples were counted in a LSC

* The project was supported by IAEA under the research contract 8079/RB and by the provincial Science and Technology Committee of Zhejiang, China.

2.4. Treatments

(a) The effect of concentration on the mineralization of methamidophos was studied by incubating soil samples treated with 0.5, 5 and 10 μg/g of mixed labeled and cold methamidophos with a ^{14}C-activity of 3.66×10^4 Bq/g soil.

(b) To assess the effect of initial soil microbial population, radiation sterilized soil was mixed with air-dried soil at ratios of 0:1, 1:3, 1:1 and 1:0. Samples of each mixture were incubated with 5 μg/50g of methamidophos with a ^{14}C-activity of 3.66×10^4 Bq.

(c) The mineralization of ^{14}C-2,4-D in the two soils (Fluvio Marine Yellow Loamy Soil and Quaternary Red Clay) was compared.

(d) The mineralization of ^{14}C-methamidophos ($3.66x10^4$ Bq), ^{14}C-2,4-D (3.92×10^4 Bq) and ^{14}C-metsulfuron (1.32×10^4 Bq) was compared in the Fluvio Marine Yellow Loamy Soil.

3. Results and discussion

3.1. The effect of concentration on mineralization of methamidophos

Concentration has a direct influence on the mineralization of methamidophos as shown in Figure 1. The accumulated activity of $^{14}CO_2$ released from ^{14}C-labelled methamidophos treated soil increases rapidly. There were differences in the rate of release of $^{14}CO_2$ during the first *5* days or so. Later the $^{14}CO_2$ release rate became almost constant. During the 24 days experiment, the accumulated $^{14}CO_2$ accounted for 8.8%, 9.2% and 8.7% of the applied radioactivity respectively for 0.5 μg/g, 5 μg/g and 10 μg/g concentrations.

3.2. Effect of initial soil microbial population on mineralization

Fig. 2 shows that the initial soil microbial population has a very significant influence on the mineralization rate. The rate in semi-sterilized soil was only half of that of nonsterilized soil. The rate in completely sterilized soil stayed very low during the experiment. The slow mineralization in the completely sterilized soil could have been caused by extra-cellular enzymes remaining after the micro-organisms were killed.

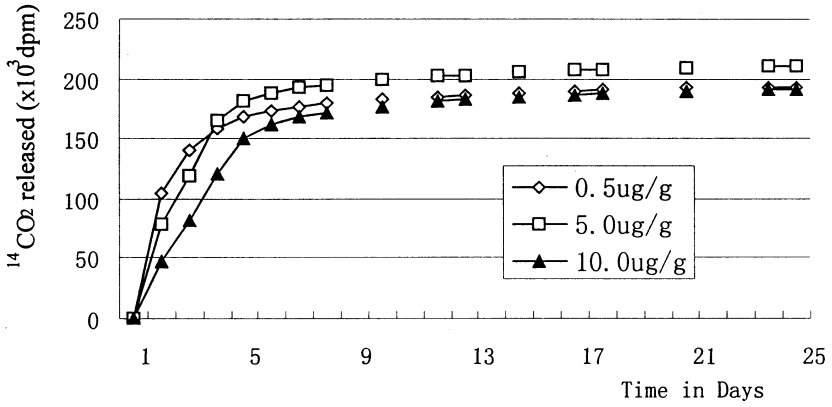

Fig. 1. Mineralization of ^{14}C-Methamidophos at different concentration.

Fig. 3 shows the accumulated $^{14}CO_2$ during the experiment. It can be seen that the initial population effect only lasts for about 20 days then it gradually disappeared. At the end of this experiment, except in the completely sterilized soil samples, the accumulated $^{14}CO_2$ activity was similar, from 9.62% to 9.80% of the total applied.

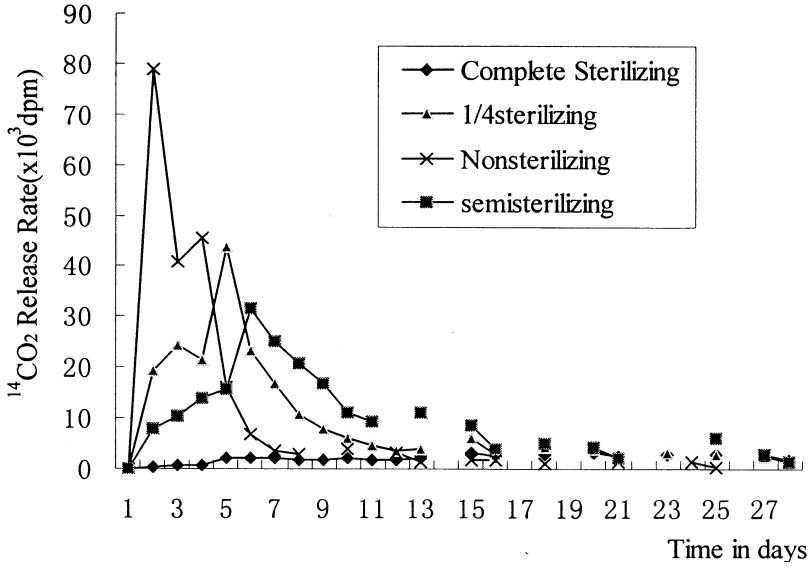

Fig 2. Effect of initial soil microbial population on mineralization of methamidophos.

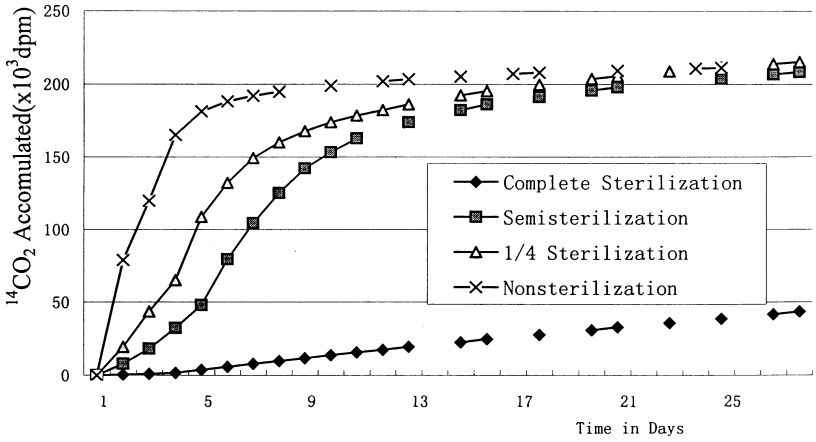

Fig. 3. Effect of soil microbial population on the mineralization of Methamidophos.

3.3. The mineralization of 2, 4-D in two type of soil

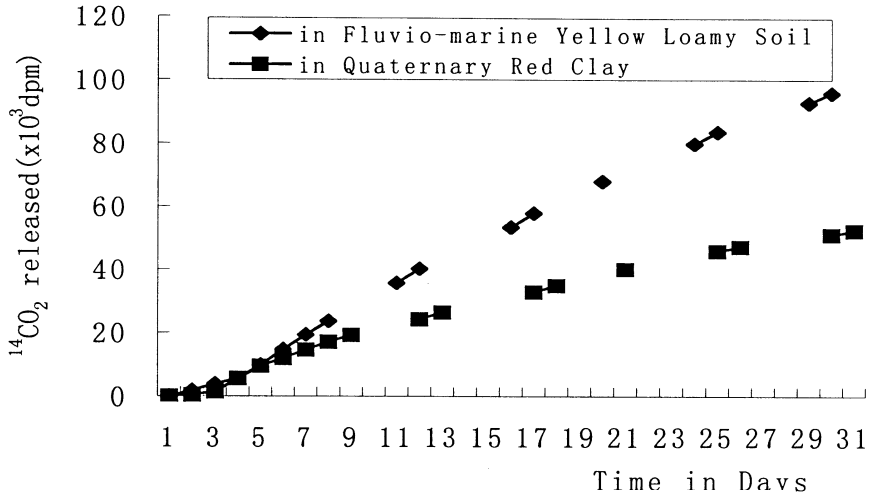

Fig. 4. Mineralization of ^{14}C-ring-2,4-D in two type of soils.

Fig. 4 shows the mineralization rate of ^{14}C-2,4-D in the two types of soil is different, presumably caused by the differences in soil properties, especially the pH value. The difference in accumulated $^{14}CO_2$ reaches around 46%.

3.4. Mineralization of different chemicals in the same soil

The rates of mineralization of the chemicals are shown in Fig. 5. Methamidophos has highest rate at the beginning and the highest degree of degradation in the experimental period. Metsulfuron showed the slowest rate. Over the experimental period, the $^{14}CO_2$ released was 8.7%, for methamidophos, 4.1% for 2,4-D, and 4.4%, for metsulfuron, of the applied radioactivity

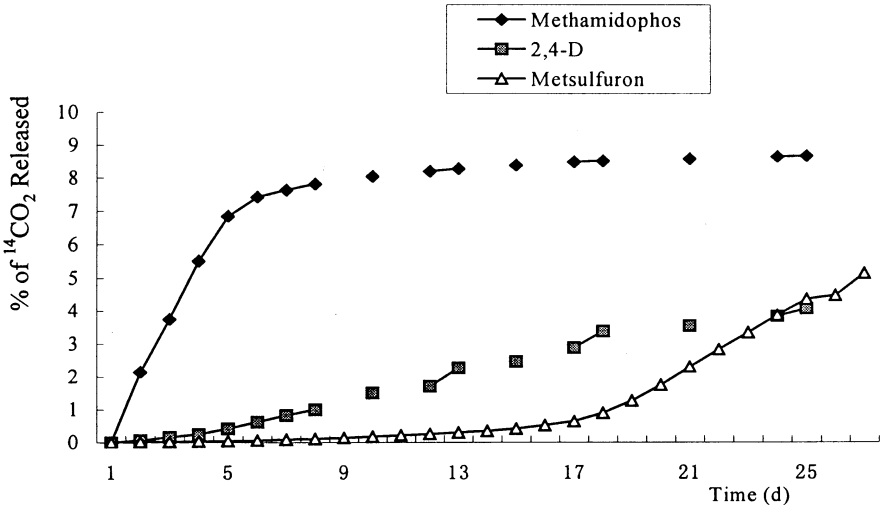

Fig. 5. Mineralization of different chemical in soil.

5. Conclusions

(a) The mineralization of agrochemicals in soil is a very complex process which is influenced by many factors, such as type of soil, concentration of pesticides in soil, nature of the chemical, population of soil microbial and so on.
(b) Generally, the extent of mineralization of agrochemicals studied here is rather low. Even though methamidophos is a small simple molecule chemical, only 8.7% of the total applied ^{14}C-activity, was released as $^{14}CO_2$ within 24 days. More than 90% of the activity remains in the soil, probably as degradation products. It seems that molecular structure is a very important factor affecting mineralization.

REFERENCES

[1] YOUCHUN FEI, Pesticide Questions and Answers (in Chinese), Chemistry Industry Press, Beijing (1997).

[2] WENXING CHEN, Soil and Environmental Microbiology (in Chinese), China Agricultural Press, Beijing, (1990).

[3] PARKER, L. W., DOXTADER, K.G. Kinetics of microbial decomposition of 2, 4-D in soil, J. Environ. Qual. **11** (1982) 679–684.

[4] ROBERTSON, B K., ALEXANDER, M. Growth linked and co-metabolic biodegradation: Possible reason for occurrence or absence of accelerated pesticide biodegradation, Pestic. Sci.**41** (l994) 311–318.

[5] AIGLABIE, J., LLOYD-JONES, G. A review of bacterial degradation of pesticides, Aust. J. Soil Res. **33** (1995) 925–942.

[6] SCOW, R.S., SIMKINS, S., ALEXANDER, M., Kinetics of mineralization of organic compounds at low concentration in soil, Applied and Environmental Microbiology, 51 (1986) 1028–4035.

[7] JAMES, T.K. et al, Degradation of primisulfuron-methyl and metsulfuron-methyl in soil, Weed Research, **35** (l995) 113–420.

[8] ZELLES, L.M.E. et al., Measurement of bioactivity based on CO_2 release and ATP content in soil after different treatments. Chemosphere **B8** (1984) 899–913.

Effect of repeated applications of pesticides used on cotton on soil properties

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Abstract. Repeated application of monocrotophos, methomyl and carbaryl for four years considerably reduced microbial counts, iron reduction, nitrification and arginine deaminase activity in soil. The microbial activities seemed to recover several weeks following pesticide application. The inhibition of enzyme activities was in general more obvious during the second to the fourth years. The maximum inhibition of iron reduction capacity and arginine deaminase activity was observed by the end of the fourth year and amounted to about 90% of control values. No pronounced effect of the used insecticides on respiration and dehydrogenase activity could be detected over the experimental period.

1. Introduction

Pesticides reaching the soil affect non-target organisms and their activities [1]. To examine these side effects, several investigations are necessary to identify possible changes in the bioactivity of soil organisms contributing to soil fertility. These organisms especially bacteria, fungi and actinomycetes decompose root residues and bring about many reactions necessary for plant growth and crop production.

Generally, pesticide residues will remain in the top 15 cm layer of the soil [2] which is the region of greatest activity of soil microflora [3], thus favouring the interaction of pesticide residues with the flora of the soil ecosystem. Most of studies on the effect of pesticides on soil microbial activity have been laboratory studies [4–7] following single applications of pesticides for short periods. The effect of repeated application in the field on activities of soil microbes and enzymes has so far received little attention.

The present work was undertaken to assess the effect of repeated application of pesticides commonly used on cotton fields in Egypt, on the activities of soil microbes and enzymes.

2. Materials and methods

2.1. Experimental plot

For this study a field plot (approx. 200 m^2) of clay soil was used. It was cultivated with cotton plants (var. Giza 85) during March (from 1995–1998). Clover was cultivated in the plot (Oct.–Feb.) as a winter rotational crop between the cotton seasons, The soil characteristics are :- sand, 32.5%; silt, 23.5%; clay, 44.0%; organic matter,1.25%; pH 7.8

2.2. Pesticides used

Plants were treated with the insecticides as shown in Table 1. Irrigation, fertilization and hand weeding were performed as usually practiced in the field.

A control field plot (6 × 8 m^2) and of similar soil characteristics to the experimental plot, was cultivated as in the experimental plot and kept untreated with pesticides.

2.3. Experimental procedures

Most of the procedures used for determination of soil bioactivity followed the protocols generated during the first RCM, Neuherberg, Germany (29 May–2 June 1995).

Table 1. Names and conditions of application of the insecticides used.

Common Name	Chemical Name	Rate of Application	Time of Application	Type of Formulation
Monocrotophos	Dimethyl-(*E*)-1-methyl-2-methyl-carbamoylvinyl-phosphate	200 mL/Feddan	June 1995–1998	E.C.[(a)]; 40% A.I.[(b)]
Methomyl	*S*-(methyl-*N*-(methylcarbamoyloxy)thioacetimidate	330 g/Feddan	July 1995–1998	Wettable powder; 80% A.I
Carbaryl	1-naphthyl-methylcarbamate	1.5 kg/Feddan	September 1995–1998	Wettable powder; 80% A.I

Samples from the plough layer (upper 15-cm zone) of the different soil types were used for assessment of soil bioactivity. A minimum of 5 cores (i.d. 2.5 cm) per plot were collected, pooled and mixed thoroughly before use. Sieved soil (5 mm mesh) samples, from experimental and control plots, were adjusted to about 55% maximum water holding capacity and kept, till use in a dessicator in a temperature regulated chamber at 24 ^{o}C. Soil samples were not stored more than two weeks.

To determine changes in microbial population, soil samples were subjected to a soil dilution plate method, using sodium albuminate agar [8] for bacteria, Jenson's medium agar for actinomycetes and rose bengal streptomycin agar for fungi [9].

In soil respiratory studies, the basal and substrate induced respiration of soil microorganisms were determined according to Anderson [10]. From these data the respiration quotient, $Q.CO_2/h/g^{-1}$ soil, could be calculated. The soil microbial biomass (C_{mic}) was calculated according to Anderson and Domsch [11] where 1 mL $CO_2/h/g^{-1}$ soil $\equiv$ 40 mg C_{mic}.

Soil dehydrogenase activity was measured by determination of formazan (2,3,5-triphenyltetrazolium formazan (TTF) formed after incubating the soil with 2,3,5-triphenyl tetrazolium chloride (TTC). The product is determined colorimetrically at 485 nm [12].

Changes in oxidation of ammonium from soil organic nitrogen were determined by nitrification. Nitrite was analyzed by a diazotization method with sulphanilic acid in sodium acetate buffer [6].

Other parameters used for studying soil bioactivity included the soil capacity to reduce ferric iron [13] and arginine deaminase activity [14].

3. Results and discussion

Repeated application of insecticides seemed to affect the microbial count in treated soil during the first year (1995). Actinomycetes were reduced by all insecticides, while methomyl suppressed mainly the bacterial count as well. Between the second and fourth years (1996–1998), the bacterial and actinomycetes counts were progressively inhibited by the used insecticides (Table 2). In general, the count of fungi was slightly affected by insecticides used over the period of the experiment.

The reduction of the low soluble ferric iron to the more soluble ferrous iron has been considered to be a measure of bioactivity of anaerobic microorganisms in soil. The results obtained during the first year indicate that the application of monocrotophos and methomyl reduces the capacity of iron reduction directly after application. Carbaryl, on the other hand, showed only a slight effect. However, the capacity to reduce ferric iron was restored slowly with time. By the end of 1996 and during the

following two years (97 and 98), a marked inhibition in iron reduction capacity was observed (Table 3). The effect of repeated insecticides application was more obvious during the third and fourth years where the inhibition reached its maximum (over 90% of control values) after application of carbaryl by the end of 1998.

The effect of the insecticides used on respiration is represented in Table 4. The respiratory quotient Q/unit microbial mass (C_{mic}) in both treated and control soils ranged between 0.027–0.028. This indicates that the insecticides used did not affect respiration to any significant extent during the experimental period.

No pronounced effect of insecticides used on dehydrogenase activity could be detected during the experimental period. The inhibition observed during the second year lasted throughout 1998 (Table 5).

During the first year of pesticide application, nitrification seemed to be apparently suppressed only by monocrotophos and methomyl, where, ammonia production was decreased to about 50% after addition of both monocrotophos and methomyl. The concentration of nitrate was reduced by about 25% only by carbaryl and methomyl. The latter, however, caused a reduction in nitrite concentration by about 50%. Further repeated insecticide applications led to considerable suppression of ammonia and nitrite concentration whereas nitrate concentration was slightly affected. By the end of the fourth year, the inhibition in ammonia production, nitrate and nitrite concentration amounted to about 71, 54 and 56%, respectively (Table 6).

The arginine deaminase activity was assayed only by the second year. The deaminase activity was considerably inhibited in treated soil. The percentage inhibition 21 days following the last application (carbaryl) amounted to about 75% of control values. During the third and fourth years, the deaminase activity was considerably inhibited after the addition of each insecticide. The percentage of inhibition amounted to 93% by the end of the fourth year (Table 7).

The results obtained indicate that repeated application of the insecticides used (monocrotophos, methomyl and carbaryl) for four successive years had a pronounced effect on several enzyme activities related to soil fertility especially microbial count, iron reduction, nitrification and deaminase activity during the experimental period. In general, the microbial activities seemed to recover at least partially several weeks following application of the insecticide.

able 2. Effect of insecticides application on soil microbial count in the field during 1995–1997.

Sampling Time	Sample from Area	Insecticide Applied	Total Count/g Dry Soil					
			Year 1995			Year 1996		
			Bacteria	Actino-mycetes	Fungi	Bacteria	Actino mycetes	Fungi
June	C	Monocrotophos	104×10^5	56×10^5	56×10^3	102×10^5	80×10^5	50×10^3
	T_1		105×10^5	54×10^5	55×10^3	180×10^4	54×10^5	50×10^3
	T_2		150×10^4	43×10^5	42×10^3	143×10^4	43×10^5	45×10^3
July	C	Methomyl	108×10^4	77×10^4	49×10^2	105×10^5	77×10^5	49×10^3
	T_1		103×10^4	63×10^4	47×10^2	160×10^4	60×10^5	45×10^3
	T_2		59×10^4	63×10^4	50×10^2	70×10^4	50×10^5	39×10^3
September	C	Carbaryl	100×10^5	88×10^5	44×10^3	100×10^5	76×10^5	45×10^3
	T_1		160×10^4	58×10^5	60×10^2	155×10^4	55×10^5	80×10^2
	T_2		140×10^4	25×10^5	44×10^3	130×10^4	30×10^5	90×10^2

C : Control
T_1 : One week before application of the insecticide
T_2 : 48h after application of the insecticide

Table 2 : (continued)

Sampling Time	Sample from Area	Insecticide Applied	Total Count/g Dry Soil					
			Year 1995			Year 1996		
			Bacteria	Actino-mycetes	Fungi	Bacteria	Actino-mycetes	Fungi
June	C	Monocrotophos	140×10^5	103×10^5	61×10^3	136×10^5	126×10^5	76×10^3
	T_1		144×10^5	111×10^5	49×10^3	115×10^5	107×10^5	60×10^3
	T_2		63×10^5	77×10^5	45×10^3	64×10^5	56×10^5	45×10^3
July	C	Methomyl	114×10^5	106×10^5	63×10^3	120×10^5	110×10^5	65×10^3
	T_1		90×10^5	85×10^5	55×10^3	80×10^5	70×10^5	53×10^3
	T_2		68×10^5	69×10^5	49×10^3	55×10^5	50×10^5	40×10^3
September	C	Carbaryl	108×10^5	103×10^5	48×10^3	110×10^5	103×10^5	60×10^3
	T_1		75×10^5	65×10^5	50×10^3	70×10^5	53×10^5	49×10^3
	T_2		69×10^5	67×10^5	49×10^3	50×10^5	45×10^5	32×10^3

C : Control
T_1 : One week before application of the insecticide
T_2 : 48h after application of the insecticide

able 3. Effect of insecticides application on iron-reduction capacity in soil during 1995–1998

Sampling Time	Sample from Area	Insecticide Applied	μg Fe^{2+} /g Dry Soil			
			Year 1995	Year 1996	Year 1997	Year 1998
June	C	Monocrotophos	2.40 ± 0.26	1.54 ± 0.03	3.23 ± 0.25	3.53 ± 0.21
	T_1		2.40 ± 0.20	1.70 ± 0.05	2.90 ± 0.20	2.90 ± 0.10
	T_2		1.32 ± 0.33	1.30 ± 0.07	1.22 ± 0.13	1.2 ± 0.05
July	C	Methomyl	2.00 ± 0.26	1.50 ± 0.08	2.75 ± 0.25	3.00 ± 0.15
	T_1		1.06 ± 0.03	1.35 ± 0.15	1.50 ± 0.15	1.60 ± 0.05
	T_2		1.20 ± 0.23	1.10 ± 0.03	0.78 ± 0.09	0.63 ± 0.07
September	C	Carbaryl	2.40 ± 0.26	1.70 ± 0.01	3.00 ± 0.08	2.85 ± 0.13
	T_1		2.20 ± 0.36	1.50 ± 0.07	0.88 ± 0.18	0.78 ± 0.09
	T_2		2.10 ± 0.17	0.33 ± 0.02	0.26 ± 0.06	0.15 ± 0.03

C : Control
T_1 : One week before application of the insecticide
T_2 : 48h after application of the insecticide

Table 4. Effect of insecticides application on soil microbial respiration in the field during 1995–1998

Sampling Time	Sample from Area	Insecticide Applied	Year 1995		Year 1996		Year 1997		Year 1998	
			Q	C_{mic}	Q	C_{mic}	Q	C_{mic}	Q	C_{mic}
June	C	Monocrotophos	0.21 ± 0.1	7.64 ± 1.0	0.27 ± 0.1	9.82 ± 1.7	0.20 ± 0.0	7.27 ± 1.2	0.26 ± 0.02	9.3 ± 0.70
	T_1		0.20 ± 0.0	7.27 ± 0.9	0.15 ± 0.0	5.45 ± 0.8	0.17 ± 0.0	6.18 ± 1.5	0.21 ± 0.01	7.6 ± 0.35
	T_2		0.23 ± 0.0	8.36 ± 1.8	0.18 ± 0.1	6.55 ± 0.5	0.19± 0.0	6.91 ± 0.6	0.17± 0.01	6.06 ± 0.23
July	C	Methomyl	0.38 ± 0.1	13.82 ± 3.2	0.38± 0.1	13.82 ± 3.0	0.23 ± 0.0	8.36 ± 0.3	0.27 ± 0.02	9.7 ± 0.51
	T_1		0.19 ± 0.1	6.91 ± 1.0	0.18 ± 0.1	6.55 ± 2.4	0.21 ± 0.1	7.64 ± 1.5	0.19 ± 0.01	6.9 ± 0.35
	T_2		0.21 ± 0.1	7.64 ± 2.4	0.16 ± 0.1	5.62 ± 0.7	0.18 ± 0.0	6.55 ± 0.6	0.16 ± 0.01	5.8 ± 0.35
September	C	Carbaryl	0.32 ± 0.1	11.64 ± 1.5	0.29 ± 0.1	10.55 ± 2.4	0.21 ± 0.1	7.64 ± 0.9	0.25 ± 0.01	9.1 ± 0.40
	T_1		0.24 ± 0.1	8.73 ± 0.8	0.18 ± 0.1	6.55 ± 1.9	0.19 ± 0.0	6.91 ± 0.8	0.17 ± 0.01	6.2 ± 0.40
	T_2		0.17 ± 0.0	6.18 ± 1.60	0.14 ± 0.0	5.10 ± 0.9	0.15 ± 0.0	5.45 ± 0.9	0.14 ± 0.02	5.2 ± 0.70

C : Control
T_1 : One week before application of the insecticide
T_2 : 48h after application of the insecticide
Q : Basal/SIR/hour/g.soil.
C_{mic} : Unit microbial mass

able 5. Effect of insecticides application on soil dehydrogenase activity during 1995–1998

Sampling Time	Sample from Area	Insecticide Applied	Dehydrogenase Activity (µg formazan/10 g soil)			
			Year 1995	Year 1996	Year 1997	Year 1998
June	C	Monocrotophos	0.15 ± 0.03	0.17 ± 0.03	0.13 ± 0.01	0.21 ± 0.03
	T_1		0.30 ± 0.02	0.17 ± 0.02	0.11 ± 0.01	0.13 ± 0.02
	T_2		0.09 ± 0.01	0.14 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
July	C	Methomyl	0.09 ± 0.00	0.17 ± 0.02	0.10 ± 0.00	0.17 ± 0.02
	T_1		0.05 ± 0.01	0.09 ± 0.01	0.09 ± 0.00	0.11 ± 0.01
	T_2		0.05 ± 0.01	0.07 ± 0.00	0.09 ± 0.00	0.08 ± 0.00
September	C	Carbaryl	0.17 ± 0.03	0.17 ± 0.02	0.20 ± 0.01	0.15 ± 0.02
	T_1		0.10 ± 0.03	0.10 ± 0.01	0.11 ± 0.00	0.10 ± 0.01
	T_2		0.14 ± 0.01	0.09 ± 0.00	0.10 ± 0.00	0.07 ± 0.00

C : Control.
T_1 : Before application of the insecticide (one week).
T_2 : 48h after application of the insecticide.

Table 6. Effect of insecticides application on nitrification in soil in the field during 1995–1998

Sampling Time	Sample from Area	Insecticide Applied	Year 1995			Year 1996		
			Ammonia mg/g	Nitrate µg/g	Nitrite µg/g	Ammonia mg/g	Nitrate µg/g	Nitrite µg/g
June	C	Monocrotophos	0.22 ± 0.02	60.0 ± 0.80	0.40 ± 0.01	0.34 ± 0.03	54.00 ± 2.80	0.53 ± 0.03
	T_1		0.22 ± 0.02	58.4 ± 0.80	0.40 ± 0.01	0.33 ± 0.04	54.53 ± 2.25	0.45 ± 0.03
	T_2		0.09 ± 0.03	56.0 ± 0.80	N.D.[(a)]	0.27 ± 0.05	49.25 ± 2.59	0.29 ± 0.03
July	C	Methomyl	0.23 ± 0.03	59.2 ± 1.60	0.41 ± 0.08	0.40 ± 0.02	51.94 ± 2.85	0.51 ± 0.05
	T_1		0.21 ± 0.01	63.7 ± 1.22	N.D.	0.31 ± 0.03	47.01 ± 2.57	0.31 ± 0.03
	T_2		0.10 ± 0.05	44.0 ± 0.80	0.21 ± 0.02	0.18 ± 0.02	44.00 ± 0.80	0.22 ± 0.03
September	C	Carbaryl	0.23 ± 0.01	58.5 ± 0.80	0.40 ± 0.08	0.37 ± 0.04	59.73 ± 2.44	0.52 ± 0.04
	T_1		0.20 ± 0.07	50.8 ± 0.96	N.D.	0.29 ± 0.02	41.86 ± 3.78	0.25 ± 0.02
	T_2		0.17 ± 0.03	44.5 ± 1.66	N.D.	0.15 ± 0.05	36.00 ± 4.20	0.17 ± 0.04

C : Control
T_1 : Before application of the insecticide (one week)
T_2 : 48h after application of the insecticide.
[(a)]N.D. : Not detected

able 6. (cont.)

Sampling Time	Sample from Area	Insecticide Applied	Year 1997 Ammonia mg/g	Year 1997 Nitrate µg/g	Year 1997 Nitrite µg/g	Year 1998 Ammonia mg/g	Year 1998 Nitrate µg/g	Year 1998 Nitrite µg/g
June	C	Monocrotophos	0.20 ± 0.01	65.60 ± 0.90	0.56 ± 0.03	0.30 ± 0.03	67.33 ± 3.05	0.52 ± 0.03
	T_1		0.16 ± 0.02	63.20 ± 0.40	0.52 ± 0.02	0.30 ± 0.02	63.66 ± 2.08	0.48 ± 0.02
	T_2		0.11 ± 0.04	56.00 ± 0.30	0.48 ± 0.03	0.20 ± 0.02	55.66 ± 2.08	0.40 ± 0.01
July	C	Methomyl	0.23 ± 0.05	62.40 ± 1.60	0.52 ± 0.05	0.34 ± 0.03	63.00 ± 3.00	0.53 ± 0.01
	T_1		0.16 ± 0.04	58.50 ± 0.60	0.50 ± 0.03	0.26 ± 0.02	57.33 ± 1.53	0.47 ± 0.02
	T_2		0.18 ± 0.01	54.40 ± 0.40	0.32 ± 0.02	0.15 ± 0.02	40.00 ± 2.00	0.30 ± 0.02
September	C	Carbaryl	0.23 ± 0.03	63.70 ± 1.00	0.54 ± 0.08	0.34 ± 0.02	61.00 ± 2.50	0.50 ± 0.02
	T_1		0.15 ± 0.02	50.90 ± 0.80	0.36 ± 0.05	0.18 ± 0.02	44.66 ± 2.08	0.44 ± 0.01
	T_2		0.09 ± 0.01	47.20 ± 0.20	0.28 ± 0.07	0.10 ± 0.01	28.33 ± 2.50	0.22 ± 0.01

C : Control
T_1 : Before application of the insecticide (one week)
T_2 : 48h after application of the insecticide.
[(a)]N.D. : Not detected

Table7. Inhibition of arginine deaminase in soil treated with pesticides for three successive years during 1995–1998.

Year	Sample from Area[1]	Insecticide Applied	Arginine Deaminase μg N/g Dry Soil[2]	Inhibition[3], %
1996*	C		0.36±0.00	
	T	Before application	0.30±0.00	17
	T_a	All insecticides	0.07±0.00	81
	T_b	All insecticides	0.09±0.01	75
	T_c	All insecticides	0.08±0.01	78
1997	C		0.62±0.07	
	T	Before application	0.50 0.04	19
	T_1	monocrotophos	0.19±0.03	70
	T_2	methomyl	0.12±0.01	80
	T_3	carbaryl	0.06±0.00	90
1998	C		0.59±0.02	
	T	Before application	0.52±0.02	12
	T_1	monocrotophos	0.17±0.01	71
	T_2	methomyl	0.10±0.01	83
	T_3	carbaryl	0.04±0.01	93

(1) Samples were taken 48h after the application of the insecticide.

(2) Readings were obtained from standard curve.

(3) Referred to control

* Assay done at the end of second year (1996) after application of all insecticide (monocrotophos. methomyl and carbaryl) , T_a-T_c were taken one week a part.
C = Control
T = One week before application of the insecticide.

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REFERENCES

[1] TU, C.M., MILES, J.R.W., Interactions between insecticides and soil microbes, Res. Review **64** (1976), 17–65.
[2] CHISHOLM, R.D., et al., DDT residues in soil, J. Econ. Entomol., **43** (1950) 941–942.
[3] ALEXANDER, M., Introduction to Soil Microbiology, Wiley, N.Y. (1961), 472.
[4] KALE, S.P., RAGHU, K., Relationship between microbial numbers and others microbial indices in soil, Bull. Environ. Contam. Toxicol, **43** (1989) 941–945.
[5] TU, C.M., Effect of some herbicides on activities of microorganisms and enzymes in soil, J. Environ. Sci. Health, **B27** (1992) 695–709.
[6] TU, C.M., Effect of fungicides captafol and chlorothalonil on microbial and enzymatic activities in mineral soil, J. Environ. Sci. Health, **B28** (1993) 67–80.
[7] ZELLES, L.,et al., Side effects of some pesticides on non-target soil microorganisms, J. Environ. Sci. Health, **B20** (1985) 457–488.
[8] WAKSMAN, S.A., A tentative outline of the plate method for determining the number of microorganisms in the soil, Soil Sci., **14** (1922) 27–28.
[9] MARTIN, J.P., Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi, Soil Sci., **69** (1950) 215–232.

[10] ANDERSON, J.P.E., "Soil Respiration" Methods of Soil Analysis Part 2. Chemical and Microbiological Properties, 2nd Ed. (PAGE, A.L., MILLER, R.H., KEENEY, D.R. eds) Soil Science Society of America Inc., U.S.A. (1982) 831.
[11] ANDERSON, J.P.E. AND DOMSCH, K.H. Soil Biol. Biochem. **10** (1978) 215–221.
[12] CASIDA JR., L.E.,et al., Soil dehydrogenase activity, Soil. Sci. **98** (1964) 731–736.
[13] ZELLES, L., et al., Comparison of methods to test chemicals for side effects on microorganisms, Ectox. Environ. Safety **12** (1986) 53–69.
[14] ALEF, K., KLEINER, D., Arginine ammonification, a simple method to estimate microbial activity potentials in soils, Soil Biol. Biochem., **18** (1986) 233–235.

Impact of repeated pesticide applications on the binding and release of methyl ^{14}C-monocrotophos and ring labelled ^{14}C-carbaryl to soil matrices under field conditions

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Abstract. The dissipation of (*O*-methyl-^{14}C) monocrotophos and U-ring labelled ^{14}C-carbaryl was monitored for over two years in the absence and presence of other insecticides using *in situ* soil columns. The dissipation of ^{14}C-monocrophos from soil treated with non-labelled methomyl and carbaryl showed a faster rate of downward movement than in control columns tagged with the labelled insecticide alone. The same trend was observed in experiments with ^{14}C-carbaryl that dissipated more readily in soil treated with non-labelled monocrotophos and methomyl. In the presence of other insecticides the percentage of bound residues was generally lower than in control experiments. The bound residues at the top of the column were released at a low rate under conditions prevailing in the field. The overall half lives for monocrotophos and carbaryl estimated from control experiment were approximately 20 and 24 weeks, respectively. The data indicate that repeated applications of pesticides might enhance the release of ^{14}C-bound residues.

1. Introduction

The soil burden resulting from repeated long term applications of pesticide chemicals necessary for protection of cotton plants is of special concern. Such treatments may suppress soil microflora and hence affect soil properties. Also pesticides may have binding potential to soil, the extent of which depends greatly on the nature of the chemical used. Data on the soil binding capacity of pesticides have been generated mainly from studies using a single application of a labelled pesticide to soil for certain periods. Useful data may be generated from investigations reflecting real situations where the soil constantly receives a multitude of pesticide chemicals. Such studies are best carried out by using field soil columns.

The present work was to study the persistence and leaching of methyl-^{14}C-monocrotophos and ring-labelled ^{14}C-carbaryl during two years exposure in *in-situ* soil columns in the absence and presence of another insecticide. This would reflect the impact of repeated pesticide applications on the binding and release of bound pesticide residues to soil matrices.

2. Materials and methods

2.1. Experimental setup

Four sets of hard PVC-cylinders (22 cylinders each 5 x 50 cm) containing clean soil were used in this study. The cylinders were driven into the soil leaving 5 cm protruding above the soil surface in order to prevent runoff. Soil columns received regular inputs of fertilizer and were kept moist and left in the open air under field conditions.

Two radiolabeled pesticides were used for this investigation, namely (methyl-^{14}C) monocrotophos and U-^{14}C-ring labelled carbaryl. The experiment began on March 1996 and lasted until July 1998. For each radio-chemical two sets of PVC-cylinders were used as follows:

(A) For labeled monocrotophos, one set received labeled monocrotophos only (set 1) and the other set received labeled monocrotophos followed by cold methomyl then carbaryl (set 2)

(B) For labelled carbaryl, one set received labeled carbaryl only (set 3) and the other received in succession cold monocrotophos, methomyl and labeled carbaryl (set 4).

Sets 1 and 3 act as controls for sets 2 and 4, respectively. The latter sets simulate actual situation of field applications. All concentrations, time and frequency of application (including the radiochemical) are similar to field conditions.

2.2. ^{14}C-Pesticide

The radiochemical was appropriately diluted with the cold compound. Each column received 10 mg of the diluted preparation/kg soil (185 kBq), dissolved in 100 µL of water or acetone and applied by means of a micropipette onto the soil surface.

2.3. Sampling and extraction

At various intervals (over 28 months) two cylinders of each set were removed, frozen and the soil columns carefully removed from the cylinders and divided into 10 cm zones. Soil of each zone was air dried and thoroughly mixed. Three 50 g samples from each column were extracted in a Soxhlet apparatus with methanol for 6h and the extractable residues determined in a LSC. Bound ^{14}C-residues were determined by combustion in a Harvey Biological Oxidizer (OX-600). The sampling dates for ^{14}C-monocrotophos are given in Tables 1 and 2 and those of ^{14}C-carbaryl in Tables 3 and 4. For each column, the overall residue load, and hence persistence, was calculated by summing increment loads of the column.

2.4. Analysis of extractable ^{14}C-residues

The nature of ^{14}C-residues in the methanol extracts was determined by thin chromatography (TLC) of samples taken 6 months following application of the radioactive insecticides (on 15/9/1996). The TLC analysis was conducted on pre-coated silica gel plates using the following systems for development:-

For monocrotophos:
System 1: methylene chloride:methanol (9:1)
System 2: ethylacetate:ethanol:acetic acid (9:9:2)

For carbaryl:
System 1: Petroleum-ether:acetone (4:1)
System 2: Petroleum-ether:ethyl acetone (4:1)

Authentic substances were run alongside as references.

3. Results and discussion

3.1. Dissipation of (methyl-^{14}C) Monocrotophos

The data given for the dissipation of ^{14}C-monocrotophos, over 28-months using *in-situ* soil columns are shown in Table 1. Two columns were analyzed at t_0 determine the actual applied dose that was considered to be 100%. Twenty four hours after application, most of radioactivity was concentrated in the upper 5-cm zone that contained about 82.5% of applied dose. Such application loss is probably due to volatilization. After 20 weeks, the amount of extractable radioactivity in the upper 10 cm zone decreased to 11.1% indicating a moderate rate of dissipation of monocrotophos from the surface soil layers under subtropical conditions. Some binding already occurred at 24h following application perhaps, indicating movement of the insecticide by diffusion into micropores. Bound residues gradually increased with time. The maximum binding was detected in the upper 10 cm zone 20 weeks following application of the insecticide (about 30% of applied dose). By that time, smaller amounts of extractable and bound ^{14}C-residues could be detected in the next 10 cm zone.

Table 1. Dissipation of ^{14}C-monocrotophos in absence of other insecticides in soil columns during 1996–1998 (control experiment)

Sampling Date	Column Zone (cm)	^{14}C-monocrotophos residues Extractable µg±S.D	%	Bound µg±S.D	%	Total µg±S.D	%	Recovery, %
1/3/96	0-5	9.7±0.0	97	3±0.0	3	10±0.0	100	100
2/3/96	0-5	7.8±0.04	78.0	0.45±0.02	4.5	8.25±0.20	82.5	82.5
15/4/96	0-5	2.67±0.10	26.7	1.33±0.01	133	4.00±0.40	40.0	75.5
	5-10	2.44±0.03	24.4	1.11 ±0.07	11.1	3.55 ±0.40	35.5	
15/7/96	0-5	0.67±0.03	6.7	2.00±0.17	20.0	2.67±0.04	26,7	48.9
	5-10	0.44±0.03	4.4	0.89±0.03	8.9	1.33±0.04	13.3	
	10-20	0.22±0.02	2.2	0.67±0.03	6.7	0.89±0.03	8.9	
15/9/96	0-10	0.90±0.04	9.0	1.80±0.03	18.0	2.70±0.04	27.0	42.0
	10-20	0.30±0.03	3.0	0.33±0.04	3.3	0.63±0.03	6.3	
	20-30	0.57±0.02	5.7	0.30±0.04	3.0	0.87±0.04	8.7	
15/11/96	0-10	0.30±0.03	3.0	1.68±0.04	16.8	1.98±0.10	19.8	38.0
	10-20	0.25±0.02	2.5	0.66 ±0.07	6.6	0.91±0.04	9.1	
	20-30	0.45±0.03	4.5	0.46±0.01	4.6	0.91±0.03	9.1	
1/3/97	0-10	0.18±0.02	1.80	1.62±0.04	16.2	1.80±0.08	18.00	31.4
	10-20	0.22±0.02	2.20	0.31±0.02	3.1	0.53±0.04	5.30	
	20-30	0.32±0.02	3.20	0.15±0.03	1.5	0.47±0.03	4.70	
	30-40	0.31±0.03	3.10	0.03±0.01	0.3	0.34±0.04	3.40	
15/7/97	0-10	0.04±0.00	0.42	0,23±0.01	2.28	0.27±0.01	2.70	11.7
	10-20	0.03±0.00	0.31	0.34±0.01	3.36	0.37 ±0.01	3.67	
	20-30	0.05±0.00	0.46	0.21±0.01	2.04	0.25±0.01	2.50	
	3 0-40	0.06±0.00	0.58	0.23±0.01	2.32	0.29±0.01	2.90	
15/3/98	0-10	0.04±0.00	0.4	0.20±0.02	2.0	0.24±0.02	2.4	8.2
	10-20	0.04±0.00	0.4	0.18±0.01	1.8	0.22±0.02	2.2	
	20-30	0.06±0.00	0.6	0.12±0.01	1.2	0.18±0.01	1.8	
	30-40	0.06±0.00	0.6	0.12±0.01	1.2	0.18±0.01	1.8	
15/7/98	0-10	0.04±0.00	0.4	0.18±0.02	1.8	0.22±0.02	2.2	7.3
	10-20	0.04±0.00	0.4	0.18±0.01	1.8	0.22±0.02	2.2	
	20-30	0.03±0.00	0.3	0.12±0.01	1.2	0.15±0.01	1.5	
	30-40	0.04±0.00	0.4	0.10±0.00	1.0	0.14±0.01	1.4	

Radioactivity applied per column = 166.5 kBq (10 mg insecticide/kg soil)
Results are mean of two columns

After one year, ^{14}C-residues were detected throughout the whole column. The highest binding was still associated with the upper 10 cm zone and amounted to about 16% of the applied dose. During the period from 15/7/96–1/3/97 (about 35 weeks), the ^{14}C-bound residues in the upper 10 cm-zone declined by 44%, indicating a gradual release under normal field conditions. After 500 days, the ^{14}C-bound residues represented the major portion of ^{14}C-activity in the column (85.5%). By that time around 90% of applied radioactivity had moved below the 50-cm leaching column. By two years, limited movement and much slower loss of ^{14}C-monocrotophos from soil columns were observed. Pesticide loss from soil is frequently quantified as a "half-life" a value which assumes it is a first order process. Often field dissipation of pesticides follows a two compartment pattern with an early rapid-loss phase followed by a later slow process. The data obtained seem to fit a biphasic pattern. The first phase is a rapid- early loss with volatalization as major process. However, leaching/penetration and adsorption/binding may also contribute to this phase. The second phase is a slow process associated with a long steady decline in concentration, probably associated with diffusion into less accessible

adsorption sites (Fig. 1). The estimated time required for loss of 50% (overall t_{50}) of radiocarbon from the whole column was approximately 20 weeks. Half-lives calculated for the initial rapid and the later slower phases were 19 and 26 weeks, respectively. The data indicate that bound residues, at the top of the column, are released at a slow rate under conditions prevailing in the field probably through soil microorganisms. Soil microorganisms are believed to play an important role in the release and further degradation of bound pesticide residues. ^{14}C-Bound DDT residues in soil were reported to be readily released also by microorganisms [3, 4].

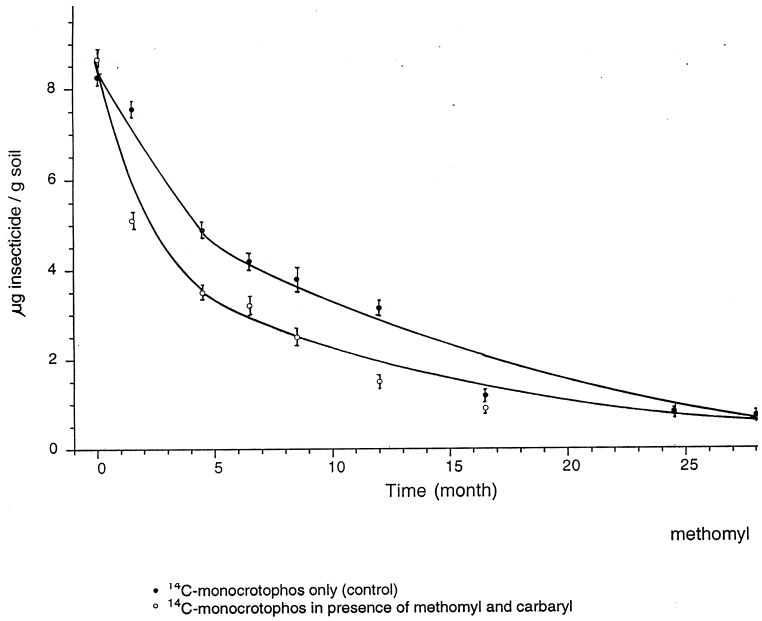

Fig. 1. Dissipation of ^{14}C-monocrotophos in absence and presence of methomyl and carbaryl in soil columns during 1996–1998.

It is worth mentioning that the rates have been calculated from radioactivity measurements which represent the net values of disappearance of the parent chemical and formation of degradation products.

The downward movement of ^{14}C-monocrotophos in columns subsequently treated with non-labelled methomyl and carbaryl is shown in Table 2. The data indicate a faster rate of downward movement of ^{14}C-monocrotophos compared to control columns tagged with the labeled insecticide alone (Table 1). Here again, the two-compartment model fits most data points fairly well and reinforces the concept of an early, rapid loss of radioactivity during the first 4-5 months. The time to 50% disappearance of ^{14}C was about 90 days for the first rapid phase and 128 days for the second slower phase. As in the case of control experiments, the maximum binding of ^{14}C-monocrotophos residues was associated with the upper 10-cm zone that constituted about 22% of applied dose 6 weeks following application. During the period between July 96 -Mar. 97, the overall ^{14}C-residues declined by about 60%. After one year, the ^{14}C-bound residues throughout the column contributed to 8.3% of the applied dose. This value didn't change to any appreciable extent after further 20 weeks. The data presented here provide an indication that repeated applications of pesticides might enhance the release of ^{14}C-bound pesticide residues. The released residues may undergo successive processes of binding and release in the lower layers of the column during their downward movement.

Table 2. Dissipation of ^{14}C-monocrotophos in presence of methomyl and carbaryl in soil columns during 1996–1998

Sampling Date	Column Zone (cm)	^{14}C-monocrotophos residues Extractable µg±S.D	%	Bound µg±S.D	%	Total µg±S.D	%	Recovery, %
1/3/96	0-5	9.5±0.0	95	0.5±0.0	3	10±0.0	100	100
2/3/96	0-5	8±0.50	80	0.66±0.04	6.6	8.66±0.26	86.6	86.6
15/4/96	0-5	1.33±0.02	13.3	1.11±0.09	11.1	2.44±0.05	24.4	51.1
	5-10	1.56±0.06	15.6	1.11 ±0.03	11.1	2.67±0.02	26.7	
15/7/96	0-5	0.22±0.02	2.2	1.33±0.03	13.3	1.55±0.03	15.5	35.5
	5-10	0.22±0.02	2.2	0.67±0.02	6.7	0.89±0,04	8.9	
	11-15	0.22 ±0.03	2.2	0.89±0.02	8.9	1.11±0.03	11.1	
15/9/96	0-10	0.30±0.01	3.0	1.65±0.07	16.5	1.95±0.07	19.5	32.0
	10-20	0.15±0.02	1.5	0.60±0.02	6.0	0.75±0.02	7.5	
	20-30	0.10±0.01	1.0	0.40±0.04	5.0	0.50 ±0.03	5.0	
15/11/96	0-10	0.20±0.01	2.0	1.00±0.08	10.0	1.20±0.04	12.0	25.0
	10-20	0.09±0.00	0.9	0.66±0.04	6.6	0.75±0.02	7.5	
	2 1-30	0.05±0.00	0.5	0.50±0.02	5.0	0.55±0.04	5.5	
1/3/97	0-10	0.10±0.04	1.0	0.31±0.03	3.1	0.41±0.04	4.1	15.0
	10-20	0.12±0.04	1.2	0.15±0.02	1.5	0.27±0.00	2.7	
	20-30	0.24±0.04	2.4	0.34±0.02	3.4	0.58±0.02	5.8	
	30-40	0.21±0.02	2.1	0.03±0.01	0.3	0.24±0.04	2.4	
15/7/97	0-10	0.10±0.01	1.04	0.30 ±0.01	2.96	0.31±0.03	3.1	9.1
	10-20	0.06±0.01	0.60	0.18±0.01	1.80	0.24±0.05	2.4	
	20-30	0.05±0.00	0.46	0.15±0.00	1.54	0.20±0.02	2.0	
	30-40	0.04±0.01	0.36	0.12±0.00	1.24	0.16±0.02	1.6	
15/3/98	0-10	0.06±0.00	0.6	0.16±0.01	1.6	0.22±0.02	2.2	7.9
	10-20	0.03 ±0.00	0.3	0.19±0.01	1.9	0.22 ±0.02	2.2	
	20-30	0.04±0.00	0.4	0.14±0.01	1.4	0.18±0.01	1.8	
	30-40	0.03 ±0.00	0.3	0.14±0.01	1.4	0.17±0.01	1.7	
15/7/98	0-10	0.06 ±0.00	0.6	0.15±0.01	1.5	0.21 ±0.02	2.1	6.9
	10-20	0.03±0.00	0.3	0.14±0.01	1.4	0.17±0.01	1.7	
	20-30	0.03±0.00	0.3	0.12±0.01	1.2	0.15±0.01	1.5	
	30-40	0.03±0.00	0.3	0.13±0.01	1.3	0.16±0.01	1.6	

Radioactivity applied per column = 166.5 kBq (10 mg insecticide/kg soil)

Monocrotophos + methomyl and carbaryl were added in succession, during 1996-1998

Samples taken two days after application of the insecticide.

The extractable residues of the whole column treated with ^{14}C-monocrotophos 6 months following application of the labeled insecticide constituted about 42% of the total ^{14}C-residues. Qualitative TLC analysis showed the presence of minute amounts of the parent compound in addition to desmethyl monocrotophos, to a lesser extent *N*-hydroxymethyl monocrotophos and two unknowns.

2.Dissipation of U ring-^{14}C labelled carbaryl

Table 3 summarizes the results of ^{14}C-carbaryl dissipation in a common Egyptian soil using *in-situ* soil columns. The downward movement of carbaryl is rather slow compared to monocrotophos. After 6 weeks, about 90% of the applied radioactivity could be recovered from the column. By that time over 50% of the applied dose was soil bound indicating a relatively high binding tendency of carbaryl. As in the case of ^{14}C-monocrotophos binding was concentrated in the upper 10 cm-zone of the column. Gradual release, followed by rebinding probably occurs with time. The percentage of bound residues increased with time and represented over 63% of total ^{14}C-residues after one year. By 13 months about

84% of the applied radioactivity moved below the 50-cm soil column. After that time no appreciable ^{14}C-activity leached from columns and the radioactivity on the column did not change much with time.

Soil columns, previously, treated with non-labeled monocrotophos and methomyl (Table 4) showed, in general, a lower percentage of binding than ^{14}C-monocrotophos but a faster rate of downward movement than in control columns. Nevertheless, the columns yielded a high rate of recovery comparable to that obtained from control columns (Table 3). After 6 weeks from application of labeled carbaryl, the ^{14}C-bound residues amounted to 30% of applied dose as compared with 51% in control columns. The overall t_{50} estimated for 50% dissipation of ^{14}C-carbaryl in the control experiment was approximately 5.5 months. In the presence of monocrotophos and methomyl, the time to 50% disappearance was about 4 months (Fig. 2). The dissipation of carbaryl was generally slower than monocrotophos. This may be associated with the remarkable soil binding capacity of carbaryl. The extractable residues of the whole column treated with ^{14}C-carboryl 6 months following application of the labeled compound amounted to 47% of total ^{14}C-residues. The TLC analysis of these residues proved to contain traces of carbaryl and α-naphthol together with 1,4 and 1,6-dihydroxynaphthalene and at least two other unknowns.

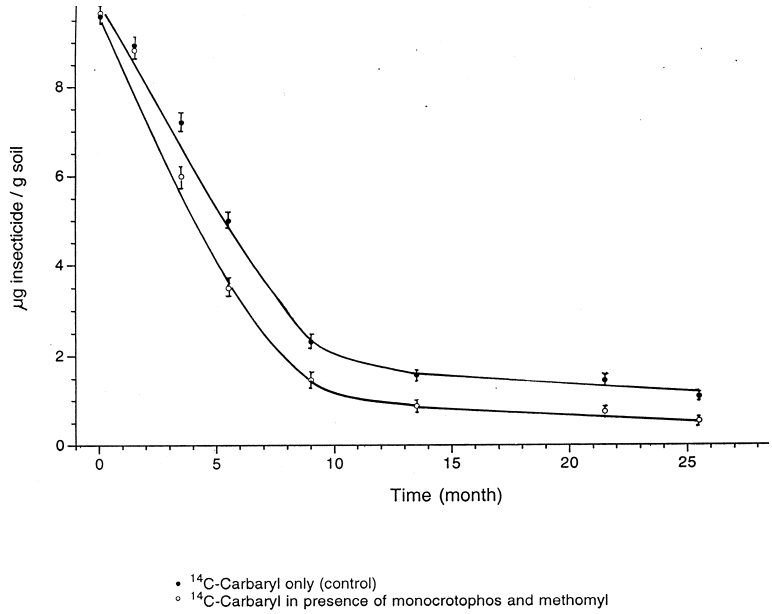

Fig. 2. Dissipation of ^{14}C-carbaryl in absence and presence of monocrotophos and methomyl in soil columns during 1996–1998.

Table 3. Dissipation of ^{14}C-carbaryl in absence of other insecticides in soil columns during 1996–1998 (control experiment)

Sampling Date	Column Zone (cm)	^{14}C-carbaryl residues Extractable µg±S.D	%	Bound µg±S.D	%	Total µg±S.D	%	Recovery %
1/3/96				NO TREATMENT				
1/4/96								
1/6/96	0-5	8.9±0.0	89	1.1±0.0	11	100	100	100
2/6/96	0-5	4.00±0.40	40.0	2.00±0.10	20.0	6.00±0.20	60.0	96.0
	5-10	2.40±0.03	24.0	1.20±0.07	12.0	3.60±0.06	36.0	
15/7/96	0-5	2.77±0.04	27.7	2.98±0.17	29.8	5.75±0.03	57.5	89.4
	5-10	0.21±0.02	2.1	1.70±0.08	17.0	1.91±0.06	19.1	
	10-15	0.85±0.04	8.5	0.43±0.03	4.3	1.28±0.04	12.8	
15/9/96	0-10	1.80±0.06	18.0	1.95±0.08	19.5	3.75±0.06	37.5	72.0
	10-20	1.20±0.04	12.0	1.35±0.04	13.5	2.55±0.05	25.5	
	20-30	0.40±0.04	4.0	0.50±0.03	5.0	0.90±0.02	9.0	
15/11/96	0-10	0.80±0.03	8.0	1.30±0.05	13.0	2.10±0.04	21.0	50.0
	10-20	0.75±0.02	7.5	1.35±0.04	13.5	2.10±0.04	21.0	
	20-30	0.35±0.03	3.5	0.45±0.04	4.5	0.80±0.02	8.0	
1/3/97	0-10	0.46±0.01	4.6	0.64±0.03	6.4	1.10±0.02	11.0	23.2
	10-20	0.33±0.04	3.3	0.47±0.04	4.7	0.80±0.05	8.0	
	20-30	0.07±0.02	0.7	0.17±0.03	1.7	0.24±0.05	2.4	
	30-40	0.04±0.01	0.4	0.14±0.02	1.4	0.18±0.02	1.8	
15/7/97	0-10	0.32±0.00	3.2	0.41±0.01	4.1	0.73±0.04	7.3	15.8
	10-20	0.21 ±0.00	2.1	0.30±0.00	3.0	0.51±0.04	5.1	
	20-30	0.04±0.00	0.4	0.14±0.00	1.4	0.18±0.02	1.8	
	30-40	0.02±0.00	0.2	0.14±0.00	1.4	0.16±0.03	1.6	
15/3/98	0-10	0.20±0.00	2.0	0.45±0.02	43	0.65±0.04	6.5	14.5
	10-20	0.14±0.00	1.4	0.36±0.01	3.6	0.50±0.04	5.0	
	20-30	0.08±0.00	0.8	0.12±0.00	1.2	0.20±0.03	2.0	
	30-40	0.02±0.00	0.2	0.08±0.00	0.8	0.10±0.00	1.0	
15/7/98	0-10	0.12±0.00	1.2	0.38±0.01	3.8	0.50±0.04	5.0	10.8
	10-20	0.10±0.00	1.0	0.30±0.01	3.0	0.40±0.03	4.0	
	20-30	0.02±0.00	0.2	0.08±0.00	0.8	0.10±0.00	1.0	
	30-40	0.02±0.00	0.2	0.06±0.00	0.6	0.08±0.00	0.8	

Radioactivity applied per column = 166.5 kBq (10 mg insecticide/kg soil). Results are mean of two columns

Table 4. Dissipation of ^{14}C-carbaryl in presence of monocrotophos and methomyl in soil columns during 1996–1998

Sampling Date	Column Zone (cm)	^{14}C-carbaryl residues Extractable µg±S.D	%	Bound µg±S.D	%	Total µg±S.D	%	Recovery, %
1/3/96				Cold monocrotophos				
15/4/96				Cold methomyl				
1/6/96	0-5	9.20±0.0	92	0.80±0.0	8	10.0±0.0	100	100
2/6/96	0-5	4.17±0.06	41.7	1.67±0.02	16.7	5.84±0.02	58.4	96.7
	5-10	3.33±0.05	33.3	0.5±0.04	5	3.83±0.02	38.3	
15/7/96	0-10	3.33±0.00	33.3	1.67±0.03	16.7	5.0±0.35	50	88.3
	10-20	1.67±0.02	16.7	0.83 ±0.02	8.3	2.50±0.20	25	
	20-30	0.83±0.03	8.3	0.5±0.02	5	1.33±0.02	13.3	
15/9/96	0-10	1.50±0.04	15.0	1.70±0.05	17.0	3.20±0.10	32.0	60.0
	10-20	1.10±0.09	11.0	1.25±0.04	12.5	2.35±0.10	23.5	
	20-30	0.20±0.03	2.0	0.25±0.01	2.5	0.45±0.02	4.5	
15/11/96	0-10	0.80±0.02	8.0	1.10±0.09	11.0	1.90±0.05	19.0	35.0
	10-20	0.40±0.05	4.0	0.55±0.04	5.5	0.95±0.04	9.5	
	20-30	0.30±0.05	3.0	0.35±0.03	3.5	0.65±0.02	6.5	
1/3/97	0-10	0.35±0.04	3.5	0.32±0.03	3.2	0.67±0.03	6.70	14.7
	10-20	0.23±0.04	2.3	0.23±0.02	2.3	0.46±0.04	4.60	
	20-30	0.06±0.01	0.6	0.13±0.02	1.3	0.19±0.01	1.90	
	30-40	0.03±0.00	0.3	0.12±0.03	1.2	0.15±0.01	1.50	
15/7/97	0-10	0.22±0.00	2.2	0.20±0.04	2.0	0.42±0.04	4.20	8.8
	10-20	0.11±0.00	1.1	0.09±0.00	0.9	0.20±0.01	2.00	
	20-30	0.04±0.00	0.4	0.10±0.00	1.2	0.16±0.01	1.60	
	30-40	0.01±0.00	0.1	0.09±0.00	0.9	0.10±0.00	1.00	
15/3/98	0-10	0.07±0.00	0.70	0.28±0.02	2.8	0.35±0.02	3.5	7.5
	10-20	0.05±0.00	0.50	0.11 ±0.00	1.1	0.16±0.01	1.6	
	20-30	0.04±0.00	0.40	0.12±0.00	1.2	0.16±0.01	1.6	
	30-40	0.02±0.00	0.20	0.06±0.00	0.6	0.08±0.01	0.8	
15/7/98	0-10-	0.08±0.00	0.80	0.17±0.01	1.7	0.25±0.02	2.5	5.4
	10-20	0.04±0.00	0.40	0.11 ±0.00	1.1	0.15±0.01	1.5	
	20-30	0.02±0.00	0.20	0.07±0.00	0.7	0.09±0.00	0.9	
	30-40	0.01±0.00	0.10	0.04±0.00	0.4	0.05±0.00	0.5	

Radioactivity applied per column =166.5 kBq (10 mg/insecticide/kg soil)
Samples taken two days after application of the insecticide.
Monocrotophos + methomyl + carbaryl were added in succession respectively during 1996–1998

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REFERENCES

[1] HELLING, C.S., et al., DDT Dissipation in Hawaiian *in-situ* soil columns, J. Environ. Sci. Health **B29** (1994) 103-117.

[2] KHAN, S.U., IVARSON, K.C., Microbiological release of unextracted (bound) residues from an organic soil treated with prometryn, J. Agric. Chem. **29** (1981) 1901-1903.

[3] HUSSAIN, A., et al., Studies on dissipation and degradation of ^{14}C-DDT and ^{14}C-DDE in Pakistani soils under field conditions, J. Environ. Sci. Health **B29** (1994) 1-15.

[4] ZAYED, S.M.A.D.,et al., Chemical and biological release of ^{14}C residues from soil treated with ^{14}C-p,p'-DDT, J. Environ. Sci. Health **B29** (1994) 169-175.

Mineralization of ^{14}C-labelled aromatic pesticide molecules in Egyptian soils under aerobic and anaerobic conditions

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Abstract. The mineralization of 2,4-D, carbofuran and pirimiphos-methyl in Egyptian soils was studied over a period of 90 days. Laboratory studies under aerobic and anaerobic conditions were conducted using ^{14}C-ring labelled pesticides. Under anaerobic conditions 10-14% of applied ring labelled 2,4-D mineralized during 90 days with no significant variations due to soil type. Under aerobic conditions, 2,4-D mineralized more readily in clay soil to reach 29-34% of applied dose within 90 days. In clay loam soil, ^{14}C-carbofuran and ^{14}C-pirimiphos-methyl mineralized at a rather slow rate to reach 12-14% and 12-13% of applied dose in 90 days, respectively under aerobic conditions. Generally, soils repeatedly treated with pesticides gave a slightly lower percentage of mineralization than control soils. In all studies, the soil extractable pesticide residues decreased with time and the bound residues gradually increased. The highest binding affinity of about 26-29% was observed with 2,4-D in clay soil under aerobic conditions in 90 days. Carbofuran, and pirimiphos-methyl, on the other hand, had lower binding capacity that did not exceed 16% of applied radioactivity.

1. Introduction

The activities of soil microflora are manifold and essential for soil fertility and the functioning of the ecosystem. Soil bioactivity covers several parameters related to aerobic and/or anaerobic activity. Mineralization represents a main route by which pesticides dissipate from soil. Through mineralization, pesticides are completely degraded by microorganisms into carbon dioxide that dissipates readily into the environment [1].

Carbofuran, 2,4-D, and pirimiphos-methyl are important pesticides that belong to different chemical groups. The carbamate carbofuran is a broad spectrum long residual insecticide and nematocide [2], known for its effectiveness against several insect pests through contact, stomach and systemic action [3]. The organochlorine pesticide 2,4-D is a well known herbicide and a plant growth regulator. The organophosphate pirimiphos-methyl is a broad spectrum, quick acting insecticide/acaricide which exerts its activity through contact action and inhalation [4].

Several studies of mineralization and degradation of pesticides in soil have been carried out during the last two decades [5–8] but most of the reports available so far originate from temperate regions of the world. Only little information is available on the mineralization of pesticides with aromatic rings in tropical and subtropical soils.

The present work aimed at studying the rate of mineralization of the above mentioned pesticides in Egyptian soil under aerobic and anaerobic conditions. For these investigations, radio-labelled laboratory soil dissipation studies were conducted, using pesticides labelled in the aromatic nucleus.

2. Materials and methods

2.1. Pesticides used

Three ^{14}C labeled pesticides were used namely:-
Ring labeled 2,4-D (2,4-dichlorophenoxyacetic acid), specific activity 2.14 MBq/mg (473.6 MBq/m mole), provided by IAEA;
Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-*N*-methyl carbamate), labelled at the 3-carbon atom of the furan ring; specific actitivity 3.11 MBq/mg (687.46 MBq/m mole), radiometric purity of

* National Research Centre, Dokki, Cairo.

88%, (purified from traces of carbofuran phenol by TLC on silica gel plates using benzene/ethyl-acetate for development), purchased from the Institute of Isotopes, Budapest, Hungary; Pirimiphos-methyl (*0,0*-dimethyl-*0*-(2-diethylamino-6-methyl-pyrimidin-4-yl)-phosphorothioate) labelled at position 2 of the pyrimidine ring, specific activity 1.98 MBq/mg (605.3 MBq/m mole), radiometric purity (by TLC) over 97%, purchased from the Institute of Isotopes, Budapest, Hungary.

The labelled pesticides were diluted with pure non-labelled chemicals to give radioactive preparations of specific activity 37 kBq/10 mg (1 μCi) of the insecticide.

2.2. Soils

Two local soils were used, a clay (soil A) and clay loam (soil B). Soils repeatedly treated with pesticides were used alongside non-treated control soils of the same type. The soil characteristics are:

Soil A (Clay soil): clay 67.0%; silt 27.3%; sand 5.7%; organic matter 1.65%; pH8.1.
Soil B (Clay loam soil): clay 43.2%; silt 23.5*;* sand 32.2%; organic matter 1.1%; pH7.7.

2.3. Laboratory mineralization experiments

The soil capacity to mineralize aromatic rings of the tested pesticides was studied under aerobic and anaerobic conditions. The mineralization of ring labelled 2,4-D was investigated in both soils and that of ring labelled pirimiphos-methyl and carbofuran was studied in the clay loam soil only.

2.3.1. Mineralization under Anaerobic Conditions

One hundred g of soil were moistened with water to about 75% of field water holding capacity and transferred to standard 250 mL biometer flasks. The side arm of the flask contained 10 mL of 1M KOH to trap evolved $^{14}CO_2$. Soil was spiked with 10 mg of labelled insecticide/kg soil. Flasks were incubated at about 25°C in the darkness for twelve weeks. At certain time intervals a sample of the alkaline solution was determined for its radioactivity and the soil in the biometer flask was analyzed for extractable and bound residues. For soil analysis duplicates were used and data were expressed as percentage of applied radioactivity.

2.3.2. Mineralization under Aerobic Conditions

Soils were incubated for 90 days, in a closed discontinuously aerated laboratory system described previously [9].

Flasks in triplicates for each soil type (with about 55% water holding capacity) were treated with the radio-labelled insecticide at a concentration of 10 mg/kg soil for carbofuran and pirimiphos-methyl and 5 mg/kg in the case of 2,4-D. Flasks were connected to a trapping line to collected $^{14}CO_2$ in 2 tubes containing 1M KOH solution, and kept at 25°C. The system was flushed with air once a day for few minutes by a pump to help trapping of the liberated $^{14}CO_2$. The liberated $^{14}CO_2$ was monitored at different time intervals over a period of 3 months. The extent of mineralization was estimated from the radioactivity of liberated $^{14}CO_2$, expressed as percentage of applied radioactivity.

2.4. Soil analysis

Soils were analyzed at specific time intervals for ^{14}C extractable in methanol and ^{14}C-bound residues. Extraction was performed with methanol water (95:5) in a Soxhlet apparatus for 4-6 hours.

Radioactivity in solutions was determined by direct counting in a Packard liquid scintillation spectrometer using a dioxan-based scintillation cocktail.

Soil bound residues were determined by combustion of soil samples in a Harvey-Biological Oxidizer (OX-600) followed by counting in the LSC.

3. Results and discussion

The biodegradation of pesticides in soil is a well documented microbial phenomenon. Microorganisms play an important role in determining the fate of organic compounds in the environment. The degradative activity of soil microorganisms includes mineralization of plant, animal, microbial and organic synthetic compounds, where the formation of carbon dioxide represents the last step of carbon mineralization.

In the present investigations, monitoring of liberated $^{14}CO_2$ from the degradation of ring labelled 2,4-D, carbofuran and pirimiphos methyl in Egyptian soils showed that considerable amounts of these pesticides are mineralized during three months. Table 1 illustrates the cumulative evolution of $^{14}CO_2$ from ring labelled 2,4-D under aerobic and anaerobic conditions in clay and clay loam Egyptian soils, respectively. The two soils did not show significant variations regarding their capacity to mineralize 2,4-D under anaerobic conditions. The percentage of mineralization showed a slight but consistent increase with time and by the end of the experiment (90 days) reached 10 and 14% of applied radioactivity in clay loam and clay soil, respectively. In general, soils previously repeatedly treated with pesticides gave a somewhat lower percentage of mineralization than soils without previous pesticide history (control soils). This correlates well with the observation that soil microbial bioactivity recovers almost completely several weeks following pesticide application [10].

Under aerobic conditions 2,4-D mineralized.in clay soil more readily than under anaerobic conditions. The percentage of mineralization reached 34% and 29% within 90 days in control and treated soils, respectively (Table 1).

Previous work on the aerobic degradation of ^{14}C-ring labelled 2,4-D in soils with no previous herbicide history showed that, over a period of 24 days, 25-31% of the applied ^{14}C was released as carbon dioxide. In soils previously treated with 2,4-D, however, the rate of mineralization was faster with approximately 50% of the applied ^{14}C-being released as $^{14}CO_2$ [11]

Under anaerobic conditions, the amount of extractable residues decreased with time, whereas the percentage of soil bound residues gradually increased to reach 30-35% of the applied dose after 90 days. The binding capacity of 2,4-D residues was slightly lower under aerobic conditions.

In almost all laboratory experiments, a good balance sheet was obtained, and the percentage recovery was generally between 91-100%. This suggests that volatilization probably does not represent a significant percentage in the dissipation of 2,4-D from soil under the tested conditions.

These results show that Egyptian soils, especially the clay soil, have a remarkably high efficiency for the mineralization of 2,4-D under aerobic conditions, as compared with other organochlorine pesticides such as DDT and hexachlorocyclohexane. Both ^{14}C-DDT and ^{14}C-DDE were reported to be mineralized in Egyptian soils at a low rate that didn't exceed 3% of applied dose within 90 days under aerobic conditions [12]. A low percentage of DDT mineralization that amounted to 2.6% in 3 months was also observed in tropical Pakistani soil [13].

The non-aromatic insecticide HCH shows a special mineralization pattern in soil. Lindane, the γ-isomer of hexachlorocyclohexane, as well as the α, β and δ isomers, has been known to undergo fairly rapid degradation in anaerobic ecosystems [14, 15].

Under aerobic conditions about 10–12% of the ^{14}C-carbon in α and γ-HCH was reported to be mineralized during 48h after inoculation with a soil bacterium *Pseudomonas* spp. In this case, lindane is utilized as a source of carbon for proliferation by aerobic γ-HCH degrading bacterium [16, 17].

Table 1. Fate of ^{14}C-2,4-D in 2 soils under laboratory conditions

Sampling Time (days)	Soil	Anaerobic Conditions ^{14}C-Residues, % Applied Dose Extractable	Bound	$^{14}CO_2$	Total ^{14}C Recovered	Aerobic Conditions ^{14}C-Residues, % Applied Dose Extractable	Bound	$^{14}CO_2$	Total ^{14}C Recovered
					Clay soil				
1	C	88	8	4	100	80	15	5	100
	T	90	7	3	100	88	11	4	103
15	C	76	9	6	91	68	21	7	96
	T	80	8	4	92	76	19	6	101
30	C	67	15	9	91	57	23	18	98
	T	70	14	8	92	65	20	16	101
45	C	64	19	10	93	45	27	25	97
	T	67	17	9	93	50	24	22	96
60	C	60	25	12	97	40	29	30	99
	T	65	22	11	98	47	25	25	97
90	C	52	35	14	101	37	29	34	100
	T	56	31	12	99	44	26	29	99
					Clay Loam Soil				
1	C	89	7	3	99	92	6	1	99
	1	89	7	3	99	95	5	1	101
15	C	84	8	5	97	84	10	3	97
	1	86	7	4	97	88	9	3	100
30	C	72	12	7	91	72	14	9	95
	T	74	11	6	91	82	10	6	98
45	C	70	16	9	95	55	19	14	88
	T	72	18	8	98	76	14	10	100
60	C	64	21	10	95	49	24	18	91
	T	68	20	9	97	61	21	16	98
90	C	54	30	11	95	45	25	22	92
	T	56	29	10	95	57	23	19	99

The ready aerobic mineralization of 2,4-D in clay soil is analogous with the behaviour of the ^{14}C-U ring labelled phenyl urea herbicide isoproturon in soil. The latter was reported to be readily mineralized under aerobic conditions, where, according to soil type, 14-23% of initial activity was liberated as $^{14}CO_2$ over 67 days. The authors reported that the mineralization rate was generally significantly correlated with soil biomass [9].

Table 2. Fate of ^{14}C-carbofuran in clay loam soil under laboratory conditions

Sampling Time (days)	Soil	Anaerobic Conditions ^{14}C-Residues, % Applied Dose Extractable	Bound	$^{14}CO_2$	Total ^{14}C Recovered	Aerobic Conditions ^{14}C-Residues, % Applied Dose Extractable	Bound	$^{14}CO_2$	Total ^{14}C-Recovered
1	C	90.0	2.0	0.9	92.9	90.0	2.3	1.1	93.4
	T	89.0	1.3	0.7	91.0	88.3	2.0	1.0	91.3
30	C	76.0	3.0	6.9	85.9	84.0	4.5	5.4	93.9
	1	73.5	2.0	6.0	81.5	76.6	3.8	4,8	85.2
45	C	68.9	3.5	7.3	79.7	77.6	5.5	8.3	91.4
	T	66.0	2.9	6.9	75.8	74.0	4.8	7.4	86.2
60	C	66.0	4.1	8.9	79.0	69.8	12.6	12.0	94.4
	T	65.5	3.7	7.4	76.6	63.0	10.0	10.9	83.9
90	C	59.6	5.5	8.9	74.0	63.0	16.0	13.9	92.9
	T	58.0	5.3	7.5	70.8	59.0	13.0	12.0	84.0

Table 3. Fate of ^{14}C-pirimiphos-methyl in clay loam soil under laboratory conditions

Sampling Time (days)	Soil	Anaerobic Conditions ^{14}C-Residues, % Applied Dose				Aerobic Conditions ^{14}C-Residues, % Applied Dose			
		Extractable	Bound	$^{14}CO_2$	Total ^{14}C Recovered	Extractable	Bound	$^{14}CO_2$	Total ^{14}C-Recovered
1	C	89	2.6	1.3	92.9	88.5	3.6	1.4	93.5
	T	88	2.4	1.3	91.7	83.8	2.8	1.3	87.9
30	C	77	8.1	9.4	94.5	76.5	7.2	9.7	93.4
	T	76	7.0	8.5	91.5	74.4	6.0	8.8	89.2
45	C	70.8	11.3	9.9	92.0	73.6	9~6	10.1	93.3
	T	70.2	11.0	9.0	90.2	71.6	8.1	9.3	89.0
60	C	64.5	13.8	10.0	88.3	70.8	10.9	12.2	93.9
	T	63.3	12.6	9.3	85.2	67.8	9.7	10.7	88.2
90	C	62.0	13.7	10.2	85.9	65.1	12.4	13.3	90.8
	T	61.5	13.0	9.2	83.7	59.2	11.0	12.0	82.2

The fate of ^{14}C-ring labelled carbofuran and pirimiphos methyl in clay loam soil is represented in Tables 2 and 3, respectively. The data indicate that during 90 days, no obvious differences could be observed between the mineralization of the two chemicals. In the case of carbofuran, the percentage of mineralization was 12-14% and 8-10% of the applied dose under aerobic and anaerobic conditions, respectively. As is the case in mineralization of 2,4-D, the untreated soil showed a slightly higher capacity of mineralization (around 2%) than treated soils (Table 2). The amount of pirimiphos-methyl mineralized during 90 days was 12-13% and 9-10% of the applied radioactivity under aerobic and anaerobic conditions, respectively.

In the case of carbofuran and pirimiphos-methyl, the amount of soil extractable residues was found to decrease with time (Tables 2, 3). Carbofuran, under anaerobic conditions showed a low potential for soil binding that amounted to 5.5% of applied dose during 90 days. Under aerobic conditions, however, a higher percentage of binding (13-16%) was observed during the period of the experiment (Table 2). The calculated total recovery from carbofuran mineralization varied between 74-94% of applied ^{14}C. The percentage of soil bound pirimiphos-methyl residues, however, ranged between 11-14% under aerobic and anaerobic conditions (Table 3). The recovery was 83-94%.

The low recovery observed suggests there could have been considerable dissipation of carbofuran and pirimiphos methyl residues through volatilization.

It is noteworthy, that enhanced degradation of carbofuran is a frequently documented problem in soil repeatedly exposed to the insecticide which has been linked to increased ability of soil microorganisms to utilize carbofuran as carbon or energy source. From such soils carbofuran-degrading *Pseudomonas* and *Flavobacterium* spp. have been isolated [18].

The results obtained in the present investigations suggest that mineralization can be used - together with other parameters - in a battery of tests for the evaluation of soil bioactivity and hence soil fertility.

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REFERENCES

[1] SCHINNER, F., et al., (eds). Methods in Soil Biology, Springer Verlag, Berlin-Heidelberg-New York (1955).
[2] FEST, C., WEGLER R. (eds), Chemie der Pflanzenschutz und Schadlingsbeckampfungsmittel, Vol 6, Springer Verlag, Heidelberg (1981), 336.
[3] VETTORAZZI, G. (ed), International Regulatory Aspects for Pesticide Chemicals, Vol. I, Toxicity Profiles, CRC-Press, Florida (1979), 19.
[4] KUHR, R.J., DOROUGH, H.W. (eds), Carbamate Insecticides, Chemistry, Biochemistry and Toxicology, CRC-Press, Cleveland, Ohio (1982).
[5] PARKER, L.W., DOXTADER, K.G., Kinetics of microbial decomposition of 2,4-D in soil, J. Environ. Qual. **11** 4 (1982) 679–684.
[6] ROBERTSON, B.K., ALEXANDER M. Growth linked and cometabolicbiodegradation, Pestic. Sci. **41** (1994) 311–318.
[7] AIGLABIE, J., LLOYD-JONES, G. A review of bacterial degradation of pesticides, Austr. J. Soil Res. **33** (1995) 925–942.
[8] SCOW, R.S.et al. Kinetics of mineralization of organic compounds at low concentration in soil, Applied and Environ. Microbiol. **51** (1986) 1028–1035.
[9] LEHR, S.et al. Mineralization of free and cell wall bound isoproturon in soils in relation to soil microbial parameters, Soil Biol. Biochem. **28** (1996) 1–8.
[10] TU C.M., Effect of five insecticides on microbial and enzymatic activities in sandy soil, J. Environ. Sci. Health **B30** (1995) 289–306.
[11] SMITH, A.E., AUBIN, A.J. Metabolites of [^{14}C]-2,4-dichlorophenoxyacetic acid in Saskatchewan soils. J. Agric. Food Chem. **39** (1991) 2019–2021.
[12] ZAYED, S.M.A.D., et al. Degradation and fate of ^{14}C-DDT and ^{14}C-DDE in Egyptian soil, J. Environ. Sci. Health. **B29** (1994) 47–56.
[13] HUSSAIN, A., et al. Studies of the effects of temperatures and solar radiation on volatilization, mineralization and binding of ^{14}C-DDT in soil under laboratory conditions, J. Environ. Sci. Health, **B29** (1994) 141–151.
[14] SETHUNATHAN, N., et al. Microbiology of rice soils, CRC. Critical Rev. Microbiology, **10** (1983) 125–172.
[15] STRAUBE, G., Microbial transformation of hexachlorocyclohexane, Zentralbl. Mikrobiol., **146** (1991) 327–338.
[16] SAHU, S.K., et al. Degradation of α-, and γ-isomers of hexachlorocyclohexane by rhizosphere soil suspension from sugarcane, Proc. Indian Acad. Sci. Plant Sci. **100** (1990) 165–172.
[17] SAHU, S.K., et el., Mineralization of α, β and γ isomers of hexachlorocyclohexane by a soil bacterium under aerobic conditions, J. Agric. Food Chem. **43** (1995) 833–837.
[18] EDWARDS, D.E., et al., Characterization and growth response of bacteria in soil following application of carbofuran, J. Environ. Sci. Health **B27** (1992) 139–154.

Long term fate and effects of the herbicide bromoxynil in soil cropped with maize

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Abstract. Bromoxynil (3,5-dibromo-4-hydroxy-benzonitrile; BO)**,** mainly used as the octanoate ester (BOO), is among the most widespread herbicides applied to maize in Germany and other countries. Effects on the microflora of soil were assessed by the determination of microbial biomass and bioactivity using microcalorimetry, of enzymatic activity using dehydrogenase activity, of potential nitrification and of phospholipid fatty acid pattern. In laboratory experiments for 3 weeks, significant effects on microbial biomass and bioactivity, dehydrogenase activity and nitrification in general were obvious only for 100-to 1000-fold of the concentration resulting from normal agricultural application rate. Differences in the composition pattern of phospholipid fatty acids were obvious as trends already at BOO-concentrations corresponding to the normal application rate and were highly significant at 10-fold application rate level. After application of BOO formulation to outdoor lysimeters at normal agricultural application rates, no significant differences in dehydrogenase activity and nitrification between treated soils and controls could be detected; however, both treated soils and controls exhibited seasonal variations between the different sampling dates. After application of ^{14}C-BOO formulation to outdoor lysimeters, the uptake of ^{14}C in maize plants was negligible (<0.08 µg equivalent to BOO per g soil dry mass); ^{14}C in leachate amounted to about 0.12 µg/L and was due only to highly polar, water-soluble products not identified thus far. Laboratory degradation experiments with ^{14}C-BO and ^{14}C-BOO in soil demonstrated mineralization to $^{14}CO_2$, transformation to the corresponding benzoate, and the formation of soil-bound residues.

1. Introduction

Bromoxynil (3,5-dibromo-4-hydroxy-benzonitrile; BO) is a herbicide used in maize and other crops, preferably as the octanoate ester (BOO; Fig. 1).

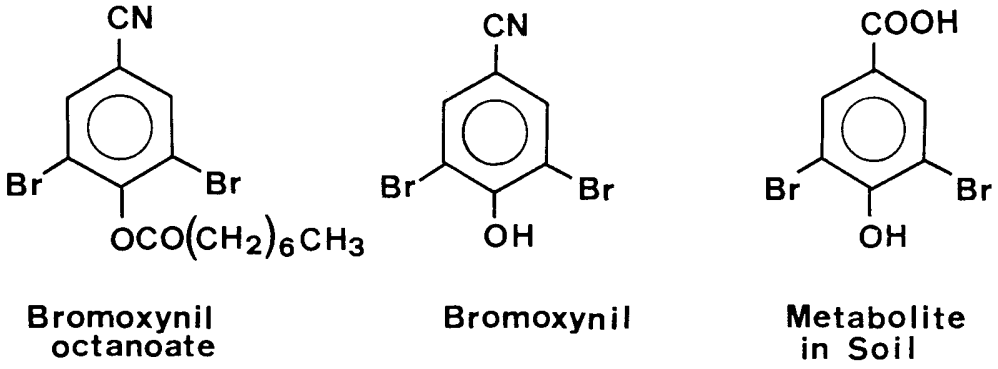

Fig 1. Formulae of bromoxynil, its octanoate ester and a metabolite in soil.

Although, according to literature data, the effects of this herbicide on soil microorganisms are small [1,2], in this paper studies are reported on effects, including more selective methods, higher concentrations, and long term exposure times under outdoor conditions. The uptake of ^{14}C from ^{14}C-labeled compound by plants and leaching of ^{14}C in lysimeters, as well as mineralization, transformation and formation of soil-bound residues are also studied.

2. Materials and methods

2.1 Soil

The soil used was a silty soil (colluvium) from an agricultural field. It was sampled from various depths, as shown in Table 1. For the laboratory experiments, soil samples down to a depth of 10 cm were used; for outdoor experiments, the horizons taken from different depths were filled in the lysimeters according to their natural sequence. The physico-chemical properties of the layers from various depths are included in Table 1. Whereas the particle size distribution as well as the pH-value did not greatly differ between the various soil depths, total organic matter and total N decreased with increasing depth.

2.2. Pesticide

For laboratory experiments determining biological effects and for two lysimeters, the commercial product "Buctril" containing the active ingredient bromoxynil octanoate (BOO) was used. Laboratory experiments for mineralization, transformation and the formation of soil-bound residues were performed with ^{14}C-ring-labeled BO and ^{14}C-ring-labeled BOO.

^{14}C-BOO was purchased from International Isotopes, Unterschleissheim, Germany, and ^{14}C-BO was prepared from ^{14}C-BOO by enzymatic hydrolysis with esterase from rabbit liver in 3.6M $(NH_4)_2SO_4$, 0.1M Tris, pH8.5, Sigma-Aldrich, Steinheim [3]. ^{14}C-BOO in a formulated form was kindly supplied by Rhône-Poulenc Agriculture Ltd., Ongar, Essex, U.K.

2.3. Laboratory experiments for biological effects

Soil taken from a 0-10 cm depth was sieved to 2 mm and treated with herbicide at various concentrations. In order to achieve a concentration corresponding with the agricultural application rate, a penetration depth of 5 cm and a soil density of 1.8 g cm^3 was assumed. This resulted in a concentration of 0.615 mg a.i. per kg dry soil matter. The herbicide formulation was applied to 100 g of soil which then was air-dried, homogenized in a mortar, added to 5 kg of fresh soil and incubated for 2 weeks.

Table 1. Properties of soil used for laboratory and lysimeter experiments

Depth (cm)	Clay (%)[a]	Silt (%)[a]	Sand (%)[a]	Organic Matter (%)[a]	Total N (%)[a]	pH ($CaCl_2$)
00-10	14	43	43	2.78	0.16	6.6
10-20	14	44	42	2.38	0.15	6.4
20-30	14	42	44	2.26	0.14	6.3
30-40	14	41	45	1.88	0.12	6.2
40-50	14	43	43	1.45	0.13	6.3

[a] % of soil dry matter sieved through a 2 mm sieve

Sub-samples were filled in 500-mL Erlenmeyer flasks or in desiccators connected to an aeration system providing moist air. At definite time intervals, aliquots were taken for the determination of various biological soil parameters reflecting effects on soil microorganisms.

Basal heat output was determined in a four-channel microcalorimeter Thermal Activity Monitor, LKB 2277, Järvalla, Sweden [4,5]. For determination of substrate-induced heat output, 1% glucose (w/w)

and 1% $(NH_4)_2SO_4$ were added to the soil samples. The metabolic heat quotient, rqheat, was calculated as the relationship between basal heat output and substrate-induced heat output:

$$rqheat = BW[\mu W/g] \times 100 \times SIW\ [\mu W/g]^{-1}$$

where BW = basal heat output
and SIW = substrate-induced heat output.

Dehydrogenase activity was determined using INT [2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazoliumchloride] as a substrate [6]. Potential nitrification was measured by determination of nitrite after incubation of the soil in the presence of sodium chlorate [7].

Phospholipid fatty acids were extracted from soil with a mixture of chloroform, methanol and phosphate buffer. The chloroform layer was dried, concentrated and separated in various fractions by solid phase extraction on Si- and NH_2-columns. The identification and quantification of fatty acids was done by gas chromatography-mass spectrometry (gc-ms).

2.4. Lysimeter experiments

In order to study the long term fate and effects of ^{14}C-BOO in the open air, six lysimeters were established. The round lysimeters - with a depth of 1 m and a circumference of 2 m (surface area 0.32 m^2) - were made from stainless steel. A wire cloth and 3 metal plates, in which holes had been punched resulting in a “hole area” of about 50% of the total lysimeter area, were fitted in the bottom of the lysimeters to allow the leachate to run out of the soil column.

The properties of soil used are presented in Table 1. The soil was sampled from an agricultural field, and each soil horizon was sampled separately. After transport in plastic bags to the lysimeter station the horizons were put into the lysimeters and pressed to the same density as in the natural soils.

A water reservoir was placed below the soil column in the lysimeter to collect the leachate for at least 4 weeks. The leachate was sucked by means of a vacuum pump into 5 L glass bottles which were stored in a refrigerator at a temperature of 4°C. Water was collected intermittently every 2 or 4 weeks. Maize was cultivated in all lysimeters.

The BOO or ^{14}C-BOO was applied to 5 kg of soil as described in **2.3**. Then, the soil was spread on the lysimeter surfaces between the maize plants. Two lysimeters were treated with formulated ^{14}C-BOO and two with non-radioactive Buctril. Two further lysimeters were kept as untreated controls.

Small soil samples were taken from the non-radioactive lysimeters, at intervals and analysed for dehydrogenase activity and potential nitrification. From the lysimeters treated with ^{14}C-BOO, during the vegetation period, the percolation water was collected when necessary and analyzed for radioactivity.

At harvest time, maize plants from the ^{14}C-lysimeters were collected. Stems, leaves and cobs were analyzed separately. They were homogenized, and ^{14}C was determined by combustion of aliquots to $^{14}CO_2$ in a Packard Tri-Carb 3060 sample oxidizer, followed by liquid scintillation counting (Packard Tri-Carb 1900 TR). Then, the samples were exhaustively extracted with methanol in a Soxhlet apparatus. The ^{14}C in the extracts was determined by liquid scintillation counting (Packard Tri-Carb 1900 TR using Packard Ultima Gold XR, as a scintillation cocktail); the ^{14}C left in the extracted plant material was determined following combustion.

2.5. Laboratory experiments for mineralization, transformation and bound residues formation

In order to determine the mineralization, transformation and bound residues formation of ^{14}C-BO and ^{14}C-BOO, 50 g soil samples were mixed with ^{14}C-BO or ^{14}C-BOO, respectively, at concentrations of 1.62 µg ^{14}C-BO per g dry soil or 2.16 µg ^{14}C-BOO per g dry soil. The soils were incubated for 60 days in a closed, discontinuously aerated laboratory system described previously [5]. Volatile organic ^{14}C-compounds and $^{14}CO_2$ resulting from mineralization were trapped separately in special absorption tubes, collected at certain time intervals, and determined in a liquid scintillation counter. At the end of the incubation time, the soils were exhaustively extracted in a Soxhlet with methanol for 24h. ^{14}C in the extracts was determined in a liquid scintillation counter, residual ^{14}C in the extracted soil, corresponding to soil-bound residues, was determined by combustion of aliquots to $^{14}CO_2$ followed by liquid scintillation counting.

The soil extracts were analyzed for BO, BOO and metabolites by hplc-ms [3].

3. Results and discussion

3.1. Laboratory experiments for biological effects

3.1.1. Substrate-induced heat production

Substrate-induced heat production is a soil parameter from which total microbial biomass may be calculated [4,5]. The results from three weeks' laboratory experiments are presented in Figure 2.

It can be seen that during the first week differences between the control and treated samples are not significant. After two weeks, the effects of the highest concentration rate (1000-fold of agricultural application rate) begin to increase and to deviate significantly from the other concentrations. It is obvious that this biological parameter representing total microbial biomass is not very sensitive against BOO.

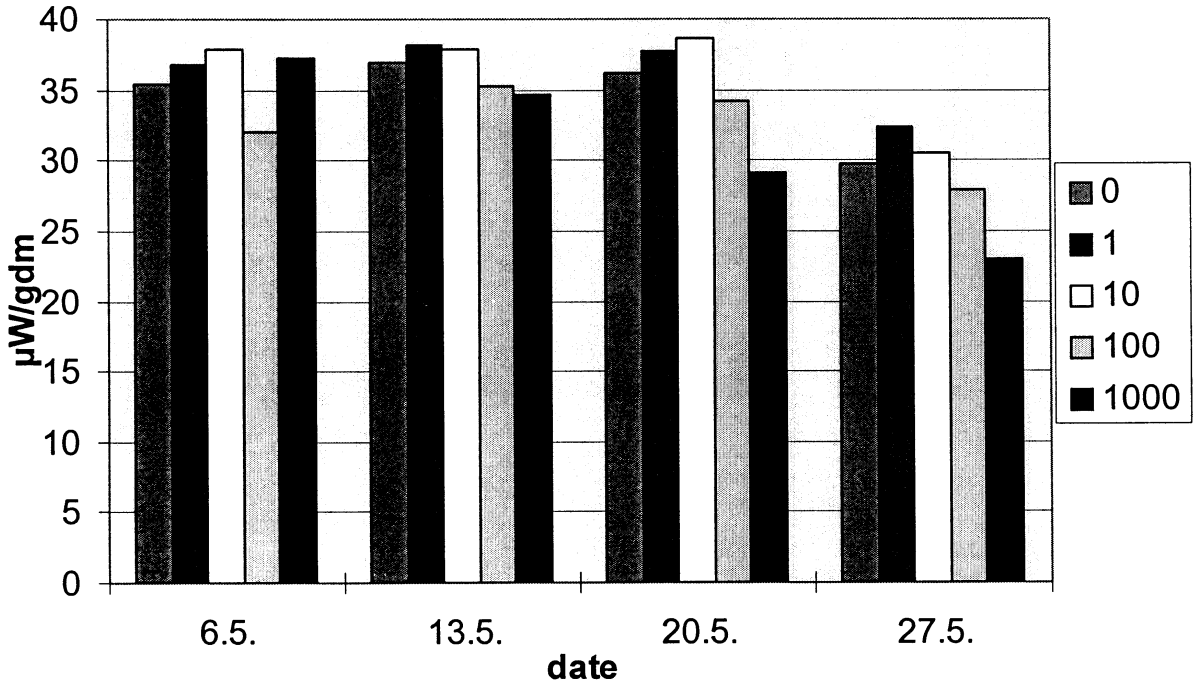

Fig. 2. Substrate-induced heat production in soil untreated or treated with BOO (Buctril); 1 = normal agricultural rate, 0.615 mg/kg dry soil; 10 = 10 fold; 100 = 100 fold; 1000 = 1000 fold gdm: gram dry matter.

3.1.2. Basal heat production

In the case of basal heat production (Figure 3), an indicator of microbial activity, also only the highest concentration causes significant depression as compared to the control. The depression is not reversible but increases with time.

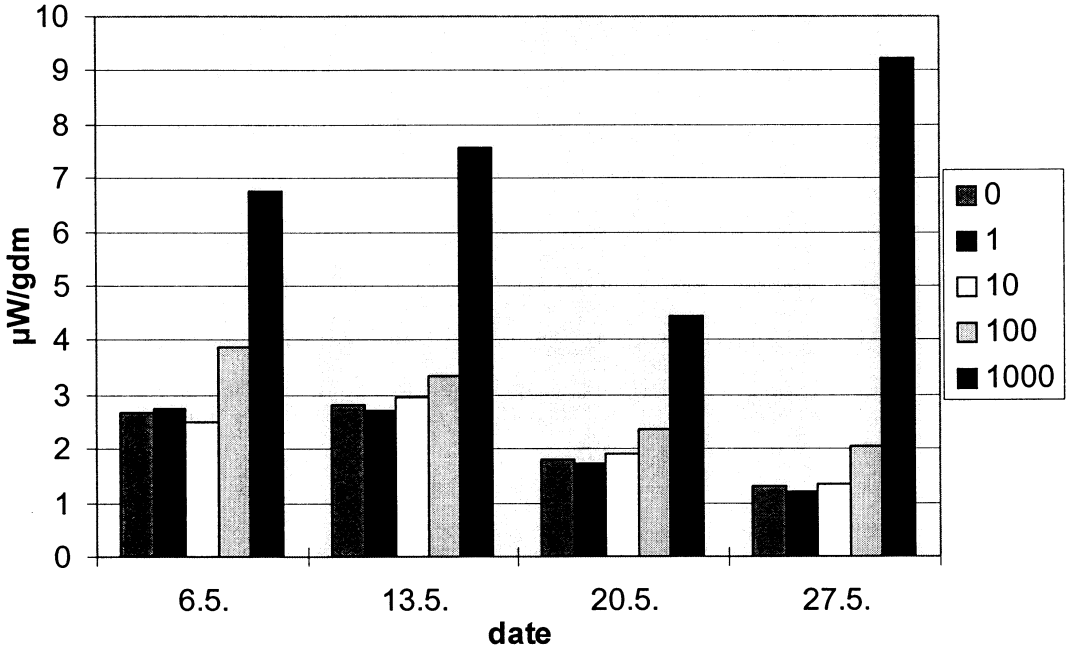

Fig. 3. Basal heat production in soil untreated or treated with BOO (Buctril); 1 = normal agricultural rate, 0.615 mg/kg dry soil;10 = 10-fold; 100 = 100-fold; 1000 = 1000-fold; gdm: gram dry matter.

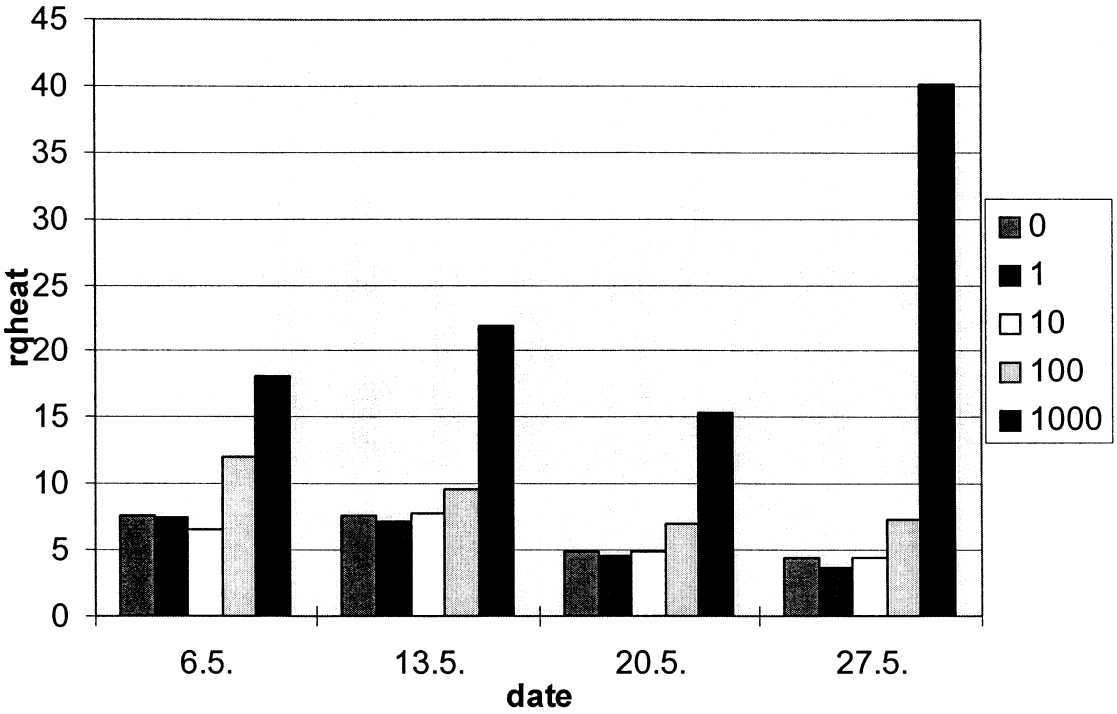

Fig. 4. Metabolic heat quotient (rqheat) in soil untreated or treated with BOO (Buctril); 1 = normal agricultural rate, 0.615 mg/kg dry soil;10 = 10-fold; 100 = 100-fold; 1000 = 1000-fold. 3.1.3. Metabolic heat quotient (rqheat).

This is an ecophysiological parameter describing the efficiency of energy utilization by microorganisms in soils. An inefficient energy utilization, characterized by an enhanced rqheat, is indicative of repair mechanisms and, thus, of an enhanced physiological activity of stressed microorganisms [9-12]. Figure 4 shows that this is the case for the highest BOO concentration; the effect increases with time.

3.1.4. Dehydrogenase activity

Dehydrogenases are oxidoreductases and are responsible for the oxidation of organic material. Therefore, the dehydrogenase activity of a soil describes the intensity of microbial oxidative metabolic processes [13,14]. Figure 5 demonstrates that dehydrogenase activity is significantly depressed by 100- to 1000-fold BOO concentrations. For 100-fold concentration, the depression is reversible, whereas the depression is increasing with time by the 1000-fold concentration, and dehydrogenase activity reaches zero after 2 weeks.

3.1.5. Nitrification

In the nitrogen cycle of the soil, nitrate has a central position since it is the most bioavailable nitrogen for plants. Therefore, nitrification is a process which is very important for soil fertility. Ammonium released from organic matter by ammonification is oxidized to nitrite by *Nitrosomonas* and then to nitrate by *Nitrobacter*. In the potential nitrification test, *Nitrobacter* is inhibited by sodium chlorate, and only nitrite is formed as a reaction product [7]. In Figure 6, the results for various concentrations of BOO are presented.

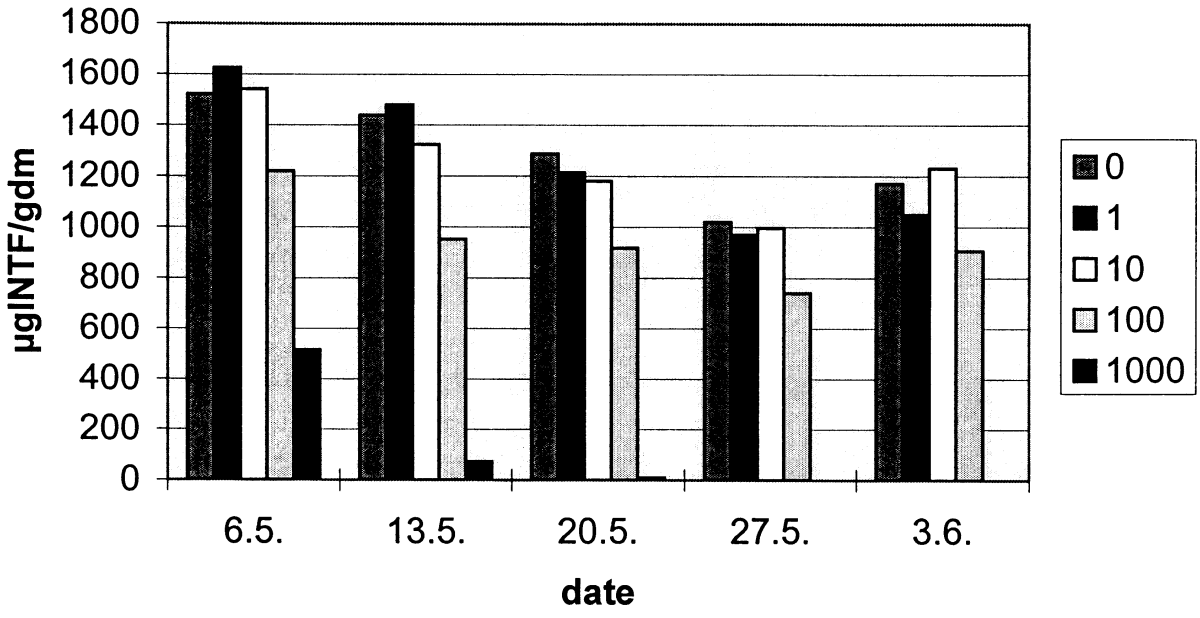

Fig. 5. Dehydrogenase activity in soil untreated or treated with BOO (Buctril) 1 = normal agricultural rate, 0.615 mg /g dry soil; 10 = 10-fold; 100 = 100-fold; 1000 = 1000-fold; INTF: iodonitrotetrazolium chloride formazan; gdm: gram dry matter; time: 2h.

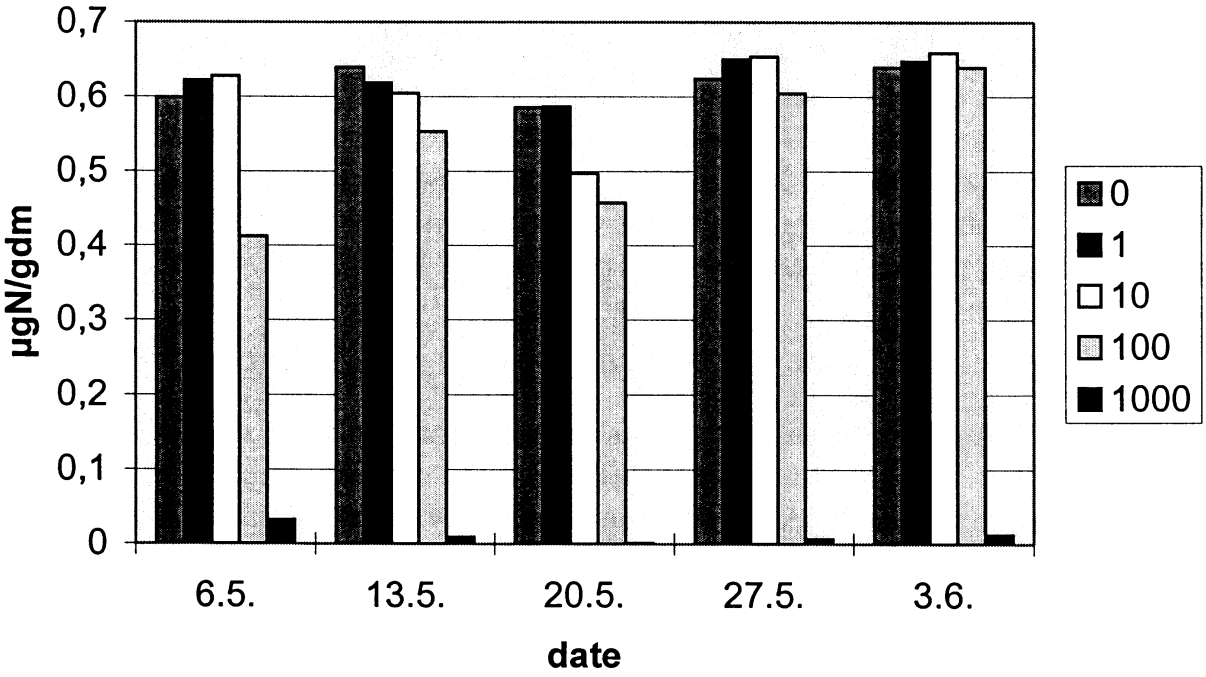

Fig. 6. Potential nitrification in soil untreated or treated with BOO (Buctril), 1 = normal agricultural rate, 0.615 g/kg dry soil;10 = 10-fold; 100 = 100-fold; 1000 = 1000-fold; gdm: gram dry matter; time: 5h.

As in the case of dehydrogenase activity, effects are significant for the 100- and 1000-fold concentrations. Whereas the effects are reversible for the 100-fold concentration, they are irreversible for the 1000-fold concentration; nitrite production within 5 hours is <100 ng NO_2^--N per g dry soil and, thus, below the limit that is indicative of soil contamination [16].

3.1.6. Phospholipid fatty acid pattern

Phospholipid fatty acids are cell wall constituents of all living cells [13]. The fatty acid profiles are fixed genetically and, thus, characteristic of individual microbial groups. The determination of the composition of the phospholipid fatty acid pattern, therefore, gives indication for the composition of the microbial community in soil. Figure 7 presents, as an example, the concentration of the group of polyunsaturated fatty acids (PUF) in soils treated with various doses of BOO.

The decrease of PUF as a consequence of BOO application can be seen as a trend at concentrations corresponding to normal agricultural application rates, and is highly significant at 10-fold concentrations. Thus, this test is more sensitive than the other tests of this study. PUF are indicative of eucaryonts and of cyanobacteria [17]. Their decrease, therefore, is an indication for shifts in the composition of soil microbial communities.

3.2 Lysimeter experiments

In lysimeter soils treated with BOO at normal agricultural application rates, no significant differences in dehydrogenase activity or in potential nitrification could be detected as compared to control soils. However, for treated lysimeters as well as for controls, significant differences between the sampling dates could be noted, indicating seasonal influences.

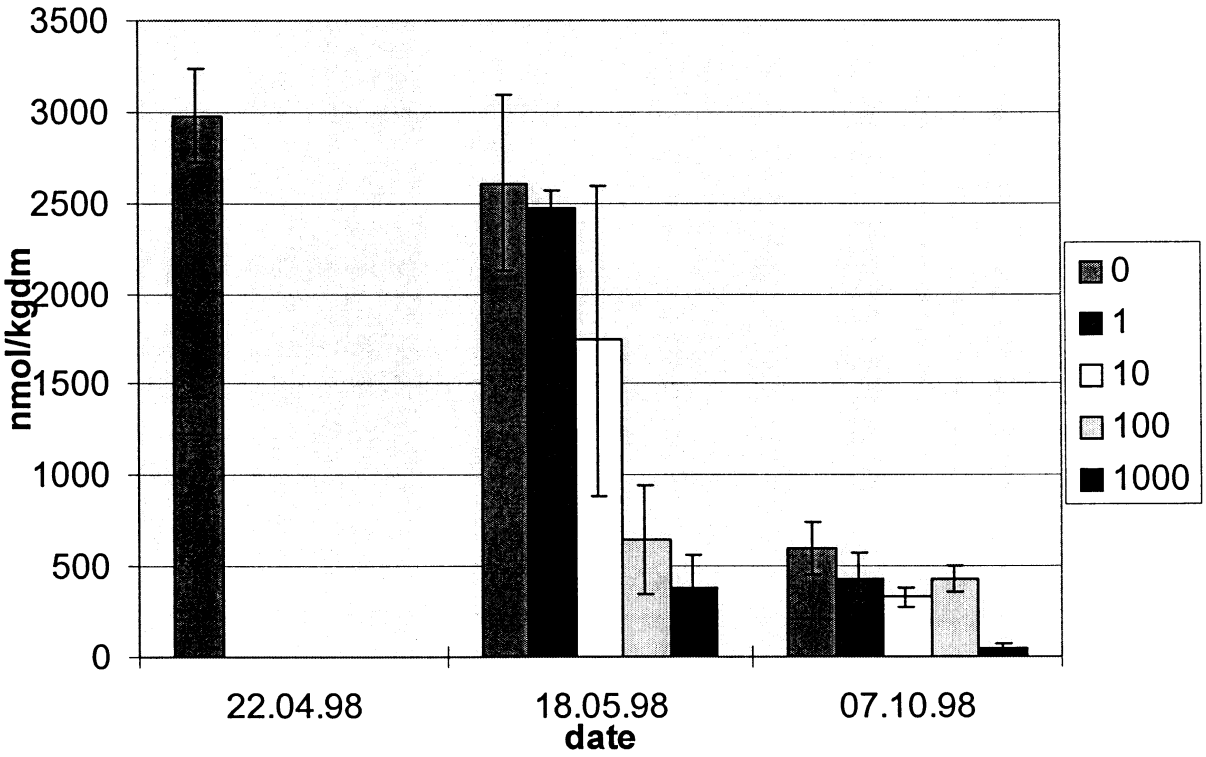

Fig. 7. Polyunsaturated fatty acids (PUF) in soil untreated or treated with BOO (Buctril), 1 = normal agricultural rate, 0.615 mg/ kg dry soil; 10 = 10-fold; 100 = 100-fold; 1000 = 1000-fold; PUF: polyunsaturated fatty acids; kgdm = kilogram dry matter.

In lysimeters treated with ^{14}C-BOO, the uptake of ^{14}C by maize plants was very low (equivalent to <0.08 µg ^{14}C, BOO, per g dry plant matter). This ^{14}C was nearly completely unextractable; the extractable ^{14}C was equivalent to <0.004 µg BOO per g dry plant matter.

In the leachate percolated through the lysimeters treated with ^{14}C-BOO, very low amounts of radioactivity were detected (about 0.12 µg/L). This radioactivity was not due to BOO but to highly polar, water-soluble products which could not be identified thus far.

3.3. Laboratory experiments for mineralization, transformation, and bound residues formation

The results of laboratory degradation experiments of ^{14}C-BO and ^{14}C-BOO are compiled in Table 2. Both substances show a high mineralization rate during 60 days, whereas their volatility is negligible. The radioactivity left in soil after incubation, to a large extent, is unextractable by organic solvents and, thus, in a soil-bound form. The extracted radioactivity from the experiments with ^{14}C-BO contained, besides unchanged parent compound, a metabolite which was identified as 3,5-dibromo-4-hydroxy-benzoate. The formation of this metabolite in soil has been reported also in the literature [18-19]. ^{14}C-BOO is rapidly metabolized to ^{14}C-BO.

4. Conclusions

By the methods available today for the determination of biological effects in soils, effects of the herbicide bromoxynil, applied at normal agricultural rates, on the microflora of soils could not be detected. Tenfold or higher concentrations, however, caused effects on total biomass, bioactivity, enzymatic activity, nitrification, and the composition of the microbial community in soil.

Table 2. Mass balance of ^{14}C in biodegradation experiments with ^{14}C-bromoxynil (^{14}C-BO) and ^{14}C-bromoxyniloctanoate (^{14}C-BOO) after 60 days incubation in soil (in % of ^{14}C applied).

^{14}C-Fraction	^{14}C-BO (%)	^{14}C-BOO (%)
$^{14}CO_2$	42.4±1.3	49.3±9.4
^{14}C volatile	0.03±0.01	0.06±0.03
^{14}C in soil, extractable	2.9±0.3	4.4±0.9
^{14}C in soil, non-extractable	52.3±2.3	44.4±3.5
^{14}C recovery	97.6±3.4	98.1±13.4

Since bromoxynil is mineralized to CO_2 and since its residues are bound in soil in an unextractable form to a large extent, its availability for uptake by plants as well as for leaching is low. However, attention should be paid to the formation of conversion products whose chemical identity is not yet known and which might have unfavourable properties. Therefore, research into this herbicide should be continued.

ACKNOWLEDGEMENT

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REFERENCES

[1] FAYEZ, M., et al., Interaction of the herbicides bromoxynil and afalon S with Azospirillum and growth of maize, Z. Pflanzenemahr. Bodenkd. **146** (1983) 741–751.

[2] RATNAYAKE, M., AUDUS, L.J., Studies on the effects of herbicides on soil nitrification, II, Pest. Biochem. Physiol. **8** (1978) 170–185.

[3] ROSENBROCK, P., et al., Biodegradation of the herbicide bromoxynil and its plant cell-wall bound residues in an agricultural soil, Pest. Biochem. Physiol., in preparation.

[4] SPARL1NG, G.P., Estimation of microbial biomass and activity in soil using microcalorimetry, J. Soil Sci. **34** (1983) 381–390.

[5] LEHR, S., et al., Mineralization of free and cell-wall bound isoproturon in soils in relation to soil microbial parameters, Soil Biol. Biochem. **28** (1996) 1–8.

[6] VON MERSI, W., "Dehydrogenase activity with the substrate INT", Methods in Soil Biology (SCHINNER, F., et al., Eds.), Springer-Verlag, Berlin Heidelberg New York (1996) 243–245.

[7] KANDELER, E., "Potential nitrification", Methods in Soil Biology (SCHINNER, F., et al., Eds.), Springer-Verlag, Berlin Heidelberg New York (1996) 146–149.

[8] ZELLES, L., "Fatty acid patterns of microbial phospholipids and lipopolysaccharides", Methods in Soil Biology (SCHINNER, F., et al., Eds.), Springer-Verlag, Berlin Heidelberg New York (1996) 80–92.

[9] ANDERSON, T.H., DOMSCH, K.H., Application of eco-physiological quotients (qCO_2 and qD) on microbial biomasses from soils of different cropping histories, Soil Biol. Biochem. **22** (1990) 251–255.

[10] PARKINSON, D., COLEMAN, D.C., Methods for assessing soil microbial populations, activity and biomass, Agric. Ecosys. Environ. **34** (1991) 3–33.

[11] HEILMANN, B., et al., Methoden zur Erfassung von Aktivitats- und Strukturveränderungen der Mikroorganismengesellschaft eines fungizid-behandelten Ackerbodens, Mitteil. Dtsch. Bodenk. Gesellsch. **71** (1993) 331–333.
[12] HEILMANN, B., et al., Methods for the investigation of metabolic activities and shifts in the microbial community in a soil treated with a fungicide, Biol. Fertil. Soils **19** (1995) 186–192.
[13] SCHLEGEL, H.G., Allgemeine Mikrobiologie, Thieme Verlag, Stuttgart (1992).
[14] VON MERSI, W., SCHINNER, F., An improved method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride, Biol. Fertil, Soils **15** (1991) 216–220.
[15] BECK, T., Die Nitrifikation in Böden, Z. Pflanzenernähr. Bodenkd. (1979) 344–364.
[16] DECHEMA, Biologische Testmethoden für Böden, Deutsche Gesellschaft für chem. Apparatewesen, chemische Technik und Biotechnologie e.V., Frankfurt a.M. (1995).
[17] HELLMANN, B., et al., Emission of climate-relevant trace gases and successions of microbial communities during open-windrow composting, Appl. Environ. Microbiol. **63** (1997) 1011–1018.
[18] COLLINS, R.F., Perfusion studies with bromoxynil octanoate in soil, Pest. Sci. **4** (1973) 181–192.
[19] GOLOVLEVA, L.A., et al., Decomposition of the herbicide bromoxynil in soil and in bacteria cultures, Folia Microbiol. **33** (1988) 491–499.

Effect of repeated pesticide applications on soil properties in cotton fields: I. Impact on microbes, iron reduction capacity and respiration

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Abstract. Soil microorganisms have a primary catabolic role in the environment through degradation of plant and animal residues. The activities of microorganisms in soil are thus, essential to the global cycling of nutrients. As these pesticides are designed to be biologically active, their continuous use might affect soil microflora either by changing their properties or their numbers, which may lead to impairment in soil fertility. Soil was analyzed for microbial numbers, iron reduction capacity and respiration. Stimulatory, inhibitory or no effects of insecticide treatments were observed on microbes and microbial activities. The insecticides used had only temporary effects on microbes and their activities which disappeared either before the next insecticide treatment was carried out or at the end of experimental period.

1. Introduction

Soil is a dynamic living system and consists of a variety of micro and macrofauna and flora viz., bacteria, actinomycetes, fungi, arthropods, crustaceans, earthworms, etc. They have a primary catabolic role in degradation of plant and animal residues in the environment which contributes to the cycling of nutrients [1]. Insecticide residues are known to have an impact on microbial populations [2,3] in soil which may lead to stimulation, decrease or modification of soil biological processes such as nitrification [4,5], ammonification [6B,7A-B], respiration [8,9,10], ATP [11,12,13], and other processes which are essential for soil health, fertility, productivity and crop yield [4].

Insecticide residues usually occur in the top 15 cm layer of soil [14,15]. It is also the region of greatest activity of soil fauna and flora [16], and provides a platform for interaction of insecticide residues with them. Microbial breakdown of pesticides is considered one of the most important activities in soil.

Soil microbiological investigations are thus well suited to determine the physical and chemical influences of insecticides on soil biology. A number of investigations have been carried out on the effects of single applications of insecticides. In general, observed effects were minor and short lived [17]. However, not much is known about the more realistic situation where different insecticides are used together one after the other. There might arise additional stress from the use of mixtures and sequences of pesticides because of the accumulation of toxic agents or the formation of new compounds (from pesticide combinations) which may be more toxic than the original substances.

In view of the heavy pesticides usage on cotton throughout the cotton growing countries including India, the present study was undertaken to investigate the impact of various sequentially applied insecticides at recommended dosages as per normal practices, on the soil health viz., on microorganisms, the biological processes they perform and the degradation of insecticide residues by soil microbes in a cotton agroecosystem and ultimately the impact on crop yield. The insecticide residues present in the soil were also estimated (see Part II).

2. Materials and methods

2.1. Insecticides

The insecticides used were typical of those associated with cultivation of cotton and were purchased locally. Their general and IUPAC names are given below:

(i) Dimethoate; *O,O*-dimethyl *S*-methylcarbamoylmethyl phosphorodithioate
(ii) Monocrotophos; Dimethyl *(E)*-l-methyl-2-(methylcarbamoyl)vinyl phosphate
(iii) Deltamethrin; (*S*)-α-cyano-3-phenoxybenzyl(1*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclo propanecarboxylate
(iv) Endosulfan; (1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylenebismethylene) sulphite)
(v) Cypermethrin; (*RS*)-α-cyano-3-phenoxybenzyl (1*RS*)-*cis-trans*-3-(2,2-dichlorovinyl)-1,1-dimethyl-cyclopropanecarboxylate
(vi) Triazophos; *0,0*-diethyl *0*-1-phenyl-1H-1,2,4-triazol-3-yl phosphorothioate.

2.2. Field site and insecticide spray schedule

Experiments were conducted at two different locations. In 1994 cotton was sown at the campus of University of Delhi. Two plots quite apart from each other; control (11 m x 4.2 m) and treatment plot (6.6 m x 3.2 m) were prepared for field experiments. In 1995, 1996 and 1998 the experiments were conducted in a farmer's cotton fields at Nurpur village, Ludhiana, Punjab (lat. 30°–54' N, long. 75°–48'E). In 1995 and 1996 three plots (500 sq.m.each) quite apart from each other were taken as control plot (no insecticide treatment), T1- treated plot (all the insecticides were applied for the first time), T2-treated plot (in the farmer's cotton field which had received insecticides for many years) whereas in 1998 investigations there were only two plots control and treated. Cotton varieties Pusa 31, F 846 and LH 900 were sown respectively at 20 kg/ha. The seeds were first delinted using 7–8 mL of conc.

Table l. Insecticide treatment schedule

Insecticide	Dosage (g a.i./ha)	Time of spray
	1994	
Dimethoate (Rogor 30 EC)	300	42 days after sowing
Monochrotophos (Nuvacron 36 SL)	500	68 days after sowing
Deltamethrin (Decis 2.8 EC)	12.5	84 days after sowing
Endosulfan (Thiodon 35 EC)	750	110 days after sowing
Cypermethrin (Cypermethrin 10 EC)	60	142 days after sowing
	1995 and 1996	
Dimethoate (Rogor 30 EC)	300	45 days after sowing
Monochrotophos (Nuvacron 36 SL)	500	60 days after sowing
Deltamethrin (Decis 2.8 EC)	12.5	75 days after sowing
Endosulfan (Thiodon 35 EC)	750	90days after sowing
Cypermethrin (Cypermethrin 10 EC)	60	105 days after sowing
Trizophos (Hostathion 40 EC)	600	120 days after sowing
	1998	
Dimethoate (Rogor 30 EC)	300	45 days after sowing
Trizophos (Hostathion 40 EC)	600	60 days after sowing
Monochrotophos (Nuvacron 36)	500	90 days after sowing
Endosulfan (Thiodon 35 EC)	750	105 days after sowing
Cypermethrin (Cypermethrin 10 EC)	60	120 days after sowing

H_2SO_4 for 250 g seeds. After 3–4 minutes they were washed thoroughly with water. Seeds were allowed to germinate in suitable laboratory conditions and sown in the field. Three fertilizers were applied: DAP at 62.5 kg/ha at the time of sowing; potash at 62.5 kg/ha at the time of initiation of squaring and urea at 50+50 kg/ha at the time of boll formation. No irrigation was applied till August. The insecticide spray schedule and application rates are given in Table l.

2.3. Soil sampling

Soil samples were taken at random by soil auger from 8–10 places from each field and mixed thoroughly to prepare one composite sample. Plant material and other debris were removed from the sample by hand and the soil was sieved using 4 mm mesh. During 1994 and 1998 experiments the soil samples were collected only from top 15 cm whereas during 1995 and 1996 samples were collected from two soil depths: 0–15 cm & 15–30 cm. Soil samples were collected at repeated intervals before and after each insecticide treatment. The last sampling was done 50 days after the last treatment. Samples were brought to the laboratory and stored at 4°C till analyses were conducted.

Temperature, relative humidity and rainfall were recorded daily throughout the experimental period.

2.4. Physical and chemical analysis of soil

The soil was an alluvial sandy loam, typical of north India. Analyses were made taking appropriate samples of soil using standard techniques. Conductivity and pH were measured using saturated soil solution (1:2.5·soil:water). Cation Exchange Capacity was determined by extraction with 1N sodium acetate, butanol and ammonium acetate. Organic carbon was estimated by the modified Walkley-Black method [18]. Available nitrogen was estimated using the procedure of Subbiah and Asija [19]. Available phosphorus was estimated by Olsen's method [20].

2.5. Soil microbial analysis

Agar media were prepared according to the directions, sterilized in autoclave at 1.05 kg cm^2 and 120°C for 30 min and cooled to a pouring temperature of about 37°C. Serial soil dilutions were prepared according to the microorganisms to be studied. One mL of the required dilution was spread evenly on an agar-media petriplate to determine population per gram soil. Thornton's medium was used for estimation of Bacteria. Jensen's medium was used for estimation of azotobacter. Knight's medium was used for estimation of actinomycetes and Martin's streptomycin — Rose Bengal medium was used for estimation of fungi. Fogg's medium was used for algal estimation.

2.6. Fe -III reduction capacity

Iron reduction capacity was determined using Olson's method [21]. Five g of the prepared soil was placed in 25 mL polyethylene vials with screw caps and 5 mL of distilled water containing 4 mg glucose/g dry soil were added as an energy source. The vials were shaken for 10h to obtain a homogeneous soil suspension and incubated under anaerobic conditions for 5 days followed by extraction of ferrous iron by shaking for 10 min with 5 mL of 1N KCl solution. After centrifugation for 15 min, 4.5mL of the clear solution were treated with 0.5 mL of conc. nitric acid and acetate buffer solution was added until pH3-4 was obtained. Ferrous iron was determined by addition of 5mL *O*-phenanthroline and it was allowed to stand for 5 min before measurement of absorbance at 512 nm. The iron concentration was obtained in an unknown sample as μg iron/g dry wt. soil from a standard curve. Controls (without glucose) were run in parallel.

2.7.Determination of respiration

2.7.1 Basal respiration

Fifty g of moist soil at 55% water holding capacity were placed in triplicate in biometer flasks. Ten mL of 0.1M KOH solution was inserted into the side arm of each biometer flask and the system was kept at 22°C for 96h. The absorbed carbon dioxide was determined titrimetrically against the 0.05N HCl. Measurements of CO_2 were made every 24h until 96h.

2.7.2. Substrate- induced respiration

Substrate-induced respiration was determined as above except that 4 mg glucose/g dry wt. soil was added along with the soil in each flask and readings were taken every hour for 4h.

2.8. Statistical analyses

Statistical analyses were done for field experiments in cotton fields using ANOVA.

3.results and discussion

Pesticides usually enter the soil environment in the order of 0.5–5 kg/ha and occasionally even lower. If the pesticide is assumed to be evenly distributed in the top 10cm of soil, the resulting concentrations will be approximately 0.5–5 mg/kg. It should thus be apparent that the amounts transferred between and within environments can be exceedingly small and in many instances may be insignificant. This, however, does not rule out that these residues can have an impact on the soil.

3.1. Physicochemical analyses of the soil studied

Soil at both Delhi (sand 58.9%, silt 26.3% and clay 14.8%) and Ludhiana (sand 68%, silt 12% and clay 20%) was sandy loam with water holding capacity 23.6% and 15–20% respectively. Physicochemical characteristics of the soil are given in Tables 2 and 3.

Table 2. Physicochemical parameters of the soil of cotton fields treated with insecticides in 1994.

*Days after first treatment	pH		CEC		Organic Carbon%		Available N kg/ha		Available P kg/ha	
	C	T	C	T	C	T	C	T	C	T
0	7.4	7.5	3.5	5.2	1.28	1.23	65.9	65.9	45.6	32.07
42	7.9	7.9	5.3	1.0	1.01	0.95	59.6	59.6	40.08	35.25
68	8.0	7.8	5.6	0.8	0.67	0.7	112.9	72.1	51.6	37.27
84	7.7	7.7	5,6	1.1	1.03	0.95	50.2	40.8	35.84	46.59
110	8.0	7.9	4.6	1.2	1.12	0.84	44.4	53.3	35.84	48.45
143	8.0	8.0	5.8	0.9	1.02	1.24	67.4	84.7	38.01	44.23
Grand mean	7.8		3.4		1.0		64.7		40.08	
For two fields	**SEm	CD	SEm	CD	SEm	CD	SEm	CD	SEm	CD
within treatments	0.05	0.15	0.7	2.1	0.07	0.2	9.2	29.1	2.2	6.96
For two treatments within fields	0.03	0.09	0.3	0.9	0.03	0.09	6.0	18.9	1.09	3.41

* Samples were taken before the next treatment.**SEm±CD (0.05)
C = Control field (No insecticide treatment). T = Treated field

Table 3. Physicochemical characteristics of the soil of cotton fields 1995–1998

	Control Field	Treated Field- T1	Treated Field T2
pH	8.8±0.23	8.85±0.06	8.96±0.07
Conductivity (μSiemens)	147.7±22.6	182.6±3.82	193.4±12.8
Organic Carbon %	0.356±0.13	0.371±0.28	0.29±0.14
Organic Matter %	0.796	0.832	0.695
Phosphorus kg/ha	11	9.4	11
Potash kg/ha	440	420	440

Control; No pesticide application; T1 First time pesticides applied; T2 Farmer's field, long history of pesticide application in cotton

The soils studied were alkaline with pH varying from 7.4 to 8.0 in Delhi and from 8.8 to 8.96 in Ludhiana (Tables 2–3). Variation in physicochemical parameters with insecticide usage was studied only in the 1994 crop period. Overall no alteration in pH of the treated fields were observed with the insecticide treatments, the only exception being a slight decrease observed 26 days after monocrotophos treatment in pH in the treated field to 7.8 compared with 8.0 in the control field (Table 2). Cation exchange capacity varied from 3.5–5.6 μSiemens in the control field whereas in the treated field it varied from 0.8 to 5.2 μSiemens (Table 2). From monocrotophos treatment onwards (i.e from 42 to 143 days after the first treatment) cation exchange capacity remained low varying from 0.8 to 0.9 μSiemens in the treated field compared to a significantly higher cation exchange capacity in the control field varying from 3.5 to 5.8 μSiemens (Table 2). Twenty-six days after endosulfan treatment organic carbon was temporarily reduced to 0.84% in the treated field which is significantly less than the 1.12% in the control field (Table 2) whereas with cypermethrin it was enhanced. Available nitrogen levels were similar in both control and treated field the only exception being significantly low nitrogen (72.1 kg/ha) observed in the treated field 26 days after monocrotophos treatment compared to 112.9 kg/ha in the control field.

3.2. Meteorological data

The data are shown in Figs. 1a-c. The weekly maximum and minimum temperature during the course of investigation fluctuated between 44.9 to 31.2°C and 25.6 and 11.0°C respectively in 1995 and between 43.0 to 30.6°C and 27.0 and 12.5°C respectively in 1996. The maximum relative humidity varied from 100.0 to 61.0 and minimum from 75.0 to 22.0 in 1995 and between 100.0 to 83.0 and 74.0 to 22.0 in 1996 throughout the course of investigation Total rainfall (mm) recorded was maximum in the 14th week in 1995 with 409.1 mm. Rainfall recorded in 1996 was less and was maximum in the 8th week being 97.8 mm.

3.3. Effect of insecticide treatments on the soil microbial population of the cotton fields

3.3.1. Bacterial population

The numbers of bacteria occurring in soils are usually higher than those of the other groups; however, because of their small size in relation to the large cell size and extensive filaments of the other groups, bacteria account for less than half of the total microbial biomass in soil [22]. Typically, there are between 10^6 and 10^9 bacteria per gram of soil. The insecticide treatment schedule used in the present investigation did not cause any adverse effect on bacterial numbers. An increase in bacterial numbers from 40.0 to 135.0 x 10^5colony forming units **(**c.f.u.)/g dry wt.soil was observed in the treated field 42 days after dimethoate treatment in 1994 (Table 4) whereas in the 1998 crop period the bacterial population in the treated field was significantly (76%) less compared to control two days after dimethoate treatment, however, in another 10 days it reached levels similar to control (Table 7).

A temporary adverse effect with a 61.8% decrease in numbers was noticed with monocrotophos treatment in 1994 (Table 4) whereas no significant change was observed in rest of the crop periods (Tables 5–7,) except for a decrease after monocrotophos in 1996, in the 15–30 cm soil (Table 6). After deltamethrin treatment 39.4% increase in bacterial numbers was observed in 1994 (Table 4) whereas a 70% decrease in T2 field in top 15 cm soil and in both the treated fields in 15–30 cm soil was observed in 1995 and 1996 respectively after deltamethrin treatment (Tables 5–6). An increase in the number of bacteria in the soil treated with deltamethrin was observed by [3]. A significant 3.85 fold increase in bacterial numbers was also observed after endosulfan treatment in 1994 (Table 4).

And in both the treated fields in 15–30 cm soil in 1995 (Table 5), however, the 13% increase observed in 1998 was statistically non-significant (Table 7).

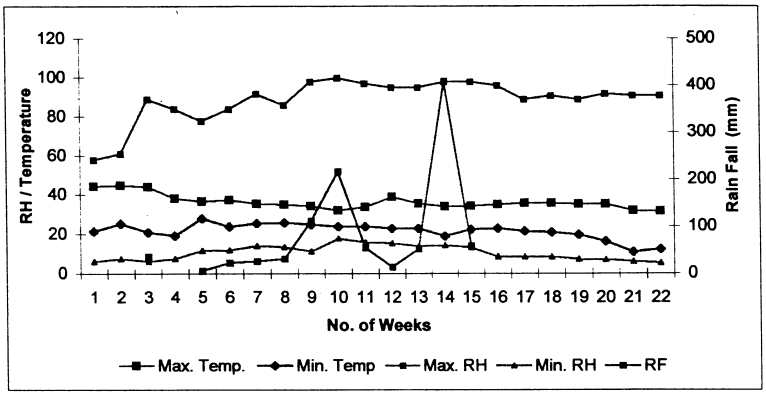

FIG.1a Meterological data for 1995 crop period

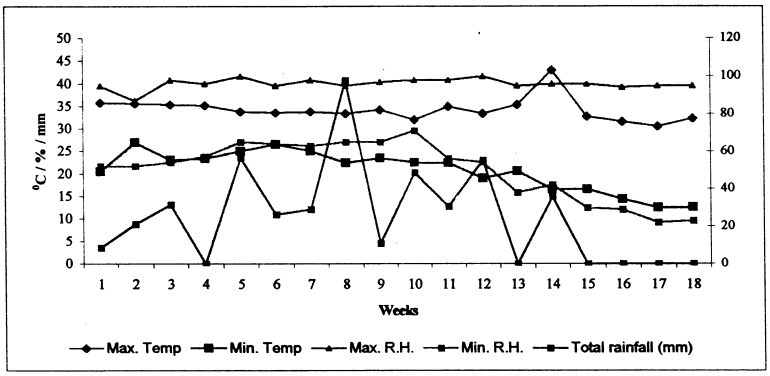

FIG.1b Meterological data for 1996 crop period

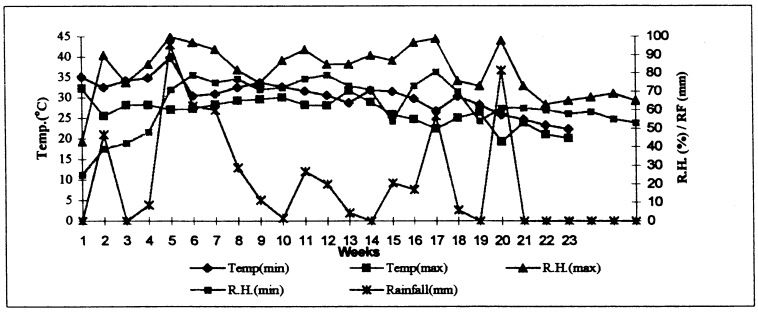

FIG.1c Metereological data for the experimental fields (1998)

The rest of the insecticides used had no effect on bacterial numbers (Tables 4–7). Thus, overall in 1994 insecticides had a positive effect on the bacterial numbers whereas in the other years only temporary fluctuations were observed with populations reaching levels similar to control at the end of the experiment. Inhibitory effects of insecticides on bacteria have been reported [23,24,25] whereas no significant effect on populations of bacteria was reported [26,27,28,29]. In contrast significant stimulation of bacteria was reported [30A,6A,B,5]. A decrease in numbers of bacteria during the first week after insecticide applications, followed by rapid increase in the second week and then gradual reversion to normal was observed by [31,32] and a significant decrease in bacterial numbers with subsequent recovery after 2 days after triazophos treatments at low concentrations was observed by [33].

Table 4. Microbial population (c.f.u./g dry wt.soil) in the soil in 1994

Days after first treatment	Bacteria (x 10^5)		Fungi (x 10^3)		Actinomycetes (x 10^4)		Azotobacter (x 10^3)		Algae (x 10^2)	
	Field type									
	C	T	C	T	C	T	C	T	C	T
0	20.0	40.0	30.0	47.0	140.0	45.0	106.5	98.0	45.0	120.0
42	30.0	135.0	17.0	23.0	175.0	130.0	86.0	105.5	105.0	95.0
68	380.0	145.0	30.0	33.0	80.0	140.0	107.5	99.0	40.0	115.0
84	330.0	460.0	27.0	33.0	130.0	195.0	104.0	141.5	125.0	165.0
110	65.0	315. 0	47.0	67.0	155.0	135.0	99.5	176.5	43.0	45.0
142	65.0	150.0	43.0	53.0	230.0	190.0	94.5	139.5	185.0	225.0
Grand mean	178.0		38.0		145.0		113.2		141.0	
	SEm	CD	SEm	CD	SEm	CD	SEm	CD	SEm	CD
For two fields within treatment	51.0	160.0	12.0	NS	16.0	50.0	7.9	24.9	74.0	232.0
For two treatments within field	21.0	65.0	6.0	NS	15.0	47.0	6.2	19.5	31.0	99.0

Table 5. Soil bacterial populations (10^5/g d wt soil) in 1995

	Depth											
	0–15cm						15–30cm					
Field	Days after first treatment											
	0	15	30	45	60	75	0	15	30	45	60	75
Control	50.0	43.0	47.0	67.0	147.0	23.0	-	23.0	33.0	40.0	130.0	47.0
TI	20.0	47.0	27.0	60.0	137.0	50.0	-	30.0	47.0	30.0	393.0	37.0
T2	47.0	17.0	13.0	20.0	150.0	57.0	-	30.0	53.0	33.0	300.0	43.0
Grand mean				57.0						85.0		
For 2 fields within treatments				SEm	CD					SEm	CD	
				16.0	47					44.0	124.0	
For 2 treatments within fields				11.0	31.0					29.0	83.0	

Table 6. Soil bacterial populations (x10^5/g dry wt. soil) in 1996

	Depth													
	0–15 cm							15–30cm						
Field	Days after first treatment													
	0	15	30	45	60	75	120	0	15	30	45	60	75	120
Control	47.0	33.0	267.0	103.0	17.0	70.0	50.0	27.0	-	720.0	227.0	27.0	17.0	113.0
T1	87.0	20.0	233.0	14.0	67.0	73.0	50.0	17.0	-	503.0	13.0	10.0	27.0	30.0
T2	67.0	23.0	307.0	117.0	63.0	60.0	67.0	50.0	-	633.0	27.0	13.0	37.0	153.0
Grand mean				93.0							147.0			
For two fields within treatments				SEm	CD						Sem	CD		
				26.0	NS						42.0	120.0		
For two treatments within fields				14.0	NS						29.0	81.0		

Table 7. Soil bacterial population (x 10^5 c.f.u./g d wt soil) in 1998

Treatments	Days after lst treatment	Control±SE	Treated
Dimethoate	(A) 2	153.3±8.8	36.66±8.8**
Triazophos	(B) 22	63.3±17.6	100.0±26.5
	(A) 24	50.0±25.1	63.3±12.0
Monocrotophos	(B) 32	156.6±64.8	130.0±15.3
Endosulfan	(B) 52	80.0±26.4	80.0±5.7
	(A) 54	100.0±15.3	113.3±6.6
Cypermethrin	(B) 64	43.33±8.8	100.0±25.1
	(A) 66	170.0±30.0	86.6±20.2
No Spray	76	113.3±17.6	76.6±18.5
Post harvest	96	56.6±8.8	63.33±14.5

**p<0.01; (B) Before treatment; (A) After treatment

Table 8. Soil azotobacter population (x10^3/g dry wt. soil) in 1995

						Depth						
	0–15 cm						15–30cm					
Field	Days after first treatment											
	0	15	30	45	60	75	0	15	30	45	60	75
C	148.7	122.0	70.3	67.3	74.3	39.3	-	93.7	147.3	80.7	36.0	76.7
Ti	127.0	76.7	59.0	61.0	92.0	86.0	-	71.3	95.7	79.0	74.0	55.3
T2	104.3	99.0	73.3	75.3	88.7	70.0	-	91.0	101.7	79.0	84.0	52.7
Grand mean				85.2			-			81.2		
For two fields within				SEm	CD		-			SEm	CD	
treatments				17.5	50.3		-			11.6	34.4	
For two treatments				10.5	30.2		-			8.0	23.9	
within fields							-					

3.3.2. Azotobacter population

Azotobacter are capable of fixing nitrogen in soil. They are very common in the rhizosphere region of plants and they maintain themselves on root exudates. The insecticide treatment schedule used in the present investigation did not cause any adverse effect on azotobacter numbers in either 1995 or 1996 (Tables 8 and 9). In 1995, 35% and 31% decreases in numbers were observed after monocrotophos in T1 and T2 fields and 1.05 fold and 1.33 fold increases were observed after endosulfan treatment in T1 and T2 fields in the 15–30cm layer.

However, in 1994 a non-significant 22.7% increase was observed in the treated field after dimethoate treatment (Table 4) and significant 36%, 77.4% and 47.6% increases were observed after deltamethrin, endosulfan and cypermethrin treatments respectively (Table 4). Thus an overall stimulation in azotobacter population was observed with insecticide treatments in 1994. This could be due to a smaller field size and differences in climate. In 1998 a significant 7.3 fold increase was observed in the treated field twenty days after dimethoate treatment (Table 10). After an initial non-significant 15.6% increase, a significant 91.7% decline was observed a further eight days after triazophos treatment (Table 10). A stimulatory effect of triazophos at low concentrations on azotobacter was observed after 2 days [33]. After monocrotophos treatment an increase was observed in the treated field which was,

however, not significant statistically (Table 10). Azotobacter numbers tended to be higher immediately after endosulfan treatment followed by a 65.8% decline in another 10 days (Table 10). Recovery was observed after cypermethrin treatment and at the end of the experimental period azotobacter numbers were statistically similar in both the fields again indicating that the insecticides used had only temporary effects followed by recovery of the population. Increase in azotobacter numbers after insecticide treatment has been observed by many workers [34,35, 30B].

Table 9. Soil azotobacter population (c.f.u. x 10^3/g d wt soil) in 1996

	Depth													
	0–15 cm							15–30 cm						
Field	Days after first treatment													
	0	15	30	45	60	75	120	0	15	30	45	60	75	120
C	69.3	47.0	100.0	181.0	79.3	67.3	23.0	55.7	34.7	31.7	57.3	59.0	22.3	122.7
T1	60.0	48.7	85.3	175.7	101.7	64.7	35.7	58.0	57.3	71.3	79.3	79.0	73.0	100.3
T2	89.0	38.3	53.7	169.3	39.0	33.0	40.0	50.0	38.3	24.0	74.0	82.0	51.0	104.0
Grand mean				76.2							63.1			
For 2 fields within same treatment				SEm	CD						SEm	CD		
				17.4	NS						16.4	NS		
For 2 treatments within fields				9.4	NS						9.4	NS		

Table 10. Soil azotobacter population (c.f.u. x 10^3/g d wt soil) in 1998

Treatments	Days after 1st treatment	Control (±S.E)	Treated
Dimethoate	(A) 2	100.0±40.0	80.0±5.6
Triazophos	(B) 22	26.6±8.8	220.0±17.3**
	(A) 24	170.0±11.5	196.6±61.1
Monocrotophos	(B) 32	160.0±51.9	13.3±3.3
Endosulfan	(B) 52	20.0±10.0	120.0±55.5
	(A) 54	76.6±20.3	180.06±50.3
Cypermethrin	(B) 64	263.3±73.1	90.0±32.1
	(A) 66	176.6±18.5	106.6±46.6
No Spray	76	143.3±12.0	133.3±8.8
Post harvest	96	100.0+40.4	110.0±20,0

**p<0.01; (B) Before treatment; (A) After treatment

3.3.3. Fungal population

Although numerically much less abundant (between 10^4 and 10^6 fungal propagules per gram soil) than bacteria, fungi are the major contributors to soil biomass and account for as much as 70% by weight of the biomass [36]. Most fungi are opportunistic. They grow and conduct their activities when environmental conditions (e.g., nutrients, moisture, temperature, aeration) are favourable. The acidic side of pH is generally more favorable for fungi. Fungi were quite resistant to the insecticides used in the present investigation as no adverse effect on fungal numbers was observed after insecticides treatments in all the crop periods (Tables 4,11–13). However, a decrease in fungal numbers was observed in the previously untreated field on the last sampling day in 1996 which shows that the population in this field could not recover either due to last (i.e. triazophos) treatment or due to some synergistic effect (Table 12). A 70.15% decrease in fungal population was also observed in the 15–30 cm soil layer in 1995 after deltamethrin treatment in the T2 field (Table 11) and 86.8% after cypermethrin treatment in the T1 field (Table 11). A non significant decrease was also observed in the

corresponding treated fields (Table 11). In the 1998 crop period, the fungal population in the treated field was 21% less than control 2 days after dimethoate treatment (Table 13). A 34.4% significant decline was also observed 2 days after triazophos treatment (Table 13). Fungi were quite resistant to monocrotophos as the populations were similar in both the fields (Table 13). After an initial significant decrease of 48% following endosulfan treatment recovery was observed in another ten days (Table 13). A significant decrease (38.1%) was also observed 2 days after cypermethrin treatment (Table 13).

Table 11. Soil fungal population ($x10^3$/g dry wt. soil) in 1995

	Depth											
	0–15 cm						15–30cm					
	Days after 1st treatment											
Field	0	15	30	45	60	75	0	15	30	45	60	75
Control	23.0	57.0	33.0	20.0	67.0	-	-	40.0	63.0	67.0	63.0	53.0
Ti	47.0	43.0	67.0	30.0	67.0	-	-	47.0	80.0	33.0	53.0	7.0
T2	47.0	37.0	43.0	30.0	37.0	-	-	37.0	97.0	20.0	57.0	23.0
Grand mean			43.0							49.0		
For two fields within			SEm	CD					SEm	CD		
treatment			12.0	35.0					16.0	45.0		
For two treatments within fields			9.0	25.0					11.0	33.0		

Table 12. Soil fungal populations ($x10^3$/g dry wt. soil) in 1996

	Depth													
	0–15 cm							15–30cm						
	Days after 1st treatment													
Field	0	15	30	45	60	75	120	0	15	30	45	60	75	120
Control	23.0	60.0	43.0	43.0	30.0	27.0	77.0	17.0	3.0	33.0	17.0	33.0	20.0	27.0
TI	43.0	50.0	33.0	53.0	43.0	27.0	17.0	10.0	20.0	43.0	17.0	60.0	23.0	7.0
T2	30.0	53.0	33.0	37.0	40.0	13.0	60.0	17.0	20.0	40.0	27.0	70.0	10.0	20.0
Grand mean			40.0								25.0			
For two fields within			SEm	CD						SEm	CD			
treatments			11.0	33.0						14.0	NS			
For two treatments within fields			7.0	2.0						8.0	NS			

Table 13. Soil fungal populations ($x10^3$/g dry wt. soil) in 1998

Treatment	Days after 1st treatment	Control±S.E	Treated
Dimethoate	(A) 2	23.66±1.76	18.66±1.76
Triazophos	(B) 2	30.0±1.53	23.33±4.66
	(A) 24	22.33±0.88	14.66±1.76*
Monocrotophos	(B)32	19.0±0.0	15.66±1.66
Endosulfan	(B) 52	14.33±3.48	15.3±30.66
	(A) 54	16.66±0.88	9.0±2.51*
Cypermethrin	(B)64	21.33±1.76	23.0±1.15
	(A) 66	81.33±1.76	50.33±1.76**
No Spray	76	27.66±3.18	26.33±0.88
Post harvest	96	13.0±1.15	11.33±0.66

**p<0.01; (B) Before treatment; (A) After treatment

Adverse effects on fungal numbers were nullified by the end of present investigation with similar populations in all the fields (Tables 4, 11–13). No effect of insecticides on fungi have been reported by many workers [5,6B,34,37,38,39]. Fungitoxic effects of many insecticides were demonstrated by [40,41] whereas stimulatory effects have been confirmed by many workers [6A,C, 30A,B,].

3.3.4. Actinomycetes population

Actinomycetes compose 10% to 33% of the bacterial population [22] and are more abundant in surface soils. They are more abundant in soils with high pH. Dimethoate had no adverse effect on actinomycetes population in all the years (Tables 4,14–16). Monocrotophos also had no effect, except for a 75% stimulation observed in 1994 (Table 4) and 45.75 and 41.2% decreases observed in T1 and T2 fields respectively in 1995 in the 15–30 cm soil layer (Table 14). Deltamethrin also increased actinomycete numbers by 50% and 2 to 2.5 fold respectively in both 1994 and 1995 (Tables 4,14). An increase in the number of actinomycetes in soil treated with deltamethrin has been observed [3]. Endosulfan and cypermethrin showed no adverse effect except a temporary 36.7% decrease observed in T1 and 39.5% increase in T2 field in 1995 and 1996 respectively after endosulfan treatment (Tables 14,15). In 1998 a 1.2 fold increase in numbers was observed 20 days after dimethoate treatment (Table 16). However, after endosulfan treatment actinomycetes numbers were significantly reduced by 65% in ten days.

Table 14. Soil actinomycetes population (no.x 10^3/g dry wt. soil) in 1995

	Depth											
	0–15cm						15–30cm					
	Days after first treatment											
Field	0	15	30	45	60	75	0	15	30	45	60	75
C	150.0	47.0	170.0	-	343.0	-	-	133.0	153.0	140.0	97.0	190.0
T1	137.0	77.0	177.0	-	217.0	-	-	160.0	83.0	140.0	93.0	193.0
T2	117.0	47.0	153.0	-	307.0	-	-	140.0	90.0	193.0	93.0	43.0
Grand mean			162.0						130.0			
For two fields within treatments			SEm 29.0	CD 83.0					SEm 16.0	CD 46.0		
For two treatments within fields			21.0	60.0					12.0	33.0		

Table 15. Soil actinomycetes population (x 10^3/g dry wt. soil) in 1996

	Depth													
	0–15cm							15–30cm						
	Days after first treatment													
Field	0	15	30	45	60	75	120	0	15	30	45	60	75	120
C	157.0	60.0	70.0	97.0	423.0	360.0	67.0	70.0	47.0	67.0	37.0	503.0	387.0	73.0
T1	110.0	37.0	137.0	113.0	437.0	350.0	37.0	60.0	57.0	67.0	50.0	467.0	363.0	30.0
T2	90.0	40.0	77.0	87.0	590.0	290.0.	83.0	97.0	27.0	77.0	63.0	553.0	317.0	90.0
Grand mean			177.0							167.0				
For two fields within treatments			SEm 41.0	CD 115.0						SEm 43.0	CD NS			.
For two treatments within fields			23.0	64.0						25.0	NS			

Table 16. Soil actinomycetes population x 10^3/g dry wt. soil) in 1998

Treatments	Days after 1st treatment	Control±S.E	Treated
Dimethoate	(A) 2	40.0±10.0	26.66±12.02
Triazophos	(B) 22	16.66±6.66	43.33±14.53
	(A) 24	20.0±10.0	33.33±8.82
Monocrotophos	(B) 32	26.66±12.02	10.0±0.0
Endosulfan	(B) 52	13.33±3.33	10.0±0.0
	(A) 54	13.33±3.33	13.33±3.33
Cypermethrin	(B) 64	66.66±12.01	23.3±6.66*
	(A) 66	46.66±6.16	10.0±0.0**
No Spray	76	40.0±5.77	33.33±14.5
Post harvest	96	40.0±15.27	33.33±8.82

*p<0.05; **p<0.01; (B) Before treatment; (A) After treatment

Actinomycete numbers tend to remain significantly less immediately after cypermethrin treatment (Table 16), however, recovery was observed in another ten days and at the end of the experimental period actinomycete numbers were similar in both fields again indicating that the insecticides used had only temporary effects. Stimulatory effects of insecticides on actinomycetes have been confirmed by [6A,C, 30A,B, 37,38,39] whereas significant and lasting reductions were reported by [2]. No effect of insecticides on actinomycetes were reported by [41]. No adverse effects of monocrotophos and its soil degradation products on soil microbial populations were demonstrated by [42,43].

3.3.5. Algal population

The algal population was studied only in 1994 (Table 4). The algal population in the treated field was more (120.0 c.f.u. x 10^2/g.d.wt soil) but statistically non significant on zero day compared to control where it was 45.0 c.f.u. x 10^2/g.d.wt soil. The algal population varied non significantly from 45.0 to 125.0 x 10^2 c.f.u. /g.dry wt. soil in the control field and from 120.0 to 165.0 x 10^2 c.f.u. /g.dry wt. soil in the treated field from zero to 84 days after first treatment. From 84 to 110 days after first treatment a significant increase in population from 125.0 to 430.0 x 10^2 c.f.u. /g.dry wt. soil was observed in control soil whereas a significant decrease from 165.0 to 45.0 x 10^2 c.f.u. /g.dry wt. soil was observed in treated soil resulting in a significantly lower population in the treated field 15 days after endosulfan treatment. From 110 to 143 days, however, the algal population increased significantly from 45.0 to 225.0 x 10^2 c.f.u./g.dry wt. soil in treated field whereas it decreased significantly from 430.0 to 185.0 x 10^2 c.f.u./g.dry wt. soil in the control field during the same period. The algal populations on the 143rd day in both the fields were not statistically different. No effect of pesticides on algae was observed by [30B] whereas an increase in algal population with insecticide treatments was documented by [39,44].

3.4. Effect of insecticides treatment on iron reduction capacity

The capacity of microorganisms to reduce iron has been used for studying the effects of pesticides on the bioactivity of microorganisms in soils [45,46]. An adverse effect of dimethoate on iron reduction capacity with 44.6% and 3 8.4% decreases was observed in T1 and T2 fields in 1995 (Table 17) whereas no effect was observed in 1996 (Table 18). No significant change in the activity was observed after monocrotophos and triazophos treatment (Tables 17 and 18). After deltamethrin treatment increases in the activity was observed in both the treated fields in 1996 which was significantly less by 52.8% in T2 (Table 18). An increase in activity in treated fields was also observed in 1995 but after fifteen days the activity in the treated fields were statistically comparable to control (Table 17). After endosulfan treatment activity was adversely affected (27.5 and 19.33% reductions in T1 and T2 fields respectively) in 1996 (Table 18), a non significant 17.9% decrease was also observed in T2 field in 1995 whereas activity was maintained at the previously increase levels in T1 field (Table 17). Adverse

effects with 39.6 and 43.3% decreases in T1 and T2 fields respectively was observed with cypermethrin treatment in 1996 (Table 18) whereas a 1.65 fold increase in activity was observed in the T1 field in 1995 (Table 17). An increase of 1.47 fold was observed in iron reduction capacity in the T2 field after cypermethrin treatment in 1995 in the 15–30 cm soil layer (Table 17) whereas a52.7% decrease was observed with the same insecticide in 1996 (Table 18).

Table 17. Soil iron reduction capacity (µg/g dry wt.) in 1995

	Depth											
	0–15 cm						15–30 cm					
	Days after first treatment											
Field	0	15	30	45	60	75	0	15	30	45	60	75
Control	62.0	125.8,	75.7	77.4	80.4	21.1	-	71.7	49.1	56.3	32.0	20.9
TI	37.1	69.6	57.4	109.4	117.0	55.9	-	38.9	37.6	66.3	42.4	44.1
T2	70.2	77.5	85.8	107.2	66.3	31.1	-	48.3	44.6	34.5	40.5	51.6
Grand mean			73.7							45.3		
For two fields within				SEm	CD					SEm	CD	
treatments				11.7	33.8					10.4	30.4	
For two treatments within				7.4	21.5					6.7	19.4	
fields												

Table 18. Soil iron reduction capacity (µg/g dry wt.) in 1996

	Depth													
	0–15 cm							15–30 cm						
	Days after first treatment													
Field	0	15	30	45	60	75	120	0	15	30	45	60	75	120
Control	12.8	14.8	13.8	77.4	45.0	61.0	12.0	14.8	14.2	13.2	45.4	38.3	72.8	9.4
Ti	13.3	14.0	14.2	69.1	32.6	36.8	10.7	12.8	15.7	14.7	48.5	34.3	48.1	11.8
T2	13.0	14.8	14.4	36.5	36.3	34.6	11.8	13.3	14.6	15.3	27.5	34.4	34,4	10.7
Grand mean			28.0								25.9			
For two fields				SEm	CD						SEm	CD		
within treatments				4.0	11.3						6.4	18.6		
For two treatments				2.3	6.6						3.6	10.4		
within fields														

Table 19. Soil iron reduction capacity (µg/g dry wt. ±SE) in 1998

Treatments	Days after 1st treatment	Control	Treated
Dimethoate	(A) 2	18.26+4.05	-
Triazophos	(B) 22	26.66±2.33	20.33±2.6
	(A) 24	13.5±3.18	9.0±2.88
Monocrotophos	(B) 32	28.83±0.73	72.33±8.11**
Endosulfan	(B)52	63.83±13.5	59.0±0.58
	(A) 54	7.33±3.38	14.33±5.81
Cypermethrin	(B) 64	28.83±1.74	24.66±1.74
	(A) 66	17.33±1.2	19.66±0.33
No Spray	76	22.0±3.51	76.5±0.86**
Post harvest	96	48.66±4.04	47.16±1.2

No overall effect of insecticide treatments was observed in either field in both years in the lower layer (Tables 17–18). In 1998 significant 1.5 and 2.47 fold increases were observed 10 days after triazophos and 12 days after cypermethrin treatment respectively in the treated field (Table 19). None of the other insecticides used had any adverse effect with similar iron reduction capacity in both the fields at the end of experimental period (Table 19). Decreases in the rate of iron reduction in soil incorporated with pesticide was observed by [46].

3.5 Effect of insecticides treatment on soil respiration

A viable part (4–49%) of the microbial biomass is active [47A-B]. Soil respiration, as indicated by oxygen consumption or CO_2 evolution, is therefore a good index of the activity of microflora involved in organic matter decomposition [48]. Respiration is mostly dependent upon the physiological conditions of the organisms as well as on environmental parameters such as temperature, illumination etc.[4]. The insecticide treatment schedule used in the present investigation caused no major adverse effect on the respiration-both basal and substrate-induced (Tables 20–25) in all the years. There were statistically significant decreases in substrate-induced respiration 15 days after monocrotophos treatment in the T1 field and in the T2 field in 15–30 cm soil in 1996 (Table 24), in basal respiration in the treated field 20 days after dimethoate treatment in 1998 (Table 22 and in substrate-induced respiration in the treated field 20 days after dimethoate treatment and 10 days after triazophos treatment in 1998 (Table 25).

Inhibition of soil respiration at higher doses is reported in the literature [50,51,52,53,54,55] whereas an increase in oxygen consumption was observed by [56,57,58A,58B,59]. An initial increase with subsequent decrease in formation of CO_2 was reported by [60].

Table 20. Soil basal-respiration μg CO_2/g dry wt. 24h) in 1995

			Depth (0–15 cm)		
			Days after first treatment		
Field	0	15	30	45	60
C	390.0	462.0	374.0	451.0	404.0
T1	469.0	437.0	433.0	455.0	428.0
T2	448.0	448.0	369.0	451.0	406.0
Grand mean			428.0		
For two fields within treatments		SEm	CD (0.05)		
For two treatments within fields		39.0	NS		
		27.0	NS		

Table 21. Soil basal-respiration (μg CO_2/g dry wt. 24hr) in 1996

						Depth								
				0–15 cm							15–30cm			
						Days after first treatment								
Field	0	15	30	45	60	75	120	0	15	30	45	60	75	120
Control	412.7	398.0	531.7	379.3	219.7	441.3	437.7	474.3	452.0	520.0	379.7	246.7	414.3	450.3
Ti	469.7	413.0	511.0	361.7	220.0	446.7	475.7	370.3	380.0	523.7	363.0	225.0	460.7	528.3
T2	475.3	420.0	530.0	368.7	233.3	450.3	488.0	323.7	383.3	542.3	326.7	224.7	443.3	478.3
Grand mean				413.5							405.3			
For two fields within treatments				SEm	CD						SEm	CD		
				53.8	NS						64.6	NS		
For two treatments within fields				28.8	NS						35.1	NS		

Table 22. Soil basal-respiration (μg CO_2/g dry wt. 24h ±S.E) in 1998

Treatments	Days after 1st treatment	Control	Treated
Dimethoate	(A) 2	318.0±15.97	302.55±0.57
Triazophos	(B) 22	319.65±11.27	289.32±4.59*
	(A) 24	208.37±30.09	201.16±27.09
Monocrotophos	(B) 32	311.35±38.56	260.45±13.24
Endosulfan	(B) 52	327.45±11.87	333.25±12.9
	(A) 54	308.35±17.64	317.8±24,97
Cypermethrin	(B) 64	201.7±31.19	200.6±27.7
	(A) 66	314.82±29.23	314.75±24.81
No Spray	76	334.45±7.7	318.45±4.22
Post harvest	96	344.75±8.35	324.75±15.7

* p< 0.05; (B) Before treatmen;t (A) After treatment

Table 23. Soil substrate-induced respiration (μgCO_2 /g dry wt. h) in 1995

	Depth (0–15 cm) Days after first treatment				
Field	0	15	30	45	60
C	425.7	483.3	327.0	486.3	347.3
T1	474.0	467.3	348.3	508.0	477.7
T2	480.0	465.7	344.3	481.7	422.7
Grand mean			436.0		
For two fields within treatments		SEm 28.7	CD NS		
For two treatments within fields		18.7	NS		

Table 24. Soil substrate~induced respiration (μgCO_2 /g dry wt. h) in 1996

	Depth													
	0–15 cm							15–30cm						
	Days after first treatment													
Field	0	15	30	45	60	75	120	0	15	30	45	60	75	120
C	427.0	420.0	439.0	463.0	254.0	463.0	333.0	464.5	406.5	457.5	467.5	242.0	458.0	325.5
Ti	493.0	423.0	388.0	459.0	250.0	464.0	295.0	463.5	401.8	348.8	447.3	240.3	464.3	317.5
T2	502.0	409.0	422.0	439.0	251.0	452.0	360.0	429.8	400.0	433.5	433.3	216.5	427.5	321.0
Grand mean			400.0							388.9				
For two fields within treatments				SEm 21.0	CD 60.0						SEm 21.6	CD 62.1		
For two treatments within fields				12.0	34.0						12.6	36.1		

Table 25. Soil substrate~induced respiration (μgCO_2 /g dry wt h^{-1} ±S.E) in 1998

Treatments	Days after 1st treatment	Control	Treated
Dimethoate	(A) 2	339.9±8.48	358.95±11.35
Triazophos	(B) 22	348.55±9.17	296.5±5.42**
	(A) 24	290.2±1.43	287.37±1.69
MonocrotophoS	(B) 32	356.1±11.66	284.15±8.03**
Endosulfan	(B) 52	355.02±8.89	344.3±12.58
	(A) 54	337.5±5.99	343.95±6.19
Cypermethrin	(B) 64	285.92±2.82	275.6±7.03
	(A) 66	358.45±1.57	364.0±13.2
No Spray	76	364.15±3.01	353.1±8.12
Post harvest	96	348.75±7.21	354.25±1.69

**p<0.01; (B) Before treatment; (A) After treatment

Decreases in fungal, actinomycetes and/or bacterial populations followed by increases and finally a return to pre-treatment population numbers in soils that were treated with insecticides was also reported by [23,57,58A-B,61]. Many workers have stressed the fact that insecticides do not in general have much effect, except at concentrations, greatly exceeding normal recommended field rates on soil microbial populations and activities [17,51,62] There may be selective inhibition of some species but others rapidly appear to replace the sensitive species [63,64,65] thus maintaining the metabolic integrity of the soil. Successive applications of the pesticides caused only slight and short lived side effects on the soil microflora and these usually disappeared before the next treatment was carried out. The results appeared to be significantly influenced by weather conditions [7A,B]. No cumulative effects on soil microorganisms [4] and on microbial activities and crop yields were observed [66].

The size and activity of the microorganism populations depend on soil organic matter quantity, quality, and distribution and have been related to soil texture [67,68], to soil pH [69], to climatic conditions [70], and to different agricultural practices [71,72]. The ratio of microbial biomass C to total organic C has proved a sensitive indicator of changes in soil organic matter due to changing management conditions [73A-B,74,75].

3.6. Plant height and crop yield

Plant height at the time of first treatment was 65 cm and plants were in the vegetative stage. At the time of the second and third treatments plants were in the flowering and reproductive stages respectively. Cotton seed yields in 1995 were 843, 1493 and 1543 kg/ha for control, T1 and T2 fields respectively and in 1996 543, 1393 and 1438 kg/ha. On average the cotton seed yield in 1995 and 1996 was higher in T2 than T1 and control. A similar trend was observed in 1998 with seed yield in the treated field 962 kg/ha compared to 298 kg/ha in the control. This could be due to the effective control of pests in the treated fields which caused less destruction of the crop compared to control where the pests caused maximum destruction leading to less yield [cf 4,75].

4. Conclusions

Insecticides in the present investigation had only temporary affects which disappeared either due to initial low deposits of insecticides on soil, less persistence of the insecticides used due to degradation by soil microorganisms. The stage of crop, spacing, canopy spread and plant leaf characteristics also influence the interception of pesticide spray by plant tops [76,77,78]. However, in the absence of proper controls (where no insecticides are used and where the crop is free from insects pests and diseases), it is not possible to conclude that the intervening inhibitions/stimulations caused by insecticide treatments really do not have any effect on the crop yield. A decrease or increase in specific microorganism(s) may affect the developmental stages of the crop which may in turn affect not

necessarily the crop yield but the quality of the crop such as thread length, colour and strength in the case of cotton. Clearly further in depth studies are required to settle this very important subject as today in many instances, even after providing many agrochemical inputs, increase in crop production is becoming increasingly difficult.

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REFERENCES

[1] DOETSCH, R.N, COOK., T.M., Introduction to Bacteria and Their Ecobiology, Univ. Park Press, Baltimore, Maryland.

[2] AMBROGIONI, L., et al., Soil biological activity under sugarbeet cultivation and pesticide application, Redia. **70** (1987) 21–50.

[3] ZHANG, L.,et al., Persistence, degradation and distribution of deltamethrin in an organic soil under laboratory conditions, J. Agric. Food Chem. **32** (1984) 1207–1211.

[4] HEINONEN-TANSKJ, H., et al., The effects of the annual use of pesticides on soil microorganisms, pesticide residues in the soil and barley yields, Pestic. Sci. **16** (1985) 341–348.

[5] TU C.M., Effect of five insecticides on microbial and enzymatic activities in sandy soil, J. Environ. Sci. Health, **B 30** (1995) 289–306.

[6A] NAUMANN K., Zur Dynamik der Bodenmikroflora nach Anwendung von Pflanzenschutzmitteln: III: Untersuchungen uber die Wirkung von Parathion-methyl auf den Mikroorganismenpopulationen von Lehm- und Sandboden, Pedobiologia **11** (1971) 227

[6B] NAUMANN K., Dynamics of the soil microflora following the application of insecticides. VI. Trials with the insecticides gamma-BHC and toxaphene, Pedobiologia **11** (1971) 286

[6C] NAUMANN K., Alterations in the bacterial flora of soil following the application of pesticides, Zentbl. Bakt. Parasitkde Infektionskrankheiten und Hygiene, Zweite Abt. **126** (1971) 530.

[7A] SCHUSTER E., SCHRODER, D., Side-effects of sequentially-applied pesticides on non-target soil microorganisms: Field experiments, Soil Biol. Biochem. **22** (1990) 367–373.

[7B] SCHUSTER E., SCHRODER, D., Side-effects of sequentially- and simultaneously- applied pesticides on non-target soil microorganisms: Laboratory experiments, Soil Biol. Biochem. **22** (1990) 375–383.

[8] ANDERSON R.N., et al., "Effect of saprophytic grazing on net mineralization", Terrestrial Nitrogen Cycles (CLARK, F. E., ROSSWALL, T., Eds.). Ecological Bulletins **33** (1981) 201–215.

[9] JENKINSON D.S., POWLSON, D. S., The effects of biocidal treatments on metabolism in soil. V, Soil Biol. Biochem. **8** (1976) 209–213.

[10] ZELLES L., M.E., et al., Measurement of bioactivity based on CO_2-release and ATP content in soils after different treatments, Chemosphere **13** (1984) 899–913.

[11] TU, C.M., Influence of pesticides on activites of invertase, amylase and level of adenosine triphosphate in organic soil, Chemosphere **11** (1982) 909–914.

[12] MALKOMES, H.P., WOHLER B., Testing and evaluting some methods to investigate soil effects of environmental chemicals on soil microorganisms, Ecotox. and Environ. Safety**. 7** (1983) 284–294.

[13] ZELLES L., et al., ATP-measurement in soil: A combination between the TCA and NRB extraction methods, Chemosphere **14** (1985) 139–148.

[14] HARRIS, C.R., SANS, W.W., Vertical distribution of residues of organochlorine insecticides in soils collected from six farms in southwestern Ontario, Proc. Entomol. Soc. Ontario **100** (1969) 156.

[15] LICHTENSTE1N, E.P., et al., Vertical distribution and persistence of insecticidal residues in soils as influenced by mode of application and a cover crop, J. Econ. Entomol. 55 (1962) 215.
[16] ALEXANDER, M., Introduction to Soil Microbiology, John Wiley and Sons, New York (1961).
[17] GREAVES, M.P.,"Side-effect testing: an alternative approach", Pesticide Effects on Soil Microflora. (SOMERVILLE, L., GREAVES, M.P., Eds) Taylor and Francis, London (l987) 183–190.
[18] WALKLEY, A., BLACK, C.A., An examination of Degtjareff methods for determining soil organic matter and a proposed modification of the chromic acid titration method, Soil Sci. **37** (1934) 29–38.
[19] SUBBIAH, V., ASIJA, G.L., A rapid procedure for the determination of available nitrogen in soils, Curr. Sci. **25** (1956) 259–260.
[20] OLSEN, S.R., et al., "Estimation of available phosphours in soils by extraction with sodium bicarbonate", U.S. Dep. Agric., Washington, D.C. (1954) 939. Methods of Soils. Plants. Waters and Fertilisers. (TANDON, H.L.S., Ed) Fertiliser Development and Consultation Organisation, India.p. 19–23.
[21] OLSON, RN., "Iron." Methods of soil analysis, Part 2, Chemical and Microbiological Methods. (BLACK, C.A., ed). ASA, Madison, WI. (1965) 966–967.
[22] ALEXANDER, M., Introduction to Soil microbiology, 2nd ed. John Wiley & Sons, New York (1977).
[23] TU, C.M., Effect of four organophosphorus insecticides on microbial activities in soil, Applied Microbiol. **19** (1970) 479.
[24] STOJANOVIC, BA., et al., Edaphic aspects of the disposal of unused pesticides, pesticides wastes and pesticide containers, J. Environ. Qual. **1** (1972) 54.
[25] SHAMIYEH, N.B., JOHNSON, L.F., Effect of heptachlor on numbers of bacteria, actinomycetes and fungi in soil, Soil Biol. Biochem. **5** (1973) 309–314.
[26] JONSSON, A., FAHRUES. G., On the effect of aldrin on soil bacteria, Ann. Royal Agr.Col.Sweden **26** (1960) 323.
[27] MARTIN, J.P., "Influence of pesticides on soil microbes and soil properties", Pesticides and Their Effects on Soils and Water. Soil Sci. Soc. Amer., AASA Special Publ. **8** (1966) 95.
[28] TU, C.M., Interactions between paraquat and microbes in soils, Weeds **8** (1968) 38.
[29] HARRIS, C.R., Laboratory studies on the persistence of biological activity of some insecticides in soils, J.Econ. Entomol. **62** (1969) 1437.
[30A] NAUMANN, K., Dynamics of the soil niicroflora following application of insecticides. I. Field trials on the effects of methyl parathion on the bacterial and actinomycetes population of soil, Zentbl. Bakt. Parasitkde Abt. **124** (1970) 743.
[30B] NAUMANN, K., Dynamics of the soil microflora following application of insecticides. II. Reaction of soil bacteria belonging to different physiological groups to field applicat ions of methyl parathion, Zentbl. Bakt. Parasitkde Abt. **124** (1970) 755.
[31] GAWADD, A.A.A., et al., Studies on soil insecticides. X. Effects of some soil insecticides on soil microorganisms. XI. Effect of some insecticides on the nitrogen transformations in treated soils, Zbl. Bakt. Abt. II, **127** (1972) 290–300.
[32] NAUMANN, K., Die Wirkung einiger Umweltfaktoren auf die Reaktion der Bodenmikroflora gegenuber Pflanzenschutzmitteln. Zbl. Bakt. Abt. II **127** (1972) 379–396.
[33] TU, C.M., Soil Biol. Biochem. **10** (1978) 451–456.
[34] JAISWAL, S.F,. Effect of some pesticides on soil microflora and their activity, J. Res. Punjab Agric. Univ. **4** (1967) 223–226.
[35] MAHMOUD, S.A.Z., et al., Effect of dieldrin and lindane on soil microorganisms, Zbl. Bakt. Abt. II **125** (1970) 134–149.
[36] LYNCH, J.M., Soil Biotechnology: Microbial Factors in Crop Productivity, Blackwell, London (1983).
[37] GUNNER, M.B., Microbial ecosystem stress induced by an organosphosphate insecticide, Mededelingen Faculteit Landbouwwetenschapen Gent. **35** (1970) 581.

[38] SIVASITHAMPARAM, K., Some effects of an insecticide (Dursban) and a weedicide (Linuron) on the microflora of a submerged soil, Ceylon Assoc. Adv. Sci. **25 (**1969) 1.
[39] SIVASITHAMPARAM,, K., Some effects of an insecticide (Dursban) and a weedkiller (Linuron) on the microfiora of a submerged soil, Riso. **19** (1970) 339–346.
[40] RICHARDSON L.T., MILLER, D.M., Fungitoxicity of chlorinated hydrocarbon insecticides in relation to water solubility and vapour pressure, Can. J. Botany **38** (1960) 163.
[41] AUDUS, L.J., The action of herbicides on the microflora of the soil, Proc. 10th Br. Weed Control Conf. (1970) 1036–1051.
[42] ROSENBERG A., ALEXANDER, M., Microbial cleavage of various organophosphorus insecticides, Appl. Environ. Microbiol. **37 (**1979) 886.
[43] LEE P.W., et al., Fate of monocrotophos in the environment, J.Agric. Food Chem**. 38** (1990) 567–573.
[44] SETHUNATHAN, N., MACRAE, I.C., Persistence and biodegradation of diazinon in submerged soils, J.Agric. Food Chem. **17** (1969) 221.
[45] WELP G., BRUMMER, G., Der Fe(III)-Reduktionstest, ein einfaches Verfahren zur Abschätzung der Wirkung von Umweltchemikalien auf die mikrobielle Aktivität in Böden, Z. Pflanzenernahr. Bodenk. **148 (**1985) 10–23.
[46] PAL, S.S., et al., Effects of benomyl on iron and manganese reduction and redox potential in flooded soil, J. Soil Sci. **30 (**1979) 155–159.
[47A] VAN DE WARF, H., VERSTRAETE, W. Estimation of active soil microbial biomass by mathematical analysis of respiration curves: development and verification of the model, Soil Biol. Biochem. **19** (1987) 253–260.
[47B] VAN DE WARF, H., VERSTRAETE, W. Estimation of active soil microbial biomass by mathematical analysis of respiration curves: calibration of the test procedure, Soil Biol. Biochem. **19** (1987) 261–265.
[48] NANNIPIERI, P.,et al., Short term nitrogen reactions following the addition of urea to a grass-legume association, Soil Biol. Biochem. **22**(1990) 549–553.
[49] CASIDA, L.E,. et al., Soil dehydrogenase activity, Soil Sci. **98** (1964) 371–3 76.
[50] SALONIUS, P.O. Effect of DDT and fenitrothion on forest-soil microflora, J. Econ. Entomol **65** (1972) 1089
[51] KALE S.P., RAGHU, K., Effect of carbofuran and its degradation products on microbial numbers and respiration in soils, Chemosphere, **18** (1989) 2345–2351.
[52] MACRAE I., et al., The fate of nitrate nitrogen in some tropical soils following submergence, Soil Sci. **105** (1967) 327–334.
[53] BARDIYA, M.C, GAUR, A.C., Influence of insecticides on CO_2 evolution from soil, Indian J.Microbiol. **8** (1968) 233.
[54] ROBSON, H., GIJNNER, H.B., Differential response of soil microflora to diazinon, P1ant Soil **33** (1970) 613–621.
[55] TATE, K.R., Influence of four pesticide formulations on microbial processes in a New Zealand pasture soil, I. Respiratory activity, N.Z. J. Agr. Res. **17** (1974) 1.
[56] BOLLEN, W.B., TU, C.M., Influence of endrin on soil microbial populations and their activity, U.S Dept. Agr. Forest Sent. Res. Paper PNW-(1971) 114, 4 pp.
[57] TU, C.M., Effect of four nematocides on activities of microorganisms in soil, Applied Microbiol. **23** (1972) 398.
[58A] TU, C.M., The effect of Mocap, N-Serve, Telone and Vorlex at two temperatures on populations and activities of microorganisms in soil, Can. J. Plant Sci. **53** (1973) 401.
[58B] TU, C.M., The temperature dependent effect of residual nematicides on the activities of soil microorganisms, Can J. Microbiol **19** (1973) 855.
[59] TU, C.M., Interaction between lindane and microbes in soils, Arch. Microbiol. **105** (1975) 131.
[60] BARTHA, R. R., et al., Stability and effects of some pesticides in soil, Applied Microbiol **15** (1967) 67.
[61] GAWADD, A.A.A., et al., Effects of some insecticides on soil microorganisms, Agrokem. Talajit. **22** (1973). 161–168.

[62] TU, C.M., MILES, J.R.W., Interaction between insecticides and soil microbes, Res Rev **64** (1976) 17–65.
[63] DOMSCH, K.H., Einflusse von Pflanzenschutzmitteln auf die Bodenmikroflora, Mitteil. Biolog. Bunds. Land-und Forstwirt. Berlin-Dahlem. **107** (1963) 1052.
[64] DOMSCH, K.H,. Soil fungicides, Ann. Rev. Phytopathol. **2** (1964) 293.
[65] DOMSCH, K.H., "Effect of fungicides on microbial populations in soil." Pesticides in the Soil: Ecology, Degradation and Movement. Internat. Svmp. on Pesticides in the Soil. East Lansing: Mich. State Univ. (1970) p. 42.
[66] HURLE K., FREHSE, H., Do soils suffer from pesticides? Proc. 7th Int. Cong. Pestic. Chem. **1** (1991) 319–327.
[67] AMATO, M., LADD, J.N., Decomposition of ^{14}C-labelled glucose and legume material in soils: Properties influencing the accumulation of organic residue C and microbial biomass C, Soil Biol. Biochem **24** (1992) 455–464.
[68] KAISER, E.A., et al. Evaluation of methods to estimate the soil microbial biomass and the relationship with soil texture and organic matter, Soil Biol. Biochem. **24** (1992) 675–683.
[69] GEHLEN, P., SCHRODER, D., Bedeutung von pH-Wert, C_{org}-Gehalt, Kultur-, Substrat- und Jahreseinfluss fur bodenmikrobiologische Eigenschaflen in einheitlich genutzten Ackerboden, VDLUFASchriftenr **30** (1989) 467–472.
[70] INSAM, H., et al., Influence of macroclimate on the soil microbial biomass, Soil Biol. Biochem. **21** (1989) 211–221.
[71] BECK, T., Mikrobiologische und biochemische charakterisierung landwirtschaftlich genutzter Boden. I. Mitteilung die Ermittlung einer bodenmikrobiologischen Kennzahl, Zeitschrift fur Pflanzenernahrung und Bodenkunde **147** (1984) 467–475.
[72] ANDERSON, T.H., DOMSCH, K.H., Ratio of microbial biomass to total organic carbon in arable soils, Soil Biol. Biochem**. 21** (1989) 471–479.
[73A] ANDERSON, T.H., KH. DOMSCH. "Carbon link between microbial biomass and soil organic matter", Proceedings of the Fourth International Symposium on Microbial Ecology. (MEGUSAR, F., CIANTAR, M. Eds.). Slovene Society for Microbiology, Ljublijana.(l986) 467–471.
[73B] ANDERSON, T.H., DOMSCH, K.H., Carbon assimilation and microbial activity in soil, Zeitschrift fur Planzenemahrung and Bodenkunde **149** (1986) 457–486.
[74] SPARLING, G.P., Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter, Aust. J. Soil Res**. 30** (1992) 195–207.
[75] ROSLYCKY, E.B., Soil Biol Biochem**. 14** (1982) 87–92.
[76] MAINI, O.S., et al., Residues from endosulfan application on cotton crop, J. Ent. Res. **6** (1982) 90–95.
[77] WILLIS, G.H., et al., Permethrin washoff from cotton plants by simulated rainfall, J. Environ. Qual. **15** (1986) 116–120.
[78] MATHEW, S., AGNIHOTRI, N.P., Fate of ^{14}C-deltamethrin applied to chickpea in a model terrestrial agroecosystem, Pestic. Res. J. **9** (1997) 46–53.

Effect of repeated pesticide applications on soil properties in cotton fields: II. Insecticide residues and impact on dehydrogenase and arginine deaminase actvities

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Abstract. Insecticides were applied sequentially at recommended dosages post crop emergence in cotton fields and soil was sampled at regular intervals after each treatment. Soil was analysed for insecticide residues and activity of the enzymes dehydrogenase and arginine deaminase. Insecticide residues detected in the soil were in small quantities and they did not persist for long. Only endosulfan leached below 15 cm. Insecticides had only temporary effects on enzyme activities which disappeared either before the next insecticide treatment or by the end of the experimental period.

1. Introduction

The use of pesticides has become an integral and economically essential part of modern agriculture. Pesticides are often applied several times during one crop season and a part always reaches the soil. The behaviour of the pesticide in soil depends upon the chemical and physical properties of the pesticide itself, environmental factors such as temperature, precipitation, wind, sunlight, soil properties such as moisture, organic matter, pH, redox status, available nutrients, interactions between solid, liquid and gaseous phases of the soil and between abiotic and biotic components. The physico-chemical nature of the soil is important for persistence, metabolism and binding of pesticides in the soil.

Several enzymes are known to be present in the soil which catalyze organic matter turnover. These enzymes are produced by various organisms and act intra- or extra- cellularly. Soil enzymes are mainly of bacterial and fungal origin. Only a small fraction is derived from plants and/or animals. Soil enzymes play a role in the degradation of litter and "foreign" substances. The enzymes most often found in soil are dehydrogenase, catalase, phosphatase, amylase, cellulase, xylanase, pectinase, saccharase, protease and urease. The role of soil enzymes, in terms of the ecosystem, is increasingly important and is defined by the relationships between soil enzymes and the environmental factors affecting their activities [1,2]. Although much soil enzyme research has evolved without thought to this ecological implication, soil enzymes are useful in describing and making predictions about an ecosystem's function, quality and the interactions among sub-systems. Perhaps the most valuable single use of soil enzymes is to assess the effects of various inputs on the relative "health" of the soil. Numerous studies have been conducted to determine changes in a soil's enzyme activities caused by acid rain, heavy metals, pesticides, and other industrial and agricultural chemicals.

2. Materials and methods

The insecticide usage, the field sites, sampling procedures, soil properties and meteorological data are the same as those described in Part I of this report.

2.1. Analysis of insecticides residues in soil

The multiresidue extraction method of [3] was used. Fifty g of soil sample in an Erylenmeyer flask was extracted with 50 mL acetone containing 1 mL 2N ammonium acetate. Sufficient water was added with continuous stirring to disintegrate the soil into small particles. The flask was shaken for 30 min

on an orbital shaker; left to stand for some time and the extracts were decanted. The extraction was repeated twice with 35 mL acetone. The extracts were filtered through a Buchner funnel which was then washed with 15 mL of acetone. The extracts were transferred to a 1 L separatory funnel, 450 mL anhydrous 2% Na_2SO_4 was added and and the extracts partitioned with 100 mL methylene chloride in three steps. The methylene chloride was reduced to 2 mL using a flash evaporator. The concentrated extract was passed through an anhydrous Na_2SO_4 column to remove any trace of water if present. Extracts were dried completely and were made to a volume of 0.5 mL or 1 mL with acetone and residues estimated directly using GLC (Hewlett Packard model 5890A Series IT gas chromatograph equipped with an ^{63}Ni electron capture detector). Table 1 shows the column specifications and other operating parameters. Two different columns were used for identification and quantification: an HP-1 column for dimethoate, monocrotophos, deltamethrin and endosulfan and an OV-101 column for cypermethrin and triazophos.

Table 1. Gas liquid chromatograph column specifications and operating conditions

Operating parameter	
Column	(i) HP-1 (Methyl Silicone Gum); 10m x 0.53mm x 2 μm film thickness (ii) 3% OV-101 Coiled glass (2m x 2mm.)
Inlet temperature	300°C
Column temperature	(i) 150°C–180°C at 5°C and then to 270°C at 10°C/min. (ii) 250°C
Detector temperature	300°C
Carrier gas	Nitrogen
Carrier gas flow rate	(i) 20 mL/min (ii) 60 mL/min
Chart speed	0.5 cm/min
Detector	ECD
Injection volume	1–5 μ1

2.2. Dehydrogenase activity

To 3g air dried soil, 1 mg glucose solution (30 mg/L) and 0.5 mL of a 3% solution of 2,3,5-triphenyl-tetrazolium chloride (TTC) were added and the volume was made to 5 mL by addition of 0.1M Tris buffer (pH7.6-7.8). After incubating at 37°C for 24h the formazan formed was extracted with 10 mL ethanol and estimated spectrophotometrically at 485nm. The concentration of formazan was calculated from a standard curve. Dehydrogenase activity is expressed as μg formazan formed/g dry wt. soil [4].

2.3. Arginine deaminase activity

Soil samples incubated with arginine produce ammonium which is extracted with potassium chloride solution and determined colorimetrically after the addition of chromogenic reagents [5]. Five g of naturally moist soil was incubated at 37°C for 3h after adding 2 mL 11.5mM arginine solution. The blank was similarly prepared, but immediately frozen at -20°C. After incubation, 18 mL 2M KCl solution was added to the soil, shaken for 30 min and filtered. Three mL KCl solution, 2 mL 0.12M sodium phenolate solution, 1 mL 0.17mM sodium nitroprusside and 1 mL sodium hypochlorite solution were added to 1 mL of the filtrate. After 30 min, the colour formed was measured spectrophotometrically against the blank at 630 nm. Enzyme activity was calculated using a standard curve.

3. Results and discussion

3.1. Insecticide residues

Residues were analysed only in 1995 and 1996. During 1995 residue analyses were carried out only in 0–15 cm soil. Initial residues were higher in 1996 than in 1995 crop period (Tables 2 & 3*)*. Residues of dimethoate declined to 21.28% and 16.87% of the initial residues in 30 days in 1995 and to 3.64% and 2.5% in 45 days in 1996 in T1 and T2 fields respectively. The dissipation pattern of dimethoate was almost same in both the years in both the fields. The half-lives of dimethoate varied from 9.12–13.44 days. No residues of dimethoate could be detected from 60 days onwards. Faster degradation of dimethoate residues has been observed by many workers [6,7]. Monocrotophos persisted until 45 days in 1995 and 60 days in 1996 [cf 8,9,10]. This could be due to high initial deposits observed in the second year. Half-lives could not be calculated as the zero day deposits were not known. Though monocrotophos is very soluble in water no residues could be detected below 15 cm layer in cotton fields [11]. Triazophos was also less persistent and no residues could be detected on 45th day. No residues were detected in 15–30 cm layer. Low persistence of organophosphorus insecticides with short half-lives ranging from 1 to 3 weeks have been observed by a number of workers [12,13,14].

Deltamethrin residues in 1995 and 1996 were detected only on day zero. Fast dissipation of deltamethrjn residues in 15 days or less has been reported [15–21]. Initial residues were higher in 1996 in the previously untreated field. Cypermethrin was more persistent than deltamethrin and larger residues were detected in 1996 than in 1995. Up to 63–67% of cypermethrin residues could be detected in 1995 15 days after treatment [cf 10,22A,22B). No residues were detected on day 105. Both the insecticides remained in the upper layer and no residues were detected in the 15–30 cm soil layer due to their highly lipophilic nature and low water solubility [23,24,25].

Insecticide residues were mainly restricted to upper 15 cm soil zone and only endosulfan leached down to 15–30cm soil [26]. The initial deposits of endosulfan in both the fields were less in 1995 than 1996. Endosulfan residues decreased to 32.78% and 58.0% of initial residues after 15 days in 1995 in T1 and T2 fields respectively whereas in 1996 it declined to 18.19% and 11.49% after 30 days in T1 and T2. Endosulfan residues in 15–30 cm soil were 1.33 and 2.65 ng/g dry wt. soil in T1 and T2 fields respectively 15 days after treatment. Endosulfan residues detected in 15–30 cm soil layer were lower than in 0–15 cm layer and decreased gradually to 11.22% in 30 days with a half-life of 9.49 days. The extent of leaching is determined by the solubility, adsorptive properties and rate of degradation of the pesticide as well as by the natural water movement in soil, and the physical and chemical characteristics of the soil. Despite endosulfan's low solubility, in a coarse textured soil, leaching apparently occurs under the influence of downward movement of rain or irrigation water. Endosulfan was more persistent in first time treated field with a half-life of 12.2days compared to 9.65 days in the field with a previous history of pesticide treatments. Dependence of endosulfan persistence on the initial concentration of residues was observed by [27]. Less persistence of endosulfan residues in soil was reported by many workers [26–30].

Interactions between pesticides applied in combination, in terms of their persistence in soils and toxicity to crops and insects, have been demonstrated [31,32]. The combinations of various pesticides may result in additive, synergistic or antagonistic effects and may deviate from the behaviour of the individual components [33]. Stage of the crop, spacing, canopy spread and plant leaf characteristics also influence the interception of pesticide spray by plant tops [34,35].

3.2. Dehydrogenase activity

Dehydrogenase activity is a measure of the intensity of microbial metabolism in soil and thus of microbial activity in soil [36]. As O_2 is excluded from the soil, the total anaerobic activity increases and is reflected in an increase in dehydrogenase activity [37].

Table 2. Insecticide residues in 0–15 cm soil layers in 1995 (ng/g dry wt. soil)

Sampling time	Field	Dimethoate	Mono-crotophos	Delta-methrin	Endosulfan	Cyper-methrin	Triazophos
0 day after	T1	16.68±3.5	-	-	-	-	-
first	T2	16.3±0.7	-	-	-	-	-
treatment							
l5 days	T1	4.08±0.07	NS**	-	-	-	-
after first	T2	3.11±0.36	NS	-	-	-	-
treatment							
30 days	T1	3.55±1.14	9.7±0.75	1.59±0.09	-	-	-
after first	T2	2.75±0.36	8.48±0.11	1.34±0.65	-	-	-
treatment							
45 days	T1	NS	7.65±0.92	ND	7.29±1.15	-	-
after first	T2	NS	5.1±0.4	ND	6.71±0.52	-	-
treatment						-	-
60 days	T1	ND*	ND	ND	2.39±0.72	1.84±0.82	-
after first	T2	ND	ND	ND	3.9±0.42	1.95±0.61	-
treatment							
75 days	T1	ND	ND	ND	ND	1.16±0.06	16.8±1.0
after first	T2	ND	ND	ND	ND	1.32±0.07	22.09±1.1
treatment							

Table 3 Insecticide residues in 0–15 cm soil layers in 1996 (ng/g dry wt. soil)

Sampling time	Field	Dimethoate	Mono-crotophos	Delta-methrin	Endo-sulfan	Cyper-methrin	Triazophos
0 day after	T1	23.35±2 3	-	-		-	-
first	T2	23.8±0.7					
treatment							
15 days after	T1	7.5±0.28	NS**	-	-	-	-
first	T2	5.86±0.24	NS				
treatment							
30 days after	T1	4.7±1.5	32.5±8.9	2.84±0.7	-	-	-
first	T2	3.67±1.5	32.3±8.0	ND*			
treatment							
45days	T1	0.85±0.16	8.95±0.35	ND*	12.64±0.34	-	-
after first	T2	0.6±0.16	8.15±0.5	ND	13.48±0.51	-	-
treatment							
60days	T1	ND	1.44±0.08	ND	2.99±0.27	NS*	-
after first	T2	ND	1.68±0.08	ND	3.12±0.15	NS	-
treatment							
75days	T1	ND	ND	ND	2.3±0.8	2.21±0.29	16.22±0.81
after first	T2	ND	ND	ND	1.55±0.2	3.04±0.5	15.11±0.61
treatment							
120 days	T1	ND	ND	ND	ND	ND	ND
after first	T2	ND	ND	ND	ND	ND	ND
treatment							

* ND; Not Detectable **NS; No Sample

No significant effect of the insecticides used was observed in 1994 the only exception being a temporary adverse effect observed after monocrotophos (Table 4). In 1995 dehydrogenase activity increased significantly in T1 and T2 field to 48.5 and 49.7 µg formazan/g dry wt.soil respectively, after dimethoate treatment in the top 15 cm soil (Table 5) whereas in 1998 no significant difference in dehydrogenase activity was observed between control and treated field two days after dimethoate treatment, however, after 22 days it reduced significantly by 40.5% in the treated field to 58.19 µg formazan/g d.wt. soil (Table 7). Inhibition in enzyme activity to 33.6 µg formazan/g d.wt. soil after monocrotophos treatment was observed in T1 in 1995 (Table 5) whereas stimulation to 53.2 µg formazan /g dry wt. soil was observed in the same field with monocrotophos in 1996 (Table 6) and to 126.53 g formazan /g dry wt. soil in 1998 20 days after monocrotophos treatment (Table 7). In 1995, after deltamethrin treatment enzyme activity in T1 and T2 fields was reduced significantly to 25.5 µg and 19.7 µg formazan/g d.wt. soil respectively in the top 15 cm (Table 5) whereas in 1996 it increased significantly to 62.1 µg formazan/g dry wt. soil in T1 (Table 6). Enzyme activity continued to remain less after endosulfan treatment in both 1995 and 1996 in T2 and T2 fields (Tables 5 and 6) whereas temporary significant stimulation to 176.6 µg formazan /g dry wt.soil was observed in the treated field two days after endosulfan treatment in 1998. Decreased enzyme activity was observed in both T1 and T2 fields (40.5% and 56.1% respectively) in 1995 (Table 5) whereas in 1998 after a temporary significant 25% stimulation, decline wasobserved 10 days after cypermethrin treatment (Table 7). However, no significant effect was observed in 1994 and 1996 (Tables 4 and 6). In 1998 temporary non-significant 17.7% stimulation was observed two days after triazophos treatment which returned to a level similar to the control field in another eight days whereas in 1996, 45 days after triazophos treatment, enzyme activity in the T1 field was significantly reduced to 23.6 µg formazan/g dry wt. soil compared to control and T2 fields in 0–15 cm soil (Table 6). In the 15–30 cm soil layer insecticides usually had no effect on the enzyme activity which could be due to no residues being present in this layer. However, stimulation in enzyme activity was observed in T2 after endosulfan treatment and a decrease observed in T1 after cypermethrin treatment in 1995 (Table 5) whereas in 1996 stimulation was observed in both the treated fields after deltamethrin treatment (Table 6), however, no residues of deltamethrin were detected in this soil layer. Inhibition or stimulation observed after the treatment could be due to the effect of the individual insecticide or it could be due to synergistic or antagonistic effect of the subsequent insecticide treatments. Overall, insecticides had no significant effect on dehydrogenase activity in 1994 and 1998 whereas an adverse effect was observed in 1995 in both the treated fields and in the T1 field in 1996. At the end of the experimental period the enzyme activity was similar in all the fields indicating that the insecticides used had only temporary effect and recovery was observed after the treatments were over. Increases in dehydrogenase activity have been reported by many workers [38,39,40].

Table 4. Soil dehydrogenase activity (µg formazan/g dry wt. soil) in 1994

*Days after first treatment	Field type	
	C	T
0	43.9	35.6
42	38.5	30.0
68	54.7	39.5
84	38.5	35.5
110	38.7	38.7
142	34.9	42.9
Grand mean	39.3	
	SEm	CD
For two fields within treatment	5.4	NS
For two treatments within field	3.6	NS

*SampIes were taken before the next treatment

Table 5. Soil dehydrogenase activity (µg formazan/g dry wt. soil) in 1995

	Depth											
	0–15 cm						15–30 cm					
	Days after 1st treatment											
Field	0	15	30	45	60	75	0	15	30	45	60	75
Control	21.5	35.6	47.7	36.3	29.9	53.3	-	31.3	11.5	27.9	17.4	53.3
TI	34.3	48.5	33.6	25.8	39.2	31.7	-	37.1	17.0	27.4	27.4	29.2
T2	20.4	!9.7	39.4	19.7	14.4	23.4	-	34.0	21.0	36.4	44.0	37.1
Grand mean				33.6						30.1		
For two fields			SEm	CD					SEm	CD		
within treatments			4.2	12.0					6.2	18.0		
For two treatments within fields			2.8	8.0					4.1	12.0		

T1- First time treated field; T2- Farmer's field

Table 6. Soil dehydrogenase activity (µg formazan/g dry wt. soil) in 1996

	Depth													
	0–15 cm							15–30 cm						
	Days after 1st treatment													
Field	0	15	30	45	60	75	120	0	15	30	45	60	75	120
C	53.3	45.	27.7	29.8	34.3	33.3	62.5	32.2	8.5	19.7	25.1	3.2	20.1	34.4
TI	52.4	46.	53.2	62.1	4.9	22.4	23.6	38.0	4.4	11.4	40.7	5.5	24.1	29.6
T2	51.6	55.	33.5	42.9	22.6	18.6	44.2	64.6	22.1	18.9	39.3	4.2	25.9	66.9
Grand mean				39.1							25.7			
For two fields within treatments				SEm	CD						SEm	CD		
					20.5						3.5	9.9		
				7.1										
For two treatments within fields				4.0	11.5						2.2	6.2		

T1- First time treated field; T2- Farmer's field

Table 7. Soil dehydrogenase activity (µg formazan/g dry wt. soil) in 1996

Treatment	Days after 1st treatment	Control±S.E	Treated
Dimethoate	(A) 2	137.64±7.96	114.16±10.65
Triazophos	(B) 22	97.77±15.34	58.19±5.12*
	(A) 24	93.60±16.66	111.66±12.3
Monocrotophos	(B) 32	94.58±6.93	85.14±7.35
Endosulfan	(B) 52	91.66±2.22	126.53±5,76**
	(A)54	146.94±6.43	176.66±4.11*
Cypermethrin	(B) 64	106.10±4.46	112.22±3.6
	(A) 66	107.22±2.22	134.16±5.4**
No Spray	76	96.10±3.73	72.08±1.64**
Post harvest	96	82.36±19.35	86.42±5.5

*p< 0.05; **p<0.01; (B) Before treatment; (A) After treatment

3.3. Arginine deaminase activity

The ammonification of arginine seems to be a common process in microorganisms [41,42]. The amount of ammonim produced depends on the C/N ratio of the amino acid, with high ammonium production at a low ratio [5]. Dimethoate did not have any affect on arginine deaminase activity in any year except for a 63.8% increase in activity in 1998 two days after its treatment (Tables 8–10). After monocrotophos treatment enzyme activity in both T1 and T2 fields decreased significantly to 0.39 and 0.36 µg N/g dry wt. soil h respectively in 1995 in the top 15 cm of soil (Table 8) whereas in 1998 enzyme activity was more (4.63 µg N/g d. wt. soil h) in the treated field compared with control 20 days after treatment. A significant increase of 24.5% in enzyme activity was also observed ten days after triazophos treatment in 1998 (Table 10). However, in 1996, 45 days after triazophos treatment enzyme activity in T1 was 1.6 µg g N/g d.wt. soil h in the top 15 cm which was significantly less than the control and T2 (Table 9). No change in enzyme activity was observed after deltamethrin and endosulfan treatment in both 1995 and 1996 (Tables 8 and 9) whereas a temporary non-significant 28.8% decrease was observed in 1998 followed by a 55.8% increase nother 10 days after endosulfan treatment. After cypermethrin treatment a 1.12 fold increase in enzyme activity was observed in T2 in 1995 (Table 8) whereas a 24.6% decrease was observed in the same field in 1996 (Table 9) and in 1998 after a significant temporary 68.4% increase, a 54.1% decline was observed in another ten days aftyer the same treatment. In the 15–30 cm soil layer the insecticides used did not show any significant effect on the enzyme activity the only exception being a decrease observed in T2 field after monocrotophos treatment in 1995 (Table 8). Enzyme activity observed in the 15–30 cm soil layer was less in all the fields compared to that in the 0–15 cm soil (Tables 8 and 9). At the end of the experimental period the enzyme activity tend to remain higher in the treated and T2 fields in 1998 and 1995 respectively whereas in other years it remained similar to control (Tables 8–10).

Repeated applications of some insecticides are known to increase the degradation of insecticides [43,44,45], however, no such thing was observed in the present investigation where the insecticides used show the same pattern of dissipation and similar half lives in both previously untreated and treated fields with a history of insecticide exposures [46,47]. Also, except for some minor differences, the field with a history of insecticide exposures did not behave differently in terms of enzyme activity from the previously untreated field.

Table 8. Soil arginine deaminase activity (µg N/g d. wt soil h^{-1}) in 1995

	Depth											
	0–15						15–30					
	Days after 1st treatment											
Field	0	15	30	45	60	75	0	15	30	45	60	75
Control	2.93	2.85	1.13	0.58	1.05	1.14	-	2.68	1.77	0.9	1.14	1.58
T1	2.63	2.38	0.39	0.39	0.46	1.52	-	2.33	1.49	0.6	0.37	1.46
T2	2.37		0.36	0.37	0.41	2.42	-	2.65:	0.63	0.44	0.34	1.81
Grand mean				1.45						1.35		
For two fields within				SEm	CD					SEm	CD	
treatments				0.23	0.66					0.31	NS	
For two treatments				0.15	0.43					0.2	NS	
within_fields												

T1- Previously untreated field; T2- Farmer's field

Table 9. Soil arginine deaminase activity (μg N/g dry wt. soil h^{-1}) in 1996

	Depth 15–30 cm Days after first treatment							0–15 cm						
Field	0	15	30	45	60	75	120	0	15	30	45	60	75	120
Control	5.1	3.6	1.2	4.9	8.5	6.5	3.0	4.7	3.0	0.9	4.5	5.5	5.1	2.3
TI	5.2	4.1	1.6	4.7	7.6	6.0	1.6	5.1	3.0	1.1	5.6	6.7	4.9	1.2
T2	5.3	3.9	1.1	3:9	7.7	4.9	2.9	4.9	3.5	1.2	4.0	6.5	4.7	2.5
Grand mean				4.4.							3.7			
For two fields				SEm	CD						SEm	CD		
within treatments				0.4	1.2						0.7	NS		
For two treatments within fields				0.3	0.8						0.4	NS		

T1- Previously untreated field; T2- Farmer's field

Table 10. Soil arginine deaminase activity (μg N/g d. wt soil h^{-1}) in 1998

Treatments	Days after treatment	Control±SE	Treated
Dimethoate	(A) 2	2.16±0.64	3.54±0.49
Triazophos	(B) 22	2.17±0.65	3.12±0.12
	(A) 24	0.84±0.17	1.3±0.47
Monocrotophos	(B) 32	2.89±0.18	3.6±0.08*
Endosulfan	(B) 52	1.72±0.15	4.63±1.82
	(A) 54	3.85±0.39	2.74±0.44
Cypermethrin	(B) 64	3.35±0.27	5.22±1.3
	(A) 66	5.28±0.15	8.89±0.48**
No Spray	76	4.49±0.68	2.06±0.09*
Post harvest	96	1.16±0.27	4.72±0.59**

*$p<0.05$; **$p<0.01$; (B) Before treatment; (A) After treatment

On average, the cotton seed yield in 1995 and 1996 was more in T2 (1543 and 1438 kg/ha respectively) than T1 (1493 and 1393 kg/ha respectively) and the control field (843 and 543 kg/ha respectively). A similar trend was observed in 1998 with cotton seed yield in the treated field 962 kg/ha compared to 298 kg/ha in the control field. This could be due to the effective control of pests in the treated fields which caused less destruction of the crop than compared to control where the pests caused maximum destruction leading to less yield [48,49]. Thus in the present investigation insecticides had only temporary affects which disappeared either due to initially low deposits of insecticides on the soil, low persistence of the insecticides used or due to degradation of the insecticides by soil microorganisms. However, in the absence of proper controls (where no insecticides are used and where the crop is free from insects pests and diseases), it is not possible to conclude that the intervening inhibitions/stimulations caused by insecticide treatments really do not have any effect on the crop yield. A decrease or increase in a specific enzyme may affect the development stages of the plant/crop which may in turn affect not necessarily the crop yield but the quality of the crop such as thread length, colour and strength. Clearly further in depth studies are required to settle this very important subject as today in many instances even after providing many agrochemicals inputs, increase in crop production is becoming increasingly difficult.

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REFERENCES

[1] BURNS, R.G.,. Enzyme activity in soil: Location and a possible role in microbial ecology, Soil Biol. Biochem. **14** (1982) 423–427.

[2] PAUL, E.A., MCLAREN, A.D., "Biochemistry of the soil subsystem", Soil Biochemistry, Vol 3, (PAUL, E.A., MCLAREN, A.D., Eds.). Marcel Dekker, New York (1975) 1–36.

[3] AMBRUS, A., et al., General method for determination of pesticide residues in samples of plant origin, soil, and water. I. Extraction and Cleanup, J. Assoc. Off. Anal. Chem. **64** (1981) 733–742.

[4] CASIDA, L.E. JR.,et al., Soil dehydrogenase activity, Soil Sci. **98 (**1964)371–376.

[5] ALEF, K., KLEINER D. Applicability of arginine ammonification as indicator of microbial activity in different soils, Biol Fertil Soils **5 (**1987) 148–151.

[6] JOSHI, B.G., RAMPRASAD, G., Residues of some common insecticides in flucured tobacco, Pesticides **3** (1969) 17.

[7] RAJUKKANNU, K., et al., Persistence of phorate and carbofuran in flooded soils, Pesticides **11** (1977) 14.

[8] TEJADA, LW.,et al., The fate of carbofuran in rice, fish and livestock farming, J. Tropical Pest Management. **39** (1990) 281–287.

[9] TEJADA,LW., et al.,. The fate and residues of pesticides in rice production, Int. J. Pest Management. **39** (1991) 281–287.

[10] PANDIT, G.K., et al.,. Persistence of a-cypermethrin in cabbage and monocrotophos in three soils of West Bengal, Pesticide Research J. **8** (1996): 132–138.

[11] LEE, P.W., et al., Fate of monocrotophos in the environment, J.Agric. Food Chem. **38** (1990) 567–573.

[12] PANDEY, S.Y., AGNIHOTRI, N.P., Effect of fertilization on the degradation of dichlorotophos, disulfoton and phorate in soil, Fertil. News **20** (1975) 32.

[13] MEHER, H.C., SETHI, C.L., Persistence of fenamiphos in soil following soil and Foliar applications under tropical conditions, Pestic. Res. J. **4** (1992) 37–41.

[14] SURI, K.S., JOLA, B.S., Persistence of chlorpyriphos in soil and its terminal residues in wheat, Pestic. Res. J**. 8** (1996) 186–190.

[15] CHAPMAN, R.A., et al., Persistence of five pyrethroid insecticides in sterile and natural, mineral and organic soil, Bull. Envt. Contam. and Toxicol. **26** (1981) 513–519.

[16] MIYAMOTO, J., MELKAMI, N., "Degradation of pyrethroid insecticides in field". In Pesticide Chemistry: Human Welfare and the Environment. Vol. II (MIYAMOTO J., KEARNEY, P.C. Eds.). Pergamon Oxford, England. (1983) 193.

[17] HILL, B.D., Persistence of deltamethrin in Lathbridge sandy clay loam, J. Environ. Sci. Health, **B18** (1983) 691–703.

[18] KHAN, S.U.,et al.,. Deltamethrin residues in an organic soil under laboratory conditions and its degradation by a bacterial strain, J. Agric. Food Chem. **36** (1988) 636–638.

[19] KUMAR, Y., N.P.AGNTHOTRI. Persistence of deltamethrin in soil, Pestic. Res. J. **3** (1991) 87–92.

[20] SUXUL, P. Dissipation of deltamethrin and fenvalerate residues in Green Gram (Vigna radiata (L.) Wilczek) under Indian climatic condition. Bull. Environ. Contam. Toxicol. **55** (1995) 562–567.

[21] MATHEW, S., AGNIHOTRI, N.P., Fate of ^{14}C-deltamethrin applied to chickpea in a model terrestrial agroecosystem, Pestic. Res. J**. 9** (1997) 46–53.

[22A] ROBERTS, T.R., STANDEN, M.E., Degradation of the pyrethroid cypermethrin NRDC 149 (plus or minus)- alpha -cyano-3-phenoxybenzyl (plus or minus)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate and the respective cis-(NRDC 160) and trans-(NRDC 159) isomers in soils, Pestic. Sci. **9** (1977) 305–319.

[22B] ROBERTS, T.R, STANDEN, M.E., Degradation of the pyrethroid insecticide WL 41706 (±) - cyano-3-phenoxybenzyl 2,2,3,3-tetra-cyclopropane carboxylate in soils, Pestic. Sci**. 8** (1977) 600–610.

[23] KHAN, S.U. Pesticides in soil environment, Elsevier Science Publication Co. Amsterdam. (1980) 24.
[24] KAUFMAN, D.D., et al., Movement of cypermethrin, decamethrin, permethrin and their degradation products in soils, J. Agric. Food Chem. **29** (1981) 239–245.
[25] SINGH, R.P., KUMAR, R., Effect of organic matter and co-solvents (acetone and methanol) on the movement of synthetic pyrethroids in soils, Pestic. Res. J. **10** (1998) 64–72.
[26] AGNIHOTRI, N.P.,et al., Persistence and movement of endosulfan in soil in a supervised field trial at Farrukhabad, Pesticide Research J. **8** (1996) 152–156.
[27] RAO, D.M.R, MURTY, A.S., Persistence of endosulfan in soils, J. Agric. Food Chem. **28** (1980) 1099–1101.
[28] MAIER-BODE, H. Residue Rev. **22** (1968) 1.
[29] VAN DYK, LP, VAN DER LINDE, A., Agrochemophysica **8** (1976) 31.
[30] STEWART, D.K.R, CAIRNS, KG., Endosulfan persistence in soil and uptake by potato tubers, J Agric Food Chem. **22** (1974) 984–986.
[31] LIANG, T.T., LICHTENSTEIN, E.P., Synergism of insecticides by herbicides: effect of environmental factors, Science **186** (1974) 1128.
[32] KAUFMAN, D.D., Biodegradation and persistence of several acetamide, acylanilide, azide,carbamate and organophosphate combinations, Soil Biol. Biochem. **9** (1977) 49.
[33] AUSPURG, B,. "Kombinationseffekte von Planzfenschtz-mitteln im Boden". Pflanzenschutzmittel und Boden, Paul Parey Verlag, Hamburg. (1985) 92–106.
[34] MAINI, O.S.,et al., Residues from endosulfan application on cotton crop, J. Ent. Res. **6** (1982) 90–95.
[35] WILLIS, G.H., L.L., et al., Permethrin washoff from cotton plants by simulated rainfall, J. Environ. Qual. **15** (1986) 116–120.
[36] TABATABAI, M.A.,. "Soil enzymes". Methods of Soil Analysis. Part 2. 2nd ed., (PAGE, A.L., et al.,Eds.). Agronomy 9, American Society of Agronomy, Madison, Wis (1982). 903–947.
[37] LADD, J.N. ''Origin and range of enzymes in soil'', Soil Enzymes, (BURNS, R. G., Ed.). Marcel Dekker, New York, (1978) 51–96.
[38] NAUMANN, K Die Wirkung einiger Umweltfaktoren auf die Reaktion der Bodenmikroflora gengenuber Pflanzenschutzmitteln, Zbl. Bakt. Abt. II **127** (1972) 379–396.
[39] MALKOMES, H.P., WOHLER, B., Testing and evaluating some methods to investigate soil effects of environmental chemicals on soil microorganisms, Ecotox. and Environ. Safety. **7** (1983) 284–294.
[40] TU, C.M. Effect of five insecticides on microbial and enzymatic activities in sandy soil, J. Environ. Sci. Health, **B 30** (1995) 289–306.
[41] SPERES, A.B. Diversity of ammonifying bacteria, Hydrobiologia. 83 (1981) 343–350.
[42] ALEF, K., KLELNER, D., Arginine ammonification, a simple method to estimate microbial activity potentials in soils, Soil Biol Biochem **18** (1986) 233–235.
[43] HUANG-XIN, FAN-DEFANG. The degradation of carbofuran after its repeated application to rice soil, Rural Eco. Environ, (China).**2** (1989) 41–44.
[44] OU, L.T., Interactions of microorganisms and soil during fenamiphos degradation, Soil Sci. Soc.Amer. J. **55** (1991) 716–722.
[45] BUHLER, W.G.,et al., Effect of enhanced biodegradation of carbofuran on the control of striped cucumber beetle (Coleoptera: Chrysomelidae) on muskmelon, J. Econ. Entomol. **85** (1992) 1910–1918.
[46] STEVENS, T.O., et al., Biodegradation of dinoseb (2-sec-butyl-4,6-dinitrophenol) in several Idaho soils with various dinoseb exposure histories, Appl. Environ.Microbiol. **56** (1990) 133–139.
[47] AHARONSON, N., KATAN, J., Delayed and enhanced biodegradation of soil-applied diphenamid, carbendazim, and aldicarb, Arch. Insect Biochem. Physiol. **22** (1993) 451–466.
[48] ROSLYCKY, E.B., Soil Biol Biochem. **14** (1982) 87–92.
[49] HEINONEN-TANSKL,. H., et al., The effects of the annual use of pesticides on soil microorganisms, pesticide residues in the soil and barley yields, Pestic. Sci. **16** (1985) 341–348.

Mineralization and volatilization of ring labelled ^{14}C- 2,4-D in three different soils

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Abstract. Mineralization and volatilization of ring labelled ^{14}C-2,4-dichlorophenoxyacetic acid in soil was studied over a period of six weeks under laboratory conditions at 25°*C* in three different soils collected from three sites, Delhi, Jaipur and Ludhiana. A very slow rate of both mineralization and volatilization was observed in all the three soils. The observed mineralization, was highest for the Delhi soil, 0.93%, followed by the Ludhiana soil, 0.73% and the Jaipur soil 0.14% in 42 days. The extent of volatilization was 0.46% for the Jaipur soil, 0.37% for the Ludhiana soil and 0.32% for the Delhi soil.

1. Introduction

Mineralization and volatilization are the two major methods for dissipation of pesticides from soil. Mineralization is the process by which pesticides are completely degraded into inorganic substances that dissipate into the environment. Volatilization, however may not be a desirable process, since, even though the pesticide is released from the soil, it still remains present in the air in undegraded form and might be harmful to susceptible species elsewhere. Low volatilization is particularly desirable for herbicides since high volatility may not only cause injury to susceptible crops nearby, but may also reduce the efficacy of the herbicide in the soil.

2,4-D is reported to be one of the most widely used herbicides worldwide, due to its low mammalian toxicity and easy degradation in soil. Most of the reports available so far on the persistence and degradation of this herbicide in soil have been from the temperate regions of the world, the climatic conditions and soil microbial population of which widely differ from those in India. The present studies were therefore, undertaken as an attempt to determine the rate of mineralization and volatilization of 2,4-D in Indian soils.

2. Materials and methods

Ring labelled ^{14}C-2,4-D with specific activity of 473.6 mBq/mmole was obtained from Sigma, USA. The unlabelled 2,4-D was received from the International Atomic Energy Agency, Vienna

The mineralization and volatilization of ring labelled 2,4-D were studied under laboratory conditions in three types of soils collected from three sites (Delhi, Jaipur and Ludhiana). The properties are listed in Table 1. Fresh soil samples (50g dry weight) were weighed into 250 mL round-bottom flasks and treated with 50 g unlabelled 2,4-D and 74 kBq of ^{14}C-2,4-D in one mL of acetone. The flasks (in triplicate for each soil type) were maintained at 25°C in a BOD incubator connected to a trapping line. Two tubes containing KOH (0.2N) solution was placed before the sample flask to absorb atmospheric CO_2. The volatile organic substances were trapped in the first two tubes after the sample flask, each containing 15 mL of ethylene glycol monomethyl ether. $^{14}CO_2$ was trapped in the last tube, in a scintillation cocktail containing 4.3 g PPO, 260 mL ethylene glycol monomethyl ether, 120 mL ethanolamine and 520 mL toluene.

The air inside the sample flask was replaced by fresh air every 24 hours. Air was sucked through the system at a flow rate of 30 mL/min, controlled by a rotameter, for 10 minutes. The experiment was run for six weeks.

Table 1. Physicochemical properties of the three soils used in the experiment.

	Delhi	Jaipur	Ludhiana
Soil Texture	Sandy loam	Silt loam	Sandy loam
Mechanical Composition			
(a) Sand %	59.3	26.3	68.0
(b) Silt %	25.9	61.4	20.0
(c) Clay %	14.8	9.8	12.0
pH	7.9±0.09	6.75±0.49	8.8± 0.23
Organic carbon (%)	0.36±0	0.49±0.016	1.3±0.02
Calcium carbonate (%)	High	1.95±0.23	-
Available P (kg/ha)	1.56±0.1	26.64±2.83	11.53
Available potash (kg/ha)	565.8±36.6	-	197.53

Sampling was carried out twice weekly in the first three weeks and once per week thereafter by collecting the solvents and replacing them with fresh solvents. KOH (0.2N) solution in each atmospheric CO_2 trap was replaced every third day.

Samples of ^{14}C volatilised organic compounds in the ethylene glycol monomethyl ether collected from the two traps were pooled together. Three aliquots of 5 mL of each were taken from the pooled sample and transferred to liquid scintillation vials. Scintillation cocktail (15 mL), Amersham BCS 104, UK, was added to each vial, the contents were mixed and radioactivity estimated in a Packard 2000 CA Liquid Scintillation Spectrometer with an automatic quench correction facility. Extent of mineralization was estimated similarly by determining radioactivity in $^{14}CO_2$ absorbing cocktail by Scintillation Spectrometer.

3. Results and discussion

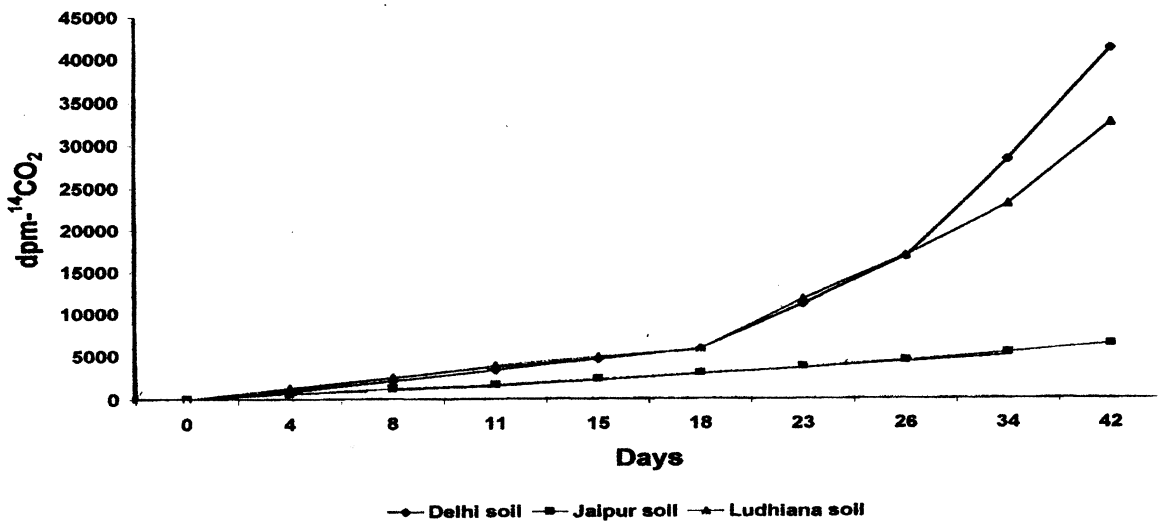
The $^{14}CO_2$ evolved from the three treated soil as a result of mineralization is shown in Table 2. The cumulative mineralization in the three soil is presented in Fig. 1. The result of the experiments show that the overall rate of mineralization in all the three soils was slow. In the first four days, the $^{14}CO_2$ trapped was 864, 579 and 1262 dpm from the Delhi, Jaipur and Ludhiana soils respectively. Similar data for 42 days were 12954, 1040 and 9552 dpm. The total mineralization at the end of 42 days was 0.93%, 0.73% and 0.14% in Delhi, Ludhiana and Jaipur soils respectively.

Almost similar observations were reported by Tejada at the second RCM of this CRP who also found that 2,4-D was quite persistent in soil and that the degradation of ^{14}C 2,4-D was generally slow with only 0.59, 0.74 and 1.3% of the applied radioactivity undergoing mineralization in acidic, neutral and basic soils respectively. It may be pointed out that the ^{14}C 2,4-D used in these experiments was from the same batch.

Table 2. Mineralization of ^{14}C 2,4-D to $^{14}CO_2$ in three different soils (ng/50 g dry wt. soil)

Days after treatment	Delhi soil	Jaipur soil	Ludhiana soil
4	77±6.6	51.50±7.68	112.3±15.56
8	103.2±5.67	50.95±7.25	103.06±8.04
11	109.3±12.75	37.87±4.24	118.43±9.77
15	111.77±6.30	60.17±3.14	92.54±8.04
18	104.09±11.78	54.73±5.73	77.87±6.98
23	480.44±34.08	62.69±4.71	533.69±66.37
26	500.73±20.02	63.89±5.69	459.33±48.89
34	1023.80±63.66	80.34±7.56	548.65±60.93
42	1152.91±144.42	92.57±6.09	850.09±42.83

Fig. 1. Cumulative $^{14}CO_2$ evolved from ^{14}C 2,4-D added to three different soils



In the microbial breakdown of any pesticide molecule, it has been suggested, that there is an initial lag phase, during which no significant breakdown of the pesticide molecule occurs [1,2,3]. This is the period during which the soil microbes adapt to the new nutrient supply and multiply to a critical size, before they can cause significant degradation of the pesticide molecules. This may be responsible for the fact that no detectable increase in the mineralization rate of 2,4-D in any of three soils studied, during the first eighteen days of the present experiment. After the eighteenth day, a rapid increase in the rate of mineralization was observed in Delhi and Ludhiana soils. The percent mineralization recorded from third to eighteenth day which had been almost constant at 0.026%/d for both the soils, increased to nearly 0.12% and 0.13% in the Delhi and Ludhiana soils respectively. This is in accordance with the pattern of microbial breakdown of the pesticide i.e. a period of rapid degradation follows the initial lag phase [2,3]. However in the Jaipur soil, no such trend was observed. The mineralization rate did not show any significant increase even after the eighteenth day, up to the end of experiment on the forty-second day. This may be due to the lack of the 2,4-D degrading microbial populations in this particular soil. McCall et al. [4] have investigated the mineralization of 2,4-D in different soil types and concluded that the variation in the extent of mineralization in different soil types was determined by the basic ability of the soil microbial population to degrade the pesticide.
From the 23rd to the 26th day, a second phase of rapid mineralization was observed in the Delhi soil and to lesser extent in the Ludhiana soil. In the former case, the increase was from 0.13% to 0.26% while in the latter case, it was from 0.12% to 0.14%. A similar second period of enhanced mineralization activity was also reported by Soulas [5] for mineralization of ring labelled 2,4-D, who offered two possible explanations for this delayed period of accelerated degradation. He said that the late development of this metabolic capacity could be related to the exhaustion of the more labile organic co-substrates which caused the microorganisms living on these to shift to and become dominant in the degradation of the xenobiotic molecule, 2,4-D. The second possibility suggested by Soulas for the second period of accelerated activity was the dissemination throughout the soil microflora of exchangeable genetic elements initially located within the more specialised microbial cells. Experimental evidence, however, for either of the two hypotheses is yet to be found.

The overall rate of mineralization in Delhi soil was little higher than that in Ludhiana soil. This can be attributed to higher organic matter content in Delhi soil, responsible for a greater microbial population

and also increased physical contact between the pesticide molecule and the microorganisms which according to Que Hee and Sutherland [6], enhances microbial breakdown.

It is obvious that mineralization is not likely to be an important factor in the dissipation of 2,4-D in the soils used. It is possible that the reduction in the availability of water in the soil as the experiment progressed may have affected the population build up of the required microbes, since the experimental design did not permit replenishment of the water content in the soil. It is also possible that the microbes were unable to adapt to the utilisation of 2,4-D as a source of energy. However, the rapid increase in the rate of mineralization observed after 18 days in case of the Delhi and Ludhiana soils may indicate that the such an adaptation had started taking place and perhaps if the experiment was continued for a longer period, the results have shown it better.

2,4-D is a herbicide known to have low volatility, which is true for both the acid and the salt forms. The ester forms, however, may vary from low to high volatility. The results of the experiments carried out for six weeks to study the volatilization of 2,4-D is given in Table 3. The cumulative volatilization of 2,4-D in the three soils is graphically presented in Fig. 2.

The overall volatilization of 2,4-D in all the three soils was very low. This can very well be attributed to the low vapour pressure of 2,4-D, since the rate of volatilization of a given compound is usually considered to be directly proportional to its vapour pressure at a given temperature [7]. Among the three soils, the maximum total volatilization over 42 days was recorded for the Jaipur soil (0.46%) followed by the Ludhiana soil (0.37%). The Delhi soil recorded the lowest volatilization of 0.32%.

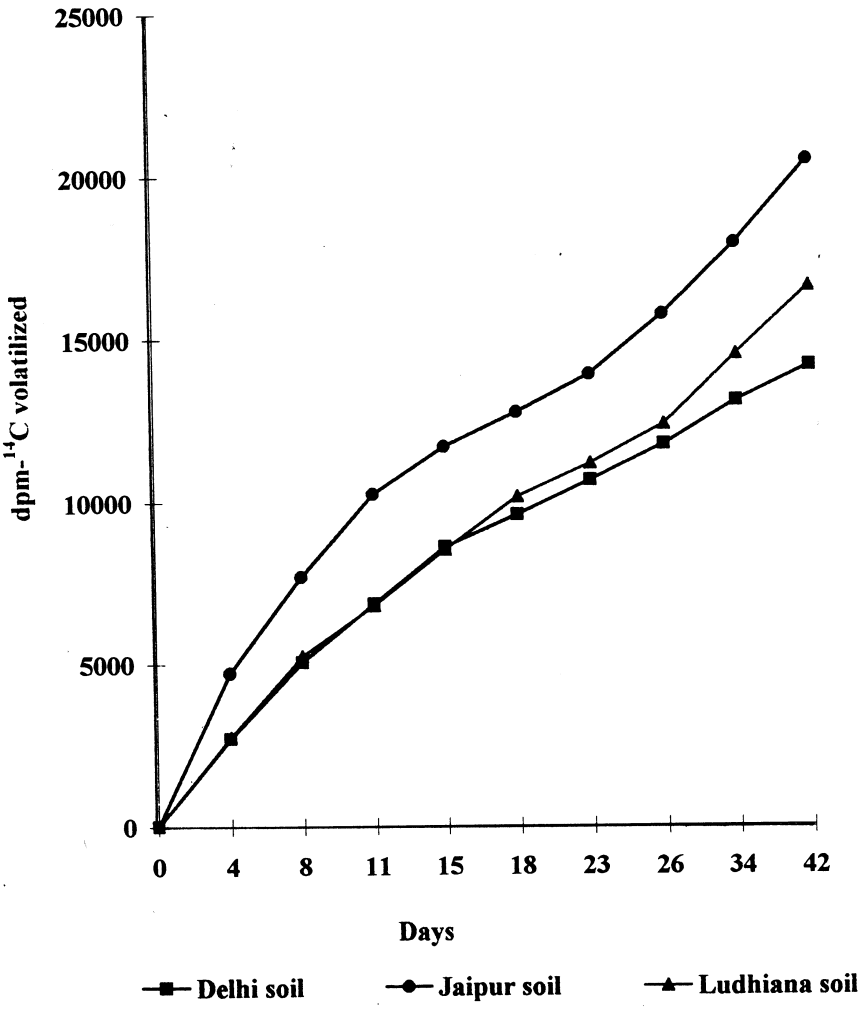
The higher volatilization exhibited by the Jaipur soil compared to the Delhi and Ludhiana soils may be attributed to the low organic matter content in Jaipur soil as well as to the fact that this soil is slightly acidic. Phenoxy herbicides such as 2,4-D being anionic, do not adsorb to the clay particles of the soil. Their principle adsorbents are organic matter [6]. Because of the low organic matter content of Jaipur soil, there is low adsorption of the herbicide, increased availability in the soil solutions and thus, higher volatilization. Also the low pH of the Jaipur soil favours the undissociated form of anionic 2,4-D, thereby increasing its potential for vapour loss as has been suggested by Plimmer [pers com].

The initial volatilization recorded in all the three cases was comparatively high. The highest value observed was for the Jaipur soil (0.1%) and for both the Delhi and Ludhiana soils it was 0.06%. On the fourth day after treatment, 2712, 4717 and 2758 dpm of ^{14}C organic volatiles were trapped from the soils of Delhi, Jaipur and Ludhiana respectively (Table 3). This was followed by a gradual decline in volatilization up to the eighteenth day in all the three cases.

Table 3. Volatilization of ^{14}C 2,4-D in soils from three different places (dpm)

Days after treatment	Delhi soil	Jaipur soil	Ludhiana soil
4	241.39±4.86	419.79±28.64	245.48±19.53
8	209.15±17.27	263.20±11.07	221.06±14.82
11	157.30±10.89	228.30±2.59	137.15±8.07
15	157.56±10.14	129.95±7.15	154.10±12.31
18	88.33±1.83	93.65±6.84	144.77±15.13
23	94.07±5.94	103.78±8.45	92.91±5.94
26	99.15±8.08	162.67±3.45	107.58±12.05
34	119.75±6.16	195.52±20.78	191.67±18.78
42	95.67±7.78	228.99±10.79	184.20±12.49

Fig.2 Cumulative ^{14}C organic volatiles evolved from 14C 2,4-D from three different soils



After 23rd day in Jaipur soil and after the 26th day in Delhi and Ludhiana soils, again a slight increase in volatilization was observed whereby 1166, 1114 and 1209 dpm were estimated on the respective days in case of Jaipur, Delhi and Ludhiana soils (Table 3 and Fig. 2). The slight increase in volatilization after the 26th day might have been due to the depletion in the soil organic matter content due to consumption by the microorganisms, as already mentioned in case of mineralization. A similar observation was reported by Soulas [5]. Therefore, reduction in soil organic matter might have caused the reduced adsorption, since the principal adsorbents for the phenoxys are the organic matter, thereby causing increased volatilization towards the end of experiment.

In many earlier studies [8,9,10], it had been shown that volatilization was the most important factor in the dissipation of organochlorine and organophosphorus insecticides and mineralization was almost insignificant in soil. However, our studies have shown that while mineralization is very low, the volatilization is even lower, being about half of the mineralization, the reasons for which can only be speculated at this time. Further studies are therefore required.

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REFERENCES

[1] WHITESIDE, J.S., ALEXANDER, M. Measurement of microbiological effects of herbicides, Weeds **8 (**1960) 204–213.

[2] GORING, C.A.I., et al., "Principles of pesticide degradation in soil". Environmental Dynamics of Pesticides (R. HAQUE, FREED, V.H., Eds). Plenum Press, New York, USA. (1975) 35–172

[3] LOOS, M.A., et al., Inexpensive soil flask for ^{14}C- pesticide degradation studies, Soil Biol Biochem. **12** (1980) 583–585.

[4] McCALL, P.J, et al., Fate of uniformly carbon-14 ring labelled 2,4,5-trichlorophenoxy acetic acid and 2,4-dichlorophenoxy acetic acid, J. Agric. Food Chem. **29** (1981) 100–107.

[5] SOULAS, G., Evidence for the existence of different physiological groups in the microbial community, responsible for mineralization in soil, Soil Biol Biochem**. 25** (1993**)** 443–449.

[6] QUE HEE, S.S. and SUTHERLAND, RU., The Phenoxyalkanoic Herbicides. Vol. I, Chemistry, Analysis and Environmental Pollution, CRC Press Inc., Florida, USA (1981) 1–4 and 245–260.

[7] QUE HEE, S.S. and SUTHERLAND, R.G., Volatilization of various esters and salts of 2,4-D, Weed Sci**. 22** (1974) 313–318.

[8] KUMAR, U. and AGARWAL, H.C., Persistence of ^{14}C-maneb and cold mancozeb on egg plants *(Solanum melongena)* under sub-tropical conditions. Pestic. Res. J. **2** (1990) 83–90.

[9] SINGH, D.K. and AGARWAL, H.C., Persistence of DDT and nature of bound residue in soil at higher altitude, Environ. Sci. and Technol. **29** (1995) 2301–2304.

[10] JINDAL, T.,et al., Dissipation of coumaphos acaricide in model cattle dipping vats and soil columns under sub-tropical climate of Delhi, IAEA-TECDOC-983 Vienna (1997) 27–37.

Dissipation and leaching of ^{14}C-monocrotophos in soil columns

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Abstract. Dissipation and leaching of ^{14}C-monocrotophos was studied in the field. Two sets of PVC cylinders were used — one set received only ^{14}C monocrotophos and the other received ^{14}C-monocrotophos along with dimethoate, deltamethrin, endosulfan, cypermethrin and triazophos at intervals of 15 days each. Each column received 37 kBq of ^{14}C-monocrotophos and 1.06 mg unlabelled monocrotophos. Both setups showed a similar pattern of dissipation with an half-life of 277.2 days. Leaching of monocrotophos was observed into the 30cm soil layer.

1. Introduction

Most of the insecticide residues present in soil originate from deliberate applications to the soil or to the foliage of crop plants and weeds, where considerable quantities reach the soil either by missing the "target" or by run-off from leaves and stems [1,2]. A proportion of the insecticides applied, eventually become incorporated into the soil. Dispersion of pesticides and their transformation products within the soil environment, or from the soil to other environments, is influenced not only by the properties of the pesticides and the soil but also the prevailing climatic conditions. Soil properties known to influence the persistence of pesticides in tropical areas include soil moisture [3] organic matter content [4,5,6], redox status [7], soil pH [8], temperature [9], sorption-desorption [10], and mineral constituents [11]. Significant interactions between pesticides applied in combination, in terms of their persistence in soils and toxicity to crops and insects, have been demonstrated [12,13]. The combinations of various pesticides may result in interactions that show additive, synergistic or antagonistic effects and may deviate from the behaviour of the individual components [14].

Leaching is an important means of dispersion of pesticides in soil. The extent of leaching is determined by the solubility, adsorptive properties and rate of degradation of the pesticide, as well as by the natural water movement in soil, and the physical and chemical characteristics of the soil. Soil organic matter content is generally inversely correlated with pesticide mobility in soil. Leaching of certain pesticides has been studied extensively by many workers [15,16,17]. A direct correlation between adsorption and soil organic matter content has been observed [18]. In the present study dissipation and leaching of ^{14}C-monocrotophos was studied individually and in combination with insecticides recommended for the cotton crop.

2. Materials and methods

2.1. Insecticides

The insecticides used were typical of those associated with cultivation of cotton and were purchased locally. Their common and IUPAC names are given below:
(i) Dimethoate; *O,O*-dimethyl *S*-methylcarbamoylmethyl phosphorodithioate
(ii) Monocrotophos; Dimethyl *(E)*-l-methyl-2-(methylcarbamoyl)vinyl phosphate
(iii) Deltamethrin; (*S*)-α-cyano-3-phenoxybenzyl(1*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclo propanecarboxylate
(iv) Endosulfan; (1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylenebismethylene) sulphite)
(v) Cypermethrin; (*RS*)-α-cyano-3-phenoxybenzyl (1*RS*)-*cis-trans*-3-(2,2-dichlorovinyl)-1,1-dimethyl-cyclopropanecarboxylate
(vi) Triazophos; *0,0*-diethyl *0*-1-phenyl-1H-1,2,4-triazol-3-yl phosphorothioate

2.2. Physical and chemical analysis of soil

Analyses were done taking appropriate samples of soil using standard techniques. Soil pH was measured using saturated soil solution (1:2.5 soil:water). Conductivity was also measured using saturated soil solution (1:2.5 soil:water). Organic carbon was estimated by the modified Walkley-Black method [19]. Plant available phosphorus was estimated by Olsen's method [20]

2.3. Soil column experiment with ^{14}C-monocrotophos

2.3.1. Layout

Polyvinyl chloride cylinders (40 cm long & 5 cm i.d.) open at both ends were used for this experiment. Cylinders were inserted in the soil in the field at the campus of University of Delhi, Delhi, India (lat. 29°N, long. 77.3°E), two weeks prior to application, with about 3 cm of the cylinder projecting above the soil surface to prevent the flow of run-off water. Two sets of PVC cylinders were used, with clean soil (with no previous insecticide history), and no plants. The first set (24 cylinders) were treated only with ^{14}C- labelled monocrotophos. The second set (24 cylinders) received ^{14}C- labelled monocrotophos plus other non-labelled insecticides. Concentrations, time and frequency of application (including the radiochemical) are given in Table 1. The first treatment was with dimethoate followed by monocrotophos (^{14}C and unlabelled monocrotophos) and others as shown in Table l. The cylinders were left until removed for sampling.

Table 1. Insecticide treatment schedule

Insecticide	Dosage (g a.i/ha)	Time of spray
Dimethoate (Rogor 30 EC)	300	0 day
Monochrotophos (Nuvacron 36 SL)*	500	15 days after first treatment
Deltamethrin (Decis 2.8 EC)	12.5	30 days after first treatment
Endosulfan (Thiodon 35 EC)	750	45 days after first treatment
Cyperrnethrin (Cypermethrin 10 EC)	60	60 days after first treatment
Trizophos (Hostathion 40 EC)	600	75 days after first treatment

*37 kBq ^{14}C-monocrotophos + 1.06 mg unlabelled monocrotophos was added

2.3.2.^{14}C-Monocrotophos

The monocrotophos was ring labelled at both methyl groups; specific activity 969.4 MBq/mol; purity by TLC 98%. Thirty seven kBq in acetone were diluted with 1.06 mg unlabelled monocrotophos and applied to each cylinder The amount used was equivalent to a concentration of 2 mg/kg in the top 15 cm of the soil.

2.3.3. Sampling

Samples were taken in triplicate 0, 90, 180, 270 and 365 days after treatment with monocrotophos. Cylinders were removed for analysis at random. The soil columns were sliced into 0–15 cm and 15–30 cm sections for analysis. The soil was air-dried, ground and mixed thoroughly and three sub-samples (from each depth) were analyzed from each cylinder. Soil was stored at -20°C till analyzed.

2.3.4. Extraction and analyses

Total residues were estimated in all samples using 300 mg aliquots in triplicate by combustion in a Harvey Biological Oxidizer (Model OX-400). Efficiency of the estimation was about 80%. A Packard 2000 CA liquid scintillation spectrometer with automatic quench correction facility was used for these estimations.

3. Results and dissussion

The soil used was a sandy loam (sand 59.3%, silt 25.9%, clay 14.8%) with pH 7.9, conductivity 152.3 μSiemens, organic carbon 0.35% and available P 1.56 kg/ha. In soil columns which received only ^{14}C-monocrotophos, 1.892 μg/g dry wt. soil was present on day zero of which only 54.88% remained 90 days after the treatment in the 0–15 cm soil layer whereas in columns treated with ^{14}C-monocrotophos along with other insecticides 1.304 μg/g dry wt. soil were present on zero day of which 74.76% remained 90 days after the treatment (Table 2). After 180 days 46% and 45.7% of the residues could be detected in both types of columns and after 365 days similar residues remained (38.32 and 39.57% of initial) in both (Tables 2,3). Total half-life calculated from 0–15 cm soil was 277.2 days for both soils. Leaching of ^{14}C-monocrotophos was observed in both experimental plots (Tables 2,3). Total residues detected in 15–30 cm soil on zero day were 0.133 and 0.155 μg/g dry wt. soil in soil columns treated with only ^{14}C-monocrotophos and in the columns which received ^{14}C-monocrotophos along with other insecticides respectively, which amount to 7.05 and 11.85% of the residues present in the 0–15 cm soil at that time (Tables 2,3).

Residues present in the 15–30 cm layer of the soil increased gradually to 0.25 μg/g dry wt. soil 180 days after treatment in the columns which received ^{14}C-monocrotophos along with other insecticides (Table 3), however, not much change in the total residues was observed from zero to 365 days. After 365 days 22.67 and 22.22% of the residues had leached to 30 cm soil. In the present experiment leaching of monocrotophos was observed into the 30cm soil layer [cf.18]. The extent of leaching is determined by the solubility, adsorptive properties and rate of degradation of the pesticide, as well as by the natural water movement in, and the physical and chemical characteristics of the soil and is generally inversely correlated with soil organic matter content [21]. The half-life of monocrotophos reported in the literature is reasonably short [18,22,23]. However, our data show that monocrotophos persists in the soil for a much longer time. This may be due in part to the leaching of monocrotophos into the soil which appears to reduce dissipation, and also the formation of bound residues which are known to persist for long periods of time in soil [24]. However, the extent of bound residues could not be estimated.

Table 2. ^{14}C-monocrotophos in soil columns treated only with monocrotophos under field conditions

Days after treatment	Total monocrotophos residues (μg/g dry wt.)	
	0–15 cm	15–30 cm
0	1.892±0.59	0.133±0.008
90	1.039±0. 18	0.147
180	0.871±0.16	-
270	0.737±0.16	0.169±0.033
365	0.725±0.05	0.164±0.021

Insecticides treatment schedule as in Table 1.

Table 3. ^{14}C-monocrotophos in soil columns treated only with monocrotophos and other insecticides under field conditions

Days after treatment	Total monocrotophos residues (μg/g dry wt.)	
	0–15 cm	15–30 cm
0	1.304±0.46	0.155±0.067
90	0.975±0.18	-
180	0.597±0.15	0.25±0.046
270	0.589±0.15	0.191±0.024
365	0.516±0.07	0.117±0.014

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REFERENCES

[1] HINDIN, E., BENNETT, P.J., Occurrence of pesticides in aquatic environments: Part I. Insecticide distribution on an agricultural plot, Tech. Ext. Service, Bull. No.317, Washington State Univ (1970).
[2] COPE, O.B. Interactions between pesticides and wildlife, Ann. Rev. Entomol. **16** (1971) 325.
[3] ROSS, D.J., Soil microbial biomass estimated by the fumigation-incubation procedure: seasonal fluctuations and influence of soil moisture content, Soil Biol. Biochem. **19** (1987) 397–404.
[4] COLEMAN, D.C.,et al., Biological strategies of nutrient cycling in soil systems, Adv. in Ecol Res. **13 (**1983) 1–55.
[5] SCHNURER, J.M. et al., Microbial biomass and activity in an agricultural soil with different organic matter contents, Soil Biol. Biochem. **17** (1985) 611–618.
[6] DUXBURY, J.M., et al., "Soil organic matter as a source and sink of plant nutrients," Dynamics of Soil Organic Matter in Tropical Ecosystems, (COLEMAN, D.C., et al., Eds.). Univ. Hawaii Press, Honolulu (1989) 33–68.
[7] SIDDARAMAPPA, R, SETHUNATHAN, N., Persistence of gamma-BHC and beta-BHC in Indian rice soils under flooded conditions, Pestic. Sci. **6** (1975) 395.
[8] AGARWAL, H.C., et al., Fate of parathion in soil under subtropical field conditions, J. Environ. Sci. Health, **B29** (1994) 189–194.
[9] YOSHIDA, T., CASTRO, T.F., Degradation of gamma-BHC in rice soils, Soil Sci. Soc. Am. Proc. **34** (1970) 440.
[10] WAHID, P.A., SETHUNATHAN, N., Sorption-desorption of lindane by anaerobic soils, J. Agric. Food Chem. **28** (1980) 623.
[11] GLASS, B.L., Relation between the degradation of DDT and iron redox system in soils, J.Agric. Food Chem **20** (1972) 324/
[12] LIANG, T.T., LICHTENSTEIN, E.P., Synergism of insecticides by herbicides: Effect of environmental factors, Science **186** (1974) 1128.
[13] KAUFMAN, D.D. Biodegradation and persistence of several acetamide, acylanilide, azide, carbamate and organophosphate combinations, Soil Biol. Biochem**. 9** (1977) 49.
[14] AUSPURG, B., "Kombinationseffekte von Pflanzenschtzmitteln im Boden", Pflanzen schutzmittel und Boden. Paul Parey Verlag, Hamburg. (1985) 92–106.
[15] KAUFMAN, D.D., et al., Movement of cypermethrin, decamethrin, permethrin and their degradation products in soils, J. Agric. Food Chem. **29 (**1981) 239–245.
[16] SOUTHWICK, LM., et al., Leaching of atrazine from sugarcane in southern Louisiana, J. Agric. Food Chem. **40** (1992) 1264–1268.
[17] AGNIHOTRI, N.P., et al., Persistence and movement of endosulfan in soil in a supervised field trial at Farrukhabad, Pesticide Research J. **8** (1996) 152–156.
[18] LEE, P.W., et al., Fate of monocrotophos in the environment, J.Agric. Food Chem. **38** (1990) 567–573.
[19] WALKLEY, A., BLACK, C.A., An examination of Degtjareff methods for determining soil organic matter and a proposed modification of the chromic acid titration method, Soil Sci. **37** (1934) 29–38.
[20] OLSEN, S.R., et al., "Estimation of available phosphorus in soils by extraction with sodium bicarbonate", U.S. Dep. Agric., Washington, D.C. Cire. 939. Methods of Soils, Plants, Waters and Fertilisers. (TANDON, H.L.S., Ed.) Fertiliser Development and Consultation Organisation, India (1954) 19–23.
[21] TEJADA, L.W.,et al., The fate of carbofuran in rice, fish and livestock farming, J. Tropical Pest Management. **39** (1990) 281–287.

[22] TEJADA, L.W., et al., The fate and residues of pesticides in rice production, Int. J. Pest Management. **39** (1991) 281–287.
[23] PANDIT, G.J.C., Persistence of α-cypermethrin in cabbage and monocrotophos in three soils of West Bengal, Pesticide Research J. **8** (1996) 132–138.
[24] SINGH, D.K., AGARWAL, H.C., Chemical release and nature of soil-bound DDT residues, J. Agric. Food Chem. **40** (1992) 1713–1716.

Impact of heavy repeated long term pesticide applications on soil properties in a cotton agroecosystem

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Abstract. A study was conducted under field conditions to investigate the effects of heavy repeated long term pesticide applications, at their recommended doses, on some biological properties in relation to the cotton agroecosystem at NIAB, Faisalabad, Pakistan. Control, test and treated soils before (BPA) and after pesticide applications (APA) were collected and analyzed at different stages of pesticide applications. The selective tests were measurements of microbial numbers, basal as well as substrate-induced respiration, nitrification, Fe-III reduction and the activities, of dehydrogenase and arginine deaminase. Endosulfan, profenophos + alphamethrin and methamidophos inhibited while monocrotophos and bifenthrin + acetamiprid enhanced the bacterial population numbers. The fungal population was depressed with endosulfan while monocrotophos, profenophos and methamidophos stimulated it. All other applied pesticides did not cause any appreciable change in total bacterial and fungal populations throughout the study period. Monocrotophos, propargite, endosulfan alone or with dimethoate and profenophos with cypermethrin or with ethion inhibited the respiration and hence affected the biomass. All other pesticides had no effect in test and treated soils compared to control soil. No pronounced inhibition or stimulation was seen in respiration after several weeks following the applications of pesticide. Endosulfan, endosulfan with dimethoate, methamidophos stimulated while profenophos + cypermethrin and bifenthrin + endosulfan inhibited the nitrification. All other pesticide applications showed similar nitrification rates in test and treated soils compared to control soil. Iron reduction capacity was stimulated by dimethoate + endosulfan and propenophos + cypermethrin and profenphos, methamidophos, propargite and diafenthiuron + profenophos reduced it. Soil dehydrogenase activity was inhibited by methamidophos, fenpropathrin, endosulfan + dimethoate and bifenthrin + ethion. Profenophos or profenophos + cypermethrin or alphamethrin stimulated the dehydrogenase activity. Arginine deaminase activity was inhibited by methamidophos, bifenthrin + acetamiprid, and endosulfan + dimethoate whereas monocrotophos, fenpropathrin, carbosulfan + fenvalerate, profenophos or profenophos with cypermethrin or ethion and bifenthrin with ethion or endosulfan stimulated it. All other pesticides did not show any effect on arginine deaminase activity.

1. Introduction

The primary role of agriculture is to produce a reliable supply of wholesome food to feed the burgeoning world population, safely and without adverse effects on the environment. The intensified agriculture in developing countries has, therefore, dictated the increasing use of agrochemicals to meet growing food demands. About 30% of the food in grown the world is lost annually because of the effects of weeds, pests and diseases [1]. In spite of the known difficulties caused as a result of pesticide usage, as yet the alternatives to practical control measures employing pesticides are still in the developing stage. Crop losses would be doubled if existing pesticide uses were abandoned. The use of pesticide chemicals has often a dramatic impact on food production. However, negative effects of pesticides on non-target organisms and their activities and on the environment must be recognized [2].

Of particular concern is the soil burden following heavy repeated pesticide applications necessary for certain crops, e.g. cotton, corn and rice. In Pakistan the major use of pesticides is on the cotton crop which accounts for 80% of the total pesticide consumption. This heavy usage may adversely affect [3] soil properties, which in turn may affect the crop yield in due course. Studies in the past have mostly

focused on fate, metabolism and impact on soil following single applications of pesticides for short periods. While useful data have been generated, investigations reflecting real situations, involving heavy repeated long term applications, have been very scanty. As soil is the most important agricultural resource, next to water, it is important to study the possible effects of specific practices on soil properties. The present study was conducted to evaluate the effects of repeated long term applications of pesticide, commonly used in cotton agrosystems, on soil properties.

2. Materials and methods

2.1. Experimental layout

The field site was located at the NIAB experimental farm. The following three plots, size 500 m^2, were established:

(a) Control soil without any pesticide treatment.
(b) Test soil (with no history of pesticide use) with pesticide treatment.
(c) Treated soil from an actual farm cotton plot.

Cotton was grown in all plots with standard fertilizer applications. Soil samples, 10 soil cores (3 cm diameter) per plot, were collected to a depth of 15 cm before pesticide application (BPA) and two days after pesticide application (APA). Soil cores from each depth were pooled, air-dried, sieved (2 mm) and stored at 4^{o}C in the dark until analyzed. The characteristics of the soils of the plots are given in Table 1.

2.2. Pesticides

The pesticides used in this study are listed in Table 2. The insecticides were applied to the plots at the recommended rates of field application. The soils collected from the three plots (a, b and c, Table 1) were monitored for the following parameters: (i) total bacterial and fungal populations; (ii) basal respiration, substrate-induced respiration, respiration quotient and biomass [4]; (iii) Fe-III reduction; (iv) nitrification; (v) enzyme activities (dehydrogenase and arginine deaminase)

The protocols developed by the First FAO/IAEA Research Coordination Meeting on "Impact of long term pesticide usage on soil properties using nuclear techniques" were used monitoring these parameters [5–6]. However, soil bacteria and fungi were enumerated by soil serial-dilution techniques and a pour-plate method [7]. One gram soil samples were used for the preparation of dilution plates in triplicate and 0.2 mL portions of appropriate soil dilutions were spread on the surface of agar plates for counts of soil microorganisms. The nutrient agar medium was used for bacteria and Rose Bengal medium for fungi [8].

Table 1. Soil physico-chemical characteristics

Soil parameters	Clean soils[a,b]	Treated soil from farm plot[c]
Saturation %	32.33	35.67
pH (saturated paste)	7.86	7.72
ECe (ms/cm)	0.19	0.21
OM (%)	0.48	0.79
Total Nitrogen (%)	0.05	0.05
Sand (%)	50.85	53.86
Silt (%)	26.36	23.12
Clay (%)	22.79	23.03
Texture	Clay Loam	Clay Loam

(a) Control soil without pesticide treatment + fertilizer + plants (cotton and rotational crop).
(b) Test soil with pesticide treatment + fertilizer + plants (cotton and rotational crops).
(c) Treated soil from an actual farm plot (cotton).

Table 2A. Pesticides used in 1997

Spray No.	Pesticides	Trade name	Formulation	Source	Dose used (L/ha)
1	Endosulfan +	Thiodan +	35EC	Hoechst	2.5
	Dimethoate	Systoate	40EC	Hoechst	1.2
2	Profenophos + Cypermethrin	Polytrin-C	440EC	Ciba Geigy	1.5
3	Profenophos + Cypermethrin	Polytrin-C	440EC	Ciba Geigy	1.5
4	Profenophos	Curacran	500EC	Ciba Geigy	2.5
5	Profenophos+ Cypermethrin	Polytrin-C	440EC	Ciba Geigy	1.5
6	Endosulfan	Thiodan	35EC	Hoechst	2.5
7	Carbosulfan +	Advantage+S	20EC	FMC	1.5
	Fenvalerate	umicidin	20EC	Granular	0.7
8	Fenpropathrin	Danital	10EC	Pan Pacific	1.5

Table 2B. Pesticides used in 1998

Spray No.	Pesticides	Trade Name	Formulation	Source	Dose used (L/ha)
1	Methamidophos	Tamaran	600 SL	Bayer	1.2
2	Monocrotophos	Novacran	40 WSC	Ciba Geigy	2.5
3	Profenophos +	Curacran +	500 EC	Ciba Geigy	2.5+
	Diafonthiuron	Polo	500 SC		0.6
4	Profenophos +	Curacran +	500 EC	Ciba Geigy	2.0+
	Alphamethrin	Bestox	5 EC	FMC	0.8
5	Bifenthrin+	Talstar +	10 EC	FMC	0.6
	Endosulfan	Thiodan	30 EC	Hoechst	2.5
6	Bifenthrin +	Talstar +	10 EC	FMC	0.6
	Acetamiprid	Mospilan	20 SP		125(g)
7	Profenophos +	Curacran +	500 EC	Ciba Geigy	2.5
	Ethion	Ethion	46.5	FMC	1.5
8	Bifenthrin+	Talstar +	10 EC	FMC	0.6
	Ethion	Ethion	46.5		3.7
9	Propargite	Omite	-	FMC	0.8
10	Bifenthrin+	Talstar+	10 EC	FMC	0.6
	Carbosulfan+	Advantage+	20 EC	FMC	1.2
	Chlorpyriphos	Lorsban	40 EC	DowElanco	2.5

The plates were incubated for 24 hours at 30±2^{o}C for bacteria and at ambient temperature (25±2^{o}C) for fungi.

3. Results and discussion

3.1. Soil microbial numbers

Pesticides applied to soil are generally considered to have minor deleterious effects on soil microorganism populations. There may be selective inhibition of some specimens but others rapidly appear to replace the sensitive species thus maintaining the metabolic integrity of the soil [9].

3.1.1.Bacterial population

Bacteria are considered to be very significant for soil fertility. The fluctuation in bacterial population may be attributed to nutritional and environmental changes, chemical pollution etc. Table 3A shows the bacterial population in test, treated and control soils ranged from 11 x 10^5 to 30 x 10^5 colony forming units (CFU)/g dry wt. soil. Fenpropathrin, carbosulfan + fenvalerate and profenophos or profenophos + cypermethrin stimulated the bacterial population in test and treated soils as compared to control soil. Maximum stimulation was observed with repeated use of profenophos + cypermethrin ranging from 11 x 10^5 to 23 x 10^5 CFU/g dry wt. soil but the bacterial population returned to the normal level in a few weeks. Endosulfan decreased the bacterial population from 23 x 10^5 to 14 x 10^5 CFU/g dry wt. soil and 23 x 10^5 to 17 x 10^5 CFU/g dry wt. soil in test and treated soils, respectively. The bacterial population in 1998 (Table 3B) ranged from 11 x 10^5 to 46 x 10^5 CFU/g dry wt. soil in test, treated and control soils. It decreased from 24 x 10^5 to 18 x 10^5 CFU/g dry wt. soil and 24 x 10^5 to 12 x 10^5 CFU/g dry wt. soil as a result of profenophos + alphamethrin and methamidophos applications respectively while monocrotophos, propargite and bifenthrin + acetamiprid increased the bacterial populations in treated soil as compared to control soil. Stimulation by profenophos or profenophos + cypermethrin and inhibition by endosulfan was also noted in our earlier study [10]. However, their populations recovered toward normal in a few days after each pesticide application. Other pesticides had no effect on bacterial numbers.

3.1.2. Fungal population

Fungi are decay organisms in the soil and have an important role in providing essential elements to higher plants. The data regarding fungal population are shown in Table 3C. Fungal populations varied from 5 x 10^4 CFU/g dry wt. soil to 6 x 10^4 CFU/g dry wt. soil in control soil showing no difference in fungal population throughout the experiment. Profenophos showed a stimulatory effect (6 x 10^4 to 9 x 10^4 CFU/g dry wt. soil) in fungal population in treated soil as compared to control soil (6 x 10^4 CFU/g dry wt. soil) while the fungal population decreased to about 35% (6 x 10^4 to 4 x 10^4 CFU/g dry wt. soil) with endosulfan application in treated soil. Similar depression was also observed in our previous study (1995–1996) where dimethoate and endosulfan depressed the fungal population. In 1998 (Table 3D) monocrotophos and methamidophos stimulated the fungal population in test and treated soils as compared to control soil. Monocrotophos showed 56% and 58% while methamidophos 56% and 48% increases over numbers before pesticide applications. All other pesticide applications did not cause any appreciable changes in the number of fungi during the season. It was observed that after insecticide applications on the cotton crop the population of fungi recovered to its normal level.

Table 3A. Impact of pesticide applications on bacterial population (x 10^5 g^{-1} dry wt. soil), 1997

	Control soil		Test soil		Treated soil	
Pesticides	BPA	APA*	BPA	APA	BPA	APA
Endosulfan+Dimethoate	23	20	17	20	12	11
Profenophos+Cypermethrin	21	15	24	18	19	19
Profenophos+Cypermethrin	15	22	19	20	19	25
Profenophos	18	18	21	19	16	16
Profenophos+Cypermethrin	18	20	21	23	10	22
Endosulfan	22	22	23	14	23	17
Carbosulfan+Fenvalerate	20	17	19	23	17	19
Fenpropathrin	22	17	20	19	17	22

*Samples taken with out pesticide at the time of test and treated samplings

Table 3B. Impact of pesticide applications on bacterial population (x 10^5 CFU/g dry wt. soil),1998

	Control soil		Test soil		Treated soil	
Pesticides	BPA	APA	BPA	APA	BPA	APA
Methamidophos	13	23	16	13	24	12
Monocrotophos	46	27	29	23	17	39
Profenophos+Diafonthiuron	17	19	22	23	14	25
Profenophos+Alphamethrin	19	32	21	20	24	28
Bifenthrin+Endosulfan	32	24	20	26	23	23
Bifenthrin+Acetamiprid	27	27	24	38	18	25
Profenophos+Ethion	26	17	18	19	11	16
Bifenthrin+Ethion	17	19	19	14	15	17
Propargite	25	20	12	23	14	23
Bifenthrin+Carbosulfan+Chlorpyriphos	27	20	18	17	15	19

Table 3C. Impact of pesticide applications on fungal population (x 10^4 CFU/g dry wt. soil), 1997

	Control soil		Test soil		Treated soil	
Pesticides	BPA	APA	BPA	APA	BPA	APA
Endosulfan+Dimethoate	5	5	6	6	6	6
Profenophos+Cypermethrin	5	5	6	6	5	5
Profenophos+Cypermethrin	5	5	6	6	6	6
Profenophos	6	6	6	8	6	9
Profenophos+Cypermethrin	6	6	5	5	7	6
Endosulfan	5	6	6	4	6	4
Carbosulfan+Fenvalerate	5	5	5	5	6	6
Fenpropathrin	5	5	5	5	6	6

Table 3D. Impact of pesticide applications on fungal population (x 10^4 CFU/g dry wt. soil), 1998

	Control soil		Test soil		Treated soil	
Pesticides	BPA	APA	BPA	APA	BPA	APA
Methamidophos	5	4	4	6	3	5
Monocrotophos	3	3	4	6	4	6
Profenophos+Diafonthiuron	5	5	5	5	5	5
Profenophos+Alphamethrin	4	4	5	5	5	5
Bifenthrin+Endosulfan	5	5	5	5	5	5
Bifenthrin+Acetamiprid	4	4	4	5	4	5
Profenophos+Ethion	4	4	5	6	5	6
Bifenthrin+Ethion	5	5	6	6	6	6
Propargite	5	6	5	6	6	6
Bifenthrin+Carbosulfan+Chlorpyriphos	5	5	6	6	6	6

3.2. Respiration

The measurement of respiration is a widely used method for the determination of overall bioactivity in soil. Respiration is mostly dependent upon the physiological conditions of the organisms as well as environmental parameters such as temperature, illumination etc [11]. In our earlier two years study it was shown that endosulfan + dimethoate and cypermethrin + profenophos showed a clear effect of inhibition of respiration in the treated soil and a return to levels similar to those in control soil in later stages [12]. The data for basal, substrate- induced respiration, metabolic quotient and biomass in 1997 are shown in Table 4A. Endosulfan showed a clear inhibition in basal, substrate-induced respiration and biomass of test and treated soils compared to control soil. Carbosulfan + fenvalerate produced an increase in biomass by decreasing substrate-induced respiration while profenophos + cypermethrin

inhibited the biomass by stimulating the basal as well as substrate-induced respiration in test and treated soils compared to control soil. In 1998 (Table 4.B) methamidophos, monocrotophos, bifenthrin + acetamiprid, profenophos + ethion, bifenthrin + ethion and bifenthrin + carbosulfan + chlorpyriphos inhibited basal as well as substrate-induced respiration. Monocrotophos, profenophos + ethion and bifenthrin + acetamiprid inhibited while bifenthrin + endosulfan, bifenthrin + ethion and bifenthrin + carbosulfan + chlorpyriphos stimulated the biomass in test and treated soils as compared to control as well as before pesticide applications. Propargite and profenophos + diafonthiuron had no effect on basal, substrate-induced respiration and biomass. No pronounced inhibition or stimulation was observed in the whole season because the biomass (Table 4C) in treated soil collected at various intervals of time, was higher than the control soil. At the time of harvest biomass was still higher in the treated soil as compared to control soil. In general, the microbial activities seemed to be recovered several weeks following the applications of the pesticide during the crop season.

3.3. Nitrification

Nitrification, the biological oxidation of reduced nitrogen to nitrite and nitrate from soil organic compounds, is the second stage of the nitrogen cycle in soil. This reaction is mainly carried out by *Nitrosomonas* spp. and *Nitrobacter* spp. In our earlier study (1995–1996) dimethoate, profenophos and carbosulfan enhanced nitrification in both the crop seasons. Monocrotophos and dimethoate in 1996 released maximum amount of nitrite and nitrate into the test and treated soils. In 1997 (Table 5A.), endosulfan and profenophos+cypermethrin inhibited while endosulfan+dimethoate enhanced nitrification. Carbosulfan+fenvalerate decreased nitrification and profenophos [13] and fenpropathrin enhanced it in test and treated soils compared to control soil. In 1998 (Table 5B.) methamidophos, monocrotophos and bifenthrin with ethion or with carbosulfan+chlorpyriphos reduced while propargite and profenophos+ethion increased nitrification in test and treated soils compared to control soil. Monocrotophos inhibited nitrification in treated soil while test soil showed no effect. Profenophos+diafonthiuron, profenophos+alphamethrin, bifenthrin+endosulfan, bifenthrin+ acetamiprid, profenophos+ethion, bifenthrin+ethion and bifenthrin+carbosulfan+ chlorpyriphos, all showed similar effects in test and treated soils compared to control soil. The changes in nitrification seemed to be affected by the environmental factors as well as management techniques [14] because similar trends were observed in control soil.

3.4. Fe-III reduction capacity

The redox potential of the soil is an important property related to soil aeration. Soil aeration influences many soil chemical reactions, which are indirectly associated with microbial breakdown of organic matter and oxidation-reduction status of the soil. The reduced form of iron and manganese are preferred by plant under tropical areas. The capacity of the microorganisms to reduce iron was used by Welp and Brumner [15] and by Pal et al. [16] for studying the effect of pesticides on the bioactivity of microorganisms in soils. The capability of anaerobic microorganisms to reduce ferric to ferrous was also affected by the applications of pesticide on the soil (Table 6A.). In the 1997 field experiments the iron reduction capacity was inhibited by the applications of dimethoate + endosulfan in test and treated soils. These results are in accordance with our earlier study in 1995–96 as well as with other workers [17]. As a result of applications of profenophos + cypermethrin, iron reduction capacity increased. Profenophos alone decreased it, however, after the application of endosulfan, the iron reduction was enhanced [18] in treated soil as compared to control soil. Carbosulfan + fenvalerate and fenpropathrin reduced the iron reduction capacity in test and treated soils. In 1998, methamidophos, propargite and profenophos with diafenthiuron or with alphamethrin decreased while monocrotophos and bifenthrin + carbosulfan + chlorpyriphos enhanced the iron reduction capacity in test and treated soils compared to control soil. The rest of the pesticides did not seem to have pronounced effects on the iron reduction capacity of soil (Table 6B.). Significant inhibition in Fe-III reduction capacity, after methamidophos applications, decreased with time and latter on its effects disappeared [19–20].

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Table 4A. Impact of pesticide applications on basal and substrate-induced respiration, metabolic quotient and biomass (1997)

Pesticides	Basal Respiration (µg CO_2/g dry wt. soil)						Substrate-induced Respiration (µg CO_2/g dry wt. soil)					
	Control soil		Test soil		Treated soil		Control soil		Test soil		Treated soil	
	BPA	APA	BPA	APA	BPA	APA	BPA	APA	BPA	APA	BPA	APA
Endosulfan + Dimethoate	21.17±1.6	22.79±2.3	24.08±1.5	21.88±1.9	22.18±0.9	23.48±4.1	40.65±3.8	39.73±1.2	40.66±4.1	39.77±1.8	35.20±1.7	35.73±4.1
Profenophos + Cypermethrin	21.43±2.4	25.53±1.9	27.45±2.3	28.72±1.2	41.34±3.2	27.12±5.2	23.19±0.9	25.03±1.4	26.56±1.3	29.20±1.4	24.30±1.4	29.64±2.8
Profenophos + Cypermethrin	25.53±1.9	26.58±3.1	28.72±3.1	29.55±1.1	21.12±2.7	28.02±1.9	25.03±2.8	36.76±2.3	29.20±2.0	42.58±1.2	19.64±2.7	44.41±1.3
Profenophos	29.50±2.1	30.03±2.1	34.03±2.7	35.63±1.4	23.40±2.8	37.22±1.5	24.60±1.7	23.31±1.5	31.96±1.8	32.49±2.8	30.69±2.1	32.60±1.4
Profenophos + Cypermethrin	15.10±2.01	16.10±1.9	17.17±1.8	20.25±1.8	15.48±0.9	18.33±2.3	28.00±3.4	30.10±1.3	31.88±1.4	54.32±1.5	29.98±3.4	49.77±1.8
Endosulfan	33.54±1.7	31.90±0.9	20.25±1.8	10.70±1.6	38.33±1.5	25.25±1.4	44.70±1.8	43.10±0.8	54.32±2.0	41.33±0.4	49.77±2.1	38.85±2.0
Carbosulfan + Fenvalerate	34.70±1.9	39.50±1.6	25.77±2.1	26.58±2.1	17.33±2.8	20.16±1.0	36.15±2.1	35.11±2.0	37.51±3.8	54.14±1.2	42.21±2.2	34.41±1.2
Fenpropathrin	30.98±1.7	32.97±1.7	26.58±1.6	31.97±1.9	30.50±0.4	31.98±2.3	29.16±1.2	30.01±1.7	31.14±2.3	32.10±1.9	31.00±1.9	31.37±3.5

	Respiration Quotient						Biomass					
Endosulfan + Dimethoate	0.52	0.57	0.59	0.55	0.63	0.66	0.23	0.26	0.27	0.25	0.28	0.30
Profenophos + Cypermethrin	0.92	1.02	1.03	0.98	1.70	0.92	0.41	0.46	0.46	0.44	0.76	0.41
Profenophos + Cypermethrin	1.02	0.72	0.98	0.69	1.08	0.63	0.46	0.32	0.44	0.31	0.49	0.28
Profenophos	1.20	1.00	1.06	1.10	0.76	1.14	0.54	0.45	0.48	0.50	0.34	0.51
Profenophos + Cypermethrin	0.54	0.54	0.54	0.37	0.52	0.37	0.24	0.24	0.24	0.17	0.23	0.17
Endosulfan	0.75	0.74	0.37	0.26	0.77	0.65	0.34	0.33	0.17	0.12	0.35	0.29
Carbosulfan + Fenvalerate	0.96	0.46	0.69	0.78	0.41	0.59	0.43	0.2 1	0.31	0.35	0.18	0.27
Fenpropathrin	1.06	1.10	0.85	0.99	0.98	1.02	0.48	0.50	0.38	0.45	0.44	0.46

Table 4B. Impact of pesticide applications on basal and substrate-induced respiration, metabolic quotient and biomass (1998)

	Basal Respiration(μgCO_2/g dry wt. soil)						Substrate-induced Respiration(μgCO_2/g dry wt. soil)					
Pesticides	Control soil		Test soil		Treated soil		Control soil		Test soil		Treated soil	
	BPA	APA	BPA	APA	BPA	APA	BPA	APA	BPA	APA	BPA	APA
Methamidophos	20.53±1.7	63.94±3.1	60.99±2.2	51.39±1.5	73.56±1.0	56.40±2.3	44.00±1.9	71.05±1.4	87.12±2.1	63.62±1.7	48.68±2.3	47.95±1.3
Monocrotophos	46.15±1.9	98.45±2.5	60.52±1.2	50.44±2.1	42.20±2.0	10.56±1.9	53.72±2.2	63.06±2.8	49.21±1.2	60.24±1.9	75.42±1.8	20.11±3.8
Profenophos+ Diafonthiuron	75.90±1.7	55.06±3.5	65.42±2.1	55.06±2.6	82.68±3.0	55.10±4.1	62.65±2.1	71.50±1.5	71.90±1.9	72.44±1.7	68.90±5.1	69.14±2.0
Profenophos+ Alphamethrin	69.87±2.0	82.40±5.0	84.88±1.2	81.28±3.0	47.53±3.1	30.66±1.8	76.77±3.4	80.29±1.2	80.78±2.4	73.99±1.7	74.32±6.5	70.64±1.8
Bifenthrin+ Endosulfan	82.41±1.2	20.13±5.2	82.40±2.6	15.80±2.6	20.78±4.1	38.90±1.3	60.64±2.1	47.93±1.4	73.99±1.6	30.10±1.6	54.44±2.9	46.32±2.9
Bifenthrin+ Acetamiprid	20.31±1.9	39.73±3.4	15.26±2.1	35.58±5.1	68.40±3.1	25.22±1.4	14.84±2.8	70.27±1.8	48.46±2.3	77.442.9	50.11±1.9	57.64±5.6
Profenophos+Ethion	15.57±1.6	30.97±1.5	15.62±0.9	15.64±1.0	38.45±4.6	25.54±1.8	49.44±2.4	54.08±4.1	34.72±1.5	49.46±.4	58.21±3.8	47.69±5.6
Bifenthrin+ Ethion	30.97±2.3	20.02±1.5	15.64±2.1	52.68±2.3	15.57±2.6	39.96±2.0	54.08±1.7	76.27±1.2	79.45±1.9	75.26±2.6	47.56±2.3	39.55±6.4
Propargite	25.30±1.0	29.94±2.3	76.32±3.0	29.74±1.0	35.30±2.5	29.06±1.2	52.65±1.9	47.52±3.9	53.30±0.9	46.04±0.6	48.04±1.7	47.04±3.5
Bifenthrin+ Carbosulfan+ Chlorpyriphos	49.41±3.1	70.08±1.9	44.61±2.0	30.40±2.0	34.84±2.5	49.73±3.5	70.58±1.2	71.50±1.1	51.93±4.1	28.94±1.6	47.42±.16	33.15±2.5

	Respiration Quotient						Biomass					
Methamidophos	0.47	0.90	0.70	0.81	1.51	1.17	0.21	0.40	0.32	0.36	0.68	0.52
Monocrotophos	0.86	1.50	1.23	0.38	0.56	0.53	0.39	0.68	0.55	0.38	0.25	0.24
Profenophos+ Diafonthiuron	1.21	0.77	0.91	0.76	1.20	0.80	0.54	0.35	0.41	0.34	0.54	0.36
Profenophos+ Alphamethrin	0.91	1.03	1.05	1.10	0.64	0.43	0.41	0.46	0.47	0.50	0.29	0.20
Bifenthrin+ Endosulfan	1.36	0.42	0.40	0.52	0.38	0.84	0.61	0.19	0.18	0.23	0.17	0.40
Bifenthrin+ Acetamiprid	1.37	0.56	0.51	0.46	1.36	0.44	0.62	0.25	0.24	0.21	0.61	0.20
Profenophos+ Ethion	0.31	0.57	0.45	0.32	0.66	0.54	0.14	0.26	0.20	0.14	0.30	0.24
Bifenthrin+ Ethion	0.57	0.26	0.20	0.70	0.33	1.01	0.26	0.12	0.10	0.32	0.15	0.45
Propargite	0.48	0.63	0.73	0.65	0.73	0.62	0.21	0.28	0.36	0.29	0.33	0.30
Bifenthrin+ Carbosulfan+ Chlorpyriphos	0.70	0.98	0.86	1.05	0.73	1.50	0.32	0.44	0.39	0.47	0.33	0.68

Table 4C. Impact of pesticide applications on basal and substrate-induced respiration(μg CO_2/g dry wt. soil), metabolic quotient and biomass (1998**)**

Sampling time	Basal respiration			Substrate-induced respiration		
	Control soil	Test soil	Treated soil	Control soil	Test soil	Treated soil
Sowing time	74.09±2.5	57.75±2.0	56.40±3.9	85.68±2.0	85.00±2.1	48.68±2.6
First pesticide application time	20.53±5.0	60.99±3.2	56.40±2.5	44.00±3.2	87.13±2.3	48.68±0.7
Second day after last pesticides application	70.08±3.9	30.40±2.6	49.73±3.6	71.51±3.2	28.95±1.2	33.15±3.6
84 days after last pesticides application	68.74±5.2	77.40±5.2	54.06±4.5	65.46±4.0	59.90±3.9	46.81±5.8
At harvest	36.34±4.5	56.85±3.8	57.29±0.6	64.27±2.0	78.75±0.9	69.45±2.8
	Metabolic quotient			Biomass		
Sowing time	0.86	0.68	1.16	0.39	0.31	0.52
First pesticide application time	0.47	0.70	1.16	0.21	0.32	0.52
Second day after last pesticides application	0.98	1.05	1.50	0.44	0.47	0.68
84 days after last pesticides application	1.05	1.29	1.15	0.47	0.58	0.52
At harvest	0.57	0.72	0.82	0.25	0.33	0.37

Table 5A. Impact of pesticide applications on nitrification (μg (NO_2+NO_3)-N/g dry wt. soil), 1997

Pesticides	Control soil		Test soil		Treated soil	
	BPA	APA	BPA	APA	BPA	APA
Endosulfan + Dimethoate	23.96±1.55	7.40±1.90	14.93±4.02	10.74±0.31	21.81±32	34.09±5.55
Profenophos + Cypermethrin	3.64±0.73	24.61±5.29	8.75±1.31	12.12±5.60	11.85±0.93	58.73±3.83
Profenophos + Cypermethrin	24.61±5.29	10.87±1.61	12.12±5.60	4.54±3.34	8.73±.3.83	11.68±3.54
Profenophos	12.73±2.03	11.05±3.20	2.15±0.55	6.16±2.20	19.27±5.93	21.75±3.09
Profenophos + Cypermethrin	15.60±3.06	17.60±5.40	5.83±2.67	12.18±7.42	24.21±3.90	13.37±8.10
Endosulfan	10.60±3.09	13.61±4.02	12.18±7.42	11.99±1.51	13.37±0.10	10.65±0.10
Carbosulfan + Fenvalerate	11.61±2.32	7.60±2.03	11.65±3.61	13.75±6.61	21.94±7.13	15.82±7.36
Fenpropathrin	19.25±3.52	18.90±5.40	13.75±6.61	25.78±6.60	15.51±5.47	23.53±5.00

Table 5B. Impact of pesticide application on nitrification (μg (NO_2+NO_3)-N/g dry wt. soil), 1998

Pesticides	Control soil		Test soil		Treated soil	
	BPA	APA	BPA	APA	BPA	APA
Methamidophos	42.20±7.0	69.35±1.7	74.80±10.1	46.50±5.5	108.43±7.4	110.69±7.2
Monocrotophos	49.39±6.1	79.25±2.4	72.23±9.6	76.62±0.9	104.59±1.1	70.91±0.4
Profenophos+ Diafonthiuron	250.20±16.7	192.17±3.7	211.27±16.6	178.40±9.4	155.91±3.2	185.55±10.4
Profenophos+ Alphamethrin	163.03±3.0	232.11±10.4	172.87±15.9	204.57±9.4	177.39±5.9	209.10±16.3
Bifenthrin+ Endosulfan	232.11±10.4	187.01±13.2	204.57±9.4	204.47±9.0	213.36±3.2	191.86±2.1

Table 5B (cont.)

Bifenthrin+ Acetamiprid	223.63±7.0	232.05±3.4	204.38±0.8	225.20±4.4	241.39±2.3	238.41±13.4
Profenophos+ Ethion	144.19±5.4	158.00±5.4	151.82±26.8	168.02±3.4	157.82±2.1	168.47±7.3
Bifenthrin+ Ethion	158.00±5.4	152.80±14.2	168.02±3.4	149.81±7.4	145.49±7.7	142.13±4.5
Propargite	142.80±5.3	118.80±6.8	145.41±16.3	163.52±3.7	143.63±1.5	159.98±2.3
Bifenthrin+ Carbosulfan+ Chlorpyriphos	170.42±8.4	172.19±2.3	169.01±7.4	166.86±6.1	182.10±3.9	164.28±8.2

Table 6A. Impact of pesticide applications on Fe-III reduction capacity (μg Fe^{+2}/g dry wt. soil), 1997

Pesticides	Control soil		Test soil		Treated soil	
	BPA	APA	BPA	APA	BPA	APA
Endosulfan + Dimethoate	0.589±0.02	1.697±0.11	1.843±0.12	1.293±0.09	2.763±0.09	1.809±0.20
Profenophos + Cypermethrin	2.602±0.06	1.238±0.04	1.241±0.06	1.403±0.02	1.669±0.06	1.741±0.09
Profenophos + Cypermethrin	1.238±0.02	2.297±0.06	1.403±0.11	2.533±0.12	1.741±0.12	2.866±0.10
Profenophos	1.205±0.03	1.422±0.01	1.450±0.09	1.408±0.09	1.985±0.25	1.484±0.20
Profenophos + Cypermethrin	1.920±0.05	2.051±0.09	2.189±0.05	1.562±0.05	2.662±0.16	1.743±0.21
Endosulfan	1.254±0.08	1.401±0.09	1.562±0.06	1.538±0.06	1.743±0.20	2.402±0.12
Carbosulfan + Fenvalerate	1.320±0.06	1.442±0.06	1.699±0.08	1.119±0.06	2.222±0.03	1.392±0.25
Fenpropathrin	1.410±0.12	1.502±0.08	1.619±0.21	1.462±0.09	1.581±0.10	1.382±0.12

Table 6B. Impact of pesticide applications on Fe-III reduction capacity (μg Fe^{+2}/g dry wt. soil), 1998

Pesticides	Control soil		Test soil		Treated soil	
	BPA	APA	BPA	APA	BPA	APB
Methamidophos	2.204±0.07	2.209±0.59	1.544±0.70	1.494±0.78	1.564±0.20	0.873±0.02
Monocrotophos	1.135±0.71	0.640±0.35	1.958±0.39	1.739±0.62	2.544±0.21	2.625±0.15
Profenophos+ Diafonthiuron	1.059±0.07	2.095±0.16	3.877±0.81	0.731±0.08	4.492±0.49	2.554±0.08
Profenophos+ Alphamethrin	2.948±0.57	2.406±0.08	0.718±0.24	0.757±0.08	3.204±0.46	1.690±0.29
Bifenthrin+ Endosulfan	2.406±0.08	0.654±0.16	0.757±0.08	3.384±0.17	0.591±0.25	0.465±0.15
Bifenthrin+ Acetamiprid	0.659±0.17	1.295±0.16	0.331±0.17	0.413±0.08	0.767±0.09	0.819±0.16
Profenophos+ Ethion	2.051±0.08	0.985±0.00	0.169±0.00	0.971±0.16	1.627±0.98	1.055±0.08
Bifenthrin+ Ethion	0.985±0.00	1.382±1.00	0.971±0.17	0.494±0.00	0.730±0.56	1.078±0.08
Propargite	0.246±0.08	0.974±0.00	2.130±0.33	0.936±0.16	2.684±0.25	0.712±0.08
Bifenthrin+ Carbosulfan+ Chlorpyriphos	1.346±0.08	0.795±0.16	1.674±0.56	1.133±0.16	2.102±0.16	2.762±0.16

3.5. Enzymatic activities

Soil enzyme activities commonly correlate with microbial parameter [21] and have been shown to be sensitive index of long term pesticide effects. Two key enzymes, dehydrogenase and arginine deaminase have been studied to know the effects of pesticides on their activities.

3.5.1. Dehydrogenase activity

Dehydrogenase activity is a measure of microbial metabolism and thus of the microbial oxidative activity in the soil. The effect of pesticides on enzyme activity is given in Table 7A. The effect of pesticides on dehydrogenase activity ranging from 53.14 to 93.65 μg formazan/g dry wt. soil in 1997 was observed in both test and treated soils. Endosulfan along with dimethoate and fenpropathrin inhibited the dehydrogenase activity while profenophos or profenophos together with cypermethrin did not inhibit but stimulated the dehydrogenase activity of the soil. The same results were also obtained in the last two years (1995–1996) study. In 1998 (Table 7B) dehydrogenase activity varied from 33 to 66 μg formazan g^{-1}dry weight soil in control, test and treated soils collected before and 48 hours after pesticide applications. Methamidophos, endosulfan and ethion along with bifenthrin inhibited the dehydrogenase activity in test and treated soils while profenophos + alphamethrin increased the dehydrogenase activity in this crop season. Dehydrogenase activity at different times in the crop season is shown in Table 7C. The dehydrogenase activity varied from 44.06 to 57.26 μg formazan/g dry wt. soil at sowing time while the time before pesticide application, it ranged from 33.37 to 49.61 μg formazan/g dry wt. soil in control, test and treated soils. When the last pesticide was applied, dehydrogenase activity decreased only in treated soil while it recovered to 52.58, 59.62 and 55.08 μg formazan/g dry wt. soil in control, test and treated soils respectively. The results showed that dehydrogenase activity was temporarily affected by the application of pesticides and later on it recovered.

Table 7A. Impact of pesticide applications on dehydrogenase activity (μg Tpf/g dry wt. soil), 1997

Pesticides	Control soil		Test soil		Treated soil	
	BPA	APA	BPA	APA	BPA	APA
Endosulfan + Dimethoate	74.19±3.60	73.63±1.03	65.64±1.02	71.21±2.36	80.77±2.30	70.55±2.54
Profenophos + Cypermethrin	73.84±8.20	73.63±2.32	80.43±5.06	80.64±5.65	73.14±1.32	75.88±2.52
Profenophos + Cypermethrin	75.02±3.06	73.63±5.09	80.64±2.23	85.76±4.65	76.55±2.60	82.19±2.13
Profenophos	74.91±4.05	74.88±0.65	85.76±1.02	93.65±3.25	56.21±2.32	82.16±2.63
Profenophos + Cypermethrin	73.57±2.06	70.60±3.52	53.90±2.65	77.24±4.23	58.71±1.32	74.23±1.32
Endosulfan	65.28±3.12	74.23±2.32	65.28±3.25	74.08±5.36	57.74±5.65	62.27±5.23
Carbosulfan + Fenvalerate	74.23±5.06	73.17±1.02	76.76±1.36	77.28±2.65	71.36±4.54	72.98±3.25
Fenpropathrin	66.61±8.01	71.70±2.02	75.10±3.25	70.93±2.03	73.72±3.65	66.77±1.02

The overall dehydrogenase activity (4 years study) varied from 25.88 to 93.65 μg formazan g^{-1} dry weight soil. With the exception of some pesticides or their mixtures (combined application) which did not have observable effects on the dehydrogenase activity, endosulfan, methamidophos and bifenthrin have inhibitory while profenophos had stimulatory effects in both test and treated soils as compared to control soil. There was a great variation in dehydrogenase activity in the control soil indicating that it was effected by many ecological factors like temperature, seasonal dynamics [22] and probably it is related to soil specific reactions (e.g. redox potential, water flow, air supply).

Dehydrogenase activity, since it requires an intact cellular electron transport system, is not found out side the microbial cell. This activity is sensitive to any change in the soil environmental conditions that would stimulate or retard microbial metabolic rates.

Table 7B. Impact of pesticide applications on dehydrogenase activity (μg Tpf/g dry wt. soil), 1998

Pesticides	Control soil		Test soil		Treated soil	
	BPA	APA	BPA	APA	BPA	APA
Methamidophos	33.37±0.69	38.58±2.69	40.03±0.81	39.92±3.58	49.62±3.03	51.10±0.79
Monocrotophos	43.32±2.82	44.41±1.03	46.39±0.90	45.76±0.74	47.98±0.42	45.70±1.37
Profenophos+ Diafonthiuron	46.88±1.31	47.69±2.41	49.63±1.17	48.28±1.15	48.69±1.12	47.09±0.50
Propfenophos+ Alphamethrin	45.71±0.38	40.42±3.33	43.20±0.51	49.22±0.58	40.52±0.96	48.94±0.05
Bifenthrin+ Endosulfan	60.42±3.33	53.03±3.08	49.22±0.58	43.54±0.29	30.95±1.23	30.14±0.59
Bifenthrin+ Acetamiprid	43.51±0.50	42.19±0.56	39.23±1.45	42.94±0.40	42.42±1.31	47.94±1.39
Profenophos+ Ethion	42.56±1.43	38.96±1.44	42.18±1.23	39.99±1.50	39.71±1.60	38.13±0.76
Bifenthrin+ Ethion	47.96±1.44	45.58±0.73	40.99±1.50	41.99±1.00	50.67±0.49	44.42±0.76
Propargite	45.07±5.25	42.93±2.98	44.87±1.11	43.31±3.16	41.91±2.42	43.88±2.96
Bifenthrin+ Carbosulfan+ Chlorpyriphos	44.22±2.99	45.60±0.96	45.62±4.00	45.35±2.86	47.43±3.82	45.98±0.34

Table 7C. Impact of pesticide applications on dehydrogenase activity (μg Tpf/g dry wt. soil), 1998

Sampling time	Control soil	Test soil	Treated soil
Sowing time	57.26±3.08	44.06±1.82	49.61±3.71
First pesticide application time	33.37±0.84	40.03±0.69	49.61±3.71
Second day after last pesticides application	48.60±1.17	44.35±3.50	40.95±0.39
84 days after last pesticides application	51.21±0.65	57.58±0.19	57.55±0.17
At harvest	52.58±0.15	59.62±5.59	55.08±2.12

3.5.2. Arginine deaminase activity

Arginine deaminase activity is strongly related to respiration (index of oxygen consumption), carbon content and poorly with microbial population fluctuations but is a potential indicator of microbial activities. In 1997 (Table 8A) fenpropathrin, carbosulfan+fenvalerate and profenophos or profenophos +cypermethrin enhanced the arginine deaminase activity in test and treated soils compared to control soil. The increase in arginine deaminase activity was similar to our earlier reported results [10] in which significantly higher deaminase activity was observed with the application of profenophos or profenophos+cypermethrin in test and treated soils in both the crop seasons. Endosulfan or endosulfan +dimethoate inhibited the arginine deaminase activity in test and treated soils compared to control soil. In 1998 field experiments (Table 8B) methamidophos, bifenthrin + acetamiprid inhibited [12] while monocrotophos, bifenthrin + endosulfan, profenophos + ethion and bifenthrin + ethion stimulated the arginine deaminase activity in test and treated soils as compared to control soil. Profenophos + alphamethrin, profenophos + diafonthiuron, propargite and bifenthrin + carbosulfan + chlorpyriphos did not show any effect on the arginine deaminase activity, their trend was similar to control soil which may be due to management techniques or environmental effect. A comparison of data collected during the whole season (Table 8C) at different time intervals did not show any significant effect on the arginine deaminase activity. Arginine deaminase activity was inhibited ~ 22% two days after the

last pesticide application in test and treated soils while at harvest values of arginine deaminase activity were similar to those at sowing time.

Table 8A. Impact of pesticide applications on arginine deaminase activity (μg N/g dry wt. soil), 1997

Pesticides	Control soil		Test soil		Treated soil	
	BPA	APA	BPA	APA	BPA	APA
Endosulfan + Dimethoate	0.319±0.23	0.602±0.10	0.996±0.09	0.470±0.00	0.268±0.01	0.201±0.06
Profenophos + Cypermethrin	1.532±0.32	1.700±0.05	1.062±0.06	3.791±0.06	1.455±0.02	1.819±0.04
Profenophos + Cypermethrin	1.700±0.01	0.462±0.09	3.791±0.01	2.243±0.02	0.520±0.01	0.675±0.02
Profenophos	0.570±0.23	0.320±0.02	1.207±0.05	2.264±0.21	0.632±0.01	0.646±0.02
Profenophos + Cypermethrin	0.775±0.10	0.680±0.04	2.252±0.02	3.981±0.30	0.318±0.02	0.958±0.06
Endosulfan	1.030±0.02	1.110±0.05	3.980±0.06	0.923±0.05	0.958±0.03	0.507±0.03
Carbosulfan + Fenvalerate	0.620±0.03	0.502±0.02	2.662±0.01	2.762±0.06	0.475±0.01	0.707±0.01
Fenpropathrin	0.703±0.10	0.610±0.10	1.128±0.10	3.161±0.09	0.705±0.02	1.384±0.02

Table 8B. Impact of pesticide applications on arginine deaminase activity (μg N/g dry wt. soil), 1998

Pesticides	Control soil		Test soil		Treated soil	
	BPA	APA	BPA	APA	BPA	APA
Methamidophos	0.626±0.02	1.012±0.08	0.909±0.01	0.627±0.28	1.119±0.01	0.856±0.08
Monocrotophos	1.081±0.08	1.038±0.14	1.052±0.10	1.112±0.01	0.745±0.09	1.221±0.01
Profenophos+ Diafonthiuron	1.136±0.02	1.087±0.00	0.929±0.01	1.024±0.01	1.093±0.05	1.051±0.03
Profenophos+ Alphamethrin	1.308±0.00	0.984±0.02	1.512±0.04	1.103±0.05	2.093±0.11	1.046±0.16
Bifenthrin+ Endosulfan	0.984±0.02	0.718±0.33	1.103±0.05	1.118±0.08	0.975±0.21	1.176±0.00
Bifenthrin+ Acetamiprid	1.095±0.07	1.329±0.01	1.640±0.06	1.206±0.09	0.531±0.26	0.262±0.07
Profenophos+ Ethion	1.381±0.08	1.272±0.00	1.273±0.10	1.324±0.05	1.151±0.04	1.351±0.03
Bifenthrin+ Ethion	1.272±0.00	1.190±0.00	1.324±0.05	1.255±0.05	0.882±0.25	1.237±0.01
Propargite	0.793±0.05	1.371±0.05	1.014±0.06	1.364±0.04	1.243±0.17	1.380±0.02
Bifenthrin+ Carbosulfan+ Chlorpyriphos	1.395±0.02	1.826±0.16	1.125±0.15	0.794±0.05	0.876±0.24	0.806±0.18

Table 8C. Impact of pesticide applications on argenine deaminase activity (μg N/g dry wt. soil), 1998

Sampling time	Control soil	Test soil	Treated soil
Sowing time	1.117±0.01	1.129±0.02	1.120±0.01
First pesticide application time	1.626±0.03	0.906±0.00	1.119±0.01
Second day after last pesticides application	1.026±0.21	0.794±0.06	0.806±0.22
84 days after last pesticides application	1.307±0.01	1.239±0.02	1.106±0.04
At harvest	1.155±0.36	0.972±0.21	1.213±0.05

4. Conclusions

4. Conclusions

Soil is a dynamic system, which is influenced by various environmental factors; all of which ultimately determined the health of the soil, which in turn affects crop production. Crop protection by pesticides results in pesticide residues in the soil, which is ultimately the sink of all these xenobiotic compounds. Accumulations of these chemicals occur as a result of deliberate use in agriculture. Chemical and biological methods were used to assess the effect of pesticide applications in a cotton agroecosystem under field conditions. The conclusions drawn from four years study are given below:

Endosulfan and methamidophos inhibited while monocrotophos and bifenthrin along with acetamiprid enhanced the total bacterial population. The total fungal population was also inhibited by dimethoate while endosulfan; monocrotophos, profenophos and methamidophos stimulated it. Methamidophos, monocrotophos, endosulfan alone or with dimethoate and profenophos along with cypermethrin or with ethion and bifenthrin with acetamiprid or with carbosulfan+chlorpyriphos inhibited respiration and hence the biomass. No inhibition or stimulation was noted several weeks after the applications of pesticide. Endosulfan, endosulfan with dimethoate, methamidophos stimulated while profenophos+ cypermethrin and bifenthrin+endosulfan inhibited nitrification. Nitrification rates following all other pesticide applications were similar in test, treated and control soil.

Some insecticides like methamidophos, carbosulfan, fenpropathrin and endosulfan+dimethoate inhibited while profenophos or profenophos+cypermethrin stimulated dehydrogenase activity. Arginine deaminase activity was also inhibited by methamidophos, bifenthrin+acetamiprid and endosulfan+dimethoate whereas monocrotophos, fenpropathrin, carbosulfan+fenvalerate, profenophos or profenophos with cypermethrin or ethion and bifenthrin with ethion or endosulfan stimulated it. All other pesticides had no effect on dehydrogenase and arginine deaminase activities. It was observed that these inhibition or stimulation effects were temporary as the enzymatic activities recovered later. All these parameters correlated and are related directly with microbial population.

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REFERENCES

[1] INTERNATIONAL ATOMIC ENERGY AGENCY, Radiotracer studies of pesticide residues in stored products, IAEA-TECDOC-405, Vienna, Austria (1987) 97.

[2] [TU, C.M., MILES, J.R.W., Interactions between insecticides and soil microbes, Res. Rev. **64** (1976) 17–65.

[3] [NANNIPIERI, P., BOLLAG, J.M., Use of enzymes to detoxify pesticide-contaminated soils and waters, J. Environ. Qual. **20** (1991) 510–517.

[4] BECK, T., et al., E., An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C, Soil Biol. Biochem. **29** (1997) 1023–1032.

[5] INTERNATIONAL ATOMIC ENERGY AGENCY, Study protocols developed at the first FAO/IAEA Research Coordination Meeting on "Impact of long term pesticide usage on soil properties using nuclear techniques", Neuherberg (near Munich) Germany, 29 May–2 June (1995).

[6] SCHINNER,F.,et al., Eds, Methods in Soil Biology, Springer Lab Manual, Springer, New York (1996).

[7] JOHNSON, L.F., et al., Methods for Studying Soil Microflora-Plant Disease Relationships, Burgess Pub. Co. Minneapolis Min. U.S.A (1959).

[8] MARTIN, J.P., Rose Bengal and streptomycin in the plate method for estimating soil fungi, Soil Sci. **69** (1950) 215–232.

[9] TU, C.M, Effect of five insecticides on microbial and enzymatic activities in sandy soil, J.Environ. Sci, Health, B**30** (3), (1995) 289–306.
[10] HUSSAIN, A.,et al., Second FAO/IAEA Research Coordination Meeting Bangkok, Thailand, 22–26 September (1997).
[11] ZELLES, L., et al., Side effects of some pesticides on non-target soil microorganisms, J. Environ. Sci. Health, B**20** (5) (1985) 457–488.
[12] BUJIN, XU, et al., Effect of methamidophos on soil microbial activity, Environmental Behaviour of Crop Protection Chemicals Proceeding of an International Symposium IAEA-SM-343/19, Vienna, (1997).
[13] AMELLA, W.T., Second FAO/IAEA Research Coordination Meeting Bangkok, Thailand, 22–26 September (1997).
[14] KANDELER, E., et al., Long term of monitoring of microbial biomass, N mineralization and enzymatic activities of a chernozem under different tillage management, Biol. Fertil. Soil, **28** (1999) 343–351.
[15] WELP, G., BRUMMER, G., Der Fe (III)-reduktionstest, ein einfaches verfahren zur abschatzung der wirkung von umweltchemikalien auf die mikrobielle aktivitat in boden, Z. pflanzenernahr. bodenk. **148** (1985) 10–23.
[16] PAL, S. S.,et al., Effect of benomyl on iron and manganese reduction and redox potential in flooded soil, J. Soil Sci., **30** (1979) 155–159.
[17] AGARWAL, H.C., et al., Second FAO/IAEA Research Coordination Meeting Bangkok, Thailand, 22–26 September (1997).
[18] XU, B.,et al., Second FAO/IAEA Research coordination meeting Bangkok, Thailand, 22–26 Sept. (1997) 45.
[19] BEHKI, R., KHAN, S.U., Second FAO/IAEA Research coordination meeting Bangkok, Thailand, 22–26 Sept. (1997).
[20] TAYAPUTCH, N., et al., Second FAO/IAEA Research coordination meeting Bangkok, Thailand, 22–26 Sept. (1997).
[21] FRANKENBERGER, W. T., DICK, W. A., Relationships between enzyme activities and microbial growth and activity indices in soil, Soil Sci. Soc. Am. J., **47** (1983) 945–951.
[22] TATE III, R. L., “Soil enzymes and organic matter transformations”, in Soil Organic Matter Biological and Ecological Effect, John Wiley & Sons, New York (1987) 69–94.

Mineralization and volatilization of ^{14}C-ring labelled 2,4-dichlorophenoxy acetic acid in Pakistani soils

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Abstract. The mineralization and volatilization of [U-ring ^{14}C] 2,4-D in three Pakistani soils was investigated under laboratory conditions using 50 g of soil and uniform distribution of 1.345 μg/g of 2,4-D. Maximum losses of $^{14}CO_2$ and volatile organic compounds occurred at day 7 and losses gradually slowed down after 21 days of incubation. The relative distribution of ^{14}C losses differed with soil type. Volatilization was higher in control soil as compared to test and treated soil in both the study seasons. The contribution of volatile material to the total loss in 1997 was highest in test soil (24.4%), and lowest in farm soil (19.8%) but in 1998 was highest in control soil (26%) and lowest in test and farm soils (7%) during 1998.

1. Introduction

The herbicide 2,4-dichlorophenoxy acetic acid (2,4-D) has been used extensively in agriculture since the mid-1940s and has been considered among the less toxic of the commonly used pesticides. The persistence of 2,4-D in soils has also been studied under field and laboratory conditions and there is considerable evidence available that 2,4-D is rapidly broken down in the soil by microorganisms [1–3]. The effects of various environmental parameters have been investigated in a variety of soil types. However, when ^{14}C tracer studies have been conducted, the tracer has usually been in the side chain so that transformations of the ring itself have been studied only in a few cases [4]. The objectives of this study were to measure mineralization and volatilization of uniformly ring-labeled ^{14}C-2,4-D in control, test and farm soils under laboratory conditions.

2.1. Materials and methods

2.1.1. Chemicals

The [U-ring-^{14}C] 2,4-D (2,4-dichlorophenoxyacetic acid); specific activity 473.6 MBq/mmol was supplied by the International Atomic Energy Agency, Vienna, Austria. The analytical grade cold 2,4 D was from Feinbiochemica. Heidelberg, Germany. All other chemicals were of analytical grade and solvents were reagent grade, freshly distilled before use.

2.1.2. Soils

The characteristics of the soils used to determine the soil capacity to mineralize ^{14}C-labelled aromatic pesticide, the [U-ring-^{14}C] 2,4-D, are given in previous paper.

2.1.3. Experimental set up.

For mineralization and volatility measurements, 50 g (dry weight basis) fresh soil samples were weighed into 250 mL round-bottom flasks and treated with 50 μg cold 2,4-D + 37 MBq of ^{14}C-2,4 D in a small amount of acetone. This produced a concentration of 1.345 μg/g. The flasks (in triplicates, for each soil type--control soil, test soil and treated soil) were kept at 22 ± 1^{o}C by a cryostat and

connected to a sequence of traps [5]. The volatile organic substances were trapped in 10 mL ethyleneglycol monomethylether and $^{14}CO_2$ was absorbed by 10 mL 1N KOH solution. The system was flushed with air once a day for few minutes by a pump to help trap the ^{14}C-products. The experiment was run for 6 weeks. Samples were taken twice weekly in the first 3 weeks and weekly thereafter by collecting the solvents from the traps and replacing them with fresh solvents.

2.1.4. Determination of radioactivity

Measurements were made in a liquid scintillation spectrometer (Nuclear Enterprise LSC- with Scale-Ratemeter (SRS). Volatilized organic compounds were counted in a toluene based mixture, containing PPO (4 g/L) and POPOP (10 mg/L). The trapped $^{14}CO_2$ was counted in Instagel. Data were expressed as % of the originally applied radioactivity.

3. Results and discussion

The data for mineralized $^{14}CO_2$ are shown in Fig. 1. Fast mineralization was observed in the first 10 days after the application of labelled pesticide and losses gradually slowed down after 21 days in both the study periods (1997–1998). Relative proportion of ^{14}C losses as $^{14}CO_2$ differed with soil type. This is understandable for $^{14}CO_2$ which results mainly from oxidative activities of soil microflora which are more numerous in the top layer of soil relatively rich in organic matter.

The data for organic volatile compounds are given in Table 1. Fast volatilization was observed in the first 7 days and latter on it gradually slowed down allowing stability after 21 days during 1997 but no volatilization was found after 21 days in control, test and treated soils during 1998. Relative distribution of organic volatiles differed with soil type. Volatilization was higher in control soil as compared to test and treated soils in both the study seasons.

Both mineralization and volatilization of 2,4-D was higher in 1997 compared to 1998. A major portion of the total loss was attributable to $^{14}CO_2$, 78–80% in 1997 and 74–93% in 1998. The rate of mineralization was same in both the study periods after 21 days. The contribution of volatiles to the total loss during 1997 was highest in test soil (24.4%) with the lowest contribution being recorded for farm soil (19.8%) but in 1998 was highest in control soil (26%) and lower in test and treated soils (7%) during 1998.

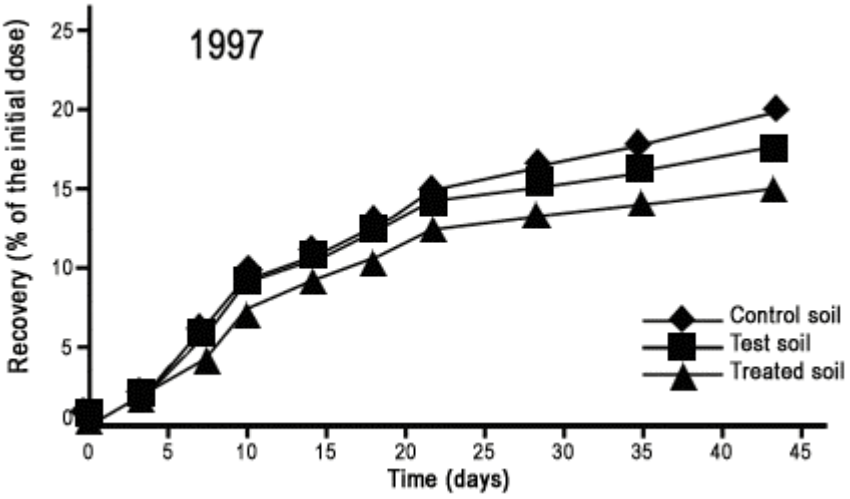

Figure 1A. Cumulative mineralization of 2,4-D during 1997

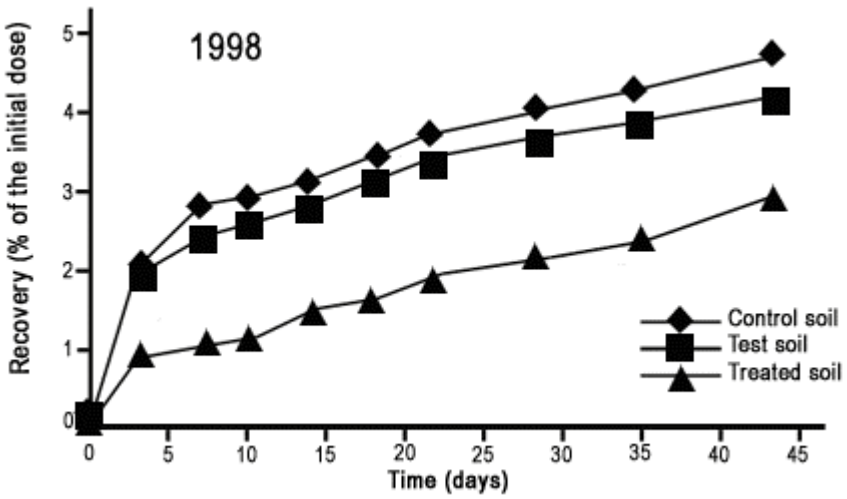

Figure 1B. Cumulative mineralization of 2,4-D during 1998

Table 1A. Cumulative volatilization of 2,4-D (1997)

	(% of applied dose)		
Time (days after application)	Control soil	Test soil	Treated soil
3	1.28±0.02	1.24±0.01	0.40±0.01
7	2.66±0.03	2.66±0.92	1.32±0.01
10	3.46±0.03	3.46±0.13	1.86±0.01
14	4.04±0.03	4.02±0.01	2.34±0.03
18	4.50±0.03	4.42±0.06	2.72±0.01
21	4.84±0.01	4.78±0.28	3.00±0.01
28	5.10±0.02	5.02±0.01	3.20±0.02
35	5.34±0.01	5.28±0.02	3.38±0.01
42	5.58±0.01	5.52±0.03	3.56±0.00

Data are means of three replicates ±S.D.

Table 1B. Cumulative volatilization of 2,4-D (1998)

	(% of applied dose)		
Time (days after application)	Control soil	Test soil	Treated soil
3	1.420±0.74	0.160±0.06	0.090±0.01
7	1.490±0.02	0.220±0.00	0.140±0.00
10	1.540±0.00	0.260±0.00	0.180±0.00
14	1.600±0.00	0.270±0.00	0.184±0.00
18	1.608±0.00	0.275±0.00	0.185±0.00
21	1.616±0.00	0.283±0.00	0.194±0.00

Data are means of three replicates ±S.D.

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REFERENCES

[1] KAUFMAN, D.D., "Degradation of pesticides by soil microorganisms", Pesticides in Soil and Water, (GUENZI, W.D., Ed.) Soil Sci. Soc. Am., Madison, Wis. (1974) 133–202.

[2] WOLF, D.C., MARTIN, J.P., Decomposition of fungus mycelium and humic-type polymers containing ^{14}C from ring and side chain labelled 2,4-D and chloropropham, Soil Soc. Am. J. **40** (1976) 700–704.

[3] McCALL, P.J.,et al., Fate of uniformly carbon-14 ring labelled 2,4,4-trichloroacetic acid and 2,4-dichlorophenoxyacetic acid, J. Agric. Food Chem. **29** (1981) 100–107.

[4] OH, K-H., TUOVINEN, O.H., Bacterial degradation of phenoxy herbicide mixtures 2,4-D and MCPP, Bull. Environ. Contam. Toxicol. **47** (1991) 222–229.

[5] INTERNATIONAL ATOMIC ENERGY AGENCY, Soil capacity to mineralize ^{14}C-labelled aromatic pesticide molecules. Study protocols developed at the First FAO/IAEA Research Coordination Meeting on " Impact of long term pesticide usage on soil properties using nuclear techniques, Neuherberg (near Munich) Germany, 29 May–2 June, 1995.

Impact of repeated pesticide applications on the binding and release of ^{14}C-methamidophos to soil matrices under field conditions.

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Abstract. The dissipation of ^{14}C-methamidophos was monitored in the absence or presence of other pesticides using *in situ* soil columns in cotton fields. Samples were taken randomly in duplicate at 0, 6, 9, 12, 18, 24 and 30 months. The soils were analyzed for total, extractable and bound residues. The dissipation of ^{14}C-methamidophos was rapid in the field environment; 3 hours after application, 12% was of the radioactivity lost due to volatilization and 88% was found in the 0–15 cm soil layer. With the passage of time bound residues in treated soil were less (11.52%) compared to those in untreated soil (13.47%). In general bound residues gradually increased with time and binding was higher in untreated soil at every sampling stage. The estimated time required for loss of 50% of radiocarbon was 73 days. In untreated samples the parent compound and three unknown spots were seen on TLC plates whereas four unknown spots with the parent chemical were found in the treated samples.

1. Introduction

Until now the use of pesticides has proved to be the only means to protect crops on a large scale. However, the effects of pesticide usage must be seen also in the context of soil pollution and sustainability of the agroecosystem. The soil burden resulting from the repeated long term applications of pesticide chemicals necessary for protection of cotton plants is of special concern. Such treatments may suppress soil microflora and hence affect soil properties. Pesticides may have potential to bind to soil, the extent of which depends greatly on the nature of the chemical used. A proportion of the pesticide applied eventually becomes incorporated into the soil. Dispersion of pesticides and their transformation products within the soil environment, or from the soil to other environments, is influenced not only by the properties of pesticides and soil but also the prevailing climatic conditions. The physico-chemical nature of the soil is important for the persistence, metabolism and binding of pesticides in soil. Data on the soil binding capacity of pesticides were generated mainly from studies using a single application of labelled pesticide on soil for certain periods. Useful data may be generated from investigations reflecting the real situation where the soil constantly receives a multitude of pesticide chemicals.

The present work was conducted to study the persistence of ^{14}C-methamidophos during 30 months exposures in *in-situ* soil columns in absence and presence of other insecticides. This would reflect the impact of repeated pesticide applications on the binding and release of bound pesticide residues to soil matrices.

2. Materials and methods

2.1. Experimental setup

Two sets of PVC cylinders with clean soil (soil which had not received pesticides for the last 20 years) were used. The first set (24 cylinders) was treated with regular inputs of fertilizer and ^{14}C-methamidophos. This set acted as the control. The second set (24 cylinders) received the same inputs plus all the other cold pesticides regularly used in the field as mentioned in the previous paper of this TECDOC. All the concentrations, time and frequency of application were similar to those used in practice for the protection of cotton plants during the crop seasons 1997–98. The cylinders were driven

into the soil with 5 cm protruding above the soil surface in order to prevent runoff. Soil cylinders were left open under field conditions [1].

2.2. ^{14}C-labelled pesticide

The ^{14}C-labelled pesticide used in this study was methamidophos. The radiochemical was appropriately diluted with the cold methamidophos to prepare a concentration of 2 mg/kg soil based as the soil present in the cylinder in a dept of 40 cm. The solution was applied with micropipette onto the surface of the cylinders. The activity received was 111 kBq per cylinder.

2.3. Sampling of cylinders

Two cylinders of each set were taken randomly 0, 3, 6, 9, 12, 18, 24 and 30 months after application and soil carefully removed from cylinders divided into 15 cm zones. Soil of each zone was air dried and thoroughly mixed. Total weight and moisture content of soil samples were also noted.

2.4. Extraction and analysis

Analyses were made for total, extractable and bound residues. For total and bound residues analysis, 500 mg soil was combusted in a Harvey Biological Oxidizer, OX 600 and counted with liquid scintillation counter (LSC). For extractable residues, 50 g (dry weight basis) soil samples were extracted in a Soxhlet extraction apparatus with methanol for 10 cycles. Methanol was concentration to 10 mL with Rotavapor, Buchi, Switzerland and 1 mL methanol was mixed with scintillator and counted with LSC. The nature of the ^{14}C-residues in the methanol extract was determined by thin layer chromatography (TLC). Silica gel 60 (20 cm x 20 cm x 0.5 mm, E. Merck) plates were used and developed in ethyl acetate and chlorine vapors (8 g $KMnO_4$ + 10 mL HCl. The plates were sprayed with a solution (*o*-toldine + KI) to show the spots and Rf values were determined.

3. Results and discussion

The data for the dissipation of ^{14}C-methamidophos, over 30 months is given in Fig. 1. Three hours after application, 88% of radioactivity was found in the 0–15 cm soil layer and 12% was lost due to volatilization in both the treated (Fig. 1) and untreated (Fig. 2) soils. The amounts of extractable and bound residues were the same in both the soils. After 3 months, 39.03 and 42.49% of the initial radioactivity was found in the treated and untreated soils, during this period more bound residues were found in untreated (8.34%) than in treated soil (5.27%) while the extractable residues were same in both the soils. After one year, more ^{14}C-activity was dissipated in treated (77% of the initial radioactivity) than untreated (72% of the initial radioactivity) soil. Bound residues in treated soil were less (11.52%) compared to untreated soil (13.47%). More reduction in bound as well as in extractable residues was observed in treated soil than in untreated soil.. Bound residues gradually increased with time and more binding was detected in untreated soil at every sampling stage.

Pesticide loss is quantified as a “half-life” value which is assumes it to be a first order process. The data obtained seemed to fit a biphasic nature. The first phase is rapid with volatilization as the major process leading to dissipation. The second phase is a slower process probably associated with diffusion into inaccessible adsorption sites and other mechanisms. The estimated time required for loss of 50% (over all half-life, t_{50}) of radiocarbon was 73 days (Fig. 3). The data indicate that bound residues may be released due to repeated applications of pesticide. Soil microorganisms are believed to play an important role in the release and further degradation of bound pesticides residues [3]. Bound ^{14}C- DDT residues in soil were reported to be readily released by microorganisms [4,5].

TLC was effective to separate and identify the parent chemical and degradation products. TLC. In the system used, the R_f value of authentic methamidophos was 0.09. On the base of this reference the parent compound with three unknown spots were seen in extracts from untreated samples whereas four unknown spots with the parent chemical were found in the treated samples.

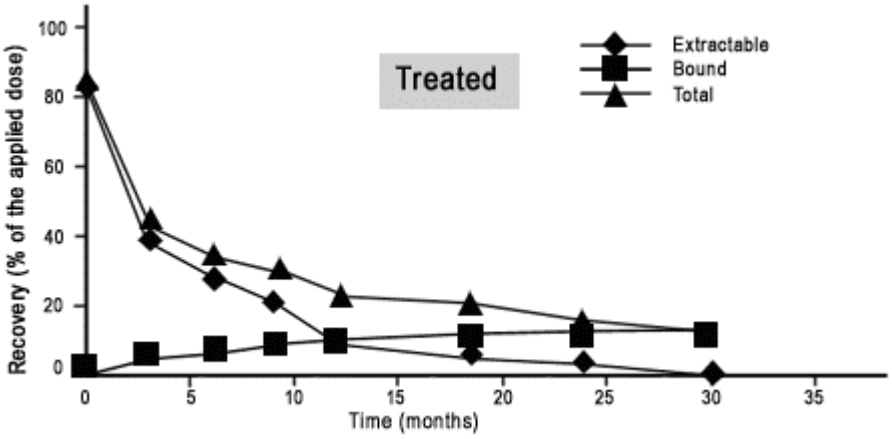

Fig. 1 Dissipation of ^{14}C-methamidophos in the presence of applied pesticides.

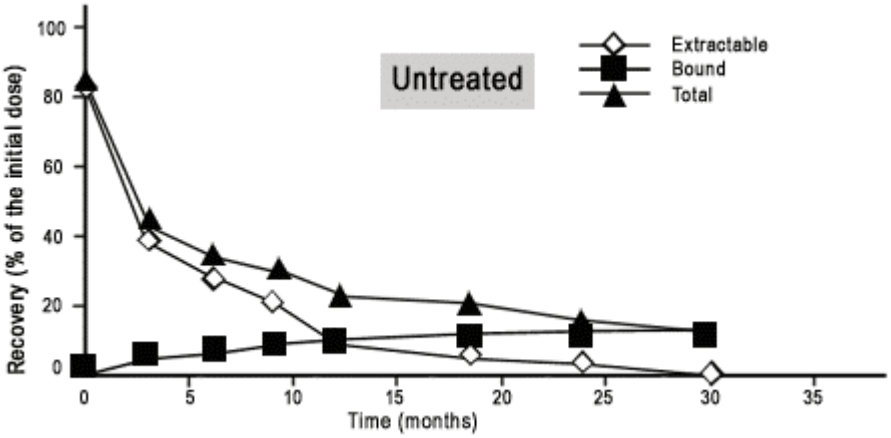

Fig. 2 Dissipation of ^{14}C-methamidophos without applied pesticides.

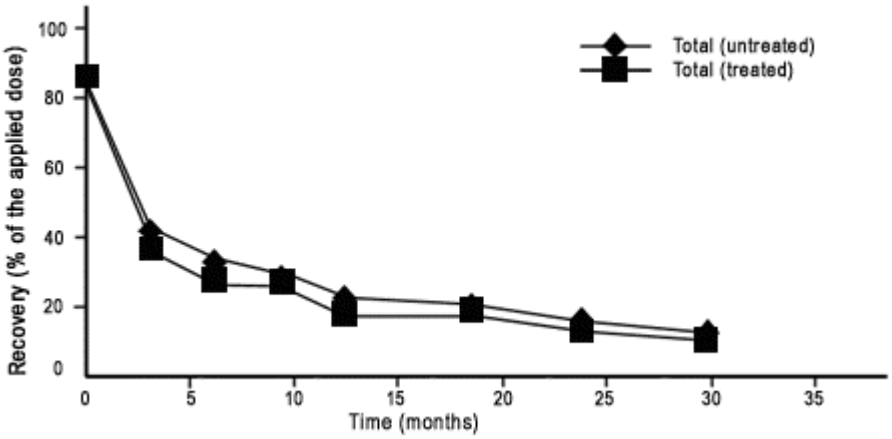

Fig. 3 Dissipation of ^{14}C-methamidophos in the absence and presence of pesticides.

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REFERENCES

[1] INTERNATIONAL ATOMIC ENERGY AGENCY, Soil capacity to mineralize ^{14}C-labelled aromatic pesticide molecules. Study protocols developed at the First FAO/IAEA Research Coordination Meeting on " Impact of Long term pesticide usage on soil properties using nuclear techniques, Neuherberg (near Munich Germany, 29 May–2 June, 1995.

[2] HELLING, C.S.,et al., DDT Dissipation in Hawaiian in-Situ Soil Columns, J. Environ. Sci. Health **B-29** (1994) 103–117.

[3] KHAN, S.U., IVARSON, K.C., Microbiological release of unextracted (bound) residues from an organic soil treated with prometryn, J. Agric. Chem., **29** (1981) 1901–1903.

[4] HUSSIAN, A., et al., Studies on dissipation and degradation of ^{14}C-DDT and ^{14}C-DDE in Pakistani soils under field conditions, J. Environ. Sci. Health, **B29** (1994) 1–15.

[5] ZAYED S.M.A.D.,et al., Chemical and biological release of ^{14}C-bound residues from soil treated with ^{14}C-p,p'-DDT, J. Environ. Sci. Health, **B29** (1994) 169–175.

Impact of continued use of profenofos on soil as a consequence of cotton crop protection

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Abstract. The same area was used since the project started in 1995 in the study to determine the impact of continued use of profenofos on soil properties in a cotton field. The effect on microbial population was minimal. Total bacterial, *Bacillus* and fungal counts generally decreased during the first spraying but recovered after the succeeding applications of profenofos. Basal respiration was not affected by profenofos treatment. Substrate induced respiration was not affected in the first spraying but was stimulated after the second and third sprayings. Movement of profenofos in the soil was slow when the soil was maintained at field capacity. It is easily degraded in the soil. The differences in organic volatiles, cumulative percent mineralization and bound residue formation of ^{14}C-2,4-D in untreated soil and farmer's field soil previously treated with cypermethrin, isoprocarb and profenofos were not statistically significant.

1. Introduction

Modern agriculture is readily associated with the use of different agricultural chemicals. Different classes of pesticides like fungicides, herbicides and insecticides are used in managing different groups of pests to maximize crop production and meet the demands for higher supplies of food, clothing and other agriculture-related necessities of the fast-growing human population.

Cotton production relies heavily on pesticides. The "Management Guide for Successful Cotton Culture" produced by the Cotton Research Development Institute (CRDI) in the Philippines listed about 30 different brands of insecticides that could be used during the different growth stages of cotton. Since cotton is a nonfood crop, farmers may not hesitate to spray more than the required amount and frequency to ensure a good harvest. Because the soil is the most important agricultural resource we have, second to water, it is important to examine the possible effects of the commonly used pesticides on soil properties.

One of the most commonly used insecticides in cotton production is profenofos (*0*-4-bromo-2-chlorophenyl-*O*-ethyl-*S* propyl phosphorothioate). It is a non systemic insecticide and acaricide with contact and stomach action used against mites, leafhoppers, thrips, aphids, mealy bugs and cotton stainer. The acute oral LD_{50} of profenofos for rats is 358 mg/kg, while inhalation toxicity (LC_{50}) is 3 mg/L a.i. The NOEL for rats is 0.02 mg/kg. LC_{50} and for rainbow trout it is 0.08 mg/L. It is toxic to honeybees and birds [1].

2. Methods

2.1. Field plot preparation

A five hundred m^2 cotton field was prepared for the experiment. It was the same area used since the project started in 1995. Standard cultural management practices for cotton were followed except for insect control where only profenofos (Selecron 500 BC) was used in one of the treatments.

2.2. Treatments

Three treatments were compared. Each treatment occupied an area of about 200 m^2. Treatment 1 was the control which did not receive any pesticide, treatment 2 was treated with profenofos and treatment

3 was a farmer's cotton field which was about 150 meters away from the first two treatments. Treatment 1 or the untreated plot was separated from treatment 2 by five border rows of cotton plants. The farmer's cotton field was previously sprayed with cypermethrin (Cymbush) and isoprocarb (Hopcin) before profenofos was applied.

Profenofos (Selecron 500 EC) was applied 30, 60, 90, 120 and 150 days after planting. at the rate of 110 mL per 16 L of water (3.00 kg a.i./ha). Each treatment was replicated three times.

2.3. Sampling

Sampling was done before spraying and two days after spraying. Soil samples were collected from ten sampling sites selected at random in each replicate of the three treatments. Samples were taken from the upper 15 cm layer of the soil. These were pooled (approximately 1 kg), air-dried, mixed thoroughly in clean black plastic bags and placed in the refrigerator. The samples were incubated for 24 hours at room temperature (3.0°C) before use when necessary.

2.4. Effect on the soil microbial population

The total bacterial count was determined by plating 0.1 mL aliquots of serially diluted samples into nutrient glucose agar (NGA). The total count of *Bacillus* spp. was determined by heating the serially diluted samples in a water bath for 10 minutes at 80-85°C prior to plating on NGA. Total fungal count was determined by plating serially diluted samples on rose bengal agar.

2.5.1 Basal Respiration

Five grams of soil sample from each treatment was placed in the test vessel. Ten mL of freshly prepared 1N KOH was placed in one trapping vial and another vial containing 10 mL 0.1N KOH was used to trap the CO_2 evolved during respiration. Air was flushed through daily for five minutes using a vacuum pump to help aerate the soil microorganisms. CO_2 in the incoming air was scrubbed in 1N KOH. The test system was maintained at room temperature (28°C) throughout the duration of the experiment and the soil moisture was maintained at 60% of water holding capacity. The CO_2 absorbed was estimated by titrating the excess KOH against 0.1 M HCl.

The amount of CO_2 evolved was calculated from:- mg CO_2/5g soil $24h^{-1}$ = (C-S) 2.2
Where:- C = mean volume of HCl consumed by controls (mL); S = mean volume of HCl consumed by soil samples (mL); 2.2 = conversion factor (1 mL of 0.1M HC1 corresponds to 2.2 mg CO_2).

2.5.2. Substrate-induced respiration

The same procedure as in basal respiration was followed except that 4 mg glucose was mixed thoroughly with 1g of soil sample prior to incubation. The carbon dioxide produced was measured every hour.

2.6. Movement of profenofos in the soil

2.6.1. Preparation of soil columns

Fourteen PVC pipes (60 cm long, 5.5 cm dia.) were packed with soil taken from the upper 30 cm layer of a cotton field located at the Experiment Station of the National Crop Protection Center, University of the Philippines Los Baños, College, Laguna. Part of the soil sample was sent to the Department of Soil Science, UPLB, for analysis and the physical and chemical characteristics are listed in Table 1. The packed PVC columns were left undisturbed for 30 days to equilibrate. Each column was treated with 100 mL emulsion containing 146 μg/mL profenofos. Each soil column was weighed every week and the amount of water lost was replenished.

Table 1. Physical and chemical characteristics of soil from cotton field.

Organic matter (%)	3.11
Total nitrogen (%)	0.14
Available phosphorous (mg/kg)	14.39
Exchangeable potassium (meq/100 g soil)	1.14
Water holding capacity (%)	85.15
pH (l:l in H_2O)	6.30
Bulk density	1.05
Textural grade	Silty clay
Sand (%)	13.43
Silt (%)	41.89
Clay (%)	44.68

2.6.2. Sampling, extraction and analysis

The first two PVC soil columns were sliced immediately (3 hours after treatment) to serve as the 0 day sample. The columns were sliced into six 10 cm layers. Each layer was air-dried prior to analysis. The remaining PVC soil columns were analyzed every month for 6 months.

One hundred grams (dry weight basis) of soil from each layer was transferred into a 500 mL Erlenmeyer flask, treated with 5 mL of 2N NH_4Ac and 100 mL acetone and shaken for 1 hour. The filtrate after suction filtration was extracted with 200 mL CH_2C1_2. The organic extract was filtered through anhydrous Na_2SO_4. The sample was concentrated to dryness using a rotary evaporator and taken up in 2 mL acetone. Profenofos residues were analyzed using a HP6890 model gas chromatograph equipped with a NP-FID detector using the following parameters:- column; HP5 (crosslinked 5% PH ME siloxane); temperatures: injector, 260°C, detector, 290°C, column, initial 80°C, first ramp 30°C /min to 178°C second ramp 2°C/min to 205°C third ramp 30°C/min to 250°C; gas flow rates: carrier (N_2) 30 mL/min, hydrogen 3 mL/min, compressed air 60 mL/min. One µL of the extract was injected after every injection of standard.

2.7. Effect of profenofos on the mineralization and volatilisation of (phenyl-^{14}C) 2,4-D

2.7.1.Sample preparation

The capacity of the untreated soil (control) and multi-pesticide-treated soil (farmer's field) to volatilize and mineralize ^{14}C-2,4-D was compared.

Five grams (dry weight basis) of soil sample from each treatment was placed in a 40 mL test vessel. Five µg cold 2,4-D was dissolved in small amount of acetone and 619.97 kBq of phenyl (U) ^{14}C 2,4-D was dissolved in 2mL acetone. A 0.1 mL aliquot of dosing solution was pipetted into a LSC vial containing 10 mL scintillation cocktail.

2.7.2. Assembly of trapping line and sampling

The test vessels (in duplicate from each treatment) were kept at ambient temperature and connected to a trapping line. The volatile organic compounds were trapped in ethylene glycol monomethyl ether and $^{14}CO_2$ was absorbed in 1N KOH solution [2]. Ten mL of the respective solutions were placed in each trap.

The system was flushed with air once daily using a pump. The trapping solutions were replaced twice a week for the first 3 weeks and once a week thereafter until the 8th week. Radioactivity in the solutions was measured using a Packard Scintillation Counter.

Extractable and bound residues

After 10 weeks, 5g of each soil (in duplicate) was extracted for 4h with 100 mL of methanol in a Soxhlet apparatus. The total volume of extract was measured and a 0.1 mL sample was added to 10 mL scintillation cocktail and counted in a Packard Scintillation Counter.

The extracted soil was dried and then burnt in a OX400 Biological Oxidiser for the estimation of bound ^{14}C residues.

3. Results and discussion

3.1. Soil microbial population

The total bacterial count in profenofos-treated plots decreased slightly after the first spraying but increased after the second and third sprayings. In the farmer's field which had received cypermethrin and isoprocarb prior to profenofos treatment, the total bacterial count decreased slightly after the second spraying but recovered after the third spraying (Table 2A).

The total Bacillus count decreased slightly from the first to second sprayings in both the profenofos-treated field and farmer's field. The population, however, recovered after the third spraying (Table 2B).

Total fungal count decreased slightly after the first spraying in both profenofos-treated field and farmer's field but recovered from the second to third sprayings (Table 2C)

These results indicate that profenofos has a minimal effect on the culturable bacteria and fungi in cotton fields. However, only about 1% of the indigenous microbial species in the soil are recovered with conventional culture techniques [3].

Table 2A. Effect of profenofos on total bacterial population.

Treatment	Total bacterial count (cfu)*					
	30 days		90 days		120 days	
	BS	2DAS	BS	2DAS	BS	2DAS
Untreated (Control)	1,550,000	1,960,000	1,450,000	1,770,000	1,120,000	1,460,000
Treated	1,980,000	1,860,000	1,910,000	2,370,000	1,710,000	2,070,000
Farmer's field	1,730,000	1,880,000	1,800,000	728,000	813,000	1,900,000

*colony forming units

Table 2B. Effect of profenofos on population of *Bacillus* spp

Treatment	Total *Bacillus* count (cfu)					
	30 days		90 days		120 days	
	BS	2DAS	BS	2DAS	BS	2DAS
Untreated (Control)	908,000	928,000	387,000	839,000	374,000	556,000
Treated	1,031,000	694,000	1,149,000	988,000	758,000	1,499,000
Farmer's field	941,000	689,000	281,000	316,000	261,000	721,000

Table 2C. Effect of profenofos on fungal population

Treatment	Total fungal count (cfu)					
	30 days		90 days		120 days	
	BS	2DAS	BS	2DAS	BS	2DAS
Untreated (Control)	292,000	319,000	247,000	399,000	622,000	660,000
Treated	317,000	222,000	233,000	318,000	378,000	576,000
Farmer's field	429,000	299,000	117,000	245,000	492,000	800,000

3.2. Basal respiration

The application of profenofos in the cotton experimental field and in the farmer's field did not affect the basal soil respiration compared with the untreated field as shown by the data from March to May, 1998 wherein three sprayings were done (Table 3). Comparisons were made between the profenofos-treated soil and untreated soil only from the fourth spraying in July to the fifth spraying in August because cotton was planted and harvested earlier in the farmers' field. A similar trend in basal respiration was observed in the two treatments. The inhibition in soil respiration after the first spray in March and after subsequent sprays in July and August could not be attributed to the profenofos treatment because a similar result was observed in untreated plots. The rainfall and temperature patterns could not help explain the results.

Table 3. Effect on basal soil respiration (μg CO_2/g soil h^{-1})

Spraying Time	Treatment								
	Control			Profenofos treated			Farmer's Field		
	BS	2DAS	Difference	BS	2DAS	Difference	BS	2DAS	Difference
March	87.33	30.50	-56.83	74.33	35.17	-39.16	71.00	42.00	-29.00
April	82.67	110.00	27.33	90.17	114.33	24.16	13.08	40.83	27.75
May	36.50	79.00	42.50	57.00	80.50	23.50	47.83	98.00	50.17
July	113.67	49.67	-64.00	112.5	67.17	-45.33	NA	NA	
August	136.67	92.33	-44.34	109.33	96.33	-13.00	NA	NA	

NA = not analyzed

3.3. Substrate induced respiration (SIR)

After the first spraying in March 1998 SIR was not affected by the profenofos treatment. From the second spraying in April to the third spraying in May, SIR was enhanced in the profenofos-treated field but was inhibited in the farmer's field (Table 4). A similar trend in SIR was observed in profenofos-treated and untreated fields after subsequent sprayings in July and August.

The stimulatory effect on SIR after the second and third sprayings may be due to adaptation and recovery of the microbial population [4]. It is also possible that the observed stimulatory effect was not due to the return of the original population but rather to the increased activity of a few resistant species [5] because in some cases the carbon supply in the soil is limiting and the microbes killed by pesticide application may serve as an energy source for other more stress-tolerant microbes [6]. The quotient of soil respiration (qCO_2) was calculated as described previously [7] and the data are summarized in Table 5.

Table 4. Effect on substrate induced respiration (μg CO_2/g soil h^{-1})

Spraying Time	Treatment								
	Control			Profenofos-treated			Farmer's field		
	BS	2DAS	Difference	BS	2DAS	Difference	BS	2DAS	Difference
March	325.00	176.50	-148.50	425.00	178.50	-246.50	427.00	288.00	-139.00
April	404.00	727.00	323.00	407.00	826.00	419.00	920.00	479.50	-440.50
May	692.00	119.50	572.50	254.00	456.50	202.50	657.00	245.00	-412.00
July	711.00	594.00	-117.00	626.00	382.00	-244.00	NA	NA	NA
August	874.00	722.00	-152.00	644.00	435.00	-209.00	NA	NA	NA

Table 5. Quotient of soil respiration (qCO_2)

Spraying Time	Treatment					
	Control		Profenofos-treated		Farmer's field	
	BS	2DAS	BS	2DAS	BS	2DAS
March	0.27	0.17	0.18	0.20	0.17	0.15
April	0.21	0.15	0.22	0.14	0.01	0.08
May	0.05	0.66	0.22	0.18	0.07	0.40
July	0.16	0.08	0.18	0.18	NA	NA
August	0.16	0.13	0.17	0.22	NA	NA

Table 6. Profenofos residue in soil layers at different days after treatment.

Soil Depth (cm)	Days After Treatment											
	0		60		90		120		150		180	
	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%
0-10	48.64	33.32	38.67	26.49	3.34	2.29	7.03	7.82	6.80	4.66	0.44	0.30
10-20	2.58	1.77	2.82	1.93	1.85	1.27	0.50	0.34	0.88	0.60	0.03	0.02
20-30	0.06	0.04	0.21	0.14	0.06	0.04	0.06	0.04	0.01	0.007	NDR	
30-40	NDR		0.04	0.03	0.12	0.08	0.01	0.007	0.01	0.007	NDR	
40-50	NDR		0.01	0.007	0.02	0.01	0.01	0.007	NDR		NDR	
50-60	NDR		NDR		0.01	0.007	NDR		NDR		NDR	
Total	51.28		41.75		5.40	3.70	7.61	5.21	7.71	5.27	0.47	0.32

NDR = no detectable residue

3.4. Movement of profenofos in the soil

Three hours after application, 33.3% of the profenofos residue was recovered at the upper 10 cm of the soil column, with only a trace amount in the 20-30 cm layer and no detectable residue in the 30-40 cm layer (Table 6). Sixty days after treatment, 26.5% of the residue remained at the upper 10 cm of the soil column and trace amount was observed in the 30-40 cm layer. This shows that when the soil is maintained at field capacity, the downward movement of profenofos in the soil is slow. Similar results have been observed with other organophosphorus compounds [8]. Ninety to 150 days after treatment only 3.3 to 6.8 μg/g (2.3-4.8%) profenofos residue was detected in the upper 10 cm of the soil column and the total residue detected in the entire soil column ranged from 3.7% to 5.3% of that applied. This is an indication that profenofos is easily degraded in the soil.

3.5. Effect of profenofos on mineralization and volatilization of (phenyl-^{14}C) 2,4-D

A slightly higher percentage of organic volatiles was observed from the untreated soil than in the farmer's field soil previously sprayed with cypermethrin, isoprocarb and profenofos (Table 7). The cumulative percent mineralization of 2,4-D ($^{14}C0_2$ evolution) was slightly higher in soil from farmer's field during the first five weeks. A faster rate of mineralization was observed in the untreated soil from the sixth to the eighth week. Higher extractable residues were obtained from the untreated soil but the bound residues were higher in soil from farmer's field (Table 8). These differences, however, are not statistically significant.

Table 7. Percent organic volatiles and mineralization of ^{14}C-2,4-D in cotton field soil (1998)

Days	% Volatiles		% Mineralization	
	Untreated	Farmer's Field	Untreated	Farmer's Field
3	0.019	0.013	0.013	0.042
7	0.038	0.010	0.013	0.011
10	0.013	0.009	0.014	0.014
14	0.013	0.009	0.013	0.008
17	0.001	0.018	0.682	0.893
21	0.013	0.012	0.089	0.030
28	0.009	0.002	0.013	0.017
35	0.004	0.003	0.270	0.184
42	0.006	0.004	0.402	0.143
49	0.003	0.002	0.045	0.043
56	0.001	0.002	0.021	0.004

Table 8. Material balance, (%) of the applied ^{14}C-2,4-D in untreated and farmer's field soil (1998).

Parameters	Soil Sample	
	Untreated	Farmer's Field
Extractable residues	47.0	38.90
Bound residues	50.0	59.00
Volatiles (within 8 weeks)	0.12	0.08
Mineralized (within 8 weeks)	1.58	1.38
Losses	1.30	0.64

The results suggest that the insecticide treatments used in the farmer's field did not have a significant effect on the microbial population that degrades 2,4-D in the soil.

In this study, only 0.45%-l.57% of the applied 2,4-D was mineralized in 56 days. This is in accord with the findings of other researchers. In the case of DDT, for example, it was found that the total $^{14}CO_2$ formed within 12 weeks accounted for only 2.56% of the applied radioactivity [8]. It was also reported in another study that the sum of volatilization and mineralization of 2,4-D after six weeks did not exceed 2% of the applied [10]. According to Khan and Iverson [11] degradation of pesticide in the soil is a slow process catalyzed by microorganisms.

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REFERENCES

[1] THE ROYAL SOCIETY OF CHEMISTRY, 'The Agrochemicals Handbook'. Second Edition., Unwin Bros. Ltd., Old Working Surrey, U.K. (1987).

[2] LEHR, S., et al., Mineralization of free and cell wall-bound isoproturon in soils in relation to soil microbial parameters, Soil Biol. Biochem**. 28** (1996) 1-8.

[3] TORSVIK, V., et al., High diversity in DNA of soil bacteria, Appi. Environ. Microbial. 56 (1990) 782-787.

[4] ROGER, P.A., BHUIYAN, S.I., "Behavior of pesticides in rice-based agrosystems", Impact of Pesticides in Farmer Health and the Rice Environment (PINGALI, C.P.L., ROGERS, P.A., Eds), Kluwer Academic Publishers, U.S.A. (1995) 111-148.

[5] DOMSCH, K.H., "Effects of fungicides on microbial population in soil", Pesticides in Soil Ecology, Degradation, Movement Symposium, East Lansing State University, Michigan (1970).

[6] SPARLING, G.P., "The soil biomass", Soil Organic Matter and Biological Activity, (VAUGHAN, D., MALCOLM, RE., Eds.) (1985) 223-262.

[7] ANDERSON, T.H., DOMSCH, K.H. Application of eco-physiological quotients (qCO_2 and qD) on microbial biomass from soils of different cropping histories, Soil Biology Biochemistry **22** (1990) 251-255.

[8] SWOBODA, A.R., THOMAS, G.W., Movement of parathion in soil columns, J. Agr. Food Chem. **16** (1968) 923-927.

[9] HUSSAIN, A., et al., Studies on dissipation and degradation of ^{14}C-DDT and ^{14}C-DDE in Pakistan soil under controlled conditions, J Environ. Sci. Health, **B29** (1994) 1-15.

[10] ESMADE, A.L., Studies on the degradation of ^{14}C-ring labelled 2,4-dichlorophenoxyacetic acid (2,4-D) in Philippine soil under controlled conditions, (Thesis, University of the Philippines Los Baños, 1997).

[11] KHAN, S.U., IVERSON, K.C., Agricultural Food Chemistry, Pentech Press, London (1989).

Impact of long term pesticide usage on soil microbial activities and ^{14}C - monocrotophos degradation

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Abstract. The effects of long term pesticide usage on soil microbial activities and degradation of ^{14}C - monocrotophos was observed under cotton field conditions. The experimental field was divided into treated and untreated plots. Pesticides were applied to treated plots at weekly intervals as in common practice in Thailand. The total numbers of applications were 11, 16 and 16 for first, second and third crop seasons, during the three years from 1996 to 1998. Soil samples at depths of 0-15 cm and 15–30 cm were sampled before and after pesticide application for the first two crops, while in the third crop season only the surface layer of soil was taken. The samples were assessed for CO_2 from respiration, soil microbial population, iron reduction capacity, and rates of nitrification. Soil biomass and microbial activities as measured from respiration and iron reduction decreased in the treated plots at both depths after each pesticide application over the three crop seasons, whereas samples from untreated plots at both depths did not show decreases. Repeated application of pesticides did not show any effect on nitrification rates of the first crop but there was inhibition in the second and third crops. Soil columns, treated with ^{14}C - monocrotophos one week after last pesticide application, were harvested after 0, 3, 6, 9, 18, 24 and 30 months. Extractable residues of ^{14}C were found only in the 0–15 cm layer. In treated and untreated plots, residues declined from 80.17 and 85.68 to 0.44% of the applied ^{14}C within 6 months. The long term usage of pesticides did not affect the half-life of ^{14}C- monocrotophos. Bound residues of ^{14}C were found at the highest concentrations, 18.94 and 12.58% of that applied, at 6 months in treated and untreated plots, thereafter the binding decreased to 4.68 and 2.74% within 30 months.

1. Introduction

The increased use of pesticide in crop production has aroused concern over possible impacts on agroecosystems, especially on the quality of soil after repeated pesticide application. Some crops, such as cotton, normally receive more than ten pesticide applications within one crop-season. Studies in the past have focused mainly on impact on soil following the single application of pesticide. Therefore, there is a need to assess the effects of multiple pesticide applications on soil properties. These experiments using labelled chemical in field soil columns were initiated to investigate the long term impact of repeated use of insecticides on soil properties to enable us to give better advice to the government on pesticide policy.

2. Materials and methods

2.1. Chemicals

^{14}C-monocrotophos, dimethyl (*E*)-1-methyl-2-(methyl carbamoyl)vinyl phosphate, (specific activity 4.26 MBq/mg) was supplied by IAEA, non-labelled monocrotophos, endosulfan, sulprofos, cypermethrin, cyfluthrin and profenofos were supplied by local pesticide manufacturers.

2.2. Experimental field plots

The experimental area was 40x40 m, divided equally into control and treated plots. The soil was a sandy loam (sand 73% , silt 8% , clay 19%, pH6.8). Cotton, variety SR-2, was planted at a spacing of 80 cm in rows 200 cm apart. During planting, carbofuran G 3% was used as a seed treatment and carbofuran ST 35% was used on young plants to protect them from leaf rolling aphids. Insecticides monocrotophos, profenophos, endosulfan, cypermethrin, cyfluthrin, sulprofos were applied singly or

in combination by spraying onto the plant as in real practice. The application were made weekly from 5 weeks after crop emergence until the plants were 4 months old. The total numbers of applications were 11,16 and 16 times in the first, second and third crop seasons, during three years of 1996 to 1998.

2.3. Application of ^{14}C-monocrotophos

One week after the last pesticide application to the first crop, PVC columns, diameter 5 cm and length 50 cm, were put into the plots leaving 3 cm protruding above the surface. Twenty four columns were inserted into each plot.

Labeled monocrotophos (185 kBq) was added to each column and columns were removed for analysis from both treated and control plots at intervals of 0, 3, 6, 9, 12, 18, 24 and 30 months.

2.4. Soil sampling and preparation

A soil auger was used to collect 10 soil cores per plot, 0–15 and 15–30 cm deep in the first and second crop seasons, while in the third season only samples from the top layer were taken. Soil was collected 8 times in each crop season and 3 times between seasons. Samples were mixed, dried and sieved through a 2 mm sieve. The soil was kept in an incubator at 22°C 3 days before determination of different microbial activities.

2.5. Soil analysis for microbial activities

These analyses followed the guidelines developed at the first RCM [1]

2.5.1.Determination of CO_2 from respiration

Triplicate soil samples from both treated and untreated plots (50 g dry wt), at 55% water holding capacity were placed in standard biometer flasks and glucose (4 mg/g dry soil) was added. Ten mL KOH solution was inserted into the side arm of each biometer flask and the system was kept at 22°C for 96h. The absorbed CO_2 was determined by titrating the excess KOH with 0.05N HCl. Measurements of CO_2 were made every 24h until 96h. Basal respiration was determined without adding glucose.

2.5.2. Determination of capacity for iron reduction

Five g of the prepared soil (dry weight basis) was placed in a 25 mL polyethylene vial with a screw cap and 5 mL of distilled water containing 4 mg glucose/g dry soil as an energy source was added. The vial was shaken for 10h and incubated for 5 days under anaerobic conditions followed by extraction of ferrous iron by shaking for 10 min with 5 mL of 1N KCl solution. After centrifugation for 15 min, 6 mL of the clear solution was treated with 0.2 mL concentrated HNO_3 and acetate buffer solution was added until pH3-4 obtained. Ferrous iron was determined by addition of 5 mL*o*-phenanthroline, the tube was allowed to stand for 5 min, before measurement of absorbance at 512 nm. The iron concentration was obtained from a standard curve as μg iron/g dry soil. A control without glucose was run in parallel.

2.5.3. Determination of nitrification

Five g soil (dry weight basis) was placed in an Erlenmeyer flask and 0.34 mg $(NH_4)_2 SO_4$ was added. Soil moisture was adjusted to 60% water holding capacity and the flask closed before incubation at 28°C for 3 weeks. A control was run without $(NH_4)_2 SO_4$. Ammonium-nitrogen was determined by adding 50 mL 2M KCl solution and 0.2 g MgO followed by steam distillation until 30 mL of distillate

was collected in 10 mL boric acid indicator solution. Ammonium-nitrogen in the distillate was determined by titration with 0.005N H_2SO_4 (1 mL 0.005N H_2SO_4 is equivalent to 70 μg of NH_4-N). Nitrate-nitrogen was determined after the ammonium-nitrogen was distilled by adding 0.2 g of Devarda's alloy and repeating the steam distillation and titration as before.

2.5.4. Determination of soil biomass

Soil biomass was estimated as respiration quotient x 0.45 [2].

2.6. Soil analysis for ^{14}C-monocrotophos residues

Soil samples at depths of 0–15 cm and 15–30 cm were taken from the PVC columns removed from the field, mixed and dried at room temperature before sieving through a 20 mesh sieve. Three subsamples were extracted by Soxhlet extraction with methanol. Non-extractable residues were determined by combustion. The total ^{14}C of extractable and bound residues were measured by liquid scintillation counting.

3. Results and discussion

3.1. Field experiment

During the three cotton growing seasons (1996, 1997 and 1998), a variety of pesticides especially insecticides were applied regularly, as in real practice. The total number of applications in the first growing season was 11, but increased to 16 in the second and third seasons due to increasing pest outbreaks in the cotton growing area.

3.2 Microbial activities

3.2.1. Respiration

The content of CO_2 from respiration in treated and untreated soil in 2 soil layers is shown in Tables 1 and 2 and in the upper soil layer only in Table 3.

Table 1. Quantity of CO_2 from respiration (mg/10 g dry wt. soil), first crop.

Sampling time (weeks)		Untreated		Treated	
		0–15 cm	15–30 cm	0–15 cm	15–30 cm
0	before	9.54±1.40	7.99±0.96	11.52±0.17	10.03±1.19
	after	9.49±0.51	7.82±1.13	10.18±0.64	9.59±1.53
3	before	10.15±1.31	8.24±1.01	11.96±0.63	10.76±1.80
	after	11.15±1.04	8.85±0.57	9.58±0.35	10.42±1.46
6	before	10.78±0.94	8.82±1.12	14.28±0.94	9.88±1.63
	after	11.35±0.59	9.43±1.17	12.47±1.22	10.60±2.44
10	before	11.35±0.69	8.96±1.05	11.81±0.54	10.46± .97
	after	10.83±0.99	9.39±1.16	12.50±0.72	10.43±1.91
13	at harvest	10.64±0.89	9.37±1.06	11.92±0.48	10.27±1.34
17	after harvest	10.48±0.77	8.32±0.81	10.23±0.42	9.63±1.43
21	after harvest	11.32±0.78	9.29±1.04	12.43±0.76	10. 50±1.21

Table 2. Quantity of CO_2 from respiration (mg/10 g dry wt. soil), second crop.

Sampling time (weeks)		Untreated		Treated	
		0–15 cm	15–30 cm	0–15 cm	15–30 cm
0	before	14.41±0.68	9.60±0.39	15.40±1.12	12.66±1.36
	after	14.40±0.66	9.53±0.44	13.31±0.54	9.39±0.19
6	before	15.18±0.77	9.38±0.32	16.82±0.43	11.32±0.54
	after	14.59±1.19	11.32±0.54	10.68±0.28	9.05±0.07
10	before	13.78±0.04	11.78±0.45	17.41±0.52	16.34±0.73
	after	15.03±0.15	9.98±0.14	15.18±0.89	13.57±0.30
13	before	14.20±0.31	10.57±0.78	15.13±0.25	14.75±1.36
	after	13.00±0.32	11.00±0.21	13.09±0.78	11.97±0.31
16	at harvest	15.47±0.67	12.61±0.03	16.81±0.62	12.69±0.90
23	after harvest	16.81±0.62	13.49±0.23	17.53±0.14	14.18±0.44
31	after harvest	16.64±0.69	11.32±0.60	20.13±1.16	14.36±2.69

Table 3. Quantity of CO_2 from respiration (mg/10 g dry wt. soil), third crop.

	Sampling time (weeks)	Untreated	Treated
0	before	12.79±0.86	13.72±0.08
	after	13.37±0.32	13.84±0.14
3	before	13.63±0.08	14.49±0.13
	after	14.95±0.36	13.23±0.31
6	before	14.75±0.43	15.51± 0.15
	after	15.07±0.06	14.35±0.11
12	before	15.65±0.30	17.36±0.15
	after	15.30±0.21	15.55±0.16
17	at harvest	15.84±0.50	17.06±0.63
21	after harvest	15.28±0.33	15.62±0.37
25	after harvest	14.82±0.10	15.30±0.28

The examination of CO_2 content released from microbial activities in treated and untreated soil revealed that application of pesticides on treated soil had some impact on microbial respiration when compared to without application or untreated soil. The inhibition of respiration in the treated plot that could be seen more clearly in the second crop might due to the increased application of insecticides from 11 times in the first year to 16 in second year. Inhibition of microbial respiration was not observed in the lower layer (15–30 cm) of treated and control soil.

Table 4. Capacity for Fe-III reduction in soil (μg/g dry wt.), first crop

Sampling time (weeks)		Untreated		Treated	
		0–15 cm	15–30 cm	0–15 cm	15–30 cm
0	before	150.00±28.50	190.30±6.00	180.00±5.00	202.30±10.60
	after	192.60±16.20	205.00±8.00	148.00±3.00	192.00±7.00
3	before	148.00±10.00	203.00±10.00	103.00±14.00	147.00±8.00
	after	152.00±5.70	161.60±6.10	92.30±2.50	135.30±3.50
6	before	114.00±15.00	138.00±7.00	118.00±15.00	148.00±8.00
	after	152.50±5.70	148.30±21.00	112.30±9.60	132.00±4.00
10	before	155.00±10.00	160.00±5.00	175.00±4.00	167.00±10.00
	after	123.00±17.00	183.00±13.00	149.00±10.00	155.55±9.00
13	At harvest	91.00±9.00	181.00±12.00	139.00±11.00	141.50±11.00
17	After harvest	109.50±6.80	107.00±8.00	124.00±4.00	131.00±7.00
21	After harvest	145.00±11.00	144.50±11.80	94.00±8.00	83.00±11.00

Table 5. Capacity for Fe-III reduction in soil (μg/g dry wt.), second crop

Sampling time(weeks)		Untreated		Treated	
		0–15 cm	15–30 cm	0–15 cm	15– 30 cm
0	before	126.93±0.31	175.58±0.78	89.07±5.81	105.32±2.90
	after	131.90±6.74	171.14±1.81	66.72±2.34	69.52±4.40
6	before	77.28±2.71	97.72±7.54	73.11±2.54	75.28±2.65
	after	83.11±2.63	118.76±1.08	41.59±5.72	51.75±0.51
10	before	103.45±4.51	140.19±6.38	82.17±4.17	69.19±4.13
	after	101.00±8.56	72.38±4.98	72.90±2.53	97.65±4.45
13	before	77.34±5.05	78.93±5.33	8355±2.24	95.30±4.69
	after	66.71±4.72	88.27±1.87	43.82±2.40	66.35±3.55
16	at harvest	70.71±4.61	100.63±4.06	69.24±2.31	80.62±5.40
23	after harvest	94.66±2.79	180.51±5.66	76.89±5.50	85.66±2.71
31	after harvest	149.16±3.26	190.31±7.22	90.66±4.36	86.5 ±4.13

Table 6. Capacity for Fe-III reduction in soil (μg/g dry wt.), third crop

	Sampling time (weeks)	Untreated	Treated
0	before	87.16±11.73	83.31±8.95
	after	90.16±14.92	31.59±3.30
3	before	111.84±4.51	75.57±3.47
	after	149.31±4.40	67.27±2.51
6	before	102.38±4.47	76.80±2.69
	after	125.92±7.72	72.50±2.75
12	before	76.07±9.14	67.44±5.67
	after	102.06± .90	49.42±4.62
17	At harvest	105.52±9.14	70.94±18.03
21	After harvest	138.42±12.25	59.40±18.64
25	After harvest	111.72±5.06	90.85±5.93

The measurements of Fe (III) reduction in treated and control soil were made to observe the effect of pesticides on bioactivity of microorganisms within 2 layers of soil in the first two years (Table 4 and, 5) and in top soil only in the third year (Table 6). In control or untreated soil, inhibition did not occur while in treated soil suppression could be observed after each pesticide application throughout the three seasons.

3.2.3. Nitrification

Tables 7, 8 and 9 illustrated the effect of pesticide use on nitrification rates in treated and untreated plots within 2 layers of soil in the first and second crops, and top soil only in the third crop.

The amounts of NO_3 N from nitrification in control soil did not decrease after application of pesticides during the first and second crop seasons but seemed to be stimulated in the third season.

3.2.4. Soil biomass

As the basal and induced respiration were higher in the upper soil layers (0–15 cm), the soil biomass in treated and untreated plots was determined only from top soil (Table 10). The estimation of biomass was made by using respiration quotient x 0.45.

The soil biomass content did not show any difference in untreated soil, before and after application of pesticides while in treated soil the biomass contents were lowered after each application of pesticides. After harvesting, soil biomass varied according to many factors such as temperature, humidity and application of fertilizer.

Table 7. Quantity of NO_3-N from nitrification (µg/g dry wt. soil), first crop

Sampling time (weeks)		Untreated		Treated	
		0–15 cm	15–30 cm	0–15 cm	15– 30 cm
0	before	9.27±2.12	9.40±1.05	8.23±1.76	5.70± 0.98
	after	5.53±0.60	7.77±1.78	7.83±1.60	7.67±2.14
3	before	6.27±2.05	7.53±1.30	9.89±2.06	9.43±2.25
	after	7.70±1.40	8.97±2.37	9.23±2.32	4.67±1.22
6	before	6.50±1.34	8.93±1.30	8.43±1.86	5.13±1.70
	after	7.77±2.15	6.73±1.14	7.67±1.50	7.53±2.61
10	before	7.27±0.86	8.37±1.95	8.17±1.60	6.60±1.93
	after	5.07±1.35	10.0±1.51	7.93±2.05	5.83±2.91
13	at harvest	6.50±1.53	8.26±0.94	8.27±0.61	6.87±1.00
17	after harvest	7.16±0.35	8.93±0.45	8.30±0.75	7.67±0.76
21	after harvest	7.35±0.42	7.85±0.62	7.82±0.62	8.20±0.95

Table 8. Quantity of NO_3-N from nitrification (µg/g dry wt. soil), second crop

Sampling time (weeks)		Untreated		Treated	
		0–15 cm	15–30 cm	0–15 cm	15–30 cm
0	before	8.50±0.04	7.70±0.36	18.07±0.35	12.60±0.36
	after	13.53±0.57	7.27±0.35	10.07±0.47	12.31±0.79
6	before	16.53±0.45	7.13±0.57	18.53±0.65	10.60±0.56
	after	14.96±0.35	8.07±0.35	12.47±0.45	6.77±0.61
10	before	11.73±0.76	8.03±0.35	20.33±0.57	12.97±0.40
	after	8.20±0.56	8.27±0.35	12.07±0.35	9.63±0.30
13	before	15.10±0.36	10.87±0.75	16.93±0.25	15.19±0.36
	after	20.23±0.55	13.17±0.25	10.40±0.70	9.00±0.36
16	at harvest	14.40±0.70	7.23±0.35	12.53± 0.65	6.77±0.25
23	after harvest	14.00±0.36	8.47±0.15	14.47±0.35	7.05±0.44
31	after harvest	14.87±0.75	10.60±0.46	18.67±0.49	15.83±0.47

Table 9. Quantity of NO_3-N from nitrification (µg/g dry wt. soil), third crop

Sampling time (weeks)		Untreated	Treated
0	before	18.43±2.64	17.73±5.72
	after	30.40±6.41	14.47 4.93
3	before	12.37±3.15	20.27±3.43
	after	33.13±7.72	14.47±1.83
6	Before	14.00±5.80	26.40±7.77
	after	31.43±5.57	18.30±5.88
12	before	17.42±1.06	17.77±2.42
	after	12.77±1.89	15.17±1.74
17	at harvest	28.20±6.37	19.60±3.75
21	after harvest	21.70±8.63	21.57±2.08
25	after harvest	28.23±4.58	25.80±0.64

Table 10. Quantity (x 10)[4]) of biomass in soil (mg/g dry wt.) in the 0–15 cm layer.

Sampling time (weeks)		First Crop		Second Crop		Third Crop	
		Untreated	Treated	Untreated	Treated	Untreated	Treated
0	before	10.94	12.24	7.69	7.80	19.12	18.48
	after	10.98	10.36	8.20	6.72	18.17	16.71
3	before	11.86	12.07			19.55	17.95
	after	12.27	9.27			21.54	12.73
6	before	12.08	13.05	8.05	9.53	16.93	17.67
	after	12.08	9.89	9.97	7.65	18.98	16.71
10	before	11.21	11.67	10.42	10.48		
	after	10.52	9.88	9.67	9.51		
12	before					13.26	14.58
	after					13.34	13.12
13	before	10.07*	12.08*	14.01	12.08		
	after			13.23	9.26		
16				12.74*	10.52*		
17	after harvest	12.11	9.92			18.90*	19.75*
21		9.19	10.32			18.55	19.32
23				8.53	8.17		
25						15.07	14.87
31				10.33	9.56		

* at harvest

3.3. Degradation of ^{14}C-monocrotophos

Table 11 shows that the amount of extractable and bound residues of ^{14}C-monocrotophos remaining in the top soil layer in the PVC columns at different times throughout the experiment. No residues of extractable and bound ^{14}C were detected in the 15–30 cm depth in any plot. Extractable ^{14}C-monocrotophos degraded rapidly from 85.68 and 80.17% of initial radioactivity from control and treated plots to 0.44% within 6 months. Calculation for half-life of monocrotophos from degradation curves resulted in 13.4 and 16.6 days in untreated and treated soil, respectively. Bound residues of ^{14}C were highest in treated and untreated plots at 18.94 and 12.58% at 6 months and declined to 4.68 and 2.74% at the end of 30 months.

Table 11. Extractable and bound ^{14}C-monocrotophos from untreated and treated soil.

Time (months)	% of initial radioactivity			
	Untreated		Treated	
	Extractable	Bound	Extractable	Bound
0 (3h)	85.68	9.61		
3	2.07	11.72	3.93	12.69
6	0.44	12.58	0.44	18.94
9	ND	11.6	ND	16.55
12	ND	10.43	ND	15.15
18	ND	8.66	ND	9.38
24	ND	6.78	ND	8.72
30	ND	2.74	ND	4.68

ND = not detectable

4. Conclusion

This long term experiment which lasted for three years (1996–1998) has shown that soil microbial activities such as respiration, nitrification, iron reduction capacity and soil biomass were suppressed by presence of pesticides from repeated application. Under these experimental conditions, long term use

of pesticides did not affect half-life of monocrotophos. Extractable ^{14}C-residues remained in the top soil (0–15 cm) and dissipated more slowly in treated than untreated soil. It may be concluded from this study that repeated application of insecticides in cotton growing field had an impact on soil microbial activities but had little effect on ^{14}C-monocrotophos degradation. However, the microbial activities seemed to recover after the end of each growing season.

REFERENCES

[1] Report on first FAO / IAEA Research Coordination Meeting on Impact of long term Pesticide Usage on Soil Properties Using Radiotracer Techniques. Neuherberg, Germany, 29 May–June (1995) 17pp.

[2] ANDERSON , J.P.E., "Soil Respiration", Methods of Soil Analysis, Part 2 , 2nd ed., (PAGE, A.L., et al., Eds.), Soil Science Society of America Inc., USA (1982) 831–871.

[3] ATLAS, R.M., et al., Assessment of pesticide effects on non - target soil microorganisms, Soil Biol. Biochem, **10** (1978) 231–239.

[4] CAMPER, N.D., Effects of pesticide degradation products on soil microflora, ACS Symp. Ser. **459** (1991) 205–216.

[5] GUTH. J.A., "Monocrotophos - environmental fate and toxicity", Reviews of Environmental Contamination and Toxicology. (GEORGE, W.W., Ed.), Springer - Verlag. New York. (1994) 75–136.

APPENDIX

Experimental protocols

This section describes the experimental procedures recommended at the 1st RCM held at Neuherberg, Germany 29 May – 2 June, 1995.

(1) Experimental layout
(2) Protocol to Determine Respiration Quotient and Biomass
(3) Protocol to Determine Capacity for Fe-III Reduction in Soil
(4) Nitrification
(5) Estimation of dehydrogenase activity in soil
(6) Determination of aryl sulfatase activity
(7) Determination of arginine deaminase activity
(8) Estimation of binding (and release) rates of ^{14}C-pesticides to soil matrices, under field conditions
(9) Soil capacity to mineralize ^{14}C-labelled aromatic pesticide molecules

The soil physical and chemical characteristics, total microbial and fungal populations may be determined by standard methods and need not to be described in detail.

1. Experimental layout

1.1. Plots

a) Control soil without any pesticide treatment + fertilizer +plants cotton or maize (and rotational crops).
b) Test soil with pesticide treatment + fertilizer + plants (cotton and rotational crops), corn or potato.
c) Treated soil from an actual farm plot (cotton, or maize)

Minimum size of each field plot: 500m^2

1.2. Soil sampling

- sampling depth: 0-15 cm (plough layer) and 15-30 cm
- sample size: minimum 10 cores (diameter 2-5 cm) per field
- minimum amount of collected soil: 1 kg per soil depth (dry wt. after sieving)
- dates of sampling: 1st (Zero) sample = before any application of pesticides
  2nd sample = 2 days after the first pesticide application
  3rd sample = immediately before the following pesticide application
  4th sample = 2 days after second application and so on
  x. sample at harvest
  y. samples during crop rotation

Mix soil samples from collected cores thoroughly (e.g. in plastic bags) and remove stones and plant debris

Note: time of sampling, weather at sampling time, last soil treatment, last fertilizer application, soil moisture content, growth stage of plants.

1.3. Transport

Samples should be transported:-

- Without loss of soil humidity but with free gas transfer (e.g. in polyethylene bags)
- In ice bags/boxes (max. 15°C)

1.4. Handling after sampling

- Determine soil water content, max. water holding capacity, pH etc.
- Divide each soil sample into subsamples
- Determine dehydrogenase activity IMMEDIATELY with a sieved, "fresh" soil subsample in triplicates.

1.5. Storage of soil

Sieve (8 mm) the soil, spread it on a plastic sheet and allow to dry overnight. Store at 4°C for not more than 3 months. Store sub-samples for pesticide residue analysis at –20°C.

1.6. Soil preparation prior to experiments

Soils stored at 4°C should be brought to 40-60% of Water Holding Capacity and incubated in the dark for 3d in plastic bags or bottles. Soils frozen for pesticide analysis should be thawed at 4°C and the moisture content determined prior to analysis.

1.7. Presentation of results

All measurements to be made at least in triplicate.
All results to be presented on the basis of soil dry weight with standard deviation.

REFERENCES TO SECTION ONE

SCHINNER, F., et al., (Eds) Bodenbiologische Arbeitsmethoden, Springer Verlag, Heidelberg (1993) 6-12.
ANDERSON, J.P.E., "Handling and storage of soils for pesticide experiments", Pesticide Effects on Soil Microflora, (SOMERVILLE, L., GREAVES, M.P., Eds) Taylor and Francis, London (1987) 45-60.

2. Determination of respiration quotient and biomass

2.1. Basal respiration

(1) Assemble standard biometer flasks which consist basically of a 250 mL Erlenmeyer flask fused to a 50 mL side-tube. Each flask is fitted with an Ascarite filter provided with a tap. Ascarite absorbs atmospheric carbon dioxide.
(2) Prepare 500 mL of 0.1M KOH stock solution and store it in a container fitted with an Ascarite filter to maintain the solution free of carbon dioxide.
(3) Place 50 g of moist soil *(*55% water holding capacity) in each of three biometer flasks.
(4) Insert 10 mL of KOH solution into the side arm of each biometer flask through the rubber stopper using a graduated syringe. During this process the stopcock is kept open and system kept at 22°C throughout the experiment.
(5) Prepare a fresh solution of 100 mL of 0.05N HCl solution.
(6) At periodic intervals an aliquot of the potassium hydroxide solution is siphoned off and carbon dioxide absorbed determined titrimetrically against the HCl solution.

(7) Sampling is continued over a period of 96 hours.
(8) To 10 mL of the collected KOH solution add 2 drops of phenolphthalein. The solution turns red (alkaline).
(9) Titrate against the HCl solution until the solution becomes colourless. Record the volume of the acid used (V_1). This gives the volume of HC1 required to neutralize KOH + the acid needed to change the carbonate to bicarbonate according to the following equations:
    (a) KOH + HCl → NaCl + H_2O
    (b) Na_2CO_3 + HCl → $NaHCO_3$ + NaCl
    (c) (the bicarbonate is acidic to phenolphthalein which is suitable for pH-range 8.3-10)
(10) Add 2 drops of methyl orange, the colour of the solution becomes yellow (bicarbonate is alkaline to methyl orange)
(11) Continue titration against HCl till the colour of the solution becomes orange. (Methyl orange is suitable for pH range 4.2-6.3).
(12) Record the volume used in step 11 (V_2). This volume is equivalent to half the carbonate present.
(13) The acid volume equivalent to CO_2 can be calculated as $V_1 - V_2$.
(14) 1 mL of 0.05N HC1 = 1.1 mg CO_2.

Results are expressed as mg CO_2/g dry weight soil.

2.2. Substrate-induced respiration

At step 3, the soil is amended with 4 mg glucose/g dry weight soil. Measurements of CO_2 produced (as above) to be made every hour up to a maximum of four hours.

2.3. Respiration quotient and Biomass

Respiration quotient = Basal respiration/substrate – induced respiration.
Biomass = Respiration quotient x 0.45

REFERENCE TO SECTION TWO

ANDERSON, J.P.E., "Soil respiration", Methods of Soil Analysis, Part 2, Chemical and Microbiological properties, 2nd ed., (PAGE, A.L., et al., Eds.), Soil Science Society of America Inc., USA. (1982) 831.

3. Determination of capacity for fe-III reduction

3.1. Extraction of soil

(1) 5 g of the prepared soil (Protocol 1) are placed in a 25 mL polyethylene vials with screw caps and 5 mL of distilled water containing 4 mg glucose /g dry soil are added as an energy source.
(2) The vials are shaken for 10h to obtain a homogeneous soil suspension and incubated under anaerobic conditions for 5 days after screwing the caps onto the vials.
(3) To extract the iron, add 5 mL of 1N KCl solution and shake the suspension vigorously (10 minutes).
(4) Centrifuge for 15 minutes.
(5) In aliquots of the clear solution, ferrous iron is stabilized by addition of conc. nitric acid (9 mL supernatant & 1 mL conc. nitric acid)

3.2. Determination of Fe^{2+}

This can be done by using *o*-phenanthrolin or 2,2′-dipyridyl which are organic bases of similar chemical properties and can chelate ferrous iron. Both reagents react rapidly with ferrous iron over a pH range of 2-9 to give orange-red (λ max 512 nm) or pink complexes (λ max 522 nm), respectively. Solutions of the complexes with phenanthroline and with dipyridyl are stable and the complexed iron is resistant to oxidation. The colour reactions are usually carried out in acetate or citrate buffer.

3.2.1. Reagents

(1) A 0.25% solution of the hydrochloride or hydrate of 1,10-phenanthroline or dipyridyl in 0.1M HCl.
(2) Standard iron solution: 1 mg/mL.
(3) A freshly prepared 10% solution. of hydroxylamine hydrochloride (This reduces Fe^{3+} to Fe^{2+} within a few minutes in a weak acid medium, pH3-4).
(4) 10% sodium citrate solution.
(5) Acetate buffer: approx. 0.4M prepared by dissolving $CH_3COONa.2H_2O$ (165 g) and glacial acetic acid (115 mL) in 1 L distilled water.

3.2.2. Procedure:

(1) Standard iron solution (1 mg/mL):- Weigh 8.635 g of ferric alum Fe NH_4 $(SO_4)_2$-$12H_2O$ and dissolve it in water containing 5 mL conc. H_2SO_4, and dilute with water to 1 L in a volumetric flask. Working solutions are obtained by suitable dilutions of the stock solution with dilute sulfuric acid (e.g. 0.005M).
(2) A sample of a slightly acidic iron solution (1 mL containing max. 120 μg) is mixed with 2 mL of the hydroxylamine solution.
(3) Add buffer till pH is around 3-4.
(4) Transfer the solution to a volumetric flask (50 mL) and add 5 mL of the complexing agent.
(5) Dilute to mark with water and mix thoroughly.
(6) Allow to stand for 5 minutes and then measure the absorbance of the coloured solution at 512 nm for *0*-phenanthroline against water (at 522 nm for dipyridyl).
(7) Determine the iron concentration in an unknown sample from the standard curve.
(8) Controls (without glucose) are run in parallel

4. Determination of nitrification

The detailed procedure was prepared by Dr. S. Khan and faxed to programme participants.

5. Estimation of dehydrogenase activity in soil

(1) To 3 g air dried soil, add 1 mL glucose solution (30 mg/L) and 0.5 mL of a 3% solution of 2,3,5-triphenyl-tetrazolium chloride (TTC). Make up volume to 5ml by addition of 0.1 M Tris buffer (pH7.6-7.8).
(2) Mix thoroughly and incubate at 37°C for 24 hours.
(3) The reaction product, formazan, has formed as a result of the enzyme activity.
(4) Centrifuge and transfer the supernatant to a flask containing 10 mL acetone.
(5) Determine optical density at 485 nm in a spectrophotometer.
(6) A calibration curve should be constructed using the reference chemical “formazan”.
(7) Dehydrogenase activity may be expressed as μg formazan formed per 10 g soil.

REFERENCES TO SECTION FIVE

SCHUSTER, E., SCHRODER, D. Side-effects of sequentially-applied pesticides on non-target soil microorganisms: field experiment, Soil Biol. Biochem. **22** (1990) 367–373.
CASIDA, L.E.Jr.,et al., Soil dehydrogenase activity, Soil Sci. **98** (1964) 371–376.

6. Determination of aryl sulfatase activity

Soil samples are incubated with *p*-nitrophenylsulfate solution which, through enzymatic hydrolysis, produces *p*-nitrophenol. The latter gives a colour, on addition of NaOH, which can be measured photometrically. The method described is a modification of the procedure published by Tabatabai and Bremner cited below.

6.1. Reagents

(1) Acetate buffer (0.5M, pH5.8); dissolve 64.0 g of sodium acetate. $3H_2O$ in 700 mL and add dropwise approx. 2 mL glacial acetic acid to pH 58. Fill up to 1000 mL with distilled water
(2) Substrate (0.02M); dissolve 0.515 g of potassium-p-nitrophenylsulfate in 100 mL buffer (always freshly prepared).
(3) Sodium hydroxide (0.5M); dissolve 20g NaOH pellets in 1 L distilled water
(4) Standards
    (a) concentrated standard; dissolve 1 g p-nitrophenol in 1 L distilled water (stable at 4°C)
    (b) working standard (0.1 mg p-nitrophenol per mL); 10 mL of the concentrated standard brought to 100 mL with distilled water.

6.2. Standard curve

(1) Pipette 0,1,2,3,4,5 mL of the working standard into small beakers and bring to 5mL with distilled water. Add 25 mL distilled water. Transfer 6 mL of the mixture into a beaker or flask and add 4 mL NaOH solution. The blank system consists of 6 mL distilled water and 4 mL NaOH solution. The calibration points will correspond to 0, 20, 40, 60, 80 and 100 µg *p*-nitrophenol.
(2) Transfer 1g of naturally-moist soil sample into a 50 mL Erlenmeyer flask and add 4 mL buffer and 1 mL substrate. Mix well and incubate at 37°C for one hour.
(3) Prepare 3 samples (with substrate) and 1-2 blanks (without substrate) from each type of soil.
(4) Add 25 mL distilled water to all flasks and l mL substrate to the blank.
(5) Shake the suspension and filter.
(6) To 6 mL of the filtrate add 4 mL of NaOH solution and measure the yellow colour formed immediately against the blank in a photometer at 420 nm.

6.3. Calculation

The enzyme activity is expressed as µg p-nitrophenol formed, calculated as follows:

$$\frac{(T-B)x30x100}{6x\%DW} = \mu g\ p \text{ nitrophenol/g}$$

where T is the mean value of test material (µg *p*-nitrophenol); B is the mean value of blank (µg *p*-nitrophenol); 30 is the extract volume (mL); 6 is the filtrate aliquot (mL) and 100/% DW is the dry wt factor.

Remarks

a) The substrate is very sensitive to light and temperature and therefore, the flask containing the substrate solution should be wrapped in aluminium foil.
b) Measurements should be made within 6 hours.
c) The standard curve is linear up to 120 µg p-nitrophenol.

REFERENCE TO SECTION SIX

TABATABAI, M.A., BREMNER, J.M. Arylsulfatase activity of soils, Soil Sci. Soc. Am Proc. **34** (1970) 225-229.

7. Determination of arginine deaminase activity

Soil samples incubated with arginine produce ammonium which is extracted with potassium chloride solution and determined colorimetrically after addition of chromogenic reagents.

7.1. Reagents

Substrate (11.5mM); dissolve 200 mg L-arginine in 100 mL distilled water (freshly prepared daily).

Potassium chloride (2M); dissolve 149 g KCl in 1 L distilled water (solution stable for several weeks)

Sodium phenolate (0.12 M); dissolve 2 g $C_6H_5ONa.3H_2O$ in 100 mL distilled water.

Sodium nitroprusside (0.17 mM); dissolve 50 mg $Na_2[Fe(CN)_5NO].2H_2O$ in 1L distilled water

Sodium hypochlorite; dissolve 5 g NaOH in 100 mL distilledwater, add 25 mL sodium hypochlorite (15% active chlorine) and make up to 1 L

Concentrated standard (1 mg N/mL); dissolve 3.8207 g NH_4Cl in 1L dist water (solution is stable for several weeks at 4°C)

Working standard (10 µg N per mL); dilute 10 mL of the concentrated standard solution to 1000 mL distilled water.

7.2. Procedure

Transfer 5 g of naturally-moist soil into a 100 mL Erlenmeyer flask and add 2 mL arginine solution (in triplicates). Stopper loosely and incubate at 37°C for 3 hours. The blank is similarly prepared, but immediately frozen at -20°C.

After incubation, 18mL KC1 solution are added to all samples which are shaken for 30 minutes on a horizontal shaker. Filter the suspension using N-free filter paper. To 1 mL of the filtrate, add 3 mL KCl solution, 2 mL sodium phenolate solution, 1 mL sodium nitroprusside solution and 1 mL sodium hypochlorite solution. Shake. After 30 minutes, the colour formed is measured in a photometer against the blank at 630 nm.

For construction of the standard curve, use 0, 0.5, 1.0, 2.5 and 4.0 mL of the NH_4Cl working standard, and bring volume to 10 mL with KCl solution. Develop the colour as described above. The values correspond to 0, 0.5, 1.0, 2.5 and 4.0 µg N per mL.

7.3. Calculation

The enzyme activity is expressed as μg N, calculated as follows:

$$\frac{(T\text{-}B)x20x100}{3.5x\%DWt} = \mu g\ N/g\ dry\ wt.\ soil\ h^{-1}$$

where T is the mean value of the test material (μg N per mL); B is the μgN/mL; 20 is the whole volume of extract (mL); 3 is the incubation period (h); 5 is the soil weight (g) and 100/% DW is the dry weight factor.

REFERENCE TO SECTION SEVEN

ALEF, K., KLEINER, D., Arginine ammonification, a simple method to estimate microbial activity potentials in soil, Soil Biol. Biochem. **18** (1986) 233-235.

8. Estimation of binding (and release) rates of ^{14}C-pesticides to soil matrices under field conditions

8.1. Layout

Twenty four hard PVC cylinders (40-50cm length, 4-5cm diameter) should be inserted in treated and control experimental plots. When inserting the cylinder into soil, two weeks prior to application, care must be taken that about 3 cm of the cylinder projects above the soil surface to prevent the flow of run-off water onto the soil surface. The first set (24 cylinders) will be treated with regular inputs of fertilizer and one ^{14}C-labelled pesticide. The second set (24 cylinders) will receive the same inputs plus all other cold pesticides regularly used in the field. All concentrations, time and frequency of application (including the radiochemical) should be similar to those used in the field. The cylinders should be left in the open under field conditions.

The radiochemical appropriately diluted with the cold chemical (to prepare a field concentration of 2 mg/kg based on the soil weight contained in a depth of 15cm) may be applied by means of a pipette onto the soil surface. Water, acetone or hexane may be used and each cylinder should receive 185 kBq, diluted with the calculated amount of the cold pesticide. Should the number of applications be large, the amount of radioactivity may be reduced to 111 kBq per cylinder.

There should be at least 3 replicates for each sampling time [0 (immediately after application of the radiochemical), 3, 6, 9, 12, 18, 24 and 30 months] and cylinders should be removed for analysis at random. Soil sections from 0-15 cm depth and 15-30 cm should be removed for analysis. Soil must be mixed thoroughly and a minimum of three subsamples (from each depth) should be analyzed from each cylinder. Soils may be frozen at -20°C until analysis.

8.2. Extraction and analysis

Soil-extractable and non-extractable (bound) residues should be determined in all samples. Soxhlet extraction should be done with methanol for 10 cycles over 4-6 hours using 50 g. of soil. Non-extractable residues may be determined (after extraction) by wet or dry combustion techniques, using 500 mg of soil. It is recommended to determine the total ^{14}C before extraction by combusting 500 mg soil. Identification of the ^{14}C-pesticide and at least one major product should be made in the extract using TLC, GC or HPLC.

Data should be expressed as mg/kg dry weight soil ± S.D. and as percentage of the zero time value.

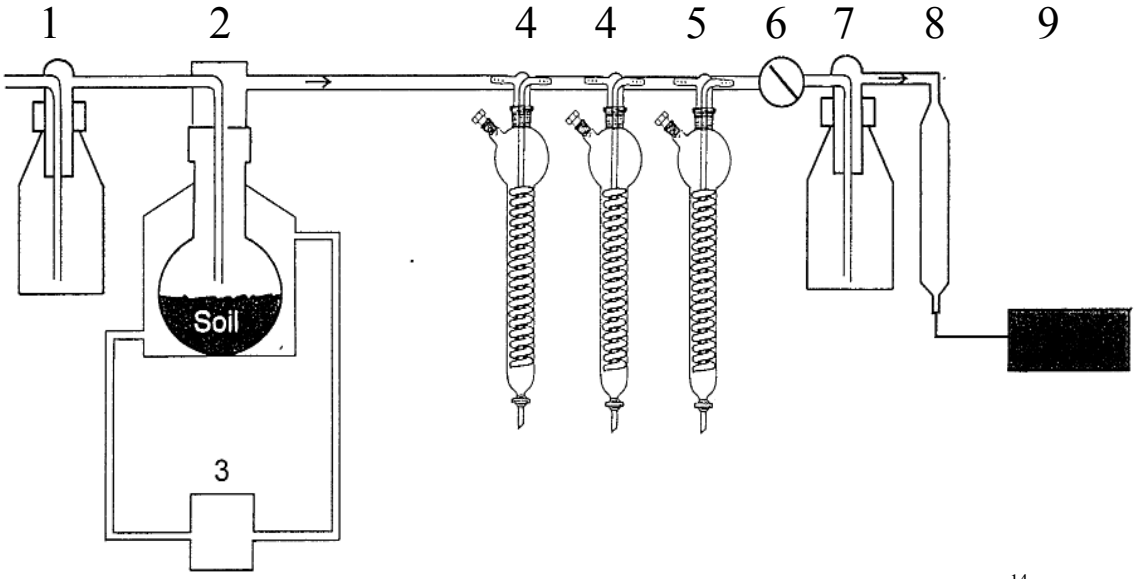
9. Soil capacity to mineralize ^{14}C-labelled aromatic pesticide molecules

(1) Use ^{14}C-ring-labelled 2,4-dichlorophenoxy acetic acid and the three types of soil identified in protocol 1.

(2) Transfer 50 g (dry weight equivalent) fresh soil into a 250 mL round-bottomed flask and add 50 µg cold 2,4-D + 37 kBq of the ^{14}C-chemical in a small amount of acetone. This will produce a concentration of one µg/g. The flasks (in triplicates, from each soil type) are kept at 22°C by a cryostat and connected to a trapping line as shown in Fig. 1. The organic volatiles may be trapped in ethyleneglycol monomethylether and $^{14}CO_2$ may be absorbed by 1N KOH solution or ethanolamine.

The system is flushed with air once a day for few minutes by a pump to help trap the ^{14}C-products. The experiment should run for 6-8 weeks or typically when the curve flattens off. Sampling may be made twice weekly in the first 3 weeks and once per week thereafter; simply by collecting the solvents from the traps and replacing them with fresh solvents.

Analyse by measuring the radioactive content of the collected solvents. Data may be expressed as % of the originally-applied radioactivity.



(1, wash bottle; 2, incubation flask; 3, cryostat; 4, absorption tubes for organic ^{14}C; 5, absorption tube for $^{14}CO_2$, 6,valve; 7, Woulff bottle; 8, charcoal filter; 9, pump)

Figure 1. Closed laboratory system for determining $^{14}CO_2$ from the mineralization of ^{14}C-pesticides in soils.

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