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Organochlorine insecticides in African agroecosystems

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FOREWORD

A rational decision on the use of pesticides in pest management should be based on cost, benefit and environmental impact. For economic reasons, the cost-benefit to the farmer has been, and still is, the dominant factor in developing countries. Consequently, cheap organochlorine pesticides are still widely used although their persistence has led to their accumulation in food chains in temperate countries with consequent potential effects on higher fauna.

Adverse effects can also appear within the agroecosystem itself. Intensive use of broad spectrum insecticides can reduce populations of insect parasites and predators; as a result, pest populations may be inadequately controlled. This, in turn, can lead to the need of more frequent use of pesticides and to the appearance of secondary pests.

We are slowly beginning to understand how natural enemies and the other non-target fauna are affected by pesticide use in a few agroecosystems in the developed countries. The knowledge about these matters in developing countries is very rudimentary.

The objectives of this programme were to increase the knowledge of how pesticides affect the agroecosystem especially pest-natural enemy interactions and the non-target fauna within and outside African agroecosystems. Chlorinated hydrocarbon pesticides were used as representing the compounds most likely to produce undesirable consequences. This TECDOC reports the accomplishments of the programme which was financed by the Swedish International Development Authority (SIDA).

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SUMMARY OF THE CO-ORDINATED RESEARCH PROGRAMME

Introduction

A series of discussions in the late 1980s between staff of the Joint FAO/IAEA Division for Nuclear Techniques in Food and Agriculture and counterparts in the Swedish International Development Agency considered mutual concerns related to pesticide use. Those of relevance to Africa gave rise to this programme.

Problem

There was a fear that indiscriminate use of cheap, but persistent organochlorine pesticides may harm the African environment, especially through effects on non-target fauna. For example, birds may suffer reduced reproductive success or be killed through direct ingestion of the toxic compounds. Aquatic life is also at risk through run-off from crop land or careless discharge of used cattle dips. The resulting depletion of the natural populations is deplorable in itself and may even harm agriculture, for example through less control of pests by predators or the absence of necessary pollinators.

The introduction and acceptance of probably less damaging alternatives, such as the use of less persistent pesticides, the introduction of pest resistant plants or organized integrated pest management is slow, largely because of the level of organization in agricultural practices required and the short-term higher cost incurred by the farmer.

Background

A number of rather effective organochlorine pesticides (mostly insecticides like DDT, lindane, heptachlor, chlordane and toxaphene) are still thought to be widely used in African countries. Most developed countries have restricted or banned their use, based on knowledge of their fairly high persistence in the environment, the common development of resistance to them by the pests, their ability to further accumulate through food chains, their reputation for interfering with reproduction of mammals and birds and, for some compounds, the extreme toxicity to aquatic life. Despite this, many developing countries have not restricted the use of these compounds, mainly because of their high efficiency/cost ratio and to the apparent non-availability of affordable alternatives. Most African countries have established an amendment to existing legislation or introduced new laws covering the distribution and the use of pesticides but implementation is not always very thorough. The introduction of the *FAO International Code of Conduct on the Distribution and Use of Pesticides* in 1986 and later (1989) the additional clause on *Prior Informed Consent* has helped a good deal in controlling the worst excesses. Although improvements are seen, the general picture is still bleak.

A decisive factor in improving the situation is the public and/or scientific interest and pressure for improvements. The general scientific knowledge of actual effects of pesticides in tropical regions is limited, especially in Africa. Scientific literature and company information, largely obtained in temperate countries, are often the only available basis for decisions in developing countries. National research is often slow and not well co-ordinated and, in many cases, too sparse to be really useful in the pesticide registration process.

The interdisciplinary approach necessary to elucidate the adverse effects of pesticidal use is not always taken by scientists. Rather the opposite is common where e.g. the chemist

may analyze a great number of samples of residues of certain pesticides (which is the most common approach) or where the biologist in an area attempts to count dead or alive individuals of one or more species or group of species. Combining the efforts of the two would tell much more of the eventual harm of identified compounds and yields more convincing results. The benefits of international scientific collaboration may be obvious to most scientists in developed countries but cannot be taken for granted in developing countries.

Objectives

The short-term objective was to co-ordinate and support African scientific research on the environmental effects of pesticides, especially persistent organochlorine compounds. The long-term objective was to diminish damage to flora and fauna through better management of pesticide use, including restrictions to the use of deleterious products, and introduction of less damaging alternatives.

Approach

The main idea was to activate national interdisciplinary research to include chemists, zoologists, botanists and ecologists. Scientific groups interested in this type of research were identified both at (agricultural) universities and in government research institutions.

The problem(s) to be examined by these groups in each participating country were to be of major importance to the national environment. The similarity in the choice of methodology, work plan(s) and goal was the common feature of the programme. It was envisaged that biologists would approach the evaluation of the adverse side effects in each ecological compartment in much the same manner, and the environmental chemists may for instance use the same radiochemical approach in determining the fate and turnover of selected compounds. The same applies to the other disciplines and, in addition, the international co-ordination provided may lead to a much better definition of why, how and when to do what.

Further considerations

The programme was originally conceived under an assumption that the necessary facilities and skills were already in place in most potential participating countries and that suitable experimental procedures were already established. This proved to be unduly optimistic and it quickly became apparent that important elements of the programme would be training and method development. In particular, possibilities for doing residue analysis were limited, validated methods for ecotoxicological studies in Africa were not available and relevant experience was scarce in most countries.

However, the positive aspects of this situation are that participants were forced to broaden their expertise in order to contribute to the programme and a number of experimental procedures were adapted to local conditions. The introduction of the FAO programme on Food Security in low income food deficit countries and the complementary IAEA Technical Co-operation (TC) Project RAF/5/036 have heightened the relevance of the work of this Co-ordinated Research Programme (CRP) as the facilities it has developed will support the monitoring activities required in these initiatives.

There is also interaction with the proposed FAO/IAEA Training and Reference Centre for Food and Pesticide Control. The difficulties faced by the laboratories in the CRP

contributed to the evidence collected which identified the need for the Centre and refined ideas about which should be the target laboratories of the Centre's programme. It is anticipated that some of them will be involved with the Centre.

Some record must be made of the constraints, natural and man made, to the programme. The most important of these was drought. In Zimbabwe, only the 1992-1993 season received "normal" rainfall and field experiments in the other years were severely restricted. In Uganda, 1992-93 was a drought season as was 1994 in Zambia. Throughout East Africa, the whole period of this programme has been relatively dry even in seasons when crops were produced and this must be borne in mind in considering the results.

Participants in Ghana were affected by a national shortage of electricity, particularly in 1994-1995 when periods of up to 3 weeks without power were experienced. Erratic power supplies also affected Nigeria but a more serious problem was strikes and lock-outs that affected the University system, the worst of which lasted for 6 months.

The Programme

The practical work was based on a "feasible" agroecosystem that could be established in all the participating countries. The maize production system was chosen for a common investigation because:

1. Organochlorine pesticides can be used;
2. It is suitable for field trials;
3. There is one major pest (stem borer);
4. There are some well known predators and/or parasites.

In some countries, other crops which met these criteria were also studied.

The programme was divided into three research areas with the maize system as a common feature:

1. The effect of pesticides on the agroecosystem
2. The fate of pesticides in the agroecosystem
3. The effect of pesticides on higher fauna.

The practical details of the procedures which were developed in the programme are set out in Appendix I.

Results

The methods used for insect assessments were modified as necessary from standard sweep net, D-Vac and pit-fall trap procedures. Gould [1], in developing statistical guidance for studies of pesticides on wild life, took a coefficient of variation (CV) of 50% as being a reasonable value for variability in observations of this type. Participants in this programme frequently reported CVs of over this value for sweepnet counts but almost all values obtained with the D-Vac were below this. Except at very low population numbers, the pitfall trap results also produced CVs of below 50% and generally were much lower. Comparable values were reported for a DDT study in Zimbabwe [2] where figures for insects extracted from soil cores gave CVs of 13-20% and for invertebrate biomass caught in pitfall traps the range was 18-37%.

Table 1 Summary of observed effects from use of organochlorines in several African agroecosystems (/ + weak effect, --/ + + moderate effect, – / + + + strong effect, 0 = no observed effect n r = not recorded)

Country	Crop	Pesticide (Years)	Non-target		Biomass	Pest	Yield
Zambia	Maize	Endosulfan (2)	Collembola	---	-	---	+ + +
			Formicidae	0			
			Spiders	0			
			Coleoptera	0			
			Diptera	n r			
Zambia	Maize	Lindane (2)	Collembola	---	0	---	+ +
			Formicidae	–			
			Spiders	--			
			Coleoptera	-			
			Diptera	n r			
Algeria	Chick pea	Lindane (4)	Collembola	---	--	--	+ + +
			Formicidae	—			
			Spiders	---			
			Coleoptera	-			
			Diptera	n r			
Algeria	Maize	Lindane (3)	Collembola	—	0	---	+ + +
			Formicidae	-			
			Spiders	--			
			Coleoptera	0			
			Diptera	n r			
Uganda	Maize	Lindane (4)	Collembola		--		+
			Formicidae	-			
			Spiders	--			
			Coleoptera	0			
			Diptera	-			
Nigeria	Maize	Lindane (4)	Collembola	---	---	--	+ + +
			Formicidae	--			
			Spiders	---			
			Coleoptera	--			
			Diptera	n r			
Nigeria	Rice	Lindane (2)	Collembola	---	---	---	+
			Formicidae	--			
			Spiders	---			
			Coleoptera	0			
			Diptera	n r			
Ghana	Maize	Lindane (3)	Collembola	—	0	0	0
			Formicidae	-			
			Spiders	0			
			Coleoptera	0			
			Diptera	n r			
Ghana	Cowpea	Endosulfan (3)	Collembola	---	0	—	+ + +
			Formicidae	-			
			Spiders	0			
			Coleoptera	0			
			Diptera	n r			
Tanzania	Maize	Lindane (4)	Collembola	--	---	—	+ + +
			Formicidae	--			
			Spiders	--			
			Coleoptera	0			
			Diptera	--			
Zimbabwe	Maize	Endosulfan	Coleoptera	0			
			Orthoptera	0			
			Spiders	0			
	Soya	Endosulfan	Coleoptera	--			
			Orthoptera	0			
			Spiders	0			

Ranking of effects of organochlorines on several non-target organisms.
0 = no observed effect, 1 = weak effect, 2 = moderate effect, 3 = strong effect. Number of countries in parenthesis

Taxonomic group	Lindane	Endosulfan
Collembola	3 (8)	3 (2)
Formicidae	2 (8)	0 (2)
Spiders	3 (8)	0 (2)
Coleoptera	1 (8)	0 (2)
Diptera	1 (2)	n.r.

Thus, the methods used in the hands of the participants in this programme gave results comparable in variability to those obtained elsewhere.

The effects observed in the various countries are summarized in Table 1. Except for the endosulfan-treated maize plots in Zimbabwe all participants recorded at least one non-target insect whose numbers were reduced by the insecticide at some point. However, none of these effects lasted from one season to the next and in most cases differences between treated and control plots were not measurable after 6 - 8 weeks. Six countries reported reduced rates of leaf litter decomposition following pesticide application but the differences were not great and of uncertain significance in relation to soil fertility. It could be argued that slowing the rate of organic matter decomposition in tropical soils could, at least in some circumstances, be beneficial.

Except in the case of lindane and maize in Ghana the insecticide reduced the incidence of pest attack although this was not always reflected in a corresponding increase in crop yield. Residues in maize grain were measured in Egypt, Algeria and Ghana, in soybean in Egypt and cowpea in Ghana. Except in the case of soybean, levels were below *Codex Alimentarius* Maximum Residue Levels. Thus, no major effects on non-target organisms were observed in this programme. This is consistent with the conclusions of the study in Zimbabwe [2] that a) wildlife populations in general were less affected by DDT than by seasonal changes in climate and spatial variation in habitat and b) important soil microbial activities were unaffected by DDT. These results are not altogether unexpected as 10-year studies in apple and corn fields in Hungary [3] and a 15-year trial in cereals and carrots in the UK [4, 5] did not identify deleterious effects from repeated pesticide use under temperate conditions where compounds are likely to persist longer than in the tropics.

Soil persistence data from field studies reported from Algeria, Ghana, Nigeria, Zambia and Zimbabwe and work with ^{14}C-labelled lindane was also reported from Algeria and Ghana. The data must be viewed cautiously because, for reasons beyond the control of the Agency's Technical Officers, only one interlaboratory comparison analysis was done and this did not produce satisfactory results. However, the trends of the results show that the persistence of lindane and endosulfan in Algeria, Ghana, Nigeria and Zambia is short compared with that observed in temperate regions. In Zimbabwe, persistence was rather longer and carry-over from one year to the next was observed. This is, no doubt, due to relatively lower temperatures in Zimbabwe combined with the dry conditions (noted in the Introduction).

The three studies with ^{14}C-labelled lindane confirmed relatively rapid rates of loss. In Algeria, where the study was done in the laboratory at 25°C, about 50% of the applied ^{14}C was lost in 50 d and breakdown was faster in soil previously treated with lindane than in control soil.

In the semi-field study in Ghana, where the temperatures, though not recorded, would have been slightly higher, 18 - 50% of the applied radioactivity remained after 50 d. In Egypt, the initial soil concentration from an application of 2.25 kg/ha was not measured but after 85 d only 0.11 μg/g was found in the top 5 cm of the soil (about 4% of the initial application if it all reached the soil). Although not totally convincing because of the analytical uncertainty, these results are consistent with those of the FAO/IAEA Co-ordinated Research Programme [6] which showed that DDT is not a very persistent chemical under tropical conditions.

There were three investigations involving higher fauna. One was concerned with monitoring water and fish for pesticide residues in areas of Ghana where organochlorine compounds are in use. Gas chromatogram peaks corresponding with the retention times of both lindane and endosulfan were found frequently in both. Residues in concentrations in fish were, as expected, higher than those in water but were lower than concentrations that might be expected to be lethal. However, the scarcity of fish in the rivers sampled suggests that some toxic agent(s) was present. Other organochlorine compounds, notably DDT, were also tentatively identified in fish and water samples. The other surveyed birds in regions of Tanzania where organochlorine compounds are known to be used. African Fish Eagles from Lake Victoria contained residues that corresponded chromatographically with p'p'-DDE and o-p'-DDE and with β-HCH. As with the fish in Ghana, the concentrations were below those that would be expected to be lethal but only living birds were sampled. The study in Egypt of the effects of lindane in a caged agroecosystem also included birds (*Asfour baladi*) and provided histological evidence of degenerative changes in liver, kidney and nerves tissue. The toxicological significance of these observations is uncertain.

Several participants also were able to obtain data on pesticide use in their countries. They are summarized in Appendix II without comment except to note that they confirm that organochlorine compounds are still in widespread use.

Conclusions

1. There was no evidence of serious environmental impacts due to these two pesticides in this project. This conclusion is based on the following research results:

   - No serious long-term effects on the natural enemies of pests were seen, although there was some depression for a period after treatment.
   - No serious effects of lindane or endosulfan on soil organisms were seen.
   - Although there was some reasonably consistent depression of rates of organic matter breakdown after treatment with the two pesticides, there appeared to be no long-term effects likely to influence soil fertility.
   - No serious uptake of the pesticides into the tissues of birds or fish was recorded.
   - Residues in harvested grain were, with one exception, within acceptable limits.

2. The data provided by the project on the persistence and fate of lindane and endosulfan support the conclusions of a previous FAO/IAEA project [6] that organochlorine insecticides dissipate much faster under hot and moist tropical conditions than under temperate conditions. This may well influence decisions on continued use and/or specific restrictions on these pesticides.

3. There is evidence in one study that repeated applications of lindane cause microbial adaptation, so that the rate of degradation was enhanced.

Outputs

1. Most participants reported a lack of trained scientists capable of working with pesticides in their countries. Through advisory visits, three workshops and during Research Co-ordination Meetings, this programme provided training in a wide range of methodologies and techniques, particularly in:

- Design and conduct of field experiments (replication; record-keeping; need for background data on soils and climatic information; location of plots and barriers between treatments; taking of yield data);
- Sampling of insects and other arthropods on crops using D-Vac, sweepnets, pitfall trapping and sticky traps; identification of key pests and beneficial arthropods (ants (*Hymenoptera - Formicidae*), spiders (*Aranea*), carabid beetles (*Carabidae*), staphylinid beetles (*Staphylinidae*)) and population assessments in relation to lindane and endosulfan treatments;
- Assessing the effects of pesticides on the key soil process of organic matter breakdown;
- Assessment of the effects of pesticides on bird and populations;
- Pesticide residue analyses (thin-layer chromatography, gas liquid chromatography), sampling (taking, size, distribution and numbers of samples) of soil, plants, earthworms, birds and fish; extraction and clean-up for assessment of lindane and endosulfan residues; the principles of analytical quality assurance and control;
- Use of radioisotope techniques in the assessment of movement, distribution and fate of pesticides.

2. The programme provided material for higher degree studies in:

Algeria	1 Master degree student
Egypt	1 Master degree student
	1 Ph.D. student
Ghana	2 Master degree students
Nigeria	2 Master degree students
Uganda	2 Master degree students
Zimbabwe	2 Master degree students.

3. Within-state, inter-state and regional links were developed, and information transferred on the environmental impacts of pesticides.

4. The countries are at different stages in developing full registration programmes but all have to depend largely upon data generated for this purpose in Europe and the USA, which may have little relevance to their own programmes. Pesticides have been banned or their use restricted, in tropical countries based on data from temperate countries, where their persistence and environmental performance may differ greatly. In this programme, highly relevant, local environmental data for registration purposes was generated in the participating countries.

5. Data were compiled on the use of organochlorine compounds in several countries.

6. Experimental guidelines were developed appropriate for the study of many aspects of the effect of pesticides on non-target organisms in agricultural ecosystems.

Recommendations

1. The data generated should be brought to the attention of competent authorities in countries in the region.

2. Programmes should not be developed without pre-proposal surveys to establish the problems that need to be addressed and the existing infrastructure and capabilities in the countries expected to participate.

3. Such programmes should have narrower, more specific aims.

4. The methods validated in this programme should be considered for use in the various programmes concerned with food security in Africa and promulgated elsewhere.

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FATE AND EFFECTS OF LINDANE IN A CHICKPEA FIELD

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Abstract

The effect of lindane on non-target organisms and the concentrations of its residues in soil and the chickpea crop were investigated over three years Lindane had adverse effects on some elements of the ecosystem Ants (*Formicidae*), spiders (*Aranae*) and beetles (*Carabidae*), to a lesser extent, were more affected than *Collembola* Organic matter, buried in non-degradable open-mesh bags in the plots, was slightly more degraded in the control plots than in the sprayed plots suggesting that the soil microflora and microfauna had been inhibited by the lindane However, it was shown by chemical analyses that lindane was degraded in both soils and plants to one tenth of the original concentrations after application in 2 months and 1 month, respectively Some concentrations (0 2-1 2 mg kg^{-1}) of lindane were found in the harvested grain of the chickpea plants

1. INTRODUCTION

The aim of pesticide use is to protect agricultural production. The possible adverse effects on non-target flora and fauna from the use of organochlorine pesticides were studied under field conditions during 1992-1994 at the Institut Technique des Grandes Cultures (ITGC) near Algiers.

This was a part of a coordinated research programme supported by FAO/IAEA/SIDA carried out in eight African countries.

2. MATERIALS AND METHODS

2.1. Materials

Formulated lindane (50 g kg^{-1} EC) was obtained from Asmidal, Algeria. Analytical standard (99.79% purity) was purchased from Rhône Poulenc.

The insecticide was applied to the plot as an emulsion in water at the rate of 1 kg AIha in 250 L of water using a back-pack sprayer (Vegatype, 20 L capacity).

2.2. Crop

Chickpea (*Cicer ariétinum*, var., ILC 482) was sown in April. The seeds were previously treated with fungicides (Carboxine and Thiram). The mean emergence was 93.3%.

2.3. Soil

The field soils have a clay texture

2.4. Experimental design

The field plot experimental design was supplied by the ITGC as follows:

1992: 2 plots with dimensions 22 m x 30 m = 660 m^2. The crop was sown with 1 m spacing between rows and 0.2 m between plants.

1993: 2 plots with dimensions 23 m x 33 m = 759 m^2. The crop was sown with 0.8 m spacing between rows and 0.2 m between plants.

1994: 8 plots with dimensions 12 m x 12.5 m = 150 m^2 with the same spacings as in 1993.

No fertilizer was applied throughout the experimental period.

2.5 Predators

Insects were collected using four pitfall traps set within each treatment and untreated, control plot with the traps set before spray application and, after spraying, at the intervals shown in Table 4. In the first study during 1992, two categories of predators were selected *F. Carabidae* and *F. Coccinellidae*. During the second year of the experiment, the two categories of predators selected were *F. Formicidae* and members of the class *Aranae*, while for the third year Collembola were selected.

2.6 Breakdown of organic matter

Batches of 50 disks (2.5 cm diameter) from eucalyptus leaves were used for the determination of the rate of breakdown of organic matter. Those batches were placed in a nylon mesh bag (mesh size 0.8 cm) which was sealed and buried at a depth of ~5 cm prior to treatment. In each subplot four bags were buried at random. The bags were dug out after 3 months, washed, dried at 60°C and weighed.

2.7 Extraction of residues

Plant material

Triplicate sub-samples of plant tissue were weighed and ground in water in an Omnimixer. Anhydrous sodium sulphate and hexane + acetone (1+1 by volume, 100 mL) was added and the mixture extracted by tumbling for 1h in screw-capped jar. The extract was filtered and the solvent placed into a separating funnel containing sodium sulphate solution (20 g L^{-1}, 200 mL) and shaken for about one minute. The upper layer containing the hexane extract free from acetone was then transferred to a C_{18} SPE column and the lindane eluted with hexane before GC analysis.

Grain

Samples (10-20 g) of chickpea flour, prepared by milling the grain, was extracted with acetone + hexane solution (1+1 by volume, 50 mL) by tumbling for one hour in a screw-capped jar. The suspension was centrifuged at 4000 rpm and the supernatant fluid was quantitatively filtered and evaporated in a Buchi rotavapor at 60°C. The residue was redissolved in hexane and then this solution was reduced in volume to 2-3 ml by evaporation at 60°C. This solution was quantitatively transferred to a C_{18} SPE column and the column eluted with hexane. The eluent was diluted to a known volume and aliquots analysed by the GC method.

2.8. Residues analysis

1992-1993: gas chromatograph: Varian 3700; column: 1 m x 4 mm i.d.; packing: OV17 + OV210 (1.5 + 1.95 by mass) on Chromosorb W-HP; temperatures: column, 200°C; injector, 230°C; detector, 300°C; carrier gas: nitrogen; flowrate, 30 mL min^{-1}; detector: ^{63}Ni electron capture. Location: CDTN, Algeria.

1994: gas chromatograph: Girdel; column: capillary; temperatures: column, 190°C; injector, 240°C; detector, 290°C; carrier gas: nitrogen; pressure: 97-110 kPa. Location: Laboratoire de Phytopharmacie de Perpignan, France.

3. RESULTS AND DISCUSSION

3.1. Crop yield

The treated and control plots during the three years of experiments (1992, 1993, 1994) gave yields of 297.5, 195.15, 382 $kgha^{-1}$ and 165.7, 135.5, 135.8 kg ha^{-1} respectively (Table 2). The ITGC standard value for chickpea is 500 kg ha^{-1}. The difference in crop yield between treated and untreated, control plots was statistically significant ($P<0.05$) for all years, indicating that lindane had a protective effect on the treated plots.

3.2. Plant damage

The values for plant damage for both treated and control plots were significantly different during the three years as shown in Table 3 which again indicates that lindane was effective. However it was observed that Fusarium disease damaged plants from both treated and control plots during 1992.

3.3. Effect of lindane on predators

The data (Table 4) obtained indicate that lindane had adverse effects on some elements of the ecosystem fauna, similar to that reported for the organochlorine pesticide DDT [1].

During 1992, two categories of insects were selected: *Coccinellidae* and *Carabidae*. Although not statistically significant, the latter was slightly affected by lindane such that at two weeks after application their numbers were reduced to about half of the initial population, whereas the untreated plot population did not change. At later intervals there were few differences in their numbers between the treated and control plots. There were no obvious effects on the *Coccinellidae* population.

During the second and third years three further categories of arthropods were selected: ants (*Formicidae*), spiders (*Aranae*) and springtails (*Collembola*). There were obvious effects on the first two of these species such that after two weeks the numbers of *Formicidae* decreased from 225 to 31 (1993) and from 14 to 6 (1994). Similar, sometimes statistically significant reductions, were also observed in the *Aranae* population (Table 4).

3.4. Breakdown organic material

The data obtained during the three years of experiments relating to loss in weight indicated that the organic matter was degraded more in the control plots than in the treated

Table 1. Mechanical and physico-chemical characteristics of soil

Depth (cm)	Mechanical Composition (%)			pH	Organic matter (%)
	Clay	Silt	Sand		
0 - 20	49.08	31.02	19.57	6.5	1.95
20 - 30	54.35	26.29	19.34	6.8	1.83
30 - 40	50.91	29.98	19.10	6.9	0.59

Table 2. Yields of chickpea in lindane treated and untreated plots

Plots	Mean yield (± S.D), ($kgha^{-1}$)		
	1992	1993	1994
Untreated	165.71 ± 1.26	135.6 ± 2.92	135.8 ± 3.2
Treated	297.54 ± 2.14	195.15 ± 33.88	382 ± 31.12

Table 3. Observed plant damage, %

Treatment interval, days	Year 1		Year 2		Year 3	
	control	treated	control	treated	control	treated
pre-treatment	4.5 ±1.53	3.95 ±1.43	1.5 ±1.1	2.0 ±0.7	1.25 ±0.8	1.25 ±0.9
15	16.4 ±4.3	5.2a ±1.1	3.75 ±1.2	3.25 ±1.2	1.75 ±0.8	1.5 ±0.5
30	60.2 ±19.3	19.6a ±3.9	5.0 ±1.4	3.75 ±0.8	3.5 ±1.1	2.0a ±0.7
45	70.2 ±21.8	31.3a ±4.8	5.5 ±0.8	3.75 ±0.8	4.0 ±0.7	2.25b ±0.4
60	77.5 ±19.1	34.9a ±5.2	6.0 ±0.7	3.75a ±0.8	4.0 ±0.7	2.25b ±0.4

a - significant difference to untreated control plants at $P < 0.05$
b - significant difference to untreated control plants at $P < 0.05$

Table 4. Total numbers of predatory arthropods caught in plants of chickpea.

Assessment interval	1992		1993		1994	
	Ctrl	Treat	Ctrl	Treat	Ctrl	Treat
Before Treatment						
1. Coccinelidae	7	10	-	-	-	-
2. Carabidae	18	16	-	-	-	-
3. Formicidae	-	-	161	225	32	14a
4. Aranae	-	-	40	36	3	3
5. Collembola	-		-	-	237	184a
After treatment						
One day	-	-	-	-	-	-
1. Coccinelidae	-	-	-	-	-	-
2. Carabidae	-	-	149	50a	-	-
3. Formicidae	-	-	29	21	-	-
4. Aranae	-	-	-	-	-	-
5. Collembola						
One week	-	-	-	-	17	13
1. Coccinelidae	-	-	-	-	2	1
2. Carabidae	-		-	-	133	59a
3. Formicidae	-	-	-	-	-	-
4. Aranae	-	-	-	-	-	-
5. Collembola						
Two weeks	13	7	-	-	-	-
1. Coccinelidae	18	9	-	-	-	-
2. Carabidae	-	-	152	31a	28	6a
3. Formicidae	-	-	32	19	3	1
4. Aranae	-		-	-	111	20a
5. Collembola						
Four weeks	6	7	-	-	23	7a
1. Coccinelidae	11	7	-	-	5	2
2. Carabidae	-	-	-	-	128	48a
3. Formicidae	-	-	-	-	-	-
4. Aranae	-		-	-	-	-
5. Collembola						
Six weeks	5	9	-	-	-	-
1. Coccinelidae	6	3	-	-	-	-
2. Carabidae	-	-	102	12a	20	9a
3. Formicidae	-	-	30	9a	5	3
4. Aranae	-		-	-	17	25
5. Collembola						
8 weeks	1	0	-	-	-	-
1. Coccinelidae	2	8	-	-	-	-
2. Carabidae	-	-	111	16a	-	-
3. Formicidae	-		36	12a	-	-
4. Aranae	-		-	-	-	-
5. Collembola						

a - differences between treated and untreated control plots significant at $P < 0.05$

plots. The differences, although small, between the treated and untreated, control plots were statistically significant ($P < 0.05$) in 1993 and 1994 (Table 5).

Table 5. Breakdown of organic material in treated and untreated plots of chickpea

Subplots	% of loss in weight (dry weight) 1992 (ns)	1993 (*)	1994 (*)
CONTROL			
Bag A	68.0	64.0	63.8
Bag B	97.3	63.4	64.2
Bag C	93.2	63.6	64.4
Bag D	92.6	64.1	64.3
Mean ± S.D	87.7±13.3	63.7±0.24	64.2±0.23
TREATED			
Bag E	59.6	59.0	58.3
Bag F	80.1	59.2	57.4
Bag G	86.9	59.5	58.7
Bag H	89.0	61.3	58.6
Mean ± S.D	78.9 ±13.4	60.0±0.39	58.3±0.5

(ns): not significant, (*) significant at $P < 0.05$

3.5. Plant and soil residues

Significant decreases during the periods after application in the amount of lindane residues measured in both soils and plants were observed (Table 6). The residues in soil were found to have declined to one tenth of the post-application concentration after 2 months owing to both physicochemical and microbial loss processes. The residues in plants delined to one tenth of their post-application concentration in a shorter time (1 month). In this case, of course, crop volume dilution effects will have assisted metabolism losses in reducing the lindane concentrations.

Perhaps surprisingly, some concentrations of lindane were also found in the harvested grain (Table 6).

For controlling locust invasions, lindane has been used in combination with malathion and fenitrothion. It will, therefore, be of interest to study the impact of malathion and fenitrothion on the fauna and the flora in Algeria using the protocols developed for lindane.

Table 6. Lindane residues ($mgkg^{-1}$, ±SD) in soils and chickpea plants.

Sampling interval	1992		1993		1994	
	plant	soil	plant	soil	plant	soil
1 day	nd	nd	14.8 ±1.04	10.6 ±0.4	5.2 ±0.35	nd
1 month	2.27 ±0.13	16.1 0.4	4.56 ±1.51	nd	0.52 ±0.14	nd
2 months	nd	nd	nd	1.01 ±0.09	**	nd
4 months	nd	nd	*	0.33 ±0.07	nd	nd

* residues in grain = 0.225±0.06 $mgkg^{-1}$ nd = not determined
**residues in grain = 1.16 ±0.83 $mgkg^{-1}$

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PERSISTENCE AND EFFECT OF LINDANE (GAMMA HCH) IN A MAIZE FIELD

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Abstract

The effects of lindane on the arthropod fauna and its persistence in soil and maize plants under field conditions were studied. Lindane significantly reduced the densities of collembola and spiders but had less significant effects on carabidae and formicidae. It decreased the damage caused by pest insects in maize plants but had no effect on the yield. Lindane dissipated rapidly from both plants and soil The residues in harvested grains were 0 2 mg kg^{-1} (year 1), 0 23 $mg.kg^{-1}$ (year 2) and 0 05 mg kg^{-1} (year 3) and below the recommended acceptable limit for grains

1. INTRODUCTION

Organochlorine pesticides may have adverse effects in the environment due to their persistence in the soil and their long term effects. Although most of the organochlorine pesticides have been banned, lindane (gamma HCH) is still being used in many countries in Africa. The metabolism and degradation of this important chemical have been studied by different authors [1-3] but knowledge of the actual effects of lindane and their impact on the environment is limited. In Algeria, there is a lack of knowledge regarding the effects of lindane on the agroecosystem itself such as beneficial arthropods and biological activity of the soil.

Trials were carried out for three years to study the persistence and effect of lindane in maize plants under field conditions.

2. MATERIALS AND METHODS

2.1. Experimental layout and method of cultivation

This experiment was carried out in the selected plot at the experimental station of Oued Smar, 20 km NE of Algiers. Two plots were used, one treated with insecticide and one untreated control. Four subplots (5m x 5m) for sampling activities were marked out in each plot. Ammonium nitrate fertilizer at a rate of 66.6 $kg.ha^{-1}$ was applied for the second year. The soil characteristics were: clay (49%), silt (31%), sand (20%), organic matter(1.95%), organic carbon (1.13%), total N (0.048%), pH, 6.5.

The site was ploughed by a tractor with a covercrop before sowing. Sowing was carried out manually with maize grains (variety, Artemis) purchased from the agriculture ministry.

2.2. Insecticide application

Lindane (Calliope, 50g.kg $^{-1}$ DC) was applied as a spray, three week post emergence, at the rate of 1 kgAI ha^{-1} in 250 litres of water.

2.3. Sampling

Pitfall traps and sweep nets were used as sampling methods at different times to determine the population of ground living arthropods and foliar living fauna. The proportion of damaged plants was determined in each subplot at different times.

In order to assess the level of microbial activities in the soil, leaves of Pittosporum (*Tobira ait*) and Eucalyptus trees were cut, weighed and 50 disks placed in a nylon mesh bag (mesh size, 0.8cm) which was sealed and buried at a depth of ~5cm, prior to treatment. Four such bags were buried at random in each subplot. The bags were dug up after 3 months and washed, dried and weighed.

Attempts were made to extract earthworms from the soil using formalin (50 mL of 40% formalin in 9 litres of water) applied in a half metre square metal quadrat.

2.4. Residues analysis

Eight cores (12 cm depth) were taken at random from each subplot and kept frozen until analysed. The cores were thawed at room temperature, air-dried, mixed well, passed through a 2 mm mesh sieve and duplicate subsamples taken for analysis of water content and insecticide residues. Samples were taken before and after insecticide treatment at 1 day, and 2 and 4 months. For residue analysis, soil (50 g) was extracted with acetone + hexane (300 mL, 1+1 by volume) in a hot Soxhlet apparatus for 6 h. The extract was filtered and its volume reduced to 10 mL using a Buchi rotary evaporature at 55°C. This solution was passed into a florisil column (30 g) that had been pre-washed with hexane (20 mL). Lindane residues were eluted from the column with a more polar solvent (150 mL) and the solvent evaporated under reduced pressure to leave a dry residue. This was dissolved in hexane (10 mL) for analysis by the GC method.

Studies on insecticide residues in maize plants were carried out by uprooting 12 plants (3 plants for each subplot) at 24h and two months after spraying. Leaves were collected and kept frozen until analysed. For analysis, leaves were thawed for one day at room temperature, chopped and mixed well. Duplicate subsamples were taken for dry weight and residue analysis. Sub-samples (10 g) were milled with water. Anhydrous sodium sulfate (40 g) was added and the mixture extracted by tumbling with acetone + hexane (300 mL, 1+1 by volume) for 1 h. The solvent extract was filtered and evaporated to dryness under reduced pressure and the residue dissolved in hexane (25 mL) and then analysed by the GC method.

Harvested grains (20 g) were taken from plants in a 2m x 2m quadrat in each subplot, ground and extracted as above for analysis by the GC method.

Lindane was determined by gas chromatography using a Varian 3700 equipped with a ^{63}Ni electron capture detector. The experimental conditions were:

glass column, OV17+OV210 (15 + 19.5 g kg^{-1}) on Chromosorb W-HP; injector temperature, 230°C; detector temperature, 300°C; column temperature, 200°C; carrier gas, nitrogen at 30 mL min^{-1}. The recovery of lindane from spiked soil samples was 85% and the limit of detection was 0.01μg kg^{-1} Corresponding figures for plant tissue were 92% and 0.01 μg kg^{-1} and for grain 86% and 0.06μg kg^{-1}.

Statistical analysis was by analysis of variance.

3. RESULTS AND DISCUSSION

The proportion of damaged plants was significantly higher in untreated plots compared with treated plots (Table I) showing that lindane had a protective effect against insect attack in the treated plots.

Lindane has a broad spectrum effect on the populations of both phytophagous insects and certain arthropods. As noted above, there was a decrease of symptoms caused by phytophagous insects such as *Agrotis* spp, *Pieris brassicae* and *Cassida viridis* but this was also accompanied by a decline in the population of Collembola in both years 2 and 3 during the 8 week period of assessment after application of the lindane (Table 2). Changes in the populations of the other orders of insects were either not significant or inconsistent.

Lindane may have adverse effects on communities of microflora and microfauna living in the soil and the composition of these communities may change considerably as has been demonstrated by Edward and Dempter [4,5]. Spraying may reduce the size of the population of the non-target fauna as seen with collembola and spiders, which seem to be very susceptible to lindane treatment, but less so with carabidae and formicidae species. Thompson and Core [6] noted that a lindane concentration of 0.5mg kg^{-1} affected 100% of *Folsomia candida* (collembola), but only 5% were affected by 50mg kg^{-1} of DDT. According to Massoud [7], the density of predators is modified for several months after the disappearance of residues.

Table 1. **Proportion (%) of damaged maize plants at intervals after treatment**

Sampling time, days	Year 1		Year 2		Year 3	
	control	treated	control	treated	control	treated
pre-treatment	0	0	3	3.25	2.8	3.2
15	1.35	0	4.25	3.5	5.35	4.7
30	3.75	0.35a	6.75	3.5	8.25	6.1a
45	4.72	0.6a	7.75	-	11.6	6.5a
60	5.6	1.02a	8.25	4a	-	-

Each value is a mean of 4 readings of 4 subplots
a = significantly different from the untreated control plants at the same interval at $P<0.05$

The data in the current experiment using formalin on earthworms was not conclusive and is not presented.

The yields from the lindane treated plots were not significantly different from those of the untreated control plots indicating that the lindane treatment had neither a positive nor a negative effect. There were no yields from the third year owing because of a drought during that summer.

Table 2. Numbers of insects trapped in the field plots at various intervals after application

Sampling time, weeks	Insect species	Year 2		Year 3	
		control	treated	control	treated
Before treatment	Collembola	161	135	127	108
	Carabidae	3	2	-	-
	Spiders	35	32	5	5
	Formicidae	-	-	11	9
2	Collembola	263	79a	107	31a
	Carabidae	3	2	-	-
	Spiders	22	9	8	6
	Formidicae	-	-	17	10
4	Collembola	131	25a	147	39a
	Carabidae	4	0	-	-
	Spiders	19	3	16	9
	Formicidae	-	-	29	15
6	Collembola	77	8a	126	66a
	Carabidae	5	1	-	-
	Spiders	4	10	15	9
	Formicidae	-	-	45	14
8	Collembola	72	14a	88	49
	Carabidae	6	0	-	-
	Spiders	41	16a	28	19
	Formidicae	-	-	26	23

a = significantly different from the untreated controls at the same sampling interval at $P<0.05$

The microbial activity, as suggested by the differences in the leaf litter remaining in the nylon bags, in the control plots tended to be slightly higher than that in the lindane treated plots, although the differences were only statistically significant in year 2.

Lindane dissipated rapidly in soil with 84 - 91% loss within 2 months of application (Table 5).

Table 3. Yields (kg ha^{-1}) of maize grain from control and plots treated with lindane

Year 1		Year 2		Year 3	
control	treated	control	treated	control	treated
3.97 (0.06)	4.48 (0.59)	4.92 (0.74)	5.23 (0.3)	-	-

Numbers in brackets are standard errors of the mean values from the 4 subplots

Table 4. Mass (g) of leaf litter remaining after incubation in the ground of the plots

Year 1		Year 2		Year 3	
control	treated	control	treated	control	treated
0.93 (0.2)	0.85 (0.11)	1.87 (0.3)	1.31 (0.28)a	0.97 (0.15)	0.96 (0.2)

numbers in brackets are standard errors of the mean values of the 4 subplots
a = significantly different from the untreated control plot at $P<0.05$

Table 5. Residues (mg.kg^{-1}) of lindane in soil taken from the treated plots

Year 1			Year 2			Year 3		
1 day	2 month	4 month	1 day	2 month	4 month	1 day	2 month	4 month
-	2.6 (0.2)	1.1 (0.26)	6.5 (0.7)	2.1 (0.35)	0.9 (0.24)	5.3 (0.6)	0.46 (0.08)	-

numbers in brackets are standard errors of the means of the samples from the 4 subplots

Table 6. Residues ($mg.kg^{-1}$) of lindane in maize plants and grain at intervals after treatment

Year 1			Year 2			Year 3		
plant 1day	plant 2mths	grain 3mths	plant 1day	plant 2mths	grain 3mths	plant 1day	plant 2mths	grain 3mths
23.2 (1.8)	2.3 (0.14)	0.2 (0.04)	-	-	0.23 (0.05)	15.5 (2.7)	1.9 (0.1)	0.05 (0.001

Numbers in brackets are standard errors of the means of 4 subplots

In maize plants, more than 85% of the lindane was degraded after 2 months when it was measured in years 1 and 3. High temperatures and intense sunlight may cause lindane to dissipate/degrade rapidly in contrast to its longer persistence under temperate zones [9].

The residue levels of lindane in maize grain harvested from the treated plot were found to be $0.2mg.kg^{-1}$, $0.23mg.kg^{-1}$ and $0.05mg.kg^{-1}$ in the years 1-3 respectively and in all cases these levels are below the recommended acceptable residue limit for grains.

In conclusion it has been shown that, throughout these experiments, lindane has some adverse effects on the fauna, including some beneficial predatory arthropods. In particular, the densities of collembola and spiders were significantly reduced. As this insecticide has been used in large quantities to control locusts for many years in the south of Algeria, and is still being used in agriculture today, the results of this study are important.

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BEHAVIOUR OF LINDANE (GAMMA HCH) IN SOIL UNDER LABORATORY CONDITIONS

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Abstract

The degradation of lindane in soil after multiple application was studied. The rate of disappearance of lindane increased at longer periods after application. The dissipation of lindane was more rapid in soil collected from treated a plot than from an untreated plot, owing to the degradation by micro-organisms suggesting that microorganisms had become adapted to degrade it. The rates of mineralization, mobility and degradation of lindane were also investigated under laboratory conditions using ^{14}C-labelled lindane. The results showed that most of the applied dose remained on the upper 6cm in the soil columns. Over a period of three months, extractable residues, bound residues and evolution of $^{14}CO_2$ were recorded. After 12 weeks, the soil contained about 50% of the initially applied ^{14}C and 20% was bound to the soil. The evolution of $^{14}CO_2$ increased with time, amounting to 3.1% in non-sterile soil and less than 1% in sterile soil. The hexane ^{14}C-extractable residues were shown, by TLC, to contain lindane as the main product.

1. INTRODUCTION

In 1991, we started a long term field experiment by applying lindane every year to the same plot with a view to obtaining more reliable information on the effect and fate of lindane in the agroecosystem. Many reports have been published on the persistence of this compound [1-3] but few have studied the dissipation in soil after multiple applications. The difference in the persistence of lindane in soil collected from both treated and untreated plots in both field and laboratory experiments are reported.

2. MATERIALS AND METHODS

2.1. Pesticide

A commercial formulation (Calliope, 50g AI kg^{-1}) of lindane (1,2,3,4,5,6-hexachlorocyclohexane) was used to study the accelerated biodegradation in soil. U-^{14}C-lindane (specific activity, 2.3 GBq/mmol) was obtained from Amersham International, United Kingdom, and diluted with technical non-labelled lindane 99% (Rhône Poulenc) to give a preparation with an activity of 7.77 MBq/mg. Radiopurity of ^{14}C-lindane, as confirmed by TLC, amounted to 96%.

2.2. Soil

Soil was collected from the experimental field near Algiers (ITGC). The soil characteristics were : clay: 49.1%, silt: 31.0%, sand: 19.6%, pH (H_2O):6.5. organic matter: 2.0%, total nitrogen: 0.05%.

2.3. Methods

2.3.1. Accelerated biodegradation

Soils were collected from both untreated and treated plots of the same field treated three times with lindane at a concentration of 1kg.AIha^{-1} per year in 250L of water during the period 1991-1993. The soils were partially air-dried at room temperature and passed through a 2 mm mesh sieve. Soil samples (20 g) were placed into separate flasks (125 mL) and a solution of lindane (12 µg) in distilled water (2 mL) was added to each soil sample. After mixing, the soil was moistened to 22% field moisture capacity, closed with parafilm and incubated in an oven (30°C). Soil samples were extracted after 1 day and 1, 2, , 6 and 8 weeks with acetone+hexane (200 mL, 1+1 by volume) using an agitator for 90 min. The extract was filtered through a glass funnel containing sodium sulfate (20 g L^{-1}) and evaporated to dryness using a Buchi rotavapor at 55°C. The residue was dissolved in hexane (20mL) and 1 µL aloquots analysed by GC .

A gas chromatograph (Girdel), fitted with an EC detector, was used to analyse lindane in the hexane solution. The operating conditions were as follows: column temperature, 200°C; detector temperature,300°C; injector temperature,230°C; carrier gas, nitrogen at 20 mL min^{-1} ; column, spiral capillary coated with 0.5 µm DC 11. Recovery of lindane from a spiked sample was 92% with a limit of detection of 5 ng g^{-1} .

Statistical analyses were conducted using Student's t-test.

2.3.2. Mineralization of lindane in soil

Soil samples (100 g, previously moistened to ~70% of field moisture capacity) were transfered to biometer flasks (250 mL). The side arm of the biometer flasks contained 10 mL of 100 g L^{-1} sodium hydroxide to trap evolved $^{14}CO_2$. The soil in each flask was treated with a radioactive preparation containing 39 kBq of lindane on 5mg of unlabelled compound. Flasks were incubated at about 22-25°C in darkness. Sampling was carried out at intervals over 3 months for determination of extractable and bound residues. The rate of evolution of $^{14}CO_2$, on the other hand, was studied over a period of 12 weeks. Parallel experiments were carried out with sterile soil, autoclaved at 120°C. Soil samples were oxidised using a Harvey Biological Oxidizer (OX 400) and both these samples and those from the sodium hydroxide traps were assayed for ^{14}C using a liquid scintillation analyzer (LSC, Tricarb Packard 1500).

2.3.3. Movement in soil

Soil samples were placed in polyvinyl chloride cylinders (18 cm length, 30 cm i.d.) that were put in pots containing the same soil. The pots were placed outside and exposed to prevailing environmental conditions. Lindane (20mg of non-labelled lindane containing 150 kBq of ^{14}C-lindane) in n-hexane (8 mL) was added to the soil surface in each cylinder. The cylinders were irrigated directly after the addition of the pesticide and were kept moist throughout the duration of the experiment. Subsequently, triplicate cylinders were taken out at various intervals over a period of 4 months and kept frozen in polyethylene bags until analysed. Each cylinder was cut in three sections of 6 cm and samples from each section were extracted.

2.4. Analysis

Sterile and non-sterile soil samples from each section were analyzed for ^{14}C hexane-extractable and bound residues. Samples of soils were extracted using a hot Soxhlet extractor using n-hexane (300 mL) for 10 cycles. Aliquots (1 mL) of the hexane extracts were analysed by LSC. The extract was concentrated to near dryness and analyzed by TLC on linear K silica gel plates using two solvent systems: hexane and hexane+chloroform (12+1 by volume). For radioscanning of the plates, increments (1cm) of the plates were scraped, added to scintillator and counted using LSC. Bound residues were determined by combustion of three soil samples using carbosorb for trapping of liberated $^{14}CO_2$.

3. RESULTS AND DISCUSSION

3.1. Accelerated biodegradation

Lindane added to the soil from the pre-treated plot was lost rapidly. About 32% and more than 86% of the applied lindane was lost within 1 and 8 weeks after incubation, respectively (Table 1). On the other, the rate of lindane disappearence was slower in the soil from the untreated control plot (24% and 64% respectively of the applied dose were lost on the same time). Interestingly, losses of lindane from the mixture of soils (column 3 - Table 1) were intermediate of those from the single soils.

The more rapid loss of lindane from the field soil pre-treated with lindane (soil B) compared with that in the untreated soil (soil A) suggested that some "lindane decomposing microorganisms" had accumulated in the pre-treated field soil.

TABLE 1. RECOVERIES OF LINDANE FROM UNTREATED AND LINDANE-TREATED FIELD SOIL SAMPLES AT VARIOUS INTERVALS AFTER RETREATMENT WITH ^{14}C-LINDANE

Sampling time,weeks	Mass of lindane remaining, μg		
	control soil -A	treated soil -B	80%A+ 20%B
0	11.1 +/- 0.5	11.7 +/- 0.4	11.1 +/- 0.6
1	8.5 +/- 0.5	8.0 +/- 0.6	8.5 +/- 0.4
2	8.1 +/- 0.2	6.2 +/- 0.15	6.7 +/- 0.42
4	7.0 +/- 0.37	3.2 +/- 0.2	6.2 +/- 0.33
6	6.2 +/- 0.4	2.5 +/- 0.1	4.3 +/- 0.3
8	4.0 +/- 0.27	1.6 +/- 0.09	2.3 +/- 0.18

3.2. Mineralization

The observed dissipation of lindane during the initial 30 days accounted for about 24% of the applied dose (Table 2). The quantity of extractable ^{14}C decreased from 90.1% to 48.9% between 1day and 90days. The data indicates a recovery that ranged from 68-92% of the applied dose. The bound residues of lindane increased with time to reach 19.7% of the initial dose after 90 days.

There was a steady increase of the amount of $^{14}CO_2$ released by microbial action from non-sterile soil.(Table 3) The increase of $^{14}CO_2$ is also reported by other authors [4,5]. In

TABLE 2. TOTAL RECOVERIES AND NATURE OF THE LINDANE METABOLITES

Sampling time, days	^{14}C-residues in the soil [a]		14C total recoveries, %
	extractable	bound	
1	90.1 +/- 4.08	2.0 +/-0.1	92.1
15	76.7 +/-3.65	6.1 +/-0.26	82.8
30	60.8 +/-3.6	15.3 +/-1.05	76.1
60	54.7 +/-3.9	17.4 +/-2.0	72.1
90	48.9 +/-5.07	19.7 +/-1.21	68.6

a) values are the mean of triplicates

TABLE 3. EVOLUTION OF $^{14}CO_2$ AS % OF APPLIED ^{14}C

Non-sterile soil	Sterile soil
0	0
0.15	0
0.2	0
0.3	0
1.0	0
1.3	0.05
1.8	0.15
2.3	0.18
2.6	0.25
2.7	0.36
2.8	0.5
2.9	0.8

case of non- sterile and sterile soils there was an abrupt increase of $^{14}CO_2$ evolution after 4 and 8 weeks respectively. After 12 weeks, about 3% of the applied radiocarbon was mineralized to $^{14}CO_2$ in non-sterile soil and less than 1% in sterile soil.

Overall, these experiments show the importance of microbial activity on the degradation of lindane.

TABLE 4. DISTRIBUTION OF ^{14}C ACTIVITY IN THE SOIL COLUMNS.

Time after treatment, months	Extractable ^{14}C,[a] % of applied			Bound ^{14}C,[a] % of applied		
	0-6 cm	6-12 cm	12-18 cm	0-6 cm	6-12 cm	12-18 cm
1	60.2 +/- 6.0	4.2 +/- 0.3	0	3.1 +/- 0.2	0.2 +/-0.02	0
2	32.5 +/- 4.1	4.9 +/- 0.5	0	2.2 +/- 0.3	0.6 +/-0.07	0
3	20.7 +/- 2.3	4.1 +/-0.36	0	0.7 +/- 0.2	0.1 +/-0 06	0
4	11.3 +/- 1.5	3.7 +/- 0.1	0	0.6 +/- 0.1	0.09 +/-0.02	0

a) Data are the means of three replicates

3.3. Movement in soil

The results (Table 4) showed clearly that most of the applied dose remained in the upper 6cm layer. The 6-12 cm layer contained some presence of both extractable and bound ^{14}C-lindane and/or its metabolites while the 12-18 cm layer contained no ^{14}C-activity indicating that there is only little downward movement of ^{14}C-lindane.

Radioscanning of the TLC plates containing the soil extracts showed the presence of lindane as the main residue (93%).

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STUDIES ON FATE OF ^{14}C-LINDANE IN SOIL AND CHICKPEA PLANTS UNDER LABORATORY CONDITIONS

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Abstract

The degradation of ^{14}C-lindane (γ-1,2,3,4,5,6 - hexachlorocyclohexane) was investigated under laboratory conditions Chickpea plants and soil were treated with ^{14}C-lindane The results indicated a decrease of lindane on the plant surface from 36 6% to 6 5% and a corresponding increase in extractable residues from within the plant from 12 5% to 34 5% during the 60 days of the trial In the soil, extractable residues decreased from 47 4% to 31 2% Bound residues in both plant and soil remained low throughout the trial After 60 days, the chickpea plants took up 16 4% of the lindane applied to the soil

1. INTRODUCTION

The use of organochlorine pesticides constitutes an important component of the efforts to control agricultural pests and vectors of disease in most areas of the developing world [1]. Man and the environment are exposed to many contaminants, either consecutively or simultaneously. Residues in soil may result from agricultural practices such as agrochemical usage, disposal of industrial wastes, etc. [2].

The rates of degradation of the insecticide ^{14}C-lindane were studied under laboratory conditions since the use of radiotracer techniques provides an attractive method in pesticide residue studies. The objective of the present work was to obtain information on the degradation rates in soil and chickpea plants sown in pots and growing under outdoor condititons.

2. MATERIALS AND METHODS

2.1. Chemicals

^{14}C-lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane, spec. act., 2.3 GBq/mmol) dissolved in solvent at at a concentration of 1.7 x 10^6 Bq mL^{-1} was obtained from Amersham International, UK. The formulated lindane (50 g kg^{-1}) was obtained from Asmidal, Algeria Solvents used were of analytical grade.

2.2. Soil

The soil was collected from the Institut Technique des Grandes Cultures (ITGC) field near Algiers, and was sieved to remove particles >2mm prior to use. The soil was a silt clay with the following characteristics: clay content, 49 1%, silt content, 31.0%, sand content, 19.6%; pH, 6 5, organic carbon content, 1.95%

2.3. Crop

Chickpea plant (*Cicer arietinum* ILC variety) seeds were treated with fungicides (carboxine and thiram) The rate of seed emergence was 95% as determined in prior laboratory studies.

2.4. Preparation of the soil pots

Soil (3 kg) was placed in PVC pots (length, 30 cm; diameter, 18 cm). Fifteen pots were used for the pesticide degradation study in soil and 3 pots for the residue study on chickpea plants with all applications on 6 June 1995.

2.5. Insecticide application

Soil trials

Lindane (1275 mg of 50 g kg^{-1} DC formulation) was added to an aliquot (1 mL) of the ^{14}C-lindane and dissolved in water (49 mL). A volume was applied by micropipette over the surface of the soil in each pot equivalent to an application rate of 1 kg ha^{-1}.

Three pots were taken at random soon after the treatment to determine the post application lindane residues. Subsequently, triplicate pots were sampled biweekly during two months.

Plant trials

Lindane (25.5 mg of 50 g kg^{-1} DC formulation) was added to ^{14}C-lindane (1 mL). An aliquot (1 mL) was added to the soil surface and to the foliage of the plant in each pot.

2.6. Experimental conditions

During the experiment period, the temperature ranged from 20°C to 35°C. The soil was kept moist during the experiment period by adding water periodically .

2.7. Determination of radioactivity

Plants

Surface lindane residues were obtained by washing the foliage surface with water (2 x 50 mL). The ^{14}C-content was assessed by liquid scintillation counting (LSC, Packard Tricarb) of triplicate aliquots (1 mL) of these washings. The washed plants were ground up and extracted with acetone+hexane (1+1 by volume, 100 mL) by stirring the suspension for 1h. The total suspension was transferred into a separatory funnel containing sodium sulfate (20 g L^{-1}, 200 mL) and shaken for a few minutes. The ^{14}C-content of the extracts was determined by counting triplicate aliquots (1 mL) of the solvent phase using LSC. The non-extractable ^{14}C-residues remaining in the solid debris after extraction were determined by complete combustion using a biological oxidiser, trapping the $^{14}CO_2$ in scintillation cocktail (Carbosorb).

Soil

Samples (10 g) of soil were extracted using acetone + hexane (1+1 by volume, 100 mL) in a similar manner to the plant samples. After extraction, samples of the soil were submitted to combustion analysis. The ^{14}C-content in the solvent extracts and in the CO_2 trap were determined by LSC.

2.8. Thin layer chromatography

Some of the extracts from the plants and soil materials containing extractable residues were evaporated to low volume using a rotavapor (Buchi) at 60°C. Aliquots were applied to

a TLC plate and the plates eluted with acetone+hexane (1+9 by volume). The radioactive spots were scraped off the plates, added to liquid scintillation cocktail and counted by LSC.

3. RESULTS AND DISCUSSION

3.1. ^{14}C residues in plants

The amount of ^{14}C-residues removed from the plant surface just after application was 36.7% and these decreased to 6.5% after 60 days whilst the amount of extractable residues within the plant increased from 12.5% to 34.5% during the same period (Fig. 1). The amounts of bound residues remained low during the experiment. The total recovery of surface, plant-extractable and plant-bound ^{14}C-compounds was 100% of the applied dose.

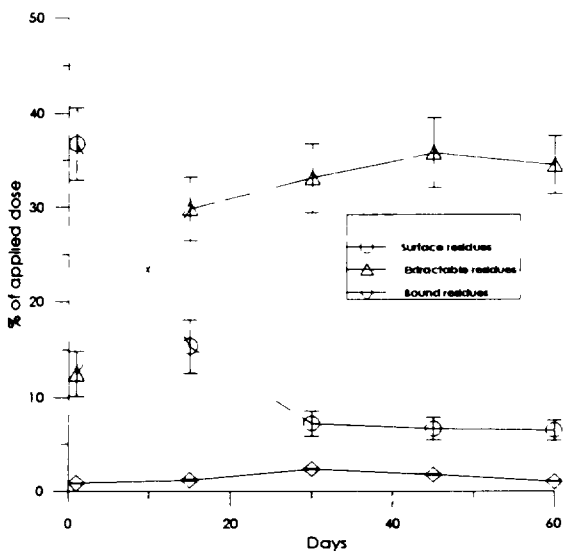

Fig. 1. Surface, extractable and bound residues in plant.

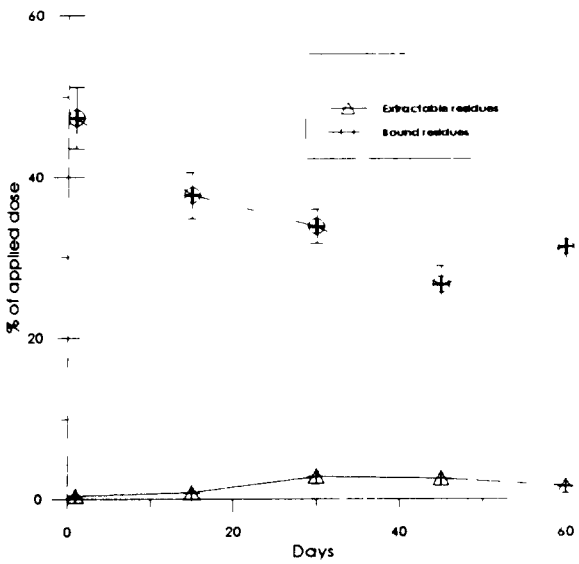

Fig. 2. Extractable and bound residues in soil.

(a) ^{14}C lindane standard.

(b) One hour after treatment.

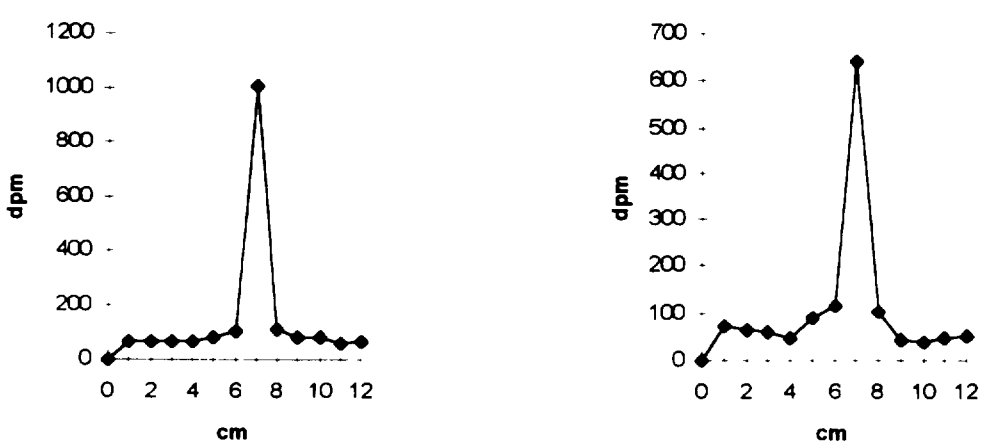

Fig. 3 Radiochromatograms.

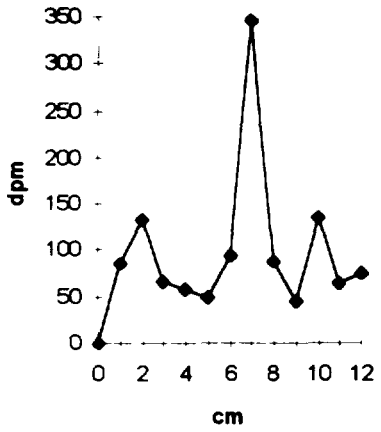

Fig. 4. 15 days after treatment.

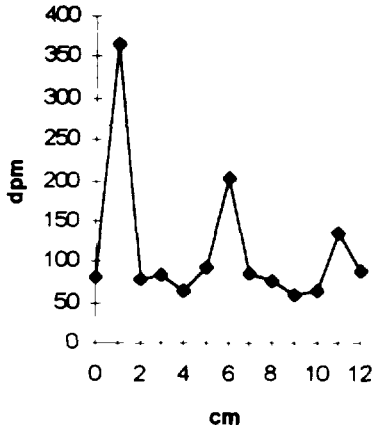

Fig. 5. 30 days after treatment.

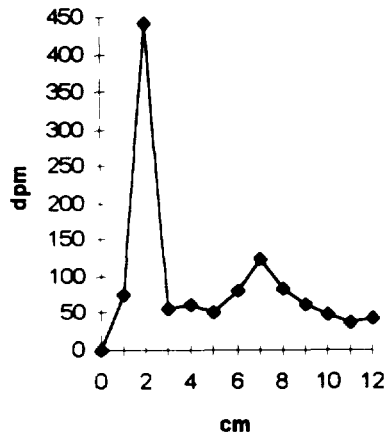

Fig. 6. 45 days after treatment.

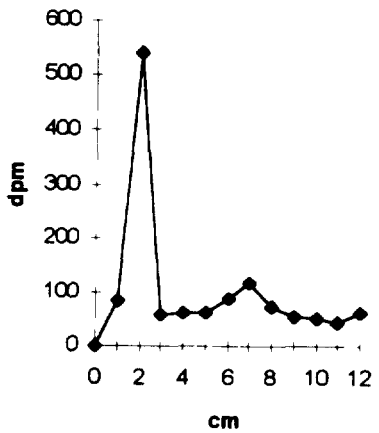

Fig. 7. 60 days after treatment.

3.2. ^{14}C-residues in soil

The amount of extractable residues decreased with time from 47.4% to 31.2% while the amounts of bound residues increased very slowly 0.14% to 1.2%. The total radioactivity from extractable and bound residues at t=0 represented 95.1% of the applied dose to the soil.

3.3. Uptake of ^{14}C-lindane from soil by plants

The uptake of ^{14}C-lindane by chickpea plants from treated soil was studied during the same period. The data indicated that, after 60 days, 16.4% of the applied radioactivity was taken up into the plant and 28.7% remained in the soil as extractable residues. The total radioactivity from both plant and soil extractable and bound residues was 45.7% of the applied dose.

3.4. Thin layer chromatography

The development of the TLC plates at the different sampling times showed the presence of lindane (Rf = 0.6) at all sampling times and was the only compound at the first sampling time. During the course of the experiment two unknown metabolites appeared from 15 days after treatment. The first metabolite (Rf =0.16) persisted over the whole experiment (Fig. 4) while the second (Rf = 0.91) disappeared after day 45 (Fig. 5).

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FATE AND EFFECTS OF ^{14}C-LINDANE IN AN AGRICULTURAL ECOSYSTEM

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Abstract

The fate of ^{14}C-lindane was studied using a terrestrial field ecosystem that included plants, soil, beetles, earthworms and one type of common bird in Egypt (*Asfur baladi*) The study was conducted on a restricted field area that was cultivated with maize plants as the target crop and soybean plants as an alternate crop The residue level in soybean seeds (3 20 μg g^{-1}) was almost 10 times more than that in dry maize seeds (0 36 μg g^{-1}) The concentration of ^{14}C-residues in beetles was 2.18 μg g^{-1} on day 60 after spraying ^{14}C-lindane, and decreased thereafter The earthworms, on the other hand, showed a progressive increase in concentration of residues with time. Birds showed the highest concentration of residues in the brain, liver and heart and histological changes were observed in these tissues.

1. INTRODUCTION

Lindane has been widely used, for many years, as a soil insecticide, foliar spray on crops, and seed dressing as well as for protection of stored grains. In some countries such as India, lindane has also been widely used for public health purposes [1]. Lindane is considered as a persistent insecticide which possesses a fumigant action. Much work has been done concerning the metabolism and degradation of lindane [2-4]. Lindane is degraded slowly in the environment by hydrolysis and biodegradation.

Lindane was accumulated preferentially in the fatty tissue of albino rats when fed with low doses of the insecticide [5]. Accumulation in the brain has also been reported [6].

Because of the extensive use of lindane, it was decided to carry out investigations on its environmental fate using radiolabelled lindane in confined field studies with the aim of highlighting critical environmental issues.

The present work describes an investigation on the fate of lindane using a terrestrial field ecosystem that included plants, soil, beetles, earthworms and birds. The distribution of lindane among the different components of the ecosystem, as well as on the toxicological potential and pathological significance of lindane residues in birds, have been investigated.

2. MATERIALS AND METHODS

2.1. The ecosystem

A restricted experimental field area of 12 m^2 was used. The plot was separated from a surrounding bigger plot on all sides by a double walled narrow mesh of plastic network. This proved to be efficient in preventing soil insects and small birds from escaping. The plot had

fresh fertile clay-loamy soil. It was cultivated with the target crop (maize) and an alternate crop (soybean).

Maize (var. Giza 2 hybrid) and soybeans (Var. Clark) were sown on May 18, 1994. Soil fertilization, irrigation, thinning of maize and handhoeing of weeds followed normal agricultural practices.

U-^{14}C-lindane of specific activity 1.01 GBq mmol^{-1} (Du Pont Chemical Company) was diluted with non-labelled lindane to give a radioactive preparation of specific activity of 6.59 MBq g^{-1}.

The wet soil was provided with a reasonable population of beetles (200; *Calosoma Chlorostictum*) and earthworms (800; *Allolobophora*) on 7th of July 1994 to allow acclimatization before spraying.

2.2. Application

Plants were sprayed twice using a laboratory spraying bottle, 23 days apart, with a solution of ^{14}C-lindane (900 mg L^{-1}) in water at an application rate of 225 mg m^{-2}. The first spray was conducted on July 11, 1994. Shortly after spraying the plants, 15 birds (*Asfur baladi*) were introduced into the ecosystem.

2.3. Sampling

At different intervals following the first foliar application, samples of different components of the ecosystem were taken as indicated later for analysis (Table 1). At maturity, grain was harvested and analysed for ^{14}C-radioactivity. Samples of young, green seeds of maize were also analyzed for ^{14}C-activity.

The total ^{14}C-activity in insects, organs of birds, plant tissues and in grains was determined by combusion of samples weighing 100-270 mg in a Harvey Biological Oxidizer (OX-600) followed by counting the liberated $^{14}CO_2$ in a liquid scintillation counter.

Extractable residues were obtained by homogenization of organs, tissues, beetles and/or earthworms with methanol (ultraturax). In the case of soil and seeds, Soxhlet extraction for 4 hours with methanol was used to isolate the extractable residues. The remaining non-extractable residues were determined by combustion.

Important organs of the birds were examined histologically. For these histological examinations, tissue samples of liver, kidney and brain were taken from the sacrificed bird, fixed in formalin, and then trimmed and embedded in paraffin according to Culling [7]. Sections about 5-7 microns thick were stained with haematoxylin and eosin for microscopical examination.

3. RESULTS AND DISCUSSION

3.1. Plants

Twenty four days after the first lindane spray, the whole plant had ^{14}C-residues of 10.1 and 25.2 μg g^{-1} in case of maize and soybean plants, respectively, with the major part of the

Table 1 : Sampling Schedule of Analyzed Samples for the 1994 experiment.

Sampling Time (Days)	Beetles	Earth-Worm	Birds	Soil	Plant		Seeds	
					Maize	Soybean	Maize	Soybean
First Spray								
1								
21	+	+	+					
		+	+					
23 Second Spray								
24								
34		+	+		+	+		
44	+	+	+					
60	+	+	+					
85	+	+	+	+	+		soft green seeds	
	+	+		+		+	mature dry seeds	mature dry seeds

residue being extractable (Table 2). After 60 days, the leaves of maize (used for fodder) has a total radioactivity equivalent to about 0.9 $g.kg^{-1}$ of lindane. The sheath of maize ears was attacked by the birds, opened and the soft grains eaten by them. Near maturity (85 days), the residues in soybean plants were reduced to low concentrations (1.4 $\mu g\ g^{-1}$).

Analysis of the target crop (maize) and the alternate crop (soybeans) showed, as expected, that plants contained considerable amounts of radioactivity shortly after treatment which then declined with time.

Table 2 : Total ^{14}C-Residues in Plants.

Sampling Day+	Sample	^{14}C-Residues ($\mu g\ g^{-1}$ lindane equivalent)++					
		Maize			Soybean		
		Total ±SD	Extract-able ±SD (%)	Non-extract. ±SD (%)	Total ±SD	Extract-able ±SD (%)	Non-extract. ±SD (%)
24	total plant	10.1 ±0.98	8.40 ±0.69 (83)	1.70 ±0.05 (16.8)	25.2 ±0.91	22.72 ±0.80 (82)	4.48 ±0.09 (17.7)
60	leaves (fodder)	0.90 ±0.04	0.73 ±0.03 (81)	0.17 ±0.02 (18.8)	N.D.	N.D.	N.D.
85	total plant	N.D.	N.D.	N.D.	1.4 ±0.04	1.12 ±0.02 (80)	0.28 ±0.03 (20)

\+ From first spray

++ Data mean of a duplicate samples N.D. not done

3.2. Seeds

Soft green maize seeds had a radioactivity corresponding to a lindane equivalent concentration of 0.56 $\mu g\ g^{-1}$. Roasting of these seeds, as usually practised for human consumption, led to only a slight decrease of 20% in the ^{14}C-residues (data not shown).

The residues in the mature dry soybean seeds were almost 10 times higher than those detected in the target crop, maize (Table 3). This may be attributed to the oily nature of soyabean seeds that could allow the dissolution and storage of a lipophilic insecticide.

Table 3 : ^{14}C-Residues in Dry Seeds.

Seeds	^{14}C-Residues (μg g^{-1} lindane equivalent)			
	Total* ±SD	Extractable ±SD (%)	Non-extractable ±SD (%)	Recovery (%)
Maize	0.36 ±0.03	0.15 ±0.026 (41.7)	0.17 ±0.02 (47.2)	88.9
Soybean	3.20 ±0.35	2.63 ±0.15 (82.2)	0.67 ±0.05 (20.9)	103.1

* Determined by combustion; Data mean of 3 samples.

3.3. Soil

At each sampling time, two soil columns (length, 150 mm; diameter, 70 mm) were taken from areas below the plants. Columns were divided in 50 mm zones, air dried to allow mixing, well mixed, sieved (0.25 mm mesh) and the samples extracted with methanol.

The results indicated a downward movement of lindane during the first 60 days after application with ^{14}C-residues found in the upper two zones (Table 4). In the period 60-85 days there were decreases in the upper two zones probably owing to volatilisation and microbial mineralisation of lindane and its metabolites.

3.4. Beetles

Samples of carabid beetles, collected and analysed 44 days after spraying, had comparatively low lindane residues. The highest residues were found to be 2.18 μg g^{-1} of lindane equivalent at 60 days after the first spray (Table 5). The fact that all beetles were alive is consistent with previous work in which it was reported that all dead beetles contained residues of lindane >2.65 μg g^{-1} [8].

3.5. Earthworms

No dead earthworms were observed. Triplicate samples were analysed for ^{14}C-activity with the results indicating an increasing accumulation of lindane and its metabolites with time up to a concentration of 5.6μg g^{-1} of lindane equivalent at 85 days after the first spray treatment (Table 6).

Table 4 ^{14}C- Residues in Soil Samples of the Ecosystem

^{14}C-Residues (μg g^{-1} lindane equivalent) [a]										
0-50mm zone				60-100mm zone				110-150mm zon		
Total [c] ±	Extract-able ±SD (%)	Bound ±SD (%)	Recovery (%)	Total(b) ±SD	Extract-able ±SD (%)	Bound ±SD (%)	Recovery (%)	Total(b) ±SD	Extract-able ±SD (%)	Boun ±SD (%)
0 22 ·0 04	0 17 ±0 02 (77)	0 04 ±0 005 (18)	95	0 15 ±0 009	0 12 ±0 01 (80)	0 03 ±0 003 (20)	100	0 05 ·0 01	0 035 ±0 004 (70)	0 01 ±0 00 (24)
0 11 ±0 01	0 08 ±0 01 (73)	0 022 ±0 003 (20)	93	0 09 ±0 002	0 06 ±0 01 (67)	0 027 ±0 007 (30)	97	0 06 ±0 02	0 036 ±0 001 (60)	0 01 ±0 00 (30)

a) mean values of duplicate samples
b) from the first spray
c) Determined by combustion

Table 5 ^{14}C- Residues in Beetles of the Ecosystem [a]

Sampling Interval [b] (days)	1 ±SD	34 ±SD	44 ±SD	60 ±SD	85 ±SD
^{14}C-Residues (μg g^{-1} lindane equiv)	0 52 ± 0 011	0 92 ± 0 014	0 66 ± 0 012	2 18 ± 0 077	0 70 ± 0 010

a) Mean values from 6 beetles (Calosoma chlorostictum) determined by extraction with methanol
b) From the first spray

Table 6 ^{14}C- Residues in Earthworms of the Ecosystem [a]

Sampling Interval (days)	1 ±SD	21 ±SD	24 ±SD	34 ±SD	44 ±SD	60 ±SD	85 ±SD
^{14}C-Residues (μg g^{-1} lindane equivalent)	1 09 ± 0 020	0 92 ±0 014	0 86 ±0 017	3 08 ±0 028	3 92 ±0 028	4 08 ±0 032	5 60 ±0 187

a) Mean values from 6 earthworms (Allolobophora)

3.6. Birds

Some birds died within 10 days of the first spray. The birds were dissected within 4 hours after death and their organs analysed for ^{14}C-radioactivity. The distribution of ^{14}C-activity among the different organs from both dead and living birds are shown in Table 7. The residues in faeces contained considerable radioactivity that ranged from 3.08 $\mu g\ g^{-1}$ for a few weeks following the first spray declining to 2.24 $\mu g\ g^{-1}$ at the end of the experiment. The major part of these radioactive residues (67%) was methanol soluble.

The radioactivity in the different organs was found to increase progressively during the first 2-3 days after spraying and then to decrease systematically during the following period up to 60 days. In dead birds, the highest level of lindane residues was detected in the liver, brain and heart (Table 7).

Table 7 : ^{14}C- Residues in Birds(+) Tissues of the Ecosystem.

Sampling Interval (days)	Sample	No. of Birds	^{14}C-Residues in Organs (++) ($\mu g\ g^{-1}$ lindane equivalent)					
			Liver ±SD	Kidney ±SD	Brain ±SD	Heart ±SD	Stomach ±SD	Intestine ±SD
1st Spray								
1	Dead	1	2.02 ±0.076	N.D.	1.57 ±0.18	N.D.	N.D.	N.D.
2	Dead	3	6.17 ±0.16	1.12 ±0.09	2.74 ±0.18	3.81 ±0.18	1.68 ±0.25	1.96 ±0.55
5	Dead	2	3.92 ±0.095	N.D.	4.20 ±0.34	N.D.	N.D.	N.D.
10	Dead	2	2.21 ±0.11	0.45 ±0.014	0.20 ±0.010	0.62 ±0.053	0.39 ±0.02	0.39 ±0.043
2nd Spray								
21	Alive	2	0.56 ±0.053	0.50 ±0.015	0.45 ±0.14	0.56 ±0.043	0.84 ±0.072	0.32 ±0.062
24	Alive	2	2.24 ±0.23	N.D.	1.12 ±0.098	0.50 ±0.01	0.31 ±0.017	0.62 ±0.055
34	Alive	2	0.39 ±0.034	0.56 ±0.036	0.54 ±0.026	0.38 ±0.026	0.37 ±0.026	0.31 ±0.017
44	Alive	2	0.22 ±0.026	N.D.	0.11 ±0.01	N.D.	N.D.	N.D.
60	Alive	2	0.11 ±0.017	N.D.	0.10 ±0.02	N.D.	N.D.	N.D.

N.D. Not done
(+) Bird : Asfour Baladi
(++) Determined by combusion; Mean values of 3 samples.

In a previous experiment [8] with Egyptian House Sparrow, the highest ^{14}C-residues in survivals were detected in the brain, liver and kidney. The accumulation of the residues in the liver and brain of birds is in agreement with the results obtained in the present investigation.

3.7. Histological Investigations

The histological investigations of tissues of birds fed on contaminated plants showed marked differences from those of control birds especially in the liver, kidney and brain. These indicated degenerative changes in these organs which, when severe, might lead to death of birds.

3.7.1. Liver

The histological examination of the liver of birds from treated plots at 21 and 24 days revealed marked differences when compared with liver of control birds fed on untreated plants (Fig. 1). Multiple focal aggregations of mononuclear cells in the hepatic parenchyma associated with coagulative necrosis were observed (Fig. 2). The hepatic sinusoids were greatly dilated and hepatic cords were dissociated. The blood vessels were congested with blood. Also, there were perivascular accumulations of inflammatory cells. The hepatic sinusoids were filled with inflammatory cells. The severity of lesions increased with longer exposure period leading to severe vacuolar degeneration of hepatic cells and early signs of fatty changes in which large spherical globules occupied the cytoplasm of the liver cells (Fig. 3), pushing the nucleus towards the periphery giving a signet-like appearance. Also, multiple and focal aggregations of mononuclear cells and increase in the activity of Kupffer cells were observed.

3.7.2. Kidney

The microscopic examination of the kidney of birds fed on treated plants did not show marked histopathological differences after 21, 24, 34 and 44 days when compared with those in untreated birds (Fig. 4). Effects on the kidneys could be observed only after exposure to lindane for 60 days, the examination revealed dilatation and congestion of renal blood vessels (Fig. 5) and interstitial haemorrhages among the renal tubules were observed (Fig. 6).

3.7.3. Brain

The histological examination of the brain (cerebrum) of birds fed on treated plants showed very mild diffuse gliosis and increase in the number of microglial cells (microphage cells of brain). The cerebral blood vessels were dilated and congested. Perivascular and intramyelinic oedema was observed (Fig. 7). The degree of diffuse gliosis was increased with an increase in the exposure period, with the appearance of perivascular oedema. Fig. 8 shows different types of nerve cells and blood vessels of brain of control birds.

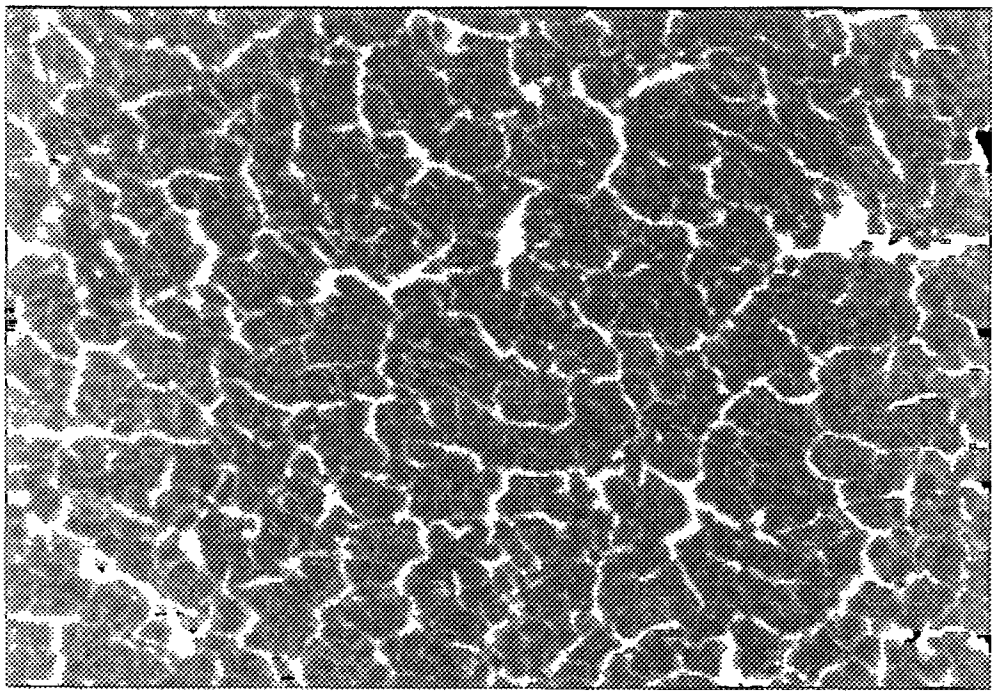

Fig. 1 : Liver of control bird showing normal hepatic cords and sinusoids (H&E, X100)

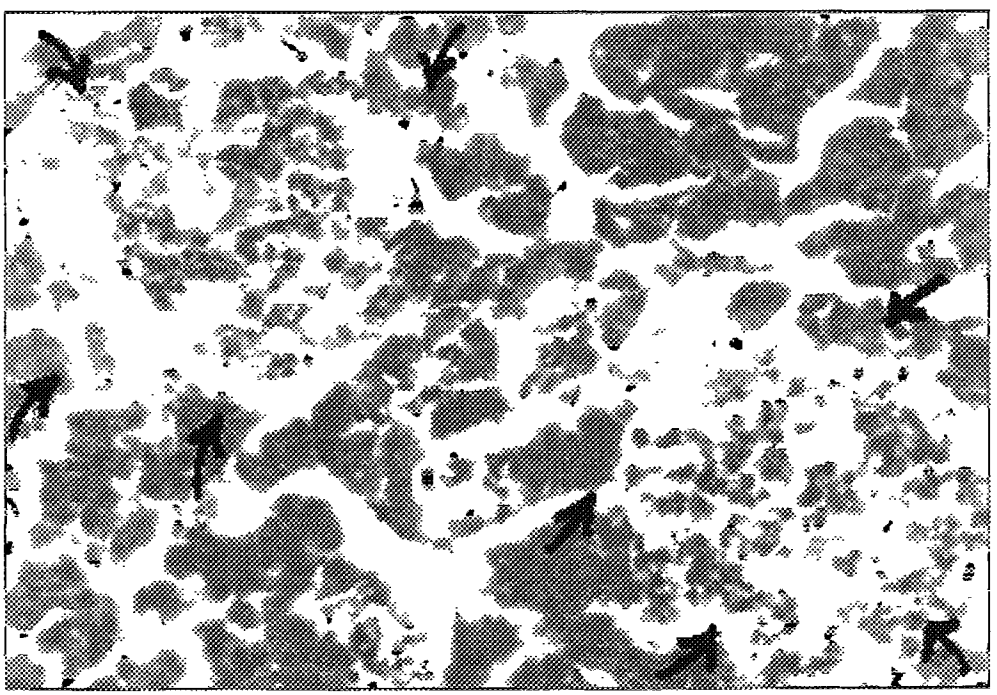

Fig. 2 : Liver of birds fed on maize showing focal aggregations of mononuclear cells with central necrosis of hepatic cells (H&E X200).

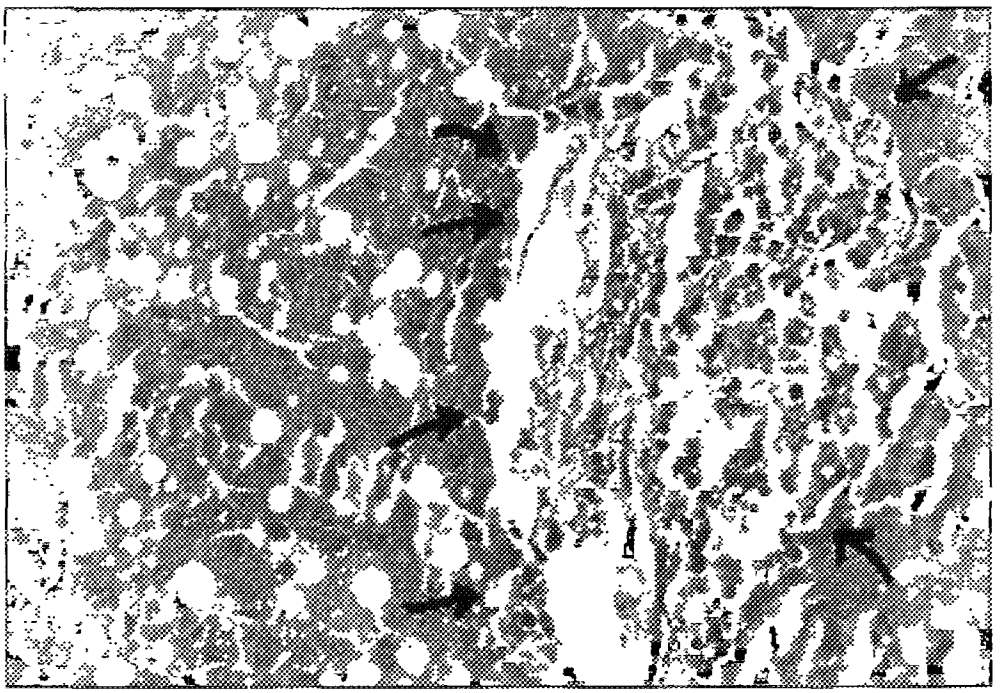

Fig. 3 : Liver of birds fed on maize showing focal aggregations of mononuclear cells and fatty change of hepatic cells (H&E, X200).

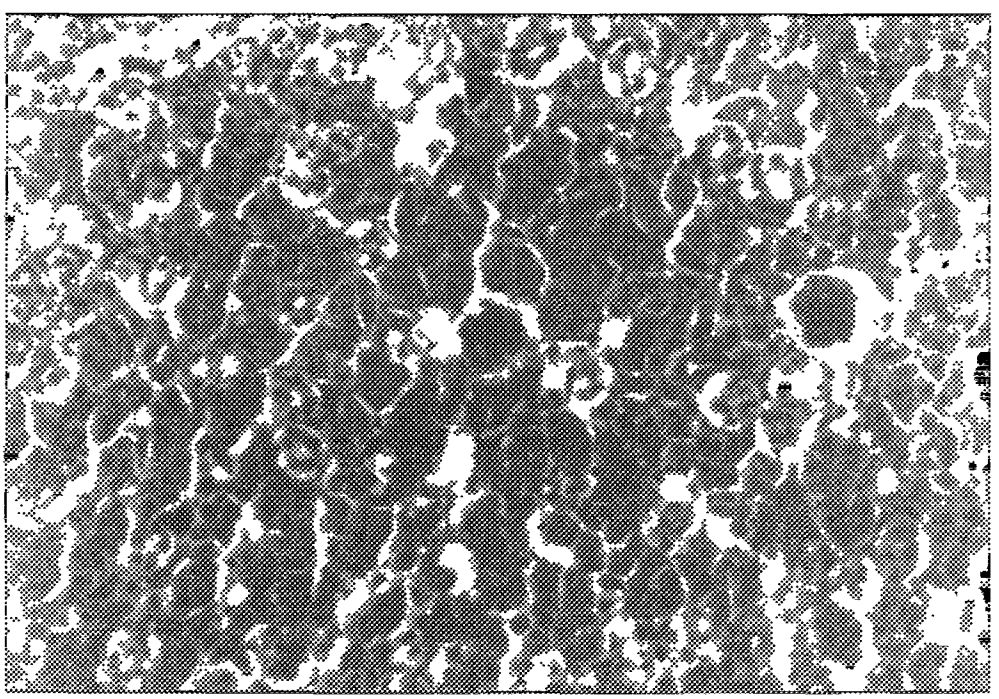

Fig. 4 : Kidney of control bird showing normal renal glomeruli and normal renal tubules (H&E, X100).

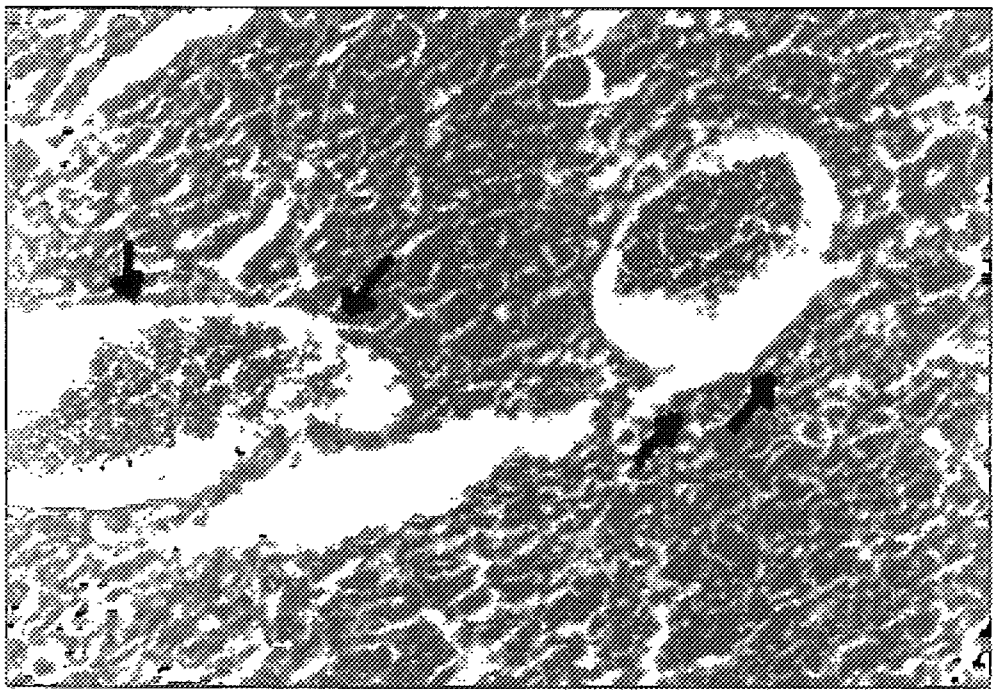

Fig. 5 : Kidney of birds fed on maize showing dilatation and congestion of renal blood vessels (H&E, X200)

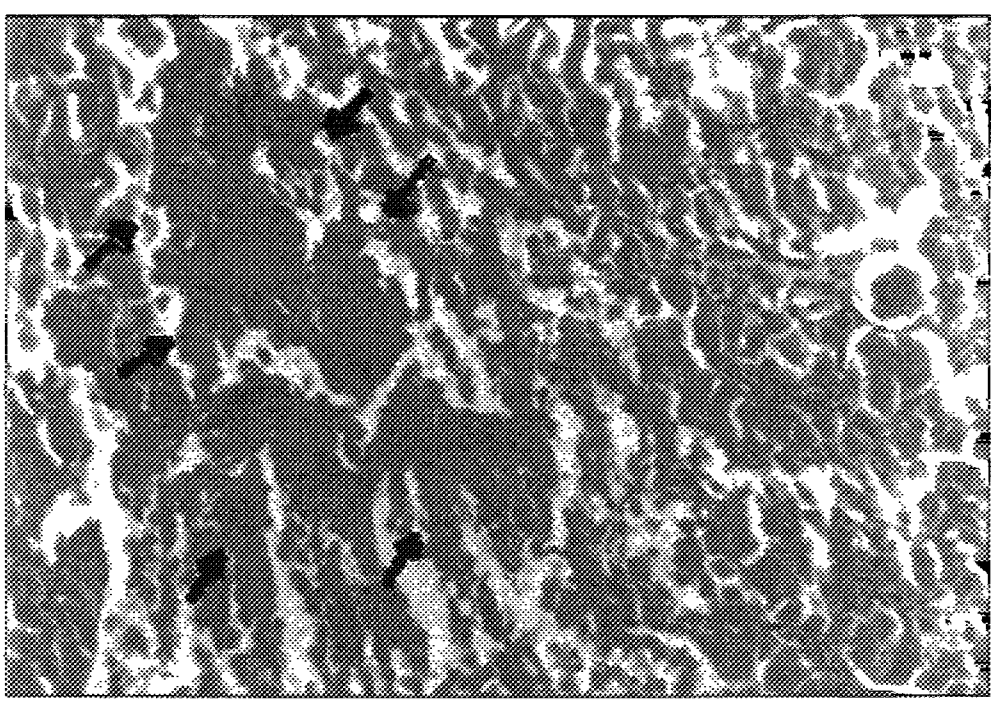

Fig. 6 : Kidney of birds fed on maize showing interstitial haemorrhages among the renal tubules (H&E X200).

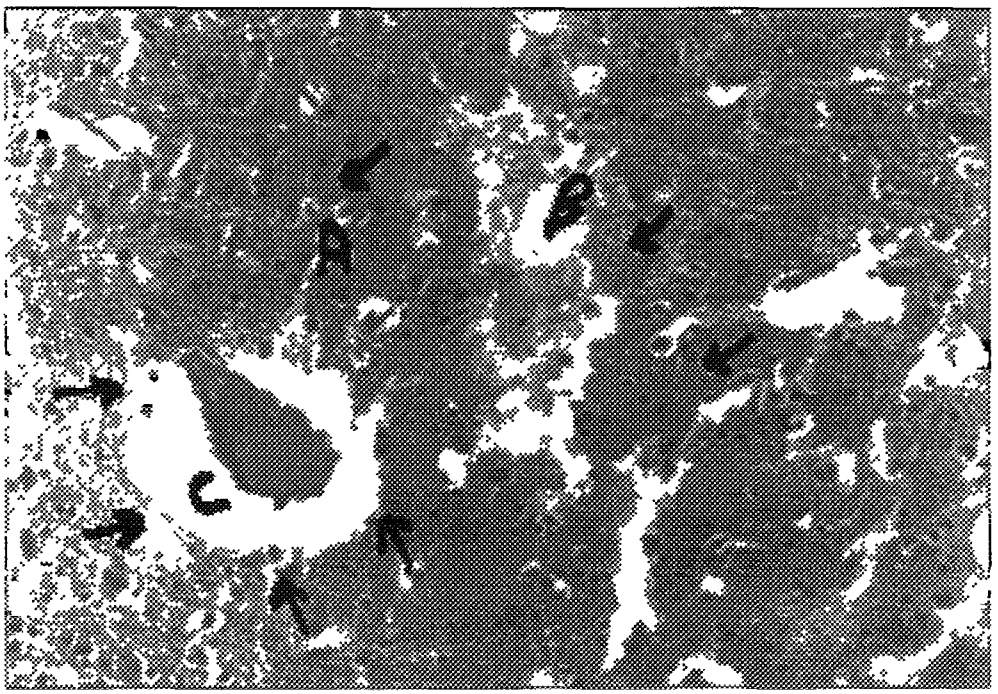

Fig. 7 : Brain (cerebrum) of birds fed on maize showing mild diffuse gliosis (A), intramyelinic oedema (B), perivascular oedema and dilated & congested blood vessels (C) (H&E, X200).

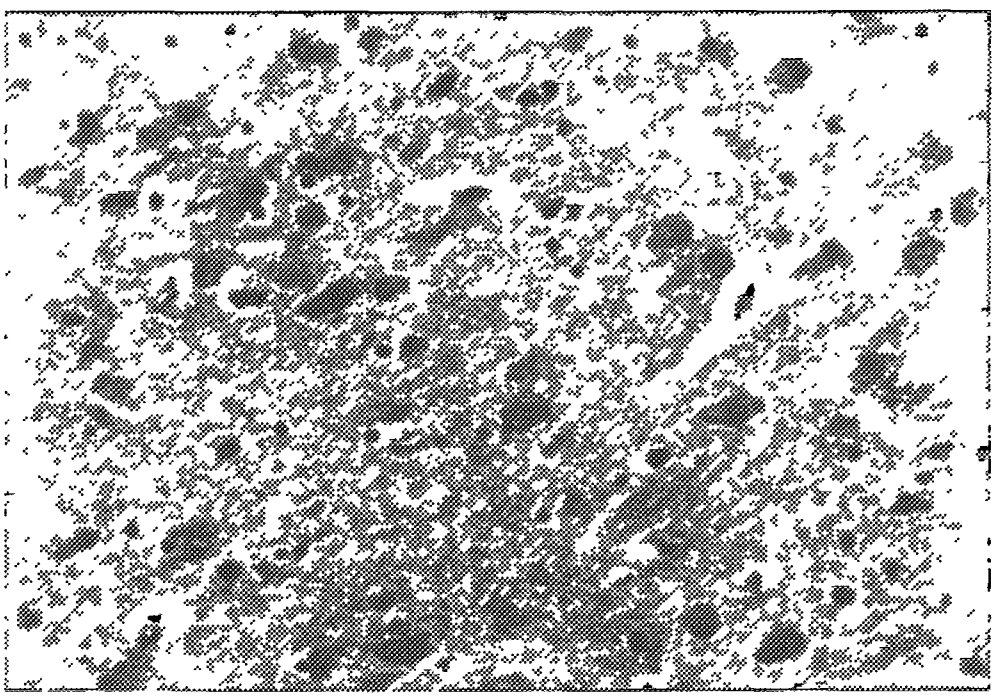

Fig. 8 : Brain of control bird, showing different types of nerve cells and blood vessels (H&E, X200).

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DEGRADATION OF LINDANE BY MICROORGANISMS. EVALUATION OF INHIBITORY EFFECT ON MICROBIAL ACTIVITY USING RADIORESPIROMETRY

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Abstract

The degradation of U-^{14}C-lindane in two types of Egyptian soil was studied under laboratory conditions The rate of mineralization of lindane was slow Evolution of $^{14}CO_2$ increased with time and amounted to 3 5-5 5% of the initial concentration within 90 days At this period both soil types contained about 88% of the applied radiocarbon; 33-37% of the initial dose being bound to the soil The methanol ^{14}C-extractables showed by TLC and HPLC analysis the presence of lindane as main product together with traces of minor metabolites In addition, the effect of different rates of application of lindane on the respiratory activity of soil microorganisms was evaluated using U-^{14}C-glucose as substrate Concentrations up to 5 mgkg ^{1} caused a short term suppression of $^{14}CO_2$ evolution A dose of 10 mgkg ^{1} significantly inhibited soil respiration as determined by $^{14}CO_2$ evolution for the 11 day period of the experiment

1. INTRODUCTION

Lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane) is a persistent insecticide which is effective against several soil insects. For several decades this insecticide has been widely used in Egypt as a soil insecticide, seed dressing and for foliar application on crops. The dissipation of lindane from soil was reported to be faster than other organochlorine pesticides such as DDT [1,2,3]. The degradation and metabolism of the insecticide have been studied by several investigators [4,5,6].

The aim of the present work is to study the fate of lindane and its effect on the activity of soil microorganisms in different types of Egyptian soils under laboratory conditions. The bioactivity of soil fauna, determined by the rate of ^{14}C-glucose utilization for respiration, was taken as a measure to assess the effect of lindane on soil microorganisms.

2. MATERIALS AND METHODS

2.1. Chemicals

U-^{14}C-glucose was purchased from Sigma Chemical Company. It had a specific activity 66.23 MBq/mg and purity > 98%. U-^{14}C-lindane (Sigma) was diluted with technical non-labelled lindane (97%, Aldrich-Chemie) to give a preparation activity of 7.4 x 10^4 Bq/mg.

2.2. Soils

Five types of Egyptian soil were used. The samples were collected from different locations such as Middle Delta, Giza and Kalyoubia. The analysis of these soils is shown in

Table 1. Samples were kept frozen till analysis. Soil was then allowed to thaw, air dried overnight and was used for starting the experiment.

TABLE 1. ANALYSIS OF SOILS

Soil	pH	Organic Carbon	Sand	Silt	Clay
A	7.9	2.3	77.0	13.5	9.5
B	7.9	0.75	93.5	4.5	2.0
C	7.7	0.75	69.8	10.7	19.4
D	7.9	1.4	40.2	24.5	35.3
E	7.5	2.5	28.2	33.1	38.7

A = **Loamy sand**
B = **Sand**
C = **Sandy loam**
D = **Clay loam (light)**
E = **Clay loam (heavy)**

2.3. Procedures

Soil was moistened to about 75% of the moisture holding capacity. The moist soil (100 g) was transferred to standard biometer flasks of 250 ml capacity. The side arm of the flask contained 20 ml of 1 M NaOH to trap the evolved $^{14}CO_2$. For studying the fate of ^{14}C-lindane in soil (Experiment 1), the soil was spiked with a lindane solution at a concentration of 10 mg kg^{-1}, containing radioactivity of about 7.4 x 10^4 Bq. Each flask was treated with 1 mg of lindane dissolved in 1 ml of acetone:water mixture (1:1). For experiment 2, dealing with the effect of lindane on microorganisms, 3.7 x 10^4 Bq of U-^{14}C-glucose in 1 ml of water for each flask was used in combination with different concentrations of the insecticide. The insecticide as well as glucose solutions were applied on the surface of soil using a micropipette followed by closure of flasks.

The flasks of experiment 1 were incubated at about 25°C in the darkness for 90 days. Parallel experiments were carried out with sterile soil, where the biometer flasks with the soil were autoclaved for 3 days at 120°. Control flasks contained all constituents except the insecticide. In experiment 2, samples and controls were incubated in triplicates at 25° for eleven days.

The amount of evolved $^{14}CO_2$ was monitored at different time intervals by determination of ^{14}C-activity of 1 ml aliquots of the alkaline solution taken by a syringe from the side arm. The cumulative evolution of $^{14}CO_2$ was expressed as percentage of the applied dose that has been converted to $^{14}CO_2$.

2.4. Radioactivity measurements

Radioactivity in solutions was determined by direct counting in a liquid scintillation counter (LSC) using a dioxan based scintillation cocktail. Readings were corrected for background and quenching.

Bound residues were determined by combustion of 100 mg soil samples followed by counting in a liquid scintillation counter. For radioscanning of TLC plates, 1 cm increments of the plates were scraped in vials, covered with the scintillator and then counted by LSC.

2.5 Analysis of residues in soil

Soils of experiments with sterile and non-sterile soil were analyzed for ^{14}C-extractable and ^{14}C-bound residues. The soil was extracted in a Soxhlet apparatus with methanol and both the extract and the extracted soils were determined for ^{14}C-activity.

The nature of ^{14}C-residues in methanol extract was determined by high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). HPLC of the extract was performed on a Porasil column using a UV-detector at 190 nm with n-heptane as the mobile phase. Analysis using a Waters-Association Model 510 equipped with a Waters Association Model U6K loop-injector and an UV-tunable absorbance detector Model 484. The concentration of the insecticide residues was determined from standard curves. TLC-Analysis of the methanol extractables was carried out on precoated silica gel plates using hexane (developing system 1), hexane-acetone (8:1, system 2) and hexane-chloroform (12:1, system 3). Spots were made visible by spraying with 0.005% ethanolic Rhodamin B solution, then with 10% sodium carbonate solution followed by scanning under UV light at 254 nm [8]. Spots of chlorinated hydrocarbons appear as brown to violet spots. By analysis of residues, emphasis was given to the parent compound (lindane).

3. RESULTS AND DISCUSSION

The distribution of ^{14}C-activity following application of ^{14}C-lindane to soil is shown in Table 2. The results indicate a gradual increase in the binding of ^{14}C-residues over time in both soils. A maximum binding of about 35% of the applied dose was observed 8 weeks following application of the insecticide in soil A and after 4 weeks in soil B. This appears to be higher than values previously reported for binding of lindane that ranged from 6% [3] to 16% [2] of the applied dose during 4 weeks. This may be attributed to difference in soil characteristics.

The amount of ^{14}C-methanol extractables, on the other hand, decreased slowly over time. Fig. 1 shows the extractable and bound ^{14}C-residues at different time intervals in soil spiked with ^{14}C-lindane. The data obtained indicate a good recovery that ranged from 87-100% of the applied radiocarbon. It is noteworthy that the behaviour of lindane did not vary much in the two types of soil used, probably because of the similarity in structure between them.

The radioactivity in the sodium hydroxide solution indicates a progressive but slow evolution of $^{14}CO_2$ over time (Fig. 2) corresponding with rates of mineralization of lindane

TABLE 2 : FATE OF ^{14}C-LINDANE IN SOIL UNDER LABORATORY CONDITIONS.

Sampling Time (days)	Soil[a] Type	^{14}C-residues in soil (% applied dose)		$^{14}CO_2$ (%)	Total ^{14}C Recovered (%)
		^{14}C-extractables	^{14}C-bound		
1	A	85.5±0.10[b]	6.8±0.20[b]	0.005±0.001[b]	92.3
	B	83.0±0.10	5.9±0.20	0.002±0.001	88.9
15	A	76.5±0.30	17.3±0.17	0.015±0.002	93.8
	B	76.5±0.30	22.0±0.10	0.020±0.001	98.5
30	A	58.5±0.20	28.5±0.35	0.100±0.005	87.1
	B	65.0±0.20	35.0±0.34	0.200±0.002	100.
60	A	60.0±0.65	35.0±0.20	3.50±0.010	98.5
	B	60.5±0.70	36.0±0.40	3.00±0.030	99.5
90	A	52.0±0.35	37.5±0.20	5.50±0.040	95.00
	B	55.5±0.20	33.0±0.40	3.50±0.035	92.00

(a) A = Loamy sand, B = Sand
(b) Data are mean of duplicate experiments ± standard deviation.

that did not exceed 5.5% of the applied dose in 3 months. Previous studies [1] have also shown that lindane is mineralized at a very slow rate (1.6% in 4 weeks).

Fig. 2 shows a lag phase during the first few weeks, where only very little $^{14}CO_2$ was collected. The $^{14}CO_2$ evolved from the sterilized soil was significantly less than from non-sterilized soil. This shows the importance of microbial action in the degradation of lindane.

TLC-Analysis of methanol extract of treated soil showed the presence of lindane as a main residue (over 90%, R_f = 0.22 in system 1; 0.7 in system 2 and 0.42 in system 3), and at least 3 unknowns. Radioscanning of TLC-plates confirms that the percentage of all lindane metabolites did not exceed 9%. HPLC analysis of the extract showed a comparable result (R_t = 18.7 min) (Fig. 3). The amount of unchanged ^{14}C-lindane, 3-months following treatment of the soil, could be determined from standard curve as 92% of the methanol extractables. These results are in line with previous reports indicating, that lindane is not readily metabolized [7].

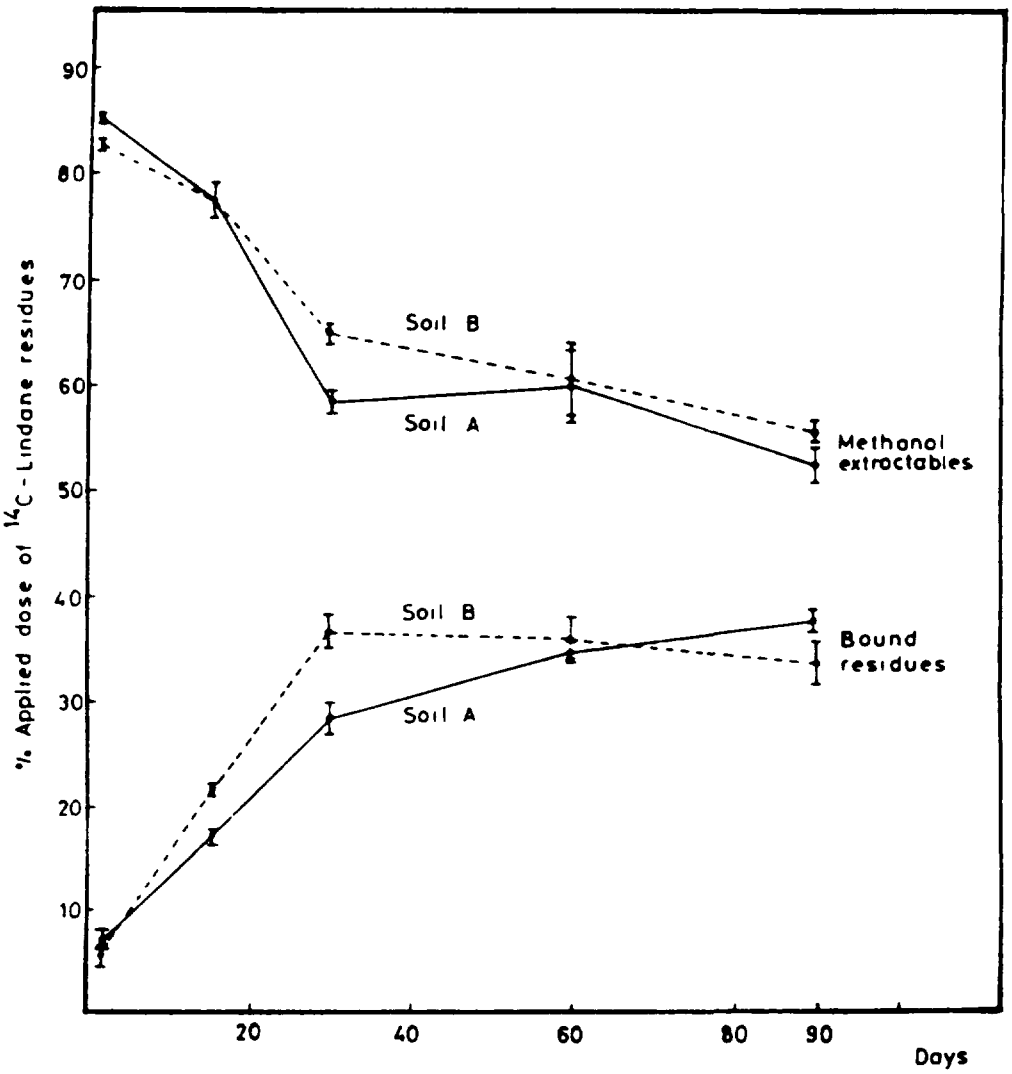

Fig 1 ^{14}C-Methanol extractables and ^{14}C-bound residues in soil spiked with ^{14}C-Lindane under laboratory conditions, Soil A = Loamy Sand Soil B = Sand

Incubation of different types of Egyptian soils with ^{14}C-U-glucose led to the liberation of appreciable amounts of $^{14}CO_2$ (Table 3). The amount of $^{14}CO_2$ evolved during eleven days of incubation period was found to depend largely on the texture of the soil used. In sandy loam soil the total amount of the evolved $^{14}CO_2$ in the absence of the insecticide was around 27% (Fig. 4) while it reached about 40% in case of the heavy soil (Fig. 6). The presence of lindane at the applied doses led to a reduction in $^{14}CO_2$ output especially during the first two days. As the incubation period increased the inhibitory effect of lindane was decreased and the recovery depend upon the dose and type of soil. Fig. 5 shows that the greatest inhibition seemed to occur in the light soil perhaps because there would have been relatively fewer organisms. On the other hand, the heavier microbial load present in the heavy soil led to a relatively rapid recovery from the initial inhibition induced by both doses. A complete recovery of microbial activity from the effects of 3 and 5 mg kg^{-1} was almost achieved after 11 days incubation (Fig. 6). The presence of lindane at a concentration of 10 mg kg^{-1} resulted in a pronounced inhibition of $^{14}CO_2$ evolution in the three different soils throughout the incubation period.

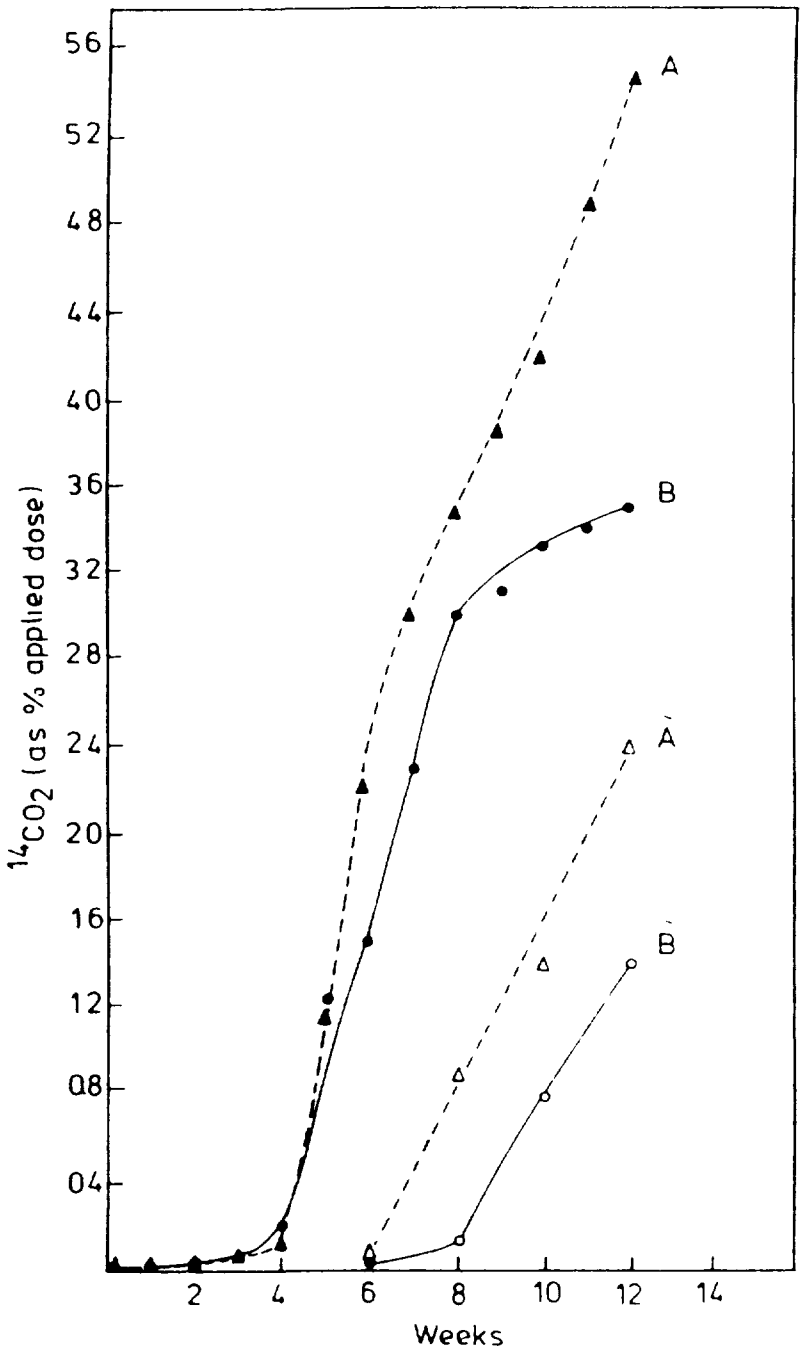

Fig 2 Rate of evolution of $^{14}CO_2$ from sterile and non-sterile soil spiked with U-^{14}C-Lindane during 12 weeks

A = Loamy sand soil , B = Sand soil ,

Ā = Sterile loamy sand soil , B̄ = Sterile sand soil

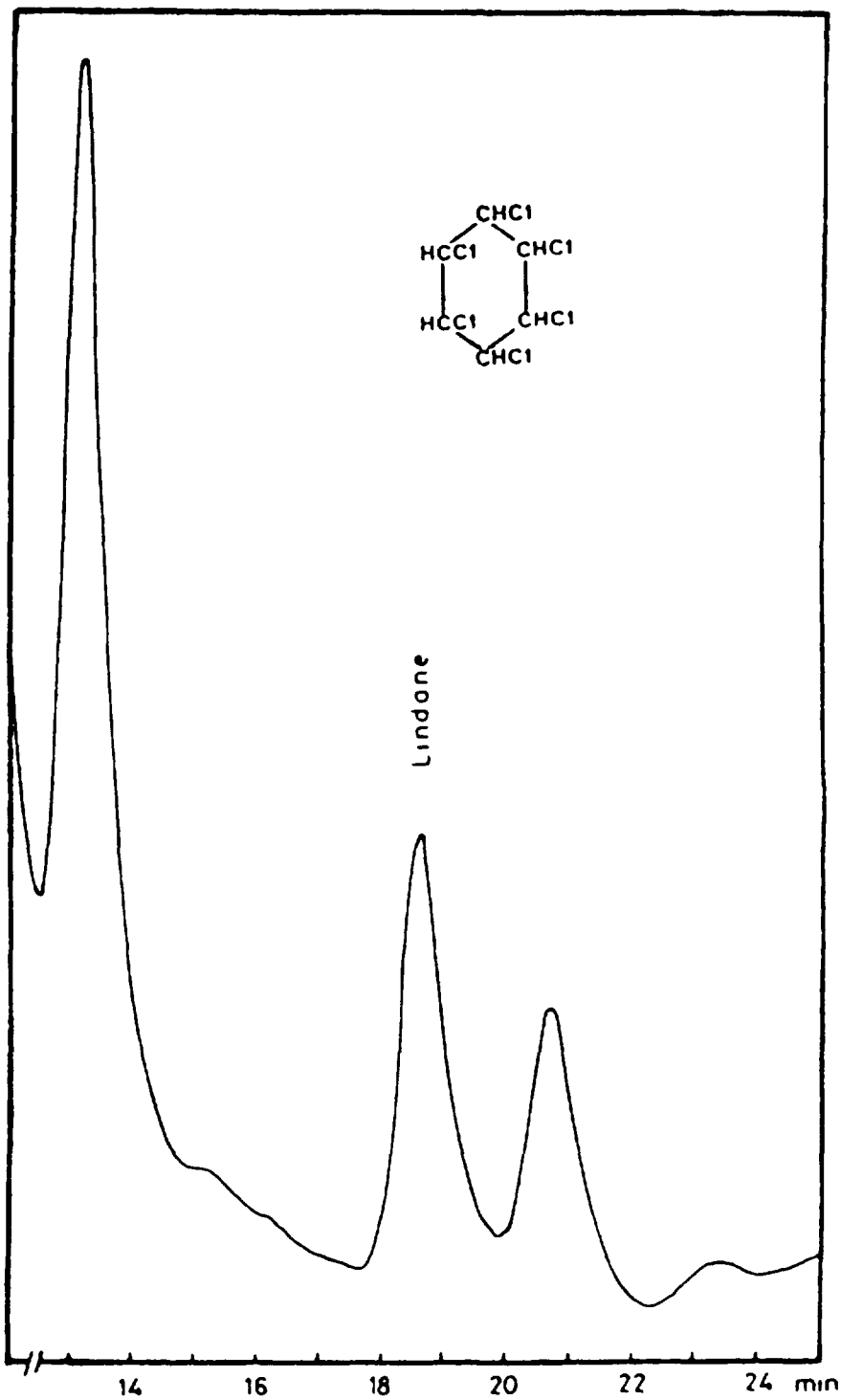

Fig 3: HPLC Analysis of the methanol ^{14}C-extractables of sand soil after 3 months

Column Porasil. Detector. UV. Wavelength 190 nm
Mobile phase n-Heptane. Flow rate. 1ml/min
Pressure. 700 PSI

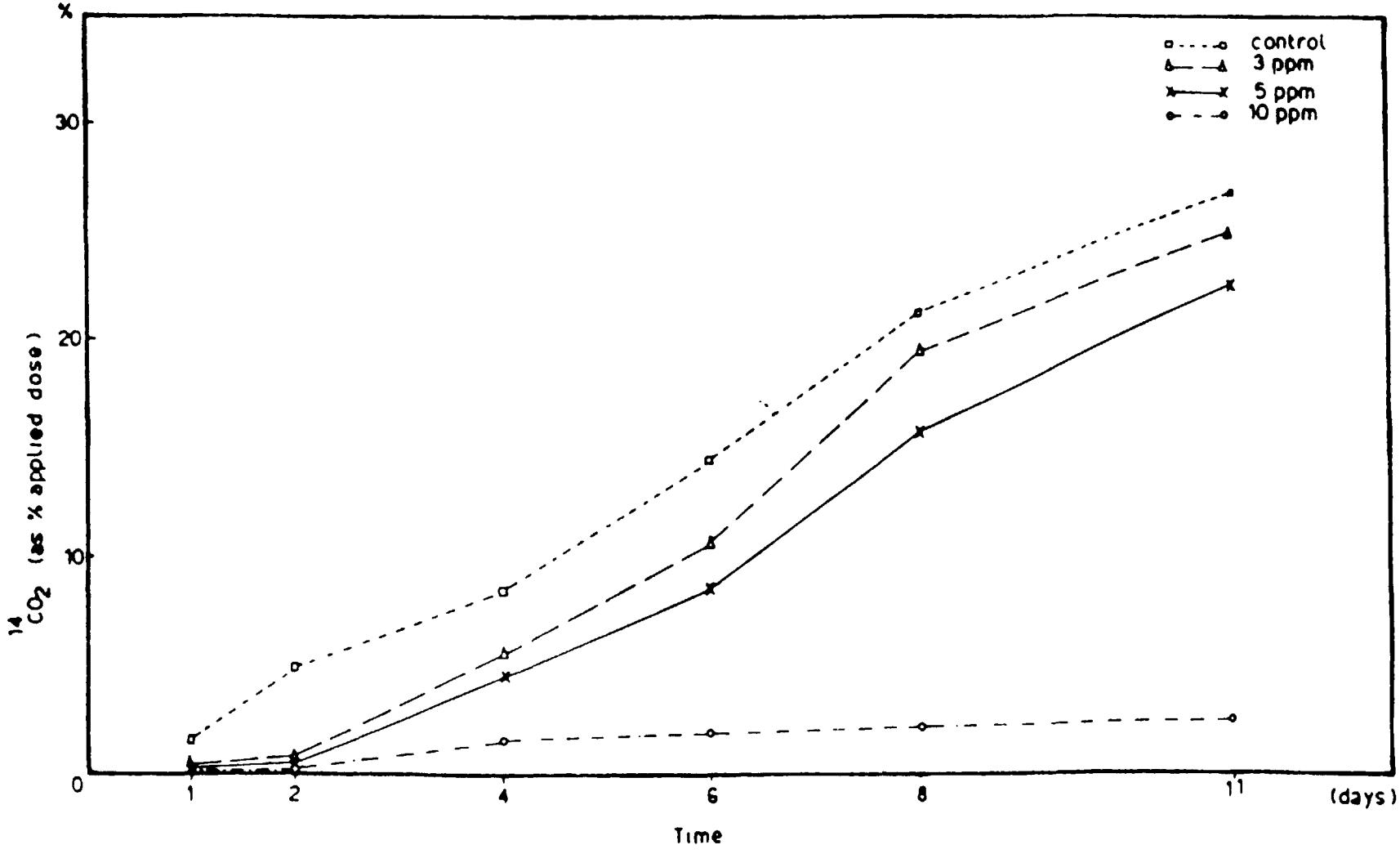

Fig. 4 : Rate of evolution of $^{14}CO_2$ after incubation of ^{14}C-glucose with soil (C) in presence and absence of lindane over a period of eleven days.

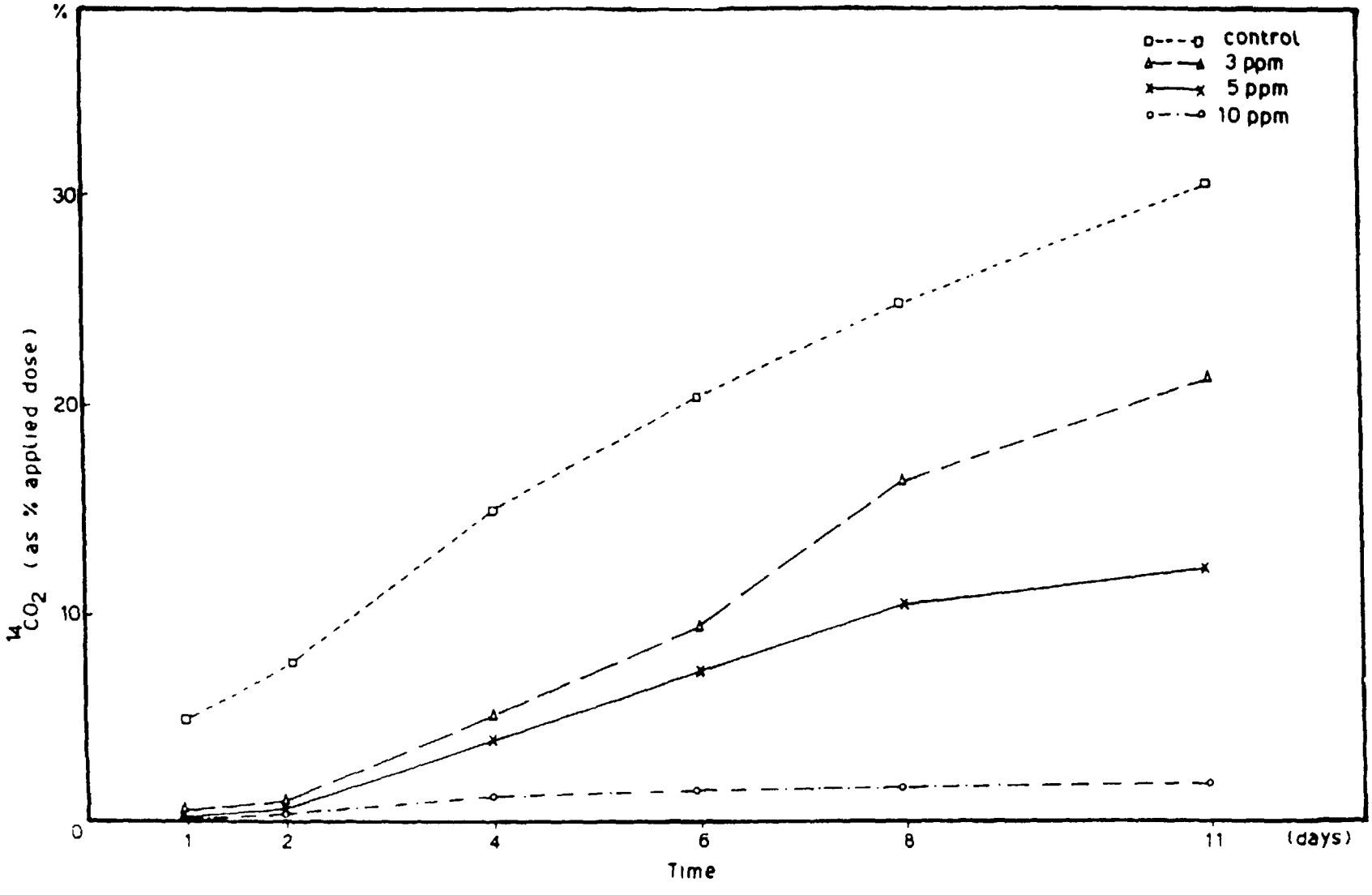

Fig. 5 : Rate of evolution of $^{14}CO_2$ after incubation of ^{14}C-glucose with soil (D) in presence and absence of lindane over a period of eleven days.

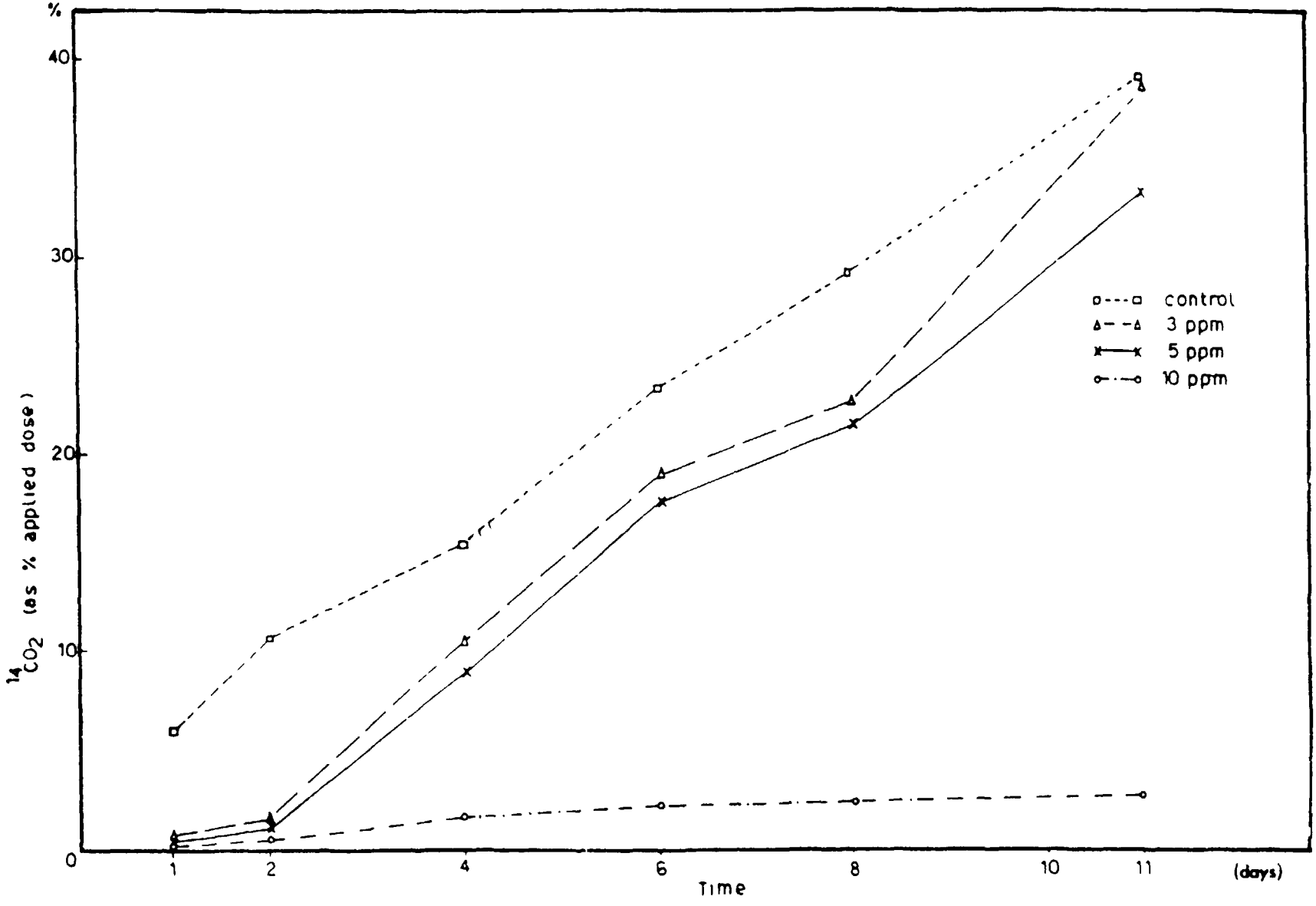

Fig. 6 : Rate of evolution of $^{14}CO_2$ after incubation of ^{14}C-glucose with soil (E) in presence and absence of lindane over a period of eleven days.

TABLE 3 . PERCENTAGE OF ^{14}C EVOLVED AS $^{14}CO_2$ AFTER INCUBATION OF ^{14}C-GLUCOSE WITH DIFFERENT SOILS IN THE PRESENCE AND ABSENCE OF LINDANE FOR 11 DAYS.

Sampling Time (days)	Soil[a]	$^{14}CO_2$-Evolved (as % Applied Dose)			
		Control	Dose 1[b]	Dose 2[b]	Dose 3[b]
1	C	1.90±0.01[c]	0.58±0.02[c]	0.50±0.02[c]	0.27±0.01[c]
	D	5.01±0.03	0.72±0.04	0.40±0.01	0.14±0.01
	E	6.31±0.02	0.72±0.03	0.50±0.03	0.32±0.02
2	C	5.00±0.04	0.68±0.01	0.77±0.04	0.72±0.03
	D	7.50±0.02	1.04±0.08	0.72±0.02	0.45±0.04
	E	10.8±0.06	1.67±0.07	1.04±0.07	0.68±0.04
4	C	8.56±0.01	5.86±0.20	4.60±0.17	1.80±0.15
	D	15.0±0.04	5.22±0.12	4.10±0.13	1.17±0.04
	E	15.3±0.02	10.8±0.13	9.98±0.29	1.18±0.06
6	C	14.6±0.04	10.8±0.17	8.56±0.13	1.94±0.09
	D	20.2±0.08	9.41±0.22	7.43±0.15	1.58±0.11
	E	23.4±0.04	18.9±0.43	17.6±0.26	2.25±0.10
8	C	21.4±0.10	19.8±0.31	15.8±0.18	2.25±0.09
	D	24.8±0.46	16.2±0.24	10.1±0.15	1.71±0.07
	E	29.3±0.29	22.5±0.43	21.9±0.30	2.30±0.21
11	C	27.0±0.18	25.2±0.36	22.5±0.21	2.48±0.15
	D	30.4±0.61	21.2±0.28	11.7±0.16	2.03±0.08
	E	39.4±0.78	39.3±0.50	33.3±0.64	2.66±0.10

(a) Soil C = Sandy loam; Soil D = Clay loam (light); Soil E = Clay loam (heavy)
(b) Dose 1 = 3 mg/kg; Dose 2 = 5 mg/kg; Dose 3 = 10 mg/kg
(c)Data are mean of duplicate experiments ± S.D.

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THE EFFECTS OF ORGANOCHLORINE PESTICIDES ON SOME NON-TARGET ORGANISMS IN MAIZE AND COWPEA AGRO-ECOSYSTEMS IN GHANA

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Abstract

In order to study the effects of organochlorine pesticides on non-target organisms under tropical conditions, a three-year study was conducted in Ghana applying lindane at 1 kg AI ha^{-1} and endosulfan at 0 75 kg AI. ha^{-1} to maize and cowpeas respectively. The endosulfan treatment was preceded by two consecutive treatments with cypermethrin at 50 g AI ha^{-1}. Lindane significantly reduced the numbers of ants, spiders and springtails trapped though the numbers of ants and spiders generally recovered within the cropping period Lindane significantly increased the numbers of leafhoppers caught from maize plots probably due to the elimination of a natural enemy Ant, spider and springtail numbers were also significantly reduced by the endosulfan treatment in cowpea plots 5) Lindane did not significantly increase maize yields in two of the three years. Endosulfan contributed to significant yield increases and reduced seed damage in cowpeas Neither lindane nor endosulfan seemed to have any significant adverse effects on the activities of soil microfauna and microflora based on the rates of decomposition of leaf discs buried in the experimental plots

1. INTRODUCTION

The development and use of insecticides during and after the Second World War resulted in increased crop yields and effective control of insect vectors of human and veterinary diseases [1]. Nevertheless, the continued use and misuse of pesticides have caught attention currently as a result of their potential negative ecological effects. These include the disruption of the agro-ecosystem through the elimination of natural enemies and other non-target organisms, the development of resistance in some insect pests leading to pest resurgence, promotion of minor pests to major pest status and the health hazards posed to agricultural workers and consumer.

Organochlorine insecticides have proved effective in controlling various insect pests. They possess broad spectrum activity and are relatively cheap. This group, which inludes BHC or lindane, contains pesticides which have been banned or severely restricted in the USA, Canada and western Europe. They have been described as being oncogenic, mutagenic and teratogenic [2] in addition to being highly persistent in the environment [1], with the associated negative effects on non-target organisms. Despite these claims, there is little documented evidence about these effects under tropical conditions, especially in most third world countries where they find ready use.

In Ghana, lindane (Gammalin 20), is the most widely used insecticide recommended for controlling capsids in cocoa, the major export crop for Ghana. Even though its sale and use is restricted, it is still used on other crops such as cowpea (*Vigna unguiculata* L.) and vegetables. It is also marketed as Gammatox for controlling veterinary ectoparasites such as ticks, mites and fleas on livestock and domestic animals.

It had been used in the past together with DDT, another organochlorine pesticide, for controlling cut worms, wire worms and stem borers in maize *(Zea mays)* [3,4,5], as well as for maize and cowpea storage [4,5].

Cowpea, which provides proteins in the diets of poorer sections of the society is also plagued by very serious insect pest problems. Endosulfan, an organochlorine, marketed as Thiodan 35 E.C. has been recommended for controlling insect pests of this crop [6]. It is also recommended for controlling insect pests in coffee plantations.

Reports from Kenya, India and Ecuador on the persistence of organochlorine pesticides under tropical conditions have shown that these chemicals are not as persistent as had been previously described [7,8,9].

As the organochlorines continue to be used in the tropics, there is an urgent need for a thorough investigation into their adverse effects on other components of the agro-ecosystems in these regions since results of work done mostly under temperate conditions should not be extrapolated to the tropical environment.

In order to investigate the effects of organochlorine pesticides in a tropical environment, a three-year study was undertaken to find out the impact of two organochlorine insecticides on non-target organisms in maize and cowpea agro-ecosystems in Ghana.

2. MATERIALS AND METHODS

2.1. Experimental site and land preparation

The study was conducted at the Ghana Atomic Energy Commission Research Farm, about 21 km north-west of Accra, Ghana. The chosen site, on a middle slope had not been cultivated for over 6 years. About 3.6 ha was initially bulldozed, stumped and harrowed.

2.2. Experimental design

In the first year, 1992, two large blocks each measuring 102 m x 102 m were demarcated. Each block was redivided into 4 plots of 50 m x 50 m, separated by 2m gaps. Two diagonally opposite plots in each block were assigned to maize while the others were allocated to cowpeas. Four subplots each of 5 m x 5 m were marked out in the centre of each plot for sampling purposes. Only one block was used in the second year for the same treatments in the second year.

In the third year (1994), the maize fields were made up of 4 blocks composed of two plots each. Each plot measured 25 m x 25 m, and was separated by a distance of 2 m. An inner sampling subplot, 10 m x 10 m was marked out in each plot. The maize crop had two treatments, control and lindane treatment.

The cowpea crop had three treatments; control, cypermethrin and cypermethrin + endosulfan. The plots were the same sizes as for the maize.

For both crops, the completely randomised block design of 4 replicates was adopted.

2.3. Soil analysis

Physical analysis of soil samples randomly taken from the site was done at the Soil Science Department of The University of Ghana.

2.4. Soil microbiology

To assess the possible effects of the organochlorine insecticides on soil microflora and microfauna, 50 leaf discs about 2.5 cm in diameter were cut out with a cork borer from the leaves of *Griffonia simplicifolia*. These tough leaves were weighed fresh and stapled into nylon mesh minibags each 12 cm x 12 cm. An initial dry weight was determined by oven-drying some of the leaves at 60^0C for 24 hr. Four each of the weighed fresh leaves were randomly buried to a depth of about 5 cm in the sampling subplots. They were retrieved after periods varying from 84 to 259 days. After retrieval, final dry weights were determined as described above and the % loss in dry weight was calculated as follows:

$$\frac{\text{(Initial dry weight - Final dry weight)} \times 100}{\text{(Initial dry weight)}}$$

2.5. Experimental crops and agronomy

2.5.1. Maize

'Abelehi', a short duration, drought resistant cultivar was sown at 80 cm x 40 cm at 2 seeds $hill^{-1}$. Weeding was done manually, usually about 3 weeks after planting. A compound fertilizer, 20:20:0, NPK, was applied at 200 kg ha^{-1} soon after weeding. A side dressing was made with urea at 50 kg ha^{-1} about 6 weeks after planting. Harvest was about 12 weeks after sowing.

2.5.2. Cowpea

An early maturing cultivar, 'Asontem', was manually sown, usually from mid to late June at 60 cm x 20 cm at 2 seeds $hill^{-1}$. Cowpeas were normally harvested at about 8 weeks after planting.

2.6. Neighbouring crops

Guard rows of maize and cowpeas were planted about 3 m away from the experimental crops. The areas beyond were mostly occupied by shrubs such as neem (*Azadiractha indica*), 'Kagya' (*G. simplicifolia*) and a number of other plants including guinea grass (*Panicum maximum*).

2.7. Maize stemborer infestation assessment

Two weeks after the first lindane treatment, 50 maize plants were randomly selected outside the sampling subplots and dissected for stemborer infestation assessment. At harvesting time, all maize stalks were also similarly treated.

2.8. Yield estimation

Yields of both maize and cowpea crops were estimated by harvesting from small plots of 2 m x 2 m, close to the sampling subplots. The moisture contents of the crops were determined by the oven method.

2.9. Insect sampling methods

In the 1992/1993 studies, two sampling methods were used., pitfall traps and sweep nets. In 1994, a D-VAC machine was used instead of the sweep net in addition to the pitfall traps. This became the standard method recommended for sampling arthropods from plants and ground.

2.9.1. Pitfall traps

In the 1992 studies, four small plastic pots of colours ranging from blue to greyish green, 13.5 cm wide and 11 cm deep, with covers were randomly buried to the brim in each subplot. A small quantity of water with a few drops of liquid soap was put into each pot and left open for 24 hours before the insecticidal treatment. Arthropods trapped were removed into vials containing 70% alcohol for storage, classification and counting. Further samplings were done at 2 weekly intervals for up to 8 weeks.

In 1993 and 1994, the pitfall traps consisted of two small, creamish-white plastic pots. One of these (11.3 cm x 8.3 cm) with a small hole at the bottom for drainage was buried randomly up to the brim in the sampling subplots. A similar plastic pot but with about 2 cm length around the rim cut off and provided with a semi-stiff wire handle was placed inside the one buried in the soil. Water with liquid soap was exposed as described above but the exposure period was extendend to 48 hr in 1993. In 1994, the traps were exposed for 7 days with 7 day intervals in between. This was done with the aim of catching more insects. A few drops of formalin were put into the pots to prevent insects from decaying and the pots were emptied as and when necessary.

2.9.2. Sweep net sampling

A day before the spray application and thereafter, fortnightly after the spraying up to 8 weeks, a sweep net was used to sample for insects in the subplots. Five sweepings were done by taking 5 steps diagonally along the subplot. The contents from the sweep net were emptied into Kilner jars with small balls of cotton wool partly soaked with chloroform. The jars were then closed with their discs and rings. In the laboratory, the catches were kept in vials with alcohol as for the pitfall trap collections.

2.9.3. D-VAC sampling machine 1994

The machine was used to sample 5 randomly chosen plants (subsamples) around each pitfall trap, each sampling lasting for about 5 seconds. Five subsamples constituted a sample. The soil around the same place was also similarly sampled. The collection at each sampling was emptied into a labelled polyethylene bag, tied up and kept in a freezer. The arthropods were later picked with a camel hair brush and kept in 70% alcohol in vials.

2.10. Pesticide application

2.10.1. Lindane

The formulation in Ghana, known as Gammalin 20 E.C or Kumakate; marketed by Chemico (Ghana) Ltd was applied to the maize crop. During the 1992 trials, lindane was applied at 1 kg AI ha^{-1}, 17 days after planting (DAP). In 1993 and 1994, the application was split into two 0.5 kg doses and the second was applied 2 weeks after the first.

2.10.2. Cypermethrin

This is a synthetic pyrethroid manufactured by Shell Chemicals, Cote d'Ivoire under the trade name - Ripcord 10 E.C. It was applied to the cowpea crop normally 30 and 40 DAP at a dose of 50g AI ha^{-1} .

2.10.3. Endosulfan

This is an organochlorine insecticide manufactured by Hoechst Aktiengesllschaft, Frankfurt, Germany under the trade name Thiodan 35 E.C. This is normally applied about 50 DAP preceded by two consecutive applications of Cypermethrin. It was applied at 750 g AI ha^{-1}.

All the insecticides were applied with a CP 15 Knapsack spraying machine using between 160 to 288 L of water ha^{-1}.

2.11. Statistical analysis

For statistical analysis of the 1992 and 1993 data, the subplots were regarded as real plots. Insect counts were first transformed by adding one to the figures and finding the log before analysing with the General Linear Model Computer Package on Minitab Release 10 programme.

3. RESULTS AND DISCUSSION

3.1. Soil analysis and meteorological data

The soil in the study area was predominantly sandy-clay loam, details are given in Table I.

Table I. SOIL CHARACTERISTICS OF MAIZE AND COWPEA PLOTS

	Maize plots		Cowpea plots	
Characterisitic	Control	Treated	Control	Treated
pH	6.21 ± 17	6.30 ± .15	6.36 ± 0.29`	6.33 ± 0.21
% Carbon	1.40 ± 0.46	i.12 ± 0.47	1.81 ± 0.65	1.26 ± 0.15
% Sand	64.2 ± 5.4	63.9 ± 7.3	57.0 ± 7.6	63.0 ± 3.2
% Silt	13.4 ± 2.8	11.1 ± 3.9	12.2 ± 2.37	11.0 ± 1.6
%Clay	24.5 ± 4.7	25.0 ± 3.8	29.0 ± 5.0	26.2 ± 4.0

3.2. Effects of lindane and endosulfan on soil microbial activity

There were no statistically significant differences ($P > 0.05$) in mean dry weights of leaf discs retrieved from the control and lindane-treated maize plots after 84 days in 1992 (Table II A), or after 84 and 154 days respectively in 1993 and 1994. Even though slightly higher percent dry weight losses were recorded in leaf discs retrieved from control cowpea plots after 259 days in 1993 and 245 days in 1994, these also did not differ significantly from those from the endosulfan-treated plots ($P > 0.05$) (Table II B). These results indicate that neither lindane nor endosulfan had had any significant adverse effects on soil microfauna and microflora which contribute to the breakdown of soil organic matter under the experimental conditions in Ghana.

3.3. Effects of spraying on maize plants, 1992

In 1992, maize plants showed signs of spray injury a day after the lindane application. The plants, however, recovered later. The split application for the subsequent years resulted in negligible levels of phytotoxicity.

3.4. Maize stemborer infestation levels

Maize stemborer assessment results are set out in Table III. At the harvesting time in 1992, a higher proportion of stemborer damage was recorded from the treated plots but there were no statistical differences between the treatments ($P > 0.05$). For the two subsequent years, there was no statistical differences between the treatments for the infestation levels 14 days after the initial treatment but in 1993, statistically higher levels of stemborer infestation were recorded from the control plots at harvesting time. Generally, stemborer damage is not very serious in maize grown during the major season in the coastal savanna area where the trials were conducted [3]. Undecomposed left-over maize stalks from the major season crop provide a reservoir of stemborers ready to attack the minor season crop which is sown about 4 weeks after the major season is crop is harvested.

3.5. Maize crop yields, 1992 - 1994

In 1992, maize yields were significantly higher in the control plots which recorded an equivalent of 4450 ± kg ha^{-1} compared to 3775 kg ± ha^{-1} from the treated plots (Table III). By

Table II. MEAN % DRY WEIGHT (± SE) OF LEAF DISCS RETRIEVED FROM PLOTS

MAIZE PLOTS

Year	Control	Treated (lindane)	Time in soil (d)
1992*	0.89 ± 0.05	0.87 ± 0.02	84
1993	50.3 ± 1.9	48.7 ± 0.8	84
1994	49.9 ± 1.1	51.3 ± 1.3	154

COWPEA PLOTS

Year	Control	Cypermethrin	Cypermethrin + endosulfan	Time in soil (d)
1993	67.8 ± 6.0	--	65.4 ± 3.2	259
1994	56.6 ± 1.2	54.4 ± 3.2	55.2 ± 2.5	245

* Dry weight of retrieved discs (g)

Table III. MEAN MAIZE YIELDS ($KG.HA^{-1}$) AND % MOISTURE CONTENT

	% Stemborer control		Maize Yields			
Year	Control	Treated	Control yield	Moisture	Treated yield	Moisture
1992	[35.8 ± 2.4}	[43.8 ± 3.5]	4450 ± 0.06[a]	16.9 ± 0.24	3500 ± 0.06[b]	17.4 ± 0.47
1993	(26.0 ± 1.6)	(18.0 ± 1.4	3125 ± 0.09[a]	22.4 ± 0.23	3285 ± 0.09[b]	21.2 ± 0,64
	[52.7 ± 4.0][a]	[31.1 ± 3.7][b]				
1994	(1.0 ± 1.0)	(1.0 ± 0.6	5500 ± 0.12	15.6 ± 4.4	5000 ± 0.18	16.5 ± 0.83
	[51.1 ± 3.4]	[45.6 ± 5.2]				

[ab] - Means within rows for the same parameter followed by different letters are significantly different ($p<0.05$)

() Infestation levels 14 d after initial treatment
[] infestation levels at harvesting time

contrast in 1993, a significantly higher mean yield equivalent of 3825 ± 0.09 kg ha^{-1} was recorded from the treated plots as against 3125 ± 0.09 kg ha^{-1} for the control plots ($P < 0.05$). This is probably because in 1992 the treated plots showed some degree of phytotoxicity and also had rather slightly higher levels of stemborer damage.

In 1994, there were no significant differences between the treatments but yields from the sprayed plots of 5450 ± 0.12 kg ha^{-1} were slightly higher than those from the control plots, 5000 kg ± 0.18 ($P > 0.05$). The comparatively higher yields in 1994 might be attributed to the higher amount of rainfall recorded that year (Table IV).

Table IV. AVERAGE MONTHLY RAINFALL (mm) AND TEMPERATURES (°C) DURING THE GROWING SEASON

	May		June		July		August	
Year	Rainfall	Temp	Rainfall	Temp	Rainfall	Temp	Rainfall	Temp
1992	174.0	27.7	75.0	26.3	29.5	25.0	4.7	25.0
1993	66.8	28.8	91.6	26.7	9.0	25.6	18.6	25.6
1994	110.4	28.1	313.1	26.4	9.4	25.3	28.9	25.5

Data for Mpehuasen village, 8 km from the Research Farm
Source; Ghana Meteorological Services Department

3.6. Arthropods caught from maize, 1992 - 1994

Formicidae (ants), Araneidae (spiders) and Collembola (springtails) were the arthropods most frequently caught in appreciable numbers. Coccinellids were not so frequent while Carabids were extremely few as to yield any reliable information. Leafhoppers were added to the list later when it was observed that numbers were higher in treated plots than in the control plots.

3.6.1. Arthropods caught with pitfall traps from maize plots, 1992

Ant catches were reduced, though not significantly, by the lindane treatments. They appeared to have recovered by 56 days after the initial treatment (DAIT) ($P > 0.05$) (Fig. 1). Lindane significantly reduced spiders 14 DAIT but recovery occurred within 29 DAIT ($P < 0.05$). The pre-treatment catches of Collembola which were significantly lower in the treated plots remained significantly less almost throughout the sampling period except that of 42 DAIT ($P < 0.01$).

3.6.2. Arthropods caught with sweep net from maize, 1992

There were no significant differences between the numbers of arthropods caught from control and treated plots. Ant catches were, however, relatively far higher in the treated plots even before the lindane treatment was applied (Fig. 2). Spiders appear to have been slightly depressed but recovered by 42 DAIT but Coccinellids were unaffected with the treated plots recording rather higher numbers.

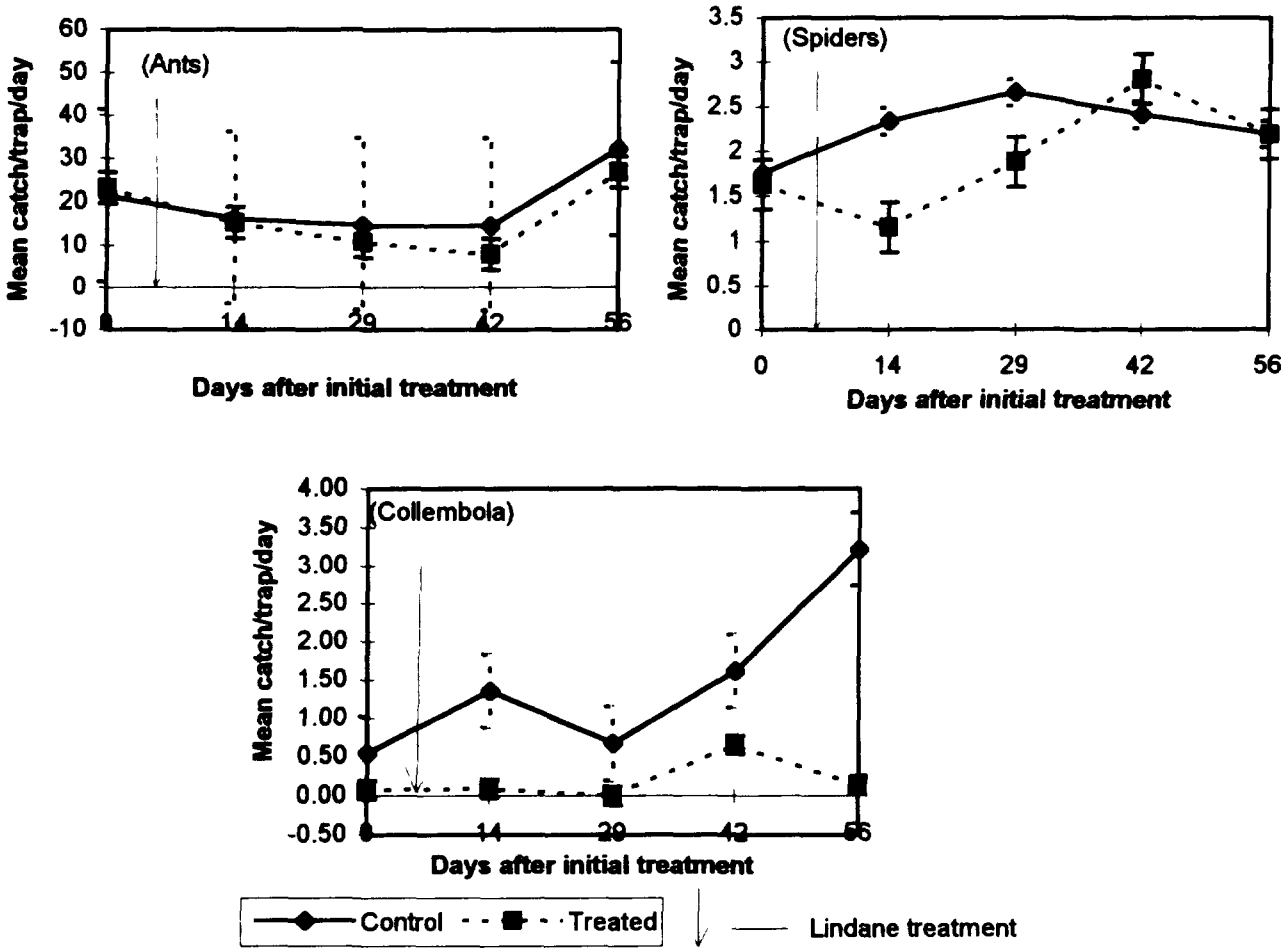

FIG. 1. Mean numbers of arthropods caught with pitfall traps from maize plots (1992)

3.6.3. Arthropods caught with pitfall traps from maize, 1993

Ants were adversely affected by lindane a day after the initial treatment ($P < 0.05$ but numbers had recovered by 56 DAIT (Fig. 3). Both lindane treatments caused slight but non-significant reduction in spiders ($P > 0.05$) but there was an upsurge 42 DAIT. The observation was similar to spiders caught with sweep net in 1992 (Fig. 2). Collembola were, however, significantly affected by lindane in almost all subsequent samplings except the 42 DAIT ($P<0.05$).

3.6.4. Arthropods caught with sweep net from maize, 1993

These included, ants, spiders and Coccinellids. While the effect of lindane on ants is not clear owing to the rather low numbers collected (Fig. 4), there was a non-significant reduction in spiders and Coccinellids caught ($P > 0.05$).

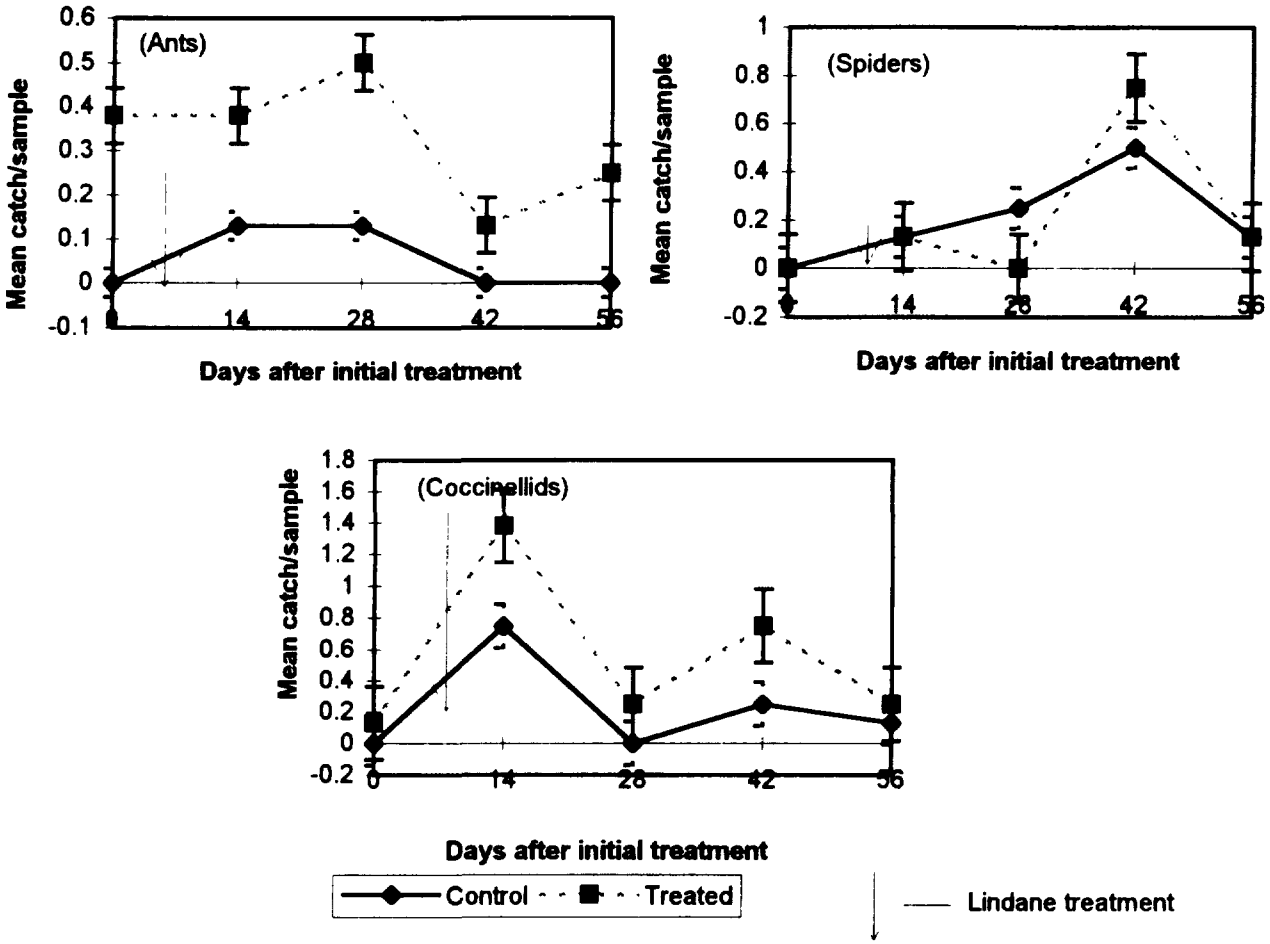

FIG. 2. Mean numbers of arthropods caught with sweep net from maize plots (1992)

3.6.5. Arthropods caught with pitfall traps from maize plots, 1994

Apart from ants, which were caught in fairly considerable numbers, other arthropods including spiders, Coccinellids and leafhoppers occurred in relatively fewer numbers. Ants were significantly higher ($P < 0.05$) in the treated plots during the pre-treatment sampling but were significantly reduced ($P < 0.05$) a day after the second treatment or 15 DAIT (Fig. 5)but recovery had occurred by 57 DAIT. A similar trend was observed in spiders where lindane seemed to have caused a statistically significant reduction 29 DAIT ($P < 0.05$). In contrast, lindane appears to have rather significantly enhanced the numbers of leafhopper caught from maize plots ($P < 0.05$).

3.6.6. Arthropods caught with D-VAC machine from maize plants, 1994

Ants, spiders and leafhoppers were caught in varying numbers with D-VAC from maize plants. The initial lindane treatment had a slight but non-significant effect on both ants and spiders ($P > 0.05$) and rather larger numbers of both were later caught from the treated plots 57 DAIT (Fig. 6). Just as in the pitfall trap catches, lindane seemed to have enhanced the numbers of leafhopper despite a slightly higher but non-significant initial catch from the control plots.

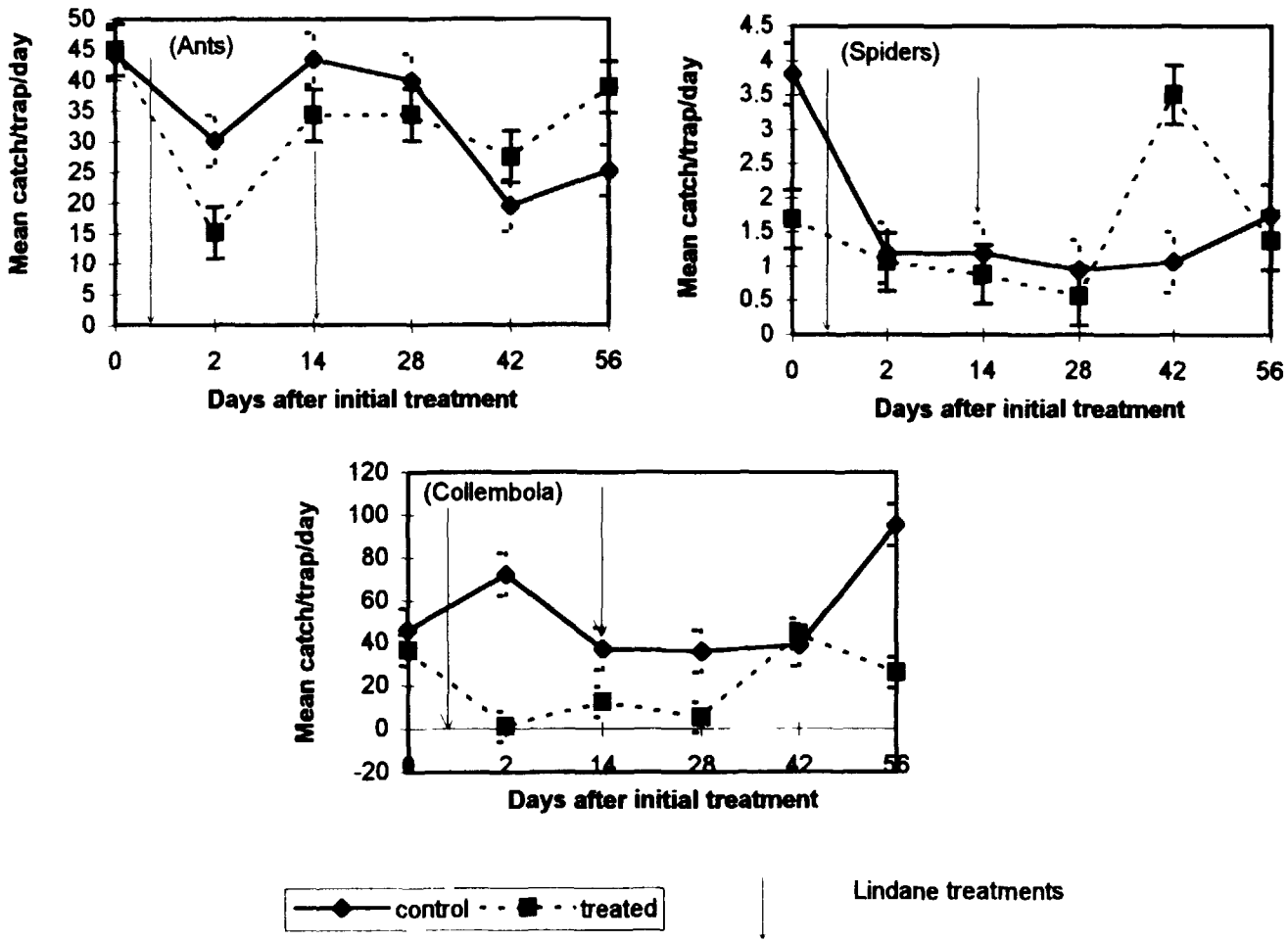

FIG. 3. Mean numbers of arthropods caught with pitfall traps from maize plots (1993)

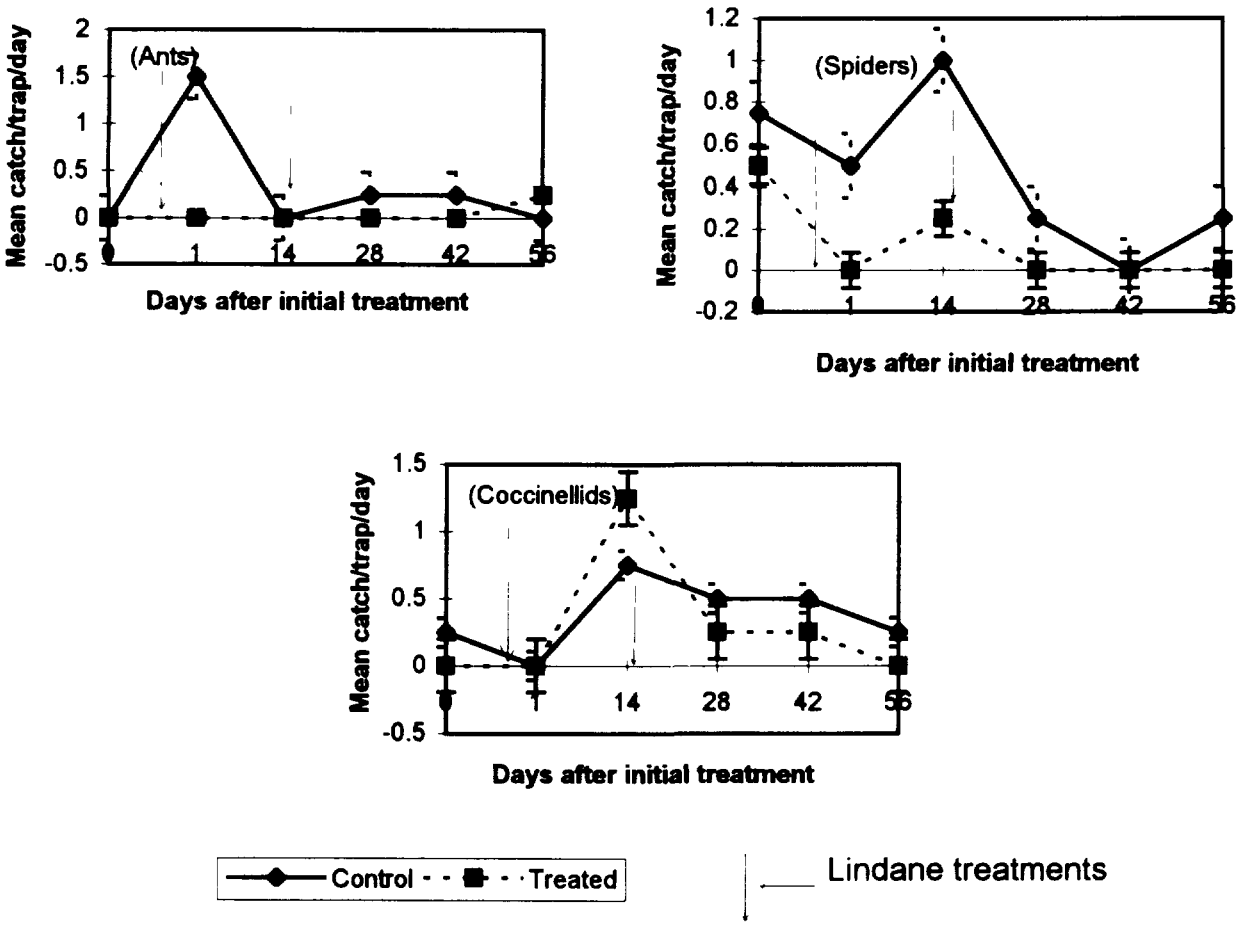

FIG. 4. Mean numbers of arthropods caught with sweep net from maize plots (1993)

3.6.7. Arthropods caught with D-VAC. machine from the ground in maize plots, 1994

Lindane did not appear to have exerted any adverse effect on ants and leafhoppers caught with D-VAC from the ground. Ants caught were rather significantly higher in the treated maize plots (Fig. 7). Spiders, on the other hand, became significantly depressed a day after the initial lindane treatment but recovered between 43 and 57 DAIT. The effect of lindane on leafhoppers was similar to that on ants and confirms the observations in the pitfall trap and D-VAC catches from maize plants (Figs 5 and 6).

3.7. Insect-damaged cowpea seeds 1992 - 1994

In 1992, 5.77% ± 0.58 of cowpea seeds harvested from control plots had been damaged by insects compared to 1.56% ± 0.28 damaged in the endosulfan-treated plots at harvest time ($P < 0.05$) Table V [A]. While in 1993, 30.33% ± 9.60 of seeds from control plots were damaged by insect as against 3.3% ± 1.14 from the plot which had had two successive cypermethrin treatments followed by endosulfan spray ($P < 0.05$). In 1994, the plots sprayed with cypermethrin and endosulfan had 0.3% ± 0.13 insect-damaged seeds compared to 0.71% ± 0.25 and 7.41% ± 1.74 respectively from plots treated with cypermethrin only and the control plots. There were no significant differences between the sprayed plots ($P > 0.05$) but both plots differed significantly from the control plots ($P < 0.05$).

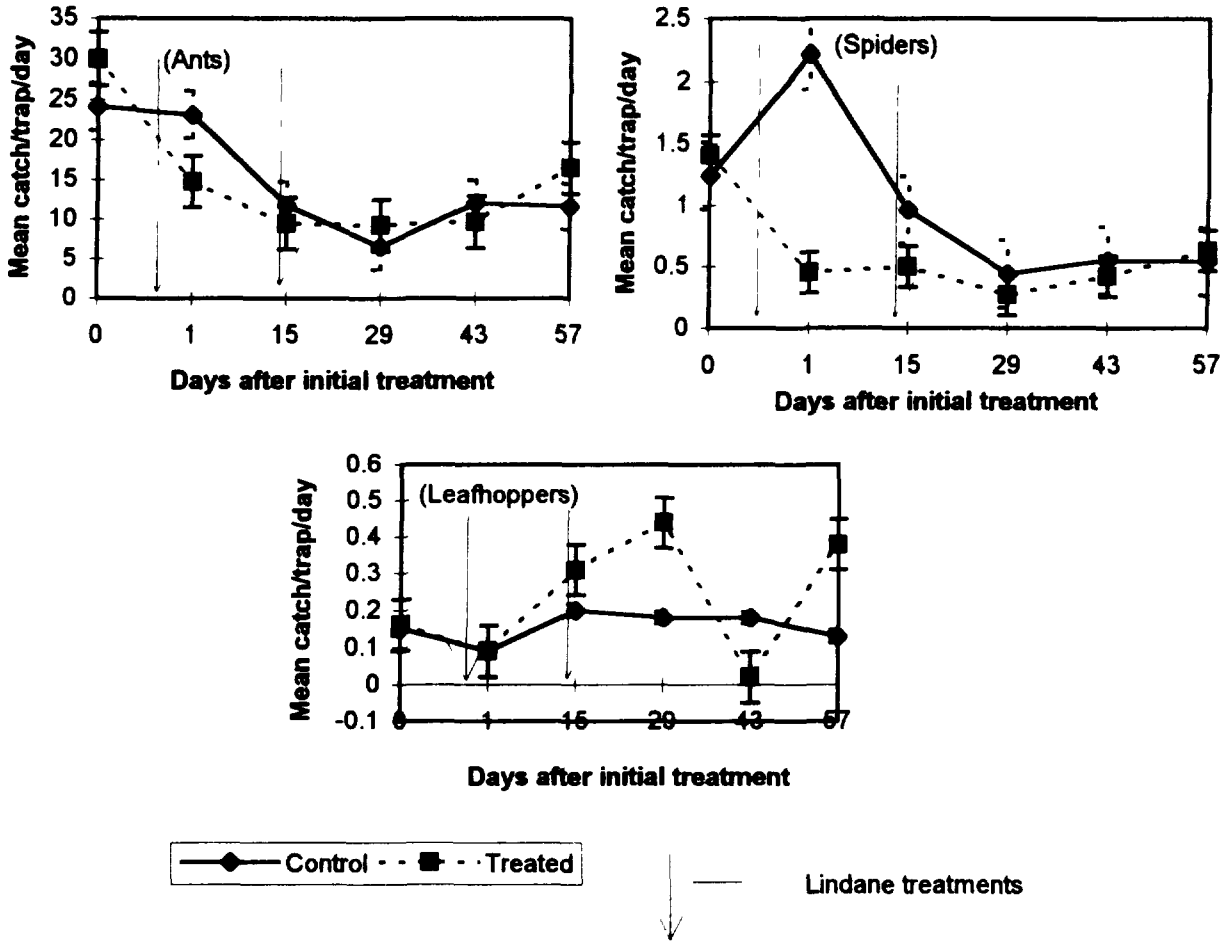

FIG. 5. Mean numbers of arthropods caught with pitfall traps from maize plots (1994)

Seed pests observed on the cowpea crop included cowpea pod borers such as *Maruca testulalis* (Geyer) (Lepidoptera:Pyralidae), *Cydia* (*Laspeyresia*) *ptychora* (Meyrick) (Lepidoptera:Tortricidae), pod bugs such as *Riptortus spp.* (Hemiptera:Alydidae) and the seed weevil, *Callosobruchus maculatus* (Fab). Thus the three endosulfan treatments in the first experiment and both the cypermethrin and cypermethrin followed by endosulfan appear to have provided significant protection to the cowpea seeds against these pests. It follows that endosulfan could also provide some degree of post-harvest protection in cowpea seeds since *Maruca* and *Cydia* spp, complete their life cycle during storage and *Callosobruchus* attack builds up after harvesting.

3.8. Cowpea yields 1992-1994

In 1992, the yield equivalent of 1250 ± 0.04 kg ha^{-1} recorded from the treated plots as against 1125 ± 0.02 kg ha^{-1} for the control plots. was not significantly different ($P > 0.05$) (Table V [B]. Reduced cowpea seed damage resulted in increased cowpea yields. It must, however, be stated that in the recommended spraying scheme for cowpeas, endosulfan is applied only once, after 2 consecutive sprays with a synthetic pyrethroid [6].

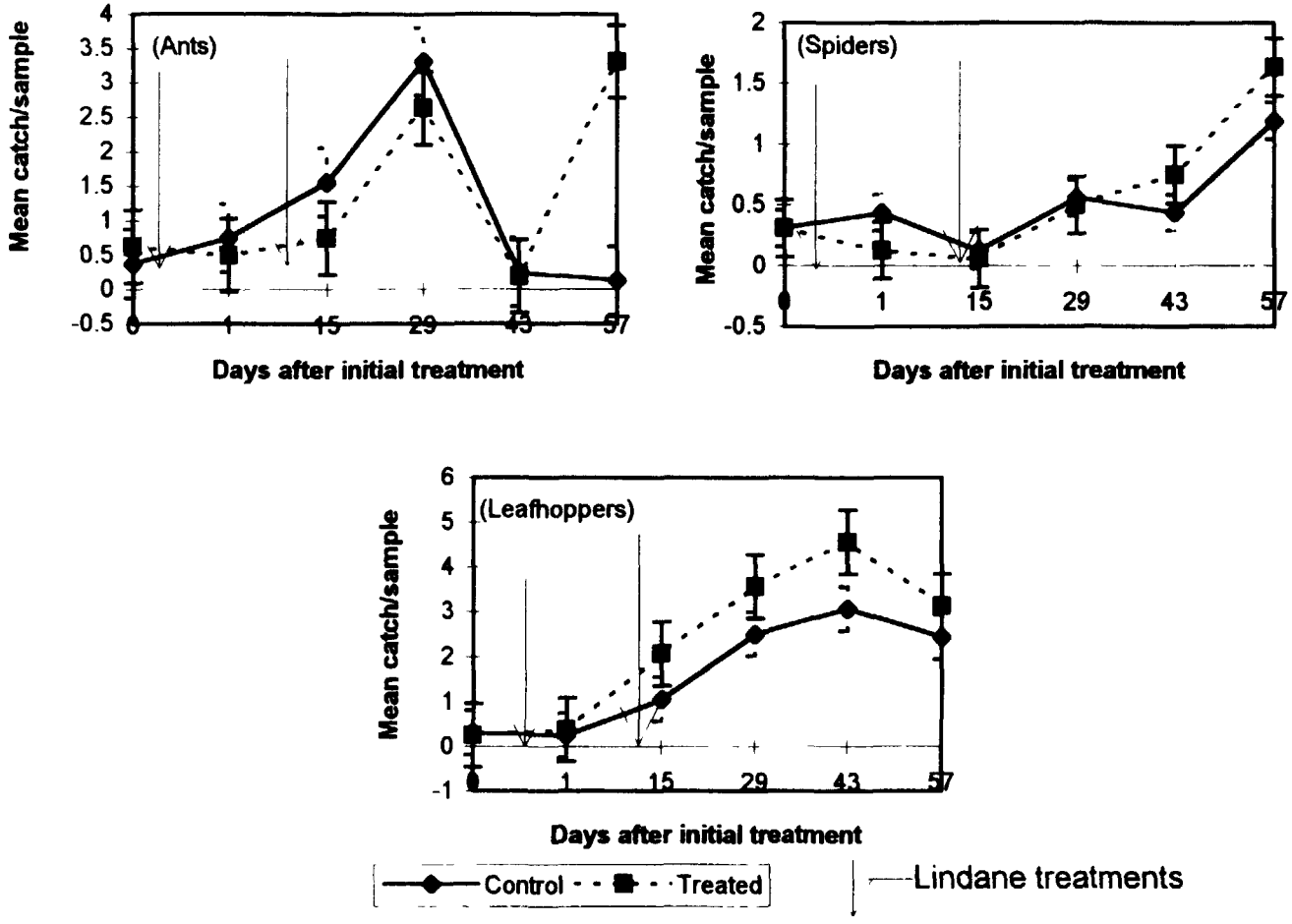

FIG. 6. Mean numbers of arthropods caught with D-VAC machine from maize plants (1994)

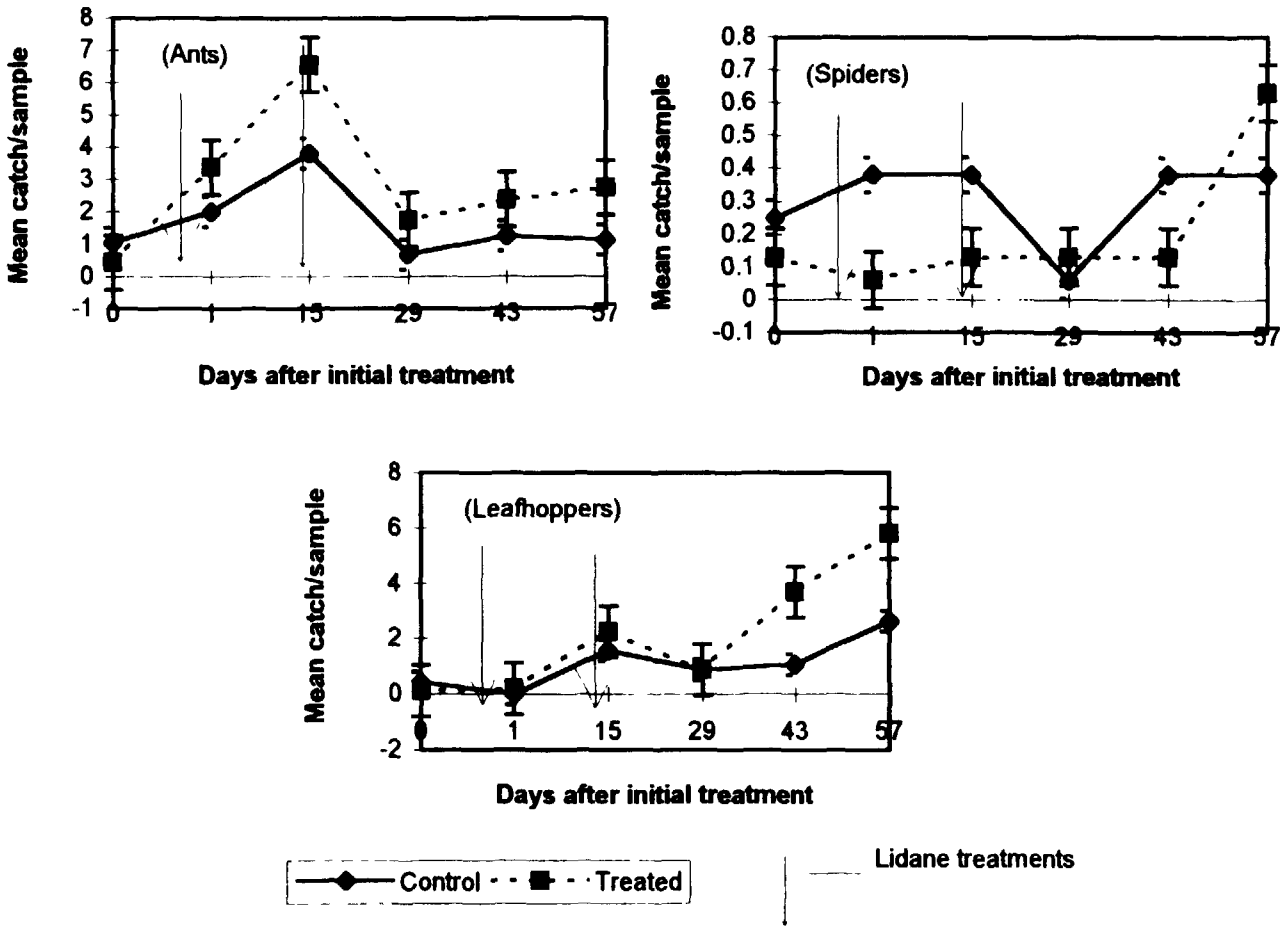

FIG. 7. Mean numbers of arthropods caught with D-VAC machine from the ground in maize plots (1994)

TABLE V. INSECT DAMAGE, YIELDS AND MOISTURE CONTENT OF COWPEA SEEDS

A. Percent insect damaged ± SE

Year	Control	Endosulfan		
1992	5.8 ± 0.6	1.6 ± 0.3[b]		
		Cypermethrin	Cyper. + endosulfan	
1993	30.3 ± 9.6[a]	_	3.3 ± 1.1[b]	
1994	7.4 ± 1.7[a]	0.7 ± 0.25[b]	0.3 ± 0.1[b]	

B. Yields (kg ha^{-1}) and moisture content ± SE

Year	Control		Endosulfan			
	Yield	Moisture	Yield	Moisture		
1992	1125 ± 0.02	16.6± 0.5	1250 ± 0.04	15.2 ± 0.6		
					Cypermethrin Yield	Cyp.+Endos Moisture
1993	500 ± 0.0[a]	15.9 ± 0.1	_	_	1450 ± 0.05[b]	16.1 ± 0.3
1994	850 ± 0.04[b]	17.3 ± 0.3	1550 ± 0.06[b]	16.7 ± 0.6	1400 ± 0.04[b]	17.0 ± 0.15

Means wtihin rows followed by different letters are statistically different ($p<0.05$)

In 1993, the mean yield equivalent of 1450 ±0.05 kg ha^{-1} recorded from treated plots was significantly higher than the 500 ± 0.0 kg ha^{-1} obtained from the control plots ($P < 0.05$). The 1993 cowpea yields from the treated plots was over 156% higher than the control plot yields and the results were statistically significant ($P < 0.05$).

In 1994, significantly higher yield equivalents of 1550 ± 0.06 and 1400 ± 0.04 kg ha^{-1} were obtained from the plots treated with cypermethrin and cypermethrin+endosulfan respectively compared to the yields of only 850 ± 0.04 kg ha^{-1} from the control plots ($P < 0.05$). Yields from the two sprayed plots were, however, not statistically significant from each other ($P > 0.05$). The highest amount of rainfall over the 3-year period was recorded in 1994 (Table IV) and this seemed to have been reflected in the corresponding higher yields from the treated cowpea plots.

Thus the highest yield from the control plots of 1125 ± 0.02 kg ha^{-1} over the three year period was recorded in the first year (1992), whilst the lowest, 500 ± 0.0 kg ha^{-1}, was obtained in 1993. The yield from the control plots for 1994 was 850 ± 0.04 kg ha^{-1}.

The three successive endosulfan treatments in 1992 and the cypermethrin alone or when followed by endosulfan contributed to the significant yield increases from the sprayed plots. The cypermethrin controls the cowpea flower thrips which are known to be capable of causing 60% to total crop losses unless controlled [6,10] and endosulfan controls pod pests and ensures good pod formation and seed development [6].

When DDT, an organochlorine pesticide was applied in very high rates of 10 kg ai ha^{-1} per season for 7 seasons in Nigeria, cowpea yields declined faster with time in treated plots compared to untreated plots [11]. The excessive rates used were, however, not recommended in practice but were applied to simulate an excessive misapplication by peasant farmers. There is thus no evidence of any progressive yield losses from the sprayed cowpea plots over the 2 successive years, 1992-1993, when cowpeas were grown on the same plots.

3.9. Arthropods caught from cowpea plots, 1992 - 1994

3.9.1. Arthropods caught with pitfall traps from cowpea plots, 1992

Pre-treatment samplings of ants and Collembola did not differ significantly but in all subsequent samplings both arthropods were significantly reduced by the three consecutive endosulfan treatments ($P < 0.05$) (Fig. 8). Spiders on the other hand did not appear to have been significantly affected ($P > 0.05$) even though the second treatment seemed to have had caused some slight reduction.

3.9.2. Arthropods caught with sweep net from cowpea plots, 1992

There were no statistical differences between ants, spiders and Coccinellids caught with sweep net from the control and treated cowpea plots ($P > 0.05$) but the first endosulfan treatment might have caused a slight reduction in Coccinellids (Fig. 9).

3.9.3. Arthropods caught with pitfall traps from cowpea plots, 1993

Ants, spiders and Collembola were all significantly reduced by the endosulfan treatment 20 DAET (Fig. 10) ($P < 0.05$).

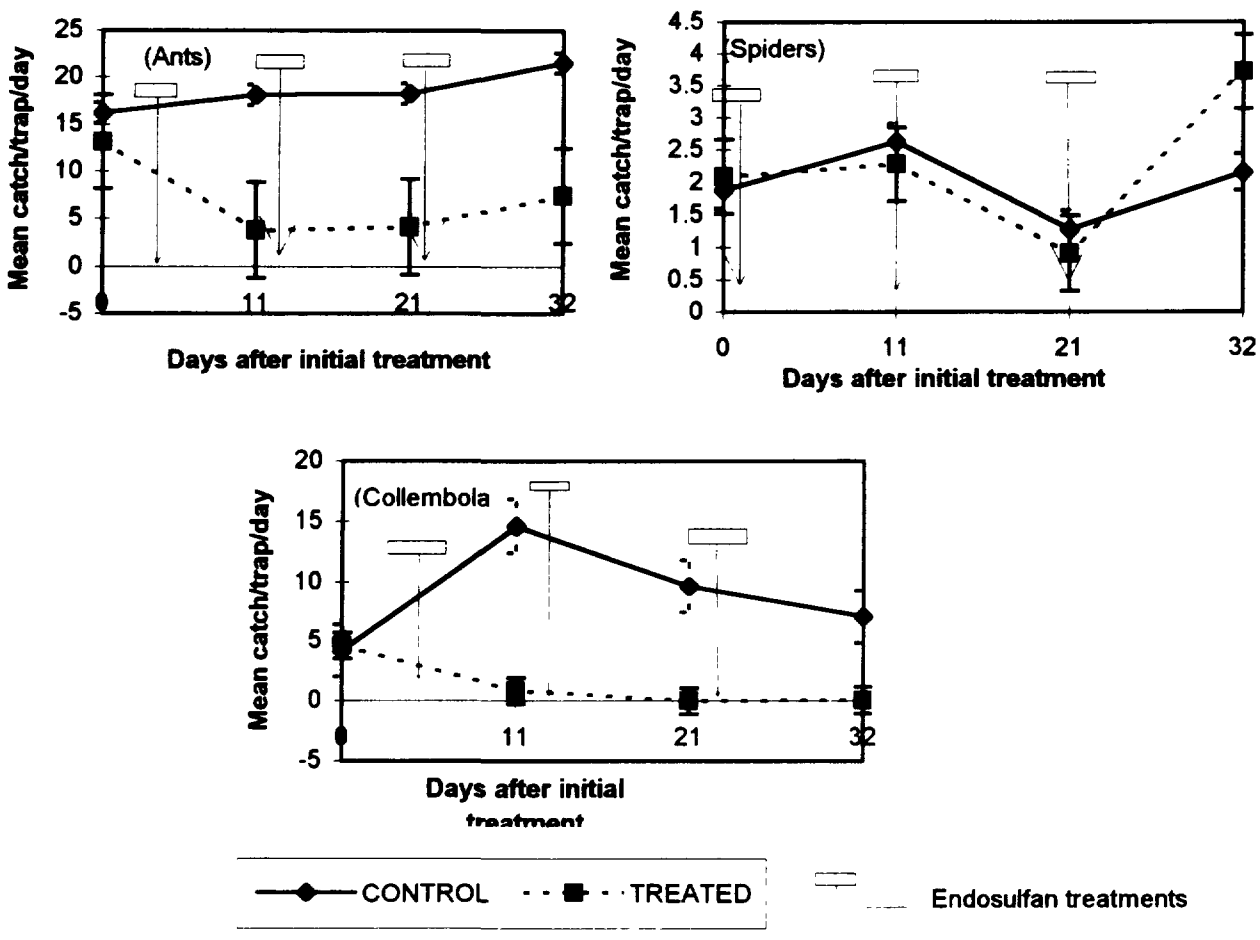

FIG. 8. Mean numbers of arthropods caught with pitfall traps from cowpea plots (1992)

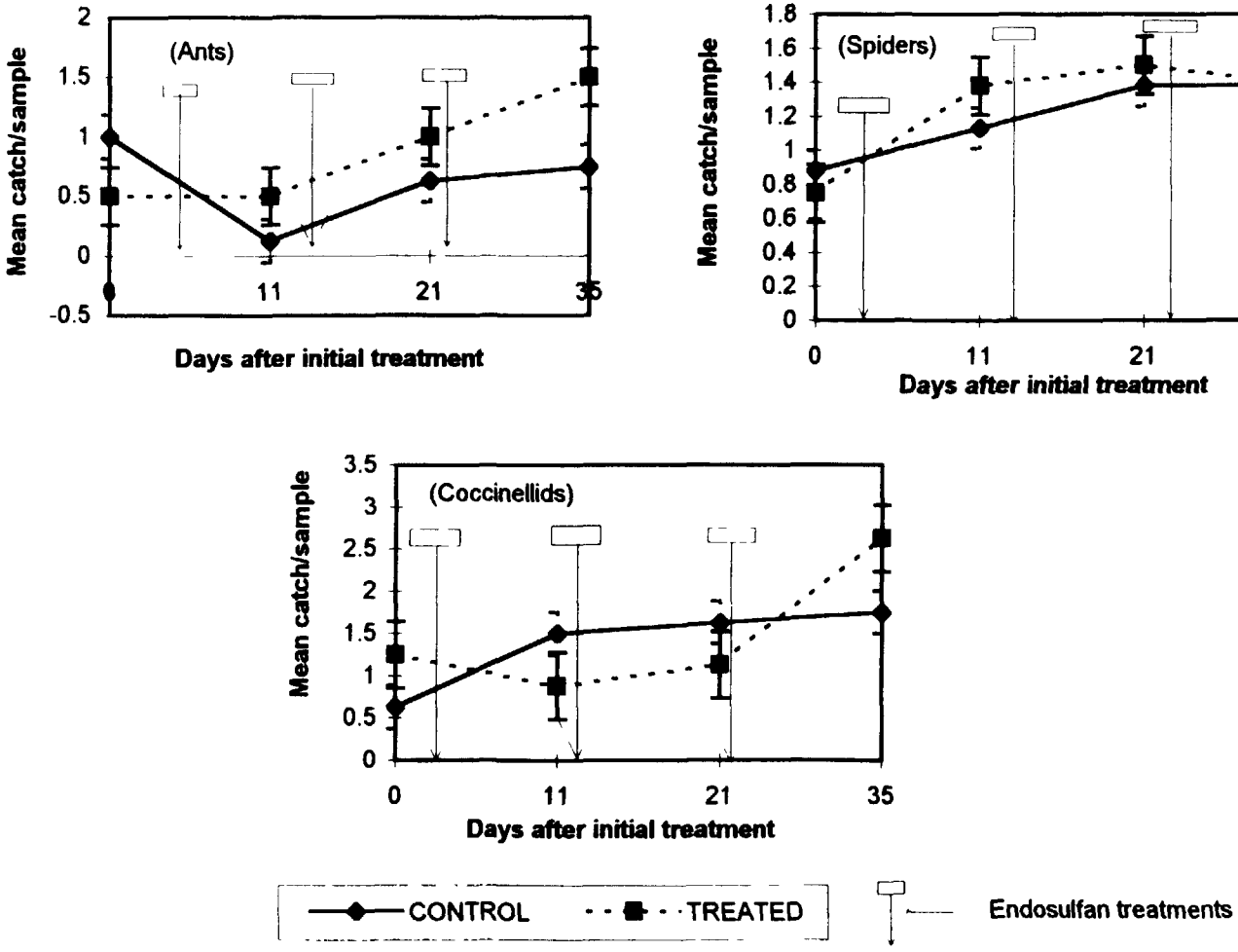

FIG. 9. Mean numbers of arthropods caught with sweep net from cowpea plots (1992)

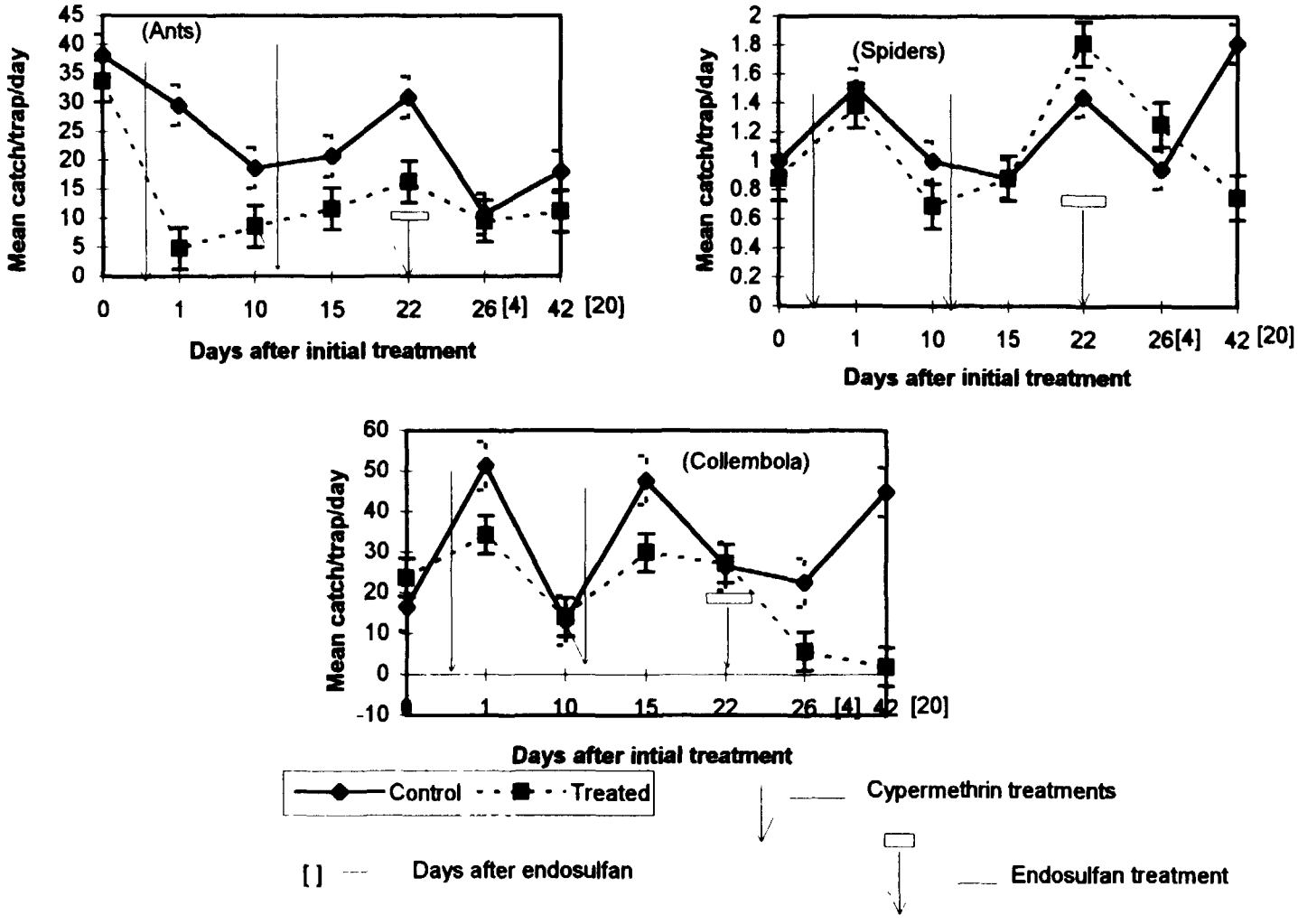

FIG. 10. Mean numbers of arthropods caught with pitfall traps from cowpea plots (1993)

TABLE VI. MEAN NUMBERS OF ARTHROPODS (± SE) CAUGHT WITH SWEEP NET FROM COWPEA PLOTS, 1993

DAIT	0		1		10		15		22		26[4]		42[20]	
	C	En	C	En	C	En	C	En	C	En	C	En	C	En
Ants	0.3 ± 0.5	0.5 ± 0.6	1.0 ± 1.2	0.3 ± 0.5	1.8 ± 3.5	0.0	0.5 ± 0.6	1.0 ± 1.4	1.0 ± 1.1	0.3 ± 0.5	0.3 ± 0.5	0.8 ± 0.9	1.8 ± 0.9	0.5 ± 0.6
Spiders	0.8 ± 0.9	0.0	0.5 ± 0.5	1.0 ± 0.8	0.5 ± 0.6	0.3 ± 0.5	1.0 ± 0.8	1.0 ± 1.4	0.8[a] ± 0.9	0.0[b]	0.8 ± 0.9	1.0 ± 1.1	0.3 ± 0.5	0.0
Coccinellids	0.0	0.0	0.3 ± 0.5	0.0	0.0	0.0	0.8 ± 1.5	0.3 ± 0.5	0.5 ± 1.0	0.0	0.3 ± 0.5	0.3 ± 0.5	0.0	0.0

DAIT; days after initial treatment; [ ] days after endosulfan treatment
Means within rows for the same day followed by different letters are statistically different ($p<0.05$)
C = Control
En = cypermethrin + endosulfan

3.9.4. Arthropods caught with sweep net from cowpea plots, 1993

The endosulfan treatment seemed to have caused reductions in ants, and spiders caught 20 DAET but the differences were not statistically significant ($P > 0.05$) (Table VI).

3.9.5. Arthropods caught with pitfall traps from cowpea plots, 1994

Arthropods collected with pitfall traps from cowpea plots included ants, spiders and Collembola. All were significantly reduced by the endosulfan treatment 14 DAET ($P < 0.05$) (Table VII).

3.9.6. Arthropods caught with D-VAC machine from cowpea plants, 1994

Endosulfan treatment significantly reduced ants caught from cowpea plants 1 DAET ($P > 0.05$) (Table VIII) but it appeared recovery commenced 14 DAET. Spiders were, however, not significantly reduced.

3.9.7. Athropods caught with D-VAC machine from the ground in cowpea plots, 1994

From Table IX, it appears endosulfan had caused a non-significant reduction in ant catches ($P > 0.05$). Prior to the application of endosulfan, comparatively higher numbers of ants had been collected from the plot. Spider catches 14 DAET were relatively lower in the endosulfan-treated plots than those from the other plots but there was no significant reduction ($P > 0.05$).

3.10. General discussion

3.10.1. Fluctuations in arthropod populations

In addition to arthropod natural enemies and other predators such as birds, lizards and frogs and microorganisms in regulating insect populations in ecosystems, the scarcity of resource and space can also cause population changes. Abiotic factors such as temperatures, light, rainfall, soil and other environmental conditions could also influence the extent of arthropod activities on day to day basis and hence the numbers trapped. These factors could partly account for the fluctuations of arthropod numbers recorded in both and unsprayed plots.

3.10.2. Apparent recoveries

Reports by other researchers [7, 8, 9] had shown that dissipation rates of the organochlorine pesticides were relatively faster under tropical conditions. The observed recoveries in some of the arthropods after the application of lindane and endosulfan could very likely be attributed to the degradation and or dissipation of the pesticides from the maize and cowpea agro-ecosystems. This is supported by the residue data reported by Yeboah et al. (this TecDoc).

TABLE VII. MEAN NUMBERS OF ARTHROPODS (± SE) CAUGHT WITH PITFALL TRAPS FROM COWPEA PLOTS (1994)

DAIT	0			11			21 [1]			35 [14]		
	C	Cy	En	C	Cy	En	C	Cy	En	C	Cy	En
Ants	15.5[a] ± 2.5	17.6[b] ± 3.7	9.4[b] ± 1.0	7.1 ±1.0	9.0 ± 1.2	6.3 ± 0.8	7.2 ± 0.7	8.6 ±1.2	6.5 ±0.6	10.1[a] ±1.2	18.7[b] ±2.8	6.7[c] ±0.7
Spiders	0.6 ± 0.2	0.6± 0.6	0.7 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.3	0.6 ± 0.1	1.0[a] ± 0.1	0.8[ab] ±0.1	0.6[b] ±0.1
Collembola	6.5[a] ±1.7	15.8[b] ± 2.2	7.9[a] ± 1.0	5.0 ± 0.5	6.0 ± 0.5	5.5 ± 0.5	8.0 ± 0.7	8.2 ± 0.7	8.3 ±0.6	7.7[a] ±0.9	10.8[a] ± 1.6	3.5[b] ±0.4

DAIT; days after initial treatment; [ ] days after endosulfan treatment
Means within rows for the same day followed by different letters are statistically different ($p<0.05$)
C = control
Cy = cypermethrin
En = cypermethrin + endosulfan

TABLE VIII. MEAN NUMBERS OF ARTHROPODS (± SE) CAUGHT WITH D-VAC MACHINE FROM COWPEA PLANTS (1994)

DAIT	0			1			11			21[1]			35[14]		
	C	Cy	En	C	Cy	En`	C	Cy	En	C	Cy	En	C`	Cy	En
Ants	4.3 ± 1.3	3.7 ± 1.1	4.8 ± 1.7	3.2 ± 0.4	2.1 ± 0.7	2.0 ± 0.6	1.1 ± 0.5	0.8 ± 0.2	0.6 ± 0.3	1.7[a] ±0.3	0.63[b] ± 0.4	0.06[b] ±0.1	0.75 ± 0.2	1.1 ± 0.5	0.75 ± 0.3
Spiders	0.7 ± 2.1	0.5 ±0.2	0.8 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.8 ± 0.4	0.9[a] ±0.5	0.1[b] ±0.1	0.3[ab] ±0.1	0.5 ± 0.3	0.7 ± 0.2	0.3 ± 0.1	1.1[ab] ± 0.4	1.8[a] ± 0.1	0.6[b] ± 0.2

DAIT; days after initial treatment; [ ] days after endosulfan treatment
Means within rows for the same day followed by different letters are statistically different ($p<0.05$)
C = control
Cy = cypermethrin
En = cypermethrin + endosulfan

TABLE IX. MEAN NUMBERS OF ARTHROPODS (± SE) CAUGHT WITH D-VAC MACHINE FROM THE GROUND IN COWPEA PLOTS (1994)

DAIT	0			1			11			21[1]			35 [14]		
	C	Cy	En	C	Cy	En	C	Cy	En	C	Cy	En	C	Cy	En
Ants	1.4[a]± 0.4	2.1[b] ± 0.4	2.3[b] ± 0.6	1.4 ± 0.4	0.7 ± 0.2	0.8 ± 0.2	0.7[a] ±0.2	1.6[b] ± 0.3	1.3[b] ± 0.3	1.1 ± 0.4	1.6 ±0.5	2.6 ± 0.6	0.7 ±0.2	1.3 ± 0.3	0.6 ± 0.2
Spiders	0.5 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.0	0.2 ± 0.1	0.1 ± 0.1	0.6 ± 0.3	0.9 ± 0.4	0.3 ± 0.1

DAIT; days after initial treatment; [ ] days after endosulfan treatment
Means within rows for the same day followed by different letters are statistically different ($p<0.05$)
C = control
Cy = cypermethrin
En = cypermethrin + endosulfan

4. CONCLUSIONS

Collembola seemed to be the most susceptible insect to both lindane and endosulfan ($P < 0.05$). Ants and spiders were also significantly reduced by these organochlorine pesticides but most often recovered from the adverse effects between 29 and 57 days in maize plots and after 21 days in cowpea plots. Lindane treatments did not consistently increase maize yields or reduce stemborer attacks and three consecutive endosulfan treatments did not significantly increase cowpea yields ($P > 0.05$). Endosulfan application preceded by two successive cypermethrin treatments significantly increased cowpea yields and reduced seed damage ($P < 0.05$). Neither lindane nor endosulfan seemed to have significantly affected soil microbial activity ($P > 0.05$) based on the rates of decomposition of organic matter buried in maize and cowpea plots and retrieved between 84 and 259 days later.

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FATE AND DISTRIBUTION OF LINDANE AND ENDOSULFAN IN MAIZE AND COWPEA ECOSYSTEMS RESPECTIVELY

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Abstract

A quantitative study is presented on lindane and endosulfan residues in maize and cowpea ecosystems respectively Both pesticides were found to dissipate very fast under the tropical Ghanaian conditions The high rate of dissipation in leaves is attributed to the fact that the leaves were exposed to sunshine and wind leading to increased volatilisation. Endosulfan was found to dissipate faster from the cowpea ecosystem than lindane did in the maize ecosystem. The mean residue levels of lindane in maize grains were 0 02 $\mu g\ g^{-1}$, whilst residue levels of endosulfan in cowpea seeds were 0 05 $\mu g\ g^{-1}$ These levels are lower than the maximum residue limits recognised as acceptable by the Codex Alimentarus Commission

1. INTRODUCTION

As a result of increasing world population, there has been a proportionate increase in the demand for food. Currently, 58 percent of the world's population is undernourished [1]. There is therefore the need to increase food production. This can be achieved through the application of such products of modern technology, as high yielding crop varieties, fertilisers, irrigation and pesticides. Pesticides help boost food production by reducing pest populations to tolerable levels. In 1986, it was found that crop loss due to pests was 40%. Cotton yields have also been found to increase three fold through effective use of chemical pesticides for pest control in the Sudan [2].

Despite the benefits derived from pesticides, some of them tend to persist in the environment, and may thus pose a hazard to living things. Organochlorine pesticides constitute one group of such pesticides that are persistent. Examples of these include DDT, lindane and endosulfan. These compounds have also been found to (i) be highly fat soluble and hence accumulate in fatty tissues of animals, (ii) accumulate in food chains, and (iii) degrade into other toxic residues which can have long term adverse effects on the environment [3,4].

In Ghana, the surviving organochlorines in use are lindane, endosulfan, aldrin and dieldrin. Lindane, formulated under the trade name Gammalin 20, has been restricted to be used only in the cocoa growing industry. However, due to mixed cropping , other crops end up by being sprayed with lindane during its application. These crops include maize, cocoyam and cassava. Under such conditions, lindane can serve to control insects that bore into stems of the maize plant.

Notwithstanding the hazards posed by organochlorine pesticides to the environment and being banned or restricted in temperate regions, developing countries including Ghana, continue to use them to boost food production. They are also used in public health to control disease vectors. Their continued use has been attributed to their high efficiency, low cost, and easy availability. Furthermore, owing to the intense heat and high humidity in the tropics, organochlorine pesticides dissipate at faster rates than in temperate regions. Consequently, it is suggested that, there should be less danger of possible accumulation of their residues in soils, plants and animal matter, that could lead to the precipitation of environmental hazards.

Whereas considerable data on pesticide residues are available in the developed countries to assist in decision making regarding the continued use of particular pesticides, knowledge on pesticide residues and their effects are rarely available in many developing countries. Hence the need to generate data on the effects of these pesticides in various agroecosystems in the tropics to allow correct decisions on their use. These should include extensive knowledge about their fate, persistence, distribution, effect on yield, and the way they affect target and non target organisms to enable an assessment of their potential for human and environmental hazards.

Maize is a staple Ghanaian food which is taken in many forms. It may be boiled, roasted, or ground into dough. Since lindane is applied to maize, a knowledge of lindane residues in maize will therefore be of immense importance to Ghanaians. Cow peas are also taken in Ghanaian homes in the boiled form. They are also ground and fried. In northern Ghana, the leaves are used to prepare soup [5].

This report therefore covers studies to identify and quantify lindane and endosulfan residues in maize and cowpea ecosystem respectively, under Ghanaian conditions.

2. EXPERIMENTAL

2.1. Apparatus

A Hewlett packard gas chromatograph series II, model 5890, equipped with a ^{63}Ni electron-capture detector and a SPB 5 capillary column (30 m × 0.5 mm id) was used for the study. The carrier gas , nitrogen, had a flow rate of 15 mL min^{-1}. The operating temperatures were: injector, 200°C; detector, 300 °C; and column, 200° C isothermal. The attenuation was set on the HP 3396A recording integrator at 8, while chart speed was 10 mm min^{-1}. Each injection had a volume of 1 µl.

Solid Phase Extraction (SPE) Bond Elute C-18 columns (500 mg in 2.8 mL) were purchased from Analytical Instruments Varian GmbH, Vosendorf, Austria.

2.2. Reagents and materials

All solvents were Nanograde , and purchased from Mallinckrodt Specialty Chemical Co. Paris, Kentucky,USA. All other reagents were Analar grade. Commercial lindane formulated as an emulsifiable concentrate under the trade name Gammalin 20 was obtained from ICI Ghana Ltd, whilst endosulfan formulated under the trade name Thiodan 35 was obtained from Hoechst Aktiengesselshaft Frankfurt, Germany. Cypermethrin was obtained from Shell Chemicals, Abidjan, Cote d' Ivoire.

The maize planted was a local variety called "Abelehi" which matures in 107 days; and the cowpea a variety called "Asutem". Lindane and endosulfan standards were obtained from the International Atomic Energy Agency (IAEA) in Vienna.

2.3. Experimental farm

The experiment was conducted on a 1.6 ha. plot situated at the Ghana Atomic Energy Commission, Kwabenya, which is about 20 km from the centre of Accra. The plot had been treated with 1 kg AI of lindane the previous year. Four plots each measuring 50 m × 50 m were marked out. Two diagonally opposite plots were sown with maize whilst the others were sown with cowpeas.

2.4. Pesticide treatment

The maize plot that had been earmarked for pesticide treatment was sprayed twice with a water emulsion of the commercial lindane at a rate of 0.5 kg ha^{-1} in accordance with local practices. The split application was done to avoid the resurgence of insect pests that may survive the first application.

Pesticide treatment of the cowpea plot was also in accordance with the recommendations of the Plant Protection and Regulatory Services of Ghana's Ministry of Food and Agriculture. Two earlier treatments were carried out with the synthetic pyrethroid cypermethrin at a dose of 50 g AI ha^{-1}. per each treatment. This application was to control insect pests that destroy cowpea flowers. These were followed by the application of endosulfan (Thiodan 35) at a dose of 750 g AI ha^{-1}. The endosulfan application was to control the pod borers that destroy cowpea seeds. The weeding, planting , spraying and harvesting schedules are as shown in Table 1.

Table 1. Schedule of activities

Activity	Crop	Date	
		Control	Treated
planting	maize	2/6	3/6
	cowpea	17-18/6	19-20/6
spraying, lindane	maize	-	17/6
	maize	-	1/7
spraying, cypermethrin	cowpea	-	21/7
	cowpea	-	31/7
spraying, endosulfan	cowpea	-	12/8
weeding, manual	maize	21/6	23/6
	cowpea	4-6/6	2-3/6
fertiliser application	maize	22/6	25/6
harvesting	cowpea	9/9	9/9
	maize	24/9	24/9

2.5. Soil characteristics

2.5.1. Sampling

The method followed the FAO/IAEA protocol [Appendix I]. Eight cores of soils were sampled at random from each sub-plot using a soil auger of 25 mm diameter and a depth of 150 mm. These were combined, ground and passed through a 2 mm sieve by mechanical shaking. Duplicate sub-samples were then taken for the measurement of pH, moisture content, organic carbon and particle size.

2.5.2. Determination of pH

The method followed that prescribed by Davies[6]. A sample (20 g) of the air dried sample was washed into of water (100 mL). The suspension was stirred with a mechanical stirrer for 5 minutes and allowed to stand for 30 minutes. The pH was determined using a meter which had been calibrated with pH4 and 7 standard buffers.

2.5.3. Determination of soil moisture

The soil sample were weighed into moisture cans and dried in an oven at 105°C to constant weight. The moisture content was determined as a percentage loss in weight.

2.5.4. Determination of organic carbon

The method followed that prescribed in Appendix I. A sample of soil (0.5 g) passed through a 0.5 mm mesh sieve was added to 1M potassium dichromate solution (10 mL). Concentrated sulphuric acid (20 mL) was added to the suspension, thoroughly swirled and allowed to stand on asbestos for 30 minutes. Distilled water (100 mL) was added, followed by orthophosphoric acid (10 mL) and finally barium diphenylamine sulphonate indicator (2 mL). The resulting solution was then titrated with 0.2M ferrous ammonium sulphate solution until the solution changed from blue to green. A blank titration was carried out to standardise the potassium dichromate.

2.5.5. Particle size analysis

Particle size analysis was determined by standard hydrometer method following dispersion with sodium hexametaphosphate, as prescribed by Charmetski [7] and Dickson [8].

2.6. Residue analysis

2.6.1. Sampling

2.6.1.1. Soils

Triplicate samples (50 g) were taken for residue analysis. For lindane, sampling was made at the following times (crop growth stages): pre-treatment (4th leaf stage), day of first treatment, 1 day (ankle stage) and 14 days after first treatment (knee high stage). Samples were also taken on the day of second treatment, followed by 14, 30 and 60 days (post tusseling stage) after the second treatment. For the cowpea plots (including the endosulfan experiment), sampling was done on the pre treatment day (flowering stage), 1, 7 (pod formation stage), 14 (pod drying stage), and 22 (harvesting stage) days post-treatment.

2.6.1.2. Leaves

For the maize ecosystem, the youngest and oldest leaves were taken off randomly chosen plants from each of the sub-plots as follows: pre-treatment day of first treatment 1, 14 days after first treatment. Samples were also taken on the day of second treatment, followed by 14 and 30 days after second treatment. With cowpeas, leaves were randomly sampled from the various sub-plots as follows: 1,7 14 and 22 days post-treatment.

2.6.2. Extraction and clean up

2.6.2.1. Soils

The triplicate samples were accurately weighed and transferred into a single thickness cellulose extraction thimble, (43 mm i.d.× 123 mm long) and extracted in a Soxhlet through 10 cycles i.e. for ~5 h with methanol (250 mL). An aliquot (5 mL) of the methanol extract was evaporated to dryness over nitrogen. The residue was taken up in methanol (1 mL) and to this was added water (2.5 mL). Prior to this, a C-18 Bond Elut solid phase extraction column had been reconditioned by flushing through two volumes of methanol followed by two volumes of distilled water. The diluted extract was poured through the reconditioned column at a flow rate of 2 mL min^{-1} and then washed with methanol + water (3+7 by volume, 1 mL) followed by distilled water (1 mL). The washed column was vacuum dried for 15 min and then the pesticide was eluted from the column with hexane (2 mL) into a glass vial. The eluent was made up to 2 mL with more hexane and then analysed by gas chromatography.

2.6.2.2. Leaves and seeds

Leaf tissue (20 g) and/or seeds were homogenised separately for 2 minutes in a Waring blender and mixed thoroughly with methanol (200 mL). The suspension was the filtered and a volume (20 mL) of the methanol extract was dried over nitrogen and the residue was dissolved in methanol (1 mL) and diluted with water (2.5 mL). The aqueous methanol extract was passed through a preconditioned SPE column at a flow rate of 2 mL min^{-1}. The column was then washed with distilled water (1 mL). The washed column was vacuum dried for 15 min and then eluted with hexane (1.5 mL) into glass vials. The eluent was made up to 2 mL with hexane and analysed by gas chromatography.

2.6.3. Recovery experiments

2.6.3.1. Soils

Samples of pesticide-free soils (50 g) were air dried and sieved as before. Volumes (1 mL) of lindane and endosulfan standards (10 μgmL^{-1}) were added to the soils. The soil was then extracted using a Soxhlet for 5 hours (10 cycles) with methanol. The extracts were then cleaned up for GLC measurements as in Section 2.6.1.

2.6.3.2. Seed and leaves

Samples (10 g) of maize grains, cowpea seeds, maize leaves and cowpea leaves were homogenised using Waring blender. A volume (1 mL) of standard solutions (10 $\mu g\ mL^{-1}$) was added to the homogenised samples (maize leaves and grains, lindane; cowpea seeds and leaves, endosulfan). Methanol (100 mL) was added to each mixture and shaken mechanically for thorough mixing. The methanol extract was filtered and a volume (20 mL) of the extract cleaned up for GLC analysis as in Section 2.6.2.

3. RESULTS AND DISCUSSION

3.1. Recoveries and soil charateristics

Though the plot had been exposed to pesticides only a year before the investigation, initial analysis before application of any pesticide showed the absence of pesticide residues within the limit of detection of the gas chromatograph (0.01 $\mu g\ g^{-1}$) . All blank samples were found to be below the detection limit. Spiked soil samples gave recoveries of 80.5 and 92.7% for lindane and endosulfan residues, respectively. Spiked leave samples gave recoveries of 95.2 and 80.5% for lindane and endosulfan residues, respectively.

The persistence of pesticides in soils is known to depend on soil characteristics. The major ones include organic matter, moisture, texture and acidity. The soil analysis showed the soils to be slightly acidic with an average pH of 6.3 and had a sandy clay loam texture (Tables 2 and 3). The average moisture content of the soils from the four subplots during the sampling period ranges from 4.78-13.3 $g\ kg^{-1}$ and 3.40-7.74 $g\ kg^{-1}$ in the maize and cowpea soils respectively.

3.2. The maize ecosystem

The dissipation of lindane in maize soils was very fast (Table 5) with 46% of the initial residue detected on the day of treatment lost one day after the first application (6th leaf

Table 2 Soil characteristics of the maize plots.

Property [a)]	Plot	
	Control (SD)	Treated (SD)
pH	6.21 (0.17)	6.3 (0.15)
% sand	64.2 (5.4)	63.8 (7.3)
% silt	13.4 (2.8)	11.1 (3.9)
% clay	24.5 (4.7)	25.0 (3.8)
% organic matter	2.4 (0.8)	1.9 (0.8)
% organic carbon	1.4 (0.5)	1.1 (0.5)

a) texture class : sandy clay loam

Table 3. Soil characteristics of the cowpea plots

Property [a)]	Plot	
	Control (SD)	Treated (SD)
pH	6.4 (0.3)	6.3 (0.2)
% sand	57.0 (7.6)	63.0 (3.2)
% silt	12.2 (2.4)	11.0 (1.6)
% clay	29.0 (5.0)	26.2 (4.0)
% organic matter	2.0 (0.4)	2.2 (0.3)
% organic carbon	1.8 (0.7)	1.3 (0.2)

a) texture class : sandy clay loam

stage), and less than 5% remained after 14 days. This may be attributable to volatilisation under the hot windy and rainy conditions of the area (Table 4). Increase in temperature is known to increase pesticide loss from soils through volatilisation, chemical degradation and bacterial decomposition, all of which are temperature dependent [9].

Rainfall might also have contributed to the pesticide loss by causing surface run-off. Furthermore, water competes with pesticides for adsorption to the soil surface [10] and lindane will therefore be more easily volatilised from surfaces of soil that have high moisture content. The moisture content of 5.8 to 13.8 observed during the period of investigation were fairly high and could have been a contributing factor to the fast dissipation rate.

About 5% of the residue detected on the day of first treatment remained in the soils 14 days (knee high stage) after application (Table 5). This is less than the 13% of the residue detected in soils 14 days after the second application (above knee high stage). This relatively slower rate of lindane dissipation after the second application might have been due to the fact that the maize plants, which had matured at the time of the second spraying provided cover over the top soil and lowered temperatures and minimised loss through volatilisation. Only 2.8% of the initial residue of lindane remained 30 days after the second application.

Thirty percent of the initial lindane residues detected in the maize leaves on the day of first treatment remained one day after application and fell to 3% 14 days after the first application (Table 5). Similarly, 14 days after the second application of lindane, the residues had fallen to 1.7% of the initial level.

Table 4. **Monthly total rainfall and mean temperatures**

	May	June	July	August	September
total rain, mm	49	81	41	17	75
mean temp. ^{0}C	28.5	26.9	25.5	25.3	26.4

Table 5 Lindane residues in soil samples and plant samples of the maize ecosystem

Sampling time	Mean lindane residue, $\mu g\ g^{-1}$ (SD)	
	Soil	Plant leaves
pretreatment	nd	nd
day of treatment	0.22 (0.04)	0.22 (0.014)
1 day after treatment	0.12 (0.05)	0.067 (0.01)
14 days after treatment	0.011 (0.008)	0.006 (0.002)
day of second treatment	0.025 (0.013)	0.24 (0.02)
14 days after second treatment	0.032 (0.006)	0.004 (0.001)
30 days after second treatment	0.008 (0.003)	nd
60 days after second treatment	nd	-

nd = none detected

Mean lindane residues levels in the maize grains were 0.02 μg g^{-1} (Table 7), which is well below the acceptable maximum residue limit for grains of 1 μg g^{-1} [10].

Table 6. Endosulfan residues in soil samples and leaf samples of the cowpea ecosystem

Sampling time	Mean endosulfan residue, μg g-1 (SD)	
	Soil	Plant leaves
pretreatment	nd	nd
day of treatment	0.29 (0.17)	0.33 (0.022)
7 days after treatment	0.022 (0.011)	0.011 (0.006)
14 days after treatment	0.008 (0.005)	0.002 (0.001)
22 days after treatment	nd	nd

nd = none detected

Table 7. Lindane and endosulfan residues in maize and cowpea grains

Crop	Mean residues, μg g^{-1} (SD)
maize	0.020 (0.002)
cowpea	0.045 (0.002)

3.3 The cowpea ecosystem

The amount of endosulfan in cowpea soils at 7days after spraying (i.e. pod formation stage) was found to be only 7.6% of the residue detected on the day of treatment, similar to that found with lindane in the maize soils (Table 6). The low persistence of endosulfan as explained in the section for the maize ecosystem can also be attributed to the low organic matter content of the soil (ranging from 1.91 to 2.18%), the fairly high temperature, the sandy clay loam texture of the soil as well as the fairly heavy rainfall (Tables 3, 4). The mean residue level of endosulfan in cowpea seeds was 0.045μg g^{-1} (Table 7). This is higher than the residue levels of lindane in maize grains and could be due to the fact that endosulfan was applied close to the time of seed formation, making it possible for endosulfan to be quickly incorporated into the seeds before it could dissipate.

CONCLUSION

Lindane and endosulfan have been shown to dissipate quickly under Ghanaian climatic conditions which are characterised by heavy rainfall and fairly high temperatures. The residue levels of lindane in maize and endosulfan in cowpea were well below the acceptable maximum residue levels.

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RESIDUES OF LINDANE AND ENDOSULFAN IN WATER AND FISH SAMPLES FROM RIVERS, FARMS IN BESEASE, AGOGO AND AKOMADAN IN THE ASHANTI REGION OF GHANA

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Abstract

Pesticide residue analyses were performed on water and fish samples from River Oda in Besease, River Aframso in Nobewam near Kumasi, River Atwetwe in Akomadan, and River Kowire at Agogo. Residues of lindane and endosulfan were found in water and fish (*Oreochromis niloticus, Tilapia zillii, Barbus trispulis, Heterobranchus sp., Tilapia busumana, Ophiocephalus obscura* and *Chana obscura*) samples. The residues of lindane varied between the years and months in the year but were in the range of 0.3 - 15 ng L^{-1} (1993-94) and 8.7-32.0 ng L^{-1} (1995) for the water samples and 0.2 - 24 ng g^{-1} (1993-93) and 8.4-120.4 ng g^{-1} (1995) for the fish samples. Residues of endosulfan in the water and the fish samples were zero in 1993-1994 but, in 1995, were in the range of 6.4-35.2 ng L^{-1} for the water samples and 5.0-267.5 ng g^{-1} for the fish samples. In all cases the lindane and endosulfan concentrations in the water were 10,000-20,000 times lower than known toxic concentration levels and therefore unlikely to cause fish toxicity problems.

1. INTRODUCTION

Organochlorine pesticides have found wider application in agriculture for the control of pests and in public health for vector control [1]. Furthermore, there has been an increase in pesticide use in Ghana over the past ten years as a result of the efforts by the goverment to boost food production.

In agriculture, organochlorine pesticides are widely used by farmers due to their cost effectiveness and their broad spectrum of activity, with a neglect of their possible impact on the environment and man. Research in the temperate regions and some parts of Africa have found the pesticides to be very persistent in the environment and remain in the soil [2] for longer periods of time resulting in serious damage to soil flora and fauna [3].

Detailed information on the effects of organochlorine pesticide residues and the amounts present in water, food, soil, flora and fauna in Africa is however, very limited. In some African countries, organochlorine pesticides have been banned based on data obtained from the developed countries in the temperate regions, without any assessment of the effect of these pesticides in the tropical environment [4].

Lindane (Gammalin 20) is widely used in Ghana in the cocoa plantations for the control of capsids on vegetables and for the control of stemborers in maize [5]. Endosulfan is used in cotton-growing areas, as well as on vegetable farms and for the control of pests in coffee plantations.

Most of the vegetable farms in the commercial vegetable producing areas in Ghana like Akomadan and Agogo are situated along rivers which act as the source water supply for farming and for drinking purposes. Pollution of rivers is known to occur through run-off of pesticides from farms during heavy rain as well as through aerial spraying of pesticides and the improper disposal and handling of the chemicals during and after spraying.

Endosulfan and lindane are known to be toxic to fish. For example, lindane has an LC50 (48h) of 0.16-0.3 mg L^{-1} for guppies [6] and an LC_{50} (96h) values of 1.2 mg L^{-1} and 0.75 mg L^{-1} for *Fundus heteroclitus* and *Oreochromis niloticus* respectively[7, 8].

This report is based on our work on the effects of organochlorine pesticide residues in water and fish, in the forest zone of Ghana, as part of the coordinated research work of the Joint IAEA/FAO research programme on the 'Adverse effects on flora and fauna from the use of organochorine pesticides on the African continent'.

2. MATERIALS AND METHODS

2.1. Experimental materials

Pesticide grade hexane was obtained from Mallinckrodt Special Chemicals, Kentucky, USA. HPLC grade methanol and acetonitrile were obtained from Aldrich Chemical Company, USA. Bonded phase C-18 SPE columns were obtained from Varian, Sunnyvale, USA.

2.2. Experimental site

This general study covered about 500,000 hectares of land in the closed forest region of Ghana which is about 34.5% of the entire land area of Ghana. Three regions of intense farming activities were selected for the residue analysis work after the general survey work had established the nature of the agricultural activities undertaken in the areas under consideration and the extent of pesticide usage.

The farming community at Akomadan is involved in large-scale vegetable farming in addition to maize farming with tomato farming constituting about 80% of the farming activity in the area. Besease and Nobewam are used mainly for rice production and are the location of the Anum Valley Irrigation Project. Close to the rivers are over 200 rice fields covering more than 400 hectares of land. Agogo is noted for its vegetable farming, rice and maize production and has other surrounding villages involved in cocoa farming.

2.3. Sampling

The pH values of the river waters were in the range 7.0-8.2 and the average temperature was 28°C. Water samples were collected at a depth of 250mm from River Oda at Besease, River Aframso at Nobewam near Kumasi, River Kowire at Agogo and River Aframso and River Atwetwe at Akomadan in the Ashanti Region in one litre sterilized bottles closed with a stopper. The duplicate samples were collected between January 1993 and July 1994 and from June 1995 to October 1995. The rivers pass through regions which have intense cocoa production and other farming activities.The initial stage of work, during 1993-94, involved random sampling in the various rivers. In 1995, a much more systematic sampling was undertaken from June to October and involved the farming communities at

Agogo and Besease. No samples were taken from Nobewam because neither lindane nor endosulfan were being used in the farming in this area. The river Oda at Besease flows through several areas of cocoa farming where lindane is actively used. Endosulfan is used in the farming at Agogo while other nearby cocoa farms use lindane.

After collection the water samples were taken to the laboratory for analysis. Fish samples for pesticide analyses were collected from River Oda near the rice fields and River Aframso in Agogo where there is large scale vegetable production. The fish samples (seven species) were obtained by a hook and line method and also with cages and nets. The fish samples obtained were immediately washed with distilled water and stored in a freezer.

The samples were later taken to the Ghana Atomic Energy Commission laboratory and the Institute of Aquatic Biology in Accra, Ghana for pesticide residue analysis.

2.4. Sampling of other species

Although several varieties of birds, rodents (rats, squirrels, grasscutter) and reptiles (lizards) were identified, the project focused on residues in fish. The rodent (grasscutter) samples, which were collected from the area, are yet to be analysed.

2.5. Laboratory analytical procedures

2.5.1. Water residue analyses

Volumes (2 x 10 mL) of methanol followed by volumes (2 x 10 mL) of distilled water were passed through an SPE C-18 column via a reservoir mounted on top of the column before conditioning. A small pressure was applied to the top of the reservoir to help force each volume of liquid to flow through the column at a rate of at least 2 mL per minute.

A volume (800 mL) of each water sample was passed through a pre-conditioned column at a flow rate of 2 ml per min. Each column was then washed with water + methanol (1 mL, 3.3 + 1 by volume) followed by distilled water (1mL) and dried under vacuum for 20 minutes. The sample which was trapped in the column was eluted with hexane (5 x 0.5 mL) to recover the pesticide residues.

2.5.2. Fish residue analyses

A sample (10 g) of each fish was homogenized using a pestle and mortar. The sample was mixed with sodium sulphate (10 g) and then transferred to a separatory funnel (250 mL). Hexane (20 mL) was added followed by acetonitrile (100 mL) which had been saturated with hexane, and the mixture was shaken for about one minute. The acetonitrile was drained into a 1 litre separatory funnel. The mixture was further extracted with portions of acetonitrile (3 x 50 mL) and the extracts combined with those in the separatory funnel. These were washed with water (500 mL) followed by saturated sodium chloride solution (40 mL) and hexane (50 mL). The mixture was allowed to stand and the aqueous layer was drained into another 1 litre separatory funnel and extracted with a further volume (50 mL) of hexane. The hexane extracts were combined and passed through a plug of sodium sulphate (10 g) into a flask. The solvents were evaporated almost to dryness and the residue dissolved in methanol (10 mL) and diluted with water (25 mL). A volume (3.5 mL) of this solution was taken for pesticide residue analysis (equivalent to 1 g of the fish sample) and passed through a

previously pre-conditioned column at a flow rate of 2 ml per minute. The column was washed, dried and eluted with hexane as described for the water samples above. Volumes (1 μL) of this hexane eluent were taken for analysis by the gas chromatographic analysis method detailed in Section 2.5.3.

2.5.3. Gas chromatographic analysis

Gas chromatograph : Hewlett-Packard Series II model 5890; column : 30 m x 0.5 mm i.d.; stationary phase : SPB-5: carrier gas: nitrogen; flow rate : 30 ml min^{-1}; temperatures: column, 200°C, injector, 250°C, detector, 300°C; detector, ^{63}Ni electron capture. The sample injection volume was 1 μL. The limit of detection in this work was 1pg mL^{-1}. Recovery of lindane and endosulfan from the fish and water samples was 90%. Lindane and endosulfan in the residues were identified only by comparison of the retention times with those of their analytical standards and confirmation by an alternative method was not attempted.

3. RESULTS AND DISCUSSION

Seven species of fish caught in the various rivers were subjected to residue analysis. The species of fish caught depended on the time of the year when the trapping was done. It appeared that due to unknown adverse effects of some material, the fish populations had decreased considerably in these rivers. There were whole days when our team could not trap any fish in some of the rivers using a combination of a hook and line method, cages and nets.

Our initial survey work indicated that farms near River Oda at Besease were sprayed up to six times during the year. Farms near River Atwetwe and River Kowire which were along tomato-producing areas were subjected to pesticide applications of up to six times over either one or two seasons. Although lindane is not used widely in the spraying of tomatoes and rice, endosulfan is one of the commonly used insecticides in these regions. Despite this, the levels of endosulfan in the water samples were below the limits of detection (1 pg mL^{-1}). The concentrations of both lindane and endosulfan, when present, were found to be significantly higher in the fish than in the water (cf Tables 1, 2 with 3, 4) though the pesticide levels were well below their lethal levels, as discussed later.

Residues whose retention times corresponded with DDT and other organochlorines were observed in the chromatogram but were not quantified since the focus of this project was on lindane and endosulfan.

Table 1. Pesticide residues in river water samples during 1993-94

Sample		Residue, ng L^{-1}	
river identity	date	lindane	endosulfan
Oda	15.01.93	14.2	0
Oda	21.12.93	15.0	0
Atwetwe	15.01.94	1.3	0
Aframso	12.03.94	3.9	0
Kowire	07.07.94	3.0	0
Aframso	22.07.94	0.3	0

Table 2. **Pesticide residues in fish caught during 1993-94**

Source of fish		Species	Residue, ng g^{-1}	
river identity	date		lindane	endosulfan
Oda	15.01.93	*Heterobranchus sp*	14.2	0
Oda	21.12.93	*Heterobranchus sp*	24.3	0
Atwetwe	15.01.94	*Barbus trispulis*	23.2	0
		Chana obscura	0.8	0
Aframso	12.03.94	*Tilapia sp*	0.2	0
		Barbus tripulis	0.2	0
Kowire	07.07.94	*Ophiocephalus obscura*	1.5	0

Table 3. Pesticide residues in river water samples during 1995

Sample		Residue, ng L^{-1}	
river	month	lindane	endosulfan
Oda	June	25.0	35.2
	July	24.0	30.0
	August	22.0	15.0
	September	32.0	14.0
	October	8.7	6.4
Kowire	September	10.0	15.4
	October	14.8	25.6
Atwetwe	September	12.5	28.2
	October	20.2	14.6

Table 4. Pesticide residues in fish samples caught during 1995

Source of fish		Species	Residue, ng g-1	
river	month		lindane	endosulfan
Oda	June	*Oreochromis niloticus*	20.0	80.2
		Barbus trispulis	120.4	158.0
		Heterobranchus sp	14.0	160.0
	July	*Oreochromis niloticus*	22.5	267.5
		Tilapia zilli	34.0	142.5
		Heterobranchus sp	35 2	64.0
	August	*Oreochromis niloticus*	36.4	20.2
		Barbus trispulis	16.8	11.4
		Heterobranchus sp	24.8	6.5
	September	*Oreochromis niloticus*	100.0	186.0
		Tilapia zilli	72.0	77 0
		Heterobranchus sp	80.0	66.0
Kowire	October	*Oreochromis niloticus*	38.2	5.0
		Barbus trispulis	22.0	10.0
	September	*Oreochromis niloticus*	8 4	12.4
	October	*Oreochromis niloticus*	13.4	22.8

In none of the samples of river water collected during 1993 and1994 was endosulfan found while lindane was found at low concentrations up to 15 ng mL^{-1} (Table1). During these two years no endosulfan residues were found in the fish samples but lindane was found at concentrations up to 24.3 ng g^{-1}. This represents over 1000 fold concentration of lindane by the fish and is in general accord with, though exceeding, the known bioconcentration factor (153) for lindane and fish in water. Although the toxic levels of lindane to these particular species of fish are not known, the fact that they were surviving is not too surprising since the actual concentrations of lindane found in the water were around 10,000 to 20,000 times below the toxic concentrations to another species of fish (guppy, LC50 (48 h), 160,000 - 30,000 ng L^{-1}) [6].

In the more complete studies of 1995, both lindane and endosulfan were found in the river water samples. Concentrations varied with the location and the month of sampling but were in the ranges 8.7-25.0 ng L^{-1} and 6.4-35.2 ng L^{-1} for lindane and endosulfan respectively. As in the previous two years, lindane residues were found in the fish samples, ranging from 8.4-120.4 ng g^{-1}. These levels were generally higher than those of the previous two years but still orders of magnitude below likely toxicity levels.

In contrast to previous years, endosulfan was found in the fish samples and often at concentrations higher than lindane. In fact this is in general accord with its known bioconcentration factor for fish (583) which is approximately four times higher than that for lindane. In the case of endosulfan the aqueous concentration toxicity levels against local species of fish are known (LC50 (96 h), 1,200,000 ng L^{-1}, *Fundus heteroclitus*; 750,000 ng L^{-1}, *Oreochromis niloticus*). It can be seen that the concentrations in the river water samples are over 20,000 times below these toxicity levels and therefore present no toxic hazard to the fish.

So, in conclusion, although both lindane and endosulfan were found in river waters and it could be seen that the fish in these rivers absorbed and concentrated these insecticides, the levels that were actually found were so far below known toxicity levels that it is unlikely that they were the cause of the perceived low populations of fish in the rivers and that must be attributed to another cause.

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STUDIES ON THE DISTRIBUTION, SALE, HANDLING AND USAGE OF AGROCHEMICALS IN ASHANTI, BRONG AHAFO AND EASTERN REGIONS OF GHANA

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Abstract

Studies were carried on the distribution, sale, handling and usage of agrochemicals in 19 major towns located in three regions noted for their intensive agricultural activities and covering 500,000 hectares of land area in Ghana.The most prevalent of the agrochemicals were insecticides (52%) followed by herbicides (28%) and fungicides (16%). Of these the organophosphates constituted 24% and the organochlorines 6% in terms of the total numbers of pesticides sold, though lindane and endosulfan were among the insecticides most frequently used by farmers. The fungicide, copper oxychloride as "Kocide", and the insecticide, λ cyhalothrin as "Karate", with 39% and 22% respectively of the sales by volume, were found to be the most widely used agrochemicals. Most of the merchants of agrochemicals and the farmers had only primary school education and had very little knowledge on the handling and safety aspects of the use of agrochemicals. The Extension Service to the farmers was inadequate and the farmers depended largely on the agrochemicals merchants for information on pesticide usage.

1. INTRODUCTION

This study was undertaken to provide additional information on the application, handling and use of pesticides in Ghana. With the launching of the Economic Recovery Programme there has been considerable emphasis on the development of agriculture in Ghana and pesticides usage has been increasing during the past ten years. Owing to costs associated with these chemicals and the lack of adequate information on the usage and application of pesticides, deleterious effects on the environment have been largely ignored. Furthermore, major law enforcement agencies like the Environmental Protection Council, Plant Protection and Quarantine Division and the Custom, Excise and Preventive Service have very little information at their disposal for effective legislation on the use of pesticides and the management of pesticides in general. Most of the data available are based on studies in developed countries. The objective of the study is to increase our knowledge of the distribution, usage and handling of pesticides in Ghana as part of the coordinated research programme on the adverse effect on the use of organochlorines (lindane and endosulfan) on the African Continent.

The study covered about 500,000 hectares of land area in the Ashanti, Brong Ahafo and Eastern Regions of Ghana located within the closed forest vegetation zone of Ghana; this constitutes about 34.5% of the total land area of Ghana. Sixteen major towns, noted for their intensive agricultural activities, within these three regions were selected for the study.

These centres were Agogo, Juaso, Konongo, Ejisu, Kumasi and Mampong in the Ashanti Region; Akomadan, Ejura, Kintampo, Sunyani, Techiman and Wenchi in the Brong Ahafo Region; and Nkawkaw, Anyinam, Koforidua, Nsawam, Suhum, Asamankase and Oda in the eastern Region.

The major crops cultivated in these areas include cocoa, palm fruits, rice, maize, cowpea and vegetables such as tomatoes, okro, eggplant and pepper.

2. MATERIALS AND METHODS

Questionnaires specifically designed for the study were used to collect data on the distribution, sale and usage of agrochemicals from the selected towns within the three Regions. Seventy-eight agrochemical sellers and one hundred and ninety-five farmers were interviewed. Besides the interviews, visits were made to selected farms to obtain additional information on the application of the agrochemicals.

3. RESULTS

3.1. Types and distribution of agrochemicals

The agrochemicals on sale and used by the farmers in the three regions were made up of insecticides (56%), herbicides (29%) and fungicides (16%) (Table 1). Insecticides always constituted the highest percentage of agrochemicals used in each of the three regions.

On the basis of their chemical composition the available agrochemicals fall into the following groups of compounds: organochlorines, organophosphates, carbamates, synthetic pyrethroids, inorganic compounds (mainly fungicides) and a miscellaneous category with a variety of chemical structures (Table 2). The organophosphates and organochlorines make up 30% of the total number of agrochemicals while the newer pyrethroids form a surprisingly large proportion (15%) of the market taking over from the older organochlorines and, to some extent, the organophosphates. DDT was one of the agrochemicals also found to be in use, though it is supposed to be banned in Ghana.

In terms of the volume of purchases the most commonly bought agrochemical was found to be Kocide, a copper oxychloride fungicide, and one of the least commonly bought was Sumithion, the organophosphate insecticide, fenitrothion (Table 3).

3.2. Socio-economic and other characteristics of merchants of agrochemicals (retailers and local distributors)

3.2.1. Educational background

The highest proportion of the merchants were middle school leavers with the remainder having secondary or other type of final education (Table 4). None of the merchants had a tertiary education.

3.2.2. Distribution of merchants by sex and age

Males (76%) were, by far, the greater proportion of the 78 merchants in the survey. The majority of them were between the ages of 20 and 40 years.

Table 1. Types of Agrochemicals with respect to their use, by farmers from three regions in Ghana.

Insecticides	Herbicides	Fungicides
Actellic (pirimiphos methyl)	Gesaprim (atrazine)	Baygon (propoxur)
Cymbush (cypermethrin)	Bellator	Caocobre
Decis(deltamethrin)	Garlon (triclopyr)	Champion
Diazinon	Gramoxone(paraquat)	Cobox(copper oxychloride)
Dimethoate 40EC	Ronstar (oxadiazon)	Dithane (mancozeb)
Dursban (chlorpyrifos)	Roundup(glyphosate)	Kocide (copper hydroxide)
Endosulfan	Samppi No3	Gastoxin
Fenitrothion	Stam F34T(propanil)	
Fenom C	Basta 20 SL (glufosinate)	
Primigram	Paracol	
Grofol	Saturnmate (thiobencarb)	
Karate (l - cyhalothrin)	Bladex (cyanazine)	
Nogos (dichlorvos)	Garex	
Ofunack (pyridaphenthion)		
Perfekthion (dimethoate)		
Phostoxin (aluminium phosphide)		
Ripcord (cypermethrin)		
Secto		
Sumithion (fenitrothion)		
Thiodan (endosulfan)		
Trebon (etofenprox)		
Gammalin 20 (lindane)		
Callifan 50CE		
Furadan (carbofuran)		
Unden (propoxur)		
Totals 25	13	7

Common names of the active ingredient of commercial products are given in parenthesis where known.

3.2.3. Sales experience and training of merchants

Most of the merchants had been in the agrochemical business for less than 5 years (Table 6). Less than 3% had been associated with the sale of agrochemicals for more than 20 years. Most (95%) claimed to have had some basic training in handling of such chemicals. Almost all of them acquired their knowledge on the job through seminars and workshops organised by the Ministry of Agriculture, Extension Services, Plant Quarantine and Protection Services and Agrochemical Companies.

Table 2. Types of agrochemicals on the basis of their chemical composition

Organo-chlorine	Organo-phosphate	Carbamate	Synthetic Pyrethroid	Inorganic Compound	Miscellaneous
Thiodan 35EC (endosulfan) Gammalin 20 (lindane)	Actellic (pirimiphos-methyl) diazinon dimethoate 40EC Dursban (chlorpyrifos) Sumithion (fenitrothion) Nogos (dichlorvos) Ofunack (pyridathion) Perfecthion (dimethoate)	Baygon (propoxur) Furadan (carbofuran) Saturnmate (thiobencarb?) Sebon (?)	Cymbush (cypermethrin) Decis (deltamethrin) Karate (λ-cyhalothrin) Ripcord (cypermethrin) Fenom C (?)	Champion (?) Caocobre (?) Cobox (copper oxychloride) Kocide (copper hydroxide) Dithsne (mancozeb)	Gesaprim (atrazine) Garlon (triclopyr) Ronstar (oxadiazon) Secto (?) Stam F34T (propanil) Callifan 50CE (?) Gramoxone (paraquat) Roundup (glyphosate) Basta 20SL glufosinate
2 (6%)	8 (24%)	4(12%)	5 (15%)	5 (15%)	9 (27%)

Table 3. Commonly used agrochemicals by volume of purchase

Chemical	Percentage
Kocide	39.0
Karate	22.0
Perfekthion	11.0
Actellic	11.0
Karate	11.0
Sumithion	6.0

Table 4. Educational background of agro-chemical sellers in the three regions.

Level of education	Number	Percentage
Middle School Leaving Certificate	41	52.6
Secondary School	30	38.4
Others	7	9
University	-	-

Table 5. Length of work experience in retail selling

Duration	Number	Percentage
0 - 5 years	36	46.2
6 - 10 years	31	39.8
11 - 20 years	9	11.5
> 20 years	2	2.5

Table 6. Source of Supply of agrochemicals to retailers and farmers in Ashanti, Brong Ahafo and Eastern Regions of Ghana

Source of agrochemical	Percentage of the total market
Chemico (GH) Limited	23.1
Ministry of Agriculture	18.0
Reiss and Co.	6.4
Imperial Chemical Company	12.8
Wienco	5.1
Retailers	23.1

3.2.4. Handling of agrochemicals by merchants

(a) Record keeping

Less than 30% of the merchants kept records of their business including financial records, expiry dates of chemicals and stock controls.

(b) Storage and display conditions

Chemicals were mostly stored in the shops in their containers and some packed in original boxes. A few of the sellers had storage rooms. Sellers in kiosks and those operating on table tops displayed some of their items in the open sun.

(c) Other activities undertaken by merchants in shops

Apart from the sale of agrochemicals over 90% of the merchants stocked other items including seeds, fertilizers, etc., in their shops. In a few instances, food items such as bread, vegetables, gari and rice were also sold in agrochemical shops.

(d) Accidents

There were some reported cases of accidents which occurred as a result of the improper repackaging of agrochemicals into smaller packages for sale to farmers. Reports of occasional headaches and other health problems were considered by the sellers to be related to the chemicals.

3.2.5. *Major sources of supply of agrochemicals to retailers and farmers*

The supply of agrochemicals to farmers was from commercial sales outlets except for a few cases in the cocoa industry where some farmers obtained chemicals directly from the Ministry of Agriculture. Most of the supply to farmers was from retailers in open market places throughout the regions.

3.2.6. *Educational background of farmers*

Nearly half (47%) of the farmers had Middle School Leaving Certificate though a reasonable proportion (17%) had no formal education at all (Table 7) and most (92%) were male.

Table 7. Educational background of farmers using agrochemicals.

Level of education	Number	Percentage
Middle School leaving Certificate	92	47.2
G.C.E. O'Level	39	20.0
Others - Post Sec, Agric. Colleges	26	13.3
University	4	2.1
None	34	17.4

3.2.7. *Tenure and size of farm*

Over one third (38%) of farmers owned their own lands with 18% based on the extended family system. The largest proportion (42%) of the land being farmed was rented but only a very small fraction (0.5%) was under the cooperative system. In general, the size of most farms for individuals was between 0.8 and 2.5 hectares. However there were a few cases where farmers owned about 15-20 hectares of land.

3.2.8. *Experience of use, application and storage of pesticides by farmers*

Most (66%) of the farmers had used pesticides actively over the past 6 years, though only about 7% had used pesticides for a period greater than 15 years (Table 9).

Most of the individual farms did not receive any Extension Service except those involved in cocoa farming. Some of the rented farms and cooperatives benefitted from those extension services provided by the Ministry of Agriculture with farms in the cocoa producing areas benefitting most from them.

Table 8. Type of farm tenure

Type of tenure	Number	Percentage of total
Owner	75	39
Family	36	19
Cooperative	1	0.5
Rented	83	43

Table 9 Duration of use of pesticides on farms

Years of Use of Pesticide on farm	Number	Percentage
0 - 5 years	128	65.6
6 - 10 years	47	24.1
11 - 15 years	6	3.1
> 15 years	14	7.2

Half (51)% of the farmers did not have any training in the use of agrochemicals although the remainder have had at least some information on the use of particular pesticides on their farm. Such information was from friends, Agricultural Extension Officers and agrochemical merchants.

Nearly all (98%) of the farmers used knapsack sprayers while a proportion (12%) of these used Mistblowers in addition to the knapsack sprayer. Less than 5% used other applicators and sprinklers.

Agrochemicals were mostly stored in the house before use though excess agrochemicals were stored under a variety of conditions. A third (31%) of the farmers used the agrochemicals immediately on the farm. A larger proportion (54%) stored them on the farm or buried the containers in the ground on the farm for future use, while a significant fraction (12%) poured them away on the farm.

A very small proportion (<5%) of the farmers used adequate protective clothing. The majority of the farmers used a variety of protective measures which appeared inadequate, though a quarter (24%) used nose protectors or cloths to cover their nose. Only a few (18%) either washed their hands after using the chemicals or used wellington boots or gloves (15%). A significant fraction (22%) indicated they did not use any safety measures at all.

There were reports of fainting (12%) after the spraying exercise and all the farmers complained of at least one ailment (fever, headache, stomach pains, chest pains) which occurred very frequently.

4. DISCUSSION

Pesticides have been in use in the Ashanti, Brong Ahafo and Eastern regions of Ghana for a relatively long time, and at least 3% of the agrochemical shops have been in operation for over 20 years whilst some farmers have used pesticides on their farms for more than 15 years.

Insecticides form the most prevalent type of agrochemical used in all three regions of Ghana and constitute about 52% of the total number of agrochemicals in use. Lindane and endosulfan are among the insecticides being used most extensively. These organochlorines can be persistent and their accumulation could pose a threat to man and the environment. Persistent insecticides, such as DDT, which have been banned are still available in shops and are used extensively on farms. This emphasises the need for a more strict enforcement of laws regarding the importation and use of such chemicals in Ghana. The danger posed by the low educational background of a large number (52%) of the agrochemical merchants needs to be seriously addressed. These merchants not only mishandle pesticides through lack of knowledge but farmers depend on them as their main source of information. There is the need to intensify training programmes to include not only the handling of such chemicals but also the disposal of stocks pesticides that have exceeded their expiration dates. The tendency for farmers to purchase such agrochemicals and even banned pesticides at relatively reduced prices is closely related to a lack of education coupled with the fact that the merchants who advise them may themselves lack the requisite knowledge about the chemicals. The relatively large number of farmers (51%) who have not had any previous training in the use of agrochemicals may further compound the problem of mishandling of these agrochemicals.

Improper record keeping also posed a big problem in the collection of data on volume of usage of these agrochemicals. The lack of an extension service to family and self-owned farms needs to be remedied. It deprives such farmers of the opportunity of benefitting from training programmes in the correct application and handling of agrochemicals.

Safety measures taken by the farmer were found to be grossly inadequate with less than 5% using adequate protective clothing while 22% did not use any safety measures. A programme of workshops, seminars and other training programmes, aimed at enhancing the farmer's capacity to handle and use agrochemicals to their full potential, needs to be set up. Furthermore, since pesticides can have adverse effects on the environment, a better knowledge of the proper handling of these chemicals from the manufacturers through retail merchantss to the farmers could ensure for safer working conditions for the distriibutor and the farmer and reduce the potential for environmental contamination.

This study presents some of the problems related to the management of pesticides in Ghana which we believe could be mitigated through the coordinated effort of policymakers and scientists on the African Continent with the support of interested International funding agencies and organizations involved in the protection of the environment.

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PERSISTENCE AND FATE OF SOIL APPLIED ^{14}C- LINDANE IN A MAIZE ECOSYSTEM

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Abstract

^{14}C-lindane applied to soil surface in a maize ecosystem (one month after planting) was taken up by the plant. Within the first 25 days of treatment, ^{14}C-lindane or its metabolites were found within the entire plant with the greatest concentration in lower leaves (from the ground level); and a sharp build up of lindane concentration towards the tip of each leaf. Radioactivity and hence pesticide concentration was uniformly distributed in the plant with time; to the extent that measurable levels of ^{14}C-compounds were detected in the tussel cob and the grain. This indicated that soil applied lindane was available to the maize plant. The persistence of ^{14}C-lindane in soils of variable organic matter content was also studied. Evidence is presented to show that ^{14}C-lindane dissipated faster in soils of lower organic matter content. Levels of surface applied pesticides that became bound in the soil increased with time after application and also with increasing organic matter content. ^{14}C-activity was mainly associated with the top soil layer (0-30 mm).

1 INTRODUCTION

The following study was designed to complement observations made in the previous paper [1].

2 MATERIALS AND METHODS

2.1. Chemicals

Uniformly labelled ^{14}C-lindane (specific activity 647 MBq mmole^{-1}) was obtained from Sigma Chemical Co. St Louis, USA, through the International Atomic Energy Agency (IAEA) in Vienna. The ^{14}C-concentration of the lindane (supplied as toluene solution) was found to be 1 kBq ml^{-1}. The scintillators 2,5-diphenyloxazole (PPO) and 2,2-phenylenebis-(4-methyl-5-phenyoxazole) (DMPOPOP) were purchased from Eastman Kodak Co., Rochester, N.Y., USA. All other chemicals were Analar grade.

2.2. Studies on the persistence of ^{14}C-lindane

Fifty-one PVC tubes (length, 300 mm; internal diameter, 70 mm) were buried in the soil in the rows between plants (in another plot), with 30 mm projecting above ground level. The tubes were arranged into groups of fifteen and in accordance with the organic matter content of the soils they contained. The soil in each of these group tubes were treated with ^{14}C-lindane (185 kBq) at the same time that plants on the field were sprayed with

commercial lindane. Two tubes in each soil were not treated with ^{14}C-lindane to serve as the control. The tubes were dug out carefully at intervals, wrapped in polythene bags and stored in a refrigerator pending extraction and determination of pesticides. The soil in each of these tubes was sprayed with ^{14}C-lindane (185 kBq) in hexane (100 mL).

2.3. Studies on the uptake of ^{14}C-lindane into plants

Twenty plastic buckets (length, 220 mm; internal diameter, 240 mm and open at both ends) were filled with soil from one of three sources, as described preciously [1]. They were buried in the crop row with 30 mm projecting above ground level. Fifteen tubes of each soil type were grouped together. Maize was planted in each bucket on the same day that planting was done in the field. The soils in the buckets were treated with ^{14}C-lindane (370 kBq) on the same day that the plants on the experimental farm were sprayed with commercial lindane. Three plants were taken periodically and analysed for uptake of ^{14}C-activity. Both anatomical and sectional distribution of ^{14}C-lindane residues in the maize plants were studied.

2.4. Extraction and analysis

2.4.1. Soils

The soil contained in the PVC tubes was scooped out from top (end exposed to the atmosphere) in successive 150 mm layers. Each soil layer was weighed, dried, ground separately (using a pestle and mortar) and sieved through a 2 mm mesh sieve. Samples (40 g) of ground soil were weighed into cellulose extraction thimbles (size 43 × 123 mm) and soxhlet extracted for 2.5 h using methanol. The extracts were concentrated at 50 ± 5 ° C under vacuum to a volume of 5 mL which was then transferred into scintillation vials and analysed for ^{14}C-activity. Recovery was 80.2%.

2.4.2. Plants

Anatomical sections of maize plants above ground level (leaves, stem, cob, etc.) were removed at intervals and stored in a refrigerator for analysis. Stem and leaves were carefully removed from points of attachments cut into 30 mm segments and analysed for ^{14}C-activity using the dry combustion method.

2.5. ^{14}C-activity measurements

Total and bound residues in soils as well as in plant parts were determined by combusting weighed samples in a model 600 Biological Material oxidiser (R.J. Harvey Instrument Corporation, New Jersey, USA). The $^{14}CO_2$ was absorbed in absorber + cocktail (1+1 by volume, 2 mL). The absorber was made up of ethanolamine + methanol (2.5 + 17.5 by volume) and the cocktail consisting of PPO (5 g) + DMPOPOP (50 g) dissolved in toluene (1 L). ^{14}C-activity was measured by liquid scintillation counting (LSC) in a Tri-Carb liquid scintillation Analyser model 100 using the sample channel ratio method for quench correction.

3. DISCUSSION

3.1. Uptake and translocation in maize

Within 25 days of pesticide application to the soil, it was observed that the plants had taken up a measurable amount of ^{14}C-activity with the greatest amount of 0.08% of applied found in the lowest leaf, i.e. the first leaf from the ground level (Fig. 1). There was then a gradual decrease in pesticide concentration in the higher leaves and the stem. The upwards movement of ^{14}C-activity in the maize plant was further shown by the gradual build up of ^{14}C-activity towards the tip of lower leaves (Figs 3, 4). With time, i.e.within 50 days after

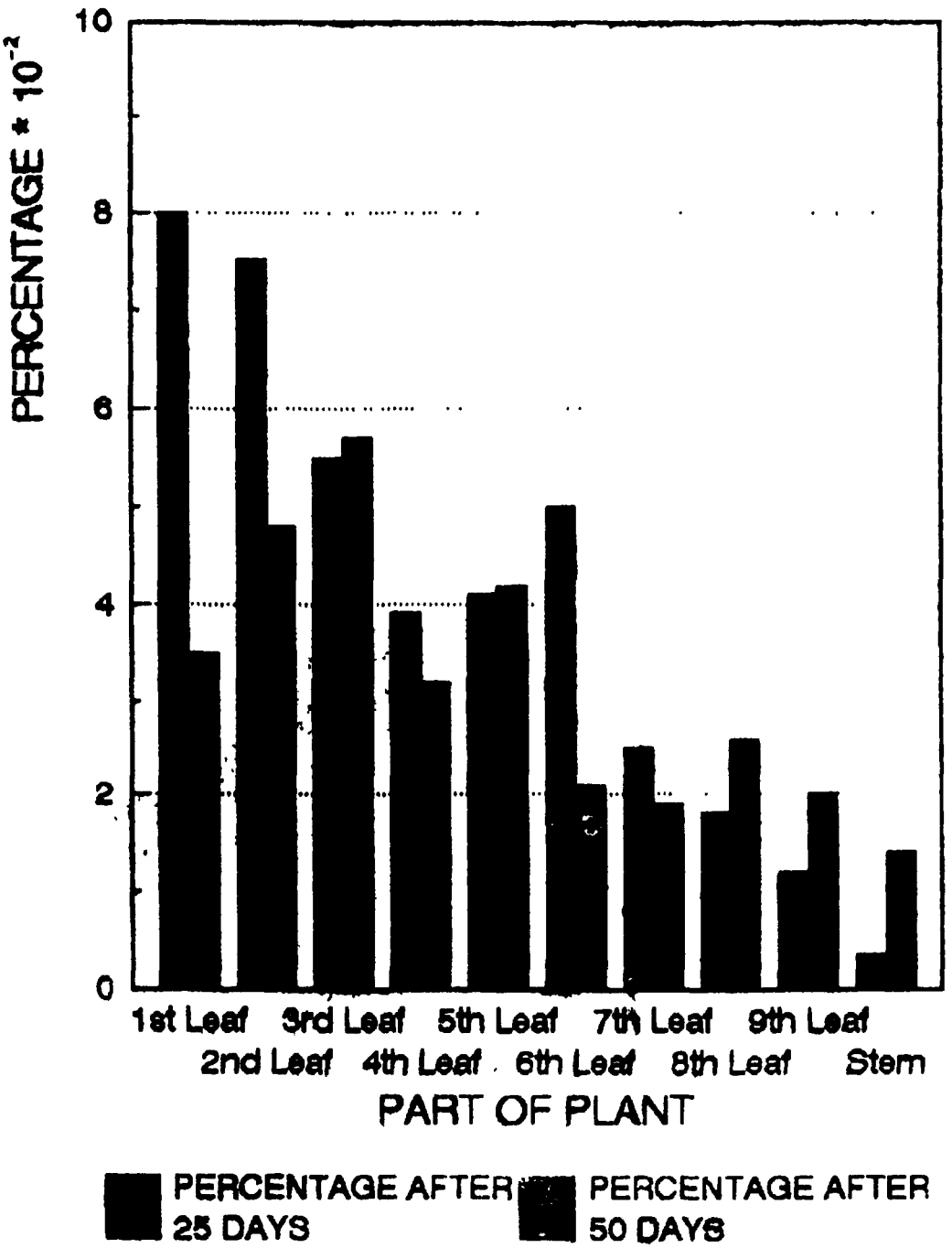

Fig 1. Percentage of initial dose recovered from leaves and stem.

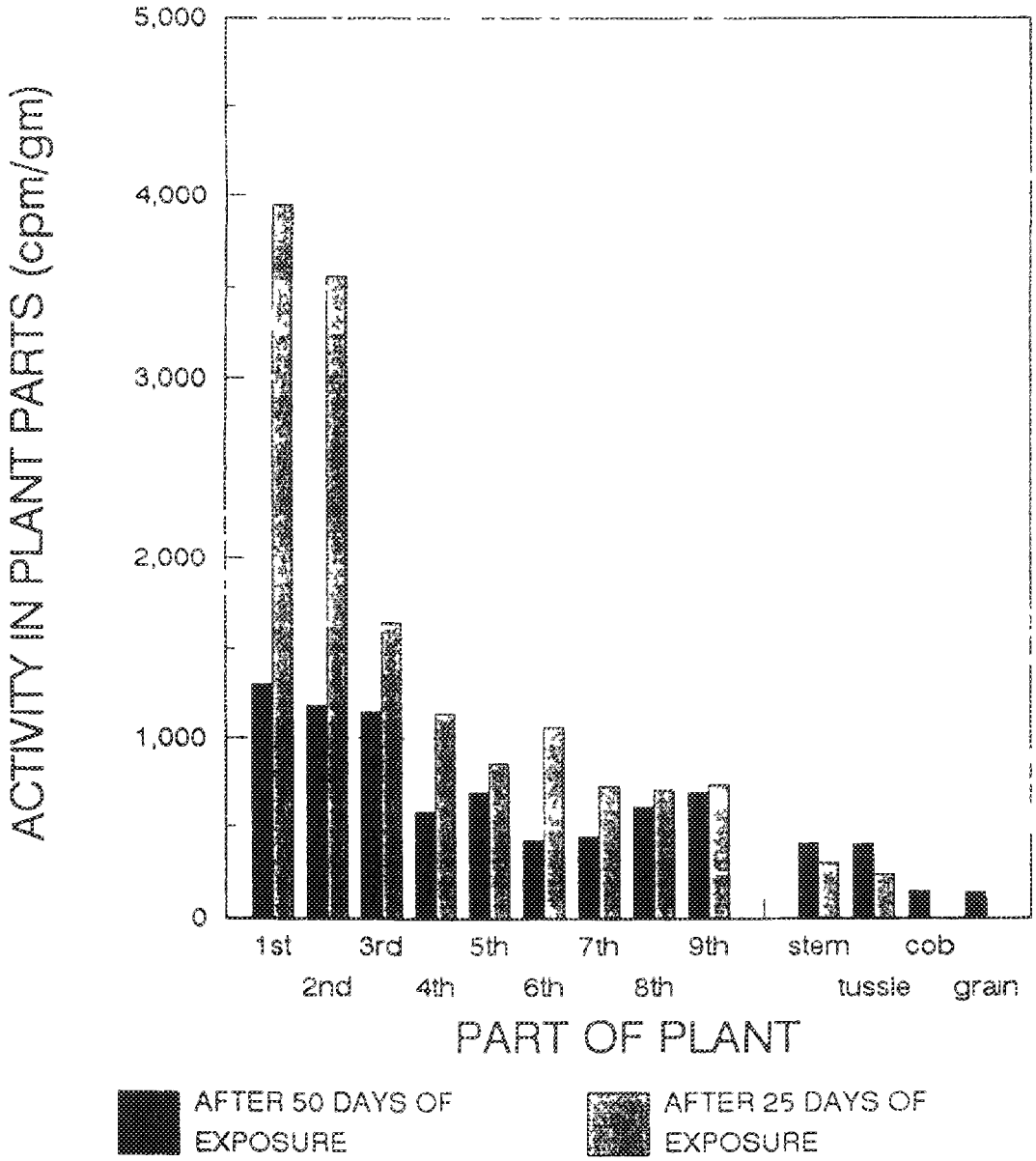

Fig 2 Anatomical distribution of ^{14}C lindane in maize plant

pesticide treatment, the ^{14}C-activity tended to spread out more uniformly throughout the plant though the greatest concentrations of ^{14}C-activity remained within the bottom three leaves with smaller ^{14}C activity in the uppermost leaves and the stem (Fig. 2) Some ^{14}C-activity was also found in the tussel, the cob and the grain 50 days after treatment (Fig 2) indicating that soil applied lindane or its metabolites were available for uptake by the plant The translocation of soil applied lindane and its metabolites have been investigated in detail [2 4, 5, 6] Neither lindane nor its metabolites were evenly distributed within the plants Comparatively high residue levels were always detected in the leaves whereas small amounts were translocated into the stems, leaves, and fruits Paasivirta et al [5] showed that in water plants, lindane concentrations were similar in both the roots and the stem Differences in residue levels have been shown to be dependent on plant species Of a series of edible crops grown in soils containing lindane carrots were shown to have higher levels than beans, tomatoes and potatoes in that order In soils with low organic matter contents, as in this study, insecticide residues are more available and hence susceptible to uptake by plants [7]

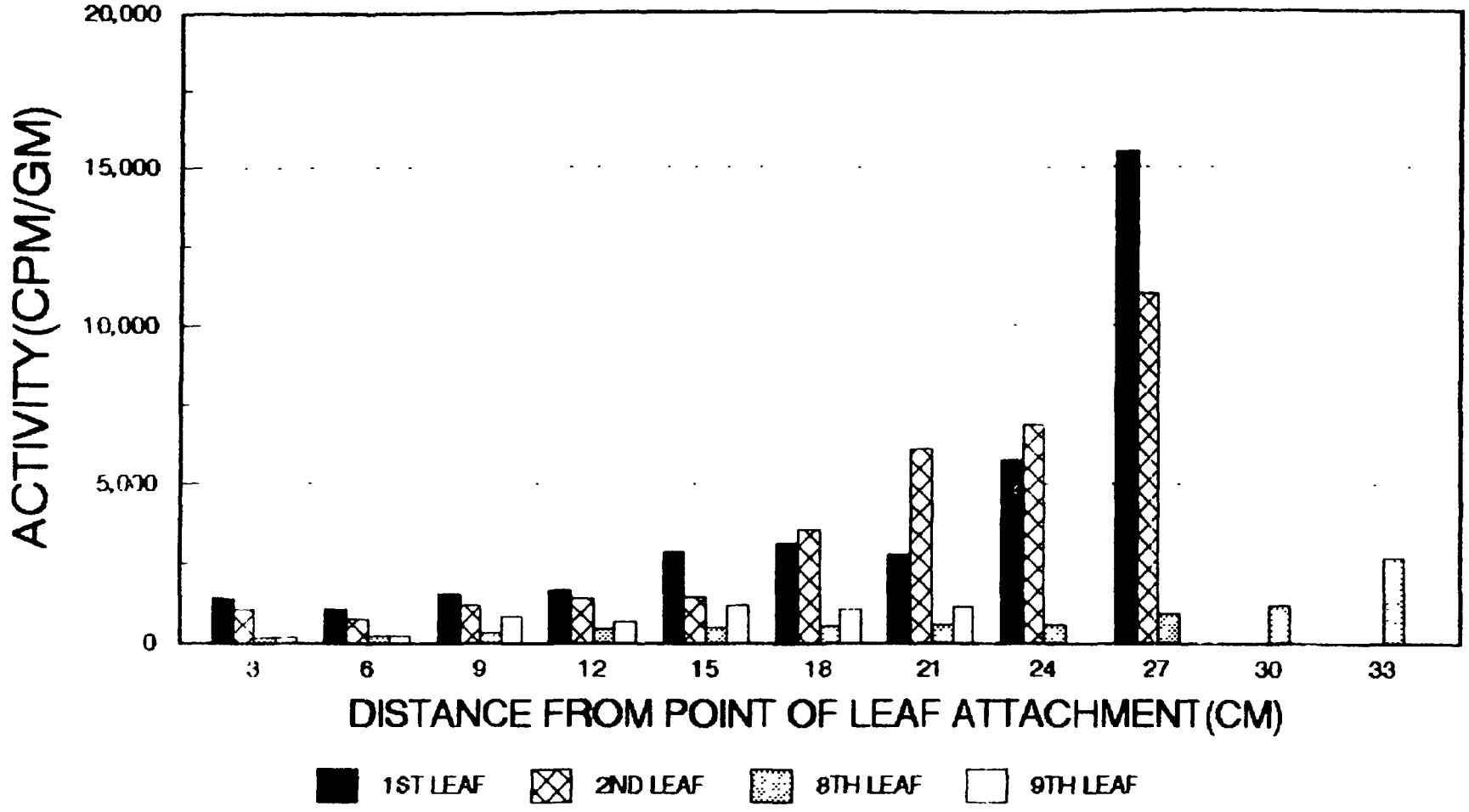

Fig 3. Distribution of ^{14}C lindane in maize leaves (25 days).

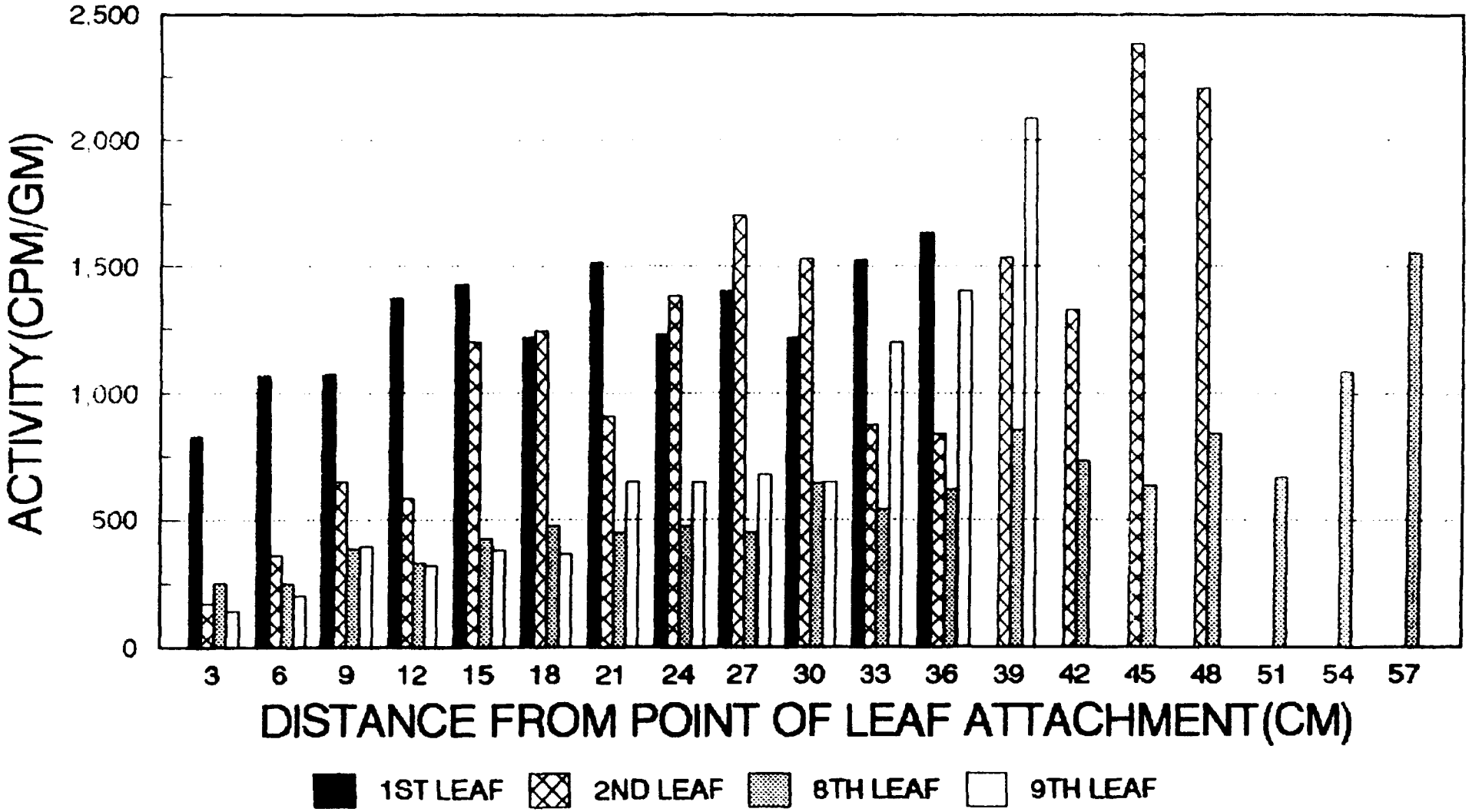

Fig 4. Distribution of ^{14}C lindane in maize leaves (50 days).

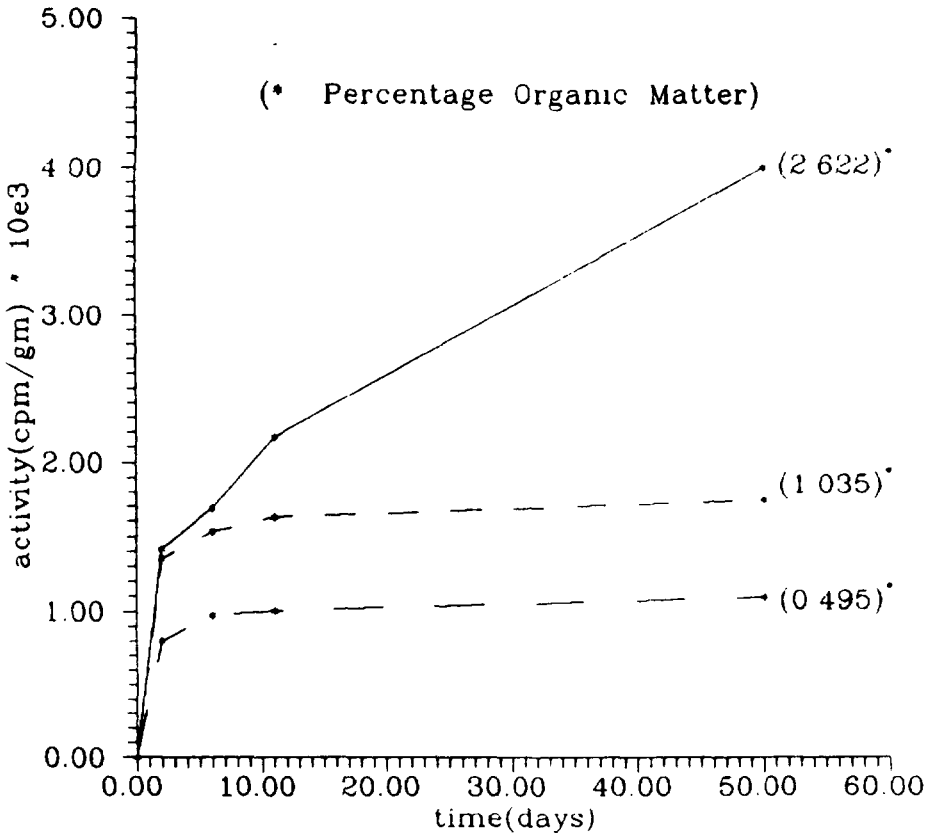

Fig 5. Bound residues of ^{14}C lindane in soils of different organic matter content.

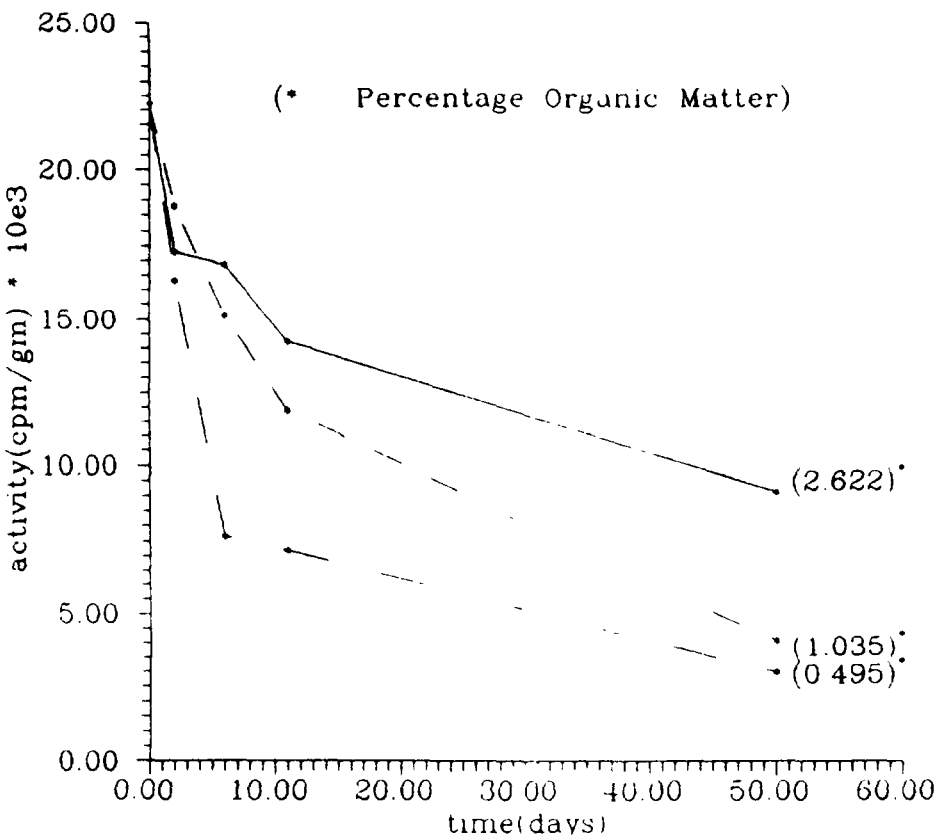

Fig 6. Dissipation of ^{14}C lindane in soils of different organic matter content.

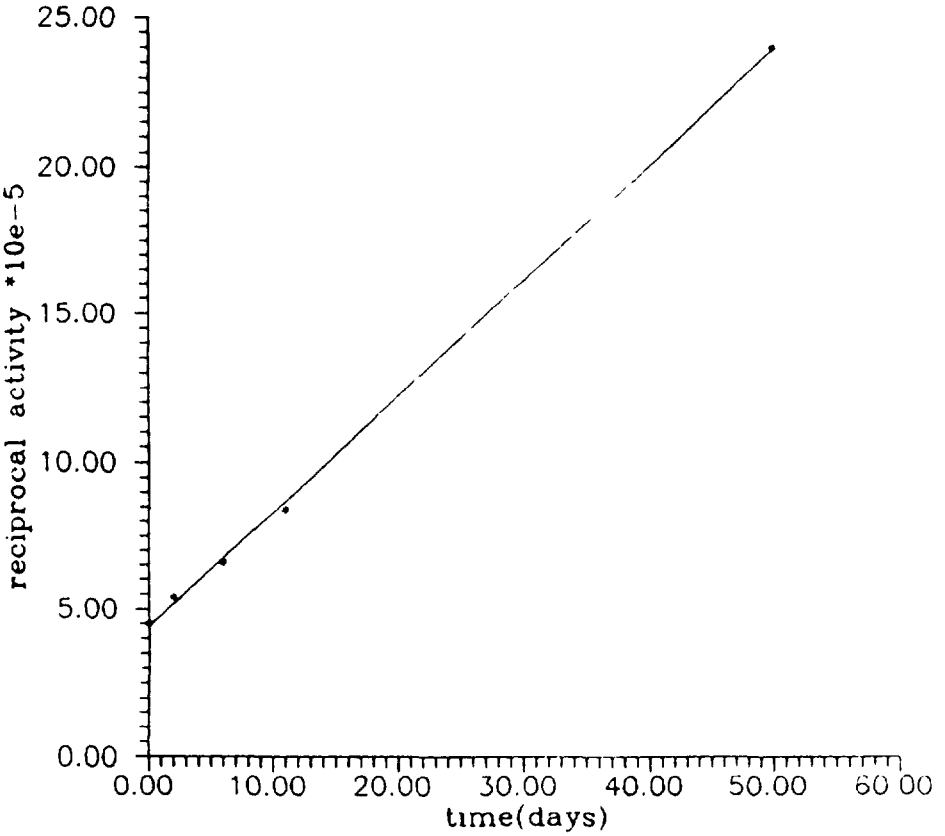

Fig. 7. Reciprocal value of activity against time.

3.2. Movement in soils

Lindane dissipated faster in soils of lower organic matter contents (Fig.5). In the three soils tested, there was a rapid rate of dissipation of ^{14}C-lindane within the first 10 days of application followed by a gradual decline in this rate. This rate of dissipation was attributed to the low organic matter content of the soils in this study. Although there were copious amounts of rainfall during the period after pesticide application (104 mm in 2 months), residues of ^{14}C-lindane were found only within the first 0-30 mm layer of the soil surface in agreement with Cliath and Spencer [7] who observed no mobility of lindane in clay soils.

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PERSISTENCE OF LINDANE IN RICE AND MAIZE ECOSYSTEMS IN NIGERIA

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Abstract

A three year field study was undertaken to examine the fate of residues of lindane in soil and crops after repeated seasonal applications of lindane to soil in which maize and rice crops were grown In the 1993 rice study, the lindane residues in the treated soil were 0 6 mgkg^{1} (1day) and 0.04 mgkg^{1} (2 months) after application respectively. In the soil from the maize plots, lindane residues were 0.19 mg kg^{-1} (1 day) and 0.16 mg kg^{1} (1month) after application and not detectable after 6 months In the 1994 maize study, the lindane residues in the soil were 0.12 (1 day), 0.10 (1 month) and 0.033 mg kg^{1} (2 months) after application respectively while in the crop they were 0.19 (1 day), 0.105 (2 weeks) and 0.05 (4 weeks) mg kg^{1}, respectively In general, lindane residues were not detected in the soil one year after application indicating that there was no accumulation of lindane in the soil during the period 1992-1994 and therefore unlikely to be any long term effects on soil fauna Lindane residues in rice and maize crops continued to decrease during the period up to harvest.

1. INTRODUCTION

Control of insect pests of rice and maize rely largely on the use of chemical pesticides. In addition to lowering the population levels of an insect, pesticides can cause changes within both the non-target organisms and co-inhabitants of its environment. Some pesticides can also accumulate in the soil and cause environmental pollution when they are leached to the underground water or runs off to streams and rivers.

Most pesticidal chemicals can be absorbed to varying degrees and in some cases can be translocated to the edible parts of plants growing in contaminated soils [1]. The presence of chlorinated hydrocarbon pesticide residues in human adispose tissue most often results from the consumption of contaminated food, either deliberately treated with chemicals for the control of pests or incidentally contaminated from environmental or industrial sources [2]. Despite the use of lindane in controlling insect pests, little is known about its fate in the soils and crops under conditions of Nigerian agriculture.

The publicity generated by the restrictions of certain pesticides, especially organochlorines, in developed countries has led to the ban of the use of these chemicals without sufficient information on their impact on other environments. Knowledge of the behaviour of pesticides and their residues in soils and crops in Nigeria is vital as it is not scientifically justifiable to make predictions of pesticidal residue accumulation using data generated elsewhere since pesticidal residue accumulation in will depend on factors such as the nature of the pesticide, soil type, moisture, soil pH, temperature, cultivation, mode of application and soil organisms [3]. The objective of the study was to determine lindane pesticide residues in soils and their absorption into crops in a field experiment after yearly applications of lindane.

2. MATERIALS AND METHODS

2.1. Materials

Lindane was obtained as the commercial formulation (200 g L^{-1} emulsifiable concentrate, Gammalin 20, ICI Ltd, Nigeria). A solution containing standard lindane was obtained from Supelco, Baltenfonte, PA by the International Atomic Energy Agency, Vienna, Austria. The standard solution was stored in the refrigerator (4^0C) until used. Analytical grade hexane, methanol and acetone were supplied by Aldrich Chemical Ltd, Gullingham, Dorset SP8 4JC. Sep Pak phase C-18 cartridges were obtained from Waters Chromatography Division, Millipore Cooperation, Milford, Massachussetts, 01757, USA.

2.2. Plot design and lindane application

The plot design and application procedures have been described elsewhere [see this Tecdoc, Umeh *et al.*].

2.3. Crop sampling

The aerial parts of 12 plants per subplot were collected 1 day and 2 months after lindane application from both the rice and maize fields in the 1992 and 1993 studies, while in 1994 the sampling intervals were 1 day, 2 weeks and 1 month after application. Amounts (~100 g) of plants were chopped into one mixture and stored in polyethylene bags in a freezer compartment until extracted.

2.4. Soil sampling

Soils were sampled at 1day pre-treatment, 1 day, 2 and 6 months after lindane application. On each sampling occasion, 12 cores of soil were taken from a 5 m x 5 m area at the side of each subplot to a depth of 15 cm using a soil auger. The samples were ground to pass 0.25 mm mesh sieve and mixed to provide one bulk sample for each subplot. Duplicate soil samples were taken for measurement of moisture content and for residue analysis. All soil samples were stored in polyethylene bags in a freezer compartment until extracted.

2.5. Residue extraction and analysis

2.5.1. Extraction of plant tissue samples

The plant tissue samples were thawed at room temperature. Samples (20 g) were homogenised with of methanol (200 mL). These suspensions were shaken mechanically for 1 h in a screw-capped jar. The suspensions were filtered to remove plant debris. Volumes (10 mL, 1993; 50 mL, 1994) were dried by aspirating with nitrogen and redissolved in methanol + water (3.5 mL,1+2.5 by volume).

2.5.2. Soil

Samples (20 g) of soil were placed in a screw-capped bottle, mixed with methanol (200 mL) and mechnically shaken for 1 h. The suspension was filtered and volumes (10 mL, 1993; 50 mL, 1994) were dried with nitrogen. Each residue was then redissolved in methanol + water (3.5 mL, 1 + 2.5 by volume).

2.5.3. *Clean up procedure*

Each aqueous methanol extract was passed through a preconditioned C-18 SPE column and then eluted with hexane. The hexane extract was dried with nitrogen and redissolved again in methanol and the clean up procedure repeated. The residue was dissolved in hexane (2 mL) and stored in a refrigerator until analyzed by the gas chromatographic method.

2.5.4. *Gas chromatographic analysis*

1992[a)] : Instrument, Shimadzu 9; column, fused silica capillary,30 m x 0.25 mm i.d.; temperatures, column, 240°C, injector, 250°C, detector, 350°C; carrier gas, nitrogen; detector, ^{63}Ni electron capture.

1993[b)] : Instrument, Varian 3700; column, fused silica capillary; temperatures, column, 190°C; injector, 220°C; detector, 270°C; carrier gas, nitrogen; detector, ^{63}Ni electron capture.

1994[a)] : Instrument, Hewlett-Packard 5890A; column, fused silica capillary, 30 m x 0.25 mm i.d.; temperatures, column, 240°C ; injector, 250°C; detector, 300°C; carrier gas, nitrogen; detector, ^{63}Ni electron capture.

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3 RESULTS AND DISCUSSIONS

The limit of detection of lindane was 0.0044 mg kg^{-1} for both crop and soil samples. The recovery from fortified soils after clean up was 93.5%. There were no detectable lindane residues before lindane application in 1992 for the rice plots and in 1992 and 1993 for the maize plots. Lindane residues were not detected in the untreated rice and maize soil because the field experimental design was oriented to avoid any contamination by erosion from treated to untreated plots. Absence of lindane residues at the beginning of the studies in each new site confirmed that lindane had not been used in the locations before the study was started.

Only data of the *extractable* lindane residues in the soil are reported. In the 1993 rice study, the lindane residues in the treated soil were 0.36 mg kg^{-1} (1 day) and 0.04 mg kg^{-1} (2 months) after application respectively. For the maize soil the residues were 0.19 mg kg^{-1} (1 day) and 0.016 mg kg^{-1} (1 month) after application and were either very low (< 0.09 mg kg^{-1}) or not detectable at 6 months after application. All these results suggest that there is no problem of long term residues of lindane building up in the soil from either rice or maize fields.

The 1994 maize study gave similar results to that of 1993 in that residues of lindane in the soil were not detectable after 6 months probably as a result of loss of lindane due to volatilization and microbial degradation. Some other studies have shown that organochlorine pesticides dissipate at higher rates under tropical and sub-tropical conditions than in temperate regions [4, 5]. The high lindane residues (0.25 mg kg^{-1}) found in maize foliage, 1 day after application, will have arisen from lindane sprayed onto the leaves, since it was applied as a surface treatment and not incorporated into the soil. The residues dissipated rapidly (0.094 mg kg^{-1} at 2 months after application) but not as fast as they did in 1993 when

they were found to be as low as 0.0033 mg kg^{-1} at the same interval after application. The cause of the differences between the years is unknown.

Table 1. Characteristics of the soils from the crop fields

Field Crop	% sand	%silt	%clay	% organic matter	pH	%N
rice [a)]	21	25	54	2.23	6.3	0.9
maize [a)]	25	20	55	1.85	6.1	0.08

a) classified as a clay

Table 2. Concentration of lindane samples taken from the rice field

Year	Crop	Sample	Lindane residues at period from spraying $mgkg^{-1}$ (SD)				
			1day before	1 day	1 month	2 months	6 months
1992	rice [a)]	soil [d)]	ND	NM	NM	0.17 (0.05)	0.09 (0.11)
		crop	-	0.53 (0.19)	NM	0.1 (0.04)	-
	maize [b)]	soil [d)]	ND	NM	NM	0.1 (0.02)	0.09 (0.01)
		crop	-	0.25 (0.19)	NM	0.094 (0.004)	-
1993	rice [a)]	soil [d)]	ND	0.36 (0.02)	NM	0.04 (0.05)	ND
		crop	-	NM	0.011 (0.01)	0.02 (0.002)	-
	maize [c)]	soil [d)]	ND	0.19 (0.013)	0.16 (0.013)	NM	ND -
		crop	-	NM	0.0033 (0.003)	NM	
1994	maize [c)]	soil [d)]	ND	0.12 (0.007)	0.103 (0.44)	0.033 (0.045)	NM
		crop	-	0.19 [e)] (0.06)	0.05 (0.03)	NM	-

ND = not detectable, NM = not measured

a) plot location, Igbariam; b) plot location, Ugwuoba; c) plot location, Enugu

d) there were no detectable residues of lindane in any of the samples of soils taken from the untreated control plots before and after application to treated plots on each occasion.

e) lindane residues at 2 weeks after application = 0.11 (SD,0.02) $mgkg^{-1}$.

4. CONCLUSIONS

In conclusion, our results indicate that lindane was not detected in the soil samples taken 6 months after application to rice and maize agro-ecosystems in Nigeria. The results also show that there was no accumulation of lindane or its metabolites in the soil during the study period thus giving little cause for concern as far as a build-up of lindane residues and long term effects on either soil fauna or transfer to the food chain is concerned. They also show that data from temperate countries should only be applied with caution to Nigerian conditions. The variability in the physical and biological properties of soil give rise to difficulties in the prediction of the fate of pesticides as their degradation is a complicated function of volatilisation loss by erosion, leaching, biotic and abiotic conversion [6].

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LINDANE RESIDUES IN FISH INHABITING NIGERIAN RIVERS

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Abstract

Analysis for residues of lindane in fish collected from various rivers close to rice agroecosystems showed that the concentrations of lindane ranged from none detectable to 3.4 mg kg^{-1} Fish from rivers where strict regulations prohibits its use had no detectable lindane residues while appreciable amounts of lindane were found in fish where such restriction was not enforced with the variation attributed to the extent of use of lindane in the area of contamination. The investigation confirms that the use of lindane in rice production in Nigeria can cause the contamination of fish in nearby rivers.

1. INTRODUCTION

Pesticides have benefitted man by reducing loss and increasing food production, but unfortunately have been a major source of pollution to fish [1, 2]. Lindane residues can be stored in tissues of birds, and mammals including man [3] and can cause death to organisms at the end of the food chain [4]. Pollution of the environment and its effects on the health of man, animals, fish, wildlife and the ecosystem in general have become a subject of concern[5]. Broad conclusions regarding lindane levels can be drawn from comparison of their distribution in biological materials from a given area. Because of the widespread distribution of fish, their opportunity for exposure to pesticides applied in rice agroecosystems, their role in Nigerian economy and their importance in the web of life, fish was chosen as a logical organism for monitoring lindane residues.

Reports show that the use of pesticides requires close monitoring of the effects on non-target organisms especially on the economically important fishery resources in rivers close to farm lands (6). In developed countries in the temperate regions there is a continous effort to monitor pesticide residues in air, water, soil, man, wildlife and fish. On the other hand, the effects of pesticide residues on non-target organisms in Nigerian agriculture and its environment is still speculative because of a lack of quantitative data. The purpose of the present study was to monitor certain rivers in Anambra State to evaluate the level of lindane contamination in fish.

2. EXPERIMENTAL

2.1. Sample collection and storage

In 1991, fish samples of different species were collected from different rivers close to rice fields that were suspected to have received lindane applications (Table 1). Fish from locations thought to be untreated were also collected. The fish were collected from the river

with a cast net and transported to the laboratory in ice buckets where they were sorted out into species, wrapped in aluminum foil and frozen until analysed.

2.2. Sample preparation, extraction and clean-up

Each frozen fish sample was ground and homogenized in a meat grinder. Samples (20 g) of the homogenized fish were blended and mixed with anhydrous sodium sulphate (80 g). The pesticide was extracted for 1 hour in a screw-capped jar with acetone + hexane (1+1 by volume, 200 mL). After tumbling, the solids were allowed to settle and the solvent extract filtered into a separating funnel containing sodium sulphate solution (20 gL^{-1}, 200 mL) and shaken for about one minute. The lower aqueous layer was discarded and the hexane extract washed twice more with sodium sulphate solution (20 gL^{-1}, 50 mL). Quadruple hexane extracts were combined and evaporated to about 10mL and then

Table 1. Residues of lindane found in fish caught at the sampling stations in Anambra State, Nigeria

River	Location	Fish species	Lindane residues, $mgkg^{-1}$
Ani	Ufuma[a)]	*Heterotis niloticus*[d)]	ND[i)]
		Clarias zariepinus[e)]	ND
		Heterobranchus bidorsalis[f)]	ND
		Oreochromia niloticus[g)]	ND
Ozzi	Enuguabo[a)]	*Heterotis niloticus*	ND
		Clarias zariepinus	ND
		Heterobranchus bidorsalis	ND
		Oreochromia niloticus	ND
Ozalla	Oyi[b)]	*Heterotis niloticus*	<1[j)]
		Clarias zariepinus	<1
		Heterobranchus bidorsalis	<1
Omoh	Oyi[b)]	*Heterotis niloticus*	<1
		Clarias zariepinus	<1
		Oreochromia niloticus	<1
Anambra	Oyi[b)]	*Heterotis niloticus*	3.1
		Clarias zariepinus	2.1
		Heterobranchus bidorsalis	3.1
		Oreochromia niloticus	2.2
Nkissi	Onitsha[c)]	*Heterotis niloticus*	3 4
		Cyprinus carpio[h)]	1.9
		Heterobranchus niloticus	2.0

Local Government area :
a) Orumba North
b) Oyi
c) Onitsha

Local name :
d) African boy tongue (Okpo)
e) Mudfish
f) Catfish (Azu isi)
g) Tilapia (Ikpokpo)
h) Carp

i) none detectable
j) detectable, but not quantifiable

transferred into vials and evaporated using a N_2 sparge. The residue was redissolved in hexane (2mL). This extract was passed through a column containing Florisil to remove non-pesticidal extracts by the procedure reported previously [7].

2.3. Gas chromatographic analysis

The hexane extracts were analysed by gas chromatography at the Department of Food Technology and Science, University of Tennesse, Knoxville, USA using a Shimadzu 9 AM; column: 30 m x 0.25 mm i.d. SPB-5 fused silica column (Supelco Inc. Belanfonte, PA); temperatures : column, 70°C for 1.5min, increased to 200°C at 10°C min^{-1} and then to 240°C for 20 min; injector, 250°C; detector, 250°C; carrier gas, helium; flowrate, 2 mL min^{-1}; detector, ^{63}Ni electron capture interfaced with a Shimadzu QP 1000 mass spectrometer. At the beginning of the analysis each day, several consecutive injections were done to prime the column [8].

3. RESULTS AND DISCUSSION

3.1. Questionnaire survey of lindane use in Anambra State

The preliminary questionnaire survey involved extensive visits and interviews with officials of the State Ministry of Agricultural Development Agencies, Pest Control Department, Office of Statistics as well as on-the-spot interviews of rice farmers in various locations in Anambra State, Nigeria. The survey revealed that there was no information on the occurrence, distribution, present usage, effects and the impact of lindane in the rice agroecosystem in Nigeria. It was observed that lindane, commonly called gammalin, is still sold in local markets even though the Federal Government of Nigeria has banned its use. Farmers still use it for pest control in various crops and also for killing fish. In some areas the Government ban is strictly observed while, in most other areas, farmers use it for pest control because it is effective and relatively cheap compared with other pesticides. The unavailability of authentic data on the usage and distribution of lindane in Nigeria as evidenced by our survey, justified the collection of fish from various rivers close to rice growing areas to begin the collection of baseline data of lindane residue levels in fish.

3.2. Lindane residues in fish

Comparison of lindane residues in fish from different rivers show that Anambra and Nkissi rivers were more exposed to pesticide contamination than the other rivers (Table 1) with the residues in fish ranging from none detectable to 3.4 mg kg^{-1}. Only small amounts of lindane were detected in fish from the Omoh and Ozalla rivers and no detectable residues in the fish from the Ani and Ozzi rivers. The absence of lindane residues in fish collected from the Ani and Ozzi rivers reflects the strong restrictions on the use of lindane for farming and fishing in these areas, while the low level of lindane found in fish from the Omoh and Ozalla rivers indicate that only small amounts of lindane, that is applied to the rice in this area, entered the rivers. These low levels of lindane are comparable to those found in areas subjected only to airbone contamination [9]. Such levels may not be harmless since it has been reported that DDT at low levels can interfere with the reproduction of certain fish species [2].

Fish obtained from Anambra and Nkissi river contained appreciable amounts of lindane probably because the river flowed through areas where lindane was frequently used

by farmers. Reports show that under certain conditions pesticide residues arising from normal agricultural use may contribute to diffuse (non-point) pollution of the aquatic environment [11]. In an experiment in Zimbabwe, all the fish sampled contained insecticide residues ranging from 0.055mg kg^{-1} in the liver of living species up to 5.1mg kg^{-1} in the liver of dead specimens, the latter presumably being the cause of death [12]. Individual fish vary in residue levels and this can be attributed to differences in fish movement, size, food habits and fat content [13]. Other field and laboratory studies showing vast differences in residue levels in fish have also been reported in Wisconsin [14]. Although a very limited number of fish samples were collected in this study, when present the levels of lindane were similar to those found in fish in the midwest of the USA, where total pesticide residue of 2.10 μgg^{-1} were recorded [15] but higher than those in Utah where only 0.05-0.096 μgg^{-1} of organochlorine residue was detected [16]. Because some fish continue to accumulate residues over a period of years, the levels observed in the fish from the Nkissi and Anambra rivers reflect the integrated history of exposure. Interviews with villagers indicate a direct contamination as the farmers in the Oyi Local Government areas, where Anambra and Nkissi rivers flow through, reported non-restricted use of the lindane in agriculture and in fish farming.

4. CONCLUSION

In the rivers studied, the extent of contamination of fish by lindane varied. Lindane which was found in fish collected from the Anambra, Nkissi and Ozalla rivers that may have originated from run-off from the rice fields or from direct contamination of the rivers by fishermen. The considerable variation in lindane residue levels in different fish samples, could be caused by variation of fish movement, food habits, species size, age and fat content. Because of these variations, there should be caution in interpretation and application of these data.

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ADVERSE EFFECTS ON FLORA AND FAUNA FROM THE USE OF ORGANOCHLORINE PESTICIDES ON THE AFRICAN CONTINENT: THE NIGERIAN CASE

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Abstract

Lindane was found to reduce stem borer damage significantly in both rice and maize plots in most years. However, a significant difference in yield was recorded only for maize in 1993 and 1995. There was a slight but steady decrease in the yield of maize over the years in the lindane treated plots although yields in these plots remained higher than in the control plots. Significant differences in the mean dry weight of retrieved leaf discs were recorded in the maize plots in 1994 and 1995. Neither insects nor spiders, were caught from plants in the treatment plots 1 day after lindane application, although almost equal numbers of insects and spiders were recovered from D-vac ground samples of treatment and control plots on the same day. Data from pitfall traps showed that lindane significantly affected the population of *Aranae*, *Formicidae*, *Collembola* and *Acarina* for up to 6 weeks after application. Its effect on Carabidae was inconsistent.

1. INTRODUCTION

Organochlorine pesticides are still widely used in Africa for agricultural and public health purposes. Their cost effectiveness and broad spectrum of activity have continued to encourage their demand and use, in spite of international pressures calling for their ban. Presently, there is no pesticide legislation in Nigeria and perhaps also, in some of the African countries resulting in unrestricted importation and use of various kinds of pesticides including organochlorines. Many organochlorine pesticides are persistent in the environment for a long time. They are also known to leave residues in the soil [1,2] resulting in the disruption of soil fauna and flora [3] in the temperate regions, and thus affecting organic matter breakdown and nutrient cycling [4,5]. Treatment of a cultivated forest soil in Nigeria with DDT resulted in a steady decline in yield of cowpea over a four year period [6] and affected activity of certain crickets, lycosid spiders and millipedes [7].

The objectives of this investigation are to determine the effects of lindane (γ-1,2,3,4,5,6 - hexachlorohexane) on:
(i) the key pests of maize and upland rice, i.e stem borers,
(ii) predatory and other non-target species in maize and upland rice agro-ecosystems,
(iii) soil biology and biological activity.

2. MATERIALS AND METHODS

The experiments were carried out from 1992 to 1995 in maize (*Zea mays*) plots, and in 1992 and 1993 in rice (*Oryza sativa*) plots. In 1992, the maize and rice plots were located at Ugwuoba, Enugu State, and Igbariam, Anambra State, respectively, the two towns being about 20 km from each other. Both towns are located in the derived rain forest zone of

Nigeria. In 1993 to 1995, the maize plots were located in Enugu, Enugu State and is at the northern fringe of the derived rain forest. These towns fall within the zone whose annual rainfall does not fall below 1,500 mm and temperature ranges between 27 and 32°C during most of the year.

2.1. Experimental plots

The plots used in Enugu and Ugwuoba were under fallow for 1 and 2 years, respectively, before the experiments began in 1993 and 1992. The plots used in Igbariam were cropped to yam (*Dioscorea* sp.), maize, egusi melon (*Colocynthis vulgaris*), cassava (*Manihot esculenta*), and various leafy vegetables in a mixed farming system. These plots had no previous history of pesticide use.

At the beginning of each experimental season, all the vegetation was ploughed into the soil which was later harrowed twice before mapping out the plots. Soil samples were taken in the three locations and found to be clayey (48-57%), acidic (pH ranges from 6.2 to 6.8), with high organic matter content (Ugwuoba: 8.0%; Igbariam: 3.8% and Enugu: 2.1%).

In 1992 (for maize and rice) and 1993 (for rice), the plot size was 50 m x 50 m each, for treatment and controls, and separated by a 3-metre row of grass. From 1993, the plot sizes for maize were 32 m x 80 m for both treatment and controls, separated by a 1-metre row of grass. In these years (1992 and 1993) there were 4 sub-plots (5 m x 5 m) located within each of the two plots 10 m away from the edge of the plots, and used for sampling. In 1994 and 1995, there was randomised block design with 4 blocks and two treatments. Within each plot was a sub-plot (10 m x 10 m). Because of the possible interference of the various sampling activities within the sub-plots with yield, a yield area plot (2 m x 2 m) was located outside but close to each sub-plot.

2.2. Planting and fertiliser application

The maize variety used throughout these experiments was TZSR yellow while the rice varieties used were FARO 43 (1992), and ITA 306 (1993). Maize was planted in rows, 1 m apart and 50 cm between hills at 2 seeds/hill in 1992. In the subsequent years, the inter-row distance was reduced to 75 cm. Rice was planted in rows by dibbling seeds, 15 cm apart both between rows and hills at the rate of 3-4 seeds/hill. A compound fertiliser (NPK 15.15.15) was applied to rice and maize soils at the rate of 200 kg ha^{-1}, 21 days after planting. A second fertiliser application of urea was made at the tasselling stage (for maize) and the booting stage (for rice), at the rate of 50 kg ha^{-1}. Weeding was done twice manually during each experimental season.

2.3. Pesticide applications

Lindane (200 g L^{-1} EC) was applied to the treatment plots in rice (1992 and 1993) and maize (1992) in a single dose of 1 kg AI ha^{-1}. From 1993 to 1995, 1 kg AI ha^{-1} lindane was applied to maize in split doses of 0.5kg AI ha^{-1}. The first lindane application corresponded to the 3-leaf stage in the maize development (stage 13 in the decimal code [8]). The second lindane application was made two weeks later. High volume sprays (120-200 L of water ha^{-1}), using a knapsack sprayer(CP15) with a red nozzle were used in the lindane application. There was no lindane application to the control plots.

2.4. Sampling

Pre-treatment samples of stem borer damaged plants, insects, other arthropods, and soil fauna were taken from the sub-plots a day before lindane application. Thereafter, samples were taken at two weekly intervals for 2 months. In 1994 and 1995, insect and other arthropod samples were also taken a day, and up to week 10 after lindane application.

2.4.1. Plant damage by stem borers

Stem borer damage was assessed by counting all the affected plants in the maize sub-plots. Because of the high plant population in the rice sub-plots, only half the plants were randomly sampled for stem borer damage. At the early stages, window panes and dead hearts were used as indices for stem borer damage. At the later stages, holes in the stem, lodging, and white heads (dried up tassels and panicles) arising from stem borer damage were also used in assessing plant damage. In 1993, the populations of stem borer larvae was estimated 4weeks after the last lindane application by dissecting 50 stalks, randomly selected outside the sub-plots, and counting all the larvae and tunnels found. Cob damage was also estimated by counting all the damaged cobs in the yield area plots.

2.4.2. Sweep netting, D-vac sampling and pitfall traps for arthropods

Insect, spider and mite populations were sampled using sweep net and pitfall traps in 1992 and 1993. In 1994 and 1995, sweep net sampling was replaced with use of a D-vac sampler which was used to sample both plants and ground. Sweep netting was carried out along two diagonal lines in each sub-plot at the rate of 5 sweeps/diagonal line. After 5 sweeps, the collections were removed and dropped into 70% alcohol until counted. Collections from the sub-plots were stored separately.

D-vac samples for plants and soil surface were taken at the rate of 4 samples per sub-plot. Each sample consisted of 5 sub-samples. For the ground samples, each sub-sample consisted of a 5 second suck with the sampling cone lightly pressed to the ground. For the plant samples, each hill containing 1 or 2 plants, chosen at random, represented one sub-sample. At the earlier stages, the D-vac cone was placed over the plants while sampling. Later, as the plants became taller, the cone was used to sweep the plants from all sides. D-vac sampling of plants was carried out up to 6 week after lindane application while ground sampling continued up to the 10th week.

Four pitfall traps were set up at random, in each sub-plot. The plastic containers used (10 cm in diameter and 10 cm deep) were buried so that their rims were flush with the soil surface. The traps were half filled with soapy water. In 1992, 1993 and 1994, and 1995, the traps were left in the field for 1 day, 2 days and 1 week, respectively, before collection. In 1995, the traps were emptied twice during the sampling week.

In 1992, the target taxa included numbers of adult stem borers, *Carabidae*, *Staphylinidae*, *Coccinellidae*, *Chrysopidae*, *Formicidae*, *Dermaptera* and *Aranae*. In 1993, stem borers, *Carabidae* and *Aranae* were sampled, while in 1994 and 1995, stem borers, *Carabidae*, *Coccinellidae*, *Formicidae*, *Aranae*, *Acarina* and *Collembola* were sampled.

2.4.3. *Breakdown of organic matter*

Fifty leaf discs (each measuring 25 mm in diameter), cut from mango leaves (*Mangifera indica*), were put in each 12 cm^2 litter bag with 10 mm apertures. The litter bag was attached to a marker peg and buried to a depth of 50 mm. Four litter bags were buried randomly in each sub-plot prior to lindane application. The litter bags were retrieved three months later and the contents of each litter bag were washed separately, oven dried at 60°C for 24 hrs, and weighed.

2.4.4. *Soil fauna*

Four soil cores, 150 mm deep and 50 mm in diameter were taken at random from each sub-plot before pesticide application in 1992 and 1993. Each soil core was cut into 6 pieces and the soil fauna present were extracted using Tullgren type extractors. A second set of soil core samples were taken 2 months after lindane application and treated similarly.

3. ANALYSIS OF DATA

The various data obtained were analysed using a two-way analysis of variance incorporating effects of treatment and time. The sub-plots in 1992 and 1993 were regarded as real plots and the data generated were analysed similarly.

4. RESULTS

4.1 Maize 1992

There was heavy rainfall (31.6 mm), which lasted several hours on June 9, 1 hr after lindane application and this seemed to have affected the experiment. The important stem borer species of maize encountered in this work were *Busseola fusca* Fuller, *Sesamia calamistis*_Hamp, and *Eldana saccharina* Wlk. Although damage caused by these stem borers was low in 1992, there was an increase in their damage with time in both treatment and control plots (Fig. 1) but the differences were only significant ($P<0.05$) at one interval (6 weeks after treatment).

A few insects and spiders were caught with the sweep net (Table I). These included specimens from *Coccinellidae*, *Formicidae* and *Aranae*. The *Coccinellids* caught were the phytophagous species, *Epilachna* sp., known to scrape leaf tissues from young maize plants. More insects and spiders were caught in the control than in the treatment plots but their differences were not statistically significant ($P <0.05$).

Pitfall trap collections included specimens from *Carabidae*, *Staphylinidae*, *Formicidae* and *Aranae* (Table II and Fig. 2). For *Carabidae* and *Staphylinidae* (Table II), there was no significant difference in the mean catches made on any of sampling dates in both treatment and control plots at a 5% level. For *Formicidae* (Fig. 2), only the second post-treatment sample, with mean catches of 3.5 and 8.0 for treatment and control plots, respectively, was found to be statistically significant. Analysis of the mean spider catches showed that significantly more spiders were trapped in the control than in the treatment plots in the 4th, 6th and 10th week after treatment. These results (Formicidae and Aranae), do not show any clear trends in their response to lindane application. Chrysopidae was caught neither in sweep nets

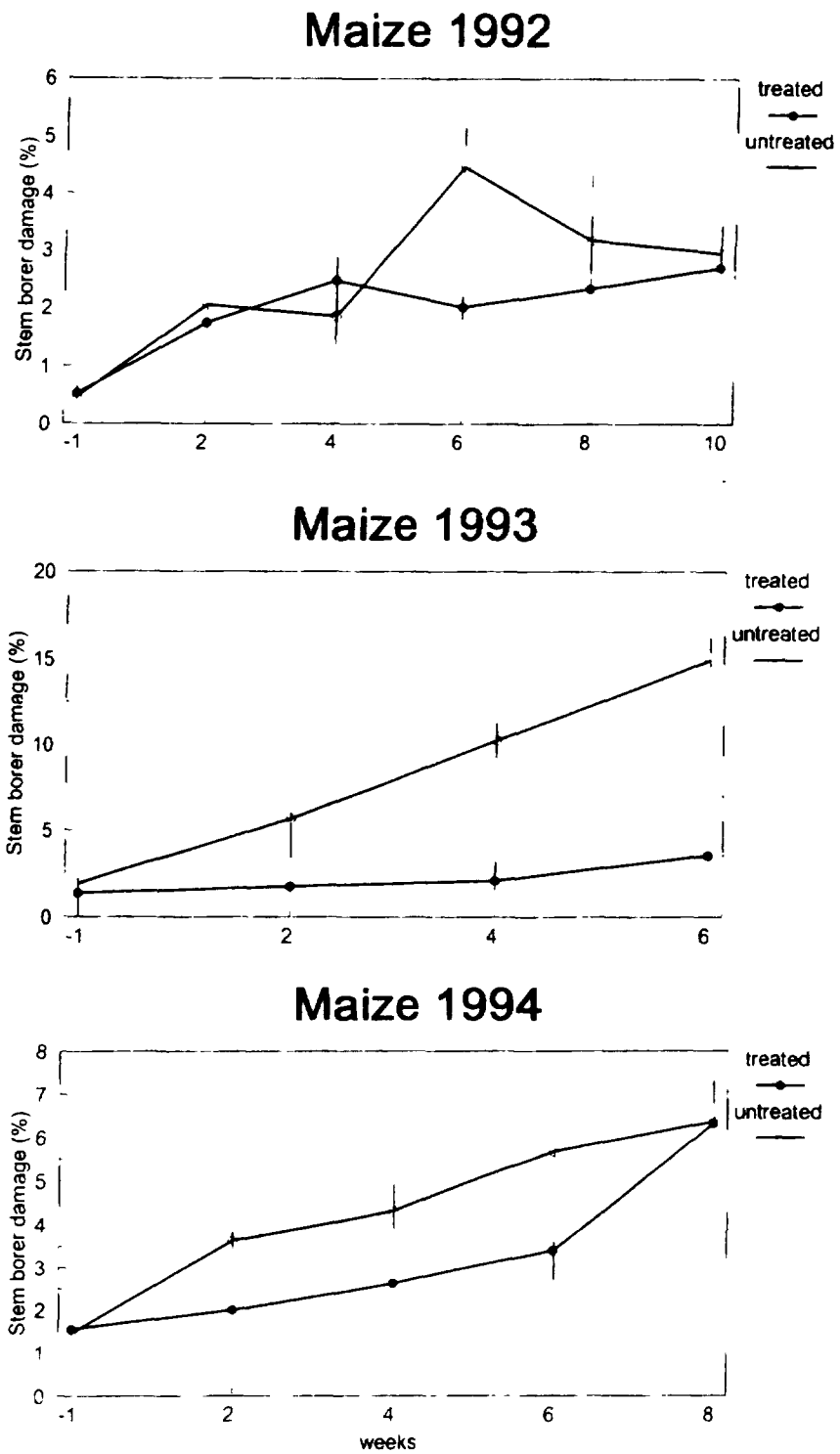


FIG. 1. Stem borer damage in maize and rice plots.

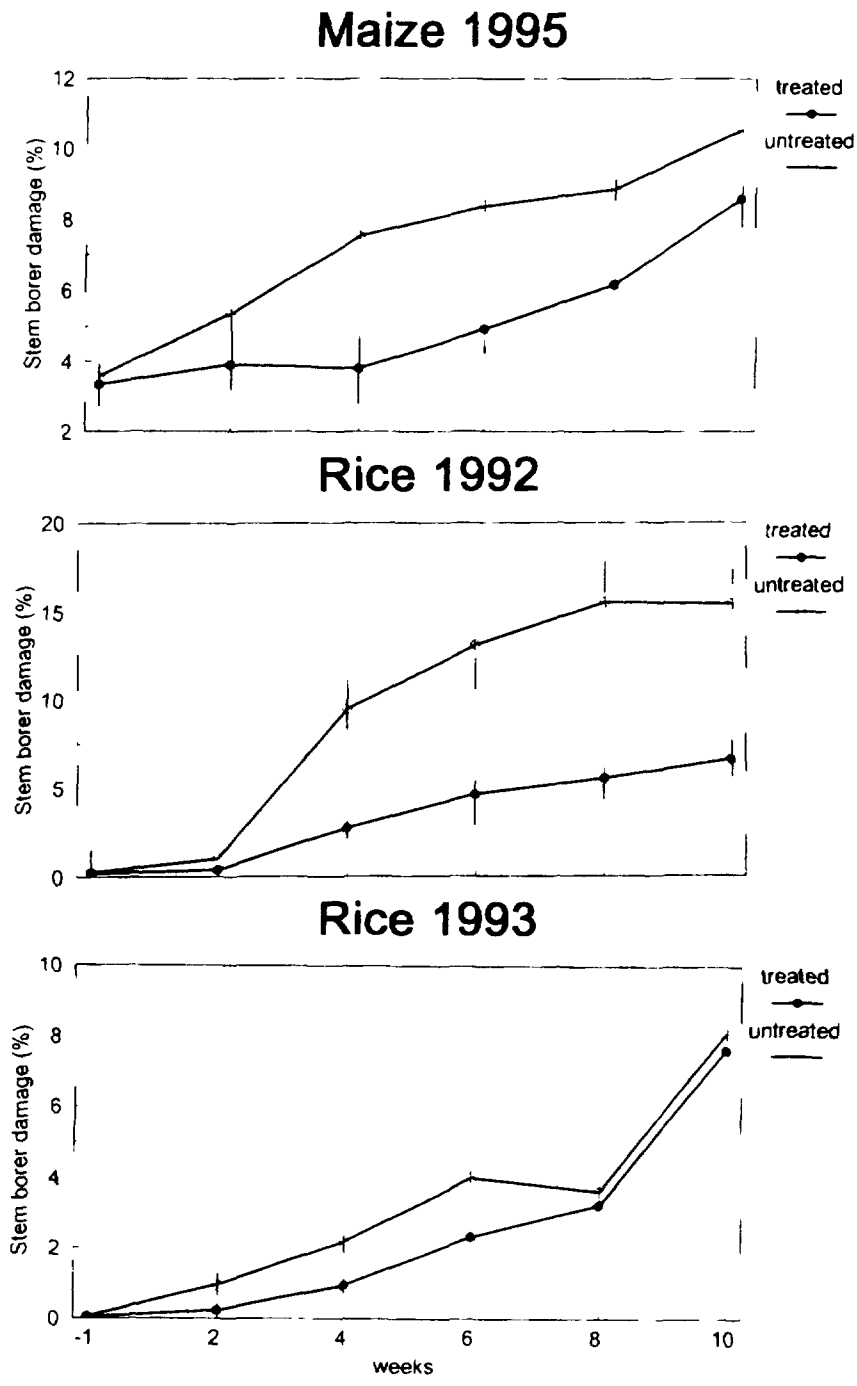


FIG. 1. (cont.).

nor trapped in pitfalls in this experiment. Overall, it would appear that the heavy rain which occurred soon after lindane application may have affected some of these results.

The soil fauna in the samples included *Collembola* (springtails), *Acarina* (mites) and other soil mesofauna Table IV). Although the pre-treatment samples showed no significant differences in the mean numbers of the various soil fauna extracted from both treatment and

control plots, post-treatment samples showed significant differences in the mean numbers of *Collembola* and *Acarina* extracted from the lindane-treated compared with the control plots. Both *Collembola* and *Acarina* are micro-arthropods inhabiting the soil and leaf litter. They would have received lindane sprays directly and may have been killed before commencement of the rain on the treatment day. Their population seemed unable to recover from the impact of lindane, even at two months after application.

Mean dry weight of leaf discs retrieved (Table V) were 4.7g and 3.8g for treatment and control plots, respectively, and this difference was not statistically significant. Yields obtained from the lindane treated plot (5.12 t ha^{-1}) was not significantly different ($P < 0.05$) from the yields obtained from control plots (4.99 t ha^{-1}). This result may be due partly to low pest infestation on maize this year but also due to the effect of rainfall which may have leached or washed away most of the lindane deposits on the treatment day.

Table I. Sweep net collections in maize and rice

a) Maize

Weeks after treatment	Mean number of specimens caught ±SE							
	Coccinellidae		*Aranae*		*Formicidae*		Stem borers	
	T	C	T	C	T	C	T	C
Pretreatment	0 00	0 00	0 00	0 00	0 00	0 00	-	-
2	0 75 ±0 5	1 50 ±1 3	0 50 ±0 6	0 50 ±0 6	0 50 ±0 5	1 25 ±0 5		
4	0 00	0 25 ±0 5	0 00	0 00	0 00	0 00		
6	0 00	0 50 ±0 7	0 00	0 00	0 25 ±0 5	0 75 ±0 5		
8	0 25 ±0 5	0 75 ±0 5	0 00	0 00	0 00	0 00		
10	0 75 ±0 5	0 75 ±0 5	0 25 ±0 5	0 25 ±0 5	6 75 ±1 0	6 50 ±1 3		

T = treated plot, C = untreated control plot

b) Rice

Weeks after treatment	Mean number of specimens caught ±SE							
	Coccinellidae		*Aranae*		*Formicidae*		Stem borers	
	T	C	T	C	T	C	T	C
Pretreatment	0 25 ±0 5	1 25 ±0 5	0 00	0 25 ±0 5	1 25 ±0 5	1 25 ±0 5	1 50 ±0 6	1 25 ±0 5
2	0 25 ± 0 5	1 00 ±0 0	0 00	0 00	0 25 ±0 5	4 25 ±1 3*	1 00 ±0 8	6 25 ±1 7*
4	0 00	0 50 ±0 5	0 00	0 75 ±0 5	2 25 ±1 3	0 00	0 75 ±1 0	3 00 ±1 4*
6	0 50 ± 0 6	0 50 ±0 6	0 25 ±0 5	0 75 ±0 5	1 25 ±0 5	3 00 ±0 8*	1 00 ±0 8	0 75 ±0 5
8	0 25 ±0 5	0 50 ±0 5	0 50 ±0 6	1 00 ±0 8	1 00 ±0 0	1 00 ±0 0	0 25 ±0 5	0 25 ±0 5
10	0 25 ±0 5	0 25 ±0 5	0 50 ±0 6	1 50 ±0 6	0 50 ±0 6	0 25 ±0 5	0 25 ±0 5	0 50 ±0 6

T = treated plot, C = untreated control plot
* significantly different from the treated plot at P = 0 05

Table II Pitfall trap collections from the maize trials

Year	Weeks after treatment	Mean number of specimens caught ±SE			
		Carabidae		*Staphylinidae*	
		T	C	T	C
1992	pretreatment	2 50 ±0 6	2 25 ±1 0	1 75 ±0 5	2 25 ±0 9
	2	1 25 ±1 0	2 25 ±0 5	0 00	1 00 ±0 8
	4	1 25 ±0 5	2 75 ±0 5	0 00	0 75 ±0 8
	6	1 25 ±0 5	1 25 ±1 0	0 50 ±0 6	1 00 ±0 8
	8	0 25 ±0 5	0 50 ±0 6	0 25 ±0 5	0 25 ±0 5
	10	2 00 ±1 1	2 00 ±1 2	0 50 ±0 6	0 75 ±1 0
1993	pretreatment	0 75 ±0 5	0 75 ±1 0		
	2	0 25 ±0 5	1 75 ±1 0		
	4	0 25 ±0 5	2 00 ±1 0		
	6	0 50 ±1 0	2 25 ±0 5		
	8	1 00 ±0 5	2 50 ±0 6		
	10				
1994	pretreatment	1 25 ±1 3	1 50 ±0 6		
	1day	1 75 ±0 5	1 75 ±1 0		
	2	2 25 ±1 3	3 25 ±1 3		
	4	1 50 ±0 6	2 25 ±0 5		
	6	1 25 ±0 5	1 25 ±0 5		
	8	1 75 ±0 5	1 50 ±0 6		
	10	1 75 ±0 5	2 00 ±0 8		
1995	pretreatment	7 25 ±5 3	7 5 ±5 1		
	1day	0 00	8 25 ±1 5		
	2	1 00 ±0 8	12 75 ±3 8		
	4	2 75 ±1 0	15 00 ±2 5		
	6	7 00 ±2 7	17 00 ±1 7		
	8	11 00 ±2 6	12 25 ±2 2		
	10	7 75 ±3 9	10 25 ±2 9		

T = treated plot, C = untreated control plot

Table III Pitfall trap collections from the rice trials

Year	Weeks after treatment	Mean number of specimens caught ±SE			
		Carabidae		*Staphylinidae*	
		T	C	T	C
1992	pretreatment	1 50 ±0 6	1 50 ±0 6	0 75 ±0 5	0 50 ±0 6
	2	1 00 ±0 0	1 75 ±0 5	0 00	0 25 ±0 5
	4	2 50 ±0 6	1 75 ±1 0	0 00	0 75 ±1 0
	6	2 00 ±0 8	2 75 ±1 0	0 50 ±0 5	1 50 ±0 6
	8	3 75 ±1 0	5 00 ±0 8	0 25 ±0 5	1 25 ±0 5
	10	4 50 ±1 3	6 75 ±3 1	2 25 ±0 5	2 00 ±0 8
1993	pretreatment	4 00 ±1 6	3 50 ±1 3		
	2	1 50 ±1 0	4 50 ±1 3*		
	4	2 50 ±1 0	4 75 ±1 5*		
	6	4 00 ±0 8	4 75 ±1 3		
	8	3 25 ±0 5	3 00 ±0 8		
	10				

T = treated plot, C = untreated control plot
* significantly different from the treated plot at P = 0 05

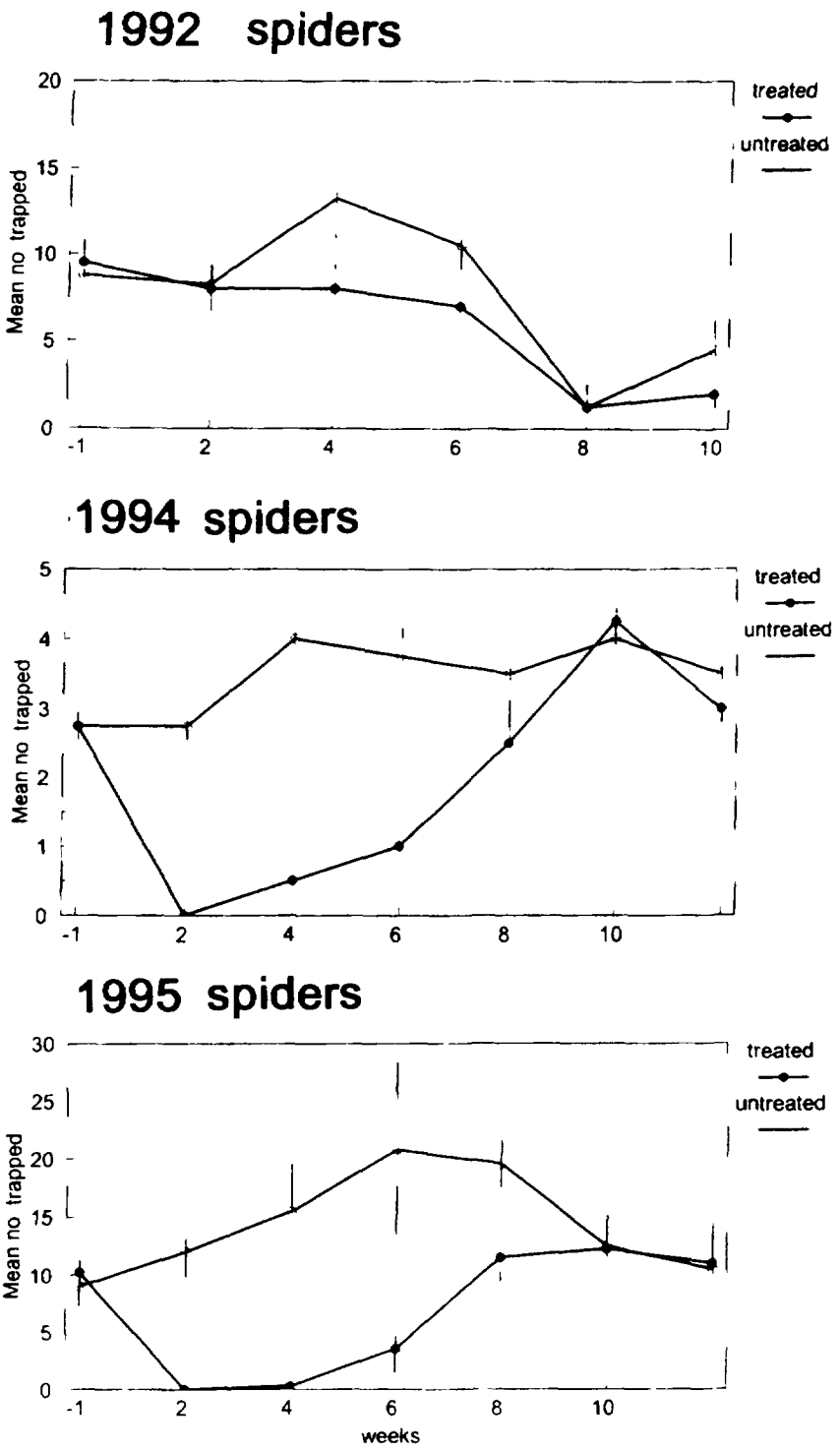


FIG. 2. Pitfall trap collections in maize plots.

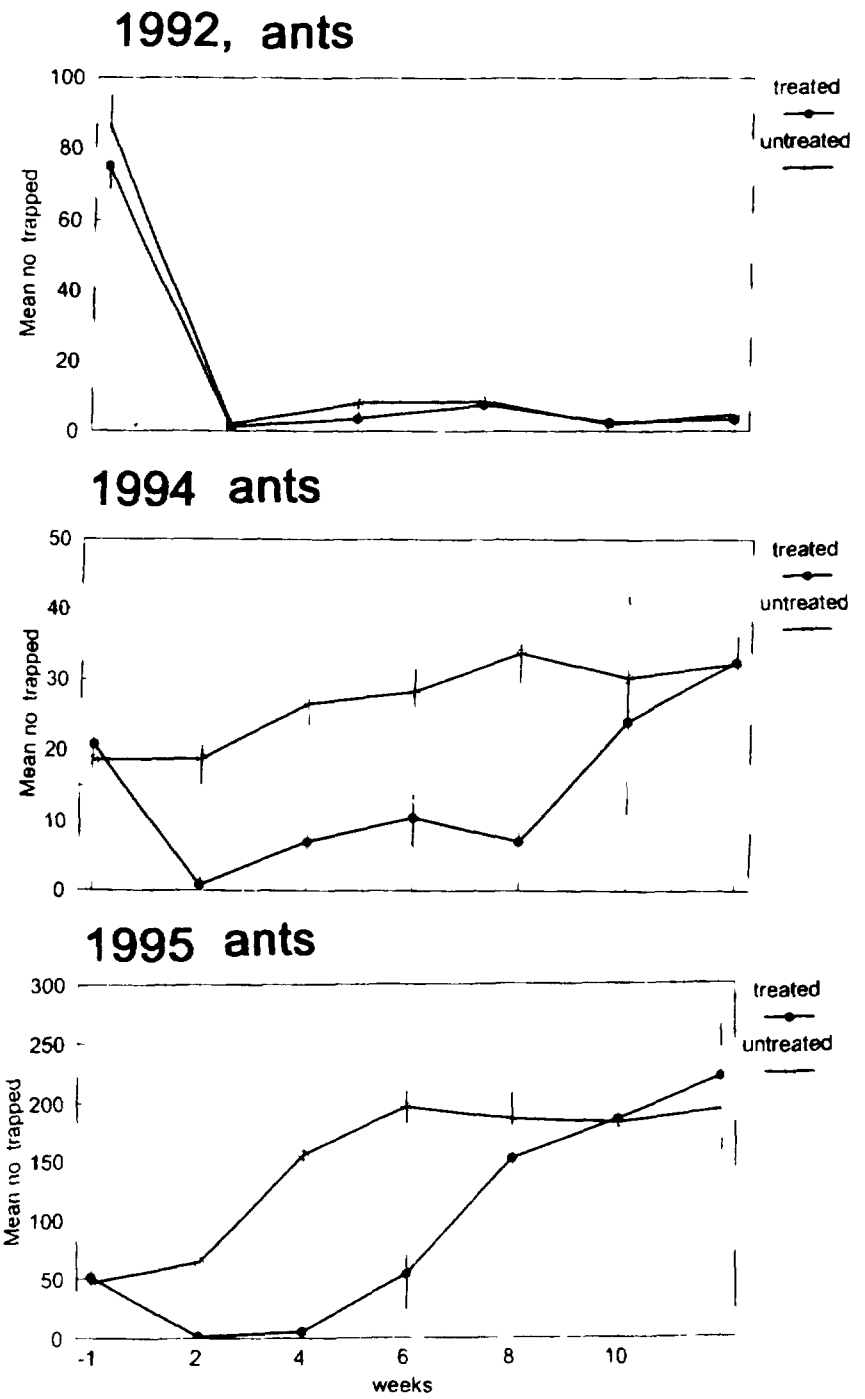

1992, ants
100
80
60
40
20
0
Mean no trapped
treated
untreated
1994 ants
50
40
30
20
10
0
Mean no trapped
treated
untreated
1995 ants
300
250
200
150
100
50
0
Mean no trapped
-1
2
4
6
8
10
weeks
treated
untreated

FIG 2. (cont.)

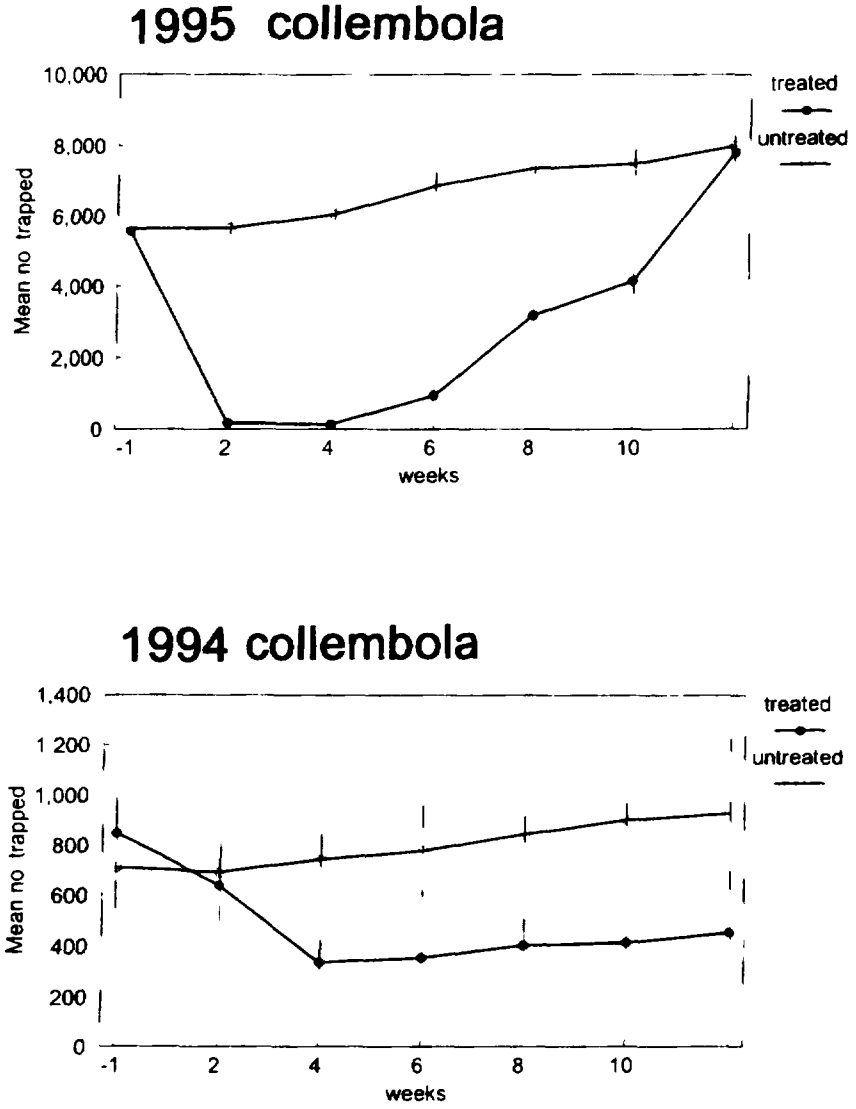
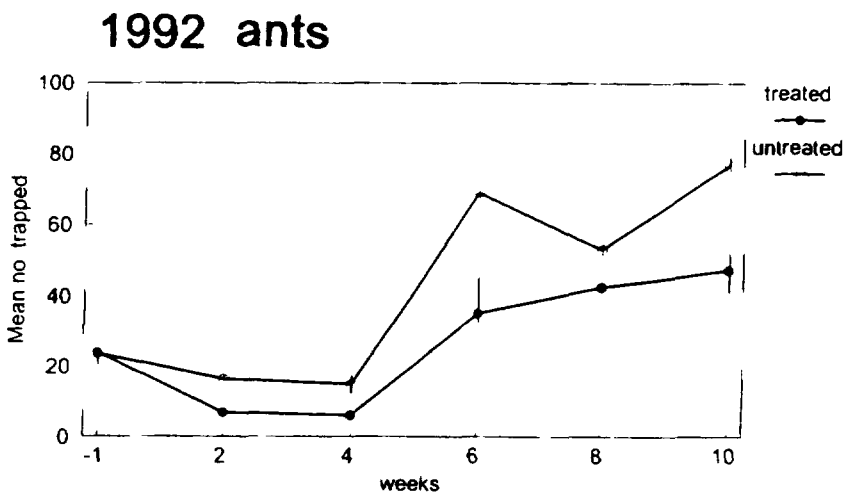


FIG. 2. (cont.)

4.2. Rice 1992

The major stem borers of upland rice are *Diopsis macrophthalma* Dalm., *Chilo zacconius* Bleszynsky, *S. calamistis* and *Scirpophaga* sp. During the vegetative stage of rice, plant damage by stem borers showed up as dead hearts while damage during the reproductive phase resulted in white heads. There was no significant difference in the mean number of dead hearts obtained in the pre-treatment, and in the week 2 samples (Fig. 1). Week 2 sample results may have been because damage attributable to the larvae arising from eggs laid in control subplots between lindane application in the treatment plots and the week 2 sample may not have been manifested. It takes about 12-20 days from egg deposition to appearance of dead hearts. Subsequent samples showed significant differences in the mean number of damaged plants recorded in the treatment compared to the control plots at 5% level.



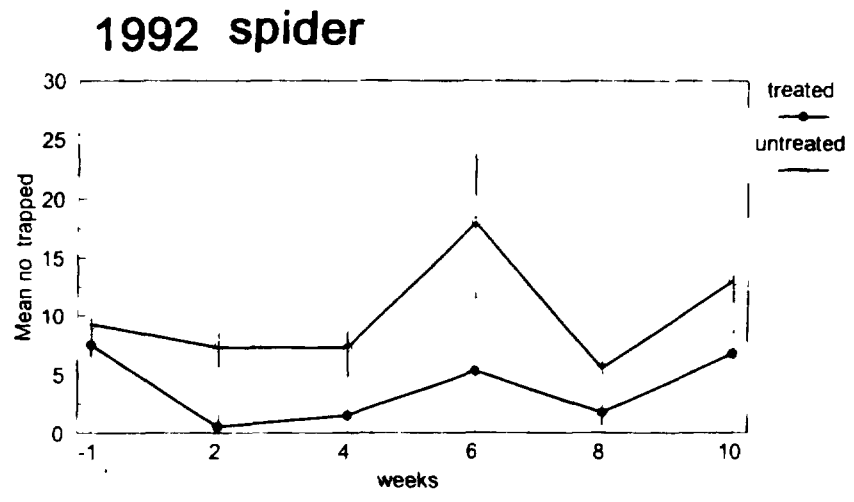


FIG. 2. (cont.)

Table IV. Fauna found in the soil cores taken from the maize and rice fields

Year	Crop	Species	Mean number of species found ±SE			
			pretreatment		posttreatment	
			T	C	T	C
1992	maize	Collembola	150 50 ±16 7	151.00 ±8 7	69 00 ±6 5	155 00 ±19 6*
		Acarina	16.50 ±5.2	16.75 ±2 5	10 25 ±1 9	11 75 ±1 7*
		others	9.75 ±3.1	10.50 ±3.9	9 75 ±3 1	11 00 ±3 4
1992	rice	Collembola	74.25 ±14.1	60.00 ±15 2	27.00 ±4 2	80 75 ±19 9*
		Acarina	10.00 ±3.2	11.00 ±1 4	4 75 ±3 3	13 50 ±6 6
		others	7.75 ±4.0	8.75 ±1.7	3.00 ±1.6	12 00 ±2 2*
1993	maize	Collembola	10.00 ±3.7	9.25 ±1.7	8 00 ±2.4	7 25 ±1 7
		Acarina	5.50 ±1.7	3.75 ±1.0	5 00 ±1.8	4 50 ±1 0
1993	rice	Collembola	52 50 ±5 1	47 25 ±3 3*	43 00 ±7 1	51 25 ±5 5*
		Acarina	12.75 ±1.7	13.50 ±1.7	18 25 ±4.1	27 00 ±3 7*

T = treated plot, C = untreated control plot
* significantly different from the treated plot at P = 0.05

Table V. Retrieval after 3 months of litter buried in the control and treated plots of maize and rice

Crop	Year	Mean retrieved weight, g ±SE	
		Treated	Control
maize	1992	4.70 ± 0.5	3.76 ±0.6
	1993	5.42 ±2.4	4.64 ±0.8
	1994	1.33 ±0.3	0.67 ±0.2*
	1995	2.40 ±0.0	1.71 ±0.2*
rice	1992	5.49 ±1.7	4.46 ±1.0
	1993	5.93 ±2.1	3.81 ±1.4*

* significantly different from the treated plot at P = 0.05

The rice plots had a richer faunal composition than the maize plots. Adult stem borers, *Coccinellidae*, *Formicidae* and *Aranae* were the main groups caught with the sweep net during the vegetative phase. At this stage, most coccinellids caught were the phytophagous species. In the later stages of rice development, a few *Carabidae*, *Dermaptera* and predaceous *Coccinellidae* were also caught in the sweep net. Statistical analyses of sweep net catches of adult stem borers showed significant differences in the mean catches from the lindane treated and the control plots during week 2 and week 4 sampling (Table I). Lindane affected stem borers of rice for up to one month after application. For all the other groups of insects and spiders, except the *Formicidae*, lindane application had no effect. The effects of lindane on *Formicidae* appeared inconsistent.

Pitfall trap collections (Table III and Fig. 2) showed that the important families trapped were *Carabidae*, *Staphylinidae*, *Formicidae* and *Aranae*. Stem borers, particularly, *D.macrophthalma* were also trapped in the pitfalls. In terms of numbers of arthropods caught in both pitfall traps and sweep nets, *D. macrophthalma* was the dominant stem borer species. Carabid beetles were trapped throughout the sample periods but no significant difference was found in their mean numbers in the lindane treated and the control plots. Similarly *Staphylinidae*, though caught on fewer occasions and in lower numbers than *Carabidae*, was also found not to be statistically significant in treatment and control plots at the 5% level of probability.

For *Formicidae* and *Aranae* (Fig. 2) significant differences in their means were recorded in treatment and control plots in all post treatment samples. Thus lindane remained effective against ants and spiders in the rice plots for more than two months after its application.

Soil fauna data (Table IV) showed a mean of 81 *Collembola* in the control plots against 27 in the lindane treated plots. These data were found to be statistically significant at the 5% level of probability. Mite populations, as well as rates of leaf disc disintegration, were unaffected by lindane in the rice plots. The difference in rice yield from treatment (2.48 t ha^{-1}) and control (2.10 t ha^{-1}) plots was not statistically significant.

4.3. Maize 1993

Stem borer damage increased steadily with time and ranged from 1.90% in the pre-treatment to 14.90% in the post-treatment control plots (Fig. 1). In lindane treated plots, plant

damage ranged from 1.35% before treatment to 3.53% after treatment. These differences between treated and control were statistically significant at a 5% level. Thus, the split dose application of 1 kg AI ha^{-1} of lindane provided protection to the plants against stem borer attack for up to 6 weeks. Larval populations determined by stem dissection showed that more larvae (X = 17) were recovered from the control plots than from the lindane treated plots (X = 4) and these were found to be statistically significant at the 5% level. Similarly stem borer damage (stem tunnelling), also determined by stem dissection, showed that a mean of 27 and 18 stems were damaged in the control and the lindane treated plots, respectively, and these were found to be statistically significant. On the other hand, more cobs were damaged in treatment plots (X = 12) than in control plots (X = 6.3) and these data were also statistically significant. None of the target families were caught in the sweep net in 1993.

Generally, pitfall trap catches were lower in 1993. There was no significant difference in the mean numbers of *Carabidae* and *Aranae* caught in the pre-treatment samples. Post-treatment samples of *Carabidae* showed inconsistent trends thus making interpretation difficult. Except the first post treatment sample, lindane application affected spider catches significantly in the treatment plots throughout the sampling period.

Mean dry weights of leaf discs (Table V) retrieved from the lindane treated plots (5.42 g) were not statistically significant from the dry weight of leaf discs retrieved from control plots (4.64 g) at a 5% level. *Collembola* and *Acarina* populations extracted from the treatment and control soil cores two months after lindane application were not significant different.

Maize yield was found to be significantly higher (5.03 t ha^{-1}) in the treatment than in the control plot (3.61 t ha^{-1}).

4.4. Rice 1993

The major stem borer species monitored was *D. macrophthalma*. However, stem borer damage reported here resulted from the combined effects of *D. macrophthalma* and other stem borers. Plant damage was found to be significantly lower in the treatment than the control plots, in all but the last two post-treatment samples. Sweep net samples showed low populations of *D. macrophthalma* in the treatment plots at 2 and 4 week after lindane application. Differences in the mean numbers of *D. macrophthalma* found in the samples from both the treated and the control plots were not statistically significant throughout.

Pitfall trap collections of *Carabidae* showed that the lindane application reduced carabid populations significantly, for up to 4 weeks after application. Post-treatment soil fauna samples showed that significantly fewer *Collembola* and *Acarina* were extracted from the lindane treated plots, and the mean dry weight of leaf discs retrieved was also significant ($P < 0.05$) in treatment compared with control plots. The yield obtained from treatment plots (2.45 t ha^{-1}) was not statistically different from that obtained from control plots (2.08 t ha^{-1}).

4.5. Maize 1994

Stem borer damage increased steadily with time although damage was lower in treatment than control plots. Damage caused by stem borers was significantly less in the lindane-treated compared with the control plots during the first 6 weeks after lindane application (Fig. 1). Another type of plant damage which became important this year was that

caused by the coccinellid, *Epilachna* sp. which feeds on the leaf tissues of young maize plants, leaving large strips of papery epidermis. Although this damage was not quantified, visual observation showed far greater damage in control than treatment plots.

D-vac samples of plants showed that *Coccinellidae*, *Formicidae* and *Aranae* were the main families caught. Pre-treatment samples showed that in these 3 families, there were no significant differences in the mean numbers caught in the lindane-treated and the control plots. Samples taken 1 d after lindane application showed that no insects or spiders were caught on plants in the lindane treated plots. Subsequent samples also showed significant differences in the numbers of insects and spiders caught in treatment compared with control plots for between 1 and 2 months after treatment (Table VI).

More faunal specimens were collected in D-vac ground samples than from plants. Specimens collected included those of *Collembola*, *Acarina*, *Carabidae*, *Formicidae* and *Aranae*. The mean number of insects, spiders, and mites collected in the pre-treatment and 1d post-treatment samples were statistically similar in all plots. Similarity in these results occurred because specimens killed by lindane the previous day were also picked up by the D-vac. Subsequent soil surface samples showed that significantly fewer *Collembola* and *Acarina* were caught in the treatment than control plots (Table VII) at the 5% level, throughout the sampling period. Carabid catches in D-vac samples were low and not statistically significant between the treatment and the control plots. Lindane application caused significantly fewer spiders and ants in the lindane treated than the control plots during the first 6 weeks of the initial lindane application.

Pitfall trap collection showed that lindane affected Collembola, Aranae and Formicidae populations adversely (Fig. 2). Spider and ant populations recovered by the 8th week. Springtail populations built up steadily in the treatment plots throughout the experiment, but on a significantly lower level than in the control plots. Pitfall catches of Carabidae were statistically similar in both plots throughout the experiment (Table II).

Table VI. Insect species caught from the maize plants by the D-VAC sampler

Year	Weeks after treatment	Mean number of specimens caught ±SE					
		Aranae		*Coccinellidae*		*Formicidae*	
		T	C	T	C	T	C
1994	pretreatment	2 50 ±0 6	2 25 ±0 5	4 00 ±0 8	4 00 ±0 8	1 50 ±1 0	1 25 ±0 5
	1day	0 00	2 50 ±0 6*	0 00	3 00 ±0 8*	0 25 ±0 5	3 50 ±1 3*
	2	0 00	1 25 ±0 5*	2 75 ±0 5	5 00 ±0 8*	0 25 ±0 5	2 25 ±1 0*
	4	1 00 ±0 8	3 00 ±0 8*	4 75 ±1 0	6 75 ±1 3*	3 00 ±1 2	5 25 ±2 2
	6	1 00 ±0 8	2 75 ±0 5*	2 50 ±0 6	3 00 ±0 8	7 00 ±1 8	11 75 ±2 4
1995	pretreatment	0 75 ±0 5	0 50 ±0 6	1 25 ±0 6	1 50 ±0 6	0 00	0 25 ±0 5
	1day	0 00	0 75 ±0 5	0 00	1 50 ±0 6*	0 00	0 00
	2	0 00	0 75 ±0 5	0 00	2 00 ±0 8*	0 00	2 00 ±0 8*
	4	0 00	1 00 ±0 5	0 75 ±0 5	3 25 ±0 5*	0 50 ±0 6	5 25 ±2 2*
	6	1 50 ±1 0	1 25 ±0 5	4 25 ±1 0	3 75 ±1 7	2 00 ±1 4	9 75 ±1 3*

T = treated plot, C = untreated control plot

* significantly different from the treated plot at P = 0.05

Table VII. Insect species caught from the soil in the maize plots by the D-VAC sampler

Year	Weeks after treatment	Mean number of specimens caught ±SE							
		Acarina		*Aranae*		*Carabidae*		*Collembola*	
		T	C	T	C	T	C	T	
1994	pretreatment	46 8 ±10 1	40 2 ±1 8	1 0 ±0 8	1 50 ±1 3	1 25 ±1 0	1 00 ±0 8	469 ±100 9	4
	1day	43 5 ±5 8	41 5 ±4 8	0 5 ±0 6	1 00 ±0 8	0 5 ±0 6	2 00 ±0 8	424 ±83 3	4
	2	12 8 ±3 6	43 8 ±4 3*	0 5 ±0 6	1 25 ±0 5*	0 25 ±0 6	1 00 ±1 0	41 8 ±3 2	5
	4	18 8 ±6 7	49 5 ±5 2*	0 5 ±0 6	1 75 ±0 5*	0 25 ±0 5	0 5 ±0 6	127 ±23 2	4
	6	30 2 ±5 8	51 8 ±2 1*	1 0 ±0 6	3 00 ±1 4*	0 5 ±0 6	0 75 ±0 5	141 ±37 2	5
	8	40 2 ±7 1	73 0 ±4 1*	2 0 ±0 8	3 00 ±0 8	0 75 ±0 5	1 75 ±0 5	160 ±43 1	5
	10	58 2 ±6 5	41 2 ±2 2*	3 5 ±1 3	2 75 ±0 5	1 25 ±0 5	1 5 ±0 6	295 ±34 2	5
1995	pretreatment	38 5 ±2 5	39 8 ±3 1	1 25 ±0 5	0 75±0 5	3 00 ±5 4	0 50 ±0 6	276 ±20 4	2
	1day	39 3 ±2 6	34 5 ±3 0*	1 00 ±0 8	0 75 ±0 5	0 00	0 50 ±0 6	276 ±19 3	2
	2	8 8 ±1 8	38 0 ±2 2*	0 00	2 25 ±1 3*	0 00	0 00	21 8 ±2 2	2
	4	8 5 ±1 3	40 5 ±1 3*	0 25 ±0 5	1 25 ±1 0	0 25 ±0 5	0 50 ±0 6	43 0 ±13 0	2
	6	20 5 ±4 1	44 0 ±5 7*	1 00 ±0 8	0 75 ±1 0	0 25 ±0 5	0 25 ±0 5	78 0 ±14 8	2
	8	35 5 ±4 1	41 8 ±3 9*	0 75 ±1 0	1 00 ±0 8	0 00	0 00	245 5 ±23 6	3
	10	40 3 ±2 4	41 0 ±4 1	1 25 ±0 5	1 25 ±0 5	0 50 ±0 6	0 00	280 5 ±27 1	3

T = treated plots, C = untreated control plots
* significantly different from the treated plot at P = 0 05

Rates of leaf disc degradation were significantly lower in the lindane treated than in the control plots. Yields, though higher in the treatment (4.53 t ha^{-1}) than in the control plots (3.51 t ha^{-1}) were not statistically different. There was a drop in yield in the lindane treated plots from 5.03 t ha^{-1} in 1993 to 4.53 t ha^{-1} in 1994.

4.6. Maize 1995

As in the previous years, stem borer damage increased steadily with time in both treatment and control plots, although at a significantly lower level in treatment than control plots (Fig. 1).

Arthropod samples (Table VI) had no significant difference in the mean numbers of specimens of the various families obtained in the pre-treatment samples from both control and treatment plots. Similar to the results of the previous year, no insects or spiders were caught on the plants in treatment plots 1 day after treatment. *Epilachna* sp. was found to be an important pest of maize seedlings this year, but was not found on the plants in the treated plots two weeks after lindane application. By the 4th week, coccinellids were recorded on plants in treated plots but their number (X = 0.75) was significantly lower than control plots (X = 3.25) at 5% level.

Like in the previous year, there were no significant differences in the mean numbers of insects and spiders obtained in D-vac ground samples of the treated and the control plots in the pre-treatment and 1 day post treatment samples (Table VI). Subsequent samples showed that the lindane application significantly reduced *Formicidae*, and *Collembola* and *Acarina* populations for about 4 and 8 weeks, respectively, after application. The effects of lindane on carabid populations was not significant. In general, few *Aranae* were caught in the D-vac this year and analysis showed no significant difference in numbers caught in treated and control plots on most sampling dates. This result may be misleading because of the few numbers involved.

Pitfall traps were left in the field for 1 week and this resulted in richer collections of *Collembola* and *Formicidae* than in the previous year. *Collembola* trapped in pitfalls in the pre-treatment samples were enormous (X = 5629 and 5555 for control and treatment plots, repectively) compared to the previous years. Samples 1 day after treatment showed that the *Collembola* population in the lindane treated plots had crashed (X=173) compared with the controls (X = 5651). The *Collembola* population remained significantly lower in the lindane-treated than in the control plots throughout the experiment. For *Formicidae*, the mean catches in the treatment plots remained significantly lower up to 4 weeks after lindane application.

The mean dry weight of leaf discs retrieved from treatment and control plots were statistically different. Higher yields (4.03 t ha^{-1}) were obtained in treatment than control plots (3.60 t ha^{-1}), and their differences were statistically significant. Decrease in yield in treatment plot was also recorded from 4.53 t ha^{-1} in 1994 to 4.03 t ha^{-1} in 1995.

5. DISCUSSION

Lindane is used extensively in Nigeria on cocoa and sometimes also in rice farms. However, it is not recommended for use on maize. Throughout, the maize crop showed phytotoxicity after lindane application and this tended to retard plant growth for a while.

Lindane protected maize and rice plants effectively from stem borer damage in most years. However, this protection was not always reflected in crop yields. Indeed, significant differences in maize yields in the lindane-treated compared with the control plots were recorded only in 1993 and 1995. Rice yield in 1992 and 1993 were not statistically significant. This would suggest that stem borers and perhaps other pests controlled by lindane were not the only determining factors in yield in these experiments. It is possible that lindane may have had an indirect negative effect on yields which declined in the treatment plots over the years. Perfect et al. [6] recorded similar decline in yields of cowpea grown on soils treated with DDT in Nigeria over a 4-year period. They postulated that DDT adversely affected the numbers and structure of soil fauna as well as the decomposition of organic matter which affected some soil properties. Their postulation was based on an earlier paper [9] which showed that elimination of soil fauna by fumigation adversely affected plant growth. The indirect effects of lindane on yield, speculated in this work, may be the result of its adverse effects on some aspects of soil biology which in turn affected yield.

The target soil fauna in these experiments were *Collembola* (springtails) and *Acarina* (mites). The mites included both predatory and detritivorous species. These soil invertebrates are micro-arthropods which, by virtue of their sizes should easily be affected by pesticides. The degree of susceptibility of these micro-arthropods to lindane in these experiments appeared to depend on the concentration of the spray solution, the quantity of the spray solution that reached the soil and perhaps also, their intrinsic ability to resist adverse effects of lindane. In the maize plots (1992) with plant distances of 1m and 500mm between rows and hills, respectively, large areas of soil surface were exposed and received lindane (1 kg AI ha^{-1}) sprays directly, and this decimated both *Collembola* and *Acarina* populations, resulting in significantly fewer numbers recovered in the lindane treated than the control plots in the post- treatment samples. In 1993, with reduced inter-row distances, a closer plant canopy was formed, which reduced the exposed soil surface thereby giving greater protection to the soil fauna. The split dose application of 1 kg AI lindane per hectare this year may also have helped these micro-fauna to resist lindane toxicity resulting in no significant differences in the mean numbers of both *Collembola* and *Acarina* recorded in treatment and control plots.

In rice (1992) with plant distances of 150 mm both between rows and hills, a close plant canopy was formed, leaving limited exposed soil surfaces which received lindane sprays (1 kg AI ha^{-1} in a single dose) directly. In this situation, the *Acarina* which probably had greater intrinsic resistance to lindane than the *Collembola* were unaffected. However in 1993, significantly fewer *Acarina* was recorded in the lindane treated than in the control plots. It is possible that rainfall in the season had spread lindane residues from where the lindane was deposited to other areas in the plot in 1992. Igbariam had a total annual rainfall of 2181 mm in 1992. Ploughing and harrowing in 1993 may have also helped to redistribute residual lindane in the farm. Samuel and Pillai [10] reported that about 15% of the initial concentration of lindane applied to an Indian soil remained as extractible and bound residues one year after application. Yeadon and Perfect [11] found that after 1year of soil treatment with DDT, 4.2% of the initial concentration was detected as residues (chiefly DDE) and after 4years the percentage of the detectable DDE rose to 24%. With 1993 spray, the level of lindane in the soil may have become high enough to also affect *Acarina*.

Micro-arthropods, particularly *Collembola*, which feed on leaf litter, are regarded as important in the fragmentation and incorporation of leaf litter into the soil. Surprisingly, in these experiments (1992 and 1993), there was no significant difference in the mean dry weight of leaf discs retrieved from treatment and control plots in spite of the significant differences in collembola population in treated and control plots. On retrieval of litter bags, they were found to be infested with young earthworms, nematodes, other unidentifed mesofauna, roots of weeds and in a few cases, ants. These agents, together with some soil microflora (bacteria and fungi) probably played more important role in litter fragmentation inside the soil than collembola.

The effects of lindane on the agents of litter fragmentation when the same plot was used over the years appeared to be cumulative. For example, in 1992 (on rice) and 1993 (on maize), the effects of lindane on litter disintegration was not significant. In subsequent years however, the effect became progressively more significant ($P<0.024$ in 1994 and $P<0.005$ in 1995). This may suggest a cumulative effect due probably to lindane residue which may have increasingly accumulated over the years to a level that drastically affected agents of litter fragmentation in sprayed plots in 1995.

Throughout these experiments, spiders and ants were consistently adversely affected by lindane. Spiders are generalist predators known to range freely over sprayed vegetation and soil surfaces in search of their preys. Critchley et al. [5] also recorded adverse effects of DDT on spiders and ants.

In general, a sweep net appeared inappropriate for sampling maize plants as very few specimens were caught in sweep net compared with the numbers caught with the D-vac sampler.

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THE DEVELOPMENT OF LABORATORY AND SEMI-FIELD METHODS TO TEST THE EFFECTS OF PESTICIDES ON PREDATORY BEETLES

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Abstract

Following the sequential testing procedure adopted by the IOBC/WPRS Working Group Pesticides and Beneficial Organisms, two simple, robust methods are presented which were designed for testing the effects of pesticides on predatory beetles. In a laboratory initial toxicity test both DDT and lindane were found harmful to the carabid *Pterostichus cupreus*, whereas α-endosulfan was 'harmless' DDT was found harmless to *P melanarius* Sub-lethal doses of both DDT and lindane incorporated in prey caused *P cupreus* females to produce smaller eggs In a semi-field test it was demonstrated that Lindane reduced the beneficial capacity of *P cupreus* Climatic conditions at the time of the test however were such that the majority of test animals in control treatments escaped Caution was therefore advised in the choice of test animal and test design for the semi-field test.

1. INTRODUCTION

Testing or screening for the side-effects of pesticides on beneficial arthropods is gaining increasing attention by research workers in different parts of the world [1]. Results from such tests enable agronomists to choose safe preparations for use in Integrated Pest Management schemes. They can also be of use to authorities concerned with pesticide registration.

Recognising the need for standardised testing methods and greater international co-operation, a Working Group Pesticides and Beneficial Organisms was formed in 1974 within the framework of the International Organisation of Biological Control (IOBC), Western Palaeartic Regional Section (WPRS) [2]. Aware of the fact that no single test method would provide sufficient information to show the side-effects of a pesticide on a beneficial organism, this group devised a sequential procedure that includes laboratory, semi-field and field methods. These methods are carried out in a particular sequence progressing from laboratory to semi-field and, ultimately, to a field test only if a preparation under examination is found to be harmful to the test organism. The reasoning behind this is that pesticides found harmless to a particular beneficial in a stringent laboratory initial toxicity test are most likely to be harmless to the same organism in the field.

In this paper we adapt a laboratory and a semi-field test method, originally designed for and used in the IOBC Working Group, to examine the direct effects of organochlorine pesticides on predatory arthropods. We also examine whether any sub-lethal effects occur when predatory beetles consume organochlorine-tainted prey. The aim has been to produce protocols for robust test methods that could be used by African colleagues participating in the FAO/IAEA/SIDA Co-ordinated Research Programme on Adverse Effects on Flora and Fauna from the Use of Organochlorine Pesticides on the African Continent.

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2. MATERIALS AND METHODS

2.1. Selection and treatment of test animals

The beetles chosen to illustrate our test methods were two medium-sized carabids, *Pterostichus cupreus* L. and *Pterostichus melanarius* (Illiger) (Carabidae, Coleoptera). Both are common in agricultural fields in Sweden and both have been shown to prey upon the most serious aphid pest in Swedish cereals, *Rhopalosiphum padi* L.[3]. Large numbers of beetles were caught in May and June 1991 in dry pitfall traps. The latter were placed on uncultivated land where no previous pesticide treatments had been made. Beetles were held in plastic containers lined with moistened tissue paper, in a refrigerator (5°C) and fed dog biscuits. All beetles were acclimatised to test conditions (15°C, 16 h light, 8 h dark, 70% r.h.) at least 72 h prior to testing.

2.2. Determination of insecticidal activity in laboratory tests

The basic test method was as follows: Individual beetles were transferred to small plastic beakers (height, 60 mm; diameter, 60 mm) containing 20 mm of even-grained sterile sand. Twenty such beakers were then placed in four rows of five on a plastic seeding tray (400 x 600 mm x 80 mm deep) in a fume cupboard. The beakers (+ beetles) were then sprayed with a hand-held sprayer connected to a compressor set at 35 kPa. The spray nozzle was passed over the beakers at a constant speed and height, calculated to deliver the recommended concentration at the IOBC standard amount (for initial toxicity tests in laboratories) of 6 mg fluid per 100 mm^2 sand. Two test runs of 20 replicates per run were performed for each pesticide/dose (see below). Controls were sprayed with water + alcohol (19 + 1 by volume), as this was used to dissolve the organochlorines tested. Between sprays, the sprayer was thoroughly washed with repeated dosings of acetone followed by water and then dried.

Treated groups were transferred to a large, well ventilated controlled climate room set at the above conditions. The small beakers were then inserted into larger ones of the same diameter (i.e.height, 60 mm; diameter, 60 mm). This ensured that the beetles a) could not escape and b) did not come into contact with excess pesticide on the beaker walls. Each beetle received one dog biscuit as food , and this was moistened daily. Beetles were examined at 1, 6 and 24 h post treatment, and thereafter daily until the sixth day whereupon the test was terminated. Numbers of beetles found dead or moribund were recorded.

Two toxicity tests were performed using this basic method. Different dilutions of DDT, starting with a 'field dose' equivalent to 1000g AI ha^{-1} (as this was the most common dose found in the literature) were tested on *P. melanarius,* and several organochlorines (i.e. DDT at a rate equivalent to 1000g AI ha^{-1}, α- and β-endosulfan : both at 1100g AI ha^{-1} and lindane : at 350g AI ha^{-1}) were screened for toxicity to *P. cupreus.* In both tests water containing alcohol (19 + 1 by volume) was used as a control treatment.

2.3. Determination of sub-lethal effects in laboratory tests

In June 1991, 50 female *P. cupreus* were selected from our field-collected stock. These were placed individually in petri dishes containing moistened pulverised peat, and divided into five groups of ten. Every third day one group of ten beetles were each fed with

one (initially two) blowfly larvae (maggots) which had previously been sprayed with a 1:32 dilution of the 'field dose' DDT used above. The other four groups of ten beetles were fed maggots that had been sprayed with dilutions (1:32) of α- and β-endosulfan , lindane, and water + alcohol (19 + 1 by volume) as control, respectively. After spraying, all but two of the maggots were frozen for later use. At subsequent feeding events, maggots were thawed and then almost cut in half prior to feeding in order to ensure that the beetles could penetrate the larval cuticle. Twice a week beetles were temporarily removed from the petri dishes and the peat substrate was replaced. The used substrate was washed through a fine-meshed sieve. Any eggs the beetles had laid in the substrate were subsequently caught in the sieve. The number of eggs found per female were recorded. Eggs were placed briefly on a filter paper to dry and then weighed.

2.4. Determination of insecticidal activity in semi-field tests

The species of beetle used in this test (*P. cupreus)* was collected and held under the same conditions as above. In June 1992, 16 plastic seeding trays (400 x 600 x 80 mm) were half-filled with potting soil and sown with three rows of spring barley at normal field seed rates (4-500 per square meter, in this case ~50 plants per 600 mm row) and at a row width of 125 mm. Trays were then placed in a greenhouse to hasten germination. The inner edges of each tray, from the soil surface to the rim, were painted with Fluon (polytetra-fluoroethylene) to discourage beetles from climbing the tray walls and escaping. When the plants had reached growth stage 32 4 four of the trays were taken outdoors and sprayed with Lindane at a 'field ' dose equivalent to 300 g AI ha^{-1}. Four trays were sprayed with a dilution (1:4) of the field dose, and a further four trays were sprayed with a second dilution (1:16). The remaining four trays were sprayed with water + alcohol (19 + 1 by volume) containing several drops of a wetting agent (Triton X-100) as controls. The trays were allowed to dry for 24 h, after which four *P. cupreus* were released onto each tray. The trays were then placed in a large, outdoor bird-proof cage and the beetles allowed to acclimatise for 48 h.

At 72 h post-treatment, each tray was provided with 100 freshly-frozen *R. padi* arranged on four moistened filter papers with 25 aphids per paper. The filter papers were placed on the soil surface and covered with a plastic roof to protect the aphids from the rain and wind. The number of aphids removed after 24 h was recorded. This procedure was repeated at weekly intervals for 3 weeks. Four fresh beetles were released onto each tray 24 h before each feeding occasion. The mean total number of aphids eaten per treatment and per feeding occasion was noted and the results examined by analysis of variance (ANOVA).

Dead beetles found on the soil surface were removed during the course of the experiment and numbers recorded. After the last feeding period, plants were cut at the stem base and removed from the trays. The soil surface was then carefully searched and surviving beetles removed with a pooter. Thereafter, the soil was sieved onto a larger tray and the beetles that had buried into the soil were recovered. The mean number of survivors per treatment was noted and the results analysed by ANOVA.

3. RESULTS

3.1. Insecticidal activities in laboratory tests

The chosen 'field' dose of 1000g AI ha^{-1} DDT caused 40% mortality in *P. melanarius* with dilutions of this field dose giving a typical dose response curve (Fig. 1). In contrast, the

same field dose of DDT caused 100% mortality in *P. cupreus*, as did the chosen field dose of lindane, whereas α-endosulfan only caused 30% mortality even at the relatively high dose of 1100 g AI ha-1 (Fig. 2).

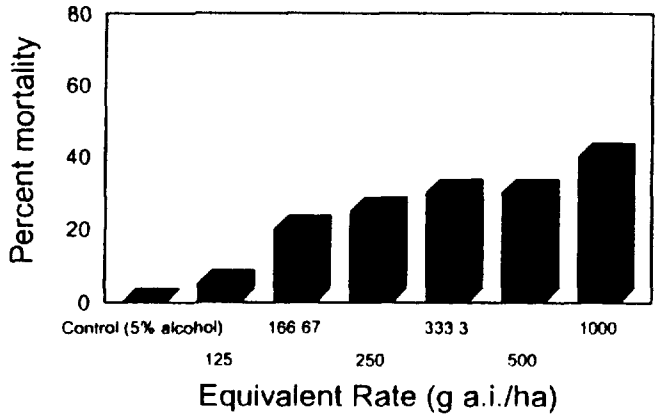

Fig 1. Initial toxicity of DDT to the carabid beetle *Pterostichus melanarius*

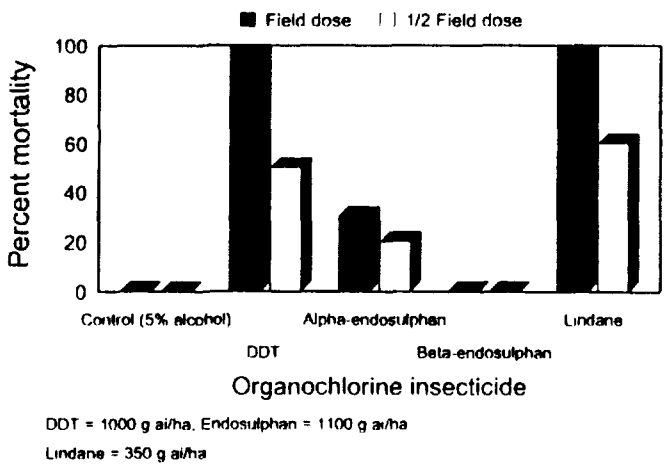

Fig 2. Selectivity of organochlorine insecticides to the carabid beetle Pterostichus cupreus in a laboratory initial toxicity test

3.2. Sub-lethal effects in laboratory studies

No significant differences were found between the number of eggs laid by female *P. cupreus* fed on organochlorine tainted prey or fed on water treated control prey ($F = 0.28$, d.f. = 50, n.s.). Eggs from female beetles fed sub-lethal doses of DDT and lindane, however, were significantly smaller (Table 1).

3.3. Insecticidal activities in a semi-field test.

The mean number of aphids eaten per treatment for each feeding occasion are given in Table 2. Mean numbers of aphids eaten in the field dose treatment were always significantly

lower than numbers in the diluted (1:4) field dose treatment. Surprisingly, mean numbers eaten in the control were not significantly different from those in either the field dose or from the different dilutions of the field dose. An explanation was found at the end of the experiment when beetles were recovered. Only one beetle of a treatment total of 48 was recovered from the control trays. There was no significant difference in the number of survivors in the treated plots (Table 3), although dead beetles were only recovered from the field dose (1:1) treatment.

Table 1. Sub-lethal effects of organochlorine tainted prey on egg weights of the carabid beetle *Pterostichus cupreus*.

Pesticide	Egg wt. (mg)	N	P
DDT	0.65	291	0.0001
endosulfan	0.70	288	n.s.
endosulfan	0.69	261	n.s.
lindane	0.63	315	0.0001
water control	0.71	254	

n.s. = not significant in a Student's T-test against control

Table 2. Consumption of aphids by the carabid *Pterostichus cupreus* in arenas sprayed either with different dilutions of lindane or with water treated controls.

		lindane (300g AI ha^{-1})		
Week after treatment	Control	1:1	1:4	1:16
1	12.9 ab	4.3 b	20.9 a	16.4 ab
2	14.3 ab	4.5 b	20.8 a	20.0 a
3	14.6 bc	11.1 c	24.4 a	24.4 ab

Numbers with the same letter are not significantly different at $P < 0.05$ (Duncan's multiple range test

Table 3. Recovery of alive and dead *Pterostichus cupreus* from arenas sprayed either with lindane dilutions or water treated controls.

		lindane (300 g AI ha-1)		
Insect status	Control	1:1	1:4	1:16
live	0.3 b	1.8 ab	3.5 a	2.5 a
dead	0	41	0	0

Numbers with the same letter are not significantly different from the control at $P < 0.05$

4. DISCUSSION

The carabid species chosen for the semi-field test, *P. cupreus*, is macropterous and can therefore fly. However, extensive behavioural studies of this species (in which beetles confined on similar arenas were video filmed at different temperatures in a controlled climate chamber 5) had revealed that the beetle rarely flies unless the soil surface temperature exceeds ~ 30°C, whereupon they climb to the top of the plants and fly away. Unfortunately the summer of 1992 was one of the hottest and driest on record in Sweden, with ambient temperatures often exceeding 26-28°C throughout the duration of the experiment! Soil surface temperatures, usually 10°C higher than ambient temperature, would therefore have exceeded the 30°C upper threshold for this species and caused it to flee. Interestingly, fewer beetles fled, or were capable of fleeing, from the 1:4 and 1:16 dilution treatments of lindane as significantly more were recovered from the arenas compared with controls. We have previously used this method successfully to screen the effects of pesticides on a small, brachyopterous carabid, *Bembidion lampros* Herbst., with good results and would therefore encourage the use of the method, though with a caution that the beneficial insect should be carefully selected.

This laboratory test has proved useful, and is robust enough to be carried out in most laboratories. The basic method can even be adapted and used as a persistence test, in which beetles are placed in the beakers at intervals post-treatment. This would be a useful exercise particularly regarding the organochlorines, and might reveal differences between, for example, DDT and lindane regarding the persistence of harmful effects. Should a macropterous species be chosen for the laboratory test, we suggest that a simple muslin cloth coated with Fluon and held in place by a rubber band over the top of the beaker will suffice to prevent escape. This was demonstrated at the CRP Workshop in Arusha where the method to screen the effects of organochlorines was used on a coccinellid species.

The increase in egg production following ingestion of a carbamate (pirimicarb) has previously been observed in *P. cupreus* [6] and is thought to be due to a hormoligosis effect [7]. Egg size has been found to affect the survival of carabid larvae [6], and first instars hatching from large eggs can withstand starvation better than those hatching from small eggs. Therefore ingestion of DDT or lindane tainted prey by female ground beetles could have significant effects on the survival of their progeny. These and other sub-lethal effects are not exclusive to the organochlorines and need to be considered when describing the overall side effects of pesticides.

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EFFECTS OF LINDANE ON A MAIZE ECOSYSTEM AT TPRI, ARUSHA, TANZANIA

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Abstract

The effects of the organochlorine insecticide, lindane, on target and non-target organisms was studied in field plots of maize at Arusha, Tanzania for 4 growing seasons A single application of 1 kg ha ^{1} caused leaf scorch to the crop but two applications of 0 5 kg ha ^{1} at an interval of 2 weeks were tolerated The insecticide reduced damage by the stem borers *Busseola fusca* and *Sesamia calamistis* in all seasons and maize yields were higher on the treated plots although the differences were not always significant (P > 0 05) Collembola and sometimes ant and spider numbers were lower in treated plots early in the season but the differences did not persist Differences in rates of decomposition of buried leaf litter were not significant

1. INTRODUCTION

The use of synthetic pesticides in crop and forestry production as well as in the control of both animal and human disease vectors has been a common practice worldwide. These pesticides are harmful to the environment particularly those which persist for long periods. Persistent pesticides include insecticides in the organochlorine group (e.g. DDT, benzene hexachloride-BHC, chlordane, heptachlor, toxaphene, methoxychlor, aldrin, dieldrin, endrin and endosulfan) which are lipophilic. While most of these have been banned from use in many countries on the basis of their persistence and accumulation in animal and human tissues, some of them are still registered for use in Tanzania [1].

Lindane (gamma benzene hexachloride) is among the organochlorine insecticides registered for use in crop protection in Tanzania. In most developed countries the environmental impact of organochlorine pesticides has been monitored and documented [2]. It has been calculated that as much as 50% of pesticide sprays applied to crops miss their target and fall on to the soil surface. Also the air can easily become contaminated with pesticides during spraying operations [3]. In most developing countries including Tanzania, very little information is available on pesticide effects on the environment. The maize stem borer *Busseola fusca* Fuller is one of the major insect pests limiting maize production in Tanzania. DDT dust and high volume sprays were recommended and commonly used by farmers but after the banning of DDT due to its persistence and hazardous effects on the environment, farmers have been using various other insecticides including lindane. The work being presented was therefore proposed to investigate the effects of lindane on field maize arthropod fauna.

2. MATERIALS AND METHODS

The studies were conducted on a 0.7 ha field at the Tropical Pesticides Research Institute, Arusha for four planting seasons starting in 1991/92 short rain season. Planting dates were based on the on-set of the rains which was November/December (this applied to 1991/92, 1992/93 and 1994/95) for the short rains and March/April for the long rains (1994 trial). Planting of the trial was done in the 1994 long rain season (which is cooler and wetter, and therefore with lower insect populations compared with the warm short rain season) due to failure of the 1993/94 short rain season crop from a severe drought in January/February 1994 The field was first ploughed and for the first two seasons animal manure (cow dung) at 3 tons

per ha was broadcasted and harrowed into the volcanic sand loamy soil which is known to be low in organic matter. The cow dung was applied to the soil a month before planting maize. The field was then divided into four equal blocks which were each further divided into two 25 m x 25 m plots. The plots in a block were each randomly assigned two treatments i.e. sprayed and unsprayed.

Maize hybrid seed CG 4141 was sown at 90 cm x 30 cm at two seeds per hole and the seedlings were thinned to one per hill at three weeks after emergence (3WAE). All field operations apart from tractor ploughing and harrowing were carried out by hand. Fertilizer top dressing using urea at 25 kg N/ha was done twice at 3WAE and 6WAE.

In each of the two plots per block, 5 m x 5 m subplots were marked out permanently for different investigations. Peripheral subplots acted as guard rows. In one subplot 4 standard pitfall traps were installed in the ground for monitoring ground fauna population activity. The traps were set for three days with a weak soap solution. They were then emptied and arthropod fauna sorted out and recorded. A second subplot was used for aerial fauna monitoring in which arthropods were swept from plants using a sweep net (three seasons) and a D-Vac in the fourth season. A third subplot was used for visual fresh plant damage assessment where all plants with fresh borer damage were counted and converted into a proportion of total subplot plant population. A fourth subplot was left undisturbed until harvest for grain yield assessment. A fifth subplot was used for organic matter decomposition studies in which four litter bags containing *Grevillea robusta* A. Cunn leaves were randomly buried for three months. Twenty plants were randomly uprooted from the remaining four subplots at each assessment day for two seasons to assess stem borer larval population and parasite incidence. The stems were each dissected for stem borer larval counts.

The crop was constantly inspected for stem borer eggs and first instar larvae to help in setting out the date for initial insecticide sprays. Spraying was carried out at 4WAE when either eggs and or first instar stem borer larvae were recorded on 0.5-1.0% of the plants. Lindane 20 EC. (Gammalin 20% from Imperial Chemical Industries) was sprayed once at 1.0 kg AI.ha^{-1} in 1991/92 and 1992/93. Because this caused severe leaf scorching the application was split and sprayed twice at 0.5 kg.AI.ha^{-1} at two week intervals starting at 4WAE in 1994 and 1994/95. CP15 and CP20 knapsack hand sprayers with polijet cone type of nozzles and operated at a pressure of 3 x 10^5 Pa (3 bar) were used at high volume rate (300 L water ha^{-1}). Arthropod populations and plant damage were monitored just before the first spray, 24 hours later and then every two weeks until harvest. All data was subjected to analysis of variance.

3. RESULTS

Field observations showed that the maize stem borer, *B. fusca* was the dominant pest early in the four seasons starting towards the end of the 3WAE. Towards the middle of each growing season, however, minor infestations of *Sesamia calamistis* Hamp. occurred and the two borers cohabited on the plants until harvest. Damage assessments were based on *B. fusca* which feeds in the leaf whorl, in the stems and on maturing seed on the cobs. *S. calamistis* normally rings the lower part of the stems and migrate to other stems or to the maturing seeds on the cobs.

The total monthly rainfall amd mean temperatures for the institute where the experiments were carried out are shown in Figures 1-4 corresponding to the four seasons. The results on beneficial arthropod population groups represented by ants, spiders, and collembola,

and plant damage for 1991/92, 1992/93, 1994 and 1994/95 are shown in Tables 1-4, respectively. Buried leaf litter weight losses are shown in Table 5 and maize grain yields in Table 6.

The results for 1991/2 show that arthropod populations were very low in that season. The one lindane application was sufficient to check borer infestations for 7 weeks by which time the plants were old enough to withstand a degree of pest attack.

In 1992/3 lindane significantly reduced Collembola numbers initially but the population recovered to a similar level to that in the control plots by the end of the season. Ant and spider numbers were essentially the same in treated and control plots but treated plots showed significantly less crop damage.

The 1993/4 short season crop failed because of drought so a crop was grown in the 1994 long rainy season. The long rainy season is normally cooler and wetter with relatively low pest insect activity but more abundant beneficial soil arthropods. Splitting the lindane treatment into two applications prolonged the depression of arthropod populations but, as in the previous seasons, they recovered by harvest time. Again, pest damage to the crop was significantly lower on the treated plots. These trends were repeated in 1994/5. In addition a gregarious *Apanteles* spp. which is a parasite of *B. fusca* larvae was observed in similar numbers intreated and untreated plots.

Buried leaf litter decomposition (Table 5) was slightly, but,not significantly, lower in treated than control plots in all three years that litter bags were buried. Grain yields (Table 6) were 31% higher on average in the treated plots over the 4 seasons although the variability was such that the differences were not significant ($p>0.05$).

4. DISCUSSION

The results indicate that lindane was effective in reducing *B. fusca* damage on maize. On the other hand it had adverse effects on most of the early season beneficial arthropod fauna monitored in the four seasons. It was especially so with Collembola, and in some seasons it also affected ants and spiders. Similar reductions in population activity for ants and spiders were observed in the humid tropical zone of Nigeria in plots treated with DDT [4].

The mid- and late-season beneficial arthropods including spiders (Araneae) and ants (Formicidae) were not significantly affected by lindane. Collembola populations were the most abundant beneficial soil microfauna at the beginning of each season but their numbers would normally decline gradually even in the controls as soil moisture decreased towards crop maturity. These results indicate that lindane could have short-lived adverse effects on the maize environment at the rate in which it was used in the warm and semi-humid climatic conditions in which the experiments were conducted. Edwards [5] observed that in a combination of wet and warm weather, the pesticides are released and may break down and disperse into the atmosphere much more rapidly than from dry soils; and that there was evidence that pesticides which persist for more than 10 years in temperate climates may disappear almost entirely in a year in the tropics..The results in this programme from Ghana, Nigeria and Zambia confirm this.

Lindane sprays in the present study seem to have reduced buried leaf litter decomposition rate (though not significantly) in the three seasons. Although there was no

Text cont. on p. 179.

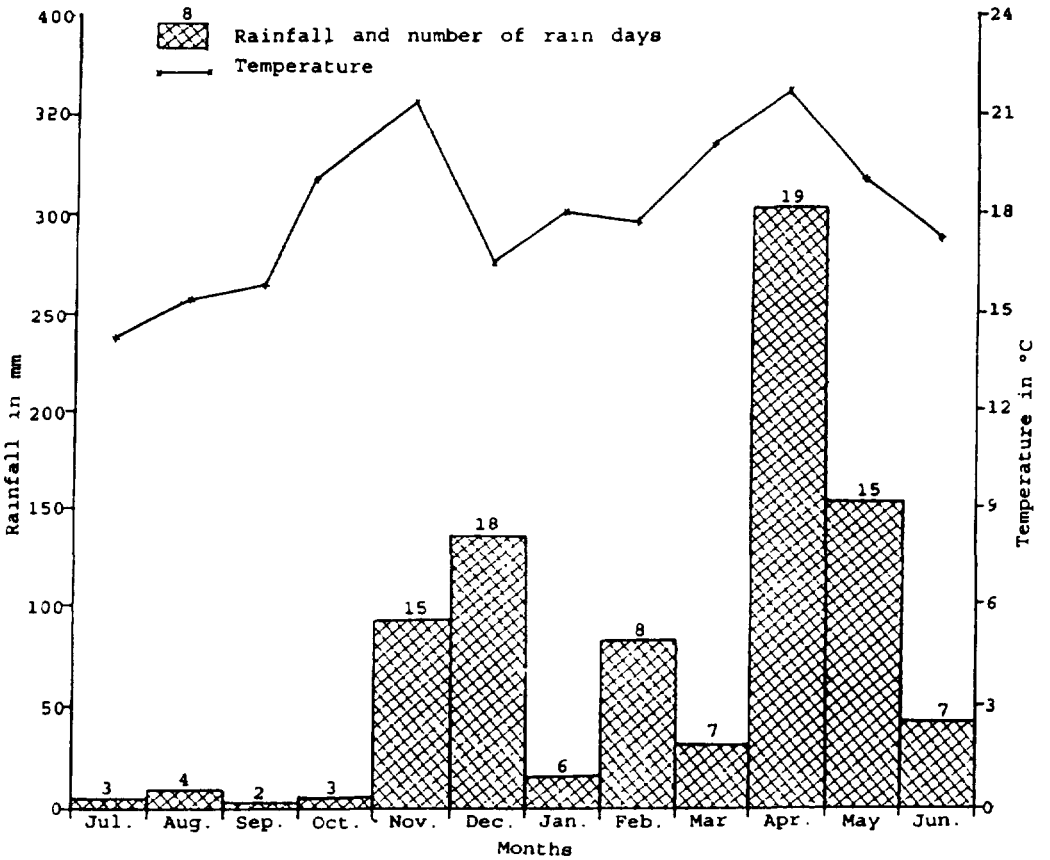

Figure 1: Total monthly rainfall (mm) at T.P.R.I. for July 1991 - June 1992

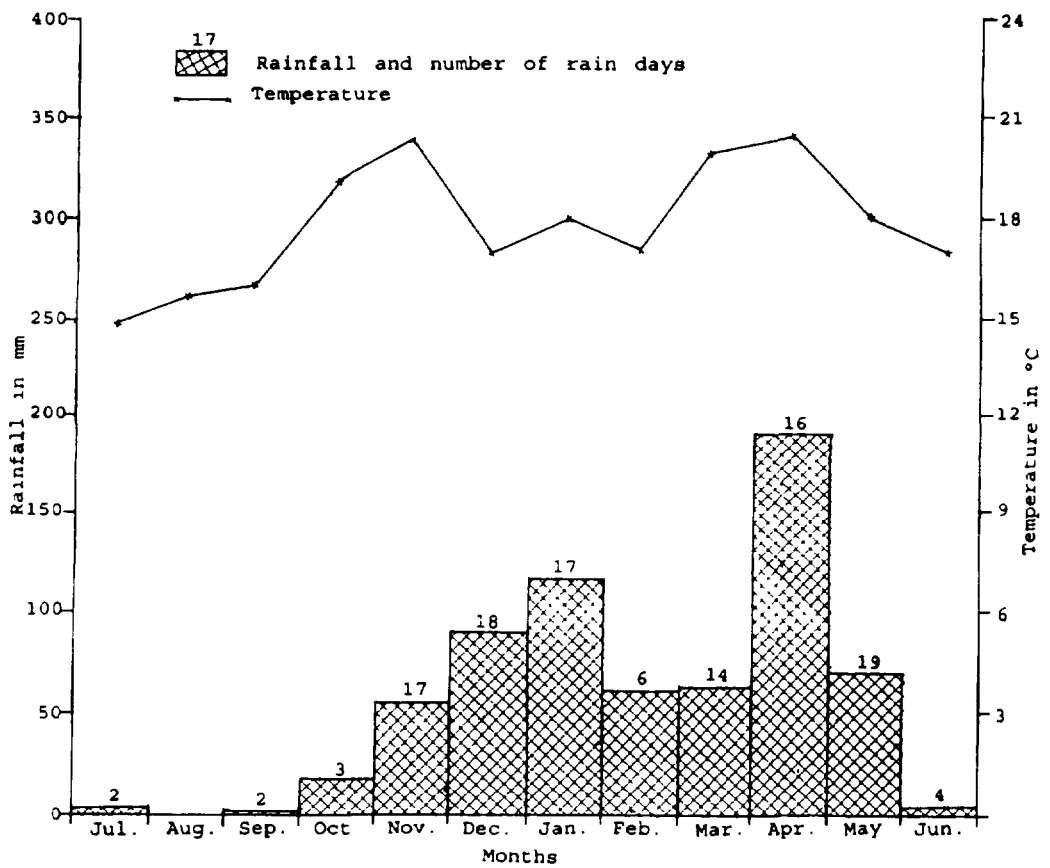

Figure 2: Total monthly rainfall (mm) and mean monthly temperature at T.P.R.I. during 1992/1993

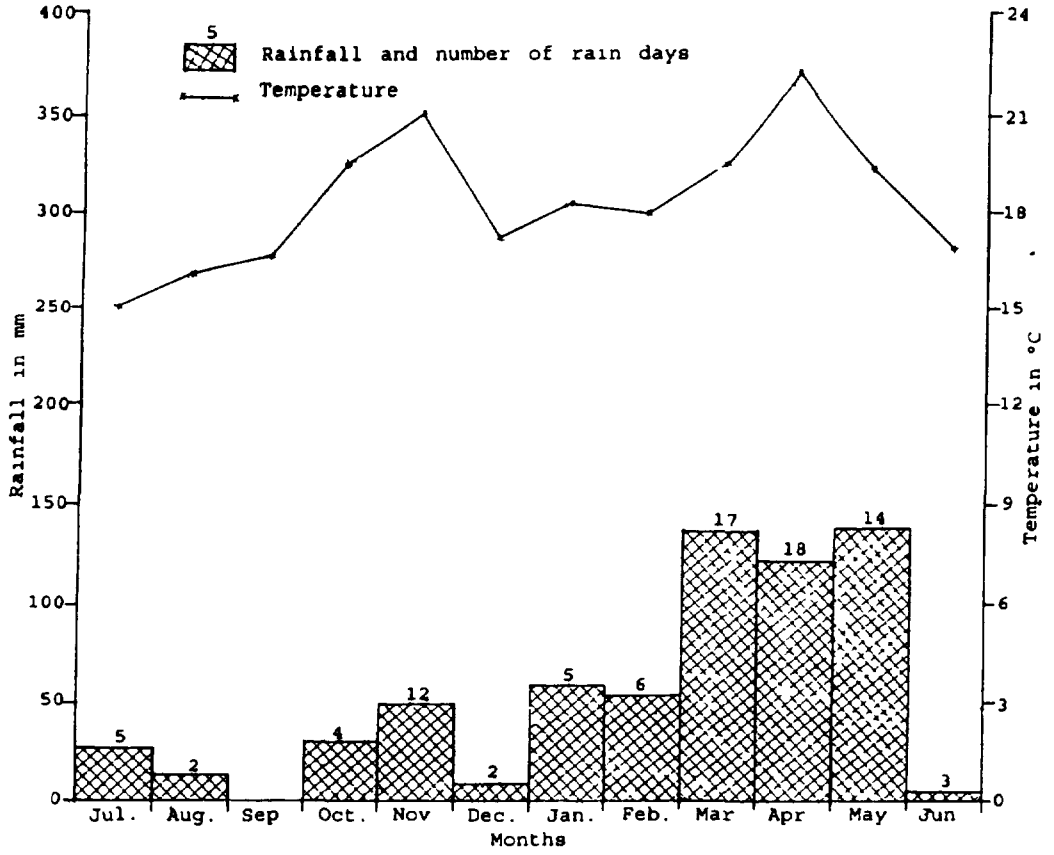

Figure 3: Total monthly rainfall (mm) at T.P.R.I. for July 1993 - June 1994

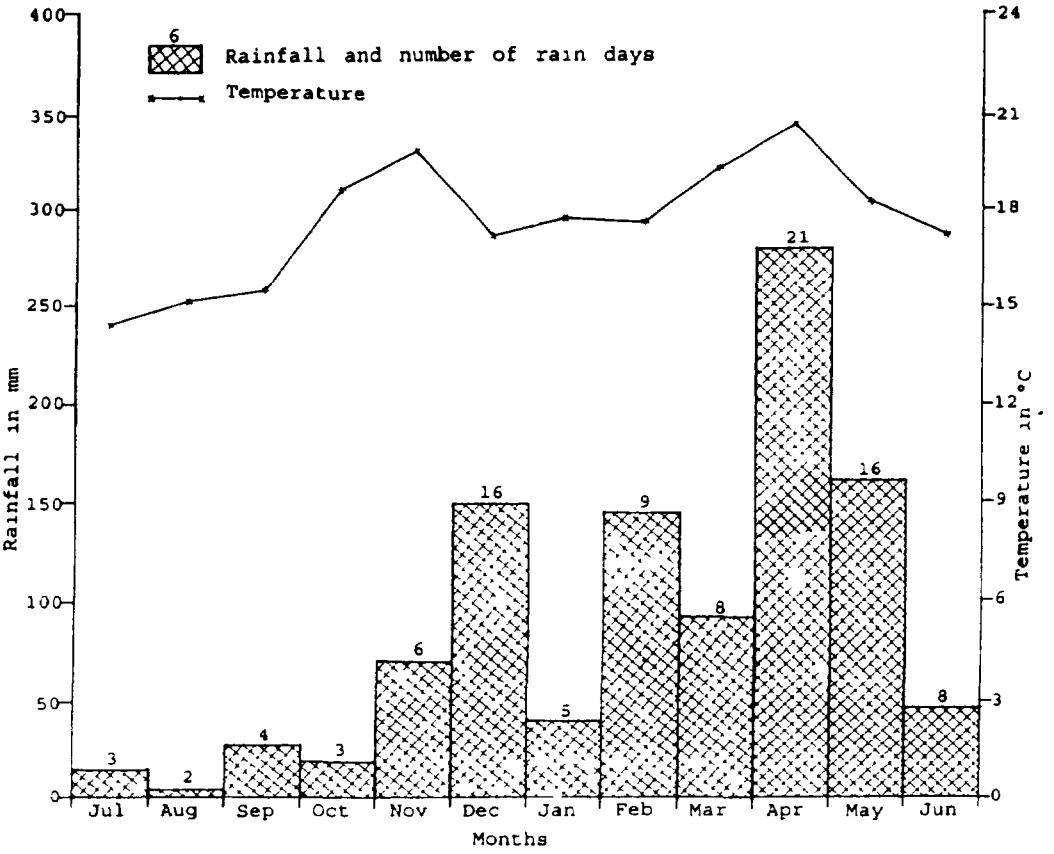

Figure 4: Total monthly rainfall (mm) at T.P.R.I. for July 1994 - June 1995

Table 1 Mean number of ants (Formicidae) populations in sprayed (lindane) and unsprayed plots

Sampling occasion	1991/92		1992/93		1994		1994/95	
	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed
1	0	0	36	16.75	56.25	65.75	7.25	11.75
2*	0	0	27.25	18.50	18.75	65.00	10.50	5.25
3	0	1.5	21.25	16.00	18.50	55.75	64.75	92.50
4**	1.75	6.0	28.25	33.25	8.00	53.50	44.75	53.00
5	3.25	0.75	52.50	47.00	27.50	47.25	22.25	25.50
6	8.75	12.00	30.00	82.25	37.00	41.75	23.75	23.00
7	0 75	11.75	61.00	54.25	65.75	52.75	0	0
8	0.0	13.00	66.00	74.25	98.25	220.00	0	0
9					113.50	238.75		
10					144.25	236.50		
Mean±SE	1 8±0.30	5.6±1.20	40.3±5.62	43.5±6.89	58.8±7.54	107.7±10.7	28.9±5.22	35.2±6.80

- Sampling occasion 2 corresponds to 24 hours after the first spray for all four seasons

** Sampling occasion 4 corresponds to two weeks after the second spray in 1994 and 1994/95

Mean values for 1992/92 are significantly different ($p<0.05$), values for other years are not

Table 2 Mean number of spider populations in sprayed and unsprayed plots

Sampling occasion	1991/92		1992/93		1994		1994/95	
	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed
1	0	0	3.00	3.75	4.00	8.25	10.5	12.00
2*	0	0	3.75	2.00	1.25	6.75	1.75	5.00
3	0	0.75	1.25	5.50	1.25	8.25	3.50	4.00
4**	0.75	2.50	2.5	3.75	1.25	12.25	8.00	11.75
5	0.50	1.50	4.25	3.00	2.25	12.00	0.50	3.00
6	2.75	1.50	7.00	6.75	5.25	11.00	3.25	3.00
7	0.75	1.50	3.25	5.50	5.25	10.25	0	0
8	2.75	2.00	3.75	6.00	7.75	11.00	0	0
9					9.25	10.25		
10					10.00	11.00		
Mean±SE	0.94±0.21	1.2±0.40	3.6±0.84	4.5±0.67	4.8±0.96	10.1±2.90	4.6±1.10	6.5±1.21

Mean values for 1994 are significantly different ($p<0.05$), values for other years are not

Table 3 Mean number of Collembola populations in sprayed and unsprayed plots

Sampling occasion	1992/93		1994		1994/95	
	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed
1	268.75	275.75	594.75	528.25	787.50	790.00
2*	190.00	262.25	228.00	603.50	17.50	12.50
3	189.25	247.50	204.50	688.00	275.00	430.00
4**	145.00	249.00	175.25	366.00	282.50	787.50
5	143.75	187.00	82.75	101.50	166.50	294.00
6	162.00	175.50	61.25	74.75	91.25	108.50
7	180.00	193.75	85.00	70.75	45.00	73.75
8	148.50	173.00	87.25	69.00	0	0
9			87.50	89.00		
10			94.50	98.50		
Mean $\pm$SE	178.4$\pm$12.8	220.5$\pm$20.6	170.1$\pm$18.7	288.05$\pm$40.63	237.9$\pm$17.5	356.6$\pm$20.6

Mean values for treated and control plots are significantly different ($p<0.05$) for all years

Table 4 Fresh borer damage (% mean) on maize plants in sprayed and unsprayed plots

Sampling occasion	1991/92		1992/93		1994		1994/95	
	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed
1	1.5	0.7	1.2	1.4	0.25	0.37	1.10	1.00
2*	0	0.7	2.9	6.8	0	0.75	0.40	1.42
3	0	5.0	2.7	5.1	0	1.12	0	2.30
4**	0	6.2	4.4	9.0	0.25	1.37	1.12	3.55
5	2.2	8.0	0.6	3.0	0.62	1.62	1.92	7.72
6	1.1	6.0	0	0.5	1.12	1.87	2.70	12.40
7	1 0	3.2	0	0	1.25	2.50	0	0
8	1.0	3.4	0	0	1.50	2.62	0	0
9					2.00	2.75		
10					2.00	3.05		
Mean $\pm$SE	0.85$\pm$0.12	4.2$\pm$0.58	2.0$\pm$0.04	4.3$\pm$0.61	0.90$\pm$0.03	1.8$\pm$0.06	0.9$\pm$0.17	3.5$\pm$0.71

Mean values for treated and control plots are significantly different ($p < 0.05$) for all years

TABLE 5. MEAN PERCENTAGE BURIED LEAF LITTER WEIGHT LOSS IN SPRAYED AND UNSPRAYED PLOTS

Season	Sprayed	Unsprayed	P value
1992/93	34.4 ± 2.6	37.6 ± 3.2	Non significant
1994	25.8 ± 2.5	27.7 ± 1.9	Non signficant
1994/95	9.4 ± 2.4	10.1 ± 1.6	Non significant

TABLE 6. MAIZE GRAIN YIELD (T HA $^{-1}$) IN SPRAYED AND UNSPRAYED PLOTS (MEANS ± SE)

Season	Sprayed	Unsprayed	%yield increase	P value
1991/92	2.20 ± 0.06	1.90 ± 0.03	15.79	Non significant
1992/93	2.40 ± 0.05	1.50 ± 0.02	60.00	Non significant
1994	2.56 ± 0.04	1.80 ± 0.04	42.22	Non significant
1994/95	2.99 ± 0.03	2.79 ± 0.04	7.00	Non significant

microarthropod extraction from the buried leaf litter in this experiment, the reduced Collembola activity in pitfall traps reported above could help to explain this slow-down in decomposition. Cook *et al.* [6] showed that Collembola and Acari account for 90-100% of fauna responsible for buried leaf litter decomposition and that their numbers were significantly reduced by DDT sprays.

The results further show that lindane reduced early season stem borer infestations thereby protecting the young maize plants at their critical stage of vegetative growth (4-8WAE). This protection is revealed by low plant damage. Protection of young maize from borer damage is important in that early infestations lead to direct plant loss due to lack of compensatory growth. Timing of spray application before borer larvae tunnel the stems is equally crucial. Delayed applications will not reach the protected larvae in the stems. Furthermore, many larvae which will have hatched from an egg batch on a single plant will have migrated to other plants if sprays are delayed until the larvae reach their third instar stage. Plant damage can be related to pest populations and insecticide applications. Severely damaged plants would certainly give poor grain yields. The insecticide sprays used in this study contributed substantially to grain yield increase in maize for the four seasons. These yield increases were however, not statistically significant in any of the seasons. Grain yield increases have been reported on cowpea after DDT applications [7]. The above results therefore show that although Lindane 20 e.c. reduced stem borer population and damage on maize thereby improving grain yields slightly, it also showed short-lived adverse effects on most of the early season beneficial arthropods which highlights the importance of detailed studies to monitor residues in the soils and on some of the beneficial arthropod fauna groups.

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PRELIMINARY STUDIES ON THE EFFECT OF ORGANOCHLORINE PESTICIDES ON BIRDS IN TANZANIA

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Abstract

Preliminary studies to investigate the effects of organochlorine pesticides on birds was conducted in Lower Moshi, NAFCO West Kilimanjaro, Arusha seed farm, Tropical Pesticides Research Institute (TPRI) farms, Manyara ranch and areas around Lake Victoria as well as in the TPRI laboratory in Tanzania. Large quantities of the pesticides particularly DDT, endosulfan, dieldrin, lindane and toxaphene are still being applied against pests of cotton, coffee, maize, beans and other crops as well as disease vectors in the country Several groups of birds including waterbirds, African Fish Eagles, Marabou storks, Oxpecker, ducks, etc were found feeding, roosting and swimming in the water and exposed to other substances that were contaminated with organochlorine pesticides and were presumably at risk. Analytical results from the tissues of the African Fish Eagles collected from Lake Victoria areas showed that the kidneys were contaminated with p,pɔ- DDE and o,pɔ- DDE at levels of 0.4 ng g^{1} and 1.45 ng g^{1} respectively. These organochlorine insecticides as well as β-HCH were also present in the brain and liver tissues. The levels of the organochlorine residues were well below the lethal and sublethal levels for bird raptors reported in the literature.

1. INTRODUCTION

Organochlorine pesticides, including DDT, dieldrin, endosulfan, lindane and toxaphene, are still being used to control agricultural pests and vectors of human and animal diseases in developing countries in the tropical and subtropical regions. Their use is greatly restricted in the developed temperate countries. This is mainly due to their high persistence in the environment and tendency to accumulate in living tissues and to move through different trophic levels in the food chains. Scientists have indicated that, compared with the temperate regions, the chemicals may behave in a different manner in tropical environments due to high temperatures, humidity, ultra-violet light, microbiological complexes etc. Information on the amount and effects of organochlorine pesticide residues present in water, food, soil, animals and birds in the tropical and subtropical countries is scanty. Talekar et al. stated that organochlorine pesticides were banned in some tropical countries without sufficient information about their impact on the local environment [1].

A number of organochlorine pesticides have been used in Tanzania for many years and are still registered for use against insect pests of coffee, especially in the Kilimanjaro and Arusha regions,and against pests of cotton particularly in the Lake Victoria zone, and those of maize and beans in many other regions as well as pests of domesticated animals and man. However, no research has been conducted to evaluate their effects on the higher fauna, particularly birds, despite the fact that in Tanzania there is a vast number of birds species including some endangered species. Therefore, studies on both the short and long-term effects of the organochlorine pesticides on the birds is very important. In the Lake Victoria zone the piscivorous and raptorial birds, particularly the African Fish Eagles, seem to be most affected by the pesticides. This is because some fishermen have been reported to pour endosulfan and other pesticides into the lake to kill fish and these pesticides, together with those washed into the lake from the crop fields during the heavy rains, accumulate in the lake so that when fish, birds and other animals that use the water they absorb the pesticides into their bodies. The African Fish Eagle (*Haliaeetus vocifer*) feeds on fish and swims in the contaminated water and hence it can be expected that high concentrations of the pesticides could accumulate in their tissues. The

population of these birds around Lake Victoria has been declining for the past few years (Mgallah, Pers. Com.). Probably the pesticides accumulate in the birds tissues and consequently can interfere with reproductive and other metabolic activities of the birds.

This study was conducted firstly, to identify the various organochlorine pesticides used in the Lake Victoria zone, Kilimanjaro and Arusha regions; secondly, to assess utilization of crop fields by avifauna in these areas; thirdly, to assess the distribution and abundance of the birds species particularly African Fish Eagles in the areas; fourthly, to investigate their feeding habits, breeding habits and success; fifthly, to determine the levels of organochlorine residues present in different tissues of the African Fish Eagles.

2. MATERIALS AND METHODS

2.1. Experimental site

Surveys were conducted in lower Moshi rice and sugarcane farms and NAFCO West Kilimanjaro (Kilimanjaro region); Arusha seed farm, Tropical Pesticides Research Institute (TPRI) farms, Manyara ranch, and Manyara National Park (Arusha region) and areas around Lake Victoria namely Bunda, Magu, Igoma, Mwanza and Butimba (Mwanza region) to investigate the pesticides used and birds species present. The experiment was started in March 1992 and took about three years to investigate the pesticides used and birds species present.

2.2. Surveys and ecological study

Pesticide shop keepers, cattle keepers, crop farmers, agricultural extension and health officers were visited and interviewed to find out the types and quantities of organochlorine pesticides used against crop pests and disease vectors in their areas. The time and persistence of the various pesticides used were recorded. The results obtained were then used to demarcate the areas into "contaminated areas", that is, those with regular use of organochlorine pesticides and" control areas" which are those areas with no such use.

Observations were made according to respective crops and pesticides spraying calendars and the numbers of dead birds and their behavioural changes during and immediately after the sprayings were recorded. The birds found in the different study sites were monitored once every month apart from those in TPRI which were monitored once every week.The birds found were sampled and their distribution and abundance, feeding habits, breeding habits and success and population dynamics were investigated. The movement behaviour of the birds was used to group them into sedentary and migrant groups. The results obtained from the ecological studies of the birds were used to determine suitable indicator species for further intensive studies.

2.3. Sampling and laboratory analysis

During the different surveys a small number of birds were sampled for residue analyses. However, because of some technical problems with GLC, only a few samples of the African Fish Eagles collected from the Lake Victoria areas could be analyzed. These birds were selected from different spots around the Lake Victoria area and shot during two different periods. The first sampling was effected at the beginning of the short rains in October 1994 and the second one in February 1995 during the heavy rainfalls. The killed birds were taken to the TPRI laboratory where liver, brain, kidney and fat tissues were removed from their bodies. The removed tissues and water which was also sampled from the same areas were then analyzed by

following the procedure outlined in the protocol provided by the IAEA but with slight modifications as shown in the technique given previously in order to suit circumstantial needs [2].

Some tissue samples were prepared for residue analysis but without using the SPE column. In this case an amount (20 g) of tissue was mixed with anhydrous sodium sulfate (four times the weight of the tissues) and ground. This mixture was tumbled in acetone + hexane (200 mL, 1 + 1 by volume) and left to settle for one hour. The suspension was filtered into a separating funnel containing sodium sulfate solution (200 mL). The hexane extract was separated and washed three times with sodium sulfate solution (50 mL, 20 g L^{-1}).

The hexane extract was concentrated to 2.0 mL and passed through a microcolumn (containing 1 g of 60 to 80 mesh silica gel for chromatography and 0.5 g anhydrous sodium sulphate) previously washed with hexane (50 mL) followed by methanol (50 mL) and dried at 75°C overnight. The column was eluted with a further volume of hexane (10 mL). The hexane extract was concentrated to 10 mL and 5 µL analysed by gas chromatography. The conditions were: column, length, 1.5 m, diameter, 6.35 mm; packing, 50 g kg^{-1} QF1 on Chromosorb WHP; temperatures, column, 220°C, injector, 260°C, detector, 260°C; carrier gas, nitrogen; flowrate, 60 mL min^{-1} ; detector, electron capture detector (^{63}Ni).

When the sample was extracted without using the SPE column broad unresolved peaks were observed. Consequently, the extracts were cleaned further by using microcolumn (or pipette column) as mentioned above and resolved peaks were obtained. The peaks were then quantitated using analytical standards after determining the retention time of the individual peaks and concentrations of the corresponding compounds. The pesticide residue concentrations were calculated as follows:

$$R = \frac{A \text{ (weight std injected)} \times B \text{ (sample peak ht/area)} \times C \text{ (final extract vol.)} \times P \text{ (std)}}{D \text{ mL (inj. vol.)} \times E \text{ (sample tissue weight)} \times F \text{ (std peak height/area)} \times 100}$$

A = weight of standard used (ng/mL)
B = Area or height of sample peak (mm)
C = Final volume of extract (mL)
D = Microlitres injected (mL)
E = weight of tissue sample taken (g)
F = Area or height of standard peak (mm)
R = Residue concentration (ng/mL)
P = Purity of standard, %

3. RESULTS AND DISCUSSION

Endosulfan, dieldrin, lindane, DDT and several cocktails of DDT and other pesticides are still recommended as cheap and effective pesticides for controlling cotton and coffee insect pests as well as disease vectors in Tanzania and were found to be sold in large quantities in the Tanganyika Farmers Association (TFA) shops and other shops. Vegetables, maize, beans, rice, sugarcane and other crop producers were buying and using the various organochlorine pesticides against their crop pests, since there were few pesticides specifically recommended for control of pests of such crops. Water which was being used to irrigate sugarcane in the lower

Moshi crop contained high concentrations of dieldrin, endosulfan and DDT which were presumably washed from the coffee farms in Kilimanjaro and Arusha regions. Endosulfan which was being sold with the trade name Thiodan 35 EC, dieldrin and DDT were being used extensively against coffee, maize, vegetables and bean pests in the Arusha seed and TPRI farms. Toxaphene was extensively used against ticks and other animal disease vectors in the Manyara ranch. Endosulfan in ULV and EC formulations was being used in large quantities to control cotton and maize insect pests in most of the areas around Lake Victoria (Mwanza region). Large quantities of lindane and DDT had also been used in the lake zone in previous years. Endosulfan and other pesticides were being used to kill fish in Lake Victoria.

The species of birds which were observed in the lower Moshi, NAFCO West Kilimanjaro, TPRI, Arusha seed farm, Manyara Ranch and Manyara National Park are listed in Table 1. The lower Moshi rice irrigation Scheme (1200 ha) and sugarcane plantation are inhabited by numerous species of birds most of which are permanent residents, and pests, of rice. Queleas visit the farms in millions between July and February and can devastate the rice crop if not managed. The majority of the waterbird species (Egrets, Herons, Ibises, Storks) were observed eating rice grains, drinking and swimming in standing water which was probably contaminated with endosulfan, dieldrin, DDT and other pesticides. During the survey conducted in the area in May 1994, immediately after extensive spraying of the sugarcane and rice fields with endosulfan and other pesticides, some birds particularly the weaver birds and fiscal shrikes were found dead and some failed to fly for long distances. The dead birds were collected for analysis of pesticides residues in the laboratory but the analyses were not done owing to problems we had with the gas chromatograph.

NAFCO West Kilimanjaro is an open Woodland area inhabited by a number of bird species including the Grey Crowned Cranes and Cattle Egrets. The birds are likely to be affected by DDT, dieldrin, endosulfan and other pesticides which are frequently applied against pests of coffee, beans, maize, vegetables and other crops. TPRI and Arusha seed farms have relatively fewer species of birds but only a few of them, including the Abdimn's and stork, are sedentary hence are likely to be affected by the organochlorine pesticides which are being used in the areas. Yellow weavers which feed on bollworms in maize crops in Arusha seed farm and TPRI experimental fields are at risk. Fiscal shrikes may be exposed to organochlorine pesticides, when feeding on insects which survive after spraying in maize, coffee and bean farms. Manyara ranch, a wooded grassland, has a high diversity of bird species and shares many bird species with lower Moshi. Several waterbirds were drinking and swimming in the five ponds which were being used for washing cattle after the water had been treated with DDT, dieldrin, endosulfan and particularly toxaphene. Scavengers, such as Marabou storks, are permanent residents; birds of prey, especially vultures, also visit the ranch. These birds as well as oxpecker, doves, weavers and other species drink the contaminated water and some may also eat cattle meat contaminated with the pesticides. The five ponds are the only sites in the area where the birds can get water. Other birds found in the ranch include ducks, geese, ibises etc., which appear to be local immigrants from nearby Manyara National Park. Queleas also utilized the acacia trees in the area as nesting and breeding sites and later migrated to feed on wheat, rice and barley in the nearby farms. The birds which are permanent inhabitants in the Manyara National Park and do not visit the areas contaminated with the organochlorine pesticides are considered to be in the "control" area. The amount of organochlorine residues obtained from the tissues of these birds (if any) can be compared with that obtained from the similar species of bird which live in the contaminated areas. The Marabou stork a raptorial and sedentary species, is a good indicator species for this kind of study as it is found in both the National Park where no pesticide is being used and the ranch where several pesticides are applied.

Table 1. Species, distribution and abundance of birds.

Species	Manyara Ranch	Lower Moshi	NAFCO-West Kilimanjaro	TPRI-Arusha seed farm	Manyara National Park
Ostrich	+				++
Little Grebe	+			+	
Long-tailed Cormorant	+	+			
Squacco heron	+	+			+
Great white Egret	+	+			
Black Heron	+	+			
Little Egret					+
Black-headed heron		+		+	
Purple Heron		+			
Cattle Egret		++	++	+	+++
Yellow billed egret		++			+
Woolly necked stork		+			+
Marabou stork	+	+			
Yellow billed stork		+			+
Hadada Ibis		+		+	+
Glossy Ibis		+			
Sacred Ibis	+	+		+	+
African Spoonbill	+	+			+
White faced Tree Duck	+	+			+
Egyptian Goose	+	+			+
Red-billed Duck	+	+			+
Knob-billed Duck	+	+			+
Maewa Duck					+
Augur Buzzard	+	+			+
Black shouldered Kite		+			+
Hilderbrandt's Francolin	+				+
Helmeted Guinea fowl	+				+
Button Quail	+	+			+
Grey Crowned Crane	+	+	++	+	+
Blacksmith Plover	+	+		+	
Black Crake		+			
Two-banded Courser		+			
Chestnut-bellied Sandgrouse	+			+	
Namaqua Dove	+	+			
Ring-necked Dove	+	+		+	
Laughing Dove	++	+		+	+
Red eyed Dove	+	+			+
White-bellied Go-away bird	+				+
White-browed Coucal	+	+			+
African Jacana		+			+
Sandpipers		+			+
Speckled Mousebird	+	+	+	+	+
Little Bee-eater	+			+	
African Hoopoe	+				
Red-billed Hornbill	+				
Long tailed fiscal	+	+		+	
Fiscal shrike	+	+	+	+	+
White Crowned Shrike	+	+			
Hilderbrandt's Starling	++		+		
Superb Starling	++		+		
Ashy Starling	++				
Yellow billed oxpecker	++				
Queleas	+++	+++	+++	+	
YellowWeavers (Ploceus ssp)	+	+++		++	
Pelicans					+
White storks				+	
Black kites				+	
Abdumn's				+	

number of birds + <100
++ 100 - 1000
+++ >1000

In Mwanza region, the African Fish Eagle, a raptorial bird was found in all the areas that were surveyed around Lake Victoria. The areas included Butimba, Igoma, Nyamahanga, Tahamu near Rubara River and Guta where the number of birds observed ranged between four and eight. The Eagles were found together with the Marabou stork, Black kite, Great Egrets, Yellow billed duck, Yellow billed stork, Black headed herons and other species of birds.

Possibly, these birds have succeeded in staying in the same place because their ecological niches do not quite overlap. The African Fish Eagle feeds on fish and small animals such as frogs living in shallow water along the lake shore and river streams. They feed during day times especially between 10.0 a.m. and 4.0 p.m. It appears that the African Fish Eagles build their nests and lay their eggs during the dry season or towards the end of the rainy season because no nests with eggs were found during the survey, which was conducted at the beginning of the rainy season. Only two young birds were spotted. These birds laid two eggs which succeeded in hatching. Their nests were 30 cm in diameter and built by using small twigs or tree branches. These were found on the highest tree tops and were not easily viewed. Such behaviour of building nests in high positions and the great parental care which the African Fish Eagles have towards their young ones, enable all their chicks to survive normally. However, because of the small number of eggs which the birds lay, their population is usually small in most areas.

The analytical results from the different tissues of the African Fish Eagles collected from Mwanza region showed that the kidney contained what is probably o,p'DDE and p,p'DDE and was more heavily contaminated with residues of DDT type than the liver and brain tissues (Table 2). Possible traces of lindane and β-HCH were also observed in the brain and liver tissues. The work on organochlorines in Africa has generally concentrated on residue levels

Table 2. Residues of organochlorine insecticides in tissues of African Fish Eagle and in samples of water taken from lakes

Sampling		Sample identity	Residues	
Time	Location		Concentration, μgg[1]	Identity
October 1994	Magu	liver	0.002	o,p'- DDE
		brain	0.024	o,p'- DDE
		kidney	0.430	o,p'- DDE
			0.022	p,p'- DDE
	Igoma (MZ)	liver	0.009	o,p'- DDE
			0.007	p,p'- DDE
		kidney	0.200	o,p'- DDE
			0.460	p,p'- DDE
	Mwanza	brain	0.010	o,p'- DDE
			0.006	p,p'- DDE
			0.019	lindane
February 1995	Mwanza	kidney	1.45	o,p'- DDE
		liver	0.001	β - HCH
		brain	0.001	β - HCH
		water	0.001	o,p'- DDE
	Kayenyeze(MZ)	water	0.114	o,p'- DDT
	Bunda	water	0.004	o,p'- DDE
	Magu	water	0.154	o,p'- DDT

without investigating the associated effects except for egg shell thinning [3]. Therefore, the side effects on birds due to the organochlorines, have been estimated by comparison with the results of studies made in other countries. This has also been difficult mainly because sensitivities to pesticides vary widely between birds species [4]. However, diurnal raptors have broadly similar sensitivities [5]. Mortalities of raptors due to DDT and its residues were reported to occur when levels of DDT exceeded 30 mg kg^{-1}[6]. In addition to raptors, DDT and its residues are reported to cause eggshell thinning and breakage as well as addling and reduction of hatchability [7]. Several studies of organochlorine pesticides in Africa have indicated that the residue levels are generally lower than those found in Europe and North America [4]. This is mainly due to a lower proportion of agricultural land in Africa and the shorter persistence of organochlorines in tropical as compared to temperate climates. Assuming that the amounts of residues obtained from tissues of the African Fish Eagles during this study can be confirmed they are well below lethal and sublethal levels calculated by other scientists [6]. However, the data obtained cannot be considered as representative results for organochlorine residues present in the African Fish Eagles found in Mwanza areas. This is because other important tissues of the bird including muscles and fats have not been analyzed for the residues. In addition, the birds were sampled only at the beginning of the rainy season, when most of the pesticides applied in the cotton fields had not been washed into the lake where the birds were feeding and swimming. Furthermore, the number of samples analyzed was small hence could not provide consistent and reliable results and the identity of the peaks has not been confirmed by GC-MS.

However, together with studying the feeding habits, breeding habits and home ranges of the indicator bird species in relation to contamination with organochlorines, the tentative data on the African Fish Eagles indicate that it is necessary to determine the levels of organochlorine residues that are present in tissues and or eggs of the representative samples of the exposed birds and to investigate their associated effects. The preliminary results presented in this paper show a need for the further study of the effects of organochlorine pesticides on birds in Tanzania such as the African Fish Eagles and Marabou storks found around the Lake Victoria areas where lindane and endosulfan are extensively used against crop pests and on the Oxpecker birds in the Manyara areas where large quantities of toxaphene are applied in cattle dips [7].

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ADVERSE EFFECTS OF LINDANE IN A MAIZE AGRO-ECOSYSTEM IN UGANDA

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Abstract

The impact of lindane, at commercial rates of application, on invertebrate fauna, soil microbial activity, earthworm populations, crop damage and yields in a maize agro-ecosystem was studied and compared with unsprayed control plots of maize using a pitfall trap, D-Vac suction, litter bag, the earthworm formalin expulsion and crop assessment methods. The findings of the study generally portrayed lindane as having very few effects on the maize agro-ecosystem.

1. INTRODUCTION

To increase food production to cope with the ever rising demands by increasing populations in developing countries, agricultural practices must become increasingly intensive and, for the foreseeable future, pesticides will continue to be used.

However, the dangers to individuals and the environment of unqualified dependence on pesticides are very well documented [1-3]. There is no doubt therefore that Third World farmers too need to be made aware of the ills of overdependence on these chemicals. Hence chemicals used in developing countries should be of the type that does not pose risks that are too serious to farmers and the environment. Many of the pesticides presently in use in developing countries are both expensive and lacking in the persistence necessary for them to be effective on tropical pests. the ones that are can be toxic and pose a hazard to the often poorly trained applicators of the Third World.

Until its use became restricted worldwide, lindane was an acceptable pesticide for the Third World mainly on account of its relative cheapness, combined with its low toxicity to applicators. However because of its persistence, its residues tend to accumulate in soil and aquatic sediments, and in plant and animal tissue which led to the restriction on its usage.

The economic viability of lindane in the tropics is however, not fully documented. Whereas its persistence as demonstrated in researches done in temperate environments is an undesirable characteristic, the extent of these negative attributes in tropical environments has not been fully investigated. In any case, some persistence can be a desirable attribute for tropical farmers.

It is well known that temperature, among other factors, influences the dissipation rates of peticides in the environment [4]. In that case, it is possible that under tropical conditions, lindane will dissipate at rates which preclude local accumulation of residues in the soil and wildlife. This implies that its use may still be acceptable, and not cause significant environmental problems in the tropics.

The research effort presented in this report is part of a the FAO/IAEA co-ordinated programme to provide more information on the merits and demerits of using lindane in the tropics. It is hoped that the data so generated will contribute to the resolution of the argument as to whether lindane should or should not be used by Third World farmers, especially given that it

possesses attributes that they probably would welcome. The following account is based on a field study that was carried out at Kawanda Research Station, Uganda, each year during the months of April-July of the years 1992-1995. The principle objective of the study was to assess the impact of lindane on the invertebrate fauna and to determine its impact on yields.

2. MATERIALS AND METHODS

2.1. Field trial design and preparation

In the study periods of April-July 1992 and 1993, two parallel fields of about 0.7 hectares were used for the experiments. The experiments were designed to have four sprayed plots in one field and four control plots in another field. The fields were at least 50 m apart, while the plots within the fields measured 10 m × 10 m and were 10 m apart and in the centre of the fields, leaving 10 m from the edges. In the study periods of April-July 1994 and 1995, one field of about 0.8 hectares was used for each of the experiments. In this case the experiments were designed to have four plots (i.e. measuring 10 m × 10 m and 10 m apart) sprayed with lindane, with another four plots left as controls. The positions of the sprayed and unsprayed plots were randomised, so as to give a randomised block experiment with four blocks and two treatments in each block.The soil properties for the years 1994 and 1995 for each of the blocks are given in Tables 8 and 9.

In all cases, the fields were ploughed by tractor and planted with dressed maize seed (*Zea Mays* var. Namulonge) at a spacing of 60 cm between rows and 30 cm within rows. Initially two seeds were placed in each hole, but these were later thinned to single plants after germination. All weeding was done manually, and the NPK fertilizer was added at the rate of 200 kg/ha when the crop was four weeks old.

The first application of lindane (bought locally as Gammalin), using a 20L knapsack sprayer at a rate of 0.5 kg AI ha^{-1}, was normally made four weeks after germination and two weeks after the laying of pitfall traps.

2.2. Pitfall traps

Following the demarcation of plots (i,e two weeks after germination), four pitfall traps (10 cm rim diameter and 10 cm deep) were randomly placed in each plot to trap any surface-active invertebrates that came by. The traps contained formalin (20 g L^{-1}) as the trap agent and preservative. Traps were emptied once a week.

2.3. D-vac sampling

The D-vac suction apparatus was used for both plant and ground sampling in the experiments conducted in the study periods of 1994 and 1995. It was normally first done three weeks after germination, and subsequently every after two weeks. For ground sampling, one sample was taken per sampling occasion per subplot, each sample consisting of four sub-samples taken from four different places in each subplot. Each sub-sample consisted of five-seconds suck with the sampling cone tightly pressed to the ground. Similarly for plant sampling, one sample was also taken per sampling occasion per subplot, each sample consisting of four sub-samples taken randomly by sucking the maize plant inserted into the D-vac cone. At the earlier stages, the D-vac cone was placed over the plants while sampling. Later, as the plants became taller, the cone was used to sweep the plants from all sides. In all cases, the

invertebrates caught were killed with chloroform and kept in formalin (50 g L^{-1}) in sample bottles for later identification and counting.

2.4. Assessment of microbial activity

In order to compare the level of microbial activity in the different plots, leaf discs of a Musanga tree (*Cecropioides* sp) were buried to a depth of about 5 cm. The discs were buried in bags made of fine mesh plastic netting, with each bag containing 20 discs of 1.5 cm diameter. The discs were oven-dried at 70^0C for at least 24 hour, before weighing both at the beginning of the experiment and after 2 months when the experiment was terminated. The loss in disc weight represents the proportion of the disc tissue that was consumed by microbes in the soil.

2.5. Earthworm sampling

The standard earthworm expulsion method was used [4]. A half-metre quadrat was placed on the soil surface in each of the fields, and 9 litres of a dilute formalin solution (50 mL of 40% formaldehyde in 9 litres of water) poured slowly within the quadrat. Earthworms would then emerge from the soil during the following 15 minutes. Two expulsion samples were taken per study period, once at the beginning of the experiment when the maize was two weeks old, and the other towards the end of the experiment when the maize had matured. Earthworm abundances at these two stages of the maize phenology were compared.

2.6. Pest damage

The maize crop was monitored by physical inspection for pest damage in all subplots throughout the experimental period. However, more intensive work was done at harvest to assess damage by *Busseola spp.*

2.7. Yield

Maize yield was estimated from four subplots (2 m × 2 m) in both sprayed and unsprayed subplots. These subplots for estimation of yield were usually located outside, but close to the subplots where sampling activities had taken place during crop growth.

2.8. Statistical methods

Students t-test was used for analysis of the data collected in the experiments conducted in the study periods 1992 and 1993. A two-way analysis of variance was employed using a statistical package (Minitab for Windows) for the data collected in the experiments conducted in 1994 and 1995.

3. RESULTS

3.1. Pitfall trap catches

Overall, there were no significant differences between the catches from the sprayed fields and those from the control fields except for fauna groups of Gryllidae and Blattidae ($P<0.05$) and Collembola ($P<0.01$) (Table 1) indicating that lindane application generally did not have a dramatic effect on the invertebrate populations in the sprayed plots.

TABLE 1. The mean catch per plot (±S.E) of selected key organisms caught using pitfall traps from sprayed and unsprayed plots during the years 1992-1995.

PRE-SPRAYING:

YEAR	FAUNA GROUP	SPRAYED (±S.E)	CONTROL (±S.E)	t-test Value	ANOVA P-value
1994	Araneida	9.8 ± 2.2	6.8 ± 1.4	-	0.207NS
	Acarina	5.0 ± 1.5	7.3 ± 3.8	-	0.516NS
	Gryllidae	4.5 ± 0.9	4.3 ± 1.3	-	0.817NS
	Formicidae	166.3 ± 56.1	233.8 ±127.0	-	0.517NS
	Collembola	102.3 ± 54.1	57.5 ± 36.2	-	0.309NS
1995	Araneida	29.5 ± 8.1	39.3 ± 4.3	-	0.226NS
	Acrididae	17.5 ± 5.6	12.3 ± 0.5	-	0.697NS
	Acarina	3.0 ± 1.5	2.3 ± 1.9	-	0.769NS
	Gryllidae	12.5 ± 1.9	17.5 ± 1.9	-	0.164NS
	Formicidae	290.3 ± 40.1	304.0 ± 61.5	-	0.783NS
	Blattidae	1.8 ± 1.4	3.0 ± 1.2	-	0.43NS
	Collembola	0.8 ± 0.8	0.5 ± 0.5	-	0.391NS

POST-SPRAYING:

1992	Araneida	21.3 ± 4.8	17.5 ± 3.8	0.42 NS	-
	Gryllidae	29.5 ± 5.5	53.8 ± 9.1	2.56 *	-
	Formicidae	577.8 ± 91.9	719.8 ± 80.1	0.88 NS	-
	Carabidae	21.3 ± 4.3	37.5 ± 7.5	0.43 NS	-
1993	Acrididae	48.0 ± 6.1	55.5 ± 7.8	0.79 NS	-
	Gryllidae	39.3 ± 8.1	67.0 ± 7.0	2.17 NS	-
	Formicidae	1056.0 ±488.0	707.5 ±126.1	1.77 NS	-
	Blattidae	14.0 ± 5.1	29.3 ± 3.4	2.35 NS	-
	Collembola	80.8 ± 30.2	7.3 ± 2.1	5.24 **	-
1994	Araneida	57.5 ± 11.4	46.3 ± 6.3	-	0.191NS
	Acarina	48.8 ± 21.6	47.5 ± 11.4	-	0.855NS
	Gryllidae	39.5 ± 2.2	34.3 ± 5.6	-	0.325NS
	Formicidae	1186.5 ±205.0	1452.8 ±500.1	-	0.528NS
	Collembola	2428.0 ±993.0	1507.3 ±171.8	-	0.064NS
1995	Araneida	66.0 ± 6.7	89.8 ± 38.8	-	0.694NS
	Acrididae	38.6 ± 11.6	28.3 ± 1.4	-	0.783NS
	Acarina	44.8 ± 26.0	31.0 ± 21.4	-	0.131NS
	Gryllidae	58.0 ± 10.2	76.3 ± 6.7	-	0.225NS
	Formicidae	1180.3 ±324.6	2382.0 ±761.1	-	0.372NS
	Blattidae	48.3 ± 8.1	60.5 ± 9.5	-	0.008*
	Collembola	17.0 ± 1.4	27.0 ± 5.9	-	0.288NS

NS= Not significant (P>0.05) *= Significant (P<0.05)
**= Significant (P<0.01)

TABLE 2. Mean number (±S.E) of selected organisms caught using a D-vac sampler from the sprayed and unsprayed control plots.

A: GROUND SAMPLES:

YEAR	ORDER	SPRAYED(±S.E)	CONTROL(±S.E)	P-Value
1994	Araneida	2.0 ± 0.4	6.5 ± 0.6	0.002 *
	Homoptera	21.8 ± 3.2	30.8 ± 4.9	0.149 NS
	Hymnoptera	14.5 ± 0.6	14.5 ± 1.3	0.995 NS
	Diptera	98.0 ±12.3	114.5 ±18.5	0.138 NS
	Coleoptera	6.0 ± 1.3	8.8 ± 1.9	0.241 NS
1995	Araneida	5.5 ± 0.9	8.5 ± 2.3	0.289 NS
	Homoptera	17.0 ± 5.3	20.5 ± 4.3	0.546 NS
	Hymnoptera	17.8 ± 3.7	18.0 ± 2.0	0.212 NS
	Diptera	68.8 ±12.2	55.0 ±15.7	0.042 *
	Coleoptera	7.5 ± 2.5	4.5 ± 1.4	0.181 NS

B: PLANT SAMPLES:

YEAR	ORDER	SPRAYED(±S.E)	CONTROL(±S.E)	P-Value
1994	Araneida	2.0 ± 0.7	3.0 ± 0.7	0.091 NS
	Homoptera	8.8 ± 2.7	9.3 ± 3.8	0.940 NS
	Hymnoptera	13.0 ± 2.3	18.8 ± 1.9	0.267 NS
	Diptera	27.5 ± 1.4	46.3 ± 5.3	0.037 *
	Coleoptera	1.0 ± 0.4	1.5 ± 0.6	0.606 NS
1995	Araneida	1.8 ± 0.9	4.0 ± 0.7	0.029 *
	Homoptera	7.5 ± 1.3	8.0 ± 1.8	0.229 NS
	Hymnoptera	15.0 ± 9.3	9.8 ± 2.9	0.628 NS
	Diptera	81.0 ±32.0	82.8 ±27.1	0.855 NS
	Coleoptera	4.3 ± 2.5	3.5 ± 1.3	0.252 NS

NS=Not Significant (P>0.05). *=Significant (P<0.05)
**=Significant (P<0.01)

TABLE 3. Mean percentage(±S.E) loss in weight of buried leaf discs of a Musanga tree over a period of two months in sprayed and unsprayed control plots.

YEAR	SPRAYED(±S.E)	CONTROL(±S.E)	P-Value
1992	63.6 ± 2.0	66.2 ± 1.5	0.383 NS
1993	24.8 ± 4.7	41.2 ± 5.6	0.001 **
1994	16.4 ± 2.6	20.7 ± 1.4	0.233 NS
1995	36.1 ± 5.5	52.3 ± 8.5	0.045

*NS= Not Significant (P>0.05) *= Significant (P<0.05)
**= Significant (P<0.01)

TABLE 4. Total number of earthworms expelled from sprayed and unsprayed control plots.

YEAR	SPRAYED	CONTROL
1992	0.0	0.0
1994	10.0	9.0
1994	6.0	5.0
1995	2.0	7.0

TABLE 5. Mean percentage cob damage by Busseola spp assessed at harvest in sprayed and unsprayed control plots.

YEAR	SPRAYED(±S.E)	CONTROL(±S.E)	P-Value
1992	4.5±0.7	5.2±1.3	0.623 N
1993	3.6±1.0	2.7±0.9	0.519 N
1994	8.4±3.3	11.5±4.5	0.224 NS
1995	6.0±1.1	9.3±3.9	0.869 NS

NS=Not Significant (P>0.05)

TABLE 6. Mean weight (Kg) of maize yield per hectare from sprayed and unsprayed control plots.

YEAR	SPRAYED x 10^4	CONTROL x 10^4	P-Value
1992	#	#	#
1993	#	#	#
1994	0.90	0.88	0.594 NS
1995	1.00	0.90	0.173 NS

#= No Data Available, NS= Not Significant, P>0.05

TABLE 7. Comparison of rainfall (mm) availability between different years for the study period January-July

	JAN		FEB		MAR		APR		MAY		JUN		JUL	
	RD	TOT	RD	TOT	RD	TOT	RD	TOT	RD	TOT	RD	TOT	RD	TOT
1990	#	#	#	#	12	284.4	14	235.7	15	304.2	5	16.6	6	1.7
1991	10	39.2	11	93.8	13	230.4	14	256.0	17	116.7	9	110.3	6	27.3
1992	7	56.9	5	30.0	9	73.5	17	97.6	14	128.3	8	113.4	7	3.8
1994	5	57.6	9	69.0	5	83.4	8	109.4	19	103.3	9	140.8	9	4.8
1995	3	18.4	5	14.3	12	223.6	14	148.7	17	150.0	8	80.6	10	4.4

= Equipment was disfunctional; RD= Rainfall days; TOT=Rainfall total

SOURCE: Kawanda Agricultural Research Institute

Table 8. Soil characteristics (1994)

Composition	Block 1	Block 2	Block 3	Block 4
Sand	58.36	64.24	62.28	60.32
Clay	27.49	27.49	25.53	31.41
Silt	14.15	8.27	12.19	8.27
% org. mat.	5.45	7.73	6.82	8.82
% nitrogen	0.09	0.10	0.11	0.12
pH	5.0	4.8	5.2	5.4

Table 9. Soil characteristics (1995)

Composition	Block 1	Block 2	Block 3	Block 4
Sand	43.0	47.0	55.0	49.0
Clay	40.0	36.0	34.0	34.0
Silt	17.0	17.0	11.0	17.0
% org. mat.	4.09	4.24	3.94	3.79
% nitrogen	0.14	0.16	0.13	0.13
pH	5.4	5.5	5.6	5.6

The overall numbers of individual groups throughout the experimental periods are presented in Table 1. The figures show that none of the invertebrate groups was ever significantly affected at any time during the cropping season. Most groups decline in numbers as the season progresses, and this happens equally in the sprayed field as in the unsprayed field.

3.2. D-vac samples

Although the individual number of invertebrates caught by both ground and plant sampling are generally higher in the control plots than in the sprayed ones, most differences were not significant ($P < 0.05$) except for Araneida and Diptera (Table 2).

3.3. Assessment of microbial activity

Soil microbial activity in control plots was generally higher than in the sprayed plots with differences significant in 1993 ($P < 0.01$) and in 1995 ($P < 0.05$) (Table 5).

3.4. Earthworm sampling

In general, few earthworms were expelled from the soil in either sprayed or unsprayed control plots on any occasion and no significant were recorded (Table 4). Soil moisture seems to have been the principal limiting factor, as the earthworms were only be expelled during the first sampling occasions when rainfall was high (Table 7). No earthworms were expelled during the second sampling occasions when the climatic conditions were rather dry.

3.5. Pest damage

The maize crop was monitored regularly to assess pest damage, being done by regular inspection of the individual plants within the demarcated plots. There were no pests on the maize during its vegetative growth period in all years. The maize stalk borer (*Busseola* sp) was the only significant pest which, however, appeared once the maize had formed cobs very late in the season. The damage recorded in the sprayed plots was not significantly different from that recorded in the control plots in all study periods (Table 5).

3.6. Maize yield

The sprayed plots gave a slightly higher, not statistically significant ($P < 0.05$), mean cropping yield compared with the control plots. In both cases, the yields were quite low, mainly due to insufficient rainfall during the cob-forming period.

4. DISCUSSION

The results of the experiments do not show great differences between the lindane sprayed and unsprayed control plots. Focusing on the macro-invertebrate fauna, it is clear that although there were some numerical differences, these were mostly not statistically significant, implying a merely moderate impact by the pesticide. Such a low impact may have been due to rapid dissipation or adsorption of the pesticide onto soil particles [4]. But it must be pointed out also that in all study periods, a few weeks of the growing season were characterised by high rainfall. This could have quickly leached away the pesticide from the maize and soil surfaces although organochlorine leaching is not considered to constitute a significant mode of loss. Whatever the cause, however, the pesticide impact was low.

The microbial activity seems to have been less in sprayed as compared to control plots. The low mean percentage weight loss in both sprayed and control plots could be explained by the rather tough leaves used, suggesting that the two months period was not long enough to allow for higher total decomposition. Although not conclusive, this observation is in line with those of other workers [5], who registered reduced microbial activity following application of DDT.

A meaningful measure of pesticide impact on an agro-ecosystem must relate to the capacity of the system in the short term to maintain crop yield [3]. In this experiment, cob damage was slightly less and the yields slightly higher, though in no case were the differences statistically significant between sprayed and unsprayed control plots.the mean maize yields were higher in sprayed than in control plots. This implied that lindane was, as expected, providing some measure of control, albeit limited, probably owing to the low pest load.

Generally, any differences in invertebrate populations, loss in weight of leaf discs, plant damage and grain yields could be attriibuted to the application of lindane since the inter- and intra-field soil factors at the Research Station, which are known to be small, could not have been responsible for such differences. The major observation however, is that the effects of lindane application were small on the population levels of macro-invertebrates or the functional capabilities of micro-organisms in the soil.

The findings of the experiments generally portray lindane as not being highly damaging to the agro-ecosystem, and therefore an acceptable option for insecticide application in the tropics.

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THE EFFECT OF LINDANE ON NON-TARGET FAUNA IN A MAIZE AGRO-ECOSYSTEM IN ZAMBIA

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Abstract

The effect of lindane on non-target fauna in a maize agro-ecosystem was studied in Zambia in 1992 and 1993. While lindane was effective against the stalk borers, a target pest, it also affected other non-target fauna. Ants, spiders and springtails were significantly reduced. However the effect was only transient and lasted for approximately two months. Lindane appeared to have no real effect on aerial non-target fauna or on soil inhabiting microorganisms

1. INTRODUCTION

Organochlorine pesticides such as DDT, endosulfan, lindane and toxophene have been widely used for the control of crop and animal pests in Zambia. These pesticides are highly persistent and may affect many of the non-target organisms. Acquiring quantitative information about the effects of pesticides on non-target fauna is fundamental in the determination of the role of insecticides in integrated pest management programmes.

In Zambia some studies have been made to determine the side effects from the use of organochlorine pesticides. However, these studies have been conducted solely in relation to tsetse fly eradification programmes [1,2]. There is no information on the effect of organochlorine pesticides within the agro-ecosystem. Thus this study was undertaken to determine the non-target effects of lindane in a maize agro-ecosystem.

2. MATERIALS AND METHODS

2.1 Experimental layout

This study was conducted at UNZA farm, 25km east of Lusaka during the 1991/92 and 1992/93 growing season. The field was cultivated by a tractor and planted with maize (Table I) at the rate of 25 kg ha^{-1} with a row to row spacing of 90 cm and a plant to plant distance of 20 cm. fertiliser, at the rate of 400 kg ha^{-1} was applied as basal dressing at planting. Urea was top dressed at the rate of 200 kg ha^{-1} when the crop was at knee height. Soil characteristics (Table I) as well as weather data (Table II) for the experimental site were recorded during each cropping season.

Two plots, each 100 m × 50 m, were marked out and separated by a distance of 100 m. About three weeks after plant emergence one plot was sprayed with Gammaxane 20 (Table I) at the rate of 1 kg AI ha^{-1} using a CP15 knapsack sprayer. The other plot was untreated and served as a control. Within each plot, four sub-plots (5 m × 5 m) were demarcated with an isolation distance of 5 m. These sub-plots were used for all sampling activities . All data were analyzed using F-test at P = 0.05 level of significance.

Table I. Crop, plot and soil characteristics of the experimental site at the University of Zambia farm for the 1992 and 1993 growing seasons.

Component	Characteristic	1992	1993
crop	variety	MM 602	MM 603
	seed	undressed	undressed
	planting date	25 November, 1991	30 December, 1992
	spraying date	7 January, 1992	20 January, 1993
	harvest date	6 May, 1992	5 June, 1993
Soil	sand	55.8 ± 1.3 %	65.6 ± 2.8 %
	silt	10.0 ± 0.4 %	14.3 ± 6.4 %
	clay	34.7 ± 1.4%	20.1 ± 3.6 %
	organic carbon	0.8 ± 0.1 %	0.3 ± 0.2 %
	organic matter	1.36 ± 0.1 %	0.5 ± 0.4 %
	pH (soil : water=1:2.5)	6.3 ± 0.1	9.7 ± 0.5
plot	cultivation	tractor	tractor
	weeding	by hand	by hand
	previous crop	fallow	maize (MM 602)
	neighbouring crop	maize	maize
	pesticide history	unknown	Gammaxane

Table II. Mean temperature and rainfall for the 1992 and 1993 growing seasons

Month	1992		1993	
	temperature, °C	rainfall, mm	temperature, °C	rainfall, mm
January	22.4	168	24.4	167.8
February	23.5	44.9	22.1	179.6
March	23.5	166.4	21.4	83.5
April	21.9	5.0	22.1	4.6
May	19.1	6.0	19.6	0.0
June	16.9	0	16.0	0.0

2.2. Sampling activities

Sampling for arthropods was done immediately pre-treatment and thereafter every two weeks for two months. Aerial fauna were swept using a standard net at two opposite diagonals across each sub-plot. Pitfall traps were used to assess fauna that inhabit the soil surface. Four pitfall traps (diameter, 50 mm; depth,100 mm) were randomly placed in each sub-plot. Each trap was filled with water to which a few drops of liquid detergent was added to reduce the surface tension. The traps were emptied after 48 hours.

To assess soil inhabiting fauna, four soil cores (diameter, 50 mm; depth, 150 mm) were randomly taken from each sub-plot prior to application of lindane and once after two months. The arthropods were extracted from the soil using Berelese funnels. Light bulbs serving as heat source were placed above the samples to obtain a temperature gradient. The soil samples were left in the funnels for 72 hours.

2.3. Determination of soil microbial activity

Prior to the application of lindane , four nylon bags (120 mm × 120 mm), each containing 50 leaf discs (25 mm in diameter) were randomly buried to a depth of 50 mm in each of the sub-plots. Three months later the bags were retrieved and the contents dried (at 60°C for 24 hours) and weighed.

2.4. Assessment of crop damage and yield

To assess damage due to stalk borers, all the plants in each sub-plot were searched and the proportion of infested plants recorded.

In each plot, maize yields were recorded from four sub-plots (2 m × 2 m) which were earmarked from the start of the experiment and left undisturbed until harvest. The cobs were dried, shelled and weighed at a moisture content of 13.8%.

3. RESULTS

3.1. Sweepnet

Due to low numbers of insects caught in the sweepnet, the data were grouped into two insect orders. In general there were no significant differences in the number of Coleoptera caught either before or after application of lindane (Table III). Prior to insecticide application, there was an apparently significantly higher number of Diptera in the "treated" plot in 1992 (Table III), for reasons unknown. After treatment, there were no significant differences between the treated and untreated plots. Overall there were no significant differences in the number of Diptera between the treated and untreated plots in 1993 (Table III).

Table III. Mean (±SE) number of insects caught using a sweepnet in the trials in 1992 and 1993.

Species	1992			1993		
	interval after spraying, days	untreated	treated	interval after spraying, days	untreated	treated
Coleoptera	pretreatment	0.8±0.3	0.6±0.3	pretreatment	0.5±0.4	0.3±0 3
	13	0.8±0.3	0.0±0.0*	15	0.5±0.4	0.5±0.5
	27	0.1±0.1	0.5±0.2	29	1.5±1.0	1.3±0 6
	41	1.3±0.3	0.6±0.1	43	1.8±0.4	3.3±1.6
	56	2.1±0.4	2.0±0.4	57	1.5±0.8	1.0±0.7
	69	1.6±0.6	0.6±0.3	71	2.0±0.7	1.5±0.5
Diptera	pretreatment	0.3±0.3	1.3±0.1*	pretreatment	0.8±0.4	0.5±0 4
	13	2.3±1.0	4.2±2.0	13	2.7±0.4	2.7±0.1
	27	3.1±0.9	0.8±0.3*	27	14.8±5.1	18.0±9 5
	41	1.6±1.0	2.1±0.6	41	2.3±1.2	5 8±1 1*
	56	1.8±0.6	2.3±1.0	56	3.0±1.1	7 5±5 1
	69	0.5±0.0	1.1±0.1*	69	2.8±1.9	1.5±1 0

* Means within rows significantly different at P = 0.05 level.

3.2. Pitfall

Overall the number of spiders caught in 1993 were higher than in 1992. Both in 1992 and 1993 the number of spiders were significantly reduced in the treated plots shortly after spraying (Table IV). Thereafter there were no significant differences between the treated and untreated plots.

In 1992 the number of ants, although not significantly different, was higher in the treated plots before the application of lindane. After spraying, the number of ants was significantly lower in the treated plots (Table IV). In 1993 the number of ants was significantly higher in the treated plots, before the application of lindane. After spraying there were no significant differences between the treated and untreated plots.

3.3. Soil core

In both years, neither springtails nor mites were recorded from the soil core samples. However, that springtails were present in the field, was confirmed by the numbers caught in pitfall traps. The number of springtails recorded in 1993 was much lower than in 1992. Spraying of lindane did significantly reduce the number of springtails in both years. The effect lasted for almost two months (Table V).

3.4. Organic matter breakdown

The losses in weight of the leaf litter bags owing to soil microbial activity were moderate with around 50% losses during the period of burial. In 1992 the organic matter breakdown was significantly higher in the treated plots (0.74 $\pm$0.33 g) than in the untreated

Table IV. Mean ($\pm$SE) number of arthropods caught using pitfall traps in the trials in 1992 and 1993.

Species	1992			1993		
	Interval after spraying., days	untreated	treated	interval after spraying, days	untreated	treated
Araneida	pretreated	0.8±0.2	0.5±0.2	pretreated	35.7±30.5	4.5±1.9
	14	1.6±0.4	0.2±0.2*	15	3.5±1.2	1.0±1.0*
	28	0.5±0.1	0.4±0.1	29	8.5±4.6	3.3±1.4
	42	0.4±0.2	1.4±1.2	43	4.8±1.4	3.3±1.4
	57	0.9±0.4	0.6±0.2	57	2.5±1.4	3.8±1.4
	70	0.6±0.1	0.2±0.1	71	4.3±1.4	7.0±4.3
Formicidae	pretreated	4.6±3.3	14.3±7.6	pretreated	10.3±10.2	113.5±31.1*
	14	23.7±3.3	9.8±0.6*	15	7.8± 3.8	15.3± 4.7
	28	9.0±2.7	6.2±2.4	29	7.5± 7.0	11.0± 2.8
	42	13.8±3.2	5.3±2.7	43	17.5±17.5	21.3± 8.9
	57	7.9±1.2	4.1±1.0*	57	42.8±25.5	34.3±12.1
	70	16.4±2.0	8.1±2.6*	71	38.5± 5.4	33.3±25.9

* Means within rows significantly different at P = 0.05 level.

Table V. Mean (+SE) number of Collembola caught in pitfall traps in the trials in 1992 and 1993.

1992			1993		
interval after spraying, days	untreated	treated	interval after spraying, days	untreated	treated
pretreatment	38.6±14.2	216.0±68.6*	pretreatment	18.0±4.7	5.8±3.8
14	75.3±17.1	0.3±0.2*	15	9.8±2.2	1.8±2.1*
28	18.4± 1.7	3.1±1.3*	29	5.8±1.5	0.3±0.4*
42	14.4± 9.1	1.5±0.7	43	9.5±5.6	3.3±1.9*
57	46.5±19.1	10.3±2.4	57	7.5±2.2	2.3±0.8*
70	31.2±10.6	22.7±1.5	71	17.8±2.4	10.8±4.2

* Means within rows significantly different at $P < 0.05$ level.

plots (0.89 ± 0.03). In 1993, this situation was reversed with a greater recovery of leaf material from the bags buried in the treated plots (0.89 ±0.19 g) than in the untreated plots (0.67 ± 0.38 g) though with the higher experimental variation in this, the difference was not statistically significant.

3.5. Plant damage

In both years a single application of lindane significantly reduced damage due to stalk borers. However, significantly higher yield of maize was only obtained in 1993 (Table VI). The results showed that about 10-12% damage could be tolerated without significantly loosing yield in 1992. Whereas, in 1993 about 8-10% damage due to stalk borers could not be tolerated without significantly affecting the yield.

4. DISCUSSION AND CONCLUSIONS

In general, the activity of insects was low probably due to the drought conditions that prevailed during the experimental period. Data from soil core samples and organic matter breakdown also indicated low faunal activity. This was not surprising considering the highly alkaline soils having poor organic matter.

Table VI. Proportion of plants damaged by stalk borers and maize yields in the trials in 1992 and 1993.

Observation	1992			1993		
	interval after spraying, days	untreated	treated	interval after spraying, days	untreated	treated
% damage	pretreat	13.6± 1.0	10.4± 0.6	pretreat	8.0± 2.2	7.1±2.4
	13	8.4±0.9	2.8±0.6*	15	13.3±3.7	2.0±0.6*
	27	15.5±2.5	2.0±0.4*	29	13.5±1.9	1.3±0.5*
	41	12.2±2.1	2.5±0.3*	43	7.5±2.2	1.3±0.4*
	-	-	-	57	2.9±1.0	0.9±0.3
yield, $kgha^{-1}$		5988±1037	8041±1016		5799±782	7395±1504*

* Means within rows significantly different at $P < 0.05$ level..

Application of lindane appeared to have no real effect on aerial fauna. Although lindane is known to be toxic to Coleoptera and Diptera [3], the lack of significance may have been due to the low activity of these insects as well as emigration and immigration. Furthermore, the effectiveness of lindane is reduced under low rainfall conditions [4] which were experienced during the present studies. In addition, because of its relative high vapour pressure, the action of lindane under tropical conditions is not as persistent when compared to lower temperatures under temperate conditions [5].

Lindane significantly reduced the number of ants, springtails and spiders, recovered from pitfall traps. These findings are in agreement with those of other reports [6]. However in the present study the impact of lindane on these arthropods lasted for about two months. These findings agree well with other concurrent studies which have shown that lindane dissipates rapidly from the soil under Zambian field conditions. About 75% of the initial concentration of lindane is lost during the first 60 days after treatment [7].

Organochlorine insecticides are known to reduce microorganisms in the soil [8] and in turn affect the breakdown of leaf litter (organic matter). Contrary to the expected findings the present study showed that lindane did not significantly affect these microorganisms and our data are difficult to harmonize with those of other studies. Probably greater breakdown of organic matter would have occurred in the untreated plots had the litter bags been left in the soil for a longer period.

In both years lindane was highly effective against the target pest (stalk borers) as indicated by a reduction in the proportion of plants damaged. The yields were relatively higher in the treated plots when compared with the untreated plots although a clear relationship between damage and yield could not be established by the results in this study.

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EFFECTS OF ENDOSULFAN ON A MAIZE AGRO-ECOSYSTEM IN ZAMBIA

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Abstract

The effect of endosulfan on non-target fauna in a maize agro-ecosystem was studied in Zambia in 1994 and 1995. Endosulfan was well tolerated by a number of beneficial arthropods such as spiders, coccinelids, carabids and ants. Springtails were significantly reduced. However the effect was only transient and lasted for at most eight weeks. While endosulfan was effective against the target pest(stalk borers) it appeared to have no real effect on the soil inhabiting microorganisms.

1. INTRODUCTION

Maize is an important cash crop, both for commercial and small scale farmers in Zambia. Over the years production of this crop has increased significantly. However, successful cultivation of the crop depends upon, among other things, the use of insecticides to control major insect pests such as the stalk borers. In Zambia [1] as, in many other African countries [2], these insecticides are applied on a prophylactic basis irrespective of pest infestation level.

Endosulfan is among the organochlorine pesticides that is still being used in Zambia for the control of maize as well as cotton, groundnuts and soyabean pests. However, its use in other African countries and the world over is being questioned because of environmental concerns. To date, there is no information on the effect of endosulfan on either the maize or the cotton agro-ecosystem in Zambia. Hence, this study was conducted to determine the non-target effects of endosulfan in a maize agro-ecosystem.

2. MATERIALS AND METHODS

2.1. Experimental layout

This study was conducted at Chalimbana (N.C.S.R.) farm, 30 km east of Lusaka during the 1993/94 and 1994/95 growing season. The field was cultivated by a tractor and planted with maize (Table I) at a rate of 25 kg ha^{-1} with a row to row spacing of 900 mm and a plant to plant distance of 200 mm. Fertiliser, at the rate of 400 kg ha^{-1} ,was applied as basal dressing at planting. Urea was top dressed at the rate of 200 kg ha-1 when the crop was at knee height. Soil characteristics (Table I) as well as weather data (Table II) for the experimental site were recorded during each cropping season.

The experimental site was divided into four blocks, each 50 m x 50 m. Each block was further subdivided into two 25 m x 25 m plots. Within each block one plot was randomly assigned and sprayed with Thiodan 350 g L^{-1} at the rate of 1 kg AI ha^{-1}, using a CP15 knapsack sprayer. The other plot was left untreated. Thus, the experiment was conducted as a randomized complete block design with two treatments within each of the blocks. Within the centre of each plot, a subplot of 10 m x 10 m was demarcated for sampling activities.

Two-way analysis of variance was used to analyze the data at P = 0.05 level of significance. Where appropriate the data were transformed using the square-root transformation ($\sqrt{Y} + 1$).

2.2. Sampling activities

Sampling for arthropods was done one day before and after treatment and every two weeks thereafter for two months. The D-vac was used to sample arthropods on the plant as well as on the ground. A total of four samples were taken from each sub-plot during each sampling occasion. Each sample consisted of 5 sub-samples. In the case of plant sampling one plant represented one sub-sample. Sampling was done by either placing the cone of the D-vac over the entire plant when plants were small or by sweeping the plant with the cone from all sides when the plants were big. With ground sampling, each sub-sample consisted of lightly placing the D-vac cone on the ground for five seconds.

Table I. Crop, plot and soil characteristics of the experimental site at Chalimbana farm for 1994 and 1995 season

Component	Characteristic	1994	1995
Crop	variety	PHB 3402	CG4144
	seed	dressed	dressed
	planting date	5/2/94	1/12/94
	spraying date	15/3/94	11/1/95
	harvesting date	- - -	7/4/95
Soil	sand, %	52 9	52 9
	silt, %	4 9	4 9
	clay, %	42 2	42 2
	organic matter, %	2 14	2 14
	pH (in $CaCl_2$)	5 1	5 1
Plot	cultivation	tractor	tractor
	weeding	by hand	by hand
	previous crop	fallow	maize
	neighbouring crop	maize	maize
	pesticide history	unknown	Thiodan

Table II Mean temperature and rainfall for 1994 and 1995 the growing seasons

Month	1994		1995	
	temperature, °C	rainfall, mm	temperature, °C	rainfall, mm
January	22 8	244	23 3	109
February	22 3	141	22 1	131
March	22 2	3	22 3	83
April	21 6	22	21 8	0 3
May	18 7	0	16 0	0
June	16 2	0	16 0	0

Pitfall traps were used to assess fauna that abound (dwell) on the soil surface. Four pitfall traps (diameter, 50 mm; depth, 100 mm) were randomly placed in each sub-plot. Each trap was filled with water to which a few drops of liquid detergent was added to reduce the surface tension. The traps were emptied after 72 hours.

To assess soil inhabiting fauna, four soil cores (diameter, 50 mm ; depth, 150 mm) were randomly taken from each sub-plot prior to application of endosulfan and once, two months later. The arthropods were extracted from the soil using Berelese funnels. Light bulbs serving as heat source were placed above the samples to obtain a temperature gradient. The soil samples were left in the funnels for 72 hours.

The breakdown of organic matter was used to assess the effect of endosulfan on microfauna and mesofauna. Prior to spraying, four nylon bags (12 cm x 12 cm), each containing 50 leaf discs (2.5 cm in diameter) were randomly buried to a depth of 50 mm in each of the sub-plots. Three months later the bags were retrieved and the contents dried (at $60^{o}C$ for 24 hours) and weighed.

To determine damage due to stalk borers, all the plants in each sub-plot were examined and the proportion of the infested plants recorded. In each plot maize yield was recorded from another sub-plot (2 m x 2 m) which was earmarked from the start of the experiment and left undisturbed until harvest. The cobs were dried, shelled and weighed at a moisture content of 14.1%.

3. RESULTS

3.1. D-vac plant sampling

Both in 1994 and 1995 there were no significant differences in the number of spiders caught on the plants either before or after application of endosulfan (Table III). In both years before treatment, the mean number of coccinellids caught in the treated plots were relatively higher, but were lower following treatment. Only in 1994 was the mean number of coccinellids significantly lower in treated plots immediately after treatment.

The number of ants recorded in 1995 were relatively higher than in 1994. In both years the number of ants, although not significantly different were higher in the untreated plots before the application of endosulfan. After treatment the number of ants were slightly lower in the treated plots than in the untreated ones though the differences were small and not significant between the treated and untreated plots.

3.2. D-vac ground sampling

Generally the number of spiders, carabids and ants recovered from the ground were relatively higher in 1995 when compared with 1994 (Table IV). Before the application of endosulfan there were no significant differences in the number of spiders, carabids and ants caught in the treated and untreated plots. After spraying, although there were no significant differences, the number of these arthropods were slightly reduced in the treated plots. Only in 1995 was the mean number of spiders and carabids significantly reduced at 4 and 6 weeks post-treatment, respectively.

Table III Mean number of arthropods caught using D-vac plant sampling in the trial plots in1994 and 1995

Arthropods	1994				1995			
	interval after spraying, days	untreated	treated	CV, %	interval after spraying, days	untreated	treated	CV, %
Araneida	pretreat	0 16	0 28	7 0	pretreat	1 34	1 53	3 1
	1	0 41	0 47	16 1	1	0 69	0 63	9 6
	16	0 09	0 28	13 2	14	0	0	0
	31	0 72	0 47	14 3	27	1 66	1 66	15 7
	52	0 56	0 72	14 5	41	1 94	1 94	7 4
Coccinellidae	pretreat	0	0 09	5 4	pretreat	0 16	0 22	12 0
	1	0 56	0 19*	4 5	1	0 69	0 47	9 9
	16	0 09	0 22	12 3	14	0 06	0 19	8 7
	31	0 78	0 69	7 6	27	0 59	0 38	14 2
	52	0 44	0 25	16 5	41	0 69	0 78	11 7
Formicidae	pretreat	0 75	0 66	10 5	pretreat	1 31	1 16	6 4
	1	0 34	0 22	8 0	1	0 94	0 34	9 6
	16	0 84	0 34	16 0	14	0 03	0 06	4 5
	31	0 28	0 41	10 3	27	2 03	2 20	4 4
	52	0 63	0 72	11 6	41	1 50	2 00*	2 9

* Means within rows significantly different at P=0 05

3.3. Pitfall trap results

Both in 1994 and 1995, there were no significant differences in the number of spiders, carabids and ants caught in the treated and untreated plots either before or after the application of endosulfan (Table V). The mean number of springtails recovered from the pitfall traps were relatively higher in 1994 when compared with 1995. Both in 1994 and 1995 the mean number of springtails were significantly reduced in the treated plots but only at 6 and 4 weeks post-treatment for the two years, respectively.

3.4. Soil core fauna results

In both years the number of mites and springtails recovered from the soil were too low to carry out statistical analysis. In 1994 only 7 mites and 6 springtails were recovered from the soil core samples Whereas in 1995, 18 mites and 18 springtails were collected from the soil. Most of these arthropods were collected from soil core samples taken after the application of endosulfan.

3.5. Organic matter breakdown

Both in 1994 and 1995 the organic matter breakdown, although not significantly different was slightly lower in the sprayed plots when compared with the unsprayed plots. In 1994 the organic matter breakdown over a period of three months was 0 39 g in the untreated

Table IV Mean number of arthropods caught using D-vac ground sampling in the trial plots in 1994 and 1995

Arthropods	1994				1995			
	interval after spraying, days	untreated	treated	CV, %	interval after spraying, days	untreated	treated	CV, %
Araneida	pretreat	0 06	0 16	8 8	pretreat	1 53	1 53	5 2
	1	0 38	0 03	10 8	1	0 94	0 34	12 3
	16	0 22	0 25	9 4	14	0 50	0 03	10 2
	31	0 44	0 28	5 8	27	0 47	0 22*	3 5
	52	0 81	0 63	18 7	41	3 28	3 47	12 5
	59	0 34	0 03	8 6	55	1 44	1 09	10 6
	72	0	0 16	3 2	71	2 81	2 63	6 5
Carabidae	pretreat	0	0	0	pretreat	1 47	1 63	11 0
	1	0 03	0 41	13 1	1	0 88	0 22	12 4
	16	0 13	0 13	0 4	14	0 28	0 03	12 6
	31	0 44	0 06	11 4	27	0 53	0 31	19 8
	52	0 13	0 22	11 1	41	5 00	3 56*	6 5
	59	0	0 16	6 7	55	1 56	1 84	8 1
	72	0 03	0 03	3 0	71	2 19	2 84	4 7
Formicidae	pretreat	0 44	0 06	9 7	pretreat	1 75	1 63	7 4
	1	0 44	0 84	18 3	1	0 84	0 44	22 4
	16	0 94	0 47	21 8	14	0 69	1 66	24 2
	31	0 06	0 03	4 5	27	1 22	1 47	19 1
	52	0 34	0 69	12 6	41	4 63	3 81	4 1
	59	0 91	1 19	18 8	55	2 16	2 63	13 5
	72	0 31	0 25	8 7	71	3 19	3 69	9 2

* Means within rows significantly different at P=0 05

plots and 0 34 g in the treated plots This represented a loss in weight of leaf material of 69 9 and 62.2% (CV 21.3%) for the two treatments, respectively. For 1995, the actual breakdown of organic matter over the period of four months was 0 61 g and 0 60 g for the unsprayed and sprayed plots, representing 72.0% and 70.5% (CV 27 0%) for the two treatments, respectively

3.6. Plant damage

In 1994 there were no significant differences in the proportion of plants damaged between the sprayed and unsprayed plots (Table VI) Sampling to assess plant damage could not be continued beyond 4 weeks post-treatment because of the effects of drought on the plants The severity of drought also negated collection of data on maize yield In 1995 a single application of endosulfan significantly reduced damage due to stalk borers. Consequently, significantly higher yields were obtained in the treated plots Endosulfan appeared to take effect about three weeks post-treatment and thereafter.

Table V Mean number of arthropods caught using pitfall traps in the trial plots in 1994 and 1995

Arthropods	1994				1995			
	interval after spraying, days	untreated	treated	CV, %	interval after spraying, days	untreated	treated	CV, %
Araneida	pretreat	1 94	5 53	40 9	pretreat	2 38	2 16	17 9
	14	4 72	4 88	14 6	14	4 44	3 25	12 7
	29	1 56	0 72	23 4	28	4 36	4 53	2 0
	43	3 06	1 72	19 9	42	4 13	3 34	13 1
	56	3 59	2 72	9 0	56	5 97	5 50	12 8
	70	1 97	2 00	12 4	69	3 59	3 44	20 9
	-	-	-		84	2 19	2 72	10 1
Carabidae	pretreat	0 41	0 47	15 7	pretreat	0 56	0 75	3 1
	14	2 31	1 38	10 7	14	1 84	2 03	12 4
	29	1 44	0 50	23 7	28	2 13	2 50	9 5
	43	5 03	3 47	12 9	42	2 38	2 69	14 1
	56	6 13	2 75	13 5	56	2 28	1 53	15 4
	70	1 88	2 63	9 6	69	1 06	1 22	12 5
	-	-	-	-	84	0 69	0 88	8 1
Formicidae	pretreat	11 25	9 50	22 6	pretreat	15 34	15 56	18 4
	14	22 91	20 03	9 8	14	10 34	10 66	16 0
	29	13 72	13 94	28 7	28	12 56	10 88	12 1
	43	24 34	27 13	16 9	42	4 13	4 31	23 4
	56	18 22	28 31	31 0	56	8 47	2 78	31 3
	70	6 09	9 66	13 0	69	13 22	11 84	23 2
	-	-	-	-	84	15 34	12 22	22 8
Collembola	pretreat	11 56	13 47	11 7	pretreat	3 44	3 44	18 0
	14	18 72	7 56	34 7	14	10 69	8 81	12 9
	29	14 94	8 09	22 3	28	12 66	5 28*	18 0
	43	23 41	3 97*	22 8	42	5 34	2 69	21 0
	56	82 91	30 03	34 9	56	11 22	4 31	26 8
	70	58 38	21 81	28 2	69	12 84	3 56	28 8
	-	-	-	-	84	6 97	6 00	11 8

* Means within rows significantly different at P=0 05

4. DISCUSSION AND CONCLUSIONS

Overall the activity of arthropods was lower in 1994 compared with 1995 Although there were no significant differences between the treated and untreated plots, the number of arthropods recovered from the treated plots after spraying were relatively low In all the experiments the number of arthropods recovered from the treated plots appeared to reach pre-treatment levels within a period of six to eight weeks

Data from D-vac sampling and pitfall traps showed no significant effect of endosulfan on spiders as well as insects such as coccinellids and carabids The results obtained from sampling Formicidae also showed no significant effect of endosulfan. This is contrary to the

Table VI. Proportion of plants damaged by stalk borers and maize yield from the field trials in 1994 and 1995

Component	1994				1995			
	interval after spraying, days	untreated	treated	CV, %	interval after spraying, days	untreated	treated	CV, %
Damage, %	pretreat	7.3	8.0	23.7	pretreat	18.8	21.1	9.2
	14	10.1	8.0	24.4	7	20.9	22.0	4.3
	35	9.2	7.4	15.0	21	31.2	23.3*	9.0
	-	-	-	-	35	33.6	24.3*	8.6
	-	-	-	-	48	34.7	24.9*	9.7
Yield, $kgha^{-1}$	-	-	-	-		4893	6995*	5.1

* Means within rows significantly different at P = 0.05.

report of Brooks who found endosulfan to be toxic to ants [3]. The rate of application of the chemical as well as immigration and emigration between plots may account for the discrepancy.

Data from pitfall traps showed that endosulfan significantly reduced the springtail populations. These findings agree well with the paper of Youdeowi and Service who stated that endosulfan is used to protect soil inhabiting arthropods such as mites and springtails [4]. However, the effect of endosulfan on springtails in the present study was transient and lasted for approximately 6 weeks. These data fit well with the findings of Mwangala et al. [5] who found that endosulfan dissipated rapidly under tropical conditions. About 65% of α-endosulfan and 70% of β-endosulfan was lost within 60 days.

There were no significant differences in the breakdown of organic matter between the treated and untreated plots as indicated by the loss of weight of the leaf litter. This was despite keeping the litter bags for longer than the usual two months period. The results indicate that endosulfan had no effect on the microorganisms that are important agents of organic matter breakdown in the soil. The data from the present study agree well with those of Gorback and Knauf who also found endosulfan to have no significant effect on soil microorganisms [6].

Data from 1995 showed that a single application of endosulfan was effective against the target pest as indicated by the proportion of plants damaged in agreement with Youdeowi and Service who have shown that endosulfan was especially effective against the maize stem borers [4]. The present study showed that one application of endosulfan was adequate to obtain a significant increase in yield.

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PERSISTENCE OF LINDANE AND ENDOSULFAN UNDER FIELD CONDITIONS IN ZAMBIA

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Abstract

The persistence of lindane and endosulfan was studied under field conditions in Zambia in 1992 to 1994 Both pesticides dissipated rapidly under field conditions. About 29% and 73% of initial concentration was lost during the first 30 and 60 days after treatment, respectively in 1992. After 180 days, about 11% of the initial concentration was recovered from the soil. In 1993, 40% of initial residues were lost during the first 30 days. At 180 days after spraying, slightly more residues (25% of the initial values) were recovered at this time than in 1992. This indicated a change in the longer term behaviour of lindane in the soil since the calculated half-lives of lindane, covering the shorter term behaviour, were 55-80 days in 1992 and ~17 days in 1993. In 1994, losses of α-Endosulfan and β-Endosulfan were 40% and 37% respectively during the initial 30 days after treatment. A further 25% of α-Endosulfan and 33% of β-Endosulfan were lost during the following 30 days. These data allow estimates of the half-lives of α- and β-Endosulfan (40 and 38 days) under the field conditions pertaining in Zambia at the time of the trials showing that this compound has only moderate persistence and unlikely to cause long term environmental problems.

1. INTRODUCTION

Pesticides are still a major component of the inputs used in agriculture in Zambia. The newer pesticides such as organophosphorus insecticides, carbamates and pyrethroids, which are more environmental friendly, are more expensive and resource-poor farmers, who are the majority, cannot afford them. Organochlorine pesticides, on the other hand, are relatively cheap and some are still effective against most pests. However, their use in Africa and the world wide is being restricted because of environmental concerns. These concerns have been based on data generated from temperate regions where organochlorine pesticides have been found to persist for longer periods [1]. In recent years, there have been indications that organochlorine pesticides may be less persistent in tropical environments than in temperate zones [2-6].

Lindane and endosulfan are among the organochlorine pesticides that are still being marketed in Zambia. Use of lindane for general agricultural purposes in Zambia was withdrawn in 1994 by the agrochemical companies, after the start of this project, but it is still being used for seed dressing. Endosulfan is still being used for control of cotton, potato, maize, soyabean and groundnut pests in Zambia. This study was undertaken to determine the persistence of lindane and endosulfan in a Zambian agro-ecosystem.

2. MATERIALS AND METHODS

2.1. Field experiments

In 1992 and 1993, a field of maize at the University of Zambia farm (east of Lusaka) was divided into two plots, 100m apart. One plot was treated with lindane (Gammexane 200 g^{-1}) at a rate of 1 kg ha^{-1}. One plot was untreated and served as a control. Each plot was divided into 5 m x 5 m subplots. The subplots were 5 m apart and at least 10 m from the edge of the large plot.

In 1994, a field of maize at National Council for Scientific Research, Chalimbana farm (east of Lusaka) was divided into four 50 m x 50 m plots. Each plot was further subdivided into two 25 m x 25 m plots. One sub-plot was treated with endosulfan (Thiodan 350 g L^{-1}) from Zambia Cooperative Federation Agrochemical Division) at 1 kg ha^{-1}.

Soil samples were taken at random over the whole sub-plots using soil core samplers (diameter, 55 mm; Eikelkamp Giesbeek soil equipment) at depth of 0-50, 50-100 and 100-150 mm on day 1, 30, 60, and 180 in 1992 and on day 0, 1, 30, and 180 in 1993 after treatment. In 1994, soil cores were collected at a depth of 0-50 mm only on day 0, 1, 30, and 60 after treatment. A minimum of nine cores were collected at each depth. Cores from each subplot, depth and sampling date were mixed together after sieving in 0.25 mm seize. The samples were stored in a deep freezer before analysis.

2.2. Moisture content

Moisture content of soil samples was determined gravimetrically by oven drying 10 g sub-samples at 110°C for 24 hours and expressing the moisture content as a percentage of water lost over weight of oven-dried soil. Soil characteristics were determined by the Department of Soil Science, University of Zambia in 1992 and 1993, and Soil Survey Section, Mt Makulu Research Station, Zambia in 1994.

2.3. Extraction and analysis

In 1992, two 20 g soil samples from each subplot were extracted with acetone+hexane (1+1 by volume,100 mL) by tumbling for 1 hour on a wrist action shaker. The extract was filtered into a separating funnel containing sodium sulphate (20 g L^{-1}, 100 mL). The funnel was shaken for 1 minute. The lower layer was discarded after the two layers separated. The hexane extract was washed twice with sodium sulphate solution (20 g L^{-1}, 50 mL). The hexane extract was dried with anhydrous sodium sulphate (5 g) and analysed by the gc method.

In 1993 and 1994, duplicate soil samples (50 g) were extracted with methanol (200 mL) in a Soxhlet apparatus for 3 hours. Aliquots (4 mL) were blown to near dryness with a stream of nitrogen and the residues were redissolved in 1mL methanol and diluted with 2.5 mL water. The samples were cleaned up on SEP-PAK C-18 cartridge columns (Waters, Milford, Massachesetts, USA). The columns were conditioned before the samples were added using methanol (3 mL), followed by distilled water (3 mL). The columns were washed with water + methanol (3 + 1 by volume,1 mL), followed by distilled water (1 mL) before elution of the column with of hexane (2.5mL) and analysed by the gc method.

The gas liquid chromatography method was as follows - column : 1 m x 4.5 mm silanised glass; packing, OV17 (16 g kg^{-1}) + OV210 (64 g kg^{-1}) on Chromosorb WHP; temperatures: column, 200°C, injector, 200°C, detector, 250°C; detector type : ^{63}Ni electron capture; carrier gas: argon. The limit of determination of lindane and endosulfan was 1 $\mu g\ kg^{-1}$ soil by the overall methods described. Recoveries from fortified samples were 83-98% for lindane and 86-96% for endosulfan and its sulfate.

3. RESULTS AND DISCUSSION

The environmental conditions that prevailed during the experimental period are presented in Table 1. The properties of the soil at the farms in 1992 to 1994 are presented in

Table 2. Moisture content of the soil on each sampling day is presented in Tables 3-5. There were no significant differences in the moisture content of soil from treated and untreated plots.

No residues were detected from the control plots in 1992 and 1993. Lindane dissipated relatively rapidly in both years (Tables 6, 7). In 1992, approximately 29% and 73% of the amount extracted on day 1 after treatment was lost during the initial 30 and 60 days, respectively from the 50 mm top layer (Table 6). After 180 days, the amount of lindane extracted from the soil was about 11% of the initial concentrations in the upper 50 mm layer. There were few variations in residue levels at lower depths and no pattern was discernible.

Table 1. **Environmental conditions that prevailed during the experimental period.**

Month	Year	Max Temp	Mean Temp	Mm Temp	Ramfall
January	1992	28 7	22 4	16 0	168 0
	1993	27 6	24 4	17 2	167 8
	1994	27 9	22 8	17 6	244 1
	1995	28 9	23 3	17 7	108 7
		(27)[a]	(21 6)	(17 0)	(203 0)
February	1992	31 1	23 5	16 0	44 9
	1993	26 7	22 1	17 4	179 6
	1994	27 9	22 3	16 6	140 7
	1995	28 1	22 1	17 1	131 2
		(27)	(21 5)	(17 0)	(177 0)
March	1992	29 9	23 5	17 2	166 4
	1993	26 3	21 4	16 4	83 5
	1994	29 7	22 2	14 6	2 7
	1995	29 1	22 3	15 5	82 7
		(27)	(21)	(16 0)	(84 0)
April	1992	29 1	21 9	14 8	5 0
	1993	28 6	22 1	15 6	4 6
	1994	28 7	21 6	14 4	21 9
	1995	28 5	21 8	15 1	0 3
		(27)	(19 9)	(14 0)	(25 0)
May	1992	26 7	19 1	11 5	6 0
	1993	28 1	19 6	11 0	0 0
	1994	26 7	18 7	10 7	0 0
	1995	24 3	16 0	7 7	0 0
		(26)	(15 1)	(10 0)	(12)
June	1992	24	16 9	8 5	0 0
	1993	23 4	16 0	8 6	0 0
	1994	24 2	16 2	8 1	0 0
	1995	24 3	16 0	7 7	0 0
		(25 3)	(17 0)	(8 0)	(1 0)

a) numbers in brackets = 30 year mean values

Table 2. Characteristics of soil in the experimental plots.

	1992	1993	1994
Sand (%)	55.8 ± 1.3	65.6 ± 2.8	73
Clay (%)	34.7 ± 1.4	20.1 ± 3.6	8
Silt (%)	10.0 ± 0.4	14.3 ± 6.4	19
Organic Matter (%)	1.60	0.51 ± 0.4	0.3
Organic Carbon (%)	0.81 ± 0.08	0.26 ± 0.2	0.15
pH (1:2.5 Soil:Water Ratio)	6.3 ± 0.1	9.7 ± 0.5	5.8

Table 3. Moisture content (%, ± SD) of soil in experimental plots at various depths in 1992.

DAY	CONTROL			TREATED		
	0-50mm	50-100mm	100-150mm	0-50mm	50-100mm	100-150mm
1	9.2 (± 1.1)	8.8 (± 0.5)	10.8 (± 0.9)	10.1 (± 0.9)	10.4 (± 0.1)	9.3 (± 0.3)
30	7.1 (± 0.3)	8.3 (± 0.3)	8.6 (± 0.2)	8.5 (± 0.3)	9.4 (± 0.2)	8.6 (± 0.2)
60	5.7 (± 0.1)	8.3 (± 0.2)	8.8 (± 0.2)	5.4 (± 0.2)	7.8 (± 0.1)	8.1 (± 0.2)
180	4.8 (± 0.1)	7.0 (± 0.6)	6.9 (± 0.3)	5.0 (± 0.1)	6.9 (± 0.2)	6.3 (± 0.3)

Table 4. Moisture content (%, ± SD) of soil in experimental plots at various depths in 1993.

DAY	CONTROL			TREATED		
	0-50mm	50-100mm	100-150mm	0-50mm	50-100mm	100-150mm
0	10.3(± 0.9)	12.4(± 0.7)	12.8(± 0.8)	8.5(± 1.1)	10.5(± 0.5)	10.8(± 1.1)
1	13.5(± 0.6)	13.5(± 1.3)	12.2(± 1.1)	11.7(± 1.5)	10.7(± 0.5)	11.1(± 0.9)
30	14.5(± 2.5)	15.2(± 1.8)	14.1(± 0.8)	13.9(± 1.2)	14.9(± 1.5)	14.6(± 3.5)
180	4.6(± 0.6)	6.4(± 0.6)	7.1(± 0.6)	4.5(± 0.8)	6.2(± 0.7)	5.9(± 1.7)

Table 5. Moisture content (%, ± SD) of soil from Chalimbana farm on each sampling day in 1994.

TREATMENT	DAY AFTER TREATMENT			
	0	1	30	60
Treated	8.15 ± 0.45	5.02 ± 0.72	2.50 ± 1.10	0.90 ± 0.76
Untreated	8.54 ± 0.72	5.00 ± 0.67	2.58 ± 1.27	0.86 ±0.78

Table 6. Lindane levels ($\mu g g^{-1}$ ± SE) in soil from University of Zambia farm during 1991/1992 season.

Depth (mm)	Day After Treatment			
	1	30	60	180
50	0.76 ± 0.02	0.54 ± 0.03	0.20 ± 0.10	0.090 ±0.006
100	0.06 ± 0	0.05 ± 0.007	0.04 ± 0.03	0.070 ± 0.006
150	0.01 ± 0	0.11 ± 0.01	0.012 ± 0.004	0.020 ± 0

Table 7. Lindane levels ($ng g^{-1}$) in soil from the University of Zambia farm during the 1992/1993 season.

Depth (mm)	Day			
	0	1	30	180
50	0.95 ± 0.07	13.5 ± 0.7	5.8 ± 0.07	3.4 ± 0.8
100	0.6 ± 0.1	4.8 ± 0.2	0.6 ± 0.2	0.8 ± 0.1
150	0.6 ± 0.1	4.0 ± 0.3	0.2 ± 0.02	0.8 ± 0.1

Table 8: α- and β-Endosulfan residues ($\mu g g^{-1}$ ± SE) in soil from the Chalimbana farm after treatment with Thiodan $350 g L^{-1}$ during the 1993/94 season.

	Endosulfan I		Endosulfan II	
Day	Treated	Untreated	Treated	Untreated
0	0.70 ± 0.12	0.52 ± 0.15	0.16 ± 0.07	0.18 ± 0.08
1	16.34 ± 1.61	1.79 ± 0.10	6.50 ± 0.37	0.85 ± 0.11
30	9.87 ± 0.69	1.31 ± 0.15	4.13 ± 0.42	0.60 ± 0.05
60	5.80 ± 0.87	1.03 ± 0.12	2.00 ± 0.23	0.47 ± 0.03

In 1993, lindane declined by more than 40% during the first 30 days after treatment in the upper 50 mm layer (Table 7). After 30 days, lindane levels declined to pre-treatment levels at 100 and 150 mm depths but the level was still high in the upper 50mm. After 180 days, 25% of the residues detected on day 1 after treatment still persisted in the upper 50 mm layer of the soil. The residues at 100 and 150 mm depths did not change and were at pre-treatment levels. The calculated half-lives of lindane in the upper 50 mm depth, during the period immediately

after application, were 55-80 and 17 days in 1992 and 1993, respectively. At later periods (> 60 days), although the data are limited, the rate of loss of lindane appeared to decline probably owing to the drier conditions of both the weather and the soil (Tables 1, 3-5) giving rise to stronger sorption of lindane and also lower microbial activity pertaining in the soil, during this period.

In the trials with Thiodan in 1994, only α- and β-endosulfan were detected from the soil and not endosulfan sulphate. During the initial 30 days, 40% and 37% of the initial amounts of α-and β-endosulfan, recorded on the day of application, were lost. Another 25% of α-endosulfan and 33% of β-endosulfan were lost after 60 days leaving residues remaining in the soil after 60 days of 36% and 31% of their initial concentrations. Both α- and β-endosulfan dissipated at the same rate with estimated half-lives of 38-40 days. Small amounts of residues were detected in the untreated sub-blocks (Table 8) possibly attributable to drift during spraying or contamination during sampling and sample handling.

The data indicate that both lindane and endosulfan dissipated rapidly under Zambian conditions. This is in agreement with other studies done in Kenya and Tanzania with DDT [3, 7]. Most of the residues were restricted to the upper 50 mm layer. There was little evidence to suggest that lindane was leached downwards to lower layers. The amount of lindane detected at the 50-150 mm depth could be due to contamination by soil particles from the top layer. The dissipation of lindane and endosulfan was possibly biphasic in nature and dependent on the varying meteorological and ensuing soil conditions during the trials. During the earlier, wetter period of the trials, particularly the first 30 days (Tables 1, 3-5), the rate of disappearance of lindane and endosulfan was fast but slowed down considerably thereafter.

It is concluded that lindane and endosulfan dissipate rapidly under Zambian field conditions. The use of lindane and endosulfan will not result in accumulation of residues. This is in agreement with other studies that have shown that the residence period of organochlorine pesticides in the soil is much shorter under tropical field conditions than under temperate zones [2, 3, 5, 7].

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SOIL PERSISTENCE, PLANT AND NON-TARGET INSECT UPTAKE OF ENDOSULFAN AND LINDANE APPLIED TO SOYA BEAN AND MAIZE IN FIELD TRIALS IN ZIMBABWE

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Abstract

The persistence of lindane and endosulfan in the soil, uptake by, and distribution in plants, and effects on and absorption by non-target insects, following application of the insecticides for the control of maize pests and soya bean respectively were determined under Zimbabwean weather conditions. No large scale effects on the non-target insects were observed though some small effects on the populations of semiloopers and orthoptera in the endosulfan treated soya bean plot were noted. Concentrations of the insecticides in spiders declined during the trial though those in grasshoppers and crickets appeared to increase.Concentrations of both insecticides in soil fell rapidly during the first 7 weeks after application but, after that, the rates of loss were much slower possibly owing to the drier conditions prevailing during this later period, reducing both physicochemical and microbial loss processes. Initial concentrations of both insecticides in all the vegetative parts of plants examined after spray application declined systematically to low levels during the 10 weeks of observation, probably owing to both metabolism within the plants and to crop volume dilution effects and will have declined to even lower levels by harvest time. Surprisingly, low concentrations of lindane and endosulfan were found in the harvested maize and soya bean seeds. At early stages after application, there were also traces of both insecticides in the vegetative parts of the plants from the untreated, control plots probably arising from uptake of soil residues from the previous year and/or spray drift but these became undetectable at later stages of growth.

1. INTRODUCTION

1.1. Background

By 1989 it was estimated that Africa accounted for 5% of the world pesticide use [1]. Most of these pesticides are used for pest control in agriculture. Although the use of pesticides in agriculture has been reported to have adverse effects on both man and other non-target organisms in the environment, agricultural development and the need to increase food production demands the use of pesticides. It is however necessary to assess the impact of the use of pesticides on the environment.

This impact will depend on several factors including temperature, rainfall, soil type, biotic activity, light intensity, land cultivation and other agricultural practices. These factors determine the persistence of the pesticide in a specific environment, and in this respect organochlorine pesticides have been found to be the most persistent as a group. Thus although the impact of pesticides on the environment has been studied extensively in the developed countries, there is still need to assess their impact in developing countries as agricultural practices in these countries may differ from those used in the developed countries.

Of major concern in Zimbabwe has been the use of organochlorine pesticides to control tsetse fly and malaria vectors. Pesticide sprays for tsetse fly control began in the early 1960s. Pesticides which have been used include dieldrin (1962-1967) and DDT (1968 to present) [2, 3]. Endosulfan and deltamethrin are also used, especially in aerial sprays [4]. In addition to its use in the control of tsetse fly and malaria vectors, DDT was used extensively in agriculture prior to 1983 when the use of DDT in agriculture was banned. Dieldrin,

endosulfan and deltamethrin are used extensively in agriculture. Table 1 shows the list of organochlorine pesticides registered for use in agriculture in Zimbabwe [5]. Contamination of non-target invertebrates and vertebrates like birds, reptiles, amphibia, fish, mice and sometimes large mammals as a result of aerial spraying of riverine and lake shore vegetation with DDT and endosulfan to kill tsetseflies in the Zambezi Valley area has been reported [6], but direct study of the effects of organochlorine pesticides on the agrosystem have yet to be undertaken in this country.

The aims of this study were: (a) To determine the persistence of organochlorine pesticides in the agrosystem, and (b) to monitor pesticide residues in the different compartments of the agrosystem. The pesticides selected for the study are endosulfan and Lindane. Endosulfan is used extensively for the control of the heliothis boll-worm, the semi-looper caterpillar and aphids in soya bean and groundnut crops, aphids in potatoes and the cutworm and red mite in maize [1] while lindane is used as for seed dressing [5]. A number of organochlorine formulations registered for use in Zimbabwe is listed in Table 1.

1.2. Persistence of lindane and endosulfan in soil

The soil is the major sink for chemicals applied to crops. Persistence patterns will vary from one climate to another and also depending on the chemical nature of the pesticide and the type of soil [7]. In cold climates degradation was generally found to be slower than in tropical climates [7, 8]. Lindane undergoes microbial degradation by dechlorination to pentachlorocyclohexane [9]. The bacteria, *Clostridium sporogenies* and *Bacillus coli*, produce benzene and monochlorobenzene from lindane [10]. Other possible transformations of lindane which occur especially in wet and submerged conditions include isomerization into the α- and β-HCH isomers of lindane [11]. The isomers were shown to be rapidly degraded

Table 1. Some organochlorine pesticides registered for use in agriculture in Zimbabwe

Trade Name	Active Ingredient	Toxicity Class*
Aldrin 40% w.p.	aldrin 40%	P
Anti-Kil	chlordane 30%	A
Razor	chlorthal-dimethyl 36%	G
Dicofol 40 E.D.	dicofol 40%	A
Kelthane	dicofol 18,5%	G
Dieldrex 50 w.p.	dieldrin 50%	P
Thionex 1% granules	endosulfan 1%	G
Thiodan 1% granules	endosulfan 1%	G
Thiodan 20 e.c.	endosulfan 20%	P
Multi Benhex	γ-BHC 12% +total BHC 75%	A
Gamatox House spray	γ-BHC 5,0%	A
Bexadust (L)	γ-B.H.C. 0,6%	G
Agri seed Dress 75%	lindane 1%	G

*P = extremely toxic; A = toxic; G= non-toxic.

in soil [12]. Endosulfan was found to undergo epoxidation which is enzyme catalyzed [13] and other transformations which include hydrolysis, reduction and hydroxylation [14]. Mechanical processes such as volatilization and run off also deplete the levels of these chemicals in soil.

The persistence of endosulfan in the soil has attracted attention from several workers. Burns studied the degradation of the insecticide in soils[15]. Kathpal et al. [16] and Martens [17] studied the kinetics of its degradation in soils and reported rates of 63% loss in 2-3 months and 5.4% in 15 weeks, respectively. Martens also studied the non-microbial degradation of the insecticide in soils under varying conditions of pH, sample wetness and temperature, and found that degradation was faster in wet samples with a higher pH. El Beit studied the kinetics of leaching of endosulfan in sediments and found that the rate of leaching was slow compared to the rate of degradation by micro-organisms [18].

1.3. Uptake of the pesticides by non-target fauna

Insects were selected for study as examples of non-target lower fauna. The uptake of organochlorine pesticides by insects following application for the control of agricultural pests in Europe and the American continents has been reported by several workers [19]. Gish found organochlorine pesticide residues of up to 0.60-0.65 $\mu g\ g^{-1}$ in beetle larvae from two agricultural fields in the USA [20]. Korschgen reported values as high as 8 ppm for aldrin and dieldrin residues in ground beetles from aldrin treated fields [21]. In Africa, DDE, DDT and dieldrin were detected in *Chiron.* L, *Corixidae*, and *Chironomidae* from Kenya by Lincer [22] and Greichus [23], while Muller reported on the uptake of dieldrin by ants, lepidoptera and isoptera following dieldrin application for the control of tsetse fly in Cameroon [24] Dieldrin, DDT and DDE were also detected in various insects by Greichus et al. in Zimbabwe [23].

1.4. Pesticide residues in crops

Several workers have studied the occurrence of pesticide residues in crops. Beestman et al. studied the translocation of dieldrin into corn from the soil and found that although 70-90% of the residues were found in the stems, appreciable quantities, up to 2%, eventually reached the seed [25]. These workers concluded further that residues measured at harvest time do not represent the total uptake by the plant during its life time because some of the absorbed insecticides are metabolized. Dormal et al. [26] and Brett and Bowery [27] found 0.3-1.5 $\mu g\ g^{-1}$ DDT residues and 0.4 $\mu g\ g^{-1}$ lindane residues respectively in beans and McCaskill et al. found 6 $ng\ g^{-1}$ lindane residues in soybean [28], while Bruce et al. reported levels as high as 0.11 $\mu g\ g^{-1}$ heptachlor in soybean [29].

1.5. Aims of the present study

The work reported in this paper was carried out between 1991 and 1995, and was conducted as part of the FAO/IAEA Co-ordinated Research Program on "Adverse Side Effects on Flora and Fauna from the Use of Organochlorine Pesticides on the African Continent". The trials conducted during the 1991/92, 1993/94 and 1994/95 growing seasons, November to March in Zimbabwe, were adversely affected by severe droughts during these periods. As a result, meaningful data were only collected during the 1992/93 growing season.

2. MATERIALS AND METHODS

2.1. Experimental field site preparation

The 1992/93 field experiments were conducted on specially designated land in the experimental section of the University of Zimbabwe Farm approximately 10 km from the University. After mechanical land preparation, the trial field of ~2 hectare was divided into 4 plots of approximately 0.25 ha each and alternate plots planted with commercially dressed soya and maize seed on 23 December 1992. The experimental plot was bordered by cattle fodder to the north and east, while plot A was bordered by a maize crop, and plot B was bordered by a roadway. The crops were separated by a 1.5 m buffer zone. This was done to simulate actual agricultural practice as closely as possible.

2.2. Pesticide application

The spraying of both soya and maize was carried out on 20 February, 1993. Thiodan (endosulfan) 50 WP (300 g) and Multi-Benhex (lindane) 75 WP (270g) were diluted in water (120 L) and applied using 15 L knapsack sprayers (Taurus Spraying Systems, Zimbabwe) to the respective plots, equivalent to application rates of 600 and 810 g AI ha^{-1}, respectively. To ensure the pesticide was applied evenly, preliminary spraying tests were carried out using water.

2.3. Sample collection

2.3.1. Soil samples

In each plot 4 subplots, each 5 m x 5 m and 10 m from the edge of the plot, were marked out. Four soil samples were collected from each of the four subplots in each plot. Each plot was divided into 4 quadrants and one soil core was collected from each quadrant. Sampling was done by means of a cylindrical soil corer 50 mm x 150 mm. Each soil core was placed into a clean polyethylene bag which was then sealed and labelled with the subplot number and the sampling point number. The samples from each plot were then placed into a single bag which was sealed and labelled with the plot letter, for transportation to the laboratory where they were stored in a deep freezer until they were taken for analysis.

2.3.2. Insect samples

Insect samples were collected using 4 pitfall traps per subplot after Critchley et. al. [30]. The traps were set 2, 4 and 10 weeks after the experimental plots were sprayed with the pesticides. Pretreatment traps were set 2 weeks before spraying. On each occasion the traps were left overnight for collection the next day. The inner cups of the traps containing the trapped insects were transported to the laboratory where the insects were classified. Each order of insects was transferred to a separate plastic vial and stored in a deep freezer without a preservative. Because of the low numbers of insects trapped, all insects of the same order from each plot were pooled together to form a single composite sample as shown in Table 4.

2.3.3. Plant samples

For residue analysis, 12 plants per plot were randomly taken. For each plot the plants were divided into stems and leaves, and roots at the field. Each of the two samples per plot

Table 2. Recovery of lindane and α- and β- endosulfan from soil samples spiked at three levels

lindane			α- endosulfan			β- endosulfan		
amount added ngg^{-1}	amount found ngg^{-1}	% recovery	amount added ngg^{-1}	amount found ngg^{-1}	% recovery	amount added ngg^{-1}	amount found ngg^{-1}	% recovery
3.01	2.50	83	2.48	2.10	85	2.53	2.20	87
6.32	5.00	80	5.00	4.20	84	5.04	4.40	87
9.32	9.04	97	9.00	8.93	99	9.83	9.77	99

Table 3. Mean residues of lindane and α- and β- endosulfan found in soil cores from the treated and untreated plots in the 1992/93 field trial

Interval after application, weeks	Mean residue concentration, ngg-1 (± SE)					
	treated plots			untreated plots		
	lindane	α-endosulfan	β-endosulfan	lindane	α-endosulfan	β-endosulfan
maize plots						
pretreatment	nd[a)]	20 ± 7	6 ± 2	nd	nd	nd
2	227 ± 71	29 ± 13	17 ± 10	1 ± 2	1 ± 2	nd
5	137 ± 33	4 ± 3	7 ± 6	nd	nd	nd
7	53 ± 13	8 ± 6	3 ± 5	-[b)]	-	-
10	37 ± 8	5 ± 4	0.4 ± 0.8	nd	nd	nd
25	24 ± 4	3 ± 2	1 ± 2	-	-	-
soya plots						
pretreatment	16 ± 7	nd	nd	-	-	-
2	3 ± 2	185 ± 5	119 ± 121	3 ± 3	1 ± 1	nd
5	6 ± 5	83 ± 10	40 ± 7	4 ± 4	2 ± 1	nd
7	7 ± 8	71 ± 1	23 ± 2	2 ± 3	4 ± 4	nd
10	-	-	-	1 ± 1	1 ± 2	nd
25	1 ± 2	40 ± 3	13 ± 2	-	-	-

a) nd = not detected b) - = analysis not conducted

was then placed in a polyethylene bag which was then sealed, labelled with the plot number and sampling date. The seed samples were randomly taken from each of the four plots at harvest and placed into polyethylene bags which were labelled as described above. The samples were then transported to the laboratory were they were stored in the deep freezer prior to residue analysis.

2.4. Residue analysis

2.4.1. Preparation of Florisil cleanup columns

Florisil (residue grade, Supelco, USA, ~5 g) was packed into a Pyrex glass column (10 mm i.d. x 110 mm) followed by anhydrous sodium sulphate (Merck, Germany, ~5 g).

Table 4. Total insect pitfall catches over 24 hours

Plot	Crop	Pesticide treatment	Insect order	Number of insects caught				
				Week / Date				
				pretreat -ment	0 20Feb	2 6Mar	3 11Mar	11 11May
A	maize	lindane	arachnida	40	43	48	41	62
			coleoptera	45	63	53	59	69
			orthoptera	17	23	15	15	28
B	soya	endosulfan	arachnida	23	24	30	21	26
			coleoptera	4	4	3	3	6
			orthoptera	62	50	58	12	29
C	maize	untreated control	arachnida	30	38	41	49	23
			coleoptera	11	21	31	49	42
			orthoptera	10	13	11	8	7
D	soya	untreated control	arachnida	48	41	38	42	49
			coleoptera	44	62	51	40	44
			orthoptera	19	15	14	18	23

The column was washed with hexane (20 mL) followed by methanol + hexane (0.5 + 9.5 by volume, 20 mL) and then dried at 180°C overnight. The column was cooled and then prewetted with hexane (10 mL).

2.4.2. Extraction and clean up of soil samples

Each soil core was taken for residue analysis. The frozen sample was thawed, ground and passed through a 2.5 mm sieve. A mass (equivalent to 1 g dry weight of the soil) was taken from the core and weighed into a beaker (10 mL). Acetic acid (0.5 mL) was pipetted into the beaker and the mixture was stirred using a glass rod. Nonane (0.5 mL) was added to the slurry and the mixture was again stirred with the glass rod. The resulting slurry was ultrasonicated for 30 minutes. The mixture was then allowed to stand and pesticide grade silica gel (Merk, 5 g) was added and the mixture stirred. The finely divided powder was transferred into a cellulose extraction thimble (Merk) containing silica gel (5 g). The thimble was then placed into a Soxhlet extraction apparatus and extracted with hexane + benzene (2 + 1 by volume) for 4 hours. The crude extract was concentrated in a Kuderna-Danish flask to 1mL. This concentrate was quantitatively transferred to a Florisil clean up column and eluted with hexane (20 mL - first fraction) followed by methanol + hexane (0.5 + 9.5 by volume, 20 mL - second fraction). The fractions were separately concentrated in the Kuderna-Danish flask (to 1 mL) and stored in glass vials (1.8 mL) with Teflon lined screw caps (Supelco, USA) at 4°C until analysed by the GC method described in section 2.4.4.

In preliminary studies soil samples (1 g) were spiked with a solution containing a mixture of lindane, α-endosulfan and β-endosulfan standards. Each spiked sample was stirred to ensure even distribution of the pesticides in the sample and then stored in the dark for 24 hours to equilibrate before extraction, clean-up, concentration and analysis as described above.

Table 5. Mean residues of lindane, α- and β-endosulfan and endosulfan sulfate in insects caught in the pitfall traps within the sprayed plots at intervals after spray application

Interval after application, weeks	Insect species	Residue concentrations, ngg^{-1}			
		lindane	α-endosulfan	β-endosulfan	endosulfan sulfate
maize pretreatment	spiders	nd[a)]	nd	nd	nd
	crickets	nd	nd	nd	nd
	semiloopers	nd	nd	nd	nd
2	spiders	5.1	0.5	nd	nd
	crickets	2.87	0.42	0.002	nd
	semiloopers	nd	0.045	nd	nd
4	spiders	1.75	nd	nd	nd
	crickets	-[b)]	-	-	-
	semiloopers	-	-	-	-
10	spiders	-	-	-	-
	crickets	20.06	6.16	nd	nd
	semiloopers	-	-	-	-
soya pretreatment	spiders	nd	nd	nd	nd
	crickets	nd	nd	nd	nd
	grasshoppers	nd	nd	nd	nd
2	spiders	nd	6.91	nd	nd
	crickets	nd	1.56	2.36	nd
	grasshoppers	0.002	nd	nd	nd
4	spiders	0.53	3.86	nd	nd
	crickets	-	-	-	-
	grasshoppers	nd	4.29	nd	nd
10	spiders	-	-	-	-
	crickets	nd	1.84	nd	nd
	grasshoppers	nd	28.16	nd	nd

a) nd = none detectable b) - = not analysed

Table 6. Recovery of lindane, α- and β-endosulfan and endosulfan sulfate from spiked plant samples

Plant sample	Spiked concentration, ngg^{-1} ; % Recovery							
	lindane		α-endosulfan		β-endosulfan		endosulfan sulfate	
	spike	% rec.	spike	% rec.	spike	% rec.	spike	% rec.
maize stems+roots	5.02	76.1	3.08	93.3	3.88	96.9	4.62	105.2
soya stems+roots	7.32	84.6	14.3	70.3	5.78	70.3	6.00	77.5
maize seed	16.0	90.3	18.0	97.1	13.2	97.8	18.4	95.7
soya seed	10.0	82.0	5.76	81.6	13.2	106.2	14.1	88.7

Table 7. Residues of lindane, α- and β-endosulfan and endosulfan sulfate in plant samples at intervals after spraying

Plant sample	Interval after spraying, weeks	Residue concentration, ngg^{-1}							
		Sprayed plots				Unsprayed control plots			
		lindane	α-endo	β-endo	endo sulfate	lindane	α-endo	β-endo	endo sulfate
maize roots	pretreat	nd[a)]	nd	nd	nd	nd	nd	nd	nd
	2	13.8	0.4	nd	nd	nd	0.2	nd	nd
	5	8.8	0.1	0.5	nd	nd	nd	nd	nd
	10	8.2	1.0	nd	nd	nd	nd	nd	nd
maize stems +leaves	pretreat	nd	nd	nd	nd	nd	nd	nd	nd
	2	19.3	0.3	nd	nd	nd	0.2	nd	nd
	5	9.5	0.2	0.15	nd	nd	nd	nd	nd
	10	1.06	0.07	nd	nd	nd	nd	nd	nd
maize seed		1.32	0.62	nd	nd	nd	nd	nd	nd
soya roots	pretreat	nd	nd	nd	nd	nd	nd	nd	nd
	2	1.72	12.7	3.31	nd	0.8	0.2	nd	nd
	5	0.13	6.55	0.4	nd	0.79	nd	nd	nd
	10	0.14	1.53	0.19	nd	nd	nd	nd	nd
soya stems +leaves	pretreat	nd	nd	nd	nd	nd	nd	nd	nd
	2	0.01	2.22	0.10	nd	0.77	0.40	0.18	nd
	5	0.07	6.57	2.02	nd	0.80	2.91	nd	nd
	10	nd	1.50	0.10	nd	nd	nd	nd	nd
soya seed		0.28	2.21	0.06	nd	0.04	0.02	nd	nd

a) nd = none detectable

2.4.2. Extraction and clean up of insect samples

A composite sample of insect species was crushed and divided into two approximately equal portions. The portions were weighed into a beaker (20 mL) and anhydrous sodium sulphate (~10 g) added. The mixture was ground using a glass rod until it was finely divided. Acetonitrile (15 mL) was added and the mixture stirred for about 10 minutes using a sonicator. The mixture was centrifuged and the supernatant transferred to a Kuderna-Danish flask. The residue after centrifugation was re-extracted with a further portion of acetonitrile and the supernatant combined with the first extract. The combined extract was then concentrated (to 1 mL), quantitatively transferred to a Florisil clean up column and eluted with hexane (20 mL, - fraction 1) followed acetonitrile + hexane (0.5 + 9.5 by volume, 20 mL - fraction 2). The two fractions were concentrated and analyzed separately as described above for soil samples.

In preliminary studies a composite sample of insects from one pitfall trap was crushed and divided into 6 approximately equal portions three of which were spiked with 18.3 ng g^{-1}, 12.1 ng g^{-1}, 1.62 ng g^{-1} and 18.2 ng g^{-1} of lindane, α-endosulfan, β-endosulfan and endosulfan sulphate respectively. The fortified samples were left to stand for 24 hours to allow the

samples to equilibrate before being extracted, concentrated and analysed as described above. The unspiked portions were used as the blanks.

2.4.3. Crop samples

The samples were ground using a Phillips grinder (Phillips, Mexico). Duplicate aliquots (5 g) were accurately weighed into a Phillips homogenizer (Phillips, Mexico) containing acetonitrile (50 mL) and homogenized at high speed until the sample was reduced to a slurry. The slurry was quantitatively transferred into 6 centrifuge tubes and then centrifuged at ~5000 rpm. The supernatants were combined into a Kuderna-Danish concentration tube. The sediment in each centrifuge tube was washed with further acetonitrile (3 mL) and recentrifuged. The rinsings were then transferred to the concentration tube containing the first supernatants. The extracts were then concentrated (to 1mL) and transferred to a prepared Florisil clean up column and allowed to stand for 2 minutes to equilibrate before being eluted first with hexane (15 mL - fraction 1) and then with acetonitrile + hexane (0.5 + 9.5 by volume, 15 mL - fraction 2). The fractions were each concentrated in a Kuderna-Danish flask (to 1 mL) and either analysed immediately or stored in Teflon stoppered glass vials at 4°C until analysed by the GC method described in section 2.4.4.

In preliminary studies the ground root, stem, leaf, and seed samples were spiked with an aliquot (1 mL) of a solution containing a mixture of lindane, endosulfan and endosulfan sulphate as shown in Table 8. Each sample was stirred to ensure even distribution of the spike in the sample and allowed to equilibrate for 24 h before homogenizing, extraction, clean up and analysis as described above.

2.4.4. Details of the gas chromatographic analysis method

Gas chromatograph : Varian Model 3300 GC (Varian AB, Solna, Sweden) fitted with a microprocessor, a split/splitless capillary injector, a Varian Model 4400 integrator and a ^{63}Ni electron capture detector (ECD); column : 30 m x 0.25 mm refined silica capillary column coated with DB-1701 (J and W Scientific, CA, USA); carrier gas: ultra pure nitrogen: flow rate : 5 mL min^{-1}; make-up gas : 25 mL min^{-1} ; temperatures : column, programmed from 100°C held for 2min and then heated to 250°C at 10°C min^{-1} and held for 3min; injector, 150°C and operated in the splitless mode during the first 30 seconds only; detector, 300°C. Quantitation was by the external standard calibration method.

3. RESULTS AND DISCUSSION

3.1. Persistence of lindane and endosulfan in the soil

Recoveries of the insecticides from the spiked soil samples were reasonable for all compounds varying between 80-97% (lindane), 84-99% (α-endosulfan) and 87-99% (β-endosulfan) (Table 2) suggesting that extraction and analysis of the field core samples would be satisfactory.

Lindane, α- and β-endosulfan disappeared rapidly during the first 7 weeks after application but declined to much slower rates in the period up to harvest at 25 weeks after application (Table 3). Losses suggested pseudo first order degradation rates over the initial 7 week stage. Similar results were obtained by Liechtenstein and Schultz following a single

application of 25 lbs of dieldrin per acre of Wisconsin soil, USA [31]. The rapid initial rate of loss may be attributed both to microbial degradation during the weeks of high rainfall when the soil was moist and to physicochemical losses by volatilisation and photodegradation. Loss of insecticide by run-off was probably minimal as shown by the extremely low levels of the insecticides detected in the untreated plots.

Lindane was also detected in plot B, while endosulfan was also detected in plot A, the plots which were not treated with that specific pesticide (Table 3). This was attributed to the fact that these plots had been treated by these insecticides in the previous year and indicates the likely extent of carry-over each year.

3.2. Variation of non-target insect populations

At the intervals examined, there were only a few obvious effects on the populations of the species of insects caught in the pitfall traps (Table 4). Firstly, there was a very low population of coleoptera in the endosulfan-treated soya bean plot (B) throughout the study, including the pretreatment period. Secondly, in this plot, the population of semiloopers declined systematically during the 10 week period of monitoring and, thirdly, the population of orthoptera was also lower at the last two intervals of observation though perhaps not outside statistical confidence limits.

It is possible, of course, that at intervals immediately following spray application there could have been larger reductions in the populations of all the species but at the time of the first assessment (2 weeks) their populations had recovered and remained unchanged during the trial, except for the coleoptera, and orthoptera in plot B.

3.3. Uptake of lindane and endosulfan by non-target insects

No insecticide was detected in any of the insects caught in the pitfall traps before spray application. At an interval of two weeks after application, lindane was detected in the insects caught in the traps from the maize plot treated with lindane but also traces of endosulfan (Table 5), presumably from cross-contamination. Similarly, both endosulfan isomers were detected in the insects trapped in the plot of soya beans treated with the endosulfan but, in this case, cross-contamination with lindane was hardly apparent (Table 5). Initially, spiders absorbed more than the other insects but the levels of both lindane and endosulfan (mainly the α-isomer) seemed to decline at later stages, though the data is limited. Spiders are known to be very susceptible to pesticides and can die from very low insecticide doses. Thus the levels in new generations or invasions of spiders decrease as the levels of the insecticides in the plot decrease with time.

Interestingly, the concentration of lindane in crickets and of endosulfan in grasshoppers appeared to increase with time reaching 20 ng g^{-1} and 28 ng g^{-1}, respectively. The reason for this is unknown but may be related to their diet (Table 5).

In contrast, no detectable residues were found in most of the insects caught in the traps in the untreated, control plots C and D (data not shown). The only exceptions were that traces of lindane were detected in grasshoppers in both plot C (0.09 ng g^{-1}) and plot D (0.003 ng g^{-1}) and α-endosulfan was detected, also in grasshoppers, in plot D (0.004 ng g^{-1}). Grasshoppers are much more mobile and these results may be due to the migration of these insects from the treated plots.

3.3. Distribution of residues in crops

In the preliminary studies, recoveries of all compounds from the spiked samples of the various parts of the crop plants were reasonable with recoveries of 76-90% (lindane), 70-97% (α-endosulfan), 70-106% (β-endosulfan) and 78-105% (endosulfan sulfate) (Table 6), indicating that the overall extraction and analysis procedures were satisfactory for examination of the field crop samples.

Lindane and endosulfan were detected in all components of the maize and soya bean plants from the treated plots. For both maize and soya bean roots, insecticide levels were initially high then declined with time (Table 7). For example, lindane concentration in maize roots was initially found to be 13.8 ng g^{-1} at 2 weeks after spraying but then declined to 8.2 ng g^{-1} at 10 weeks after spraying. In soya bean roots, endosulfan concentration was initially at 12.7 ng g^{-1} at 2 weeks after spraying and declined to 1.53 ng g^{-1} at 10 weeks after spraying. Lindane concentrations in maize stems and leaves was initially higher than in the roots at 2 weeks after spraying but, as the volume of crop foliage increased its concentration declined to low levels and below the level in the roots at 10 weeks after spraying.

The trend for endosulfan in soya bean stems and leaves is different to that of lindane in maize stems and leaves. Endosulfan in soya stems and leaves is initially low at 2.22 ng g^{-1} at 2 weeks after spraying then increased to 6.55 ng g^{-1} at 5 weeks after spraying before dropping to a low concentration of 1.53 ng g^{-1} at 10 weeks after spraying. The decline in pesticide levels with time is a complex combination of absorption, metabolism and crop volume dilution effects. The concentrations obtained for lindane in maize and soya bean grain at harvesting time were 1.32 and 2.21 ng g^{-1} respectively. These concentrations, although low, are still surprising since neither compound is expected to be transported to the storage sinks of plants.

Occasionally, trace levels of insecticide were detected from plants growing in the untreated control plots in the interval shortly after application, possibly as a result of drift during spraying (Table 7). The plant samples from the treated plots show higher levels of alien insecticides than the untreated plots (Table 7). This may be because the crops were rotated from the previous year's trial and so any higher level of an alien insecticide from plants in these plots could have resulted from both drift and carry-over from the previous year's pesticide application.

4. CONCLUSIONS

It has been shown that applications of lindane and endosulfan to maize and soya bean crops cause few medium-term changes in the populations of non-target insect species with only possible cause/effect relationships between endosulfan and coleoptera in soyabean. Analysis showed that insecticide concentrations in these species declined for spiders but increased for grasshoppers over the period of observation with both trends probably dependent on the feeding habits of these insects.

Concentrations of the insecticides in the soil initially fell rapidly but this rate of loss declined at later intervals probably as the soil dried out reducing both physicochemical and microbial mechanisms of degradation. Concentrations of both lindane and endosulfan in crop plants also declined to low levels during the monitoring period up to 10 weeks and will have declined even further in the period up to harvest. Surprisingly, low concentrations of both lindane and endosulfan were found in the harvested seeds of the treated crops.

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Appendix I

EXPERIMENTAL GUIDELINES

1. FIELD EXPERIMENT

This experiment was carried out in maize as a common crop in all participating countries and (optionally) in one other crop chosen according to local conditions. The two crops are hereafter called maize and alternative crop respectively. If the crop is not specified in the following the comments are valid for both crops. The term pre-treatment applies only to the first treatment.

1.1. Experimental layout

1.1.1. Experimental design

Use a randomised block design with four blocks and two treatments (sprayed and unsprayed) and hence eight plots. A plot size of 25 m by 25 m. gives an experimental area of 0.5 ha. Randomise the position of sprayed and unsprayed plots within each block. Where a uniform field is not available establish the experiment so that most of the variation is between blocks and as little as possible between treatments within the same block. If there is a known gradient in the field, (e.g. soil type or slope), place the blocks perpendicular to the gradient (see Fig. 1).

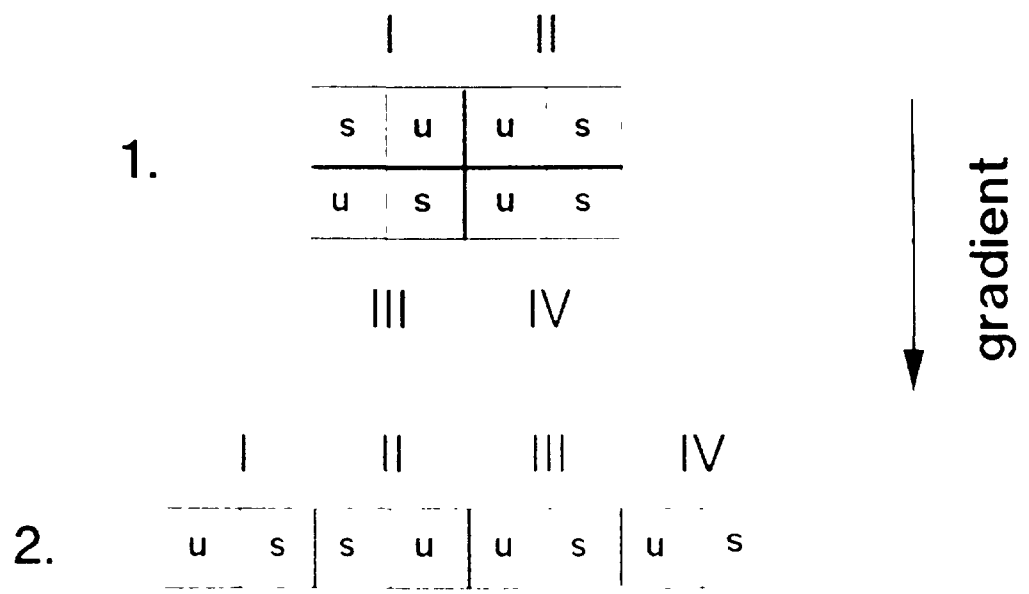

Fig. 1. Experimental design. Two examples of the layout are shown in the figure. The actual layout can be modified slightly to suit local conditions. The thick lines indicate demarcation between blocks. Blocks are marked with roman numerals. s=sprayed plot; u=unsprayed plot. The actual position of sprayed and unsprayed plots within each block is not fixed but must be randomised.

Mark out one subplot (10 m x 10 m) in the centre of each plot for sampling activities (see Fig. 2). Take samples for yield outside this subplot. Mark out other subplots for destructive sampling, e.g. plant and soil samples for residue analyses. Do not take samples within the outer 5 metres of the plot.

Analyse results by two-way analysis of variance.

1.1.2. Pesticides

1.1.2.1.Compounds

Lindane in maize and an appropriate pesticide in the alternative crop.

1.1.2.2. Rate of pesticide application

1 kg (AI) lindane divided in two applications in maize and according to local recommendations in the alternative crop.

1.1.2.3. Method of pesticide application

As a high volume (200-400 L ha^{-1}) spray in maize and according to local recommendations in the alternative crop. Choose the time of spraying to minimize spray drift (usually early in the morning) and keep the nozzle as low as possible.

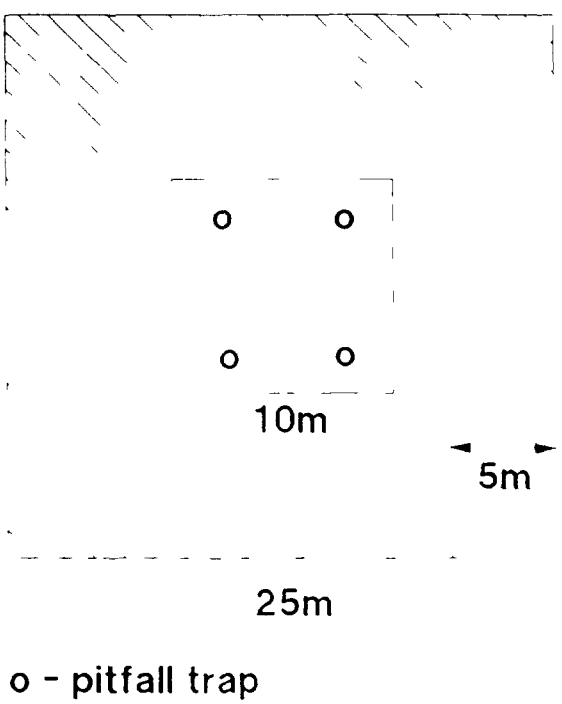

Fig. 2. Plot layout. Do not sample in the hatched outer area. The 2.5 metres surrounding the 10m *by 10 m subplot can be used for destructive sampling,e.g. soil samples and yield.*

1.1.2.4.Timing

Maize: The first application of lindane at the three leaf stage and the second two weeks later at a rate of 0.5 kg AI ha^{-1} on each occasion.

Alternative crop: according to local recommendations.

1.1.3. Field maintenance

1.1.3.1. Weeding

Mechanical

1.1.3.2. Seed dressing:

Accepted if necessary to secure the crop.

1.1.4. Background information to be recorded

1.1.4.1. Pesticide:	Formulation Manufacturer (including trade name) Dose rate Application details (type of sprayer nozzle type and identification letters/numbers, concentration of spray solution, volume rate of application)
1.1.4.2. Soil:	Mechanical composition (% sand, silt & clay) organic carbon content pH (specify soil/water ratio of slurry)
1.1.4.3. Plot:	Size Method of cultivation Previous cropping Neighbouring crops Previous pesticide history (if known) Fertilizers applied
1.1.4.4. Crop:	Variety If seed dressed specify dressing Seed rate Sowing date Density of established crop Spraying date Harvest date
1.1.4.5. Weather:	As much as is available regarding rainfall and temperature. Ideally on a daily basis. At least monthly rainfall and mean, maximum and minimum temperatures.

1.2. Monitoring pests

1.2.1. Species

Stem borers in maize and one chosen key pest in the alternative crop.

1.2.2. Sampling

(i) Maize crop: search all plants in the subplots for plant damage and record the observations as proportion of damaged plants in each subplot. An estimate of the population of stem borers in maize is optional, made 2-4 weeks after the last spray by dissecting 50 stalks, chosen randomly outside the subplots, and counting all larvae and tunnnels.

(ii) Alternative crop: according to the standard method for crop and pest.

1.2.3. Sampling intervals

One immediately pre-treatment and thereafter every two weeks for two months. Dissection of stalks to be done only once.

1.3.Monitoring non target arthropods

The objectives are to get good estimates of both ground living and foliar living predators. It is, therefore important that appropriate methods for these categories are used. The ground living fauna can be assessed with pitfall trapping or ground search and the foliar living fauna with sweep netting or D-vac sampling. None of these methods is perfect. Pitfall traps are rather easy to use but the catches are dependent on both number of insects and their activity. Ground searches are more tedious but give a more reliable estimate of predator density if performed correctly. Pitfall traps, because they are relatively easy to use, are suggested as the standard method for ground living predators.

The best method for foliar living arthropods is D-vac sampling. However, the apparatus is expensive and not readily available. Sweep netting is not an exact method but should give a reasonably accurate estimate of predator density. Sweep netting is suggested as the standard method for this study. However, those institutes that can use a D-vac sampler can substitute sweep netting with D-vac sampling.

It is very important that time and place of catch of each sample is identified. Each sample must, for statistical reasons, be counted and stored separately. It is not necessary to identify the catches beyond the indicated categories. However, it is recommended that all the listed categories are identified. If possible, store the samples in 50 - 70 % alcohol in airtight containers for future identification of important species.

1.3.1. Soil living insects

The following taxa should be identified and quantified where possible:

Order Collembola (spring tails)
Class Aranae (spiders)
Fam. Coccinellidae (lady bird beetles)

Fam. Formicidae (ants)
Fam. Carabidae (ground beetles).

Other groups (e.g. parasitoids, crickets, etc.) could be added if they are considered important natural enemies and/or are caught in substantial numbers.

1.3.1.1. Pitfall traps

Populations of ground living arthropods can be assessed periodically using simple pitfall traps. Plastic pots or beakers (7~10 cm in diameter; 10- 5 cm deep) buried with the upper rim at soil surface level can be used. It is important that the rim does not protrude above the soil surface (see Fig 3). Four traps are placed in each subplot (see Fig 3). The traps should normally be kept covered with lids or boards. The traps are uncovered and filled to half volume with water with a few drops of detergent (e.g. washing up liquid) at each sampling occasion. The traps are emptied after 48 hours.

Do not use coloured traps (they may attract or repel certain species). Use white or grey traps.

The best way to collect the catches from pitfall traps is to flush the whole contents of the trap trough a sieve (ca 1 mm mesh). The catch is then washed down with 70% alcohol (use a wash bottle) through a funnel into a container (this can be done in the field with some training). The arthropods are kept in 70% alcohol for later identification.

1.3.1.2. Ground search

Half metre square, wooden or metal quadrats are used for sampling. The quadrat is placed at random locations in the subplot. The vegetation and soil surface is carefully inspected

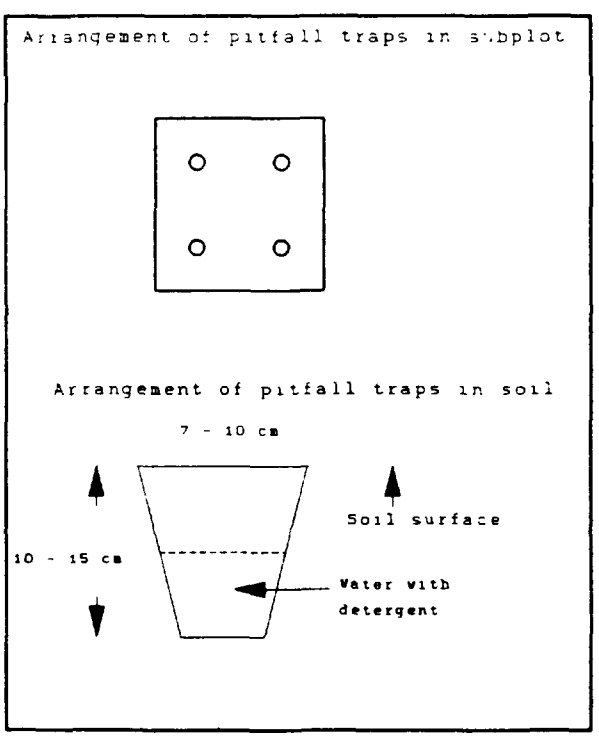

Fig 3. Arrangement of pitfall traps.

and any arthropods are caught (use a soft forceps or a pooter) and stored in 70% alcohol for later identification. Ground search should be done in the morning a few hours after sunrise, and always at the same time of the day.

NOTE! Ground search should ideally involve removal of the vegetation and inspection of the soil down to 5 cm to be of real value. However, in view of the small subplots, this would be too destructive to other sampling activities.

1.3.1.3. Pooter

The pooter or aspirator is convenient for the rapid collection of small insects in sweep nets, during ground search, etc. (Fig. 4). The catching end is placed above the insect and, by sucking the mouthpiece, the operator can catch the insects inside the bigger glass tube. By removing one of the corks the pooter can be emptied into a container.

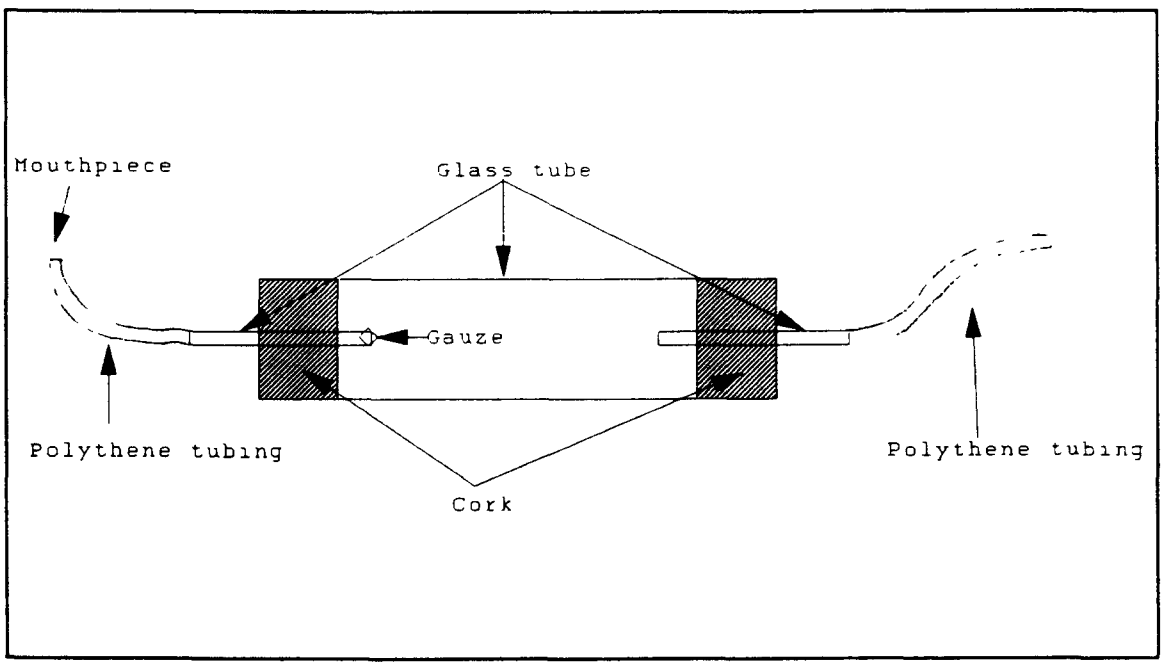

Fig. 4. The pooter.

NOTE! It is important to place a piece of gauze at the end of the glass tube leading into the mouth in order to avoid the risk of inhaling small insects or other particles.

1.3.1.4. D-Vac

The D-vac can be used for both plant and ground sampling. The cone with the biggest opening (1 sq. ft.) is for ground sampling and the smaller (1/3 sq. ft. opening) is for plant sampling.

Take ground samples at 4 different places in each subplot. A sample consists of five subsamples. Each subsample consists of a 5 second suck with the sampling cone lightly pressed to the ground. Take subsamples at intervals of two or three metres until 5 subsamples have been taken without emptying the collecting bag or switching off the

engine in between. After the 5th subsample, remove the collecting cone and empty the collecting bag into a plastic bag. Leave the engine running until the catch is secured in the plastic bag to prevent the insects flying away. Two persons are needed and prior training is necessary. It is recommended to carry a screw driver so that the air duct can be loosened from the engine to recover the bag in the event it is dropped. Repeat the whole procedure of 5 subsamples at 4 different places (e.g. near each pitfall trap) in each subplot.

1.3.1.5. Soil cores

A soil corer 5 cm in diameter and at least 20 cm long is recommended. Ideally it should be possible to divide the sample into sections, for example using a design like that of Tanton [1] (Fig. 5) but this is not essential. Four soil cores (5 cm in diameter 15 cm deep) should be taken from each subplot prior to treatment and again after two months.

The arthropods in the soil sample can be extracted in a Berelese-Tulgren funnel (see Fig. 6). The diameter of the funnel can be between 15-40 cm but the angle should be steep. Laboratory glass funnels are suitable but plastic should be avoided because of potential problems caused by static electricity. The mesh size of the metal gauze should be about 3mm. Some kind of heat source (e.g. a light bulb) should be placed above the sample or the extraction should be done in a warm room. The purpose is to obtain a temperature gradient of 10-20°C from top to bottom of the sample. The sample should be broken into

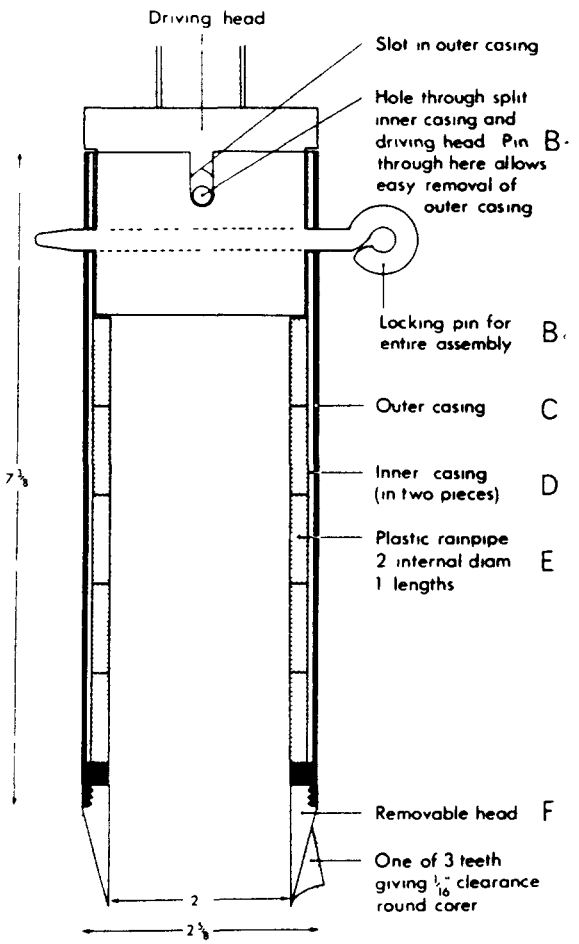

Fig. 5. Soil corer with split liner.

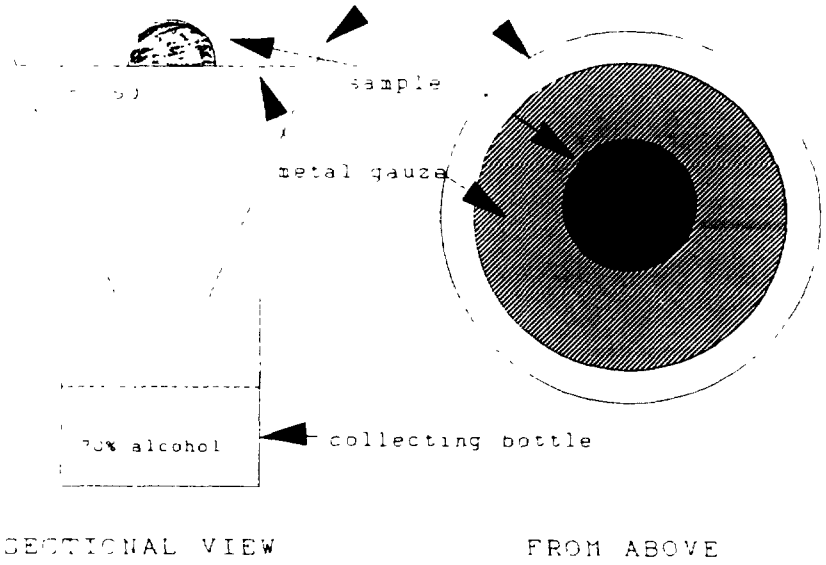

Fig. 6. The Berlese-Tullgren funnel.

smaller sections before extraction. The sample should, however, be at least 1 cm thick to avoid it drying out. Leave the sample in the funnel for 72 h. Extraction should be done as soon as possible after sampling but it is still possible for up to 4 months if the sample is stored at 4-6°C.

1.3.2. Foliar living insects

1.3.2.1. Sweep net

A standard sweep net consists of a cloth bag fitted into a wire loop (ca 40 cm in diameter) and attached to a 50-60 cm long handle. The net bag is cone shaped and approximately 60 cm deep. As you sweep, tilt the lower rim of the net so it is ca. five cm in advance of the upper rim to catch insects which drop from the plant. Be careful to keep the net below the tops of the plants until the end of the sweep. A single sweep consists of one 180° arc as you step forward. Raise the net at the end of each sweep and reverse the direction of your swing alternately. Take five sweeps for each diagonal. Two diagonals (= two samples) is taken per subplot (see Fig. 7). The catch can be collected with a pooter or put directly in a container with 70% alcohol.

1.3.2.2. D-Vac

As with the ground search, plant samples consist of 5 subsamples. In this case each subsample consists of one plant. Take a total of 4 samples in each plot. To make the sampling as exhaustive as possible place. the cone over the whole plants when they are small; when larger, sweep the plants with the cone from all sides. Since the method is impractical on large plants, sample plants only in the first 6 weeks.

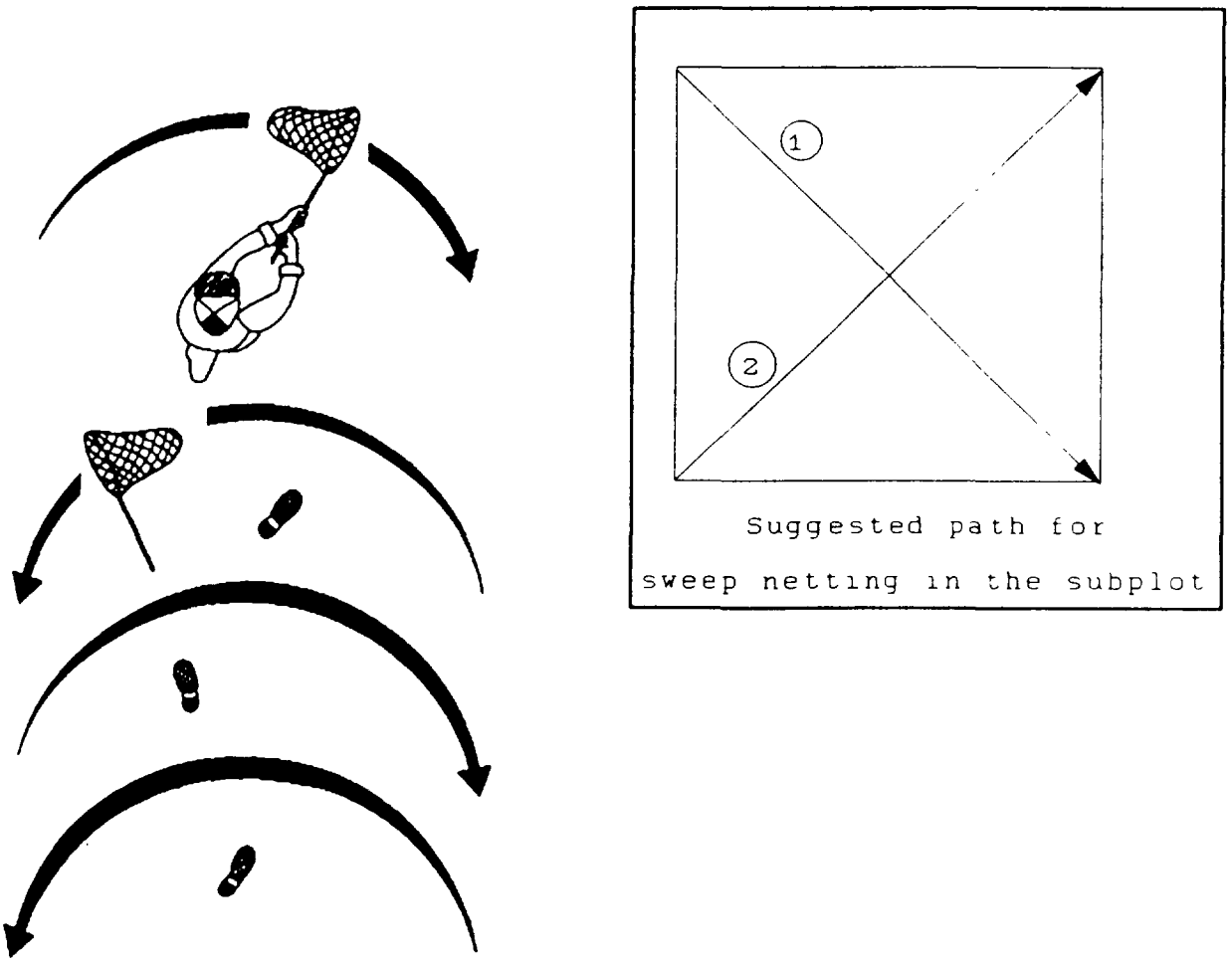

Fig. 7. Use of the sweep net.

1.3.3. Soil biology

1.3.3.1. Categories

Breakdown of organic matter (compulsory), earthworms and soil fauna (optional).

1.3.3.2. Sampling methods

(i) Breakdown of organic material: Cut disks (2.5 cm diameter), using a cork borer or similar tool, from suitable leaves collected from trees of a single, identified spp growing nearby the experimental plots. The leaves should be rather tough, uniform and growing on the same side of the tree. Place batches of fifty disks in nylon mesh (7-10 mm aperture) bags (ca. 12 cm square), closed with staples and attached to a marker peg with thread. Bury four bags per subplot at random to a depth of ca. 5 cm prior to treatment. Dig up the bags after 2-5 months and wash the contents, then dry in an oven (ca. 600 C for 24 hours) and weigh. Dry and weigh two additional batches of fifty leaves prior to treatment. Present the results in two ways: (a) percent loss in weight (the freshly dried disks are 100%) and (b) the actual weight of the content.

Note! It is important that the bags are left in the soil for an "appropriate", time that is when approximately 50% of the organic matter has been broken down. It is therefore recommended to bury two extra bags in two control plots to be used to decide when to dig up the rest of the bags.Thus one extra bag can be dug up after 1-2 months and the second after 2-3 months.

(ii) Earthworms: Use half metre square, wooden or metal quadrats for sampling. Lay them on the soil surface and apply nine litres of dilute formalin (50 mL of 40% in 9 litre water) gradually with a fine spray watering can so that the soil does not become flooded. Most of the worms come to the surface within 15 minutes, and can be picked off and collected into jars containing 5% formalin for storage and eventual identification to species. Take two samples per subplot prior to treatment and again after two months.

1 3.4. Yield

1.3.4.1. Sampling

Estimate yield from 4 subplots (2 m × 2 m) in each plot. Because sampling activities may have an effect on the yield, locate the subplots for yield outside, but close to, the subplots for sampling. Note the moisture content in the crop at harvest.

1.3.5. Residues

1.3.5.1. Categories

Crop (compulsory), soil, earthworms and insects (optional).

(i) Crop sampling: For basic analysis a sample of 12 plants per plot is suggested. Collect the leaves only 1 day pre-treatment, 1 day post-treatment, after 2 weeks and after 4 weeks. Weigh, chop, preferably mechanically, and thoroughly mix. Take duplicate subsamples for dry weight and residue analyses.

(ii) Soil sampling: Take 12 cores at random from each subplot. Cores 2.5 diameter and 15 cm deep are advised. Weigh the cores before and after removal of any stones and grind to pass a 0.25 cm sieve. Mix the ground cores thoroughly to provide one bulk sample for each plot by successive quartering of the soil on a plastic sheet or in a mechanical mixing device. Take subsamples in duplicate for measurement of moisture content and for residue analysis pre-treatment, one day post- treatment and after 2 and 6 months from both treated and untreated plots.

(iii) Earthworms: Worms can be obtained for residue analyses either by the formalin method or by digging. If formalin is used, drop the earthworms into a bucket of clean water immediately they emerge on to the soil surface. Then leave for one day on a damp filter paper in covered petri dishes to allow them to empty their intestines. Then wash, remove surplus moisture with clean tissue paper, and place in clean petri dishes in a deep-freeze and leave until brittle. Take samples pre-treatment and after two months.

1.4. Higher fauna

It is not possible to make up detailed protocols for this area at present. The methods must be adjusted to local conditions and to the range of species that are chosen.

Basically two different approaches can be adopted:

1. Field trial
2. Extensive survey.

In both cases there must be a choice of suitable "indicator" species to study. The criteria could be:

species that are particularly likely to be exposed to the chemical;
species thought to be particularly sensitive to exposure;
species for which an adverse effect would be especially damaging;
abundant species permitting large samples for data collection;
relatively sedentary species;
species whose ecology and behaviour provide easy opportunities to measure effects.

It is unlikely that a single species will fulfill all these roles, and it may be desirable to study a range of species from several categories, at least initially.

1.4.1. Field trial

This type of study requires rather large areas of land (several hectares) where the use of pesticides can be supervised. There must also be some kind of control data to enable comparison. This could be done in two ways: to look for changes before and after treatment on the same site or to compare the treated area with an untreated. A combination of both approaches is recommended.

The measurement should include:

estimation of population numbers (e.g. trapping for mammals and nest counts for birds);
reproductive success;
corpse counts;
residue analyses (corpses, blood, eggs);
biomarkers (if applicable).

Although dead animals give the clearest evidence of a damaging effect this may not always be the most useful approach to detect adverse effects from pesticide use. Carcass searching should, therefore, be complemented with a measure of other effects, e.g. estimation on population changes and reproductive success. If possible a biomarker should be used such as egg shell thinning or repression of enzymatic processes.

1.4.2. Extensive survey

This survey should be done as a comparative study between an area with regular use of organochlorine pesticides (contaminated area) and an area with no such use (control area).

This should include:

background survey, i.e. information on pesticide use, information on residues in higher fauna and identification of indicator species;
identification of contaminated area and control area;
collection of samples for residue analyses.

One crucial step is the choice of indicator species (see above). For this type of study it is important that relatively sedentary species are chosen. For birds, it is recommended to chose one or a few predatory birds e.g. falcons and one or a few insect eating birds, e.g. shrikes.

1.4.3. Sampling for residue analyses

1.4.3.1. Carcasses

Carcasses may have been shot or simply found dead in the field. They should be put in plastic bags, labelled and taken to the laboratory (preferably at 0-4°C) where they should be dissected as soon as possible. Carcass weights and fresh organ and/or tissue weights should be recorded. Liver samples should always be analysed and where appropriate brain, breast muscle and fat should also be sampled. Whole tissues or organs should be homogenised and subsamples taken as needed. If storage is necessary samples or subsamples should be kept at -20°C (or lower) in glass tubes or vessels fitted with tight sealing caps.

1.4.3.2. Eggs

After weighing, eggs should be carefully blown to remove all the contents. The content should be weighed and stored, if necessary, at -20°C. The shells should be retained to assess shell thickness either by the weight/volume ratio method or by direct measurement of sections.

A very useful publication for this area is Sommerville, L. & Walker, C.H. [2].

2. RESIDUE ANALYSES

2.1. Residue analyses (unlabelled pesticides)

If chlorinated hydrocarbons are analysed routinely, follow routine procedures. If not, the following notes can be used as a basis to develop a procedure. No extraction and cleanup procedure can be guaranteed to work first time. Always check the recovery through the procedure by 'spiking' samples (at least 3) of untreated substrate with a known quantity of analyte. The easiest way to do this is to use ^{14}C labelled pesticides, 3.7 mBq (0.1 g Ci) per sample is enough.

Always run control (untreated) samples of substrate through the procedure to establish thechromatographic background. This is necessary to determine the limit of determination. There are a number of statistical procedures that have been proposed but for most purposes the 'rule of thumb' approach of setting the limit of determination at twice the blank value is satisfactory.

2.1.1. Extraction

2.1.1.1. Plant and non-fatty animal tissue

Homogenise 10-20 g tissue in 200 mL methanol. Filter if necessary. Take a known volume of filtrate (up to 142 mL) and add 2.5 times that volume of water to give a solution containing not more than 40% methanol. The final volume should not exceed 500 mL.

2.1.1.2. Fatty animal tissue and fish

Homogenise 10 g tissue, add 20 g Na_2S0_4 and mix thoroughly. Transfer to a 250 mL separating funnel and add 50 mL hexane or petroleum ether followed by 100 mL acetonitrile saturated with hexane or petroleum ether and shake 1 minute. Drain the acetonitrile into a 1 L separating funnel. Repeat with 3 further portions of 50 mL acetonitrile and combine the extracts. (For reasons of economy you could check the recovery using smaller quantities of acetonitrile.) Add 500 mL water, 40 mL of saturated NaCl solution and 50 mL petroleum ether or hexane. Shake 30 seconds, allow layers to separate and drain aqueous layer into a 2nd 1 L separating funnel. Shake aqueous layer with 50 mL petroleum ether or hexane. Discard aqueous layer and combine the petroleum ether/hexane extracts. Drain through a plug of 10 g Na_2SO (held in a funnel for example) into an evaporating basin or a rotary evaporator. Evaporate just to dryness and dissolve residue in 10 mL methanol. Dilute with 25 mL water.

2.1.1.3. Soil

Extract 50 g soil with methanol in a Soxhlet apparatus for 10 cycles (2-4 hours). Dilute the methanol extract with 2.5 volumes of water as above.

2.1.2. Cleanup using solid phase extraction (SPE) columns

A number of suppliers produce SPE columns. They vary slightly and different batches from the same manufacturer may also vary, so the procedure needs to be checked. Recovery of standard from the column should be at least 90%. For this analysis 6 mL C-18 SPE columns are needed.

2.1.2.1. Column conditioning

Pass two column volumes of methanol, followed by two column volumes of distilled water through the column.

2.1.2.2. Sample addition

Add 200 mL of water diluted methanol extract to the extraction column(s). The flow rate through the column(s) should be 30-40 mL/min. Residual particulates can be removed by placing a 6 mL filtration column and adaptor between the extraction column and reservoir.

2.1.2.1. Column wash

Remove reservoir(s) and wash with one column volume of distilled water. Air dry column under vacuum for 10 minutes.

2.1.2.3. Sample elution

Elute with three 500 µL aliquots of hexane and make up eluate to 2 mL with hexane.

2.1.3.Gas chromatography

There are many sets of GLC conditions cited in the literature. Examples include:

Glass column 5 feet long, 1/4 in diameter packed with 5% QF I on 60-80 mesh Chromosorb G. Column temp. 220°C, detector temp 235°C carrier gas 20 mL/min [3].

Glass column 165 cm × 2 mm packed with 1: 1 mixture of 10% DC-200 and 15% QF-1 on Gas Chrom G. Injector temp 235°C, column 220°C, detector 275°C. Carrier gas 70 mL/min [4].

Glass column 1 m × 3 mm packed with 5% SE30 on Chromosorb W. Column temperature 200°C [5].

2.2. Analysis of (^{14}C) organochlorine pesticides in maize

(i) Homogenize the maize sample with methanol as in 2.1.1.1.

(ii) Take three replicate samples of not more than 300 mg dry weight and combust in a biological material oxidizer, trapping evolved $^{14}CO_2$ in Carbosorb. Analyze the trapped $^{14}CO_2$ in LSC to give total radioactivity in the sample.

(iii) Weigh a 5g sample of maize homogenate into a flask and add 20mL methanol. Shake for 30 min. then sonicate for 5 min. in an ultra sonic bath. Transfer the sample to a centrifuge tube, centrifuge for 10 min. at 3000 RPM and remove supernatant. Determine total volume and count 2 × 1 aliquots in LSC for calculation of total extractable radioactivity. Dry the residue and combust to determine unextractable radioactivity. Determine total recovery.

SUMMARY OF SAMPLING PROGRAMME

Type of sampling	Compulsory/Optional	No. of pre-treatment samples	Intervals of post-treatment samples
Plant damage	Comp	1	2, 4, 6, 8, 10 weeks
Dissection of stalks	Opt	0	ca. 8 weeks
Pitfall traps	Comp	1	0*, 2, 4, 6, 8, 10 weeks
D-vac (ground)	Comp	1	1 day, 2, 4, 6, 8, 10 weeks
D-vac (plant)	Comp	1	1 day, 2, 4, 6 weeks
Breakdown organic matter	Comp	-	2-5 months
Earthworms	Opt	1	ca. 2 months
Soil fauna	Opt	1	ca. 2 months
Yield	Comp	-	-
Residues in crop	Comp	1	1 day, 2, 4 weeks
Residues in soil	Opt	11	1 day, 2, 6 months
Residues in earthworms	Opt	1	ca. 2 months

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