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The effects of mancozeb and lindane on the soil microfauna of a spruce forest: A field study using a completely randomized block design

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Summary. The effects of mancozeb (fungicide) and lindane (insecticide) were investigated in active soil ciliates, testaceans, rotifers, and nematodes. The effects were evaluated 1, 7, 15, 40, 65, and 90 days after application of a standard and a high (10×) dose. Individual numbers were estimated with a direct counting method. Mancozeb, even at the high dose, had no pronounced acute or long-term effects on absolute numbers of the taxa investigated. The number of ciliate species, which decreased 1 day after treatment with the normal dose ($0.05 < P < 0.1$), soon recovered. However, the community structure of ciliate species was still slightly altered after 90 days. Testaceans were not reduced before day 15 at the higher dose or before day 40 at the normal one ($0.05 < P < 0.1$). A normal dose of lindane caused acute toxicity in ciliates and rotifers ($P < 0.05$) but the latter soon recovered. The number and community structure of ciliate species were still distinctly altered after 90 days ($0.05 < P < 0.1$), indicating the critical influence of lindane. Testaceans were reduced only after day 15, and nematodes only on day 40 ($0.05 < P < 0.1$). At the high dose of lindane severe long-term effects occurred in soil moisture, total rotifers ($P < 0.05$), total nematodes ($0.05 < P < 0.1$), and in the structure of the ciliate community. Generally, there were marked differences in the effect of the normal and the high dose of lindane but not with mancozeb. Ciliates showed very pronounced changes after the pesticide applications, indicating their usefulness for testing biocides under field conditions. Testaceans were more resistant than ciliates.

Key words: Microfauna – Ciliates – Testaceans – Rotifers – Nematodes – Mancozeb – Lindane – Forest soil

Well-founded knowledge of the effects of pesticides on soil organisms is a prerequisite for the evaluation of possible hazards to the biosphere and man (Ottow 1982). Usually, effects are assessed under standardized laboratory conditions in single-species toxicity tests which are, however, sometimes ecologically unrealistic. In addition, most studies have dealt only with prokaryotic organisms (bacteria and fungi) and have been restricted to agricultural soils. However, protozoa also seem to be a good tool because they are at the base of the eukaryotic food chain and are important in soil processes. Foissner (1987) found, from a review of the available literature, that protozoa are equally or even more sensitive to pesticides than other eukaryotic organisms which are often too mobile or reproduce too slowly.

Due consideration must be given to a proper experimental design of field studies. It is not sufficient to provide for one treated area and one untreated reference plot. Though widely used, the statistical evaluation of this type of experiment, even if repeated samples are taken, yields only site differences between two plots (Hurlbert 1984). For more accurate statistics, several independent plots are necessary for each treatment. This can be best achieved with a completely randomized block design. The monitoring period should be at least 90 days in order to detect persistent pesticide effects in the field (Domsch et al. 1983).

In the present work we incorporated a completely randomized block design in a study of the effects of a fungicide and an insecticide, both widely used in forestry, on active ciliates, testaceans, nematodes, and rotifers. We used a spruce forest soil, in which these organisms occur in high numbers (Petz and Foissner 1988).

Materials and methods

Site description. The experimental area is situated in a spruce forest, *Piceetum nudum*, in Oberhaag near Aigen (Böhmerwald, Upper Austria, 860 m above sea level). The stand is about 100 years old and the average yearly air temperature is 4.5 °C; yearly precipitation is about 1000 mm. The soil is an acidic brown earth on granite with

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distinct signs of podzolization. The 0–30 mm soil horizon consisted mainly of fresh and slightly decomposed needles (L and F layers), with a pH ($CaCl_2$) of 2.6.

Experimental design. The plots were arranged in six completely randomized blocks (Köhler et al. 1984) on flat terrain. Each block contained five plots each 1 m^2 (four treatments, one control). A 0.6 m high frame was used, to avoid contamination of neighbouring plots during spraying of the pesticides. To avoid contamination by horizontal leaching or washing out, e.g. after rainfall, roof tiles were buried vertically at a distance of about 0.2 m from the plots. In addition, the plots were about 1 m apart. The minimal distance between two blocks was about 2.5 m.

The commercial formulations of two pesticides were applied once in a normal and once in a tenfold dose (further referred to as "high dose") from a sprayer; the higher dose of the insecticide was poured from a watering can. The control plot was irrigated with 1 litre of tap water, which was the average amount of liquid the other plots received. Dithane M-45 (Rohm and Haas, USA), a non-systemic fungicide, is a wettable powder and contains 80% mancozeb, a complex of Zn ions and Mn ethylene-bisdithiocarbamate. The normal dose (manufacturer's instruction) is 1.2 kg ha^{-1} in a 0.2% aqueous suspension. This is equivalent to 0.096 g m^{-2} active ingredient in the normal and 0.96 g m^{-2} in the high dose. Mancozeb belongs to toxicity class 4(5) in Austria, defined as "hardly toxic to warm-blooded animals" (Smidt and Ferenczy 1977). A lethal dose (LD 50) in rats (oral) amounts to >7000 mg kg^{-1} body weight; it is practically insoluble in water (Anonymous 1982). Stammschutzmittel Gamma (trunk protectant; Chemie Linz AG, Austria), a chlorinated hydrocarbon insecticide, is a liquid and contains 200 g $litre^{-1}$ lindane, that is γ-1,2,3,4,5,6-hexachlorocyclohexane. To protect cut spruce from bark-beetles, 0.3 litre m^{-2} of a 10% aqueous emulsion is recommended by the manufacturer. This is equivalent to 6 g m^{-2} active ingredient in the normal and 60 g m^{-2} in the high dose. According to Austrian standards, lindane belongs to toxicity class 3, "moderately toxic to warm-blooded organisms". It acts as an inhalation, ingestion, or contact poison (Smidt and Ferenczy 1977). The LD 50 in rats (oral) amounts to 88–225 mg kg^{-1} and (dermal) 500 mg kg^{-1} body weight; its solubility in water is 7×10^{-4}g 0.1 $litre^{-1}$ (Anonymous 1982). Lindane is rather persistent. In the laboratory, 50% degradation occurs within 600 days (Laskowski et al. 1983).

The experiment was conducted in two parts, each consisting of three blocks, from mid-June to the end of October 1986. Samples were taken 1/2, 7/8, 15/16, 40/41, 65/66, and 90/91 days after application of the pesticides. For brevity, only the 1st number is used for each date. Plots treated with the fungicide were not investigated on day 65.

Sampling. Five subsamples were taken randomly with a spatula from the 0–30 mm soil depth in each plot. From each subsample, 0.1 g litter was removed and pooled. Out of this 0.5 g, 0.1 g litter was suspended in a few ml sterile soil solution and immediately inspected under the microscope at 40× total magnification. Active ciliates, nematodes, and rotifers were enumerated with a direct counting method (Lüftenegger et al. 1988). Usually, four replicates were inspected for each plot in one block within 2 days, that is 0.4 g soil per date and plot. Subsequently, the next block was studied.

Samples for testaceans were prepared in the same manner, but after staining with phenolic aniline blue and a series of dilutions, 0.0025 g litter was examined at 100× total magnification. Unreplicated samples were investigated 1 (six blocks), 15 (six blocks), 40 (three blocks), and 90 days (six blocks) after the pesticide applications.

The soil moisture content (% wet mass of soil) was ascertained by air-drying and soil pH was determined in 0.01 *M* $CaCl_2$ solution with a glass electrode.

Statistical analysis. The means of the four replicates from each plot were calculated. According to the block design, these data were evaluated with a two-way analysis of variance with soil moisture as covariate. Values of ciliates and rotifers had to be square-root transformed to meet the assumptions of this procedure [normality was tested with the Kolmogorov-Smirnov test (Sachs 1984) and homogeneity of variances with the Bartlett test (Köhler et al. 1984)]. If a treatment effect was suggested, pairwise comparisons followed between each pesticide treatment and the control and between both doses of one compound with the two-way (soil moisture) analysis of variance, or with a one-way analysis of variance if a block effect was not pronounced. Single testacean counts were compared using the Mann-Whitney *U*-test if the requirements of the one-way analysis of variance were not met. Percentages of ecological groups of ciliates were log t $(x+1)$ transformed, those of ciliate species were square-root transformed. Dominances of testacean species were used untransformed because the assumptions of the analysis of variance were already met.

Cluster analyses of protozoan community data, for instance, Jaccard's species similarity index and the species-abundance index of Bray-Curtis (dissimilarity type), were performed with the unweighted pair-group method using arithmetic averages. Calculations were aided by a computer using MINITAB, SPSS-X and CLUSTAN software packages.

For Shannon-Weaver's diversity index and evenness see Mühlenberg (1976).

Results

Soil moisture

A pronounced increase (about 10% on the average) in soil moisture content on all sampling dates followed the high lindane application relative to the normal dose and to the control ($P<0.05$). This is in accord with the findings of Andren and Steen (1978) who reported reduced permeability of water after elimination of the soil fauna by pesticides. Minor increases were observed with the normal dose of lindane after 40 ($0.05<P<0.1$) and 65 days ($P<0.05$). Soil humidity was slightly decreased in plots treated with the higher dose of mancozeb after 15 and 90 days ($0.05<P<0.1$; not shown).

Ciliate numbers

Mancozeb had no appreciable detrimental or stimulating effect on ciliate numbers, except 90 days after the high-dose treatment, when their abundance increased ($0.05<P<0.1$; Fig. 1). Application of the normal dose reduced the number of active ciliate species immediately ($0.05<P<0.1$; Fig. 1). However, after 7 days this depression in species number had disappeared (Fig. 1). The normal dose of lindane reduced both the numbers of active ciliates and the numbers of species 1–15 days after treatment ($P<0.05$; Fig. 1); species numbers were still considerably less 40 and 90 days after spraying ($0.05<P<0.1$; Fig. 1). Following application of the high dose, a more persistent depression occurred both in abundance and species number.

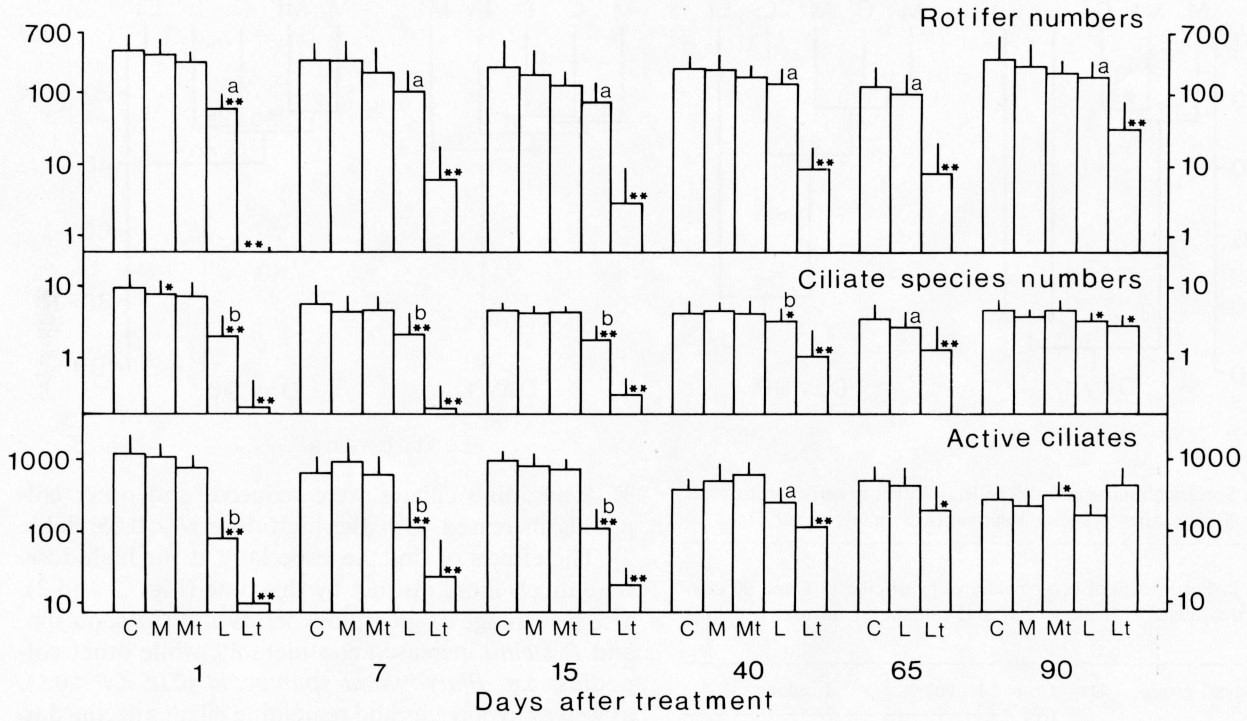

Fig. 1. Arithmetic means (±SD) of the abundance and species number of active ciliates and of numbers of rotifers at 0–30 mm soil depth following pesticide treatments. Control (*C*); normal dose: mancozeb (*M*), lindane (*L*); high dose: mancozeb (*Mt*), lindane (*Lt*). Numbers (log *e*) are given as individuals g^{-1} dry mass of soil. $*0.05<P<0.1$, $**P<0.05$, differences from control; *a*, $0.05<P<0.1$, *b*, $P<0.05$, differences from high dose

Table 1. Percentage of the dominant species of active ciliates and testaceans 1 and 90 days after treatment with mancozeb and lindane at normal and high doses

Species	Day	Control	Mancozeb		Lindane	
			1×	10×	1×	10×
Ciliates:						
Hausmanniella discoidea (Gellért)	1	23.5	44.3**	37.4	15.2**	0.0**
	90	41.3	45.8^{+}	42.9	26.7^{++}	0.0**
Platyophrya spumacola (Kahl)	1	19.4	12.7	21.7	12.1	0.0**
	90	13.1	9.8	13.6	22.4^{+}	4.4**
Pseudoplatyophrya nana (Kahl)	1	18.5	10.0*	6.0	15.2	0.0**
	90	10.8	13.6	17.7	24.2**	35.8**
Paracolpoda steinii (Maupas)	1	4.5	1.4	4.0	6.1	x^{a}
	90	0.0	0.0	0.7	2.5^{++}	44.8**
Colpoda inflata (Stokes)	1	0.1	1.0	0.5	0.0	0.0
	90	0.8	0.0	0.7	0.6^{+}	9.3**
Testaceans:						
Corythion dubium (Taranek)	1	48.1	74.6*	63.3	46.0	51.3
	90	47.9	52.2	48.0	39.7	32.7
Trinema lineare (Penard)	1	11.3	7.5	0.0	18.7	12.2
	90	9.6	17.5	17.8	18.3**	15.7
Schoenbornia humicola (Schönborn)	1	0.0	0.0	2.8	3.0	8.7
	90	9.6	9.7	6.4	6.9	17.0

[a] High value, not representative because only two individuals were found to be active

$*0.05<P<0.1$; $**P<0.05$; differences from control

$^{+}0.05<P<0.1$; $^{++}P<0.05$, differences from high dose

However, the total number of individual ciliates insignificantly exceeded that of the control 90 days after the use of lindane, but the species richness was still distinctly lower ($0.05<P<0.1$; Fig. 1). In fact, the high numbers of active ciliates in these plots may be attributed to only two species, *Paracolpoda steinii* and *Pseudoplatyophrya nana* (Table 1).

Ciliate community structure on day 1

For brevity, clusters of Jaccard's index are omitted because they are very similar to those obtained with the Bray-Curtis index (list of species in Petz et al. 1988). Changes in the community were much more pronounced in the lindane treated plots than in the mancozeb-treated ones (Fig. 2, Tables 1 and 2). Likewise, diversity drastically decreased due to the reduction of species and a reduced equitability (Fig. 3). The proportion of hypotrichs and, as might be expected, the fungus-feeding grossglocknerids (*P. nana* $0.05<P<0.1$; Table 1) decreased in the mancozeb-treated (fungicide) plots ($P<0.05$ and $0.05<P<0.1$; Table 2). At the normal dose of mancozeb, the remaining ciliates ($P<0.05$) as well as other colpodids ($0.05<P<0.1$; Table 2) increased, mainly due to

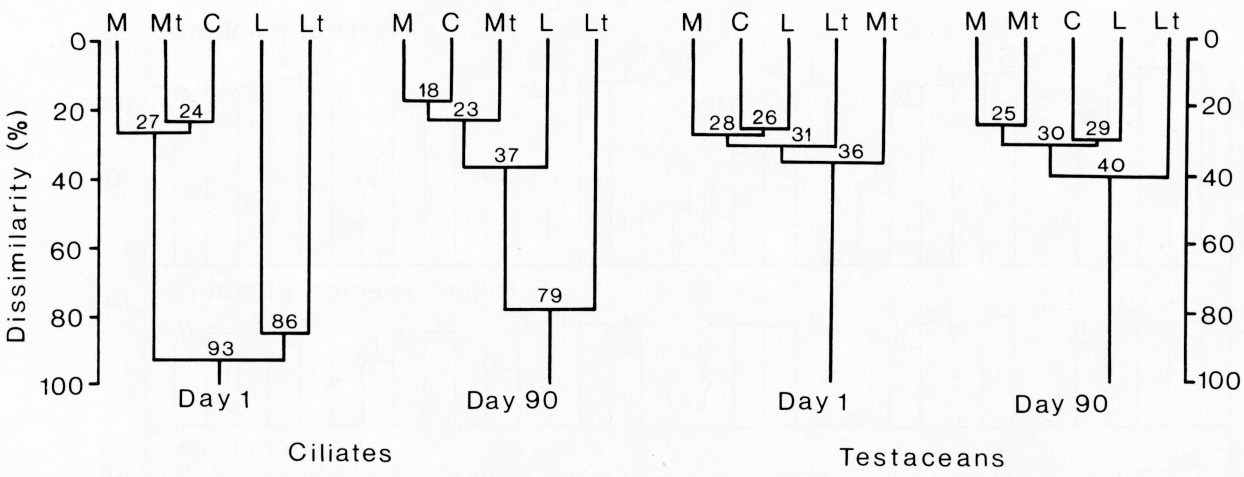

Fig. 2. Species-abundance index of Bray-Curtis in active protozoans 1 and 90 days after pesticide treatments. Symbols as for Fig. 1

Table 2. Percentage of ecological groups of ciliates 1 and 90 days after treatment with mancozeb and lindane at normal and high doses

Ecological group	Day	Control	Mancozeb		Lindane	
			1×	10×	1×	10×
Grossglocknerids	1	18.5	9.6**	6.7*	15.3^{+}	0.0**
(mycophagous)	90	14.8	15.2	20.6	26.0	39.1**
Colpoda spp. and *Paracolpoda steinii*	1	4.8	2.2	4.2	5.6	x[a]
(bacteriophagous)	90	1.5	0.9	1.3	4.1^{++}	53.6**
Other colpodids	1	40.2	55.3*	55.3	26.4^{++}	0.0**
	90	52.8	53.4	53.5**	46.7^{++}	4.3**
Hypotrichs	1	9.6	7.4^{**+}	8.7	6.9^{+}	0.0**
(omnivorous)	90	19.2	15.2	11.0	16.6^{++}	0.8**
Remaining ciliates	1	23.6	23.9**	19.6**	38.8	x[a]
	90	10.7	13.9	10.0**	4.1^{++}	1.0**

[a] High value, not representative because only two individuals were found to be active
*$0.05<P<0.1$; **$P<0.05$, differences from control
$^{+}$ $0.05<P<0.01$; $^{++}$ $P<0.05$, differences from high dose

Hausmanniella discoidea ($P<0.05$; Table 1). The remaining ciliates decreased at the high dose ($P<0.05$; Table 2). Following the normal-dose lindane treatment, *H. discoidea* was depressed ($P<0.05$; Table 1). Only two specimens of each of *P. steinii, Vorticella astyliformis*, and *Arcuospathidium vermiforme* were found active on day 1 in the samples after the high-dose application of lindane (Tables 1 and 2).

Ciliate community structure on day 90

The proportions of species changed gradually into those found at the end of the study. Cluster analyses showed scarcely any influence of mancozeb though diversity was still lower than in the control (Figs. 2 and 3). Remaining ciliates were reduced, and other colpodids increased with the high dose ($P<0.05$; Table 2). The effects of lindane, especially at the high dose, were much more distinct by this time (Figs. 2 and 3). The percentage of grossglocknerids and *Colpoda* spp. and *P. steinii* increased considerably, while other colpodids, e.g. *Platyophrya spumacola* ($0.05<P<0.1$), as well as hypotrichs and remaining ciliates declined to near extinction at the higher dose ($P<0.05$; Tables 1 and 2). A shift in dominance occurred, from the other colpodids to *Colpoda* spp. and *P. steinii* (Table 2). *P. nana* increased markedly at both doses; *P. steinii* and *Colpoda inflata* increased only at the high one ($P<0.05$; Table 1). Other species recovered only very slowly from the high dose; *H. discoidea* is entirely absent (Table 1), and *P. spumacola* appeared again after 65 days.

Testacean numbers

For brevity, only data from days 15 and 90 are shown in Table 3. The high dose of mancozeb depressed active testaceans after 15 days ($0.05<P<0.1$; Table 3). At both doses the abundance and species number of viable testaceans (active + cystic + precystic specimens) were decreased only at day 40 ($0.05<P<0.1$ and $P<0.05$, respectively). On day 1, comparatively fewer active testaceans were found under the normal dose than under the high dose of mancozeb ($0.05<P<0.1$). After 90 days, more viable specimens were counted under the standard dose of mancozeb than under the high one ($0.05<P<0.1$; Table 3). Both doses of lindane had decreased active and viable testaceans by day 15 ($0.05<P<0.1$ and $P<0.05$; Table 3), viable specimens were also reduced under the normal dose after 40 days ($0.05<P<0.1$). Similarly, the number of viable testacean species had decreased 15 and 90 days after application of the high dose ($0.05<P<0.1$ and $P<0.05$; Table 3) and on day 40 at both doses

($P<0.05$). After 15 days, more parasitized testaceans occurred under the high dose of lindane than in the control plots ($0.05<P<0.1$; Table 3). Compared with the high dose, considerably higher numbers of cystic individuals and species of viable testaceans were counted under the normal dose on day 15 ($P<0.05$) and of viable species on day 90 ($0.05<P<0.1$; Table 3).

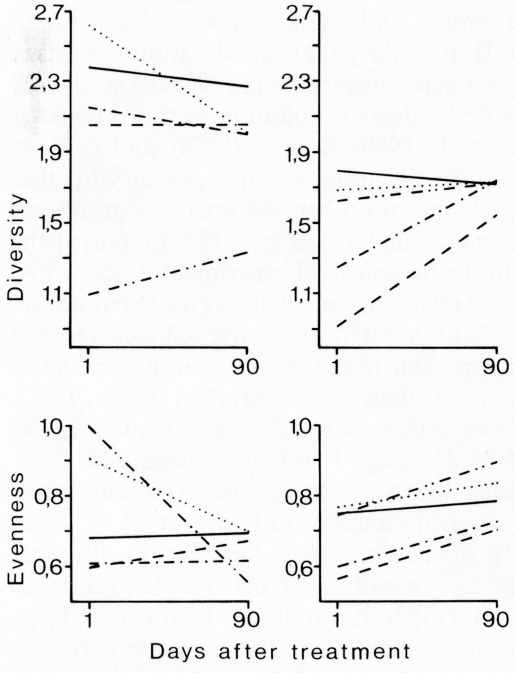

Fig. 3. Diversity (Shannon-Weaver) and evenness of active protozoans 1 and 90 days after pesticide treatments. Control ———; mancozeb: normal dose – – –, high dose – · – · –; lindane: normal dose · · · · ·, high dose · · – – · ·. Ciliates (*left*); testaceans (*right*)

Testacean community structure on day 1 and 90

For brevity, clusters of Jaccard's index were omitted because they are rather similar to those obtained with the Bray-Curtis index (list of species in Petz et al. 1988). No conspicuous differences were found in testacean communities between the treated and the untreated plots (Fig. 2), nor after 15 and 40 days (not shown). A slightly higher percentage of *Corythion dubium* was recorded under the normal dose of mancozeb on day 1 ($0.05<P<0.1$; Table 1). The diversity and evenness of testaceans were considerably lower immediately after the application of mancozeb compared with the control and the lindane-treated plots. This difference disappeared after 90 days (Fig. 3). However, at this date there was a slight distinction between the higher dose of lindane and the other treatments with the Bray-Curtis index (Fig. 2). *Trinema lineare* had increased considerably under the normal dose of lindane on day 90 ($P<0.05$; Table 1).

Rotifers (Fig. 1)

Both fungicide treatments decreased the total rotifer numbers slightly. However, P was always >0.1. Considerably fewer rotifers were counted 1 day after the normal-dose lindane application and on all dates following the high-dose application ($P<0.05$). Differences from the control were not very distinct 7, 15, 40, and 90 days after the normal dose ($0.1<P<0.2$). Numbers differed markedly between both lindane treatments during the entire experiment ($P<0.05$).

Table 3. Arithmetic means (±SD) of the abundance of active (A), precystic (B), and cystic (C) testaceans, of the abundance (D) and the species number (E) of viable (active + precystic + cystic) individuals (g^{-1} dry mass of soil) and of the abundance of parasitized specimens (F) 15 and 90 days after treatment with mancozeb and lindane at normal and high doses

Days after application		Control	Mancozeb		Lindane	
			1×	10×	1×	10×
15	A	7833 ±5029	8200 ±2854	6513 ±2817*	3817 ±1636*	6596 ±6113*
	B	1988 ±1828	2681 ±2366	1638 ±1534	2537 ±2684	743 ±1025
	C	1645 ±2487	1720 ±1696	1383 ± 597	1203 ± 570^{+}	182 ± 446
	D	11467 ±3593	12600 ±3076	9534 ±2300	7557 ±1581**	7522 ±5926**
	E	5.2 ± 0.8	6.5 ± 1.3	5.4 ± 0.5	5.2 ± 0.8^{+}	3.7 ± 1.4*
	F	6342 ±2062	6193 ±4963	3788 ±2361	6677 ±3860	7465 ±3307
90	A	5404 ±4343	8407 ±4639	6136 ± 646	6223 ±2625	3898 ±2956
	B	1725 ±2443	2057 ±1508	1906 ±1576	0 ± 0	545 ± 970
	C	2569 ±2108	3488 ±2782	1781 ±2294	1419 ± 664	1263 ± 796
	D	9698 ±7398	13952 ±4270^{+}	9823 ±2425	7641 ±2873	5706 ±4212
	E	5.4 ± 1.8	6.4 ± 2.1	4.8 ± 1.2	4.5 ± 1.3^{+}	2.8 ± 1.2**
	F	3539 ±1260	5160 ±4833	3582 ±2574	4819 ±1730	5560 ±2200

*$0.05<P<0.1$; **$P<0.05$, differences from control
$^{+}$$P\leq0.05$, differences from high dose

Nematodes

Mancozeb did not affect total nematode numbers. Yet, 90 days after application of the high dose they increased slightly ($0.05 < P < 0.1$; control $\bar{x} = 810 \pm 221$, mancozeb $\bar{x} = 961 \pm 555$ individuals). Total nematode numbers were reduced by lindane at the normal dose only by day 40 ($0.05 < P < 0.1$; control $\bar{x} = 788 \pm 346$, lindane $\bar{x} = 761 \pm 223$ individuals); the higher dose caused a pronounced decrease between day 15 ($P < 0.05$; control $\bar{x} = 667 \pm 348$, lindane $\bar{x} = 388 \pm 189$ individuals) and day 90 ($0.05 < P < 0.1$; control $\bar{x} = 810 \pm 221$; lindane $\bar{x} = 506 \pm 470$ individuals; not shown).

Discussion

In his review, Foissner (1987) concluded that (1) soil protozoa react to pesticide stress in a way generally similar to that of other organisms; (2) many protozoan species seem to be just as sensitive to pesticides as other more commonly used test organisms; and (3) insecticides are generally more toxic than herbicides but fungicides are most harmful to protozoans, especially to ciliates. Points (1) and (2) are confirmed by the present field study. Ciliates were more reduced in numbers than either total rotifers (Fig. 1) or nematodes. However, this field study is unable to show whether the reductions were caused by direct or indirect pesticide action. There is an indication that at least some effects are indirect, e.g. the reduction of grossglocknerids. These are strictly mycophagous ciliates, and their depression for at least 2 weeks after the fungicide treatment was probably caused by a reduction of their food (Table 2). Likewise, the decrease in parasitized testaceans in the fungicide plots after 15 days ($0.1 < P < 0.2$; Table 3) can be explained by a reduction of parasitic fungi. In contrast, the strong depression of the ciliates immediately after the lindane application suggests a direct effect (Fig. 1), though ciliates are able to encyst rapidly under unfavourable conditions or food restrictions. The present study does not corroborate earlier results (Dive et al. 1980) showing that fungicides are more toxic to ciliates than insecticides (Figs. 1–3, Tables 1–3). However, some changes in the ciliate community structure even 90 days after the high-dose application of mancozeb and the continuing lower diversity under both doses indicate that the fungicide had a long-lasting influence, at least on some species (Table 2, Fig. 3).

Doneche et al. (1983) reported about 80% degradation of mancozeb in vitro within 15 days and complete elimination within 3 months. This is in accord with the short duration of depressive effects on total numbers of the microfauna found in the present study (Fig. 1, Table 3). Mitterer et al. (1981) reported an initial stimulation of microbial activity after the application of mancozeb. Therefore, the increase in total nematodes, active ciliates, and testaceans on some occasions was perhaps caused by a more abundant bacterial food supply (Fig. 1, Table 3).

Reports on the effects of lindane on ciliates and total protozoans are somewhat confusing (Lal and Saxena 1982; Smith and Wenzel 1947; MacRae and Vinckx 1973). It is likely that this situation has arisen from the poor experimental design of most studies or from using an inadequate counting method (Foissner 1987). Dive et al. (1980), Komala (1978), and Zelles et al. (1985) concluded from toxicity tests in vitro that freshwater ciliates and other soil microorganisms are rather resistant to lindane. Wiger (1985) reported the opposite. In the present field experiment, lindane had an acute toxic effect on soil ciliates (Fig. 1) but not on testaceans. Within 90 days, active ciliates reached numbers comparable to the control, but the community structure was still distinctly disturbed (Fig. 1, Tables 1 and 2). Obviously, some species were favoured by lindane after 90 days, e.g. *P. steinii, P. nana* and *C. inflata*, perhaps due to a reduced competition and their *r*-selected survival strategy (Lüftenegger et al. 1985; Table 1). *H. discoidea*, highly dominant in the other plots, was very sensitive (Table 1). According to Domsch et al. (1983), this indicates a critical and persistent stress of lindane on the ciliate community, caused by the very long persistence of lindane in soils [>3 years, calculated by applying Blume and Brümmer's (1987) method to our study site]. Testaceans were not inhibited before day 15, a result shared with the mancozeb treatment (Table 3). No pronounced long-term effect of lindane could be observed (Figs. 2 and 3, Tables 1 and 3).

Total rotifers and nematodes were barely influenced by the normal doses of both pesticides (Fig. 1), although freshwater rotifers seem to be more sensitive (Lay et al. 1987). Total nematodes are reported to be rather resistant to organochlorine insecticides (Radu et al. 1974) and to other pesticide classes (Seastedt et al. 1987). In the present experiment, however, total nematodes were persistently depressed from day 15 onwards under the high dose of lindane and were slightly depressed on day 40 under the normal dose.

Differences in the effect of the normal and high doses were generally not pronounced with the fungicide, indicating that the effects were only weakly dose-dependent (Figs. 1–3, Tables 1–3). This is probably due to the water insolubility and/or the mode of action. To have an effect, mancozeb has to be taken up by the organisms, because it acts on functions involved in energy production (Kaars Sijpesteijn 1984). With lindane, differences between the normal and high dos-

es were more pronounced (Figs. 1–3, Tables 1–3). Perhaps its solubility increases in the soil solution.

Our data show that ciliates can be useful in testing biocide effects in the field. This has also been proposed for amoebae (Pussard et al. 1980). Testaceans are comparatively resistant to mancozeb and lindane.

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