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Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella julkisesti tarkastettavaksi yliopiston Villa Ranan Blomstedt-salissa joulukuun 14. päivänä 1996 kello 12.

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ABSTRACT

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Effects of harmful chemicals on soil decomposer communities, decomposition processes and soil fertility were studied in laboratory microcosms containing coniferous forest soil. Pentachlorophenol (PCP) and triazine herbicide (active ingredient was terbuthylazine) were used as contaminants. Humus soil sorbed chemicals efficiently. Hence high concentrations of chemicals were needed before lethal effects on soil organisms were observed. Microbial biomass was reduced by PCP which led to reduced densities of animals at higher trophic levels due to lowered food resources. Patchy PCP contamination affected distribution of soil organisms via lowered preference of the contaminated patches. PCP contamination reduced average size of organisms, and lowered biodiversity and biomass of decomposer community. Fungivorous animals, enchytraeids and predatory mites were sensitive to PCP contamination while some bacterial-feeding animals were indifferent or they even increased their numbers in the contaminated soil. PCP lowered decomposition rate and altered nutrient cycling in the soil. PCP lowered primary production through direct toxicity and altered nutrient cycling. Herbicide stress reduced biomass and diversity of soil decomposers. Although the herbicide was toxic to the predatory mites in the toxicity tests, no lethal effects were observed in a long lasting experiment. However, herbicide indirectly affected microbial feeding animals via lowered hunting activity of stressed predators. Herbicide also altered nutrient cycling in the soil. Decomposer food webs in the heterotrophic microcosms appeared mainly to be bottom-up controlled although predators could occasionally affect some populations of their prey and have cascading effects down to the microbes and their activity. Results clearly showed that due to complex interactions between direct and indirect effects of harmful chemicals on the decomposers, system level monitoring are needed for proper ecological risk assessment in the soil.

Key words: Harmful chemicals; soil organisms; community structure; decomposition; ecosystem function; risk assessment.

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CONTENTS

List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I Salminen, J., Haimi, J., Sironen, A. & Ahtiainen, J. 1995: Effects of pentachlorophenol and biotic interactions on soil fauna and decomposition in humus soil. - Ecotox. Environ. Safety 31: 250-257.

II Salminen, J. & Sulkava, P. 1996: Distribution of soil animals in patchily contaminated soil. - Soil Biol. Biochem. (in press).

III Salminen, J. & Sulkava, P. 1996: Decomposer communities in patchily contaminated soil: Is altered community regulation a proper tool in ecological risk assessment of toxicants? - Manuscript.

IV Salminen, J. & Haimi, J. 1996: Effects of pentachlorophenol in forest soil: A microcosm experiment for testing ecosystem responses to anthropogenic stress. - Biol. Fert. Soils (in press).

V Salminen, J., Erikson, I. & Haimi, J. 1996: Effects of terbuthylazine on soil fauna and decomposition processes. - Ecotox. Environ. Safety 34: 184-189.

VI Salminen, J., Setälä, H. & Haimi, J. 1996: Regulation of decomposer community structure and decomposition processes in herbicide stressed humus soil. - Manuscript (submitted).

Selvitys Janne Salmisen osuudesta hänen väitoskirjansa osatutkimusten suunnitteluun, toteutukseen ja tutkimuksista laadittujen käsikirjoitusten kirjoittamiseen

Osuuteni jokaisen väitöskirjani osatutkimuksen suunnittelussa ja toteutuksessa on ollut keskeinen tutkimusten kaikissa vaiheissa. Olen myös pääosin vastannut tutkimuksista laadittujen julkaisujen sisällöstä.

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Janne Salminen

1 INTRODUCTION

Anthropogenic trace substances, as harmful chemicals, can stress biological systems. This external stress can affect the performance of exposed individuals and communities at both ecological and evolutionary time scales (Calow 1989, Grime 1989). Presence of anthropogenic stress at community and ecosystem levels may be seen as reduced size of organisms, lowered diversity, reduced complexity of food webs, and/or altered nutrient cycling, decomposition and primary production (Odum 1985, Rapport et al. 1985, Shindler 1987, Havens 1994). Both structural and functional properties of the system should therefore be measured, when the effects of harmful chemicals in ecosystems are monitored (Barrett et al. 1976).

Structure of a certain trophic level in a community can be regulated by competition and predation (Connell 1983, Schoener 1983, Sih et al. 1985). Abiotic environmental stress can also have important role in community regulation (Menge & Sutherland 1976; 1987, Menge & Olson 1991). In stressed conditions, differences in stress tolerance of predators and their prey affect which one, predation or competition, that will regulate "lower" trophic levels in the system.

Hairston et al. (1960) proposed decomposers to form mainly a donor controlled part of the ecosystems: soil organisms should mainly be controlled by litter and root exudates in-puts from above-ground vegetation. Some populations in decomposer communities, however, can be controlled by their predators (Hairston et al. 1960). Recently, many studies have shown that grazing of soil animals affects the biomass and activity of soil microbes. Hence, soil animals, not only microbes, are important in decomposition of organic material, nutrient cycling and fertility of soils (Anderson & Ineson 1984, Allen-Morley & Coleman 1989, Setälä 1990, Haimi 1993). Moreover, soil organisms can have great influence on primary production of terrestrial ecosystems, and concomitantly on their own resources via increased litter in-put (Setälä & Huhta 1991, Bengtsson et al. 1995). On the other hand, soil decomposer communities have high species richness and complex food web structure (e.g. Moore & Hunt 1988). According to theories about structural complexity and stability of the ecosystems, soil decomposer system should be sensitive to external disturbances (May 1973).

Soils are the sink of many anthropogenic toxic substances (see Sheehan et al. 1985). In northern forest soils harmful chemicals are expected to have high sorption rate to the humus rich organic soil layer. Therefore high chemical concentration should be needed until acute toxic effects on organisms can be observed. On the other hand, due to low temperature the degradation rate of chemicals is low and they persist long in the environment. Because of natural variability of soil structure (physical, chemical and biological heterogeneity), harmful chemicals can be discontinuously distributed in the soil. Hence distribution of organisms can vary along the contamination gradients (Bengtsson et al. 1994).

In the present study, pentachlorophenol (PCP), pure terbuthylazine and a commercial herbicide preparation (terbuthylazine was the active ingredient) were used to cause chemical stress in the soil. PCP is a world-wide used biocide with toxic effects on most organisms (Rao 1978, Hobbs et al. 1993). In Finland, PCP was used as one active compound in preservatives against rot and bluestaining of wood by fungi in sawmilling industry. In the sawmills, timber to be treated was dipped in the chlorophenol-water solution and let to dry on the soil surface. Hence chlorophenols spread over the soil surface. The use of chlorophenolic compounds as a wood preservatives was banned in 1988 in Finland. However, the soils close to hundreds of sawmills are still highly contaminated due to low the degradation rate of chlorophenols (Valo et al. 1984, Kitunen et al. 1987, Knuutinen et al. 1990).

Terbuthylazine is a triazine herbicide. In Finland it is used in both agriculture and silviculture. Herbicides have mainly indirect effects on soil organisms via lowered above ground plant biomass and concomitant changes in microclimate and litter in-put. However, triazine herbicides have been found to affect soil organisms also directly (Edwards & Thompson 1973, Subgja & Snider 1981, Wardle 1995). Terbuthylazine also slowly degrades in the soil (Bowman 1989, Blume & Ahlsdorf 1993).

During the two last decades there has been a growing interest in using soil organisms as indicators of pollution of terrestrial environments (see Donker et al. 1994). Changes in numbers or activity of decomposer organisms, and concomitantly altered decomposition processes are assumed to be sensitive indicators of soil pollution. Some standardized single species toxicity tests have also been developed (for an earthworm and a collembolan species). However, there is a large variety of organisms in the soil (see Swift et al. 1979). Microbes, protozoans, nematodes and oligochaets are semiaquatic organisms with more or less permeable cell wall or cuticle. They are dependent on soil pore water and can live in the deeper soil layers. On the other hand, arthropods have chitinized less permeable cuticle, and many of them (e.g. predatory mesostigmatid mites) can thus stand the low moisture at the soil surface. Life history patterns also vary greatly: e.g. generation time of bacteria can be calculated in hours while some large oribatid mites have life cycles of one year or more. Differences in morphology, behaviour and life history of organisms evidently affect species specific sensitivity to chemical contamination (see Eijsackers 1994, van Gestel & van Straalen 1994). Thus, results from single species toxicity tests done by a couple of taxa are not enough for proper ecological risk assessment in the soil (van Straalen et al. 1994).

Many scientists have emphasized that ecotoxicological studies should include more ecological aspects, as well as studies at higher organisation levels of biological systems (communities and ecosystems) (Sheehan et al. 1985, Levin et al. 1989, Moriarty 1990, Calow 1993, Forbes & Forbes 1994). To assess anthropogenic effects on the higher levels of biotic systems in the field is difficult due to great natural variation e.g. in population densities and abiotic conditions (Underwood 1989). On the other hand, clear mechanistic exposure-effect relationships can seldom be found in these studies (see Cairns 1985). However, effects of chemicals on ecosystem properties or processes can not be predicted only via responses in structural parameters at lower level of the system (i.e. using data from single species tests one can not predict changes in biodiversity or nutrient cycling). Thus, studies at the system level should be done for proper risk assessment procedures (see Cairns 1985, Forbes & Forbes 1994). Some of the problems in field monitoring can be solved in microcosms [(e.g. pseudoreplication; see Underwood (1989)] while some new problems will arise (small spatial and time scales, no natural migration of organisms etc.). Though simplification, microcosm techniques have shown to be a relevant method to study both structure and function of the soil ecosystem (Teuben & Verhoef 1992).

Results from microcosm studies where harmful chemicals were used as contaminants and stressors of soil decomposer communities are reported in the present thesis. The main purpose was to evaluate changes in inter-species interactions in the stressed decomposer food webs, and their possible consequences for soil processes and fertility of soil. Both direct and indirect effects of chemicals were under study. Concomitantly, possible applications of community and ecosystem level monitoring in the microcosms for risk assessment procedures were evaluated.

2 MATERIALS AND METHODS

2.1 Experimental designs

Experiments were done in laboratory soil microcosms. In the experiments there were controls which were not treated with chemicals, and identical systems stressed with PCP or the herbicide. One or more concentrations of the chemicals were used. In some experiments the soil of the microcosms was patchily contaminated (II, III). The experiments were performed using raw humus from coniferous forest (I), or raw humus together with litter material (V, VI). For some experiments a coniferous forest floor with mineral, humus and litter layers was simulated (II-IV). A simplified decomposer community (including selected soil animal taxa) (I, V, VI) or a natural and diverse soil community (II-IV) was present in the microcosms. Experiments were carried out without or with primary producers (birch seedlings and mosses, IV).

2.2 Microcosms, soils and incubation conditions

The area of the microcosms varied from 33 to 840 cm^2. They were prepared from glass (I, V), acrylic (II, IV) or plastic (III, VI) vessels. On their top there were lids with air holes covered with a tight mesh. Some microcosms had holes in the bottom with removable septa for collecting leachates after water irrigations (IV, VI). The soil materials collected from coniferous forest near the town of Jyväskylä were homogenized and defaunated with liquid nitrogen, or were put into the microcosms without any defaunation (III). Microcosms were incubated in climate chambers at constant temperature and in darkness (I, II, V, VI), or with diurnal cycles of temperature and illumination (III) or with both seasonal and diurnal cycles of temperature and illumination (IV). Evaporated water was replaced by adding distilled water to the microcosms. Experiments lasted from one month to over one year.

2.3 Soil communities

Microbes were first reinoculated into the microcosms (if defaunated). Faunal manipulations in the simplified communities were: 1) nematodes (several taxa) alone, nematodes with collembolans (*Willemia anopthalma* Börner) or with enchytraeids (*Cognettia sphagnetorum* Vejdovsky) and nematodes with both collembolans and enchytraeids (I), 2) known taxal composition of oribatid (Oribatida) and mesostigmatid (Mesostigmata) mites, with diverse nematode and tardigrade fauna (V) and 3) two selected trophic structures (selected oribatids, various nematodes and tardigrades with or without predatory mesostigmatid mites) (VI). Animals were first extracted from the soil samples taken from the same site as the raw humus. The collembolan species was taken from a laboratory culture (I). Various compositions and densities of nematodes, tardigrades, mites, collembolans and enchytraeids were present in the diverse soil communities (II-IV). Large spiders, ants and beetles were removed after extraction from the inocula. Community structure at the beginning of the experiments was studied by taking samples from inoculated fauna.

2.4 Contaminants and acute toxicity tests

Sodium pentachlorophenate (PCP) used in studies I-IV was synthesized at the Department of Chemistry (University of Jyväskylä). Pure terbuthylazine (only in the acute toxicity test; V) and a herbicide preparation (active ingredient was terbuthylazine) (V, VI) were offered by Ciba-Geigy.

PCP and herbicide preparation were added into the soil in distilled water solution/suspension. Terbuthylazine was added into the soil as powder. PCP was mixed into the whole humus layer by hand at the beginning of the experiments (I, IV). When the effects of patchy contamination were studied, PCP was added to the soil in mesh baskets which were inserted in a uncontaminated soil matrix (II, III). Two nominal concentrations of PCP (50 and 500 mg kg^{-1} of dry soil) and an uncontaminated control were used. Herbicide preparation was added to the surface of the soil or mixed into the soil by hand. Pure terbuthylazine powder was mixed into the soil by hand. Four (V) or one (VI) concentrations of herbicide preparation were used.

Acute toxicity tests for selected soil animals were performed in raw humus (I, V) and on filter paper (V). Acute effects of two PCP concentrations were tested with a collembolan (*W. anopthalma*) and an enchytraeid species (*C. sphagnetorum*) (I). Dose related mortality of selected oribatid mites (Oribatida; Oppioidea), mesostigmatid mites [*Veigaia nemorensis* (Koch) and *Lysigamasus lapponicus* (Trägårdhi)] and enchytraeids (*C. sphagnetorum*) were tested with pure terbuthylazine and the herbicide preparation (V). Toxicity of the herbicide preparation to oribatids was also tested on filter paper [contact test, modified from OECD test no. 207; see OECD (1984)].

2.5 Analyses and measurements

Microarthropods were extracted using a modified high gradient apparatus (Macfadyen 1961) from subsamples taken from the soil of the microcosms. Nematodes, tardigrades and enchytraeids were extracted using wet funnels [methods: see Sohlenius (1979) for nematodes and tardigrades, and O'Connor (1962) for enchytraeids]. Microbial biomass was measured by analysing ATP content (I-V) [method: see Vanhala & Ahtiainen (1994)] and/or substrate induced respiration (SIR) (IV,VI) [method: see Anderson & Domsch (1978) and Nordgren (1988)] of the soil. Ergosterol content of the soil was used as an indicator of fungal biomass (III, IV) [method: see Pietikäinen & Fritze (1995)]. Biomass of the birch seedlings and coverage of mosses (% of total soil surface) were determined (IV).

Concentrations of NH_4^+-N, NO_3^--N and PO_4^{3-}-P in the soil were analysed photometrically from KCl-extracts (2M). Nutrient concentrations of leaching water were analysed, too. pH of the soil samples (in distilled water) and the leachates was measured. Nitrogen content of birch leaves were measured using the Kjehldal method. Water content (mass loss at 70 °C) and organic matter content (loss on ignition at 550 °C) were analysed from the soil samples. Soil respiration (CO_2-evolution) was measured by taking air samples from the microcosms and measuring CO_2-content of the air samples with an infrared carbon analyser (I, V, VI). Concentrations of the contaminants were analysed from the soil samples and from the leaching water (after specific extractions) (IV) using gas chromatography-mass spectrometry.

3 RESULTS

3.1 Fate of the chemicals in the soil and acute effects on soil organisms

PCP was strongly sorbed to the soil and its degradation was very slow (I-VI). Terbuthylazine was only partly sorbed to the soil and some of it leached out of the microcosms at the irrigations (VI). However, concentration of terbuthylazine decreased slowly during the experiments (V, VI).

50 mg PCP kg^{-1} in the soil was not acutely toxic to the soil organisms. PCP was acutely toxic to the enchytraeid *C. sphagnetorum* but not to the collembolan *W. anopthalma* at the concentration of 500 mg kg^{-1}. PCP possibly repelled some soil animals, and the animals avoided the contaminated soil patches (II, III). PCP seemed to be toxic to microbes at both concentrations used (II) or only at the concentration of 500 mg kg^{-1} (III).

Pure terbuthylazine powder was not acutely toxic to soil animals tested. However, the commercial herbicide preparation was acutely toxic to *C. sphagnetorum* and two mesostigmatid mites (NOEC-values were between 1-5 g active ingredient m^{-2}). Herbicide preparation was toxic to oppioid mites tested on filter paper (LC_{50} was 14.5 g a.i. m^{-2}) but no dose-related mortality was observed when mites were maintained in the soil. Four weeks' exposure to high concentration of the herbicide preparation (50 g a.i. m^{-2}) caused significant increase in the numbers of the nematode *Plectus* sp. (V).

3.2 Decomposer communities in the contaminated soil

PCP changed the community structure of soil decomposers at the concentration of 500 mg kg^{-1} dry soil. The lower PCP concentration (50 mg kg^{-1}) had only minor effects on decomposers, and these effects were mainly observed in patchily contaminated soil (II, III).

Total microbial biomass was reduced at the PCP concentration of 500 mg kg^{-1}, and both bacteria and fungi suffered from PCP (I-IV). PCP tended to lower the diversity of the whole soil community (IV). Extinction of enchytraeids caused drastic decrease in the animal biomass in the most contaminated soils (III, IV).

Total numbers and biomasses of nematodes were not affected by PCP but in the patchily contaminated soil they were occasionally reduced in the most contaminated patches (III, IV). Fungivorous nematodes suffered from PCP contamination and after one year exposure they had become extinct from the most contaminated soils (I, IV). Numbers of bacteriovorous nematode *Acrobeloides* sp. increased in the soil patches with 500 mg PCP kg^{-1} (II).

The number of microarthropod taxa were significantly reduced due to PCP in the patches with 500 mg PCP kg^{-1} of dry soil (III). However, diversity of microarthropods recovered slowly in these patches. After one year in the homogeneously contaminated soil no differences in taxa numbers were observed between the different PCP concentrations. Response of microarthropods to PCP contamination varied considerably between species. Collembolans mainly suffered from PCP and they also tended to avoid the most contaminated soil paches (I-IV). However, some collembolans were indifferent to PCP (III). The most numerous oribatid mite group in the experiments was oppioid mites (Oppioidea, including *Suctobelba* spp.) (II, III, IV). These mites were sensitive to PCP and they escaped from or died in the most contaminated patches (III). However, after one year exposure in homogeneously contaminated soil, the numbers of oppioids were even higher in the soil with 500 mg PCP kg^{-1} than in the uncontaminated soil (IV). Numbers of some large and sclerotized oribatids increased in the soil patches with 500 mg PCP kg^{-1} (III). After being exposured one year they seemed to be indifferent to PCP in homogeneously contaminated soil (IV). Predatory mites (Mesostigmata) seemed to suffer from the highest PCP concentration (III, IV). No clear differences in habitat preference were observed in predatory mites in the patchily contaminated soil (II). Due to increased numbers of oppioids and other small mites (mainly Astigmata), average size of organisms in the community became reduced in the soils with 500 mg PCP kg^{-1} (IV). Total biomass of microarthropods did not change (IV). No clear evidence of altered predator-prey interaction due to PCP was found, but reduction of microbes in the most contaminated soils was supposed to reduce densities of microbivorous animals (I-IV).

The commercial herbicide had only minor effects (increase in nematode *Plectus*) on the simplified decomposer community in the four week dose-response study (V). In the long-term exposure the herbicide seemed to lower predation mediated regulation of other decomposers although predators maintained their populations longer in the herbicide stressed than in the uncontaminated soil (VI). Finally, predators became extinct in both unstressed and stressed soils. Diversity of prey community and densities of oribatids were reduced by the herbicide only some weeks after the spraying. Microbes, nematodes and tardigrades were not affected by the herbicide.

3.3 Decomposition, nutrient cycling and plant growth in the contaminated soil

The low PCP concentration (50 mg PCP kg^{-1}) as well as varied soil animal community structure had no or only minor effects on decomposition, nutrient cycling and plant growth (I, III, IV). Higher PCP concentration (500 mg PCP kg^{-1}) reduced microbial activity (soil respiration) (I). More nutrients leached from the soil at the irrigations, and there were more KCl-extractable nutrients in the soil with the highest PCP concentration (I, III, IV). PCP had only minor effects on soil pH (I-IV). There were no differences between the PCP concentrations in the nutrient content of the soil in the microcosms with birch seedlings (IV). However, leaves of the birch seedlings grown in the soil with 500 mg PCP kg^{-1} contained less nitrogen than those of the seedlings in the uncontaminated and in the less contaminated soils. Birch growth was reduced in the most contaminated microcosms during the first growing period, while the growth partly recovered during the second period. However, after two growing periods total plant biomass was lower in the most contaminated microcosms than in the other treatments. 500 mg PCP kg^{-1} in the soil reduced coverage of mosses (*Polytrichum* spp.) on the soil surface.

The commercial herbicide product did not affect microbial activity (V, VI). In the experiment with two different food webs microbial activity was lower in the systems with predatory mites than without them (VI). At the high concentrations the herbicide tended to increase KCl-extractable NH_4^+-N content of the soil (V). In the long term experiment NH_4^+-N and NO_3^--N contents of the soil and leachates were higher in the herbicide stressed than in the uncontaminated soil (VI). Soil pH and pH of the leachates were also higher in the stressed soil than in the uncontaminated soil (VI). Predatory mites had only minor effects on nutrient content of the soil (VI).

4 DISCUSSION

4.1 Fate and toxicity of chemicals in the humus rich soil

PCP as a highly lipophilic compound was rapidly and strongly sorbed to the organic fraction of the soil (Largas 1988, Banerji et al. 1993). Sorption seemed to be strong enough to prevent leaching of PCP from the humus layer to the deeper soil layers (Salminen & Haimi 1996). It is also possible that strong sorption together with low pH and temperature lowered microbial metabolism/degradation of PCP in the soils of the present experiments (Valo et al. 1985). Obviously strong sorption, not the metabolism or degradation, caused the low recovery of PCP.

Sorption to the organic particles reduced the toxicity of PCP to soil organisms (van Gestel & Ma 1988, van Gestel & van Dis 1988). Thus, nominal concentrations as high as 500 mg PCP kg^{-1} were needed before any harmful effects on the soil organisms were detectable. PCP was harmful to semiaquatic organisms like microbes, nematodes and enchytraeids while microarthropods were mainly indifferent to PCP (except predatory mites and some small oribatids). In the patchily contaminated soils, as usually is the situation at contaminated sites in the field, some soil animals seemed to actively avoid the most contaminated patches and thereby they could avoid exposure to PCP (Gruttke et al. 1988).

Terbuthylazine was also sorbed to the humus and slowly degraded in the soil (Blume & Ahlsdorf 1993). However, some of the added terbuthylazine leached out from the microcosms at the irrigations. Due to high persistence and moderate mobility of terbuthylazine (Bowman 1989), there is a potential risk for leaching of the herbicide from the humus rich soils to deeper soil layers and ground water.

Toxicity tests using humus as substrate showed that the commercial herbicide preparation was acutely toxic to the animals tested in or close to the concentrations recommended for normal field applications. Thus, harmful side-effects of the herbicide on soil organisms can be expected after successive applications. Due to differences in foraging strategies or exposure routes and rates between the animals tested, considerable variation in the species specific toxicity values (e.g. NOEC) was observed. Both predatory mites and enchytraeids were more sensitive to the herbicide than oppioid mites. Predatory mites and enchytraeids are known to be sensitive to many kind of anthropogenic disturbances including pesticide applications (Edwards & Thompson 1973, Didden 1993). Hormetic effects of the chemical (sublethal stress stimulated reproduction) could possible have caused the increase of nematodes in the herbicide treated soil (see Van Straalen et al. 1994).

4.2 Community and ecosystem level effects of the harmful chemicals

Community structure of decomposer organisms changed in the chemical stressed soils as hypothesised earlier by Odum (1985) and Rapport et al. (1985). Species diversity of animal communities was reduced by PCP and the herbicide (III, IV, VI). Dominance relations in the nematode assemblage were also altered in the PCP stressed soil patches (II). The most sensitive nematode species became extinct and more tolerant ones [e.g. *Acrobeloides* which is known to be quite tolerant to PCP (Kammenga et al. 1994)] even increased their numbers due to altered competitive interactions. Increase in numbers of small, r-strategic species was observed in the PCP stressed soil. Increase of species with small size and short generation time was mainly connected to the altered productivity of the stressed system (IV). It is also possible that the chemical stress affected differently the bacterial and fungal based resource compartments of the soil food web (Moore & Hunt 1988). Although no drastic reduction in fungal biomass was found in the stressed soil, it is possible that reduction of fungivores (nematode and collembolan species) was a consequence of altered fungal biomass and quality (PCP is an efficient fungicide) (I-IV). Fungal based energy channel in the soil food web is known to be more sensitive to anthropogenic disturbances than bacterial based channel (Moore & Hunt 1988). Sensitiveness of predatory mites could have caused shortening of the decomposer food web, if chemical stress had lasted long enough [after one year only few predators were alive in the most stressed soils with birch seedlings, IV; see also Salminen & Haimi (1996)]. Contrary to earlier hypotheses predatory mites persisted longer in the herbicide stressed soil than in the unstressed soil. Possibly due to behavioural avoidance mechanisms (preferring deeper soil layers) or physiological mechanisms (lowered activity) predatory mites could stand the herbicide contamination which should be lethal to them (V, VI). A stress induced dormancy of predators could have been the reason for better survival in the stressed conditions (see also below). Hence, they could avoid the depressing effects of the small spatial scale of the microcosm (for long-term maintenance of predatory mites larger microcosms are needed).

No clear changes in predator-prey relationship in the PCP contaminated soil were found, although PCP evidently affected the predators directly. However, e.g. in the microcosms with birch seedling, low numbers of predators can partly explain increased numbers of some of their potential prey species (IV). In the patchily contaminated soils it was possible that migration of predators and their prey between the patches prevented possible top-down effects on the prey community to be observed, i.e. the consumed prey individuals were continuously replaced by ones from the surroundings. To observe these short-term effects of predators on the prey densities, individual level observations of animals would be needed (Kareiva 1986). On the other hand, direct toxic effects of PCP were more or less equal at all trophic levels (non-selectivity of PCP) and this could have masked possible predator mediated indirect effects of PCP on the structure of the decomposer community.

In the heterotrophic systems studied (i.e. the systems without primary producers), chemicals were toxic to microbes and indirect effects of soil contamination were seen in lowered biomasses of the microbivores and their predators (III, VI). In the last experiment (VI) predatory mites could occasionally influence the development of their prey populations. These results support the hypotheses that decomposition system in the soil is mainly bottom-up controlled, and only some decomposer populations can be controlled by their predators (Hairston et al. 1960, Pimm 1982). The herbicide spraying altered trophic relationship between the predators and their prey but not via reduced predator density. It was supposed that lowered hunting activity of the stressed predators was the reason for the altered trophic regulation in the herbicide sprayed soil (Edwards & Thompson 1973, Everts et al. 1991). On the other hand, it turned out that exact trophic structure of the decomposer systems (even in artificially constructed food webs) was difficult to determine and hence traditional food web analyses were not possible to utilize (Pimm 1982, Pimm et al. 1991) (VI). High degree of omnivory and high species richness in the decomposer food webs (e.g. Wardle 1995) can obscure the concept of trophic levels (Strong 1992).

PCP as an efficient microbiocide clearly affected the biomass and activity of microbes (Valo 1990). Thus, carbon mineralization was reduced in the most contaminated soils (microbes are mainly responsible for carbon mineralization). The commercial herbicide product did not directly affect microbes and carbon mineralization (see Nurmi 1992). Neither varying community structure of microbial-detritivores did affect the activity of microbes (I) but adding a trophic level on the top of the system reduced it (VI). This showed that soil animals at the higher trophic positions can have cascading effects on the microbes and decomposition processes in the soil (cf. Carpenter et al. 1985).

Chemical contamination increased accumulation and leaching of mineral nutrients as pointed out by Odum (1985) and Rapport et al. (1985). Reasons for the accumulation can be disturbed mineralization and immobilization processes due to quantitative and qualitative changes in microbial communities. In the systems without plants, microbes were the only biotic component capable of releasing and immobilizing high amounts of mineral nutrients. In the systems with plants no differences in nutrient contents between contaminated and uncontaminated soil were found because of efficient nutrients uptake by the plants. Altered animal community in the PCP contaminated soil patches did not significantly affect the net mineralization rate of nutrients (III). However, some evidence was found that reduction of animals such as enchytraeids in the PCP contaminated soils could reduce mineralization and be partly responsible for lowered growth and nutrient content of the birch seedlings (see Setälä & Huhta 1991) (I, IV). PCP could also directly disturb nutrient uptake via stressed root-microbial system and thereby cause lowered plant growth in the PCP contaminated soil. Irrespective of the reason, direct inhibition or indirect effect via lowered mineralization, the net primary production of the stressed system was lowered (IV).

Both plant growth and the structure of the decomposer community were partly recovered during the experiments although microbial biomass at the bottom of the food web did not recover (III, IV). Microbes seemed to be more sensitive to PCP than soil animals but in the case of terbuthylazine the situation was opposite. It is also possible that chemicals, which are strongly sorbed to the humus can become available for the microbes during the decomposition of contaminated organic material.

4.3 Risk assessment in the soil

There was a large variation in the responses of organisms to the chemicals used, and it was difficult to separate direct and indirect effects of the contaminants. Responses were mostly depressive but also positive responses were observed, obviously due to altered competitive interactions or direct stimulatory effects of the chemicals (II, III, V). Species/group specific responses to contamination indicate that single species tests are not very representative when assessing effects of chemicals on the structure and function of the whole soil community. In addition, due to complex interactions among decomposers and primary producers, it was impossible to predict effects of the chemicals on the productivity of the system from the responses of certain species (Forbes & Forbes 1994). On the other hand, single-species tests were more sensitive in the dose-response analysis than the ecologically short term multispecies test. Duration of the multispecies tests should be longer than the generation times of the organisms tested before ecologically relevant tests on the effects of chemicals on biotic interactions in the communities can be done (Connell & Sousa 1983, Yodsis 1988). In the context of "the most sensitive species" (cf. Cairns & Smith 1989), it seems that enchytraeids and predatory mites are proper canditates for test species. Both these groups have also been shown to be important in the function of the decomposer system and fertility of the soils. Thus, they fulfil criteria put for suitable test species (Eijsackers 1994).

It emerged that restricted spatio-temporal scale of the microcosms and lack of primary producers can cause some problems in the experiments: e.g. animals such as predatory mites suffered from the small size of the microcosms, unnormal accumulation of nutrients occurred in the soil, and the systems without plants had no energy input and hence they began to degenerate over time. These problems could be solved by building systems which are closer to the field conditions [e.g. large and autotrophic mesocosms, Lawton (1995)] but then unprofitable cost-benefit ratio in routine monitoring procedures could be met.

It has been pointed out by Parmelee et al. (1993) that trophic structure analysis of the decomposer community can be a sensitive assay for testing the indirect effects of chemicals. Results of these experiments confirmed that altered trophic interactions, both top-down and bottom-up types, can be found in the chemical stressed soil. However, due to complexity of soil food webs (e.g. high degree of omnivory can blur the concept of trophic levels), it can be problematic to use direct trophic interactions or food web configurations (Menge & Olsen 1990, Pimm et al. 1991) as an ecotoxicologial tool. On the other hand, harmful chemicals can have important sublethal effects on the predators and their prey. As it was supposed, changes in the behaviour of the predators caused by the chemical stress can have important effects on the structure of the food web (Abrams et al. 1995). In addition, behavioural changes in the organisms can be more sensitive indicators of chemical stress than the trophic relationship itself.

Ecosystem level parameters, e.g. decomposition rate, biodiversity and total biomass of the organisms, were clearly changed in the contaminated soils, and could be used as end points in ecotoxicological assays. Unfortunately, without further manipulations of the decomposer community, mechanistic explanations for the altered community structure and soil processes under chemical stress could not be found. Finally, as pointed out by e.g. Forbes & Forbes (1994) it seems that ecological risk assessment at the community and ecosystem levels can be properly done only by doing experiments directly at those particular levels of the system. However, data about behavioural and evolutionary changes in the organisms are evidently needed before the mechanistic base of the effects of chemicals in the soil can be understood. Microcosm experiments seem to be a relevant method for assessing both direct and indirect effects of chemicals in the soil.

5 CONCLUSIONS

The microcosm experiments show that the coniferous forest soil is well buffered against effects of harmful chemicals. However, at high concentrations the chemicals have both direct and indirect effects on decomposer communities, decomposition processes and nutrient cycling. Plant production is also reduced in the contaminated microcosms. Responses of the decomposer system support the early hypotheses about changes in anthropogenic stressed ecosystems.

If the effects of harmful chemicals on the function of the ecosystems are to be monitored, toxicity tests with only few species are not enough for reliable ecological risk assessment. Because of the complex nature of the soil processes as well as the function of the whole terrestrial ecosystem, effects of chemicals should be directly studied at the system level. Such system level experiments are evidently difficult to standardize as ecotoxicological test procedures. However, soil microcosm studies may be useful for the improving ecotoxicological risk assessment. Proper candidates for system level end points in the soil microcosms are changes in decomposition rate, nutrient cycling, biodiversity and total biomass of organisms.

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YHTEENVETO

Haitallisten kemikaalien vaikutukset maaperäeläinyhteisöihin ja hajotustoimintaan

Ihmisen luontoon päästämät haitalliset kemikaalit aiheuttavat stressiä eliöissä ja voivat vaarantaa kokonaisten eliöyhteisöjen olemassaolon. Eliöyhteisöissä ja ekosysteemeissä ihmisen aiheuttaman häiriytymisen merkkeinä on pidetty pienikokoisten eliöiden runsastumista, biologisen monimuotoisuuden laskua ja ravintoverkkorakenteen yksinkertaistumista sekä ravinnekiertojen, hajotustoiminnan ja perustuotantokyvyn muuttumista. Näitä systeemitason häiriövaikutuksia arvioitaessa on tutkittava samanaikaisesti sekä systeemin rakenteen että toiminnan muuttumista. Tämän tutkimuksen yhtenä tavoitteena oli arvioida, voidaanko maaperäeliöillä tehtäviä mikrokosmoskokeita hyödyntää kemikaalien riskinarviointityössä.

Maaperän hajottajaravintoverkkoa on perinteisesti pidetty resurssirajoitteisena systeeminä, joissa ylemmän trofiatason kautta tapahtuvaa säätelyä (saalistus ja laidunnus) tapahtuu vain poikkeustapauksissa. Tutkimuksissa on kuitenkin osoitettu, että myös maaperässä pedoilla ja laiduntajilla on vaikutuksensa koko hajottajaravintoverkon rakenteeseen ja toimintaan. Toisaalta maaperän ravintoverkko on hyvin monimuotoinen ja teoreettisesti siten herkkä ulkopuolelta tuleville häiriöille.

Tutkimuksessa mikrokosmoksien runsashumuksista metsämaata altistettiin pentakoorifenolille (PCP) ja eräälle triatsiiniherbisidille (pelkälle tehoaineelle, terbutylatsiinille sekä sen käyttövalmisteelle). Vaihtelevan kokoisiin ja muotoisiin mikrokosmoksiin laitettiin joko pelkää metsähumusta tai sitten humuksen pinnalle lisättiin karikekerros ja alapuolelle mineraalimaakerros. Osassa kokeista käytetyistä maannoksista tapettiin eläimet nestetypellä ja niihin koottiin vartavasten halutuista eläinlajeista yhteisö tai niihin palautettiin maaperän luontainen monimuotoinen eläinyhteisö. Osassa kokeista eläimiä ei tapettu vaan maa asetettiin mikrokosmoksiin pelkän mekaanisen homogenisoinnin jälkeen. Maaperän luontainen mikrobisto oli mukana kaikissa kokeissa. Eräässä kokeessa mikrokosmoksiin istutettiin koivuntaimi sekä sammalkasvusto. Kahdessa kokeessa PCP oli levitetty laikuttaisesti maahan. Eliöyhteisörakenteen ja hajotustoiminnan muutoksia mikrokosmoksien maannoksissa seurattiin pitkäaikaisaltistuksissa, ja toisaalta kemikaalien suorat vaikutukset eräille valikoiduille maaperäeliöille testattiin. Kemikaalien pitoisuuksia maannoksessa ja maannoksen läpi valuneissa kasteluvesissä seurattiin. Kokeiden kesto vaihteli yhdestä kuukaudesta yli vuoteen.

PCP ja herbisidi sitoutuivat metsämaan humukseen tiukasti, jolloin niiden myrkyllisyys maaperäeliöille aleni. PCP oli akuutisti myrkyllinen änkyrimadoille mutta ei testatulle hyppyhäntäiselle pitoisuudessa 500 mg $\cdot$ kg^{-1} humusmaata. PCP:lla oli myös eläimiä karkottava vaikutus saastuneissa maalaikuissa. Herbisidin pelkkä tehoaine ei ollut akuutisti myrkyllinen testatuille eläimille, kun taas käyttövalmiste oli myrkyllinen petopunkeille ja änkyrimadolle kun maassa oli 1-5 g tehoainetta $\cdot$ m^{-2}. Kuoripunkeille käyttövalmiste ei ollut myrkyllinen humusmaassa mutta suodatinpaperin päällä tapahtuvassa altistuksessa LC_{50}-arvoksi saatiin 14.5 g tehoainetta · m^{-2}.

Yhteisötason vaikutuksia näkyi lähes pelkästään korkeimmassa käytetyssä PCP pitoisuudessa (500 mg · kg^{-1} maata). Alemmalla pitoisuudella (50 mg PCP · kg^{-1}) oli vain vähäisiä vaikutuksia maaperäeliöstölle. Maaperän hajottajaeliöyhteisön biologinen monimuotoisuus ja biomassa laskivat sekä pienikokoiset ja nopeaelinkiertoiset mikroniveljalkaiset lisääntyivät, kun maassa oli PCP:a. Hajottajaravintoverkon alapäässä mikrobien biomassat, yhtälailla sienten ja bakteerien, laskivat PCP:lla saastutetuissa maissa. Erityisen herkkiä maaperäeläimiä PCP:n vaikutuksille änkyrimatojen lisäksi olivat sieniä syövät sukkulamadot ja hyppyhäntäiset. Näyttäisi siltä, että sieniä syövistä maaperäeliöistä koostuva hajottajaravintoverkon osa olisi herkempi PCP:lle kuin bakteerien syöjistä koostuva. Myös petopunkit vähenivät saastuneimmissa maissa. Selviä viitteitä petojen vähenemisen vaikutuksesta saaliseläinten määriin ei saatu. Osa eläimistä ei reagoinut PCP:n läsnäoloon mitenkään, ja eräiden PCP:a sietävien lajien yksilömäärät jopa nousivat lähinnä vähentyneen kilpailun takia.

Herbisidillä (maahan oli lisätty 28.7 g tehoainetta · m^{-2}) oli suoria haitallisia vaikutuksia hajottajayhteisön rakenteeseen pitkäaikaisessa altistuksessa (koe kesti 57 viikkoa), kuoripunkkien yksilömäärät laskivat ja koko yhteisön lajistollinen monimuotoisuus aleni. Toisaalta herbisidi vaikutti epäsuorasti mikrobien syöjiin alentuneen saalistuspaineen kautta (alentunut saalistusaktiviteetti, ei petojen yksilömäärä). Stressatut pedot eivät alentaneet saalisyhteisön yksilö- ja lajimääriä yhtä tehokkaasti kuin puhtaassa maassa eläneet pedot. Mikrobeihin, sukkulamatoihin ja karhukaisiin herbisidi ei vaikuttanut.

PCP alensi mikrobien hajotusaktiivisuutta (maahengitys) korkeimmassa pitoisuudessa, kun taas eläinyhteisön rakenteella ei ollut vaikutusta siihen. PCP:lla saastuneissa maissa maahan akkumuloitui enemmän mineralisoituneita ravinteita kuin puhtaissa maissa. Kastelujen yhteydessä saastuneimmista maista myös huuhtoutui enemmän ravinteita pois. Koivun kasvu hidastui ja niiden lehdet sisälsivät vähemmän typpeä kun kasvatus tapahtui korkeimmassa PCP pitoisuudessa. Häiriytynyt hajotustoiminta ja ravinnekierot sekä PCP:n suorat myrkkyvaikutukset kasveihin alensivat systeemin perustuotantokykyä.

Herbisidin ruiskuttaminen maahan ei vaikuttanut maan mikrobiaktiivisuuteen. Petopunkkien lisääminen maahan sen sijaan alensi mikrobiaktiivisuutta. Tulos vahvisti käsitystä siitä, että maaperässä ravintoverkon yläpään rakenteella on vaikutus alempien trofiatasojen säätelyssä. Herbisidi lisäsi maan KCl-uuttuvan ammonium- ja nitraattitypen määrää sekä kasteluveden mukana poistuvan typen määrää.

Tutkimus osoitti, että kemikaalien vaikutusten arviointi maaperässä ei voi perustua pelkästään harvoilla eliölajeilla tehtävään toksisuustestaukseen. Suorien ja epäsuorien vaikutusten monimutkaiset yhteydet ja maaperän hajottajaeliöyhteisön monimuotoisuus ja monimutkainen ravintoverkkorakenne vaikeuttavat vaikutusten ennustamista. Tutkimuksia kemikaalien vaikutuksista tulisikin tehdä useilla ekologisilla hierarkiatasoilla alkaen stressattujen yksilöiden käyttäytymisen seuraamisesta ja päätyen ekosystemin tuottavuuden muutosten tutkimiseen. Huolimatta tila- ja aikamittakaavan rajallisuudesta aiheutuvista ongelmista sekä ongelmallisesta standardoinnista kemikaalien testausta varten, mikrokosmostutkimukset näyttäisivät olevan hyvä menetelmä vaikutustutkimusten tekemiseen systeemitasolla.

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ORIGINAL PAPERS

I

Effects of pentachlorophenol and biotic interactions on soil fauna and decomposition in humus soil

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Janne Salminen, Jari Haimi, Anne Sironen & Jukka Ahtiainen

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II

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III

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IV

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V

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VI

Regulation of decomposer community structure and decomposition processes in herbicide stressed humus soil

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