Effects of modern pesticides on bacterial activity and denitrification in lake sediment

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by

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Abstract

Microcosm studies were performed to observe the effects of modern pesticides on denitrification and bacterial activity in lake sediment. Ecotoxicologically relevant test concentrations were retrieved from a Dutch study that presents Negligible Concentrations (NC) and Maximum Permissible Concentrations (MPC) for modern pesticides, i.e. concentrations critical for the environmental compartments water, soil, and sediment. Four functionally different pesticides, isoproturon (herbicide), deltamethrin and pirimicarb (insecticides) and captan (fungicide) were tested.

None of the four tested pesticides had a consistent negative effect on bacterial activity or denitrification. Bacterial activity was, however, affected positively by pirimicarb. No other treatment effects were found for bacterial activity. Apparently, denitrification was affected positively by pirimicarb, isoproturon and deltametrin. Denitrifying bacteria seem to be able to use the tested pesticides as a carbon source when carbon deficiency prevails. A general decrease in denitrification over time for all treatments, especially in the controls, supports the theory that the denitrifying bacteria suffered from carbon depletion. Therefore, the addition of a carbon source in ecotoxicological studies with bacteria is recommended

Introduction

Pesticides are used in a number of human activities to be able to maintain high production efficiency. There is a constant demand for a stable crop production in order to support the growing human population and the future usage of pesticides can therefore be expected to increase. Most agrochemicals are designed to affect only specific target organism groups or processes, but many of them have general toxic effects. The undesired effects that can arise as these substances are transported to lakes and streams are poorly studied and the effects on benthic microbes in aquatic systems are particularly neglected. Evaluation of possible harmful effects on non-target organisms and ecosystem processes is an important element in the regulation and approval of pesticides (Källqvist 1994).

Pesticides in the environment originate from agriculture and forestry, but also from industry and from common households. Persistent chlorinated pesticides like DDT and PCB have to a great extent been replaced by modern pesticides. The critical values that exist for these modern pesticides are frequently obtained from a human health perspective. Even if the concentrations of modern pesticides are below these critical values, it is possible that sublethal effects on ecological function exist. Another problem, reported by Hessel et al. (1997), is that existing analysing methods are not applicable to many of the modern pesticides at ecotoxicologically relevant concentrations. The detection limit for a compound may be higher than the concentration that effects the biota. For example, for pesticides registered for agricultural use in Sweden analysing methods only exist for 60% of the herbicides, 50% of the fungicides and 70% of the insecticides (Hessel et al. 1997). Reliable data on pesticide concentrations in the environment are therefore often hard to obtain.

Pesticides are often spread in low concentrations over a long period of time. Some are relatively immobile thus deposit in soil. However, shortly after application, pesticides will leave the application site in dissolved form (Kreuger et al. 1999). Hydrophobic pesticides or pesticides with strong ion-exchange to clay minerals might also enter the surface water runoff associated to suspended particles (Wauhcope 1978). Hydrophobic pesticides are relatively water-insoluble, nonpolar compounds that have high octanol-water partition coefficients (log K_{ow} > 2) (Elzerman and Coates 1987). Phase-transfer processes can move the pesticides from one environmental matrix to another, with sorption and volatilisation being the most important processes (Larson et al. 1997). For example, in most cases the log K_{ow} increases with increasing hydrophobicity of the pesticide and amount of organic matter in the sediment. The availability of particle-associated pesticides to benthic organisms depends on different sediment characteristics, chemical and physical processes as well as biological processes such as feeding habits (Kukkonen and Landrum 1996).

Bacteria are critical for the turnover of carbon, nitrogen and other nutrients in lake sediment. One process of considerable ecological importance is denitrification, which involves reduction of NO_3 and NO_2 to the nitrogenous gases NO, N_2O and N_2 and mineralisation of organic matter under anaerobic conditions. Denitrification is the only process that eliminates nitrogen from the aquatic system (Jansson et al. 1994). It is carried out by a heterogeneous group of bacteria and disturbances of this process is therefore likely to have severe effects on the sediment ecosystem.

Pell et al. (1998) showed that 11% out of 54 tested pesticides had stimulatory effects on denitrification in soil, whereas 15% had inhibitory effects. In lake sediment, Svensson and Leonardsson (1992) showed that the fungicide fenpropimorph inhibited denitrification and that the negative effect increased after prolonged exposure to the pesticide. Shand et al. (1995), on the other hand, reported that some pesticides were metabolised by different soil bacteria, indicating that pesticides can be used as a carbon source by bacteria.

Microbial responses have been recommended as an early warning indicator of ecosystem stress, because of the quick response to changes in environmental conditions (e.g. toxicant exposure) (Burton 1991). Bacteria are probably sensitive to contaminant exposure in freshwater sediment, but there is little information on the functional response of these organisms. For pesticides, the different functional groups determining the mode of action, i.e. their toxicity, may affect the microbiota in different ways. For example, a fungicide presumably has a higher toxicity to microbiota than a herbicide, and it is therefore relevant to study how pesticides with different functional groups affect the microbial community (Walker et al. 1996).

Only a few comprehensive studies have addressed the effects of modern pesticides from

an ecotoxicological point of view. In the present study, the effects of modern pesticides on sediment living bacteria were studied in laboratory microcosms. Four functionally different pesticides (one herbicide, two insecticides and one fungicide) were tested. Test concentrations for these pesticides were retrieved from a Dutch study that presents ecotoxicologically relevant concentrations (i.e. concentrations critical for the environmental compartments water, soil and sediment) of 70 modern pesticides (Crommentuijn et al. 1997). The objective was to investigate how pesticide exposure at these concentrations affect bacterial activity and denitrification in lake sediment i.e. if these variables can be used as response variables for screening acute toxicity effects in the benthic environment.

Materials and methods

Test sediment

Sediment was sampled in Lake Erken, a mesotrophic lake situated 60 km NNE of Stockholm. Lake Erken has been extensively studied for limnological research and there are restrictions concerning the agricultural activities around the lake. The sampling was done at four occasions 1) 2nd November 1999 (isotope saturation study) 2) 7th December 1999 and 3) 20th January 2000 (bacterial activity experiment) 4) 22nd February 2000 (denitrification experiment). Sediment was collected with a core sampler at a depth of approximately 16 m. The cores with sediment and overlaying lake water were aerated in a climate room at a temperature of 4-10˚C for 1-2 weeks before usage, allowing the sediment to acclimatise to laboratory conditions. Prior to the experiments, the 0-3 cm sediment-layer was retrieved from the cores and used in the experiments.

Sediment analyses were performed after each sampling occasion. The water content of the sediment was determined by drying exact quantities of sediment at 105˚C over night. 0.5 g aliquots of dried sediment were transferred to plastic scintillation vials and kept in a desiccator for later analysis of the C and N content. Loss on ignition was determined after combustion of the sediment subsamples over night at 550˚C, using a Nabertherm oven (Mod N54E). The C and N content was quantified using an elemental analyser (LECO CHNS-932). None of the measured parameters varied considerably between the sampling occasions (Table 1).

Table 1. Sampling date, water content (Water %, mean ± 1 sd), organic matter content (OM, mean ± 1 sd), C and N content for surface sediment (0–3 cm) from Lake Erken.

Date	Water (%) n=16	OM (%) n=14	C (%)	N (%)
2 Nov-99	92.0 ± 0.31	19.0 ± 0.81	9.8	1.3
7 Dec-99	91.8 ± 0.10	19.8 ± 0.59	9.6	1.3
20 Jan-00	91.6 ± 0.14	21.0 ± 0.80	9.5	1.3
22 Feb-00	91.8 ± 0.07	19.2 ± 0.78	9.6	1.3

Pesticides and pesticide exposure

A recently published Dutch study, by Crommentuijn et al. (1997), compiled on behalf of the Directorate-General for Environmental protection, Directorate for Chemicals, External safety and Radiation within the project "Setting Integrated Environmental Quality Objectives", presents ecotoxicologically relevant concentrations of 70 modern pesticides. The concentrations presented in this study are Maximum Permissible Concentration (MPC), i.e. the highest permissible concentration of a specific substance before ecotoxicological effects can be expected and Negligible Concentration (NC), i.e. the highest concentration having no effect on the biota. NCs and MPCs for water and soil were derived from ecotoxicological data using extrapolation methods, whereas the limits for sediment-living organisms were obtained using the equilibrium partitioning method, as sufficient toxicity data for these organisms were not available.

In the present study, four functionally different pesticides were used, two insecticides (pirimicarb and deltamethrin), one fungicide (captan) and one herbicide (isoproturon). The pesticides and test concentrations are presented in Table 2.

Pirimicarb is an insecticide that disables the nerve system of insects by inhibition of acetylcholinesterase (KEMI 1997). It is mostly used against aphids on garden and agricultural crops (Roberts 1998). Pirimicarb is slowly hydrolysed in water and the degradability in soil is moderate. The biodegradation of pirimicarb often results in equally active degradation

Table 2. Pesticides, active ingredients, and partition coefficients (log K_{ow}), as well as test concentrations NC, MPC, 100*MPC (as µg/kg(DW)) derived fromCrommentuijn et al. (1997).

Pesticide	Active ingredient	logK_{ow}	NC (µg/kgDW)	MPC (µg/kgDW)	100x MPC (µg/kgDW)
Captan	N-trichloromethylthio cyklohex-4-n-1,2-dicarboximid	2.8	0.013	1.3	130
Deltamethrin	(S)-a-cyano-3-phenixibenzyl(1R,3R) -3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropanecarboxylate	4.7	0.013	1.3	130
Pirimicarb	2-(Dimethylamino)-5.6-dimethyl-4-pyrimidinylcarbamat	1.7	0.22	2.2	220
Isoproturon	3-(4-isopropylphenyl)-1, 1-dimethylurea	2.5	0.053	5.3	530

products, which extends the total halflife considerably (KEMI 1997). Deltamethrin belongs to the pyrethroids, a group of insecticides that interrupts proper functioning of the sodium channels in cell membranes, thus causing a disruption of nerve signals (KEMI 1997). Deltamethrin is applied on a broad range of agricultural and domestic crops. Chemical degradation of deltamethrin is very slow. It has a high log K_{ow} of 4.7 thus adsorbs strongly to particles in water (Roberts 1998). Deltamethrin is readily degraded by microorganisms in soil and the degradation products are less toxic than deltamethrin (KEMI 1997). Captan is a fungicide used to control spores of *Botrytis*, *Fusarium*, *Fusicoccum* and *Phytium* (Martínez-Toledo et al. 1997). Captan is a N-Trihalomethylthio derivate that works through a non-specific reaction with cell components, particularly thiols, though the exact mechanism is uncertain (Roberts 1998). Captan is of intermediate lipophilicity (log K_{ow} 2.8) and will be moderately sorbed to the sediment (Roberts 1998). Isoproturon is a phenylurea herbicide that inhibits the photosynthetic electron transportation system in plants (Roberts 1998). It is used to control annual grasses and broad leafed weeds in small grain cereals. Microbial degradation is thought to be the main degradation pathway of isoproturon (KEMI 1997). Ehrenstorfer Reference Substances manufactured all tested pesticides (determined purity: deltamethrin 99%, pirimicarb 98%, isoproturon 99%, captan 98,4%).

DMSO (dimethylsulfoxide) was used as organic solvent for the pesticide solutions. DMSO is used in the Ames test as its toxicity to bacteria is thought to be negligible compared to other solvents like acetone and ethanol. In the bacterial activity experiment, pesticide test concentrations (NC, MPC and 100*MPC) were obtained by adding 20 µl of a stock solution (DMSO + pesticide) to glass scintillation vials containing 1.5 ml of sediment. In the denitrification experiment 40 µl of pesticide stock solutions were added to 3 ml of sediment.

Bacterial activity

Bacterial activity was measured as ^{3}H-leucine incorporation according to van Duyl and Kop (1994), which is a modification for sediment samples based on Simon and Azam (1989). An isotope saturation test was performed to test the appropriate amount of leucine that should be added to the test sediment (Lake Erken, 16 m depth). Due to the high labelling of bacterial protein, radioactive leucine (hot) was diluted with non-radioactive leucine (cold). Cold leucine was prepared by adding L-leucine (0.093 g/l) (biopur 99%, Merck) to filter-sterilised Milli-Q water. A solution with the same concentration of hot leucine (L-[4,5 ^{3}H]-leucine, radiochemical purity 97.9%, 141 Ci/mmol, Amersham) was prepared and the two solutions where mixed (15% hot and 85% cold). 15, 25, 35, 45 and 55 µl of the leucine solution was added to 0.25 ml sediment in Oak Ridge centrifuge tubes. Triplicate incubations were run in the dark at 10°C and terminated after one hour with 1 ml 80% ethanol. 5 blanks, where the bacterial activity was stopped with 80% ethanol directly after adding the isotope, were also included to determine the adsorption of the isotope to the sediment particles. Samples were processed by rinsing the sediment twice with 5 ml 80% ethanol and 4 times with 2 ml 5% ice

cold TCA (trichloric acid). Ethanol rinse is essential to remove leucine bound to lipids. Between washes the tubes were centrifuged (20 minutes, 10 000 g) in a cool centrifuge and the supernatant was decanted. In the next step the bacterial cells were hydrolysed in 2 ml 2 M NaOH for 2 hours at 100°C. It is assumed that cold-TCA-insoluble macromolecules (protein) is precipitated in the NaOH hydrolysis (Riemann et al. 1990). After cooling, the samples were centrifuged (20 minutes, 10 000 g) and 250 µl of the supernatant was transferred to plastic scintillation vials. 10 ml scintillation fluid (Optiphase Hisafe 2) was added and the scintillation vials were carefully shaken. The samples were left to stabilise over night. On the next day, radioactivity was measured by liquid scintillation counting (LKB-Wallac 1217 Rackbeta).

The results showed that isotope saturation was reached at the addition of 55 pmol leucine solution (Figure 1). Based on these results, 50 pmol leucine was added per 0.25 ml of sediment in subsequent incubations for bacterial activity determination. The binding of ^{3}H-leucine to the sediment was found to be negligible (< 2 %) and was therefore not considered.

To study the effects of pesticides on bacterial activity, microcosms were established by transferring 1.5 ml of sediment to 35-ml glass scintillation vials using a 5-ml cut-off pipette tip. The exact quantity of sediment was verified by weighing. Stock solutions of the different pesticides were used to obtain NC, MPC and 100*MPC in the sediment. Four replicates were run for each test concentration. The microcosms were incubated for 1, 4, 8, 24 and 48 hours in the dark at 10°C. Three controls (without pesticides, containing the same concentration of DMSO as the treatments) were included for each incubation time. DMSO-controls (without DMSO) were also incubated for 48 hours to determine the possible effect of the solvent. All microcosms were covered with aluminium foil to prevent evaporation.

For measuring bacterial activity in the pesticide exposed sediment, 250 µl subsamples of sediment were transferred from the microcosms to 10-ml Oak-Ridge centrifuge tubes using a 1-ml cut-off pipette tip. Again, each aliquot was weighed to allow correction for the variation in sediment volume. 50 µl of the leucine solution (15% ^{3}H-leucine and 85% cold leucine) was added, the tubes were gently shaken, and incubated in a water bath at 10°C for one hour. Incubations were terminated by adding 80% ethanol to the tubes. Samples were stored in a

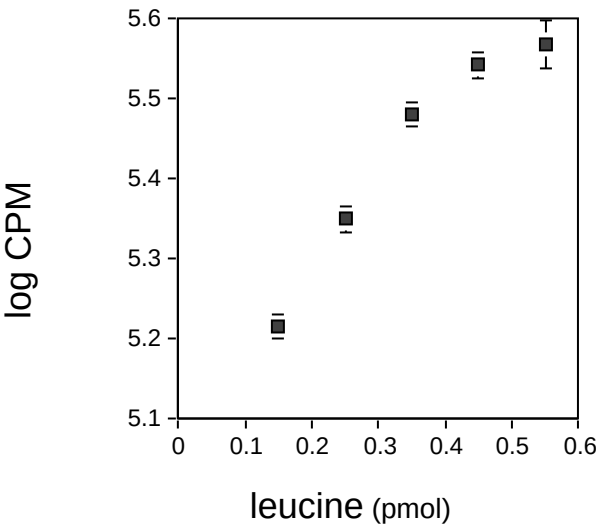

Figure 1. Leucine saturation in sediment from Lake Erken. Log CPM (counts per minute) presented (mean ± 1sd) as a function of pmol added leucine-solution (15% hot and 85% cold leucine).

refrigerator and processed within 2-3 days. Sample processing followed the same procedure as described for the isotope saturation experiment (see above).

Denitrification

Denitrification was quantified using the ^{15}N-isotope pairing technique according to Nielsen (1992), with a few modifications by Svensson (1998). Water from Lake Erken was filtered (GF/C) to remove plankton algae and other particles. Two litres of the filtered water were then saturated with N_2-gas (bubbled over night) to reach anoxia. ^{15}N-labelled KNO_3 (p.a., 99%, IsoChem) was added to the anoxic lake water to a final concentration of 1000 µg NO_3-N/L. Microcosms were established by transferring 3 ml sediment to 35-ml glass scintillation vials. The exact quantity of sediment was determined by weighing. Stock solutions of pesticides were added to the microcosms so that NC, MPC and 100*MPC (four replicates of each concentration) were reached in the sediment. Two sets of samples were incubated in the dark for 12 and 24 hours, respectively. Four replicates of controls with DMSO and four controls with sediment only were run parallel to the treatments in a time series of 6, 12, 18, 24, 30 hours. This was done to investigate possible DMSO-effects on denitrification and to see if $^{15}NO_3$ was response-limiting. Subsamples of 250 µl were then transferred to 12-ml glass exetainers sealed with

screw caps containing a semipermeable membrane. Again, each aliquot was weighed to allow correction for the variation in sediment volume. The anoxic water containing $^{15}NO_3$ was transferred to the exetainers under minimal air exposure using a 50-ml syringe and the exetainers were sealed air-tight. Incubations were made in the dark at 10°C. During incubation, the exetainers were placed horisontally on a shaking table to maximise the contact between the water and the sediment. The incubations were terminated by injecting 250 µl 50% $ZnCl_2$ through the semipermeable membrane using a 1-ml syringe. Samples were kept at 4°C for two weeks prior to analysis.

The samples were further processed by replacing 2 ml of the water phase by helium. After vigorous shaking the exetainers were put upside down to prevent gas from leaking through the membrane. N_2 has a low solubility in water and N_2 produced by denitrification will be therefore be transferred to the gaseous phase in the exetainer. Denitrification was measured by injecting 50 µl of the gaseous phase in the exetainer into a GCMS, a gas chromatograph connected to an isotope-ratio mass spectrometer (Hewlett-Packard 4100 GCMS). In the GCMS separation and quantification of the possible fractions of N_2 ($^{14}N^{14}N$, $^{14}N^{15}N$, $^{15}N^{15}N$) formed by denitrification is accomplished.

Production of single-labelled ($^{14}N^{15}N$) and of double-labelled ($^{15}N^{15}N$) dinitrogen pairs are used to calculate d15 and d14, which are the rates of denitrification of $^{15}NO_3$ and $^{14}NO_3$, respectively (Nielsen 1992):

$$(^{14}N^{15}N) = (^{14}N^{15}N)sample - (^{14}N^{15}N)bkg$$

$$(^{15}N^{15}N) = (^{15}N^{15}N)sample - (^{15}N^{15}N)bkg$$

where bkg is the background of the different N_2 fractions in the water from Lake Erken. d15 is the N_2 produced from denitrification of the $^{15}NO_3$ in the waterphase and d14 is the N_2 produced from denitrification of the $^{14}NO_3$ that is formed through nitrification. d15 and d14 were calculated by the following equations:

$$d15 = (^{14}N^{15}N) + 2(^{15}N^{15}N)$$

$$d14 = (((^{14}N^{15}N)/(2(^{15}N^{15}N)))d15$$

d14 and d15 represents the surplus of $^{14}N^{15}N/^{14}N^{14}N$ and $^{15}N^{15}N/^{14}N^{14}N$ respectively, compared to the natural concentration of N_2 in the water. The natural concentration of N_2 in water at 20°C is 495 µmol/L (Weiss 1970), and therefore:

$$d15\ (\mu M) = d15\ area * 495 * 0.01$$

and

$$d14\ (\mu M) = d14\ area * 495 * 0.01$$

where 0.01 = 10 ml in the exetainer.
The total denitrification was calculated by summing d14 and d15. Finally, total denitrification was normalised for sediment net fresh weight and expressed as nmol N_2/g*h.

Statistical analyses

All data were ^{10}log-transformed prior to statistical analysis. Bacterial activity and denitrification data were analysed using two-way and one-way ANOVA (α=0.05). For pairwise comparisons Scheffe's post-hoc tests were run.

Results

Bacterial activity

The insecticide pirimicarb had an overall stimulatory effect on bacterial activity (Two-way ANOVA, P = 0.001) (Table 3). The bacterial activity in the treatments with pirimicarb varied from 0.35 ± 0.02 (mean ± 1 sd) to 0.47 ± 0.03 pmol/g(FW)*h (Figure 2A). The treatments with the fungicide captan showed a significant interaction between time and treatment (Table 3). No treatment effect was found for captan, even though the P-value was close to 0.05 (Table 3). When analysing the separate incubation times for captan, a treatment effect was found only after one hour of incubation (One-way ANOVA P = 0.037). Pairwise comparisons showed that the 100*MPC-treatment was lower than the MPC-treatment after one hour of incubation (Scheffé's, P = 0.044) and that the controls were higher than the MPC treatment after 24 h of incubation (Scheffé's, P = 0.006). Bacterial activity in the sediment exposed to captan varied from 0.29 ± 0.01 to 0.46 ± 0.11 pmol/g(FW)*h (Figure 2B). For sediment treated with the herbicide isoproturon no effect was observed at any of the experimental treatments. Bacterial activity in the treatments with isoproturon ranged from 0.28 ± 0.02 to 0.40 ± 0.04 pmol/g(FW)*h (Figure 2C). Correspondingly, the insecticide deltamethrin had no significant effect on bacterial activity. Bacterial activity vari-

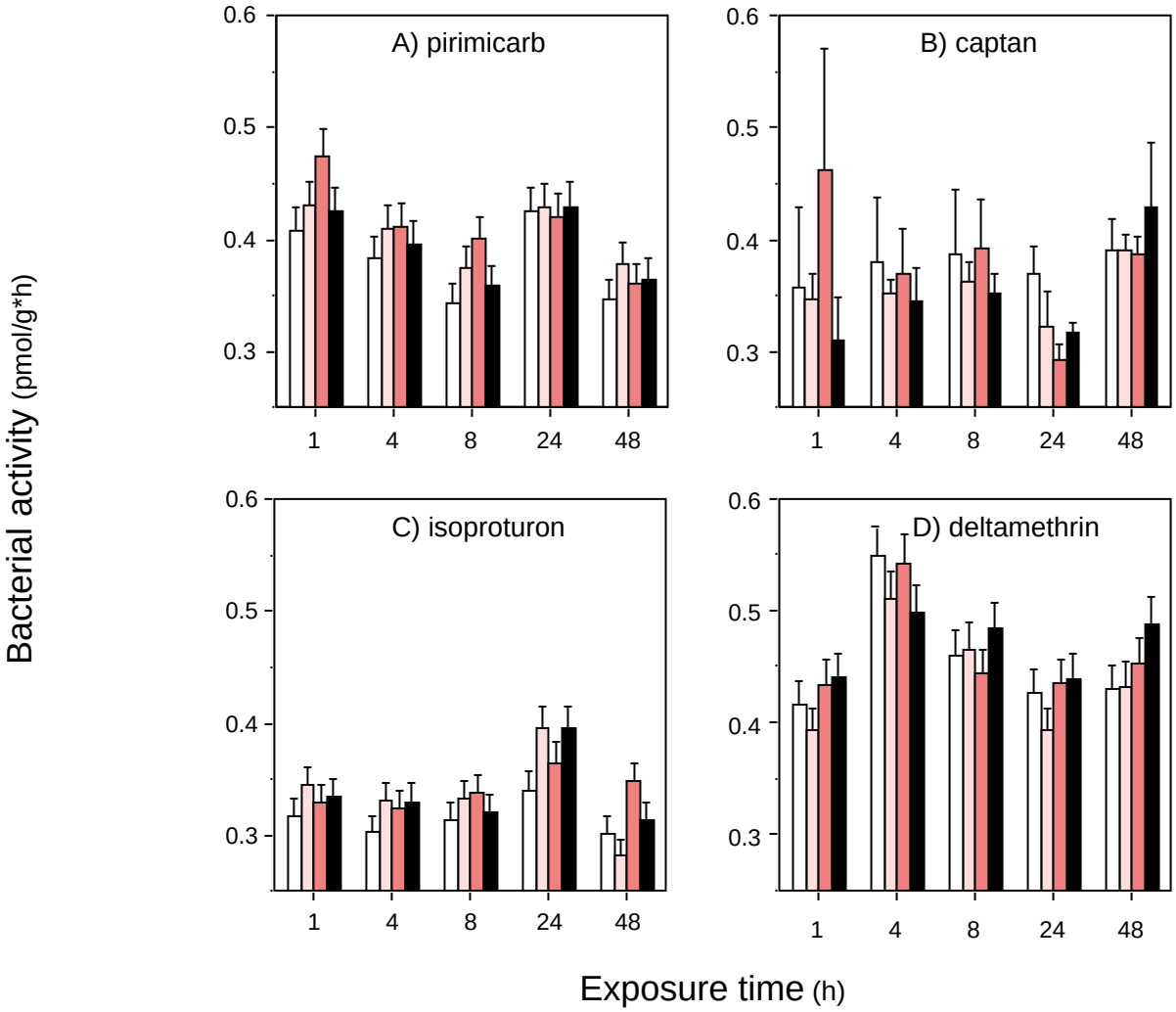

*Figure 2. Sediment bacterial activity (leucine incorporation rate) (mean ± 1sd as pmol/g FW*h) for controls (white bars), NC (striped bars), MPC (grey bars) and 100*MPC (black bars) after exposure to A) pirimicarb, B) captan, C) isoproturon and D) deltamethrin.*

ed from 0.39 ± 0.06 to 0.55 ± 0.01 pmol / g(FW)*h in treatments with deltamethrin (Figure 2D). A significant overall time effect (Two-way ANOVA, $P \leq 0.05$) was found for all four pesticide treatments (Table 3).

Denitrification

No negative effects of pesticides on denitrification were found. Surprisingly, denitrification was positively affected by pirimicarb, isoproturon and deltamethrin in some of the treatments (Figure 3). A general decrease in denitrification from 12 to 24 hours of incubation was found in all pesticide treatments except for the insecticide pirimicarb (Two-way ANOVA, $P \leq 0.05$).

For pirimicarb, a positive treatment effect on denitrification was observed after 24 hours of incubation (One-way ANOVA, $P = 0.043$) (Table 4). Pairwise comparisons showed that the 100*MPC-treatment was higher than the control after 24 hours of incubation (Scheffe's $P = 0.043$). No differences between treatments were observed after 12-hours of exposure to pirimicarb. Denitrification rates in pirimicarb treatments ranged from 4.24 ± 0.52 to 5.09 ± 1.39 nmol N_2 / g(FW)*h after 12 hours of incubation and from 2.96 ± 0.42 to 7.60 ± 2.64 nmol N_2 / g(FW)*h after 24 hours of incubation (Figure 3A).

The herbicide isoproturon also had a positive effect on denitrification after 24 hours of incubation (One-way ANOVA, $P = 0.0002$) (Table 4) and post-hoc tests showed differences between all treatments and controls, after 24 hours of incubation (Scheffe's, $P \leq 0.039$). Isoproturon had no effect on denitrification after 12 hours of incubation. Denitrification in sediment treated with isoproturon varied from

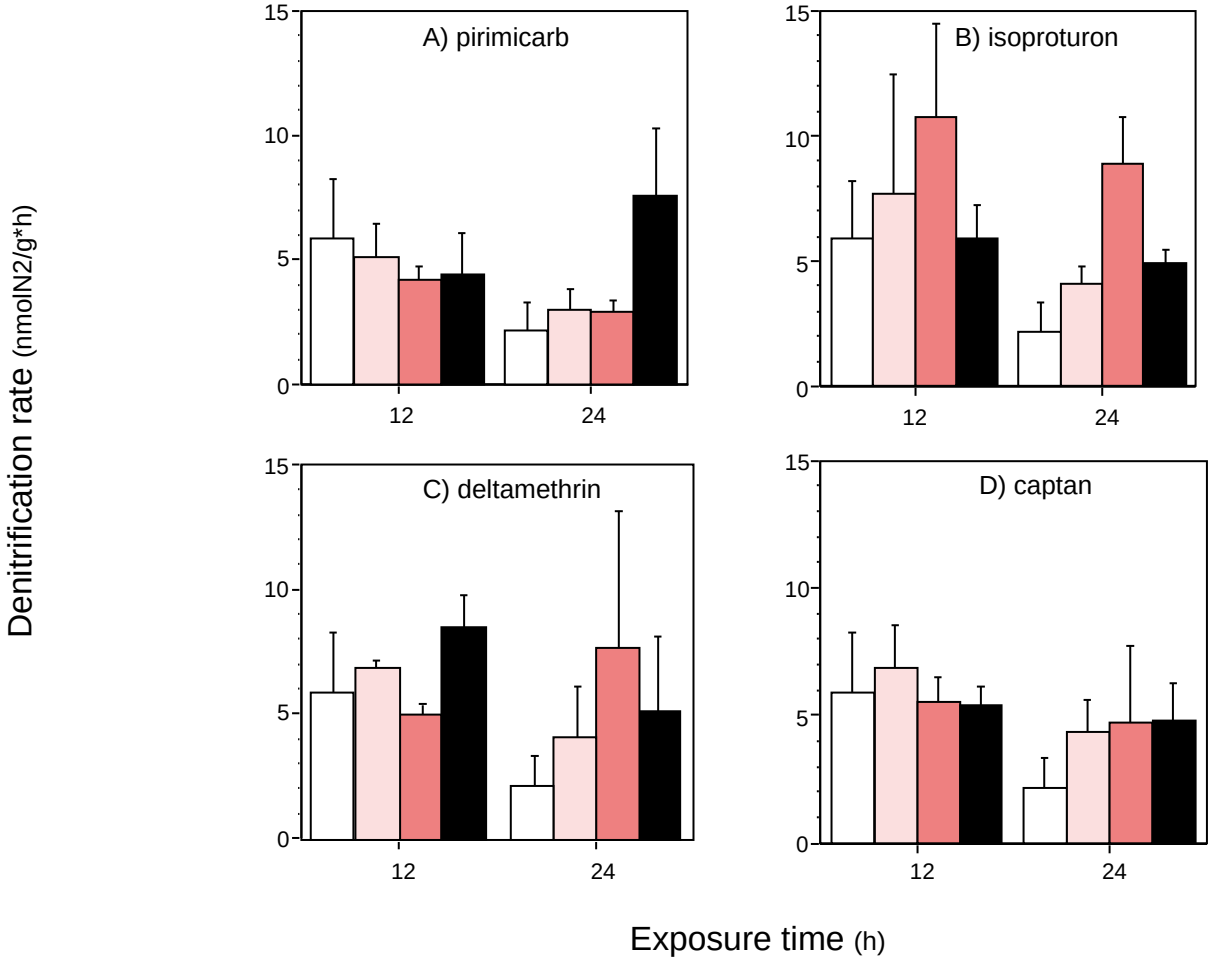

*Figure 3. Denitrification rate in the sediment (mean ± 1sd as nmolN_2/g FW*h) for controls (white bars), NC (striped bars), MPC (grey bars) and 100*MPC (black bars) after exposure to A) pirimicarb, B) isoproturon, C) deltamethrin and D) captan.*

5.86 ± 1.39 to 10.76 ± 3.69 nmol N2 /g(FW)*h after 12 hours of incubation and from 4.13 ± 0.64 to 8.91 ± 1.81 nmol N2/g(FW)*h after 24 hours of incubation (Figure 3B).

The insecticide deltamethrin positively affected denitrification (One-way ANOVA, P = 0.016) (Table 4), yet this difference appeared after only 12 hours of incubation. Denitrification was highest in the MPC-treatment of deltamethrin after 12 hours of incubation. Surprisingly, a decrease in denitrification in the 100*MPC-treatment compared to the MPC-treatment was observed (Scheffe's, P ≤ 0.003). No effect was found in the 24-hour incubation with deltamethrin. Denitrification in the sediment treated with deltamethrin varied from 4.98 ± 0.38 to 8.38 ± 1.31 nmol N_2/g(FW)*h after 12 hours of incubation and from 4.08 ± 1.99 to 7.44 ± 5.52 nmol N_2/g(FW)*h after 24 hours of incubation (Figure 3C).

The fungicide captan had no effect on denitrification. Denitrification rates for captan

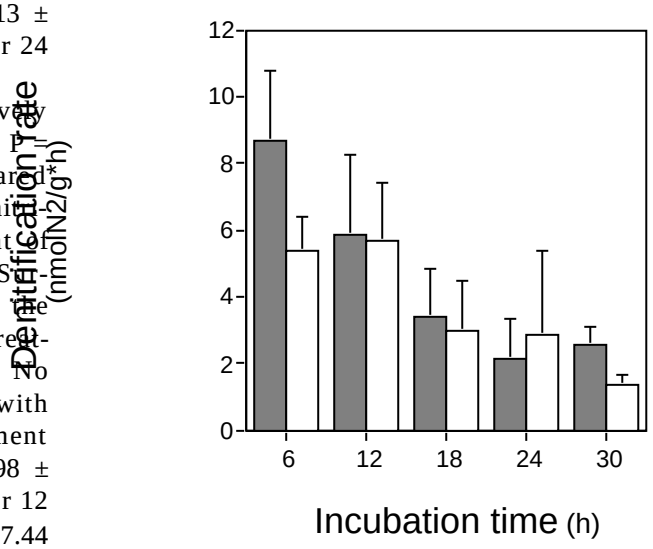

*Figure 4. Denitrification rate (mean ± 1sd as nmolN_2/g FW*h) in controls (with DMSO, grey bars) and in DMSO-controls (without DMSO, white bars).*

Table 3. Two-way ANOVA table for bacterial activity presenting degrees of freedom (df), P-value (P) and power for treatment and time effects and the interaction between time and treatment.

		Captan		Isoproturon		Deltamethrin		Pirimicarb	
	df	P	Power	P	Power	P	Power	P	Power
Treatment	3	0.058	0.610	0.103	0.513	0.139	0.458	0.001	0.953
Time	4	<0.0001	0.996	<0.0001	0.998	<0.0001	1.000	<0.0001	1.000
Interaction	12	0.003	0.980	0.378	0.554	0.605	0.423	0.254	0.693

Table 4. One-way ANOVA table for denitrification presenting degrees of freedom (df), P-value (P) and power for treatment effects after 12 and 24 hours of pesticide exposure.

		Captan		Isoproturon		Deltamethrin		Pirimicarb	
	df	P	Power	P	Power	P	Power	P	Power
12 h	3	0.544	0.162	0.187	0.362	0.016	0.811	0.504	0.175
24 h	3	0.091	0.508	0.0002	1.000	0.064	0.578	0.043	0.657

ranged from 5.41 ± 0.70 to 6.85 ± 1.71 nmol N_2/g(FW)*h after 12 hours of incubation and from 4.33 ± 1.24 to 4.80 ± 1.48 nmol N_2/g(FW)*h after 24 hours of incubation (Figure 3D).

A decrease in denitrification over time was found for both controls (with DMSO) (One-way ANOVA P = 0.013) and DMSO-controls (without DMSO) (One-way ANOVA P = 0.004). No effect of DMSO on denitrification was observed (Two-way ANOVA P = 0.085). Denitrification rate varied from 2.16 ± 1.17 to 8.67 ± 2.07 nmol N_2/g(FW)*h in controls and from 1.37 ± 0.29 to 5.67 ± 1.76 nmol N_2/g(FW)*h in DMSO-controls (Figure 4).

When analysing the effects of pirimicarb on denitrification, some outliers had to be omitted prior to the statistical analysis, to avoid misinterpretation of the results. The criterion for omission was that if a value was at least 6 times higher than the mean, it was excluded. 3 out of 24 values were excluded according to this criterion. Two more values in the pirimicarb treatment were excluded due to an error in the GC-MS measurements (the total peak for N_2 was too low compared to the separate peaks of $^{15}N^{15}N$, $^{14}N^{14}N$ and $^{14}N^{15}N$, causing errors in the final calculations).

Discussion

The results from the present study show that none of the four tested pesticides had a consistent negative effect on bacterial activity or denitrification. However, both positive (stimulatory) and negative (inhibitory) effects were observed in some instances. The use of bacterial activity as an endpoint for detecting possible toxic effects resulted in the most inconsistent response. Bacterial activity was affected only by pirimicarb. In some cases stimulatory effects on bacterial activity could be seen for NC and MPC-treatment, but not for the 100*MPC treatment with pirimicarb. No other treatment effects were found for bacterial activity. Consequently, consistent trends could not be observed from the bacterial activity data.

The study of denitrification showed that some of the pesticides affected denitrification positively. For example, all treatments with isoproturon gave higher denitrification rates than the controls after 24 hours of incubation. The denitrifiers were probably carbon limited and used isoproturon as a carbon source. This theory is applicable on treatments with pirimicarb as well, where denitrification in the 100*MPC treatment was higher than that in the control after 24 hours of incubation. Also, general decreases in denitrification over time for all treatments, especially in the controls, supports the theory that the denitrifying bacteria suffered from carbon depletion. More straightforward pesticide treatment effects might be obtained if carbon depletion is minimised by adding a labile carbon source to the sediment prior to

Table 5. MPCs and NCs (for water) of isoproturon, pirimicarb and deltamethrin (Crommentuijn et al. 1997) compared to maximum weekly mean values measured in Vemmenhögsån in southern Sweden during 122 weeks 1992-97 (Kylin et al. 1998). The number of weeks that pesticide concentrations exceeded MPC is also presented.

Pesticide	MPC (µg/l)	NC (µg/l)	Max conc. (µg/l)	No. Weeks > MPC
Isoproturon	0.32	0.003	10	37
Pirimicarb	0.09	0.0009	7	3
Deltamethrin	0.0003	0.0000003	Nd*	-

* The detection limit for deltamethrin was 0.1 µg/l, thus no conclusions about the possible problems with this substance could be drawn (Nd = not detected).

the experiment.

Several studies with soil bacteria have shown that pesticides stimulate denitrification (Yeomans and Bremner 1985 ; Pell et al. 1998). In a denitrification test without the supply of an external energy source the lack of energy is probably the most rate-limiting factor. Stimulatory effects might be an effect of killing and subsequent lysis of organisms by the pesticide, thus providing denitrifiers with an easily available energy source (Pell et al. 1998). An increase in activity may also be an effect of stress in response to the pesticide, e.g. causing an increase in metabolism. Alternatively, stimulatory effects on denitrification as a response to exposure of various pesticides may also be due to cell growth and increases in cell numbers (González-López et al. 1992, 1993).

The drop of denitrification at 100*MPC for isoproturon and deltamethrin in the present study, however, could be the result of a toxic response. Pell et al. (1998) observed inhibitory effects on denitrification by several pesticides. In their study on soils, fungicides caused the most pronounced inhibition. Previous studies on soil bacteria have shown that application of captan enhances the number of denitrifying bacteria whereas nitrifying bacteria are negatively effected (Martinez-Toledo et al. 1997). In the present study no effect of the fungicide captan on denitrifying bacteria was shown. Svensson and Leonardsson (1992), on the other hand, reported that the fungicide fenpropimorph inhibited denitrification in lake sediments. Thus, it is possible that application of fungicides may result in a disturbed microbial equilibrium followed by an altered nitrogen economy.

The isotope pairing technique used for measuring denitrification is an easy assay with minimal deviation from in situ conditions (Nielsen et al. 1992). This technique has been successfully used by Svensson (1998) for measuring denitrification in eutrophic lake sediment with high organic content (36% of DW). According to Jansson et al. (1994) denitrification in lakes is usually highest during spring and summer when the conditions are optimal i.e. high nitrate supply, sufficient amounts of reducable organic substances, low oxygen concentrations and high temperature. Jansson and co-workers observed large differences in denitrification between summer and winter, with the March values being only 10% of the summer values, implying that the conditions were only suboptimal for denitrification during winter (i.e. low water temperature and sometimes lack of organic carbon). The sediment samples for the present study were collected in a mesotrophic lake from November to February and the organic content was relatively low (20% of DW). This may explain the low denitrification rates in this study compared to those reported by Svensson (1998).

The pesticides pirimicarb, isoproturon and deltamethrin are still in use in Sweden for controlling agricultural pests. The quantities of these pesticides sold during 1999 were 39 tons for pirimicarb, 120 tons for isoproturon and 0.9 tons for deltamethrin (KEMI 2000). Captan is no longer in use in Sweden. It was banned by the Swedish National Chemicals Inspectorate in 1988, but is still in use in many other European countries. A major emission route for many pesticides is volatilisation, which leads to transportation of the active ingredient over large distances from the sprayed plot (Kurtz 1990). Consequently, it is likely that captan is transported to Swedish lakes and streams through long range transport. Kylin et al. (1998) recently detected pirimicarb and isoproturon in Vemmenhögsån in southern Sweden. The concentrations were higher than the MPCs (for water) set by Crommentuijn et al. (1997) at several occasions during the sampling period (1992-97) (Table 5).

To avoid ecotoxicological effects, pesticide concentrations in the environment should not exceed NC. In periods with pesticide concentrations higher than NC and MPC there is an evident risk for ecotoxicological effects. Comparison of MPCs and NCs with actual concentrations in the environment is, unfortunately, for

many compounds hampered by the lack of sensitive analytical methods. For many pesticides the detection limit is higher than the NC or even higher than the MPC.

The test concentrations and pesticides tested in this study were extracted from Crommentuijn et al. (1997). In this study, the concentrations for water and soil were first based on ecotoxicological data using extrapolation methods and then harmonised using the equilibrium partition method. However, since no ecotoxicological data were available for sediment-dwelling organisms, their MPCs and NCs were derived using the equilibrium partitioning method only. The MPC for sediment-living species, using equilibrium partitioning, was calculated using the following formula:

$$MPC_{(sedEP)} = MPC_{(water)} * Kp_{(sed)}$$

In which: $MPC_{(sedEP)}$ = Maximum Permissible Concentration for sediment species using the equilibrium partitioning method, $MPC_{(water)}$ = Maximum Permissible Concentration for aquatic species, and $Kp_{(sed)}$ = partition coefficient for standard sediment in L / kg.

Three assumptions are made when applying this method. Firstly it is assumed that bioavailability, bioaccumulation and toxicity are closely related to the pore water concentrations. Secondly, it is assumed that sensitivities of aquatic organisms are comparable to those of organisms living in the sediment. Thirdly, it is assumed an equilibrium exists between the chemical sorbed to the particulate sediment organic carbon and the pore water and that these concentrations are related by a partition coefficient, K_{oc} (=organic carbon normalised partition coefficient in L / kg). These three assumptions imply that the exposure for toxicants of sediment-dwelling organisms is comparable to the exposure of aquatic organisms and that the bioavailability of the toxicant is determined by the concentration in the pore water. However, in reality there might not always be an equilibrium situation among the different environmental compartments in the ecosystem, due to kinetical constraints (Belfroid et al. 1996). For example, it is likely that no consistent relationship between pore water and whole sediment will ever exist due to the multitude of physico-chemical and biological process variables (Burton 1991). Since bacteria utilise carbon compounds solved in the water or excrete exo-enzymes that interact with compounds bound to particles, the sensitivity of bacteria might be different from that of other aquatic organisms that have different feeding habits. The exposure of bacteria to xenobiotic compounds is therefore somewhat more complex than the equilibrium partitioning method implies.

When performing microcosm studies, one has to consider that laboratory manipulation inherently involves disruption of the ecosystem processes when natural materials are removed from the field and enclosed in a vessel. A study on the effects of sediment containerisation by Kurtz et al. (1998) showed, on the other hand, that the microcosm system provides an appropriate model for studying microbial processes, such as metabolic functioning. Carefully designed microcosm experiments can reduce some of the biological, mechanical, or meteorologic variability normally associated with field experiments. It is also important to try to reduce the costs and the time consumption of the experiments. Microcosm studies using short incubation times might therefore provide a useful tool when estimating ecotoxicological responses of relatively short-lived organisms.

Incitements for using the microbial community in toxicity studies is both due to its ability to respond quickly to changes in environmental conditions and due to the important role it plays in ecosystem biogeochemical processes. These are compelling reasons for continuing the development of reliable test systems involving microbes. A clear-cut conclusion regarding the environmental consequences using test concentrations based on equilibrium partitioning is difficult to draw since these concentrations are regarded as less reliable than concentrations based on ecotoxicological data. It is consequently important to produce more data to improve the modelling of MPC for sediment living organisms.

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