Stimulation Programme System-oriented Ecotoxicological Research

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The NWO Stimulation Programme System-oriented Ecotoxicological Research started in 1999. The most important aims of the Programme are to promote scientific knowledge and understanding of the way ecosystems react to chemical pollution of a chronic and diffuse exposure. And secondly to make use of fundamental and relevant knowledge to assist in formulating and implementing policy with respect to the ecological risks of chronic and diffuse pollution of the environment with a mixture of substances.
The Newsletter gives information about the ongoing research and other relevant issues; it will be published each half year.

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Illustrations to the papers (photos, tables, other graphics) are highly appreciated.
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Newsletter

First project ended:

The first research project in the Stimulation Program System-oriented Ecotoxicological Research is ended. Dr. T.H.M. Hamers gives an overview of the outcome of his project. For more information contact can be made with Tinka Murk from the Wageningen University.

Toxicological evaluation of complex mixtures of diffuse pollutants including PAHs in slugs and small mammals: Assessment of exposure, bioavailability, internal dose and (adverse)effects using bio-assays and biomarkers.

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The project 'Toxicological evaluation of complex mixtures of diffuse pollutants including PAHs in slugs and small mammals: Assessment of exposure, bioavailability, internal dose and (adverse) effects using bioassays and biomarkers' was completed in January 2003. This article describes the objectives and main results of the programme.

In the Netherlands, the bulk of environmental pollutants is emitted to the atmosphere. Emissions of organic compounds to the atmosphere exceed emissions to water by a factor 10 and emissions to soil by a factor 300. The pollutants originate from various national and international sources, such as traffic, industry, households and agriculture. The resulting mixture of air pollutants is transported through the atmosphere, even to more or less clean, remote regions. As a result, the population and the environment of the Netherlands are continuously exposed to a grey veil of air pollution, consisting of a complex mixture of thousands of substances. Up to now, there was no good method available for measuring these complex mixtures and the potential adverse effects on people and the environment. The research objective had four spear points:

1. to measure the exposure to diffuse pollution by testing the toxic potency of samples taken from the environment in biological tests with cells, bacteria or enzymes that react specifically to certain types of toxicity;
2. to determine internal exposure by measuring biomarkers (specific changes) in animals (mice and slugs) who had been exposed in situ;
3. to perform a chemical analysis to check whether the biologically measurements of exposure match the more expensive and more complicated chemical assays;
4. to work out a stepped plan on the basis of the methodology developed here enabling a toxicological risk assessment of diffuse air pollution to be performed in situ.

Eventually, investigation methods for four groups of diffuse toxic substances have been developed:

1. substances that can damage DNA (genotoxic substances) such as polycyclic aromatic hydrocarbons (PAHs);
2. substances that can activate the dioxin receptor (AhR), such as persistent polychloro-dibenzo-*p*-dioxins (PCDDs) and polychloro-biphenyls (PCBs)
3. substances that can mimic the female sex hormone estradiol (estrogenic compounds), such as some plasticizers, raw materials for plastics, detergents and some pesticides;
4. insecticides that can interfere with the operation of (esterase inhibitors) such as organo- phosphate and carbamate insecticides.

The main results of the study are:

1. There are not really any 'control' areas left in the Netherlands, i.e. areas without diffuse air pollution. Even in more or less remote areas, air pollution is only a factor 10 lower than near busy highways or in areas with glasshouse market gardening. When there is an easterly wind, these differences were even smaller and the composition of the pollution was different.
2. Concentrations of lead and dioxins in the atmosphere have declined considerably since waste incinerators have been cleaned up and leaded petrol was prohibited.
3. Measured exposures in mice populations near highways were found to be slightly higher but do not cause worrying effects. However, these conclusions cannot be extended to potential bronchial effects in humans or longer-lived large animals.
4. The esterase-inhibiting potency of rainwater, which was established using enzymes from bees' heads, was found to match the analysed concentrations of individual insecticides, but the biological method made it possible to measure more substances, corresponding to about a 50% increase in activity.
5. The estrogene potency of rainwater was found to be highest in spring. This had never been shown before. This increased concentration may well affect the organisms living in small streams that are fed solely by rain.
6. The thesis confirmed that bioassays could be valuable tools to qualify and quantify exposure to diffuse pollution.
7. Orange slugs (Large road slugs) were found to be fairly insensitive to the impacts of the substances studied, and are therefore not a suitable species for monitoring PAKs.
8. Herbivorous pink voles are apparently mainly exposed to air pollutants (particles) that fall on plants, whereas carnivorous forest shrews are mainly exposed to soil pollutants that build up (by bio-accumulation) in their prey, such as earthworms. Both mice species are basically suitable pollution indicators in their ecosystems, but, in view of their short lifespans, may not be proper models for larger, longer-lived animals.
9. In the Biesbosch area, it was the most difficult to find locations without diffuse soil pollution. Pollution is worse in areas that are regularly flooded, compared to reclaimed polder areas, but the total concentrations of persistent pollutants, such as heavy metals and PCBs, are relatively high in comparison with other normal areas in the Netherlands, and the differences between them are only minor.
10. The relatively high concentrations of soil pollutants in the Biesbosch area were not reflected in increased exposure levels in earthworms and mice. This low biological availability may be due to the ancient pollution in the polder areas and the relatively high humus and silt concentrations in the areas that are flooded regularly.

House shrew

Effects of heavy metals on substrate utilisation capacity and structure of terrestrial microbial communities

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The Demmerikse polder, in the Ronde Venen, like other peat areas in the central western parts of the Netherlands, was intensively used for digging lumps of peat from the 14th to the 19th century (Rutgers and Bogte, 2002). Parts of the region were used for farming, usually an extensive form of cattle farming. The poor soil quality and hydrological conditions, aggravated by oxidation of the peat, were tackled by applying a mixture of garbage from surrounding cities, dredging sludge, farm-yard manure and sometimes dune sand, to improve the fields. This practice was called 'toemaaken'. Nowadays, the fields are covered by a 15-to-50-cm-thick completely anthropogenic layer, the so-called 'toemaakdek', containing a high fraction of sand particles, and elevated levels of heavy metals, mainly Zn, Cu, Cd and Pb. As part of the SSEO, a research project was undertaken to study the effects of metals on the soil ecosystem in the Demmerikse polder. The basic hypothesis of this study was that the observed correlations between structural and/or physiological changes in communities of soil organisms and metal concentrations, demonstrate that metals directly or indirectly affect the organisms. The aim of this research is to find out whether any effects of these metals can be observed in the 'toemaakdek'

The presence of confounding factors such as pH and organic matter makes it difficult to causally link the effects observed to the presence of contamination. Several techniques were combined in this project. Structural and physiological changes of heterotrophic bacterial communities were investigated. Community-level physiological profiling (CLPP) was used to obtain insight in the physiological changes of the heterotrophic bacterial communities, and denaturing gradient gel electrophoresis (DGGE) was used to investigate the structural changes of the bacterial communities. However, it was not possible to causally link shifts in CLPP and DGGE patterns to the presence of a particular metal. Therefore, pollution-induced community tolerance (PICT) was used to link community effects to the presence of metals. It is generally accepted that observations of PICT provide higher causality in ecological field studies (Blanck *et al.*, 1988; Boivin *et al.*, 2002).

Approximately 90 soil samples were taken from the upper 10 cm surface layer of the Demmerikse polder in October 2001. Of these 90 samples, 30 were selected: 15 samples that showed the lowest Zn, Pb and Cu concentrations and the 15 samples that showed the highest Zn, Pb and Cu concentrations. This grouping was based on total metal concentration. Both total metal concentration and metal concentration in pore water was measured. Previous results have not yet given any indication which type of information provides the best correlation with possible effects on microbial communities in the field. Therefore, no attention will be given to distinguish between the different metal information. The samples were divided into three parts. The first part was used for abiotic measurements (field humidity, pH, dissolved organic carbon (DOC), total metal concentration and pore water), the second part was used for CLPP and PICT analysis (newsletter 4; Garland, 1997; Garland *et al.*, 1999; Lehman *et al.*, 1997;

Rutgers *et al.*, 1998) and the third part was used for DGGE analysis (newsletter 5). Before CLPP and DGGE analysis, the soil was sieved using a sieve of a 4 mm pore size and was brought to WHC50 (water holding capacity of 50%). The samples were then incubated for 2 weeks in the dark at 10^0C. Principal component analysis (PCA) and redundancy analysis (RDA) were used to analyse the CLPP and DGGE data. A PCA analysis is an indirect analysis, which means that all differences measured between samples are addressed (differences in Biolog substrate utilisation capacities per community independent of abiotic parameters). RDA analysis is a direct analysis, which means that only differences explained by the environmental variables are addressed. RDA was used in our case only to calculate the importance of the different abiotic parameters measured on the biotic differences between the samples. A Monte Carlo permutation test was performed using 9999 permutations; the different abiotic parameters were separately tested. For the PICT analysis the same frozen (-70^0C) bacterial suspensions were used as for the CLPP experiments (newsletter 6; Rutgers and Breure, 1999; Blanck, 1988; Boivin *et al.*, 2002). The EC_{50} were finally compared using a T-test ($\alpha = 0.05$).

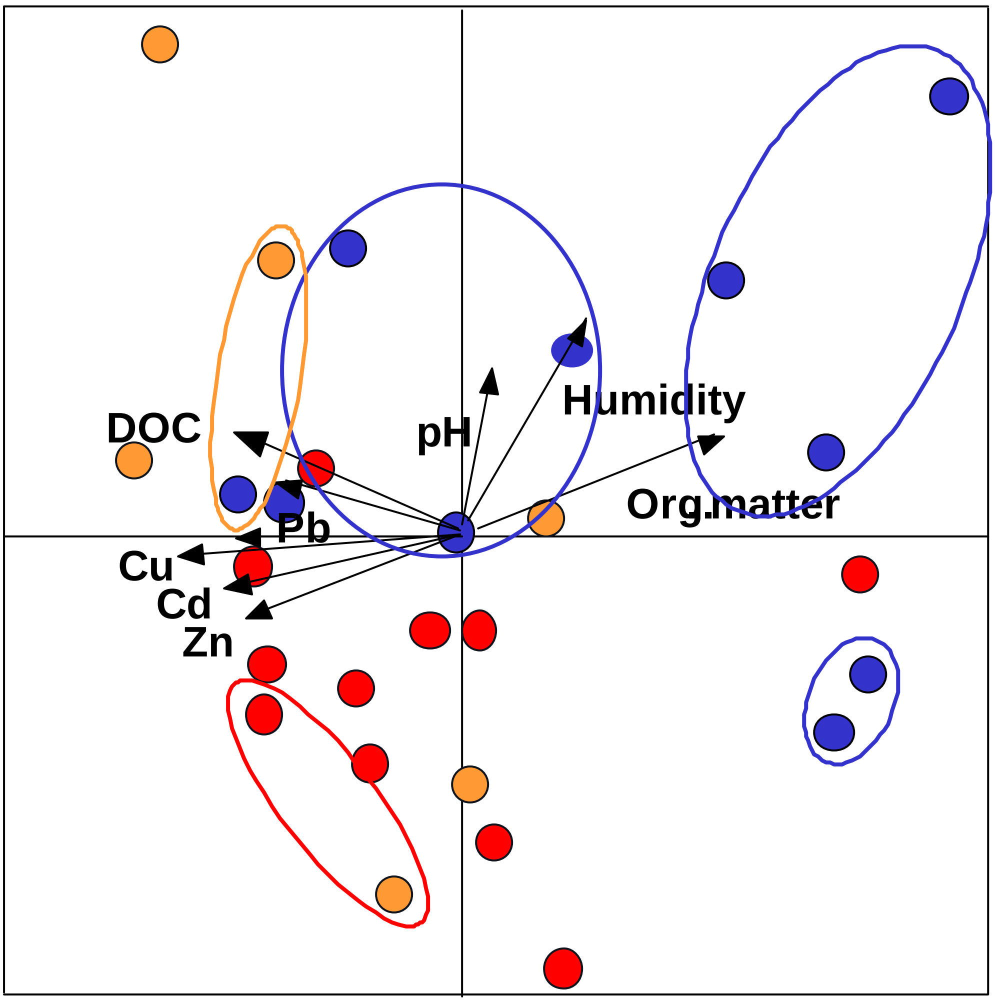

Fig. 1. Principal component analysis (PCA) of CLPP of different soil samples from the Demmerikse polder. Colours represent the degree of pollution (based on Zn, Pb, Cu and Cd). Bleu: relatively clean; Orange: relatively polluted; Red: polluted. Axis1: 16.8%; Axis2: 13.9% and Axis3: 9.8%

To investigate the influence of metals on the substrate utilisation capacity of heterotrophic bacterial communities, CLPPs were made. Figure 1 shows a PCA based on CLPPs of different samples taken in the Demmerikse polder. The first two axes explain 30.7% of the variation between the different samples. The pattern suggests that there is a correlation between the CLPP of the heterotrophic bacterial communities and the metals present in the field. Polluted samples (red markers) and cleaner samples (bleu markers) tend to group together. When RDA is performed, Zn ($p = 0.008$) and Cd ($p = 0.013$) are shown to have a significant influence on the substrate utilisation capacity of the bacterial community. Field humidity, pH, DOC and organic matter did not show any significant influence on the substrate utilisation capacity of the community ($p > 0.05$). What is also interesting to note, is that samples taken closer together showed more metabolisation similarities than samples taken further apart (encircled symbols indicate samples taken within 2 metres from each other).

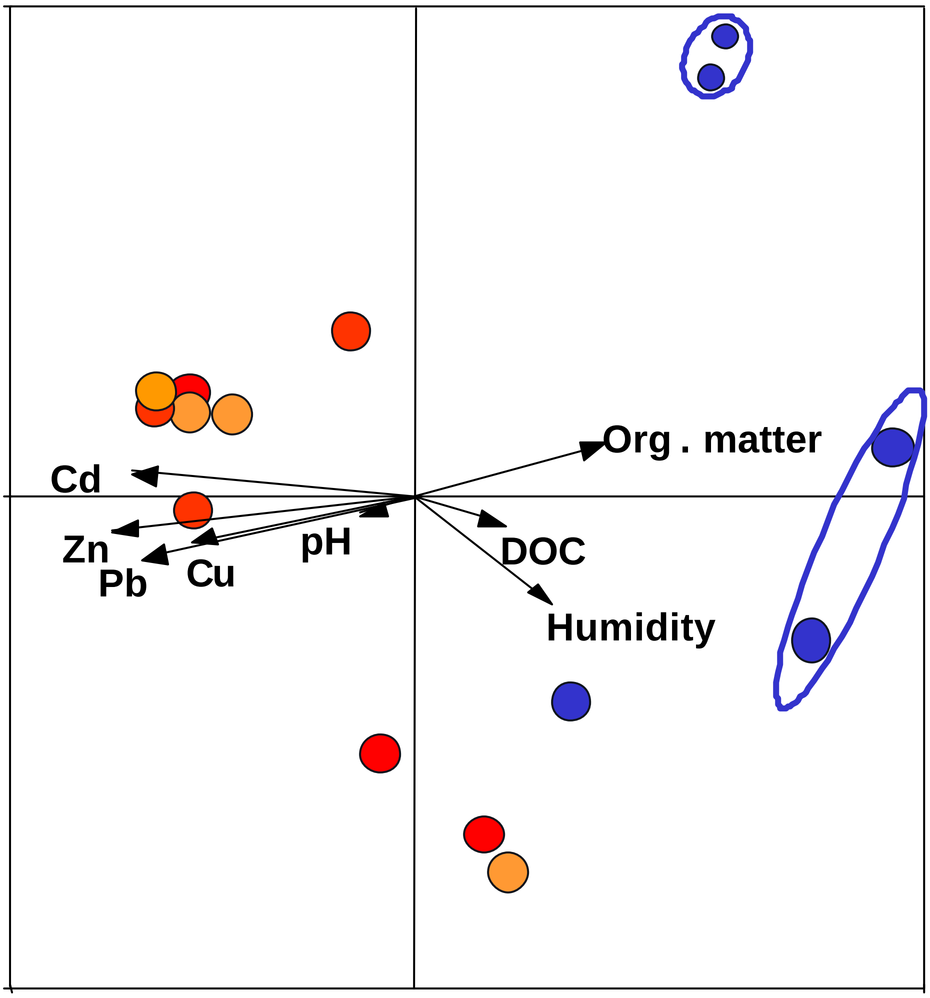

Fig. 2. Principal component analysis (PCA) of DGGE patterns of different soil samples from the Demmerikse polder (the densiometric data were used for comparison). Colours represent the degree of pollution. Bleu: relatively clean; Orange: relatively polluted; Red: polluted. Axis1: 42.6%; Axis2: 19.7% and Axis3: 8.4%.

The influence of metals on the bacterial community structure in the Demmerikse polder was also investigated using DNA extraction and DGGE. The same trends were observed for the structure of the different bacterial communities (Fig. 2) as for the CLPPs. This means that metals apparently also have an influence on the structure of the bacterial communities. The first two axes explain 62.3% of the differences between the different samples. The Monte Carlo Permutation test indicates that Zn ($p = 0.012$), Cd ($p = 0.03$) and Cu ($p = 0.03$) apparently affect the structure of the bacterial communities significantly. Other abiotic parameters, such as field humidity, organic matter, pH and DOC did not significantly affect the structure of the community ($p > 0.05$). The two samples taken within 2 metres of each other were more structurally similar than those taken further apart.

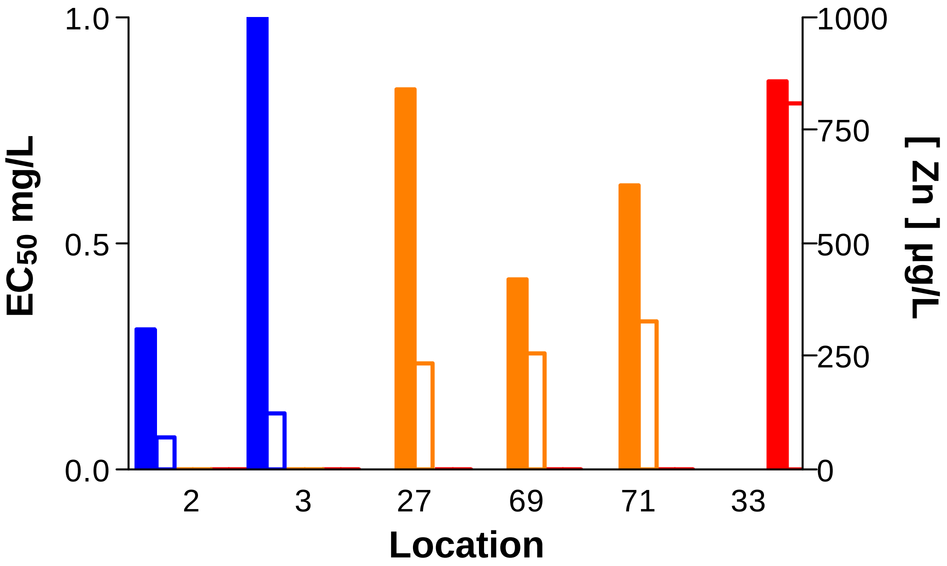

Fig. 3. Histogram representing zinc concentrations in pore water (open bars) and EC_{50} values (solid bars) from those locations.

To expand the links between metal contamination, substrate utilisation capacities and structure of the bacterial communities in the Demmerikse polder, PICT was investigated. Figures 3 and 4 represent the sensitivity shifts of the different heterotrophic bacterial communities. When we look at the data for Zn (Fig. 3), PICT was observed between the sample having the lowest Zn concentration (location 2) and the sample showing the highest Zn concentration (location 33). However, the samples with in-between Zn concentrations did not show trends of PICT. On the other hand, when we look at the data for Pb (Fig. 4), the PICT responses showed logical trends. The samples taken from the most heavily Pb-polluted area showed a development of tolerance to Pb compared to samples taken from cleaner areas. All these samples (2-3-71-27 and 69) were not significantly different from one another, but significantly different from sample 33, which also shows the highest Pb concentration (pore water).

The Demmerik area shows a very patchy and heterogeneous distribution of heavy metals. Within about 5 metres, relatively clean spots are found as well as relatively polluted spots (Rutgers and Bogte 2002). However, if we take heterogeneity into account, the location showed patterns of metal distribution. The centres of the fields are generally more polluted than the sides of the ditches. This might be due to the fact that sediment from the ditches was dredged and layered along the sides of the ditches. Another explanation might be a relatively higher peat oxidation in the middle of the field, this could be due to differential hydrological conditions. Furthermore, the patterns of substrate utilisation capacities and also the genetic structures of the microbial communities are more similar for samples taken close together than for samples taken further apart.

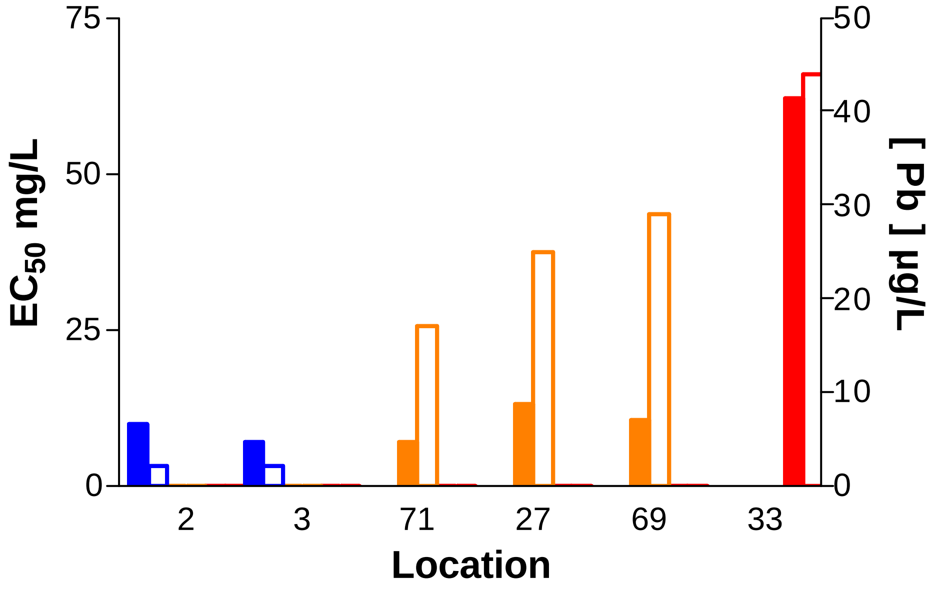

Fig. 4. Histogram representing Pb concentrations in pore water (open bars) and EC_{50} values (solid bars) from those locations

The main objective of the research described in this paper was to investigate the influence of heavy metals on microbial communities. The research was performed in collaboration with teams of the Free University of Amsterdam (VU) and Wageningen University and Research Centre focussing on Terrestrial Model Ecosystems (TMEs) and the nematode communities in the Demmerikse Polder, respectively. Their results will be presented elsewhere. The main outcomes of the present research are the apparent CLPP and DGGE shifts and the correlation of PICT, with the presence of metals. The PCA, which was made from the CLPPs, shows two major clusters representing clean and polluted locations. Based on statistical test (Monte Carlo permutation), it seems that Zn and Cd have the highest correlations with CLPP shifts, especially compared to other abiotic parameters such as pH and organic-matter contents. These outcomes are in agreement with Bosveld *et al.* (2000) who observed that Zn, Cd and Pb affected CLPP shifts most. They did not observe any impact of pH or organic-matter content on CLPP from Demmerik samples either, which is in agreement with our results. These results should be further investigated to find out whether a decrease in biodiversity can be observed on the basis of DGGE and CLPP patterns, and also to find out which functions and species are actually disappearing or are stimulated in locations that are polluted with metals.

The occurrence of PICT in the Demmerik area is premature. The pattern observed for Zn is not what we expected. The EC_{50} values are variable except for the lowest and highest Zn concentrations. On the other hand, PICT-Pb was observed for the highest soil Pb concentration. Apart for the limited amount of experimental data, these vague results may be due to method limitation: were the bacteria extracted and preserved properly, was the laboratory metal exposure adequate, was data treatment correct? The impact of pH and organic matter on the availability of metals in the field may also influence the results.

Except for method limitation, it could be difficult to quantify relevant field exposure to metals, simply because it may be too difficult to estimate this from standard methods such as total destruction, pore water or $CaCl_2$ extraction, and model calculations. In that case, PICT will provide more direct clues to metal effects than any metal concentration determined in field samples. More samples should be investigated to better understand the PICT observations in the Demmerikse polder.

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Assessing ecosystem functions under metal stress in a grass-land area

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The nature-reserve area "De Ronde Venen", located 30 km southeast of Amsterdam is a grassland area, which is extensively grazed by sheep and is one of the three areas selected for SSEO research. It was selected, because from the 16 th to the 20th century, municipal garbage from cities was dumped in this area to enrich the land. This resulted in a new soil profile: sand/clay on peat. Nowadays, these soils are considered to present a potential risk, because they contain elevated levels of lead, copper, zinc and cadmium. Given the fact that the area is a nature reserve, ecological implications of these levels are a grave concern and are therefore being investigated by a consortium of RIVM, WUR and VU. The scope of this research was described in SSEO Newsletter 4 and presented at the SETAC Europe meeting in Vienna, Austria. Preliminary results were shown at last year's meeting of the SSEO programme in Utrecht, The Netherlands.

The project started by compiling an inventory of the field situation. Metal concentrations and abundances of some dominant species present (microbes, nematodes and enchytraeids) were determined in these grasslands. Subsequently, an indoor experiment was designed to investigate functional implications of heavy metal pollution. It was hypothesised that ecological functions would be affected by pollution, as key species would have disappeared or adapted. The functional variables that we monitored in these experiments were: primary production (i.e. plant growth), decomposition (i.e. CO_2 production) and nitrogen mineralisation (nitrate and ammonium levels). Bait-lamina sticks containing a cellulose & bran mixture were inserted into columns to calculate consumption by primarily enchytraeids and earthworms. Resistance to stress is considered another important function and this was assessed by applying an increasing level of extra stress to intact soil cores from locations with different levels of metal pollution. The cores were incubated in the laboratory under standard climatic conditions and the extra stress applied was an increased level of zinc chloride in rainwater. A complete ecological assessment of the area will be made by closely monitoring system-related variables during this period and determining structural variables, e.g. nematodes, bacteria, enchytraeids and earthworms.

Results of the monitoring under stress revealed that total plant production did not change by the application of zinc. No obvious results were found of a range of different treatment levels. Because several plant species are present in these grasslands, it is premature to draw firm conclusions about the Zn concentration, although it is generally considered vital in ecosystem functioning. Decomposition (~average CO_2 production) was found not to have changed by the extra stress factor either, although variation increased with increasing treatment levels. Likewise, nitrate and ammonium levels did not show a consistent pattern. It was concluded from these results and from the fact that no structural changes were observed that none of the functions showed any response to the extra stress. We propose changes in methodology because the desired level of stress was not achieved which may limit the firmness of this conclusion. Following experiments will use another stressor to assess these functions and will be performed in 2003 again by the same consortium.

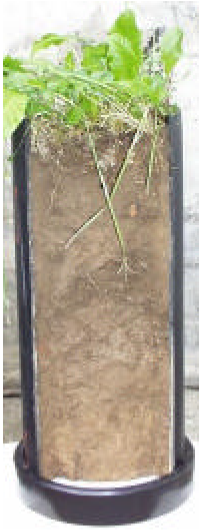

Fig. 1: A cut-open example of the columns used for this project (above)

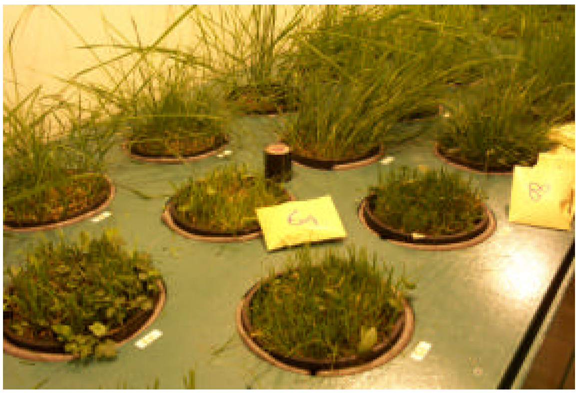

Fig. 2: Determination of functions (here: total plant production) in a standardised incubation room (above)

Even though the ecological functions examined in this experiment did not show any changes with increasing stress application, some fascinating observations were made. It was shown that not only different heavy metal concentrations existed the within a single field, but also different a-biotic factors such as pH, soil humidity, total carbon and total nitrogen levels which could affect the outcome of functional variables. The responses of structural aspects revealed that for example the abundance and diversity of enchytraeids did indeed correlate negatively with increasing metal levels. This could be an explanation for the observed reduction in consumption of the bait-lamina sticks in the more polluted columns. Noteworthy was the fact that this was also consistent with increasing treatment levels of zinc, so this was the only variable that showed any response.

This result was not expected, as the zinc treatment did not cause any changes in diversity or total numbers of earthworms or enchytraeids. The combined research effort of RIVM, WUR and the VU will be further integrated this year to make a full ecological assessment of these grassland areas. In co-operation with the same partners, an intensive field campaign will be conducted this year to relate more detailed information on structural and functional aspects. This field sampling campaign involving a grid to spatially plot the relationships between different grassland areas and heavy-metal pollution levels will take place in the polluted Demmerikse Polder, as well as other locations to enable comparisons to be made.

Ecosystem responses to heavy metal pollution: analysis of food-chain transfer and effects on structure and functioning in herbivore-plant communities in contaminated terrestrial tidal sediments in the Biesbosch.

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This project investigates the transfer of heavy metals through a terrestrial food chain in contaminated terrestrial sediments in the Biesbosch. The selected food chain includes the plant species *Urtica dioica* and the herbivorous leaf-eating snail species *Cepaea nemoralis*. Our own experiments demonstrated that fresh *Urtica* leaves are indeed preferred by this herbivore. The project started in 2001 with an extensive field survey in the Biesbosch area. Samples were taken from the soil-, plant- and snail component and analysed for Zn, Cu, Cd and Pb concentrations. A paper on the results of this survey is in preparation. On the basis of this survey, we planned an experiment to focus on the transfer of metals from plants to snails. The experiment aimed at determining food-chain transfer of Cd from nettle leaves to *Cepaea* snails. It was expected that higher leaf concentrations would result in higher Cd concentrations in snails. To keep the experiment close to the field situation, stinging nettle leaves were used. We aimed at creating leaf concentrations equal to and somewhat higher than the concentrations in the Biesbosch leaves. The highest Cd concentration found in Biesbosch leaves was about 0,18 µg/g dw.

The nettle plants were grown in a greenhouse on nutrient solutions with six different nominal Cd concentrations. The plants were grown from seed bought from a seed company. The plants were transplanted onto nutrient solution (based on the Hoagland recipe) when they were approximately two to four centimetres high. After an adaptation period of one week, the addition of the Cd treatments was started. The Cd concentrations in the nutrient solution were (µM $CdSO_4$) 0; 0,05; 0,1; 0,2; 0,4 and 0,8. Per treatment, 20 nettle plants were grown in four basins, with five plants per basin (Fig. 1).

Fig. 1: Urtica dioica plants on a Cd-enriched nutrient solution.

The Cd concentrations in the leaves of the different treatments were monitored twice before the start of the snail experiment. When enough leaves were available, measurable Cd concentrations were indeed detected in the leaves. This was the time the feeding experiment started. The experimental snails were sampled in the clean reference site 'Torenvalkweg' close to Lelystad. Per Cd treatment, seven snails were kept individually in plastic boxes in a climate room (16 °C and 75% humidity) (Fig. 2).

Fig. 2: Cepaea nemoralis snails in test boxes with cut nettle leaves as food.

Wet sponges were inserted into the boxes to keep the humidity at a maximum. The sponges had to be removed after the third week of the experiment because the snails started eating them. The leaves for the snail food were harvested per treatment, and comprised three leaves from three different levels of the plants (bottom, middle and top section). All leaves were pooled together per treatment, cut into pieces and mixed. A sub sample of about 1 gram (fresh weight) was given to each snail. One such sample per treatment was used to determine the wet weight: dry weight ratio of the food. The average ratio per food series was used to calculate the amount of dry weight consumed by the snails. Three sub samples per Cd treatment were analysed for their Cd concentration and used to estimate the average Cd concentration in the leaves per treatment per food series. The food was replaced with a fresh food ratio after 3-5 days. The boxes of the snails were cleaned and the faeces were collected. It was planned to run the experiment for about two months, but unfortunately the experiment had to be discontinued after five weeks (eight food series) because of white fly pest in the greenhouse. Moreover, the nettle plants were severely affected by the harvesting and it became very difficult to find suitable leaves. The snails were left without food for two days to empty their guts. After these two days, they were killed by boiling them and dissected into two parts: the mid-gut gland and the 'rest'. Cd concentrations were measured by means of graphite furnace AAS.

The data are still being analysed and the results reported here are not complete. The Cd concentrations in the leaves increased with increasing nominal concentration in the nutrient solution. However, there are no detectable differences between the three low-concentration treatments. The three highest Cd treatments produced leaves with Cd concentrations over 0,18 µg/g dw. The concentrations in the leaves per treatment decreased in time. Leaf consumption by the snails decreased with increasing leaf Cd concentration (p= 0,024 and R= -0,347 Spearman rank correlation) (Fig. 3). The Cd intake (leaf consumption x average leaf Cd concentration) shows an increasing relationship with leaf Cd concentration (p=0,000 and R=0,955 Spearman rank correlation) (Figure 4).

No straightforward relationship was observed between the leaf Cd concentrations and the total snail concentrations. Correlation analyses show a significant linear relationship, but this seems to be caused by some influential points.

The Cd concentrations of the faeces play a crucial role in the elaboration and explanation of the results. We know that Cd has gone into the snail and because we cannot detect it in the snail it must have been excreted. Our next step is to measure the Cd concentrations in the faeces of the snails. The faeces of the lowest and the highest nominal Cd concentrations will be analysed first.

Because this study is not yet complete, it is too early too draw conclusions or to be able to discuss the results. The data so far, however, provide a comprehensive picture of Cd transfer and once the faeces data come in, it will be possible to calculate a Cd budget.

Some results of this study can be related to the potential impact of pollution on the functioning of the snails, an interesting topic for future experiments.

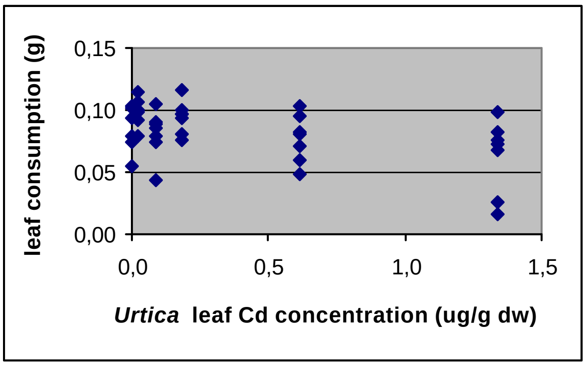

Fig. 3: Average Urtica leaf Cd concentrations versus leaf consumption by C. nemoralis, averaged over time (eight food series). Spearman rank correlation: p=0,024 and R=-0,347.

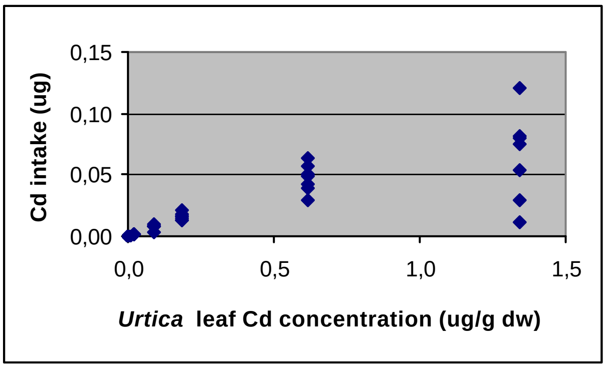

Fig. 4: Average Urtica leaf Cd concentrations versus Cd intake by C. nemoralis, averaged over time (eight food series).Spearman rank correlation: p=0,000 and R=0,955.

Transfer functions of metals in soil invertebrates

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Introduction and objective:

The general challenge of our research is to improve our understanding of metal uptake mechanisms in soil invertebrates, by matching the supply of chemicals to the soil with the biological uptake by invertebrates and species physiology. Various studies on metal uptake by soil biota have been reported in literature. Earthworms are one of the key species used to investigate soil ecosystems. Earthworms may take up chemicals from the soil and from pore water, both through their skin (the dermal route) and by ingestion (the oral route). It remains unclear, however, what the relative importance of these pathways is. To assess bioavailability of pollutants in soil to earthworms, it is necessary that the contributions of both pathways be known.

How to block an earthworms' mouth?

In our experiment, *Lumbricus rubellus* were sealed by means of medical histoacryl glue, to block ingestion of soil particles and pore water. Histoacryl glue (Braun aesculap, Germany), made of enbucrilate, was developed for medical purposes, e.g., to seal human tissues. The glue is odourless and not corrosive. Histoacryl glue is applied easily, by dipping the earthworm's mouth in the glue (see Fig. 2). The glue dries within 30 seconds. Liquid or mucus has to be removed before applying the glue to the worm's mouth, since this will inhibit the drying process. In this way, oral uptake is excluded. Before the earthworms could be analysed for metals, they had to be sacrificed by boiling in water for 2 seconds, and the glue cap had to be removed from their mouths, to exclude possible interference of glue in the analyses.

Sealing earthworms with medical glue does not require any extreme dexterity. The sealed earthworms showed good survival and vitality for six days; they could dig naturally in the soil. When we checked for soil ingestion, using ashing techniques, and control systems without organic matter, no soil ingestion could be detected. After six days, the medical glue started to dissolve in water in some cases, and the experiments had to be stopped.

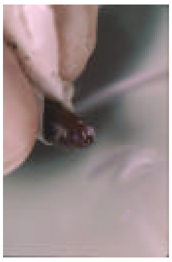

Fig 1: the earthworms' mouth is dipped in the medical glue

Fig 2: photograph of a sealed earthworm

Fig 3: the remaining glue-caps after sacrificing the earthworms

Metal accumulation:

Sealed and unsealed earthworms were exposed to a metal-polluted field soil, and metal kinetics were determined by analysing earthworms at different time intervals. Uptake rates of Cd, Cu and Pb in sealed and unsealed earthworms exposed to two contaminated field soils were similar. Uptake and elimination kinetics of Zn were significantly lower in sealed earthworms exposed to one of the two field soils. Therefore, body concentrations of Cu and Pb could be attributed completely to the dermal route. For internal Cd and Zn concentrations, however, 0% - 17% and 21% - 30% respectively were derived from ingestion.

In a second experiment, when sealed and unsealed earthworms were exposed to an inert matrix continuously flushed with metal-contaminated water, we saw that the two different treatments of earthworms did not exhibit different metal-uptake kinetics. These results demonstrate that pore-water uptake via ingestion has a negligible contribution to metal accumulation.

Conclusions:

We concluded that the dermal route is the main uptake route for metals. The experimental data presented in the present study constitute the first experimental evidence of the contributions of different exposure routes to metal uptake in earthworms. Moreover, the sealing method described here may be useful in a variety of earthworm nutrition and contamination effect studies.

A paper on this research is published:

Vijver M.G., Vink J.P.M., Miermans C.J.H., Gestel C.A.M. 2002. Oral sealing using glue: a new method to distinguish between intestinal and dermal uptake of metals in earthworms. *Soil Biology and Biochemistry* 35 (2003) 125-132.

NWO has a new director

Prof. L.A.A.M. Coolen is performing the function of general director at NWO since March the 1st. Professor Coolen came from the KPN. Before he came to NWO he was already chairman of the National Research Institute for Mathematics and Computer Science (a NWO institute) and member of the board of the technology foundation STW.

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