Centimeter-scale vertical variability of phenoxy acid herbicide mineralization potential in aquifer sediment relates to the abundance of tfdA genes

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MSCN. The concentrations of parent compounds as well as metabolites were analysed by means of HPLC-MS/MS. The results and their implications for the environment are under evaluation.

P39: CENTIMETER-SCALE VERTICAL VARIABILITY OF PHENOXY ACID HERBICIDE MINERALIZATION POTENTIAL IN AQUIFER SEDIMENT RELATES TO THE ABUNDANCE OF *TFDA* GENES

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Centimeter-scale vertical distribution of mineralization potential was determined for the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D), 4-chloro-2-methylphenoxyacetic acid (MCPA) and 2-(4-chloro-2-methylphenoxy)propanoic acid (MCPP) by 96-well microplate radiorespirometric analysis in aquifer sediment sampled just below the groundwater table. Mineralization of 2,4-D and MCPA was fastest in sediment samples taken close to the groundwater table. Considerable variability was exhibited at increasing aquifer depth, more so with 2,4-D than with MCPA. This suggests that the abundance of MCPA degraders was greater than that of 2,4-D degraders, possibly due to the fact that the overlying agricultural soil had long been treated with MCPA. Mineralization of 2,4-D and MCPA was followed by increased abundance of *tfdA* class I and class III catabolic genes, which are known to be involved in the metabolism of phenoxy acid herbicides. *tfdA* class III gene copy number was approximately 100-fold greater in samples able to mineralize MCPA than in samples able to mineralize 2,4-D, suggesting that *tfdA* class III gene plays a greater role in the metabolism of MCPA than of 2,4-D. Degradation rate was found to correlate positively with *tfdA* gene copy number, as well as with the total organic carbon content of the sediment.

P40: TRANSPORT OF BAM DEGRADING BACTERIA FACILITATED BY FUNGAL HYPHAE INCREASES BAM MINERALIZATION IN SAND

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Leaching of pesticides and their metabolites pose a great threat to the groundwater resources. The most frequently detected pollutant in Danish groundwater wells is the pesticide metabolite 2,6-dichlorobenzamide (BAM), which originates from the herbicide dichlobenil. Bioaugmentation has been suggested as a possible means to clean pesticide contaminated sites, as several strains capable of degrading e.g. BAM have been identified. There are however some challenges to overcome for bioremediation to be successful. It is difficult to ensure survival of introduced strains, and moreover, accessibility of the bacteria to the contaminants is often limited.
The aim of this study was to test whether presence of the zygomycete *Mortierella* sp. LEJ701 affected BAM mineralization by *Aminobacter* sp. strain MSH1 in sand with different moisture contents. The hypothesis was that fungal growth facilitates transport of bacteria, and thus increases the bioavailability of BAM, leading to an increased mineralization.
Mineralization was determined using ^{14}C-labeled BAM and measuring production of $^{14}CO_2$. Furthermore, Thin Layer Chromatography (TLC) was performed to determine the fate of the BAM that had not been mineralized. Transport of *Aminobacter* sp. strain MSH1 was detected and quantified by PCR and QPCR respectively.
Results showed an increased mineralization rate by the *Aminobacter-Mortierella* consortia at all moisture contents. Moreover an overall greater mineralization was obtained by the consortia at the lower moisture contents. TLC results support these findings. Preliminary results of the QPCR indicate there had been a greater transport of *Aminobacter* through the sand columns when *Mortierella* was present.

P41: FUNGAL DEGRADATION OF THE PHENYLUREA HERBICIDE DIURON

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The majority of studies on microbial pesticide degradation have focused on bacteria, whereas fewer examine the role of fungi. However, it has been shown that fungal species/strains have the ability to degrade phenylurea herbicides.
The objective of this study is therefore to give new insights into the role of fungi in degradation of the phenylurea herbicide diuron. We compare several strains of *Mortierella* for their ability to degrade diuron to clarify the significance of phylogenetic relationships between fungal pesticide degraders. Furthermore, we investigate if fungi can utilize diuron as carbon or nitrogen source, or whether degradation is caused by co-metabolism.
Fungal strains were isolated from a soil previously treated with phenylurea herbicide. Subsequently, DNA extraction, PCR and sequencing of the isolates were carried out. Degradation experiments were conducted in liquid media to test degradation potential of five different *Mortierella* strains and the effect of substrate C and N concentration.
Our results show that three of the five *Mortierella* strains have the ability to degrade diuron. These fungi belong to a group of closely related strains, indicating that the ability for diuron degradation by *Mortierella* might be limited to this group. With regard to substrate effects our results show that diuron degradation is faster in medium with a high content of C and N compared to in a C or N limited medium. This indicate that *Mortierella* do not utilize diuron as carbon or nitrogen source. Finally, diuron degradation is followed by formation of the metabolites DCPMU and DCPU. In addition an unknown metabolite is observed. In conclusion, this work underlines the relevance for including fungal degradation of pesticides in future research.

P42: EFFECT OF SOIL AMENDMENTS (ACTIVATED CHARCOAL, BIOCHAR AND COMPOST) ON PHENANTHRENE DESORPTION AND BIODEGRADATION

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Soil amendments such as activated charcoal (AC), biochar and compost can reduce the aqueous concentration of PAHs. On the one hand this might limit the bioavailability and uptake by organisms leading to reduced toxicity, but on the other hand this might also decrease biodegradation. Such carbonaceous materials are use since longtime in water purification and plant nutrient regeneration systems.
The aim of this study was to determine the influence of soil amendments such as activated carbon (AC), biochar (charcoal) and compost on the desorption and mineralization / biodegradation of low concentrations of freshly sorbed phenanthrene (> 5 µg l-1). A first set of experiments was done to measure abiotic desorption rates of phenanthrene sorbed to suspensions of the soil amendments in different media (minimal salts medium or tryptic soy broth) into a dominating silicone sink. The tests were conducted in 20 mL vials and lasted for 6 days. By a second set of experiments, the phenanthrene mineralization due to biodegradation by Sphingomonas sp. 10-1 (DSM 12247) was determined over 12 days using a 14C-respirometric method. In the desorption experiments, highest fractions remained sorbed to AC (75%), followed by charcoal (26%) and compost (1%). The phenanthrene mineralization as reflected by radioactivity measurements of 14CO2 was similar for all soil amendments (60% of initially applied radioactivity). HPLC analysis showed only very minor amounts (< 5%) of residual phenanthrene remaining in the suspensions, indicating almost complete biodegradation. Desorption from soil amendment was not rate limiting for biodegradation. This suggests that phenanthrene sorbed to AC and charcoal was only partly desorbable but fully biodegraded, which would imply a reduced toxicity risk but still potential for bioremediation. In contrast, phenanthrene sorbed to compost was both fully desorbed and degraded. In conclusion, using only 14C-respirometric methods to measure bioavailability and biodegradation rates may lead to misinterpretations of the environmental fate of low concentration of bound hydrophobic organic pollutants. In this study, the estimation of bioavailability was different for the silicone O-rings and by 14C-respirometric methods as the latter overestimated the bioavailable fraction.