SESSION 5
DEGRADATION

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Improving the exploitation of microorganisms in environmental clean-up

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ABSTRACT

There are few if any natural materials that cannot be degraded by at least one type of microorganism making microbes potentially very valuable for the clean-up of man-made chemicals introduced in the environment. This review identifies the physical and microbiological constraints to environmental clean-up processes, and describes the rationale behind manipulating the chemical and biological environment for enhanced remediation.

INTRODUCTION

Microorganisms have several important features that make them potentially very valuable for the clean-up of man-made chemicals introduced in the environment—amongst these is their extreme metabolic diversity. There are few if any natural materials that cannot be degraded by at least one type of microorganism and this is likely to be the case for most man-made compounds (xenobiotics). In many cases, only a select few microbial isolates have demonstrated the ability to metabolise a particular xenobiotic. In even fewer cases have the complex biochemistry and genes involved in the catabolic pathways been resolved. As well as being metabolically diverse, microorganisms are genetically adaptable organisms, which enables them to rapidly respond to new situations and stresses, such as pollution events. Consequently, when pollutants enter pristine environments, microorganisms that can degrade them are either already present or can "acquire" the necessary genetic capacity through mutation, gene re-assortment or horizontal gene transfer.

Despite the metabolic versatility of microbes and their wide distribution, they are not infallible, evident by the thousands of hectares of contaminated land still present. The reasons for the poor recovery of an impacted site are complex, but may be summarised as:

- The functional microorganisms or genes required for degradation are present in very low numbers or absent.
- The chemical conditions of the pollutant are unfavourable to microbial degradation - for example, the concentration of pollutant is so high it is toxic or too low to stimulate degradation.
- The environmental conditions are biologically unfavourable for microbial growth and activity – for example pH, water logging or inadequate nutrients.

Considerable attention in recent years has been given towards furthering the understanding of the microbiological and chemical component of bioremediation, however, much remains obscured by the physical complexity of the environment. The purpose of this review is to identify the physical and microbiological constraints to clean-up processes, and to describe

the rationale behind manipulating the chemical and biological environment for enhanced remediation. For convenience we have divided this review into 3 sections.

1. Assessing microbial diversity and genetic potential for natural attenuation.
2. Identifying the populations responsible for attenuation and the constraints to activity.
3. Manipulating microbial communities to overcome the constraints.

ASSESSING MICROBIAL DIVERSITY AND THE GENETIC POTENTIAL FOR NATURAL ATTENUTION

We are largely ignorant of the diversity and identity of microorganisms responsible for environmental detoxification and clean-up processes. For example, we know little of the overall function or the extent of the diversity of component microorganisms involved, even in intensively studied systems such as sewage processing plants. This ignorance is largely due to the lack of suitable methods of assessing the composition and population dynamics of microbial communities. Traditionally, microbial community profiling has been based on culture-based methods that require the isolation and growth of microorganisms on artificial media. Typically, these methods qualitatively and quantitatively underestimate the composition of the microbial community. The isolation method can also be hampered by uncertainties caused by the selective enrichment procedure itself. Because of this limitation, bioremediation and natural attenuation studies have been carried out using a "black box" approach, whereby the disappearance of substrates indicates microbial degradation, with little understanding of the microbial communities responsible for the process.

An important advance in studies aimed at understanding and exploiting microbial populations for the remediation of environmental xenobiotics, has been the application of molecular biology. The foundation of recent work in environmental molecular biology has been exploitation of the differences in the nucleotide sequence of an ubiquitous set of genes within the ribosomal RNA (rRNA). A considerable database of sequences for the small sub-unit RNA, 16S rRNA, is available and is rapidly growing, thereby facilitating the identification of bacterial isolates. The analysis of 16S rRNA often elucidates the presence of previously unknown members of the microbial community, thereby contributing valuable information to an understanding of the overall attenuation picture. However, the key advance these methods bring in terms of assessing microbial diversity, is that they preclude the need for culturing and in so doing, provide a more comprehensive assessment of the composition of the microbial populations present. Two key methods, denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) (Muyzer 1999) have been widely adopted for monitoring microbial diversity. These techniques generate a genetic fingerprint or community profile, based on the small differences present in the 16S rRNA sequence of specific bacteria. Total nucleic acids (DNA and RNA) are extracted from a soil sample, and amplified by PCR using primers specific to a particular gene sequence, i.e. 16S rRNA. The amplimers generated are then separated due to their sequence differences by gradient-gel electrophoresis. In a complex mixture of soil DNA, typical of an environmental sample, specific amplimers migrate to different positions down the gel matrix resulting in banding patterns similar to a bar code. The methods enable the diversity of communities to be measured and contrasted. In this way, microbial population dynamics can be followed in natural and perturbed soil environments (Torsvik *et al.*, 1998). An improved understanding of the community composition enables directed manipulation of the microbes responsible for pollutant degradation. Members of a microbial community may

be monitored for their activity and response to pollution perturbation, thereby indicating the efficacy of attenuation processes (Whitely & Bailey 2000; Whitely *et al.*, 2001). Molecular techniques have the important advantage in that they are relatively rapid to perform (Griffiths *et al.*, 2000). DGGE and TGGE have been applied by a number of groups to interrogate the dynamics of a wastewater treatment plant (Curtis & Caine 1998), and to detect, isolate and characterise the active bacteria in a sample (Watanabe *et al.*, 1998). Another key advance over previous methods is that the approach can be used to monitor specific taxonomic groups of bacteria (Stephen *et al.*, 1998), the presence of functional gene sequences (Rosado *et al.*, 1998) and identifies microbial consortia involved in specific clean-up processes (Boon *et al.*, 2001).

IDENTIFYING THE POPULATIONS RESPONSIBLE FOR ATTENUATION AND THE CONSTRAINTS TO ACTIVITY.

Molecular studies have revealed considerable diversity within microbial populations and are helping to elucidate their complex interactions with contaminants. By comparison of microbial community profiles provided by DGGE, it is possible to determine the response of specific populations to chemical and physical changes in contaminated sites. By further analysis it is possible to identify the populations responsible for the biodegradative process. The utility of 16S rRNA oligonucleotide probes enable the rapid screening of populations for the presence or absence of the selected nucleotide sequence. These probes may be fluorescently tagged, and are available at a number of different wavelengths, allowing a mixture of probes to be used simultaneously. The technique of fluorescent *in situ* hybridisation (FISH) has the advantage of both enabling rapid *in situ* detection and enumeration of microorganisms with homology to the probe. Furthermore, it is possible with the combined use of different phylogenetic probes and substrate addition, to determine the contribution of key functional groups to a particular process. This has been illustrated in systems examining phenol and metal working fluid degradation (van der Gast *et al.*, 2001; Whitely & Bailey 2000). FISH and similar methods used to interrogate remedial processes provide significant insights into the key functional bacterial groups. When used in tandem with chemical analysis, molecular probing can provide valuable information for monitoring pollution and its temporal and spatial dynamics.

The generation of probes for measuring specific gene activity (i.e. RNA rather than DNA) *in situ*, arguably, provides the most useful measure of the biodegradative potential and activity within a contaminated site. Gene probes are being used to monitor the presence of specific genes in the population and their level of expression. The true utility of this technology is when it is correlated to degradative activity as demonstrated by pollutant mineralisation. This has been particularly effective in the analysis of naphthalene degradation, associated with BTEX compounds, were *nahA* mRNA expression has been correlated to enzyme activity and [^{14}C] naphthalene mineralisation rates (Fleming *et al.*, 1993). Despite a number of similar studies with equally encouraging results, there are many cases where molecular signatures do not correlate with monitored degradation rates (Laurie & Lloyd-Jones 2000). These failures are in part due to the misleading assumption that genes identified in laboratory-derived bacterial isolates are solely responsible for the biodegradation observed in the field. To be an effective measure of attenuation, it is therefore essential that the genes to be monitored fully represent the true complement of genes responsible for the pollutant's degradation. In this respect, a novel application has been developed, stable isotope probing

(SIP), which exploits the fact that DNA from microorganisms can become ^{13}C-labelled following growth on a ^{13}C-enriched carbon source (Radajewski *et al.*, 2000). The method is elegant in its simplicity. The ^{13}C-labelled DNA of replicating organisms is heavier than DNA containing the more abundant ^{12}C. The difference in density (due to isotopic atomic mass difference) between 'heavy' ^{13}C- and 'light' ^{12}C-DNA can be resolved by equilibrium centrifugation in CsCl-ethidium bromide gradients. Thus, after purification and sequence analysis of the heavy DNA the active members of the microbial community responsible for the pollutant's degradation can potentially be identified. Theoretically, any labelled substrate can be applied, illuminating the catabolically active member(s) of the population. This will eventually lead to a better understanding of how genetically diverse groups share a common function or co-operate in the degradation of recalcitrant compounds.

Another way in which molecular biology is being used to enhance understanding of the biodegradative process is through the use of biosensors. A typical approach is the modification of an indigenous strain to express a novel phenotype, which is used to indicate the presence or absence of a chemical. The most common reporter gene used is derived from naturally bioluminescent organisms, such as fire-flies or small group of marine bacteria - yielding quantitative light emissions. The biosensors themselves fall broadly into two categories, specific and generalised. Specific sensors have been developed for the detection of one or more related compounds. The sensor utilises reporter gene constructs that are expressed from inducible promoters, which respond to the bioavailability of specific chemicals (Corbisier *et al.*, 1996). Generalised sensors have more widespread utility since they can monitor the impact of toxicity on the overall physiological state of the cell; for example, a decrease in physiological activity of the cell correlates to an increase in toxicity (Boyd *et al.*, 1997; Paton *et al.*, 1995). Reporter based biosensors are therefore a powerful tool in identifying biological and physical constraints on cellular metabolism, such as impaired bioavailability or the presence of inhibitors, elevated concentrations of toxins or the effect of co-contaminants.

MANIPULATION OF CONTAMINANTS AND MICROORGANISMS IN SOIL

Molecular and conventional bacterial isolation techniques are capable of demonstrating the presence of xenobiotic degraders in a soil. However, in a majority of the cases, these populations are either not metabolically active or too few in numbers to significantly contribute to natural attenuation. One viable solution to this problem is to relieve one of the major constraints to microbial activity, the nutrient limitation (carbon or nitrogen). Thus, when this is alleviated through the use of fertilisers (Lindstrom *et al.*, 1991), significant pollutant degradation is often observed. However, alleviating the nutrient limitation is only part of the solution. Xenobiotic biodegradation in the environment is also restricted by the accessibility of the microorganism to the pollutant, termed bioavailability.

The bioavailability of a chemical in soil is influenced by a number of factors, including concentration, solubility, type and quantity of clay and organic matter, pH, temperature, and the compound's chemical characteristics (Alexander 1999). The phenomenon of sorption and desorption, or the partitioning of a chemical from one phase to another (i.e., aqueous to solid), helps to define a chemical's location in the soil, and thus its bioavailability. Typically, as a compound's hydrophobicity, solubility and affinity to organic matter and clay increases, its bioavailability declines. Biounavailable chemicals are often sorbed within small soil pores, typically in clay particles or organic matter, where they remain largely inaccessible to

microorganisms. This sorption process often entails an initially rapid and reversible phase, where the highest bioavailability can be found, followed by a period of slow sorption, which can continue indefinitely (Hatzinger & Alexander 1995). It is the remediation of the desorption-resistant fraction, which makes clean-up a significant and in some cases seemingly insurmountable challenge. It has been commonly observed that the desorption-resistant pollutant pool increases with time as the chemical remains in the soil (MacLeod & Semple 2000). The slow diffusion into and out of clay and organic matrices contributes to the phenomenon of ageing—the declining bioavailability and extractability of a pollutant over time.

It is only recently that researchers have begun to quantify the bioavailable fraction. A number of studies have demonstrated the utility of different extraction solvents and biological indicators in quantifying and qualifying the bioavailable fraction of a pollutant in soil (Reid *et al.*, 2000; Tang *et al.*, 1999). The key to improving the efficacy of bioremediation is to understand and address the factors that influence the dynamics of this bioavailable pool. The current understanding of bioavailability suggests that, if the aqueous concentration of a pollutant is increased (i.e. partitioning from the solid to the aqueous phase), a greater contact between the microbe and the pollutant can be attained, thereby increasing the pollutant's bioavailability. A primary method for increasing the aqueous concentration of a pollutant has been with the use of surface-active-agents, or surfactants (Willumsen & Karlson 1997).

A surfactant molecule typically contains two or more moieties of varying polarity, and by definition lowers the surface tension of a liquid. Surfactants are classified as cationic, anionic or nonionic, referring to the presence of a positive, negative, or neutral charge, respectively, in the molecule. Much of the experimental evidence suggests that the increase in pollutant solubility afforded by surfactants, dramatically increases the rate and extent of pollutant biodegradation (Aronstein & Alexander 1992; Mulligan *et al.*, 2001). As well as increasing the solubility of pollutants, some surfactants also act as nutrient sources for the inoculum and/or indigenous microorganisms (Lajoie *et al.*, 1992; Singer *et al.*, 2000; Singer *et al.*, 2001). In addition to the use of synthetic surfactants, a rapidly developing field is the use of bacterial species that excrete surface-active or emulsifying agents, which decrease the surface tension of water and increase the apparent solubility of the pollutant (Holmberg 2001; Willumsen & Karlson 1997). Biosurfactants are renewable resources and in some cases have been shown to be superior to synthetic surfactants in reducing the surface tension of water, a measure of a surfactant's efficacy (Yakimov *et al.*, 1995).

In addition to alleviating nutrient limitations and pollutant bioavailability, supplementing the soil with inducers and co-metabolites has also been demonstrated to dramatically improve the rate and extent of pollutant biodegradation. The introduction of readily degradable compounds with similar structure to the recalcitrant pollutant has been shown to stimulate degradation of otherwise highly recalcitrant pollutants. This practice has been termed analogue enrichment. For example, biphenyl is readily utilised as a cometabolite, serving as a carbon source and inducer for polychlorinated biphenyl (PCB) degradation. It was observed in the 1990's that non-structurally related compounds could also stimulate recalcitrant xenobiotic degradation (Gilbert & Crowley 1997; Singer *et al.*, 2000). For example, carvone and a number of other plant-derived compounds (eg. terpenes and flavones), were shown to stimulate PCB degradation at equivalent rates to that of biphenyl, the classically used cometabolite (Gilbert & Crowley 1997). Inducers are typically plant-

derived compounds. Since the compound is natural and is not required at high concentrations, it is much safer to use, and has been demonstrated to be as effective as a cometabolite (Gilbert & Crowley 1997).

Future bioremediation projects will likely require the incorporation of surfactants, inducers, and/or cometabolites, as part of an integrated clean-up system. To be competitive with current remediation technologies, the system will need to be applicable to very large-scale sites. Current technologies are too cost prohibitive for large impacted sites, leaving the door open for a novel, environmentally friendly alternative. A promising new technology has taken off in recent years, which is highly suitable to large-scale contaminations; it is the use of plants to assist in stimulating biodegradation—phytoremediation. The available research has suggested planted soils significantly outperform similarly treated fallow soils (EPA 2001). Potential future avenues may lie in the application of plants to contaminated sites that produce inducing compounds, *in situ*, thereby alleviating the need for repeated inducer applications (Crowley *et al.*, 2000).

Natural attenuation and bioremediation hold great promise for efficient and cost effective means for cleaning contaminated environments. By applying methodologies that examine ecological events at their molecular level, we should improve our ability to predict the impact of pollution, the intrinsic ability of natural microbial communities to degrade the contaminant, provide a reliable estimate of recovery time, and obtain an indication of the effectiveness of remedial measures. The quality of the information we are now getting from the environment is improving, and is approaching the point of being able to determine the genes present in a contaminated site, which population contains the genes, and the factors that constrain the expression of the genes. What we now need to do is effectively combine this microbial information with the mass of data on the chemistry, hydrology and geology of a site and develop integrated predictive models. Extrapolation into the future should find us in the position where we can rapidly predict from a small sample the fate of a contaminant, the genetic potential for self-cleaning, the constraints on microbial activities, and the need for manipulations, such as the addition of surfactants or inducers.

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Sorption and degradation of glyphosate and dichlobenil in fractured clay

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ABSTRACT

Sorption and degradation of glyphosate, dichlobenil and 2,6-dichlorobenzamide (BAM) were studied in a Danish topsoil (0-0.45 m) and in the underlying, fractured clayey till (0.45 - 4.10 m). Sediments from fracture walls and the adjacent matrix were carefully transferred to flasks containing dichlobenil or ^{14}C-glyphosate. Mineralisation of glyphosate was measured by trapping the evolved $^{14}CO_2$ in an alkaline solution while accumulation of BAM from degradation of dichlobenil was measured using an immunological assay. Glyphosate mineralisation was observed in all samples, but the extent of mineralisation was highly variable (6-21% CO_2 recovery) and no correlation between depth and the extent of mineralisation was seen. The highest amount of $^{14}CO_2$ was seen in experiments with fracture wall material sampled at a depth of 2.4 m. Sorption coefficients (K_d) for glyphosate ranged between 65-147 l/kg with the highest values in the topsoil and at depths below 2.8 m. Dichlobenil was transformed to BAM in samples above 0.6 m, only. Sorption of dichlobenil was high in topsoil (51 l/kg), but decreased by a factor of ten in the underlying sediment. K_d values for BAM followed the same pattern, although they were one order of magnitude lower (0.2 - 0.7 l/kg).

INTRODUCTION

Sorption of xenobiotics to soil or sediments may impede contamination of the groundwater; however, certain soils such as clay often contain fractures that may facilitate rapid transport to underlying aquifers. The only way to eliminate xenobiotic compounds from the environment is by complete degradation to CO_2, a process known as mineralisation. Glyphosate is among the most widely used herbicides, but due to its strong sorption, it is not expected to be found in groundwater. In contrast, 2,6-dichlorobenzamide (BAM) which is a product of dichlobenil degradation (Figure 1) is among the most frequently found pollutants in Danish groundwater and has caused closure of many drinking water wells.

Figure 1. Structural formulae of pesticides studied: Glyphosate and transformation of dichlobenil to 2,6-dichlorobenzamide (BAM).

We studied sorption and degradation of glyphosate, dichlobenil and BAM in a Danish topsoil and in the underlying fractured clay sediment. The scope was to compare potential degradation and sorption in the fracture fillings and the adjacent matrix sediment.

METHODS AND MATERIALS

Topsoil and sediments were sampled from the Avedøre field site located Southwest of Copenhagen, Denmark (McKay *et al.*, 1999). The site consists of 7-8 m of clay rich till which overlies a major chalk aquifer. The upper 3-4 m of the till is oxidized and highly fractured with widely spaced fractures persisting to at least 5 m depth. The samples were obtained from a 5 x 5 m hole dug to a depth of about 5 m below the surface (m.b.s). Matrix and fracture wall sediments were carefully sampled from different depth using a sterile spatula.

Bacterial numbers was determined by extraction of bacteria from the sediment and counted as colony forming units by plating onto R2A agar.

Transformation of dichlobenil to BAM was assessed in 10 ml vials with 2 g wet sediment and 10 ml of a mineral salt solution. Dichlobenil was added in 20 µl aliquots of aqueous solutions to reach a concentration of 25 µg/l and the vials were incubated at 10 °C. The amount of BAM produced was measured using an immunological assay (Bruun *et al.*, 2000). Mineralisation of glyphosate was studied in small test tubes with 1 g wet sediment and 10.000 DPM ^{14}C-glyphosate equivalent to 33 µg/kg. To trap the evolved CO_2 the test tubes were placed in 10 ml scintillation vials containing 2 ml of 0.5 M NaOH. At times of sampling, the test tubes were removed to new vials and the remaining NaOH was added to 10 ml OptiPhase HiSafe scintillation fluid and were counted for 10 min on a Wallac 1409 liquid scintillation counter.

Adsorption and desorption were determined using ^{14}C-labelled herbicides. Aliquots of 1, 2 or 5 g fresh soil were transferred to 15 ml Pyrex flasks with Teflon sealed caps followed by 10 ml groundwater with 1 mg/l unlabelled herbicide and 50000 DPM labelled herbicide. NaN_3 was added to 0.1 M to prevent microbial degradation during the experiment. The flasks were incubated on a tilting table at 20°C in the dark. After a short centrifugation, water phase samples (100 µl) were collected after 2, 24, 48 and 96 h and analysed on a Wallac 1409 liquid scintillation counter as described above. The herbicide-containing groundwater was then replaced by uncontaminated groundwater and after a period of 182 h the amount of herbicide desorbed to the water phase was quantified.

RESULTS

The pH values of the sediments were in the range of 7-8 with a maximum pH at a depth of 0.45 m (Figure 2). The organic matter content was highest in the topsoil (8.5% of dry weight) decreasing to less than 1.5%, an exception being the matrix sediment from a depth of 1.5 m which had an unusual high concentration of carbon. The amount of phosphate was high in the topsoil (118-163 mg phosphate/kg soil) decreasing to almost zero below 0.5 m, an exception being the depth of 3.9 m where 162 mg phosphate/kg soil was measured. This high value is

probably due to transition from oxidised to more reduced conditions at this depth, which may lead to release of bound phosphate. The carbonate content was low (<25 mg/g soil) to a depth of 0.6 m below which a stable concentration of about 200 mg/g soil was measured (Figure 2).

The number of culturable bacteria in the topsoil was in the range of $2 \cdot 10^5 - 1.1 \cdot 10^6$ cells/g soil (Figure 3). At a depth of 0.6 m fractured till became evident and was shown to support a very heterogeneous distribution of bacteria. Compared to the matrix sediments, the fracture walls contained a higher bacterial cell number, except at a depth of 2.4 m and 3.2 m where the numbers were in the same range. The highest bacterial community of $1.1 \cdot 10^6$ cells/g was obtained at the fracture wall at a depth of 2.4 m.

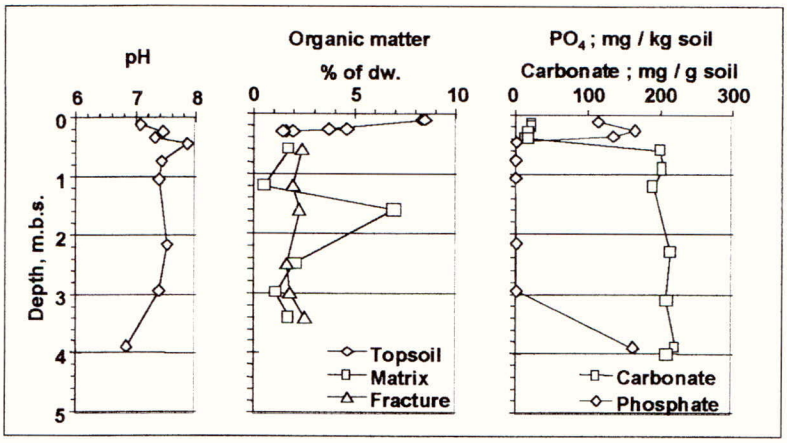

Figure 2. Analysis of groundwater chemistry. Left; pH in pore water. Middle, Organic matter measured as loss after ignition. Fractures were visible only below 0.6 meter. Right; phosphate and carbonate in bulk samples.

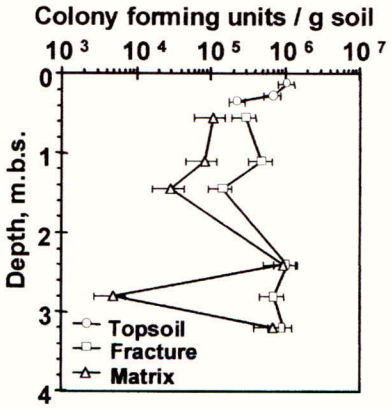

Figure 3. Number of colony forming units.

Glyphosate was mineralised without a lag-phase in all samples from the topsoil (Figure 4). The mineralisation was most rapid during the first 5-8 days after which the rate decreased. The maximum recovery of added ^{14}C as $^{14}CO_2$ amounted to 15.0 % and was found at the depth of 0.25-0.30 m. A potential to mineralise glyphosate was also seen in sediments from lower depths (Figure 5). The highest recovery of $^{14}CO_2$ was obtained with sediment from the fracture wall sampled at a depth of 2.30-2.50 m. The fracture wall sediments in general, however, did not seem to have a higher potential to mineralise glyphosate compared to the matrix sediment.

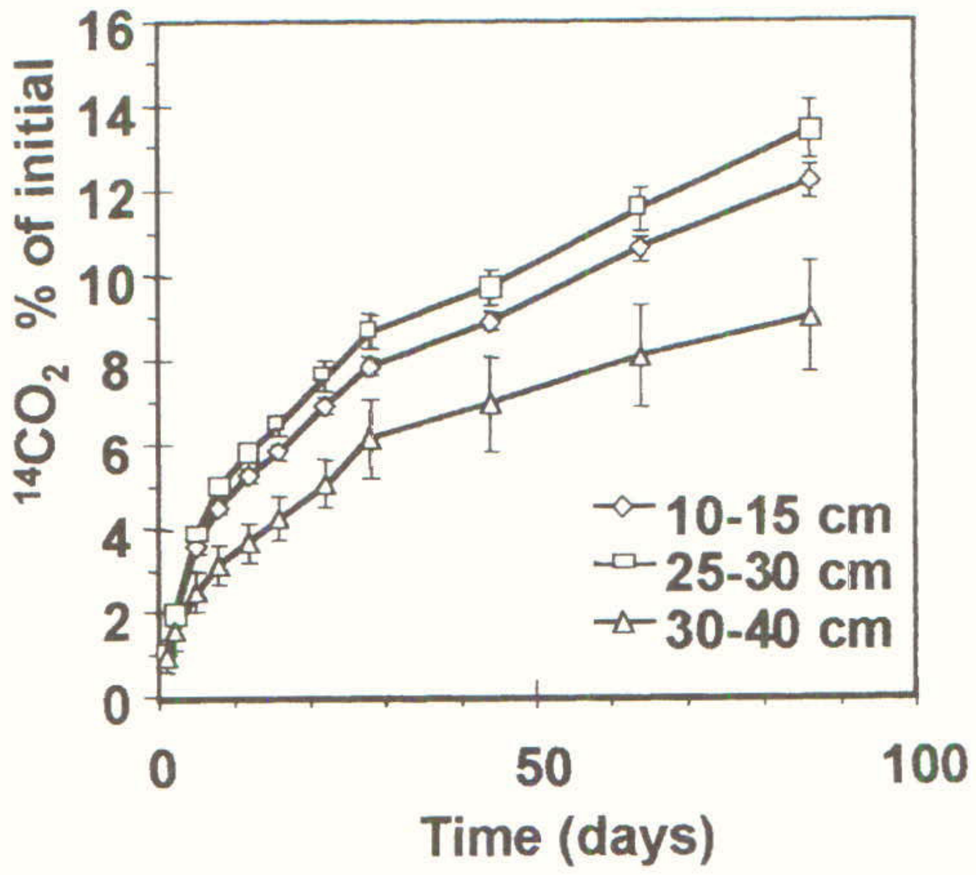

Figure 4. Cumulative $^{14}CO_2$ from mineralisation of [P–methylene–^{14}C] glyphosate in topsoils.

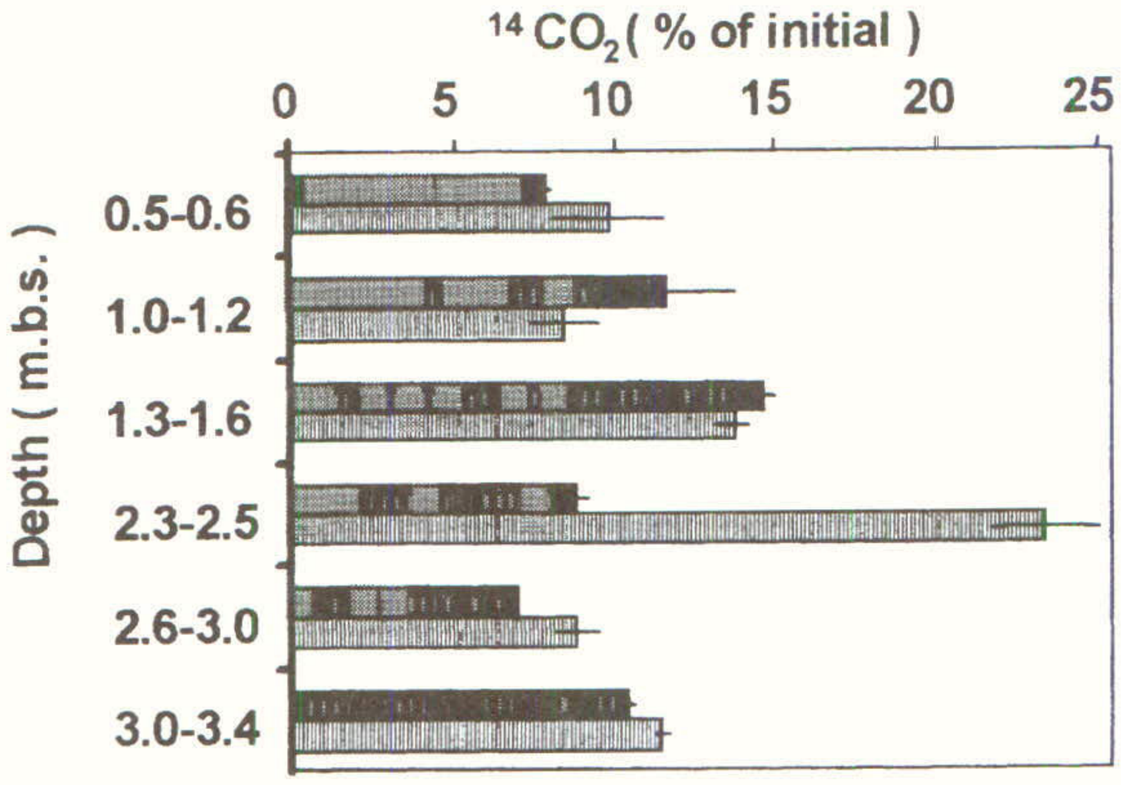

Figure 5. Recovery of $^{14}CO_2$ from [P–methylene–^{14}C] glyphosate in sediments incubated at 10 °C for 110 d. Dark grey bars: Matrix sediments, striped bars: fracture wall sediments.

Transformation of dichlobenil to BAM was only observed in the topsoil (0.1-0.45 m) and in the matrix sediment at a depth of 0.50-0.60 m, but very large standard deviations between replicates were seen (Figure 6). BAM did not accumulate in vials with autoclaved soil, indicating that the transformation is microbially facilitated. However, more research is needed to elucidate the mechanism of dichlobenil transformation to BAM.

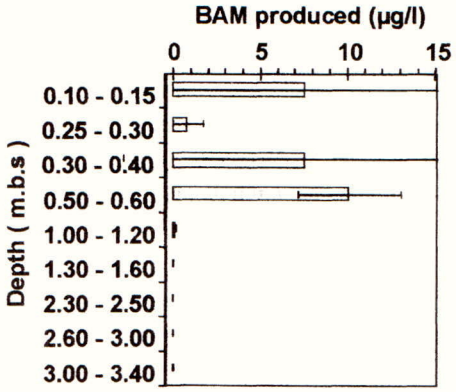

Figure 6. Accumulation of 2,6-dichlorobenzamide (BAM) in top soils and matrix sediments incubated at 10 °C for 64 d. No accumulation of BAM was seen in the fracture wall sediments (data not shown).

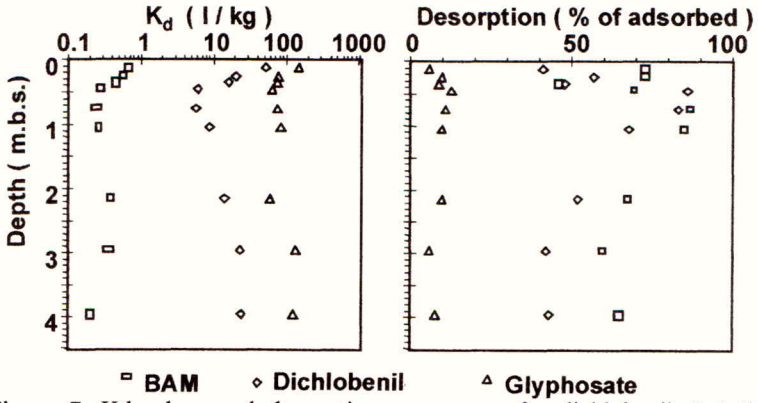

Figure 7. Kd-values and desorption percentage for dichlobenil, 2,6-dichlorobenzamide and glyphosate.

Glyphosate was the herbicide showing the highest sorption to both the clayey till and the organic topsoil. K_d-values were in the range between 65 and 147 l/kg (Fig 7). Dichlobenil also sorbed to soil, but to a lesser extent than glyphosate. K_d-values were in the range between 7 and 51 l/kg with the highest value in the uppermost soil. In practise no sorption of BAM was seen. The measured K_d-values were in the range between 0.2 and 0.8 l/kg with values decreasing from the topsoil towards the lower depths. The desorption study showed that only about 10 % of the sorbed glyphosate was released to the aqueous phase. The highest desorption was seen for BAM. Up to 87% of the sorbed BAM could be released to the aqueous phase which shows that BAM is a highly mobile compound in clayey tills (Fig 7).

DISCUSSION

The higher cell number in the fracture wall compared to the matrix sediment may be explained by bacterial growth on organic compounds preferentially transported through fractures

towards the groundwater. Not all fractures are hydrological active (Vinther *et al.*, 1999) and it is possible that a thorough study identifying the active fractures would have revealed even more distinct bacterial enrichments on the active fracture walls. A potential to mineralise glyphosate was seen at all depths. In contrast previous studies have shown more rapid mineralisation of e.g. mecoprop and isoproturon in topsoils compared to deeper sediment (Larsen *et al.*, 2000). The high potential for mineralisation of glyphosate at greater depths may be explained by the relatively high biomass found at those depths. Dick & Quinn (1995) isolated several bacteria able to use glyphosate as a phosphorus source. It is possible that a preferential use of phosphate in the topsoil inhibit glyphosate mineralisation. On the other hand, the topsoil also contained much organic carbon, which may serve as a primary carbon source for the glyphosate mineralising organisms. Glyphosate showed the highest sorption of the herbicides studied and only limited desorption was seen. Slow desorption may limit the degradation of glyphosate.

Contrary to glyphosate, transformation of dichlobenil to BAM was only associated with the topsoils and the upper part of the till. The process is probably microbially mediated, but more research is needed to elucidate environmental factors governing the transformation rate. Dichlobenil is now banned in Denmark, but it has been found in large quantities in many soils. Compared to dichlobenil BAM is very mobile and is a threat to the groundwater quality for many years.

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