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Influence of diesel concentration on the fate of phenanthrene in soil

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Diesel influenced the fate of [^{14}C]phenanthrene by both physical displacement/sorption factors and by altering the functional activity of the microbial community.

Abstract

The aim of this study was to investigate the influence of diesel on the loss and bioavailability of soil-associated [^{14}C]phenanthrene with time. In addition, the temporal development of phenanthrene catabolic activity and the impact of co-contaminant mixtures on the soil microflora were also assessed. With respect to compound fate, the results suggested that competitive effects between dissimilar co-contaminants did influence [^{14}C]phenanthrene loss. Where diesel was present at a concentration of 0, 20, 200 and 2000 mg kg^{-1}, increased phenanthrene loss was observed with increasing diesel concentrations. In the 20 000 mg kg^{-1} diesel treatment, however, a significantly higher amount of the initial [^{14}C]activity remained after 225 days. Furthermore, initial degradation of phenanthrene in this treatment was retarded as a result of repressed phenanthrene catabolic activity. These results were complemented by a 4-fold increase in total culturable bacterial cell numbers in the 20 000 mg kg^{-1} treatment when compared with the 2000 mg kg^{-1} after 225 days of incubation time.

Keywords: Bioavailability; Catabolism; Phenanthrene; Non-aqueous phase liquid

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous hydrophobic organic contaminants (HOCs) and constitute a group of priority pollutants, introduced into the environment mostly through anthropogenic sources (Sims and Overcash, 1983). Their genotoxic and mutagenic properties are of increasing environmental concern (Bekaert et al., 1999; Bispo et al., 1999; Cerniglia, 1992; Henner et al., 1999). Much research has been conducted to elucidate the fate of PAHs in soils and sediments (Carmichael and Pfaender, 1997; Hatzinger and Alexander, 1995; Wild and Jones, 1993). More recently, simple single contaminant studies have been superseded by studies with two or more analytes of interest. In these multi-contaminant systems, compound fate can be influenced, in a physical/chemical sense, in two related but fundamentally different ways. Firstly, for example, where an aged contaminant is already present in a soil the addition of a 'fresh' dose of compound can result in a competition driven desorption of the 'native' contaminant. Several studies by White and co-workers have examined competitive displacement of aged phenanthrene by a co-solute, generally pyrene (White and Pignatello, 1999; White et al., 1999a,b). In each case, the availability of aged phenanthrene to bacteria and to solvent extractants was

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increased in samples amended with pyrene compared to non-amended samples (White and Pignatello, 1999; White et al., 1999a,b). In addition to competitive desorption effects, co-contaminants can also compete for sorption sites. This is arguably a more important scenario if, for example, co-contaminants are introduced at the same time. Where one of these contaminants is present as a non-aqueous phase liquid (NAPL), interactions between sorbent and NAPL are possible whereby the NAPL acts as an additional sorption phase for HOCs in soils and sediments, consequently affecting their fate. A study by Jonker et al. (2003) showed that sediment–water distribution coefficients (K_d) for six deuterated PAHs spiked in fresh and weathered oil resulted in sorption-reducing competitive effects at low oil concentrations and sorption-enhancing effects at high oil concentrations (i.e. above the critical separate phase concentration, CSPC). Values of NAPL CSPC in the literature range from concentrations of approximately 1000 mg kg^{-1} (Jonker et al., 2003; Sun and Boyd, 1991) to approximately 4000 mg kg^{-1} (de Jonge et al., 1997). Where these values have been normalized to soil or sediment organic carbon content, CSPC for oil approximates 15% (w/w) (Jonker et al., 2003). In addition to physical/chemical effects, the concentrations and ratios of co-contaminants can also perturb the soil (micro)biology. This in turn has consequences for contaminant biodegradation. Several studies have shown that the rates and extent of mineralization of phenanthrene in a NAPL to be dependent on the degradability of that NAPL (Efroymson and Alexander, 1994; Labare and Alexander, 1995; Morrison and Alexander, 1997). These studies show that when present in a more readily degradable NAPL, phenanthrene was mineralized more slowly and to a lesser extent than when present in a poorly or non-degradable NAPL. Morrison and Alexander (1997) showed no significant difference in the rates of mineralization of phenanthrene present in different NAPLs of ranging biodegradability where the NAPL concentration was 500 mg kg^{-1} but a decrease in rates with increasing NAPL biodegradability where the NAPL was added at 10 000 mg kg^{-1}. This phenomenon was attributed to microorganisms competing for a limited supply of inorganic nutrients in the presence of excess carbon. Indeed, it has been shown that the addition of nitrogen and phosphorous doubled the rates of phenanthrene mineralization when the NAPL was highly degradable but had no effect when the NAPL was non-degradable (Morrison and Alexander, 1997). Alternatively, it is probable that the HOCs are retained within the NAPL that then precludes HOC-microbe interactions and therefore biodegradation.

Despite measuring biodegradation (the result), most of these studies have not considered the temporal development of catabolic activity (the process) with respect to PAHs (e.g. phenanthrene) in the presence of a NAPL. Yet the persistence of pollutants in the environment is dependent on the intrinsic biodegradability of these pollutants and the ability of the indigenous microflora to adapt by developing metabolic capacities towards these pollutants. Furthermore, given the occurrence of subsurface contamination through spillage of multi-component NAPLs, of which PAHs are a major component, there is a need for investigations to be carried out to elucidate the fate of hydrophobic organic compounds (HOCs) in multiple contaminant systems where compounds are of dissimilar classes. In this study we investigated the influence of increasing diesel concentrations on the loss and bioavailability of soil-associated phenanthrene with time. In addition, we assessed the temporal development of catabolic activity and the impact of these co-contaminant mixtures on the soil microflora.

2. Materials and methods

2.1. Chemicals

Phenanthrene (98% purity) and its ^{14}C-radiolabelled analogue ([^{14}C]9-phenanthrene, radiochemical purity >98%), cyclohexamide and hydroxypropyl-β-cyclodextrin (HPCD) were obtained from Sigma–Aldrich, UK. Diesel fuel was purchased from a local British Petroleum filling station. Toluene (99.5% purity) was obtained from BDH Chemicals, UK. GF/A filter paper was obtained from Whatman, UK. Sample oxidizer scintillation cocktails (Carbosorb-E and Permafluor-E), Combustaid and the scintillation cocktail Ultima Gold were all obtained from Perkin–Elmer Life Sciences, UK. Micro Agar was obtained from Duchefa, Netherlands. Phosphate-buffered saline (PBS) (Dulbecco A) was obtained from Oxoid, UK.

2.2. Soil

Soil (sandy loam) was collected from a permanent pasture field (University of East Anglia, Norwich, UK, TG199073) from the A-horizon. Air-dried soil was passed through a 2-mm sieve to remove stones and roots. The organic matter content was established to be 3.3 ± 0.4% (mass loss on ignition on a dry-weight basis). The soil had a pH of 8 and the following particle size distribution: 75% sand, 18% silt, 7% clay. The water-holding capacity (WHC), relative to dry-weight, was 21.2 ± 1.0%.

2.3. Soil spiking and storage

Air-dried soils were spiked following the single spiking/rehydrating procedure for dry soil described by Reid et al. (1998). Sufficient water to rehydrate