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The influence of a NAPL on the loss and biodegradation of ^{14}C-phenanthrene residues in two dissimilar soils

A.L. Swindell, B.J. Reid *

School of Environmental Sciences, University of East Anglia, Norwich NR4 7TJ, UK

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Abstract

This study was carried out to assess the influence of diesel, applied over a log concentration range, on the loss and extractability of phenanthrene (measured as putative ^{14}C-phenanthrene residues) in two different soils. The influence of diesel on the ability of a cyclodextrin based extraction method to predict the microbial bioavailability of ^{14}C-residues was also assessed. An increase in loss of ^{14}C-residues with increasing diesel concentration from 0 to 2000 mg kg^{-1} was generally observed with time in both soils. It is suggested that this trend is attributable to competitive sorption for soil sorption sites and to a lesser extent to displacement of ^{14}C-residues from soil sorption sites by diesel resulting in greater compound availability and therefore greater loss by degradation via the actions of indigenous microorganisms. However, in the 20000 mg kg^{-1} diesel treatments of both soils, results indicated a delayed loss. It is suggested that this retarded loss was due to the formation of a discrete NAPL-phase into which ^{14}C-phenanthrene residues partitioned, thereby decreasing their availability and as a consequence their degradation. Furthermore, it is suggested that nutrient limitation may have slowed down degradation rates as diesel concentrations increased. Comparison between cyclodextrin-extractability and microbial mineralisation supported the use of cyclodextrin to assess microbial bioavailability of ^{14}C-residues after 50 d or more ageing up to diesel concentrations of 2000 mg kg^{-1}. However, results suggested that at high diesel concentrations (specifically 20000 mg kg^{-1}) co-extraction of ^{14}C-phenanthrene residues may have occurred as a result of the combined solvating powers of both the cyclodextrin and the diesel. Furthermore, mineralisation of ^{14}C-phenanthrene residues may have been affected by extreme nutrient limitation in this treatment.

Keywords: NAPL; Diesel; Bioavailability; Cyclodextrin; Ageing

1. Introduction

Many factors govern the fate of hydrophobic organic contaminants (HOCs) in soil; these include soil properties, contaminant physicochemical properties and environmental conditions such as temperature and precipitation (Reid et al., 2000a). In addition, soil-compound interactions also affect the fate of HOCs. Indeed, studies by Alexander and co-workers have shown that HOCs that persist in soil exhibit reduced extractability and bioavailability to soil biota with increasing contact time (Hatzinger and Alexander, 1995; White and Alexander, 1996; Kelsey et al., 1997; Tang and Alexander, 1999). It is widely accepted that this 'ageing' is controlled by interactions between the contaminants and the soil matrix (i.e. mineral and organic matter fractions), resulting in compound sequestration. Two main mechanisms of sequestration have been proposed, namely, intraparticle diffusion (Wu and Gschwend, 1986; Nam and Alexander, 1998) and intraorganic matter diffusion (Xing and Pignatello, 1997; Cornelissen et al., 1998).

Chung and Alexander (1999) reported the relationship between nanoporosity and other soil properties. They observed that % silt offered better correlation coefficients to total pore volume (for pores <10^4 nm) ($r = 0.65$) than % sand ($r = -0.46$), % clay ($r = -0.11$) and % organic carbon ($r = 0.54$). Consequently, our study considered two soils with similar pH, % clay and % organic matter but

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* Corresponding author. Tel.: +44 1 603 592357; fax: +44 1 603 591327.
E-mail address: b.reid@uea.ac.uk (B.J. Reid).

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dissimilar % sand and % silt with a view to corroborating the results of Chung and Alexander (1999).

Furthermore, sorption is reported to be affected by the presence of other compounds. Indeed, recent studies by White and co-workers have examined the competitive displacement of phenanthrene from sorption sites by a co-solute (pyrene) (White and Pignatello, 1999; White et al., 1999a,b). They observed an increase in the availability and extractability (by a mild solvent) of aged phenanthrene from samples amended with pyrene or anthracene compared to non-amended samples. In addition, NAPLs have also been shown to affect the fate of HOCs in soils and sediments. 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)).

In addition to these competitive sorption/desorption effects, NAPLs can also perturb the soil (micro)biology. Several studies have shown the rates and extent of phenanthrene mineralisation in a NAPL to be dependant on the concentration and biodegradability of that NAPL (Efroymson and Alexander, 1994; Labare and Alexander, 1995; Morrison and Alexander, 1997). Indeed, Morrison and Alexander (1997) showed that at a NAPL concentration of 500 mg kg^{-1}, there was no significant difference in the rates of mineralisation of phenanthrene, irrespective of NAPL biodegradability but a decrease in rates was observed with increasing NAPL biodegradability where the NAPL was added at 10000 mg kg^{-1}. This phenomenon was attributed to microorganisms competing for a limited supply of inorganic nutrients in the presence of excess carbon.

The aim of this study was to assess the influence of diesel applied over a log concentration range on the loss and extractability of ^{14}C-residues (putative ^{14}C-phenanthrene) in soil. With a view to providing a comparison with more traditional single contaminant studies on dissimilar soils (Hatzinger and Alexander, 1995; White and Alexander, 1996; Kelsey et al., 1997; Tang and Alexander, 1999) this study was conducted using two different soils, to compare the influence of diesel concentration on the loss and extractability of ^{14}C-residues. Finally, the ability to predict the extent of microbial degradation of ^{14}C-residues in soil in the presence of a NAPL by a chemical means was also assessed using an aqueous solution of hydroxypropyl-β-cyclodextrin (HPCD) (Reid et al., 2000b).

2. Materials and methods

2.1. Chemicals

Phenanthrene (98% purity), its radiolabelled analogue (^{14}C-9-phenanthrene, radiochemical purity > 98%) and HPCD were all obtained from Sigma–Aldrich, UK. Diesel fuel was purchased from a British Petroleum filling station. Toluene (99.5% purity) was obtained from BDH chemicals, UK. GF/A filter paper was obtained from Whatman, UK. Sample oxidiser chemicals (Carbosorb-E and Permafluor-E), Combustaid and the scintillations cocktails Ultima Gold and Ultima Gold XR were all purchased from PerkinElmer life sciences, UK.

2.2. Soils and their properties

Two different soils (A horizon) were collected from fields in Norfolk, UK. The soils were air-dried for 14 d and passed through a 2 mm sieve. The soil properties (based on Eldridge, 1980; Hodge et al., 1984) and the sampling location are listed in Table 1.

Nitrate values were calculated from total oxidised nitrogen measured in aqueous soil extracts using the Kone Lab 30 Analyser. Soil samples (5 g) were extracted into MilliQ water (25 ml) and the resultant solution collected by filtration under vacuum. The extract was then reduced using a combined hydrazine-copper reagent (hydrazinium sulphate (3.3 mM) copper sulphate pentahydrate (14 μM)). Nitrite present in the resultant solution was then converted into a red azo-dye using a second combined reagent (sulphanilamide (29 mM) and *N*-(1-naphthyl)-ethylenediamine dihydrochloride (20 nM)). Adsorbance at 540 nm was then determined (Kone Lab 30 Analyser). Nitrate was calculated by subtracting nitrite concentration determined in the sample prior to reduction from the value obtained using the method above (representing nitrite plus nitrate).

2.3. Soil spiking and storage

The air-dried soils were spiked following a single step spiking/rehydrating procedure for dry soil described by Reid et al. (1998). Sufficient water to rehydrate the air-dried soil to 65% water holding capacity was placed in a beaker. Phenanthrene was added to the water as ^{12}C-phenanthrene (100 μl) and ^{14}C-phenanthrene (100 μl). Standards were prepared in toluene that delivered a phenanthrene concentration of 21 mg kg^{-1} and an activity of 14 Bq g^{-1}, relative to soil dry weight. Diesel was then added at either 20, 200, 2000 and 20000 mg kg^{-1}, relative to soil dry weight. Control treatments containing only phenanthrene (21 mg kg^{-1}) were also prepared, as were analytical blanks (soil not

Table 1
Soil properties and sampling locations

Soil series	Grid reference	% Sand[a]	% Silt[a]	% Clay[a]	% LOI[b]	pH	Soluble nitrate as NO_3[e] (mg kg^{-1})
Moulton	TG199073	63[c]	25[c]	12[c]	3.3	7.7	5.8 ± 3.2
Sheringham	TG113355	47[d]	44[d]	9[d]	3.1	7.2	45.4 ± 10.8

[a] Values for residue after ashing.
[b] LOI: loss on ignition (dry weight basis).
[c] Data from Hodge et al. (1984).
[d] Data from Eldridge (1980).
[e] Data presented as mean ± SEM ($n = 5$), detection limit is 1 mg kg^{-1}.