Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Mineralisation of ^{14}C-phenanthrene in PAH-diesel contaminated soil: Impact of *Sorghum bicolor* and *Medicago sativa* mono- or mixed culture

Anthony C. Umeh[a,b], Gabriela M. Vázquez-Cuevas[a], Kirk T. Semple[a,*]

[a] Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, United Kingdom
[b] Global Centre for Environmental Remediation (GCER), University of Newcastle, Callaghan, NSW 2308, Australia

ARTICLE INFO

Keywords:
PAH mixtures
Diesel oil amendment
Phytotoxicity
Sorghum bicolor
Medicago sativa
^{14}C-phenanthrene mineralisation

ABSTRACT

Plant-assisted biodegradation can offer a cost-effective and sustainable approach for the bioremediation of PAHs in soil. As such, selecting the most appropriate plant species is important. The potential for plant-assisted biodegradation of complex PAH-diesel mixtures in soil by sorghum (*Sorghum bicolor*) and alfalfa (*Medicago sativa*) grown as monocultures and mixed cultures using ^{14}C-contaminants has not been widely reported. The objective of this study was to assess ^{14}C-phenanthrene mineralisation profiles in mixtures of PAH-diesel in soil in the presence of *Sorghum bicolor* and *Medicago sativa.* Soil was spiked with PAHs and diesel, after which *M. sativa* and *S. bicolor* were introduced and grown as mono- or mixed- cultures. The toxicity of the PAH-diesel oil mixture in the planted treatments, as well as its effect on the mineralisation of ^{14}C-phenanthrene were evaluated. Monocultures of both plant species tolerated the complex PAH-diesel mixtures based on growth and survival, and increased rates and extents of ^{14}C-phenanthrene mineralisation in soil. The influence of PAH concentration on ^{14}C-phenanthrene mineralisation profiles varied in planted and unplanted treatments. The rates and extents of ^{14}C-phenanthrene mineralisation tended to decrease in diesel amended soil, especially at low PAH concentrations. To the best of the authors' knowledge, this is the first report of ^{14}C-phenanthrene mineralisation patterns in complex PAH-diesel oil mixtures contaminated soil especially with respect to the specified plant species. The findings offer new insights on mono- and multi-species phytotoxicity as well as plant-assisted biodegradation of PAH mixtures in soil which may be useful in the risk assessment and remediation of contaminated sites.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic organic contaminants with two or more fused benzene rings together. Generally, these compounds are of concern to human and environmental health due to their carcinogenicity, toxicity, and persistence in the environment (Juhasz and Naidu, 2000). The USEPA has classified 16 PAHs as priority pollutants including phenanthrene (Phe), benzo[a] anthracene (BaA), and benzo[a]pyrene (BaP) (USEPA, 2008). Although PAHs are released into the environment from natural combustion of organic matter, anthropogenic activities constitute the most important sources (Wilson and Jones, 1993). For example, burning of fossil fuel, coal, and wood, vehicular emissions, heating, and accidental spills of crude oil and other petroleum products among others are well known sources of PAH release into the environment.

Soil is considered a major sink for PAHs in the environment (Wild and Jones, 1995). PAHs can be found as complex mixtures in soil, where they associate with other chemicals such as phenols, aliphatic hydrocarbons and metals (Allan et al., 2007; Thavamani, 2012). PAHs also exist in co-contamination with non-aqueous phase liquids (NAPLs) such as transformer oil from electrical cables and diesel oil from deliberate and accidental oil spillage around petroleum hydrocarbon contaminated sites (Molina-Barahona et al., 2004). The implication is that co-contamination is likely to change the fate and behaviour of PAHs in soil (Lee et al., 2003; Couling 2010). This effect has been previously observed under a range of conditions by different authors such as Swindell and Reid (2006) or Towell et al. (2011).

Considering the environmental implications of the presence of these contaminants in soil, various studies have reported the potential of plant-assisted biodegradation of PAHs in soil (Banks et al., 2003; Meng 2011; Chen et al., 2016; Deng and Zeng, 2017). Although the mechanisms promoting plant assisted biodegradation of PAHs and other hydrophobic organic contaminants are not fully understood, different processes have been observed to affect the biodegradation process (Oyelami et al., 2013). Among these, plant identity (Panchenko et al., 2016) and root exudates have been hypothesised to play an important

* Corresponding author.
E-mail address: k.semple@lancaster.ac.uk (K.T. Semple).

https://doi.org/10.1016/j.apsoil.2017.12.010
Received 22 March 2017; Received in revised form 20 November 2017; Accepted 11 December 2017

role (Fan et al., 2008; Wenzel, 2009; Gao et al., 2017). Mixed cultures of two or more plant species can enhance rates and extents of biodegradation (Chen et al., 2016), potentially due to nutrient- and metabolites-richer rhizosphere, when compared to their corresponding monocultures (Wenzel, 2009). Since the effectiveness of plant-assisted biodegradation may differ with plant species (D'Orazio et al., 2013), finding appropriate plant species mix may represent a confounding factor for phytoremediation (Panchenko et al., 2016; Thijs 2017).

Plant-assisted biodegradation of complex PAH-diesel oil mixtures in soil, measured through a ^{14}C-PAH mineralisation approach, in the presence of mono- or mixed- cultures of *Medicago sativa* L. (Fabaceae) and *Sorghum bicolor* (L.) Moench (Poaceae) has not been previously reported. Plant-assisted biodegradation in this present study was used to imply increased microbial mineralisation of ^{14}C-phenanthrene, or microbial activities, in planted soils when compared to corresponding unplanted controls. For this study, it was hypothesised that (i) both *M. sativa* and *S. bicolor* would show tolerance in PAH-diesel oil mixture contaminated soil, regardless of PAH concentration or diesel amendment; (ii) Increases in PAH mixture concentration, and diesel amendment, would decrease rates and extents of ^{14}C-phenanthrene mineralisation in soil; (iii) rates and extents of ^{14}C-phenanthrene mineralisation would be greater in planted treatments (monocultures or mixed cultures), and (iv) rates and extents of ^{14}C-phenanthrene mineralisation in treatments associated with mixed cultures would be greater than those of monocultures. To address these hypotheses, the following objectives were set: (i) to assess the tolerance (growth and survival) of *M. sativa* and *S. bicolor* in PAH-diesel oil mixture contaminated soil; (ii) to assess microbial mineralisation of ^{14}C-phenanthrene in soil spiked with a mixture of three PAHs and amended with diesel oil, and (iii) to evaluate and compare microbial mineralisation of ^{14}C-phenanthrene in PAH-diesel oil mixture in planted and unplanted treatments.

2. Materials and methods

2.1. Chemicals and other materials

Non-labelled phenanthrene (> 98%), benzo[a]pyrene (> 97%), benzo[a]anthracene (> 95%), sodium hydroxide (reagent grade), plate count agar (Fluka analytical), and toluene were purchased from Sigma-Aldrich, UK. [9-^{14}C] phenanthrene (3.7 MBq/ml) was obtained from American Radiolabeled chemicals, Inc., USA, Goldstar liquid scintillation cocktail (LSC) from Meridian, UK, general purpose agar (agar-agar), general purpose grade Ringer's solution tablets, acetone (HPLC grade), as well as the chemicals used for preparing minimum basal salts (MBS) solution were acquired from Fisher Scientific, UK. Seeds of *M. sativa* and *S. bicolor* were purchased from Moles Seeds Ltd., UK and Chiltern Seeds, UK respectively. Commercial diesel was obtained from a local UK petrol station.

2.2. Soil preparation

A pristine agricultural soil was collected from a depth of 5–20 cm, from Myerscough Agricultural College, Preston, Lancashire, PR3 0RY, UK. The soil was a clay-loam (Dystric Cambisol) (FAO, 1988). Soil was air-dried and then passed through a 2 mm sieve. Thereafter, the sieved soil was stored in the dark at 4 °C until needed. Soil properties have been previously determined (Couling et al., 2010) and are presented in Table SI 1. Air-dried soil was spiked with ^{12}C-PAH standard (ΣPAH = Phe + BaP + BaA) at 100 mg kg^{-1} and 300 mg kg^{-1}, as well as diesel (0.1% w/w) when applicable. Spiking was done using an inoculum approach following the protocol described by Doick and Semple (2003). Briefly, the soil was rehydrated to approximately 35% moisture content with deionised water, after which 3 batches of 250 g soil were placed in a mixing bowl and spiked with ^{12}C-PAH standard in acetone:toluene (1:1, v/v) carrier solvent mixture. Solvent was allowed to disperse in soil and vented off in a fume cupboard. Soil was then thoroughly homogenised and distributed in pots according to the treatments described in Table 1.

Table 1
Experimental treatments.

Treatment	Characteristics
C1	Control = un-spiked soil + carrier solvent + diesel + plants
C2	Control = un-spiked soil + carrier solvent + plants
C3	Control = untreated soil + plants
C4	Control = un-spiked soil + no plants
T1	100 mg kg^{-1} ΣPAH + carrier solvent + diesel + plants
T2	300 mg kg^{-1} ΣPAH + carrier solvent + diesel + plants
T3	100 mg kg^{-1} ΣPAH + carrier solvent + plants
T4	300 mg kg^{-1} ΣPAH + carrier solvent + plants
C5	Control = 100 mg kg^{-1} ΣPAH + carrier solvent + diesel + no plants
C6	Control = 300 mg kg^{-1} ΣPAH + carrier solvent + diesel + no plants
C7	Control = 100 mg kg^{-1} ΣPAH + carrier solvent + no plants
C8	Control = 300 mg kg^{-1} ΣPAH + carrier solvent + no plants

2.3. Plant-assisted biodegradation test

2.3.1. Assessment of seedling emergence and phytotoxicity

Seedling emergence and growth test of both plant species was conducted following relevant OECD and USEPA guidelines (OECD, 2006; US EPA, 2012) with slight modifications. Prior to the growth test, a seed viability test was conducted using seeds (n = 10) of each species placed on a moistened filter paper in a petri dish. The petri dish was covered and placed in a controlled temperature room (21 ± 1 °C) and assessed daily for germination. The pot experiment was conducted in a glasshouse and had a completely randomised block design with three replicates. The specific treatments are described in Table 1. Plastic pots (90 mm) were filled with 50 g soil, with a disc of filter paper fitted at the bottom to avoid soil loss. In addition, individual pot trays were fitted under each pot to control any leachate and avoid cross contamination. For monocultures, 10 seeds were sown into the pots, whereas 5 seeds each were sown for the mixed cultures (i.e. *M. sativa* + *S. bicolor*). Germination, survival and general visual detrimental effects were assessed daily, while percentage seedling emergence and growth was determined after 21 days. Further, weekly measurements of plant heights were made while other visual toxic effects were also observed. At the end of the growth assay, planted treatments were destructively sampled in order to determine plant biomass. The shoots were harvested from the soil surface while the roots were carefully harvested after inverting the pots on a clean polythene sheet. Afterwards, roots were gently rinsed to detach soil from the surface and then dried with a clean paper towel. The fresh weights of the shoots and roots were measured, after which they were oven-dried at 60 °C for 24 h and their dry weights assessed. The root/shoot biomass ratios were then calculated.

2.3.2. ^{14}C-Respirometry assay

To assess plant-assisted biodegradation, evolution of ^{14}C-phenanthrene mineralisation in planted (after 0 and 21 days) and unplanted soils (after 0, 21, and 42 days) was monitored in 250 ml modified Schott bottles (n = 3) at 20 ± 1 °C, following the methods described by Reilley et al., 1996; Reid et al. (2001). The biodegradation parameters assessed in this study include (i) the lag phase (defined as the time taken to reach 5% mineralisation); (ii) the fastest rate (%$^{14}CO_2$ days^{-1}), and (iii) the cumulative extent of mineralisation expressed as a percentage of the initial ^{14}C-phenanthrene, which has been mineralised to $^{14}CO_2$ during each sampling time.