Ecotoxicology and Environmental Safety 147 (2018) 594–601

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Impact of nitrogen-polycyclic aromatic hydrocarbons on phenanthrene and benzo[*a*]pyrene mineralisation in soil

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ARTICLE INFO

Keywords:
Mineralisation
N-PAHs
Benzo[*a*]pyrene
Phenanthrene
Bioavailability

ABSTRACT

When aromatic hydrocarbons are present in contaminated soils, they often occur in mixtures. The impact of four different (3-ring) nitrogen-containing polycyclic aromatic hydrocarbons (N-PAHs) on $^{12/14}$C-phenanthrene and $^{12/14}$C-benzo[*a*]pyrene (B[*a*]P) mineralisation in soil was investigated over a 90 d incubation period. The results revealed that both $^{12/14}$C-phenanthrene and $^{12/14}$C-benzo[*a*]pyrene showed no significant mineralisation in soils amended with 10 mg kg $^{-1}$ and 100 mg kg $^{-1}$ N-PAHs ($p > 0.05$). However, increases in lag-phases and decreases in the rates and extents of mineralisation were observed, over time. Among the N-PAHs, benzo[*h*]quinoline impacted ^{14}C-phenanthrene mineralisation with extended and diauxic lag phases. Furthermore, $^{12/14}$C-B[*a*]P and ^{14}C-benzo[*a*]pyrene–nitrogen-containing polycyclic aromatic hydrocarbons (^{14}C-B[*a*]P-N-PAHs) amended soils showed extensive lag phases (> 21 d); with some ^{14}C-B[*a*]P-N-PAH mineralisation recording < 1% in both concentrations (10 mg kg $^{-1}$ and 100 mg kg $^{-1}$), over time. This study suggests that the presence of N-PAHs in contaminated soil may impact the microbial degradation of polycyclic aromatic hydrocarbons (PAHs) and the impact was most likely the result of limited success in achieving absolute biodegradation of some PAHs in soil.

1. Introduction

Exploitation of the biodegradative capabilities of microorganisms for the clean-up of contaminated land has become widely accepted as an alternative to both physical and chemical methods because of its comparatively low cost and environmental sustainability (Sayer and Ripp, 2000; Sutherland et al., 2009; Doley et al., 2017; Menga et al., 2017; Li et al., 2017). Polycyclic aromatic hydrocarbons (PAHs) are among the most abundant organic contaminants with a potential to be degraded. Thus, attention has been paid to the degradation of PAHs and their catabolic pathways are well documented. However, most of these investigations on PAHs mineralisation have focused on single organic contaminants (Grosser et al., 1991; Yoon et al., 2002; Chauhan et al., 2008; Rhodes et al., 2008; Menga et al., 2017; Li et al., 2017). Studies have shown that PAHs degradation processes result in the formation of carbon dioxide (CO_2), water (H_2O) and simple inorganic compounds (maleic acid, fumaric acid, maleanic acid, glutaric acid, acetoacetyl-coenzyme). However, the kinetic efficiency of the pathway, type of reaction and bio-transformed metabolites produced depends on the number of aromatic rings and only limited success has so far been achieved in the biodegradation of some PAHs (Kästner and Mahro, 1996; Juhasz and Naidu, 2000; Lundstedt et al., 2003; Bamforth and Singleton, 2005; Menga et al., 2017; Doley et al., 2017). The reasons for the limited success are not well understood but could be constrained by one or more of the following process: (a) the solubilization of the PAHs, (b) their transport into the cell, (c) the expression of the degradative genes (d) the enzymatic breakdown of the PAHs (e) bioavailability and (f) the presence of other contaminants (Semple et al., 2007; Chauhan et al., 2008; Anyanwu and Semple, 2015b; Menga et al., 2017). Furthermore, interactions between low molecular weight (LMW) and high molecular weight (HMW) PAHs and their metabolites have been reported to play an important role in the induction of the catabolic enzymes, and such interactions can be either synergistic or antagonistic (Demanèche et al., 2004; Mohan et al., 2006; Couling et al., 2010). In the former case (synergistic), the metabolites produced in the degradation of aromatics in one strain may enhance the induction of catabolic enzymes of other aromatics in other strain(s) (cross-induction) (Whitman et al., 1998; Couling et al., 2010; Doley et al., 2017). In the latter case (antagonistic), aromatics or their metabolites may affect degradation due to substrate competition and/or microbial toxicity (Demanèche et al., 2004; Granato et al., 2017). Yet, the impact of more polar and soluble aromatics (such as 3-ring N-PAHs) on LMW and HMW PAHs mineralisation is not well known.

In a similar way, studies on biodegradation of nitrogen-containing

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http://dx.doi.org/10.1016/j.ecoenv.2017.09.019
Received 19 July 2017; Received in revised form 30 August 2017; Accepted 9 September 2017

polycyclic aromatics in soil have focussed mainly on indole, quinoline, isoquinoline, carbazole and acridine (Pereira et al., 1988; Fetzner, 1998; Millette et al., 1995; Lundstedt et al., 2003; Salam et al., 2017). However, biodegradation of the phenanthrolines (1,10-phenanthroline, 1,7-phenanthroline, 4,7-phenanthroline and benzo[*h*]quinoline) have not been recorded in literature.

Nitrogen-containing polycyclic aromatic hydrocarbons (N-PAHs) which are not as much reported can exist, often at high concentrations in soil (Webber, 1994; Švábenský et al., 2009). N-PAH contamination, emanating from petrogenic, pyrogenic and biogenic processes, constitutes major pollution and toxicological problems within the environment (National Toxicology Program, 2001; US Environmental Protection Agency, 2001; IPCS Environmental Health Criteria, 2003; Hazardous Substance Data Bank, 2010; Environment Canada, 2011; IARC, 2012; US Environmental Protection Agency, 2017). Despite the physico-chemical properties of N-PAHs, there is lack of information on the biodegradative impact of N-PAHs on LMW and HMW PAHs in the soil environment, over time.

Biodegradation is a major route of loss for organic contaminants in soil. It is widely known that polluted sites contain mixtures of compounds which vary in their impact to soil microbial community and as well, biodegradation of other aromatics. Irrespective of this, the impact of 3-ring N-heterocyclic aromatics (1,10-phenanthroline, 1,7-phenanthroline, 4,7-phenanthroline and benzo[*h*]quinoline) on phenanthrene and benzo[*a*]pyrene mineralisation has not been systematically studied in the soil environment. Conceptually, biodegradation of PAHs in soil by indigenous microbial communities may be enhanced or inhibited by the presence of other chemicals in contaminated sites. Thus, this study investigated if the presence, concentration and contact time of a more polar and soluble heterocyclic aromatics (3-ring N-PAHs) could enhance phenanthrene and B[*a*]P mineralisation in soil.

2. Materials and methods

2.1. Chemicals

Phenanthrene (Phen), 1,10-phenanthroline (1,10-Phen), 1,7-phenanthroline (1,7-Phen), 4,7-phenanthroline (4,7-Phen), benzo[*h*] quinoline (B[*h*]Q), benzo[*a*]pyrene (B[*a*]P) (Table 1) were obtained from Sigma-Aldrich, UK and ^{14}C-phenanthrene, ^{14}C-benzo[*a*]pyrene was obtained from ARC, UK. Goldstar liquid scintillation cocktail was supplied by Meridian Biotechnologies Ltd, UK.

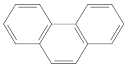
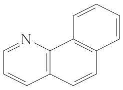
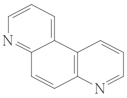
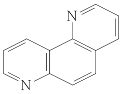
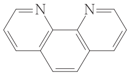
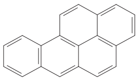
2.2. Soil preparation

Soil from Myerscough Agricultural College, UK was prepared for this study. The soil was collected from a depth of approximately 5–20 cm. The soil texture was sandy-loam (19.5% clay, 60.4% sand, 20.0% silt); organic matter content 2.7% and pH 6.5 (Doick et al., 2003). The soil was air dried at room temperature, sieved through a 2 mm mesh and rehydrated back to 45% of the water holding capacity. Soil was amended with PAHs and N-PAH compounds using the method reported in Doick et al. (2003). Soils were placed in bowls: $^{1}/_{3}$ of the soil (100 g, n = 3) was spiked with individual standards of phenanthrene, B[*a*]P, benzo[*h*]quinoline, 1,10-phenanthroline, 1,7-phenanthroline or 4,7-phenanthroline dissolved in toluene (2 ml) to give concentrations of 10 mg kg $^{-1}$ and 100 mg kg $^{-1}$ dry soil. The carrier solvent was allowed to volatilise from the soil for 3 – 4 h in a fume hood, after which soils were mixed with the remaining $^{2}/_{3}$ of the soil (200 g). Samples were prepared using soils amended with toluene (only) to serve as a control. The amended soils were then kept in amber glass jars (500 ml) and incubated in the dark at 21 ± 1 °C for 1, 30, 60 and 90 d. Soil moisture content was checked regularly and lost water was replenished with deionized water. Recoveries of phenanthrene and its nitrogen-containing analogues from soil over time has been reported (Anyanwu and Semple, 2016a, 2016a, 2015b).

2.3. Mineralisation of 14C-PAHs in soil

The ability of soil indigenous microflora to mineralise ^{14}C-PAHs to $^{14}CO_2$ was assessed at 1, 30, 60 and 90 d soil-contact time. Respirometric assays were carried out in modified 250 ml Schott bottles incorporating a Teflon-lined screw cap containing 1 M NaOH to trap any $^{14}CO_2$, 10 g (n = 3) of soil (wet wt) and 30 ml sterile minimal basal solution (MBS) (Doick and Semple, 2003). Standards prepared in toluene to give a ^{12}C-PAH concentration of 10 mg kg $^{-1}$ and 100 mg kg $^{-1}$

Table 1
Physical and chemical properties of phenanthrene, N-PAH isomers and benzo[*a*]pyrene. Source: www.chemspider.com/Chemical-Structure

Chemical	Chemical formula	Chemical structure	Molecular mass	Boiling point	Solubility 25 °C (mg/L)	Vapour pressure 25 °C (mm Hg)	Henry's law (atm-m^3/mole)	Log K_{ow}	Log K_{oc}	Log K_{oa} (m^3/µg)
Phen	$C_{14}H_{10}$		178.2	340.00	1.15	1.21E − 04	1.50E − 05[a]	4.46	4.32	9.12E − 06[b]
B[*h*]Q	$C_{12}H_9N$		179.2	339.00	78.70	2.08E − 05	1.11E − 06[a]	3.43	4.32	0.001[b]
4,7-Phen	$C_{12}H_8N_2$		180.21	361.20	38.04	2.39E − 05	1.85E − 07[a]	2.4	4.32	0.022[b]
1,7-Phen	$C_{12}H_8N_2$		180.21	365.10	30.64	2.39E − 05	1.85E − 07[a]	2.51	4.32	0.022[b]
1,10-Phen	$C_{12}H_8N_2$		180.21	365.10	30.64	2.39E − 05	1.85E − 07[a]	2.51	4.32	0.022[b]
B[*a*]P	$C_{20}H_{12}$		252.31	377.00	0.17	2.44E − 006	4.648E − 006	5.99	5.40	7.823

[a] Henrys Law Constant (VP/WSol estimate using EPI values).
[b] Kp Octanol/air model (particle/gas partition coefficient).