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Two-liquid-phase system: A promising technique for predicting bioavailability of polycyclic aromatic hydrocarbons in long-term contaminated soils

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HIGHLIGHTS

- Extraction by two-liquid-phase system is a promising method for PAHs bioavailability assessment in contaminated soil.
- Desorption kinetics of PAHs from four long-term contaminated soils are presented.
- Applying sufficient nutrients in bioremediation of field contaminated soil by indigenous microorganisms is crucial.

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ABSTRACT

A two-liquid-phase system (TLPS), which consisted of soil slurry and silicone oil, was employed to extract polycyclic aromatic hydrocarbons (PAHs) in four long-term contaminated soils in order to assess the bioavailability of PAHs. Extraction kinetics of six PAHs *viz.* phenanthrene, fluoranthene, pyrene, benzo(*a*)anthracene, benzo(*a*)pyrene, dibenzo(*a,h*)anthrancene were selected to investigate as they covered the susceptible and recalcitrant PAHs in soil. A parallel experiments were also carried out on the microbial degradation of these PAHs in soil with and without biostimulation (by adding $(NH_4)_2HPO_4$). The rapidly desorbed fraction of fluoranthene, as indicated by the two-fraction model, was found the highest, ranging from 21.4% to 37.4%, whereas dibenzo(*a,h*)anthrancene was the lowest, ranging from 8.9% to 20.5%. The rapid desorption of selected PAHs was found to be finished within 24 h. The rapidly desorbed fraction of PAHs investigated using TLPS, was significantly correlated ($R^2 = 0.95$) with that degraded by microorganisms in biostimulation treatment. This suggested that the TLPS-assisted extraction could be a promising technique in determining the bioavailability of aged PAHs in contaminated soils. It also suggested that applying sufficient nutrients in bioremediation of field contaminated soils is crucial. Further work is required to test its application to more hydrophobic organic pollutants in long-term contaminated soils.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants from natural or anthropogenic origin. They are of great environmental concern because of their toxic, mutagenic and carcinogenic properties (Cioroiu et al., 2013; Lau et al., 2014). Due to their low water solubility, PAHs tend to interact with non-aqueous phases and soil organic matter (SOM) and, as a consequence, become potentially unaccessible for biodegradation (Johnsen et al., 2005; Ouvrard et al., 2014). For risk assessment and remediation of contaminated soils, it is generally relied on the vigorous extraction by organic solvents which result in an overestimation of the bioavailability of the pollutants (Kelsey and Alexander, 1997; Kelsey et al., 1997; Hu et al., 2014). This demands determination of the bioavailable fraction of the soil contaminants rather than the total concentration (Liste and Alexander, 2002; Ortega-Calvo et al., 2015).

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On the other hand, bioassays are considered as the appropriate means for assessing the bioavailability of the organic contaminants (Liste and Alexander, 2002; Katayama et al., 2010). However, these techniques are expensive, have low precision and are time-consuming (Kelsey et al., 1997; Doick et al., 2003). Therefore attention has been focused on devising chemical or physical assays for assessing the bioavailability of organic pollutants (Kelsey et al., 1997; Liste and Alexander, 2002; Katayama et al., 2010). Until now, many methods have been suggested to estimate the bioavailability of organic pollutants in the contaminated soils (Liste and Alexander, 2002; van der Heijden and Jonker, 2009; Cui et al., 2013). These methods included pore-water analysis (Houx and Aben, 1993), solid-phase extraction such as Tenax beads and C_{18} membranes (Tang et al., 1999; Bernhardt et al., 2013) or extracting contaminants by using organic solvents such as tetrahydrofuran (Tang et al., 1999), alcohols (Kelsey et al., 1997) and *n*-butanol (Liste and Alexander, 2002), surfactants such as hydroxylpropyl-β-cyclodextrin (HPCD) (Hartnik et al., 2008; van der Heijden and Jonker, 2009), high temperature desorption kinetics (Cornelissen et al., 1997), supercritical-fluid extraction (Björklund et al., 1999), etc. However, the feasibility of a two-liquid-phase system (TLPS) in predicating the bioavailable of organic pollutants in aged soil has not been reported.

A TLPS which commonly consists of aqueous phase and a water-immiscible, non-volatile organic phase was used to degrade organic pollutants or to select xenobiotic-degrading microorganisms (Ascon-Cabrera and Lebeault, 1993; Villemur et al., 2000; Wang et al., 2013). The organic phase acts as a reservoir for hydrophobic compounds to diffuse from the organic phase to the aqueous phase, which assess the microbes to metabolize the compounds at the interface of two phases and/or in the aqueous phase (Ascon-Cabrera and Lebeault, 1993). More importantly, the organic phase can improve the mass transfer of hydrophobic organic contaminants (HOCs) and oxygen transfer rate to microbes and thus enhancing the biodegradation of HOCs (Villemur et al., 2000; Aldric et al., 2009; Muñoz et al., 2012). Silicone oil is often selected as organic delivery phase due to its advantages such as i) it is superior partitioning abilities for the high-molecular-weight PAHs than other organic solvents (Vandermeer and Daugulis, 2006). ii) it is highly stable, hydrophobic and chemically resistant to oxidative attack, and iii) it is biocompatible but not bioavailable to microbes (Muñoz et al., 2006; Mahanty et al., 2010). The TLPS has been effectively used to degrade organic pollutants such as PAHs, chlorobenzenes, styrene etc. (Ascon-Cabrera and Lebeault, 1993; El Aalam et al., 1993; Mahanty et al., 2010; Wang et al., 2010a). A TLPS consisting of soil slurry and silicone oil could also promote desorption of HOCs from soil and increase their bioavailability (Villemur et al., 2000; Wang et al., 2010a). However, only a little work has been reported on the relationship between bioavailability of aged PAHs in field soils and their extractability using TLPS.

In this paper, we study the desorption kinetics using TLPS and to assess the bioavailability of selected PAHs *viz.* phenanthrene (Phe), fluoranthene (Fln), pyrene (Pyr), benzo(*a*)anthracene (BaA), benzo(*a*)pyrene (BaP), dibenzo(a,h)anthrancene (DBahA) in aged soils. Main aim of the study was to investigate if the TLPS extraction could be used to predict the bioavailability of aged PAHs in the long-term contaminated soils.

2. Experimental procedure

2.1. Materials and methods

Four soils were used in this study. The soil samples were collected from two parks (soil S1 and S3) and a site near steal plant (soil S2) in Nanjing city, and a sample from a chemical plant (soil S4) in Wuxi city of China. These sites were selected because of different time periods of PAH-contamination in the soils (Table 1). Surface soils (0–15 cm) were collected, sieved (<2 mm) and stored at 4 °C. Prior to the biodegradation experiments, the samples were equilibrated for 2 weeks at room temperature (~22 °C) with 60% of the water holding capacity (WHC). Selected physicochemical properties of the soils are given in Table 1.

Silicone oil (polydimethylsiloxane, fluid type (molecular weight, 2000; viscosity, 50 centistokes; density, 0.96 g cm^{-3}) was purchased from Sinopharm, China. The PAHs namely phenanthrene (Phe), fluoranthene (Fln), pyrene (Pyr), benzo(*a*)anthracene (BaA), benzo(*a*)pyrene (BaP), dibenzo(a,h)anthrancene (DBahA) with purity of 99.9% were obtained from Supelco Corporation, USA. All the other chemicals were analytical grade and were purchased from Tianjin, China.

2.2. Bioavailable PAHs extracted by TLPS

Ten gram equilibrated soil (dry weight equivalent) was transferred to a 250-mL flask. Then 90 mL sterilized distilled water and 40 g silicone oil were added to the flask. Sodium azide (200 mg L^{-1}) was added as a biocide. The treatments were performed in triplicates and were magnetically stirred at 28 °C at 500 rpm under dark conditions for ~200 h (Wang et al., 2010a). The oil was sampled periodically and the concentrations of the above mentioned PAHs were determined following the method described by Villemur et al. (2000). Briefly, 0.5 mL silicone oil sample was vortexed with 1 mL of *N, N*-dimethyl formamide (DMF) for 2 min and centrifuged at 1150 *g* for 1 min to separate the two phases. A volume of 0.5 mL of the upper phase (DMF) was mixed with 0.5 mL of acetonitrile containing 0.1% acetic acid and analyzed using high performance liquid chromatograph (HPLC, LC-20A, Shimadzu, Japan) equipped with a fluorescence detector and a Supelco (USA) PAHs special chromatographic column according to the program as described by Yin et al. (2008). The extraction efficiency of selected PAHs from silicone oil was great than 96% (the method for determination of PAHs recovery was shown in the Supplementary Material).

2.3. Biodegradation experiments

In the first experiment, 500 g soil (oven dry weight basis) was used for the biodegradation studies. The soil was transferred to a 1000-mL bottle with a plug coated with aluminum foil and the moisture content was adjusted to 60% of the WHC. The bottles were incubated at 28 °C in the dark for 5 months when no biodegradation was observed. Four replicates for each treatment were run. The soil in the bottle was stirred weekly to increase oxygen exchange. At the end of the incubation, 2 g soil was sampled from the bottle for determination of the PAHs concentrations.

Another experiment was conducted to study the effect of biostimulation on biodegradation by adding $(NH_4)_2HPO_4$. In this experiment, S2 soil was selected because our preliminary studies showed that a mixture of PAHs was degraded well by the consortium from this soil (Wang et al., 2010a). Briefly, a fertilizer $(NH_4)_2HPO_4$ was amended to bring the soil to an approximate C/N ratio of 15/1, in order to facilitate optimal growth conditions (Wang et al., 2010b). Then the amended soil was adjusted to 60% of the WHC and incubated at the same conditions as described above for the first experiment.

2.4. Determination of total PAHs concentrations in soil

Two gram soil was homogenized with 5 g diatomaceous earth and the mixture was extracted by Accelerated Solvent Extraction (ASE 200, Dionex, USA). The extraction was done at 100 °C and 1500