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Variations in the bioavailability of polycyclic aromatic hydrocarbons in industrial and agricultural soils after bioremediation

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HIGHLIGHTS

- Variation of PAH bioavailability in original and post remediated soils was studied.
- Bioavailability of 3- and 4-ring PAHs were decreased in MPG and agricultural soils.
- Rapidly desorbing fractions of 5- and 6-ring PAHs changed little in MGP soil.
- Tenax was proved to be a more sensitive method than SPME in agricultural soil.
- Tenax and SPME have the potential to assess bioavailability of PAHs in MGP soil.

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ABSTRACT

The aim of this study was to demonstrate the variations in bioavailability remaining in industrial and agricultural soils contaminated by polycyclic aromatic hydrocarbons (PAHs) after bioremediation. After inoculation of *Mycobacterium* sp. and *Mucor* sp., PAH biodegradation was tested on a manufactured gas plant (MGP) soil and an agricultural soil. PAH bioavailability was assessed before and after biodegradation using solid-phase extraction (Tenax-TA extraction) and solid-phase micro-extraction (SPME) to represent bioaccessibility and chemical activity of PAHs, respectively. Only 3- and 4-ring PAHs were noticeably biodegradable in the MGP soil. PAH biodegradation in the agricultural soil was different from that in the MGP soil. The rapidly desorbing fractions (F_{rap}) extracted by Tenax-TA and the freely dissolved concentrations of 3- and 4-ring PAHs determined by SPME from the MGP soil decreased after 30 days biodegradation; those values of the 5- and 6-ring PAHs changed to a lesser degree. For the agricultural soil, the F_{rap} values of the 3- and 4-ring PAHs also decreased after the biodegradation experiment. The Tenax-TA extraction and the SPME have the potential to assess variations in the bioavailability of PAHs and the degree of biodegradation in contaminated MGP soils. In addition, Tenax-TA extraction is more sensitive than SPME when used in the agricultural soil.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a priority group of environmental contaminants that pose a threat to human health and ecological security owing to their toxic, carcinogenic, and mutagenic properties (Haritash and Kaushik, 2009). PAHs are found at high levels in the soils of industrial sites worldwide (Barnier et al., 2014), but some agricultural soils are also contaminated with PAHs, resulting in threats to food safety and human health in the contaminated areas (Li et al., 2008; Duan et al., 2015). In 2006, Xu et al. estimated that approximately 25,000 tons of total PAHs (ΣPAH, in this case the sum of the 16 PAHs listed by the U.S. EPA as priority pollutants) were generated annually in China, with the burning of biomass and coal, and the coking industry contributing more than 90% of the total emissions. Both Wang et al. (2013) and Xu et al. (2006) reported ΣPAH concentrations in soils were higher in the industrialized east of China, than in the un-

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derdeveloped west of the country, and higher in urban and roadside soil than in agricultural soil. The source of PAH contamination in agricultural soils (organic wastes or wastewater used as compost and fertilizers) are different to those of industrial sites (coal-related energy production, and oil used for transport fuel), and there is therefore an urgent need to implement remediation processes for both agricultural and industrial contaminated sites (Larsson et al., 2013).

A number of bioremediation methods to degrade PAHs in industrial and agricultural polluted soils have been developed and applied (Llado et al., 2013). Typically total extractable (bulk) soil PAH concentrations are used to measure and describe the amount of soil contamination and bioremediation efficiencies. However, it is not bulk PAH concentration in a soil that is important from an ecological risk assessment and bioremediation perspective, but rather the fraction of the total amount of PAHs and specific PAHs that are available to soil biota (Gomez-Eyles et al., 2011; Cebron et al., 2013). In the context of bioremediation of PAH-contaminated soils, bioavailability may be defined as the extent to which a contaminant is available for biological conversion (Juhasz and Naidu, 2000). PAH sequestration and PAH bioavailability can have a significant influence on the efficiency of bioremediation processes. In short, ecological risks may often be overestimated if assessments are based on the bulk PAH concentration in the soil (Alexander, 2000; Semple et al., 2003; Gomez-Eyles et al., 2010). For the purposes of bioremediation studies, it is important to investigate the bioavailability of PAHs in soils (Juhasz et al., 2014a, 2014b).

Biological methods are traditionally regarded as the most appropriate methods for quantifying bioavailability, but their application may be time consuming and laborious, and the results are species dependent (Cui et al., 2013). With this in mind, a large number of efficient and cost-effective chemical methods have been developed to estimate the bioavailability of PAHs (Bergknut et al., 2007; Gomez-Eyles et al., 2011; Spasojevic et al., 2015). Two major processes, bioaccessibility and chemical activity, control the physicochemical component of bioavailability (Reichenberg and Mayer, 2006). Bioaccessibility refers to the amount or portion of contaminant that is or can become available within a given time span, and can be measured by depletive non-exhaustive extractions (such as solid-phase Tenax extraction). Whereas chemical activity depicts the energetic state of a chemical and quantifies the potential for spontaneous physicochemical processes such as diffusion and partitioning, and it uses non-depletive biomimetic methods (such as solid-phase micro-extraction (SPME)) to measure freely dissolved concentrations (Cachada et al., 2014). Tenax-TA extraction is based on the principle that water is the exchange medium in bioaccumulation and that organisms use compounds from the aqueous phase (Cui et al., 2013). SPME is a partitioning-based method that depends on the hydrophobicity of PAHs and their affinity for the fiber coating relative to soil organic matter (Mayer et al., 2014). Both methods are inexpensive, efficient, and solvent free for the extraction stage from the soils, they have been used to estimate the bioavailability of PAHs (Ter Laak et al., 2006; Bergknut et al., 2007), and to predict the accumulation of contaminants in organisms under laboratory conditions (Liu et al., 2011; Yang et al., 2013; Bielska et al., 2014).

There is little information about changes in PAH bioavailability after bioaugmentation treatments on industrial and agricultural soils. As such, to fill this gap in knowledge, we carried out a bioaccessibility assessment using Tenax-TA extraction and a chemical availability assessment using SPME on the original and post remediated soils. A bacterial strain and a fungal strain were chosen as the bioremediation agents in this study, since they are both able to degrade PAHs in soils under different conditions (Su et al., 2006, 2008; Wang et al., 2012). *Mycobacterium* species are highly adapted to using a variety of PAHs, and several studies have reported their role in PAH degradation, relative gene expression, and *Mycobacterium* community profiles in soil (Karabika et al., 2009; Badejo et al., 2013; Li et al., 2013; Mueller-Spitz and Crawford, 2014). However, to date, few studies have reported PAH degradation by *Mucor* strains (Grimmer et al., 1996; Ravelet et al., 2000), and very few studies have used *Mycobacterium* strains to remediate field MGP soils contaminated with high levels of PAHs. SPME and Tenax extraction have been little used to demonstrate variations in the bioavailability of PAHs in post-remediated soils in which the PAHs are in the process of biodegradation. Sun et al. (2013) studied the variations in methyl-β-cyclodextrin-enhanced bioremediation of PAH-contaminated soil using Tenax extraction. Hu et al. (2014) recently investigated the bioavailability of toxic contaminants in PAH-contaminated MGP soil before and after biological treatment, but the variations in the freely dissolved concentrations were not studied by them. In our knowledge, to date, there have been none comparisons of the bioremediation performance of the two methods for PAH-contaminated industrial and agricultural soils. The main aim of this study was, therefore, to demonstrate and compare the variations in the bioavailability of PAHs in industrial and agricultural soils following biodegradation using two strains of microorganisms and using both SPME and Tenax-TA extractions. The Tenax desorption data were fitted to a two-compartment model to calculate the rapidly and slowly desorbing fractions. A further aim of the study was to investigate whether SPME and Tenax-TA extraction methods and data could be used to estimate the quantity of PAHs that was lost from the industrial and agricultural soils.

2. Materials and methods

2.1. Soils, microorganisms, and degradation experiments

Two PAH-contaminated soils were used in this study. An industrial soil was sampled from a manufactured gas plant (MGP) site in Beijing, China at depths of 0–20 cm. This soil had an organic carbon content of 1.3%, and the proportions of clay, silt, and sand were 65.8%, 14.9%, and 19.3%, respectively. An agricultural soil was collected from the top 20 cm of a wastewater-irrigated site in the suburban area of Shenyang, China. The organic matter content of this soil was 2.4%, and the proportions of clay, silt, and sand were 61.4%, 28.5%, and 10.1%, respectively. The soil samples were sieved through a 2-mm mesh to remove small rocks and plant material, homogenized, and kept in the dark at 4 °C until analysis. For a summary of the methods used to quantify the concentrations of individual PAHs and total PAHs (sum of 12 PAHs) in the soil, see Sections 2.2 and 2.5; for pre-experimentation concentrations of individual PAHs and total PAHs in the soil see Table S1.

The microbial strains used in this study were *Mycobacterium gilvum* (genbank accession number DQ512892) and *Mucor mucedo* (China Centre for Type Culture Collection: CCTCC M 2011092), and both strains were isolated from contaminated soil. The strains were maintained at 4 °C and were periodically sub-cultured on agar plates. Pre-inocula were grown under liquid conditions for 7 days at 28 °C in 250 mL Erlenmeyer flasks. Cultures were then homogenized, and 20-mL aliquots of the strain suspension were added to sterilized solid media in glass bottles. The media contained 4 g sucrose, 30 g bran, 4 g pulse flour, 120 mg KH_2PO_4, 300 mg $MgSO_47H_2O$, 1 g $(NH_4)_2HPO_4$, 1 g quicklime, and 160 g corncob. The media were moisturized to 70% (w/w) water content. After inoculation, the cultures were covered and grown for 2 weeks at 28 °C under constant dark conditions.