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

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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.

Keywords: bioavailability; biodegradation; Tenax-TA extraction; solid-phase micro-extraction (SPME); manufactured gas plant 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 25000 tons of total PAHs (Σ PAH, 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 Σ PAH concentrations in soils were higher in the industrialized east of China, than in the underdeveloped 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; Cebren 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 over-estimated 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; Juhasz et al., 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 and a fungal strains 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; Su et al., 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 in *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 on 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 section 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 KH₂PO₄, 300 mg MgSO₄·7H₂O, 1 g (NH₄)₂HPO₄, 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.

The industrial and agricultural soils were incubated in glass jars with 100 g of either the immobilized inoculum of *Mycobacterium* sp. or *Mucor* sp. per kg of dry soil. Throughout the incubation, sterile deionized water was periodically added to maintain the moisture content of each microcosm at a constant level (about 55% of maximum water-holding capacity (WHC)), and the glass jars were covered to avoid excessive water loss. Each microcosm, namely the

combination of the inoculum carrier, strain, and soil, was prepared in triplicate, and incubated in the dark at 28 °C for 30 days. All the treatments were mixed manually every three days to enhance oxygenation, and kept moisture homogenization. Samples were taken at the start time and after the degradation experiment, analyzed for their bulk and bioavailable PAH concentrations.

2.2 Soil PAH extraction

PAHs in the contaminated soil were extracted using a mechanical chemical extraction procedure (Gong et al., 2010; Wu et al., 2010). Contaminated soil (1 g) was added to a glass bottle, and then 1.5 g of sodium chloride, 10 mL of deionized water, 20 mL of acetone, and 15 mL of dichloromethane were added. The bottle was capped and shaken on an orbital shaker at 160 rpm for 16 h. The organic phase was concentrated and cleaned up by passing it through on a chromatography column filled with 1 g of activated silica gel (heated at 130 °C for 16 h) and 0.5 g of deactivated aluminum oxide (deactivated by addition of 15% water). The collected sample was then dried under nitrogen gas, before being re-dissolved in acetonitrile for analysis by high-performance liquid chromatography (HPLC).

2.3 PAH extraction via Tenax-TA

Tenax-TA (60–80 mesh, 177–250 µm; Alltech Associates, Inc., Chicago, Illinois, USA.) is a porous polymer based on 2,6-diphenyl-p-phenylene oxide. Before use, the Tenax-TA was ultrasonically cleaned with water, then hexane, and then acetone (three times each; 10 mL solvent g⁻¹ Tenax-TA) and dried at 110 °C overnight. The Tenax-TA extraction experiment was carried out using the method described by Liu et al. (2011). A soil sample (1 g dry weight) was added to a 50 mL glass screw-capped centrifuge tube. A 0.2 g sample of Tenax-TA, 38 mL deionized water, and 1 mL of 1 mg mL⁻¹ NaN₃ were added to the tube. The tubes were then capped and shaken in a mechanical shaker at 120 rpm and 25 °C. At periodic intervals (i.e., 2 h, 4 h, 7 h, 12 h, 24 h, 2 days, 4 days, 7 days, 12 days, and 30 days), the tubes were centrifuged at 1609×g for 20 min until the Tenax-TA beads floated to the surface. The Tenax-TA was then collected using a stainless steel scoop and ultrasonically extracted three times for 30 min each time with 20 mL of a hexane-acetone mixture (3:1 v/v). The tubes were replenished with 0.2 g of new Tenax-TA beads and the shaking of the sample continued. The three extracts were combined for each sample and

concentrated via rotary evaporation, dried under a nitrogen gas stream, dissolved in 2 mL acetonitrile, and measured via HPLC.

2.4 PAH extraction by PDMS fibers

Before use, a disposable SPME fiber, coated with polydimethylsiloxane (PDMS 28.5 μm thick, glass-core diameter of 110 μm ; Poly Micro Technologies, Phoenix, AZ, USA) was cut into 4-cm pieces and cleaned by heating at 275 $^{\circ}\text{C}$ for 16 h under a constant helium flow. The SPME with PDMS fibers were examined using the method described by Ter Laak et al. (2006). Four thermally cleaned fibers (4 cm long) were exposed to 4 g (dry weight) of soil in 14.2 mL amber vials with 4 mL of a 10 mM NaN_3 solution. Fibers were exposed to the original soils for 5, 8, 12, and 15 d to obtain uptake curves and equilibration times for all compounds, and a 10-d exposure time was then used in the tests for all soil samples. Four fibers were exposed to soil samples and shaken on a mechanical shaker (120 rpm) at 25 $^{\circ}\text{C}$ for 10 d. The fibers were then wiped with wet tissue and were subjected to Soxhlet extraction for 10 h with 100 mL dichloromethane at 55 $^{\circ}\text{C}$. The extracts were collected and concentrated in a rotary evaporator, dried under a stream of nitrogen gas, dissolved in 2 mL acetonitrile, passed through a 0.22 μm membrane filter, and PAHs concentrations measured via HPLC.

2.5 HPLC analysis

Samples were analyzed with an HPLC (Agilent Technologies 1200 series) equipped with an ultraviolet detector, a fluorescent detector, and a C-18 column (Agilent Technologies, HPLC-Cartridge 250-3). The separation was performed with the following gradient: acetonitrile and water were used as the mobile phase at a flow rate of 0.5 mL min^{-1} ; for the first 35 min, acetonitrile in the mixture was linearly increased from 60% to 100% and maintained at that composition for 10 min; for the following 10 min, acetonitrile was linearly decreased to 60%, and this composition was held for 5 min. Detection was conducted at selected excitation/emission wavelengths. In all cases, 10 μL of sample was withdrawn and injected into the HPLC by the autosampler. An external standard mixture (M-8310, AccuStandard, New Haven, Connecticut, USA) was used for PAH calibration and quantification. Reproducibility of all measurements was tested by comparison of independent replicates. Samples were not analyzed for four low molecular

weight PAHs, naphthalene, acenaphthylene, acenaphthene, and fluorene, because of their volatile characteristics and poor reproducibility during HPLC analysis.

2.6 Data analysis

The Tenax desorption data were fitted to an empirical, two-compartment first-order kinetic model, used by Cornelissen et al. (1997), as follows:

$$S_t / S_0 = F_{rap} e^{-k_{rap}t} + F_{slow} e^{-k_{slow}t} \dots\dots\dots (1),$$

in which S_t and S_0 (mg kg^{-1}) are the soil-sorbed PAH concentrations at time t (d) and at the start of the experiment; F_{rap} and F_{slow} are the rapidly and slowly desorbing fractions; and k_{rap} and k_{slow} (d^{-1}) are the rapid and slow rate constants for the two desorption compartments. It was generally assumed that the two defined fractions covered the entire amount of PAHs, therefore F_{rap} plus F_{slow} were equal to one.

PAH concentrations in soil pore water, namely PAH freely dissolved concentrations, can be calculated using equation (2):

$$C_w = C_f / K_f \dots\dots\dots (2),$$

where C_w is the PAH concentration in soil pore water ($\mu\text{g L}^{-1}$), C_f is the PAH concentration in the fiber coating ($\mu\text{g L}^{-1}$), and K_f is the fiber-water partition coefficient.

The experimental results were subjected to a one-way analysis of variance and least significance difference using SPSS 16.0 statistical software at 95% confidence. SigmaPlot 12.5 was used to generate the graphs. Besides, all the parameters in equation (1) were determined by direct curve fitting in SigmaPlot 12.5.

3. Results and discussion

3.1 PAH concentrations in industrial MGP and agricultural soils

Total PAH (the sum of 12 compounds) concentrations in the MGP and agricultural soils were 178.2 mg kg⁻¹ and 7.8 mg kg⁻¹, respectively (Table S1). Three-ring PAHs dominated (62.2%) in the MGP soil, and phenanthrene accounted for 59.2% of the total PAH concentration. In the agricultural soil, 4-ring PAHs (62.5%) dominated, and fluoranthene represented 20.4% of the total PAH concentration. The proportion of low-molecular-weight (LMW) PAHs in the MGP soil in this study were similar to those reported by Hu and Aitken (2012) (>50% for 3-ring PAHs and 30% for 4-ring compounds) and Sánchez-Trujillo et al. (2013) (62% and 24% for the 3- and 4-ring compounds, respectively). Whereas natural attenuation processes resulting from farming activities might be preferentially removing LMW PAHs from the agricultural soil (Barnier et al., 2014). PAHs environmental behaviors in industrial and agricultural soils are rather different due to the different physicochemical properties, concentrations, and aging.

Table S1 PAH concentrations in the experimental soils (C_s).

Compound	Rings	MGP soil (mg kg ⁻¹)	agricultural soil (mg kg ⁻¹)
phen	3	105.50±3.55	0.66±0.04
ant	3	5.38±0.21	0.30±0.05
flt	4	27.58±0.88	1.59±0.13
pyr	4	13.86±0.49	1.19±0.03
baa	4	6.38±1.36	1.21±0.04
chry	4	4.57±0.69	0.89±0.09
bbf	5	4.08±0.09	0.66±0.03
bkf	5	1.91±0.03	0.23±0.01
bap	5	1.77±0.05	0.29±0.02
daha	5	0.41±0.01	0.06±0
bghip	6	3.58±0.09	0.38±0.05
icdp	6	3.18±0.06	0.30±0.03
Total PAH		178.21±6.76	7.81±0.16

3.2 Microbial degradation of the PAHs in industrial MGP and agricultural soils

Overall, the amount of PAH biodegradation was similar for the bacterial and fungal strains (Fig. 1). For instance, there was up to 71% and 72% removal of total PAHs from the *Mycobacterium* and *Mucor* treated industrial MGP soils, respectively, whereas removal of total PAHs from the

agricultural soils in the *Mycobacterium* and *Mucor* treatments was similar at 56 % and 62%, respectively.

Fig. 1 Degradation of the PAHs by the *Mycobacterium* sp. and *Mucor* sp. in (a) the MGP soil and (b) the agricultural soil. The same letter indicates no significant difference between treatments ($P > 0.05$), and different letters indicate a significant difference ($P < 0.05$).

The total PAH degradation in the MGP soil was higher than that in the agricultural soil. This is because 95.8% and 93.2% of phenanthrene, and 77.2% and 83.7% of anthracene were biodegraded by the *Mycobacterium* and *Mucor* treatments, respectively, in the MPG soil, and these two 3-ring PAHs accounted for more than 90% of the total PAH concentrations (Fig. 1a). The other PAHs in the MGP soil were not degraded as significantly as phenanthrene and anthracene. However, there are greater adverse effect on soil organisms associated with the high-molecular-weight (HMW) PAHs, implying that higher total PAH removal by the microorganisms does not necessarily mean that the adverse effect was reduced by a greater extent.

The degradation pattern for individual PAHs in the agricultural soil was very different to that for the MGP soil (Fig. 1). The higher 4- to 6-ring PAHs were degraded in the agricultural soil by the *Mycobacterium* and *Mucor* treatments, perhaps because the comparatively lower concentrations of these HMW PAHs or adaptation of the microorganisms in the agricultural soil might facilitate their biodegradation. However, the differences in the degradation of individual PAHs in the agricultural soil were not as pronounced as those in the MGP soil (Fig. 1b), which is perhaps a reflection on the different PAH profiles and concentrations in the MPG and agricultural soils.

Overall, the results demonstrate that the *Mycobacterium* sp. and *Mucor* sp. inocula used in this study were capable of PAH degradation, albeit that this ability was limited to HMW PAHs in the industrial MGP soil. Sterilization was not undertaken because destroying the natural microbial ecosystem and then using a pure strain of bacterium or fungus for PAH biodegradation does not represent the actual behavior of the environmental microorganisms during bioremediation of natural PAH-contaminated soils (Janbandhu and Fulekar, 2011). Therefore, biodegradation of the PAHs in the two soils in this study was dependent on the co-operative metabolic activities of both the inoculated and indigenous microbial populations. The *Mycobacterium* and *Mucor* strains were immobilized in carriers, and because of their good growth, they overcame the adverse environmental conditions that normally threaten microbial survival (Su et al., 2006).

3.3 PAH bioaccessibility assessment using Tenax-TA extraction

Kinetic curves for 3-, 4-, 5-, and 6-ring PAHs of the industrial MGP soil, and 3- and 4-ring PAHs of the agricultural soil, before and after 30 days of the *Mycobacterium* and *Mucor* treatments were modeled using the two-site distribution model (Figs. S1 and S2). The kinetic curves for 5- and 6-ring PAHs of the agricultural soil are not presented, because the comparatively lower concentrations and highly hydrophobic characteristics of HMW PAHs resulted in very small desorbed amounts of PAH that were not detectable, and/or the variation in the S_t/S_0 ratio with time was too small. The two-compartment model gave a direct value of the rapidly desorbing fraction. The shapes of the desorption kinetics obtained with the industrial MGP soil and the agricultural soil (Figs. S1 and S2) were similar to those observed by Barnier et al. (2014). However, the rates and extents of desorption differed among the individual 3- to 6-ring PAHs, and there were also differences between the industrial MGP soil and agricultural soil for 3- and 4-ring PAHs. The kinetic curves of the PAHs highlighted the differences between the original and post-remediated soils, indicating the change in the rapidly desorbing fraction and bioavailability after the biodegradation experiment.

Fig.S1 Desorption kinetics of 3-, 4-, 5-, and 6-ring PAHs in the MGP soil before and after biodegradation with consecutive Tenax extractions. S_t/S_0 was the PAH residual fraction in the soil at desorption time (d).

Fig.S2 Desorption kinetics of 3- and 4-ring PAHs in the agricultural soil before and after biodegradation with consecutive Tenax extractions.

The fitted values of the rapidly desorbing fractions of the two-site model for individual ring PAHs

(F_{rap}) for the industrial MGP and agricultural soils before the biodegradation experiments ranged from 0.16 to 0.70, and from 0.29 to 0.54, respectively (Tables 1 and 2). These values were higher than those reported by Barnier et al (2014) in their assessment of the desorption kinetics of PAHs from aged industrial soils using the Tenax extraction method, but even higher values for the rapidly desorbing fractions have been reported for the desorption kinetics of spiked phenanthrene and benzo(a)pyrene from soils (Rhodes et al., 2010; Spasojevic et al., 2015). Values of F_{rap} for 5- and 6-ring PAHs were significantly lower than those for the 3- and 4-ring PAHs. Furthermore, most F_{rap} values of 3- and 4-ring PAHs decreased after *Mycobacterium* and *Mucor* treatments in the two soils. F_{rap} values are not available for the 5- and 6-ring PAHs in the agricultural soil but the data indicates that there was very little change in the F_{rap} fractions of the 5- and 6-ring PAHs in the MPG soil after biodegradation treatments.

Table 1 The rapidly desorbing fractions (F_{rap}) of PAHs in the MGP soil before and after microbial degradation.

PAHs	before degradation	after degradation	
		<i>Mycobacterium</i> sp.	<i>Mucor</i> sp.
phen	0.46±0.03	0.34±0.03	0.27±0.04
ant	0.47±0.03	0.40±0.02	0.23±0.03
flt	0.49±0.02	0.47±0.02	0.34±0.05
pyr	0.70±0.03	0.52±0.03	0.44±0.06
baa	0.53±0.01	0.51±0.01	0.39±0.04
chry	0.46±0.02	0.44±0.03	0.34±0.03
bbf	0.40±0.03	0.35±0.02	0.37±0.02
bkf	0.37±0.03	0.35±0.02	0.37±0.02
bap	0.33±0.03	0.31±0.01	0.32±0.01
daha	0.20±0.03	0.11±0.01	0.18±0.01
bghip	0.16±0.01	0.16±0.01	0.16±0.01
icdp	0.19±0.02	0.18±0.01	0.21±0.01

phen: phenanthrene, ant: anthracene, flt: fluoranthene, pyr: pyrene, baa: benzo(a)anthracene, chry: chrysene, bbf: benzo(b)fluoranthene, bkf: benzo(k)fluoranthene, bap: benzo(a)pyrene, daha: dibenz(ah)anthracene, and bghip: benzo(ghi)perylene, icdp: indeno(cd)pyrene, the same below

Table 2 The F_{rap} of PAHs in the agricultural soil before and after microbial degradation.

PAHs	before degradation	after degradation	
		<i>Mycobacterium</i> sp.	<i>Mucor</i> sp.
phen	0.54±0.04	0.46±0.04	0.44±0.03
ant	0.50±0.01	0.21±0.03	0.19±0.01
flt	0.42±0.03	0.38±0.03	0.36±0.02
pyr	0.44±0.04	0.21±0.02	0.24±0.03
baa	0.49±0.05	0.23±0.02	0.20±0.01
chry	0.29±0.03	0.18±0.01	0.11±0.01

The F_{rap} values of 3- and 4-ring PAHs in the MGP soil were lower for the *Mucor* treatment than for the *Mycobacterium* treatment (Table 1). Moreover, the rate constants of the rapid desorption (k_{rap}) of 3- and 4-ring PAHs of the MPG soil after the *Mucor* treatment were much higher than those after the *Mycobacterium* treatment (Table S2). The former values increased after the biodegradation experiment, whereas there were no notable increases in the latter. There were only very slight changes in k_{rap} for 5- and 6-ring PAHs of the MGP soil after the biodegradation experiment. In contrast to the MGP soil, there were no notable differences in F_{rap} or k_{rap} of 3- and 4-ring PAHs for the agricultural soil between the *Mycobacterium* and *Mucor* treatments (Table 2), even though the k_{rap} of the 3- and 4-ring PAHs in the agricultural soil increased after the biodegradation experiments (Table S3).

Table S2 The rate constants of the rapid desorption (k_{rap}) of PAHs in the MGP soil before and after microbial degradation.

PAHs	before degradation	after degradation	
		<i>Mycobacterium</i> sp.	<i>Mucor</i> sp.
phen	1.706	1.612	5.892
ant	1.655	0.936	8.204
flt	1.019	1.274	5.672
pyr	0.987	1.374	4.996
baa	0.743	1.025	2.028
chry	0.942	0.812	2.627
bbf	0.537	0.574	0.924
bkf	0.613	0.654	0.780
bap	0.626	0.717	0.804
daha	0.543	0.988	0.726
bghip	0.534	0.852	0.704
icdp	0.520	0.649	0.648

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Table S3 The rate constants of rapid desorption (k_{rap}) of PAHs in the agricultural soil before and after microbial degradation.

PAHs	before degradation	after degradation	
		<i>Mycobacterium</i> sp.	<i>Mucor</i> sp.
phen	0.528	0.680	0.842
ant	0.468	3.865	3.666
flt	0.078	0.128	0.126
pyr	0.419	1.936	2.414
baa	0.099	0.285	1.252
chry	0.083	0.215	1.590

341

342 Overall, the degradation percentages were slightly higher than the F_{rap} of most PAHs extracted by
 343 Tenax-TA from the contaminated industrial MGP and agricultural soils (Fig.1, Tables 1 and 2).
 344 This is similar to the findings of Cornelissen et al., (1998) and Sun et al., (2013), and may reflect
 345 the fact that a small portion of the slowly desorbing fraction has been degraded during the
 346 biodegradation process.

347

348 **3.4 Assessment of PAH chemical activity using SPME**

349 The chemical activity quantifies the potential for spontaneous physicochemical processes. It is
 350 linearly related to the freely dissolved concentrations (C_w) and can be measured by SPME fibers
 351 (Cachada et al., 2014). The individual and total concentrations of freely dissolved PAHs (C_w) in
 352 the original MGP soil were calculated using the $\log K_f$ values reported by Ter Laak et al. (2006)
 353 and equation (2). The C_w values of the individual PAHs ranged from 0.06 to 195.28 $\mu\text{g L}^{-1}$ (Table
 354 3), and the total concentrations in pore water were dominated by 3- and 4-ring PAHs (99.5%).
 355 Phenanthrene accounted for 78.1 % of the total concentration. Consequently, the concentrations of
 356 the HMW PAHs were low, and only approximately 0.06 $\mu\text{g L}^{-1}$ of freely dissolved
 357 indeno(cd)pyrene was detected. Freely dissolved PAH concentrations of most of PAHs in the
 358 agricultural soil were not detectable. This is consistent with Barthe et al. (2008) who carried out an
 359 evaluation of PAH availability in sediments with various levels of contamination, and
 360 demonstrated that the SPME method was unable to predict PAH availability in minimally
 361 contaminated sediments in which biological factors like digestible organic matter were important.

Table 3 The freely dissolved concentrations (C_w) of individual and total PAHs in the MGP soil.

Compound	Rings	C_w ($\mu\text{g}\cdot\text{L}^{-1}$)
phen	3	195.28 $\pm$ 2.63
ant	3	13.66 $\pm$ 0.46
flt	4	20.64 $\pm$ 0.95
pyr	4	13.75 $\pm$ 0.50
baa	4	2.79 $\pm$ 0.16
chry	4	2.64 $\pm$ 0.06
bbf	5	0.38 $\pm$ 0.01
bkf	5	0.18 $\pm$ 0.01
bap	5	0.40 $\pm$ 0.02
daha	5	0.08 $\pm$ 0.01
bghip	6	0.14 $\pm$ 0.01
icdp	6	0.06 $\pm$ 0.01
Total PAH		250 $\pm$ 4.80

363

364 The SPME technique for assessing chemical activity implies the potential of hydrophobic organic
 365 contaminants (HOCs) to partition into organisms at equilibrium according to the equilibrium
 366 partitioning theory. It has been suggested that this can be used to predict PAH accumulation in
 367 earthworms and plant roots (Jonker et al., 2007; Gomez-Eyles et al., 2012), and so bioremediation
 368 endpoints may be evaluated on the basis of freely dissolved concentrations. The assumption is that
 369 changes in the bioaccumulation potential of PAHs in soil biota after soil remediation processes can
 370 be described using SPME analysis. Consequently, we hypothesized that the freely dissolved
 371 concentrations of all the PAHs would decrease in the pore water of the MPG soil after the
 372 biodegradation experiment. For the most part this is what was observed, and our data is consistent
 373 with that of Thomas et al. (2014), who reported reductions in pore water concentrations in
 374 remediated sediments. However, the data shows that the C_w of some HMW PAHs increased.
 375 Therefore, variations between the freely dissolved concentrations of individual and total PAHs
 376 were calculated based on the original and post-remediation concentrations, with positive and
 377 negative values indicating reductions and increases in C_w after the biodegradation experiment,
 378 respectively. The *Mycobacterium* and *Mucor* treatments achieved reductions of 65% or 70% in C_w
 379 of total PAHs (Fig. 2), mainly because of reductions in phenanthrene, anthracene, and
 380 fluoranthene. However, concentrations of several 5- and 6-ring PAHs increased, especially those

of benzo[a]pyrene, dibenz[a,h]anthracene, and benzo[ghi]perylene, perhaps due to the low initial C_w of these HMW PAHs or the metabolism of microorganisms. The microorganisms may produce biosurfactants that enhanced the bioavailability of HMW PAHs (Haritash and Kaushik, 2009), or they may have active efflux mechanisms that transported the PAHs out of the cell (Bugg et al., 2000), which had an important role in releasing these highly recalcitrant PAHs into the aqueous phase.

Fig. 2 Variations of freely dissolved concentrations of individual and total PAHs after microbial degradation in MGP soil. The same letter above the columns indicates no significant difference between treatments ($P > 0.05$), and different letters indicate a significant difference ($P < 0.05$).

3.5 Correlations between PAH losses and bioavailable PAHs

Linear correlations were calculated between the losses of individual PAHs (mg kg^{-1}) in soils and the bioavailable PAH contents determined by SPME ($\mu\text{g L}^{-1}$) and Tenax-TA (mg kg^{-1}) before biodegradation. The Tenax-TA regression equations for the industrial MGP and agricultural soils were $y=0.87x$ ($R^2=0.73$) and $y=1.47x$ ($R^2=0.79$) (Fig. 3), respectively. The fitted rapidly desorbing fractions in this study correspond well to the biodegradation of PAHs. Previous research has shown that a best-fit line with a slope of 1 is ideal for predicting soil-associated organic contaminant bioavailability (Reid et al., 2000). As such, the F_{rap} values for PAHs in the industrial MGP soil were much closer to the expected values, whereas those in the agricultural soil underestimated the biodegradation. Cornelissen et al. (1998) noted that the Tenax extraction method was a good indicator of the extent of degradation when the ratio of PAH degradation and

the rapidly desorbing fraction was 1.4 ± 0.5 . This is consistent with the results for this study (0.87 and 1.47). The regression equation of the SPME method for industrial MGP soil was expressed as $y = 0.54x$ ($R^2 = 0.73$) (Fig. 4). Yang et al. (2009) also found a good correlation (0.48–0.91) between the freely dissolved concentration of phenanthrene measured by SPME and its degradation by a PAH degrading bacterium PYR-1 in spiked soils. There was little research focusing on the relationship between biodegradation and C_w in original PAH-contaminated soils. Our data suggested that the SPME and Tenax-TA extraction methods can be used to predict the extent of biodegradation in MGP soils in this study.

Fig. 3 Correlations between the rapidly desorbing fractions of PAHs extracted by Tenax-TA before bioremediation and the amounts of PAHs degraded by the *Mycobacterium* sp. and the *Mucor* sp. in (a) the MGP soil and (b) the agricultural soil.

Fig.4 Correlations between the PAH concentrations in soil pore water and the amount of PAH degradation by *Mycobacterium* sp. and *Mucor* sp. in the MGP soil.

Comparison of the PAH degradation by the Tenax-TA extraction and SPME shows that Tenax-TA

extraction and SPME provide useful tools for assessment of PAH degradation in the MGP soil. Besides, the PAH degradation/rapidly desorbing fraction ratio value close to 1. The Tenax-TA extraction also provided some bioavailable data for the 3- and 4-ring PAHs in the agricultural soil, whereas the SPME data were not applicable for some PAHs in the agricultural soil. Correlations between the PAH biodegradation and the bioavailable concentrations of PAHs determined using SPME and Tenax-TA extraction for the agricultural soil have not previously been reported. The results from this study demonstrate that Tenax-TA was a more sensitive method than SPME for predicting PAH biodegradation in contaminated industrial and agricultural soils. The success of methods used to reflect PAH bioavailability may depend on the test conditions, the species, and the type of soils. Several studies have investigated the ability of SPME and/or Tenax-TA extraction to predict the actual bioaccumulation in field-contaminated soils (Trimble et al., 2008; Gomez-Eyles et al., 2010; Ruus et al., 2010; Yang et al., 2013). However, bioaccumulation and biodegradation are different processes, and similar methods used in different conditions with different target species can produce different results.

4. Conclusion

There were differences between the biodegradation of PAHs in the MGP and agricultural soils. High biodegradation was obtained only for the 3-ring PAHs, whereas there was no significant degradation of the HMW PAHs in the MGP soil. The kinetic curves, rapidly desorbing fractions, and the rate constants of the two-site model determined by the Tenax-TA extraction method for PAHs changed after the biodegradation experiments, with decreases in most of the F_{rap} values of the 3- and 4-ring PAHs in the two soils. There were minimal changes in the F_{rap} values for the 5- and 6-ring PAHs in the MGP soil after the biodegradation treatments, and these values were not available for the agricultural soil. The freely dissolved concentrations of total PAHs in the MGP soil determined by the SPME method decreased by 65% and 70% after the *Mycobacterium* and *Mucor* treatments, respectively. The SPME method was not able to measure bioavailable concentrations of PAHs in the agricultural soil because of the low PAH concentrations. SPME and Tenax-TA extraction have the potential to assess bioavailability of PAHs and predict the extent of biodegradation in the MGP soil. Only the Tenax-TA extraction could be used to assess the bioavailability of the 3- and 4-ring PAHs in the agricultural soil. These findings demonstrate that Tenax-TA was a more sensitive method than SPME to assess PAH biodegradation in contaminated industrial and agricultural soils.

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