Polycyclic aromatic hydrocarbons bioavailability in industrial and agricultural soils: linking SPME and Tenax extraction with bioassays

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Abstract: The aims of this study were to evaluate the bioavailability of polycyclic aromatic hydrocarbons (PAHs) in industrial and agricultural soils using chemical methods and a bioassay, and to study the relationships between the methods. This was conducted by comparing the quantities of PAHs extracted from two manufactured gas plant (MGP) soils and an agricultural soil with low level contamination by solid-phase micro-extraction (SPME) and Tenax-TA extraction with the quantities taken up by the earthworm (*Eisenia fetida*). In addition, a biodegradation experiment was conducted on one MGP soil (MGP-A) to clarify the relationship between PAH removal by biodegradation and the variation in PAH concentrations in soil pore water. Results demonstrated that the earthworm bioassay could not be used to examine PAH bioavailability in the tested MGP soils; which was the case even in the diluted MGP-A soils after biodegradation. However, the bioassay was successfully applied to the agricultural soil. These results suggest that earthworms can only be used for bioassays in soils with low toxicity. In general, rapidly desorbing concentrations extracted by Tenax-TA could predict PAH concentrations accumulated in earthworms ($R^2=0.66$), while SPME underestimated earthworm concentrations by a factor of 2.5. Both SPME and Tenax extraction can provide a useful tool to predict PAH bioavailability for earthworms, but Tenax-TA extraction was proven to be a more sensitive and precise method than SPME for the prediction of earthworm exposure in the agricultural soil.

Keywords: bioaccumulation; biodegradation; earthworm; rapidly desorbing fraction; solid-phase micro-extraction (SPME); Tenax extraction

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a ubiquitous class of organic pollutants that pose threats to human health and ecological security (Gong et al. 2015, Haritash & Kaushik 2009). The bulk concentrations of PAHs in soils extracted by traditional chemical methods may lead to an overestimation of their environmental and human health risks, because only the biologically available fractions of PAHs can be taken up by organisms (Alexander 2000, Cachada et al. 2014, Tao et al. 2009). The fraction of PAHs accessible for bioaccumulation or biodegradation by organisms is referred to as the available or bioavailable fraction (Cvancarova et al. 2013, Lu et al. 2010). Consequently, the assessment of bioavailability in contaminated soils is traditionally performed using bioassays that measure PAH biodegradation or accumulation in organisms (Cui et al. 2013).

Bioassays incorporate all factors affecting the net contaminant levels in organisms and are considered as the most accurate approach for quantifying bioavailability. The earthworm, *Eisenia fetida*, is the most common organism used for this purpose (Bergknut et al. 2007, Yang et al. 2013), but its application may be time consuming and laborious, and it can only be used within a limited concentration range for some substances (Cui et al. 2013, Cachada et al. 2014). For instance, Cvancarova et al. (2013) had to use diluted soils to investigate the toxicity of PAHs to earthworms. In response to the limitations of bioassays, in the past few years several efficient and cost-effective chemical methods have been developed to predict PAH bioavailability to bacteria, earthworms, and plants, including the use of semipermeable membrane devices (SPMDs) (Tao et al. 2009, Wang et al. 2015), solid-phase micro-extraction (SPME) (Jonker et al. 2007, Van der Wal et al. 2004), supercritical carbon dioxide extraction (Kreitinger et al. 2007), and extraction with Tenax (Li et al. 2015, You et al. 2011) and cyclodextrin (Spasojevic et al. 2015). Among these, the measurement of chemical activity (freely dissolved chemical concentrations) by SPME and the estimation of bioaccessibility (rapidly desorbing fraction) by Tenax-TA extraction have been successfully applied to predict the bioavailability of contaminants in organisms under laboratory conditions (Bielska et al. 2014, Liu et al. 2011, Yang et al. 2013). However, most of these studies have been carried out using laboratory-spiked soils or field-contaminated sediments (Arp et al. 2009, van der Heijden & Jonker 2009), or using SPME and Tenax extraction to estimate

bioavailability of other hydrophobic organic contaminants (HOCs) (You et al. 2011), such as polychlorinated biphenyls (PCBs) (Trimble et al. 2008) or pyrethroids (Harwood et al. 2013). Pollutant dynamics and bioavailability greatly differ between soil and sediments (Vasseur & Bonnard 2014), and there are very few studies to date that have focused on testing SPME and Tenax-TA extraction methods in a comparative manner to assess bioavailability in historically PAH-contaminated soils, especially not in agricultural soils (Cachada et al. 2014).

PAHs are found at high levels in soils at manufactured gas plants (MGP) (Arp et al. 2014; Barnier et al. 2014), and some agricultural soils are also contaminated with PAHs (Bayat et al. 2015, Duan et al. 2015, Guo et al. 2017). Generally, PAH bioavailability is often assessed in industrial contaminated soils (Gomez-Eyles et al. 2012, Hu & Aitken 2012). There have been no studies predicting the bioavailability of PAHs in field contaminated agricultural soil to earthworms, although the bioavailability of PAHs at relatively low concentrations (up to 9.9 mg kg⁻¹) has been studied in urban soil and sludge (Oleszczuk 2009). It should be noted that there are some variations in the distribution and environmental behavior of PAHs between industrial and agricultural soils as a result of differences in sources, concentrations, age of contaminants, and exposure pathways. The primary sources of PAH contamination to agricultural soils are wastewater irrigation and atmospheric deposition, while those of industrial soils are coal-related energy production and oil used for transport fuel. In agricultural soils, bioaccumulation into soil biota, plants, or humans has been recognized as the main exposure pathway of soil contaminants (Bayat et al. 2015, Lu et al. 2010); for industrial soils, such as those of MGP, desorption should be considered as a priority risk factor as human contact with contaminated soil is the major exposure pathway. Thus the study of PAH bioavailability in MGP and agricultural soils is important for accurately performing environmental and human health risk assessments.

To our knowledge, the bioavailability of PAHs in agricultural soil has only been investigated using earthworms in one study (Beck et al. 1996), where agricultural soil was amended with sewage sludge. In the present study, we assessed the potential of both SPME and Tenax-TA extraction to predict PAH bioaccumulation in earthworms (*E. fetida*) from two MGP soils and an agricultural soil. In doing so, the applicability of chemical and biological methods in evaluating PAH bioavailability in soils and the internal links between them was demonstrated. This study

80 therefore provides information for environmental risk assessment based on PAH bioavailability in
81 contaminated MGP and agricultural soils.

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2. Materials and Methods

2.1 Soils

Three PAH-contaminated soils were used in this study. Two industrial soils were sampled from MGP sites operated between 1958 and 2006 in Beijing, China. Soil from the first site (MGP-A) had a total organic carbon (TOC) content of 1.3%, the percentages of clay, silt, and sand were 65.8%, 14.9%, and 19.3%, respectively. Soil from the second site (MGP-B) had a TOC content of 1.6%, and the percentages of clay, silt, and sand were 62.3%, 15.8%, and 21.9%, respectively. The agricultural soil was collected from a wastewater-irrigated area, which was used to grow grain and vegetables in a suburb of Shenyang, China. The TOC of this soil was 2.4%, and the percentages of clay, silt, and sand were 61.4%, 28.5%, and 10.1%, respectively. All the soil samples were collected from the surface layer (0-20 cm) of the sites and sieved through a 2-mm mesh to remove small rocks and plant material, homogenized, and kept in the dark at 4°C until analysis.

2.2 Soil PAH extractions

The bulk concentrations of PAHs in the contaminated soils were estimated by high-performance liquid chromatography (HPLC) after extraction using a mechanical chemical extraction procedure (Gong et al. 2010, Wu et al. 2010). In short, contaminated soil (1 g of MGP soil or 5 g of agricultural soil) 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 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 evaporated to dryness under nitrogen gas, before being re-dissolved in acetonitrile for analysis by 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. Prior to 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 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 d, 4 d, 7 d, 12 d and 30 d), the tubes were centrifuged at 1609×g for 20 min, then the Tenax-TA was collected using a stainless steel scoop, 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 °C for 16 h under a constant helium flow. The SPME with PDMS fibers was conducted according to the method described elsewhere (Guo et al. 2016). Briefly, 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 NaN₃ solution, and shaken on a mechanical shaker (120 rpm) at 25°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°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 measured via HPLC.

2.5 Biodegradation experiment

A biodegradation experiment was conducted on MGP-A soil to clarify the relationship between PAH removals by biodegradation and variations in PAH concentrations in soil pore water. 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); both strains were isolated from contaminated soil and were capable of PAH degradation (Guo et al. 2016, Wang et al. 2012).

The strains were maintained at 4°C and were periodically sub-cultured on agar plates.

Pre-inocula were grown under liquid conditions for 7 d 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 constituents are described elsewhere (Guo et al. 2016). After inoculation, the cultures were covered and grown for two weeks at 28°C under constantly dark conditions.

The industrial MGP-A soil was incubated in glass jars with 100 g (dry weight) of either an inoculum of *Mycobacterium* sp. or *Mucor* sp. per kg of 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 growth medium, strain, and soil, was prepared in triplicate, and incubated in the dark at 28°C for 30 d. The soils were sampled after the degradation experiment and were analyzed for their bulk and bioavailable PAH concentrations.

2.6 Earthworm Bioassay

Earthworms (*E. fetida*) were obtained from Shenyang Agriculture University (Shenyang, China). Only adult earthworms with a clitellum were used. The bioaccumulation study was conducted in triplicate following the method described by Tang et al. (1998) with minor modification. Five worms (average weight 0.3 g each) were exposed to 20 g of PAH-contaminated soil, at a moisture content of 60% of field capacity, in the dark at 20°C for 14 d. After exposure, the worms were rinsed with water and kept on wet filter paper in a glass petri dish for 24 h to allow for depuration. Then the recovered worms were dried with paper towels, weighed, frozen, freeze-dried, and homogenized with 25-30 g of anhydrous Na₂SO₄ with a mortar and pestle prior to Soxhlet extraction. Concentrated extracts of earthworm tissue were divided for both analysis of PAHs and determination of lipids. PAHs in the earthworms were extracted for 24 h with 100 mL dichloromethane and n-hexane (1:1) at 60°C (Tao et al. 2009). The extracts were passed through silica gel to remove cholesterol and other interfering compounds, 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 measured via HPLC. Lipid contents of the subsamples of earthworms were determined gravimetrically by Soxhlet extraction in a 1:1 acetone/hexane

solvent mixture and evaporation to dryness at 65°C for 16 h (Gomez-Eyles et al. 2012). The difference in weight divided by the initial weight of the subsamples was the lipid content of the earthworms.

2.7 HPLC analysis

PAHs extracts from the soil samples were analyzed with the HPLC (Agilent Technologies 1200 series) equipped with an ultraviolet detector, a fluorescence detector, and a C-18 column. The separation and selected excitation/emission wavelengths are described elsewhere (Guo et al. 2016). In all cases, 10 µL of sample was injected into the HPLC by an autosampler. An external standard mixture (M-8310, AccuStandard, New Haven, Connecticut, USA) was used for PAH calibration and quantification.

2.8 Data analysis and Quality Assurance

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

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

where 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, respectively; F_{rap} and F_{slow} are the rapidly and slowly desorbing fractions, respectively; and k_{rap} and k_{slow} (d⁻¹) are the rapid and slow rate constants, respectively, for the two desorption compartments. It was generally assumed that the two fractions encompassed all of the PAHs, therefore F_{rap} plus F_{slow} is equal to one. All the parameters in Equation (1) were determined by direct curve fitting in SigmaPlot 12.5 (SYSTAT Software Inc., San Jose, USA).

PAH concentrations in soil pore water, namely PAH freely dissolved concentrations, were calculated using the following equation:

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

where C_w is the PAH concentration in soil pore water (µg L⁻¹), C_f is the PAH concentration in the fiber coating (µg L⁻¹), and K_f is the fiber-water partition coefficient.

Concentrations of individual PAHs in earthworms were predicted by the equilibrium partitioning theory and bioconcentration factor (BCF), derived from the K_{ow} , using $\log \text{BCF} = \log K_{ow}$ (Jonker et al. 2007), as shown in the following equation (Gomez-Eyles et al.

2012):

$$C_{\text{worm predict}} = C_w * \text{BCF} \dots\dots\dots (3)$$

The results of the Tenax-TA extraction and SPME methods were compared to earthworm bioaccumulation by comparing the concentrations obtained by the Tenax-TA extraction to the concentrations accumulated in earthworm, and by predicting concentrations in *E. fetida* from the concentrations extracted by SPME using equilibrium partitioning calculations. The relationship between the PAH removals by degradation in MGP-A soil and the variations in PAH concentrations in soil pore water was also compared.

Both bioassays and chemical extractions were replicated three times. The procedural blank was determined by performing the same extraction and purification procedures for each series of samples. To estimate the recoveries of bioassays and chemical extractions, a mixture of PAHs was spiked into soil or earthworm and extracted using the procedures described in sections 2.2-2.4 and 2.6. Average recoveries for three replicates from soil were 83-105%, and 74-98% from earthworms. The recoveries of the SPME fibers and Tenax-TA extraction were 89-108% and 98-110%, respectively. All data were expressed as mean $\pm$ standard deviation (n=3). The differences between groups were compared using one-way ANOVA and least significant difference at the 95% confidence level ($p < 0.05$) using SPSS 16.0 (SPSS Inc., Chicago, IL, USA)

3. Results and discussion

3.1 PAH bulk concentrations in soils

In this study we examined the PAH bioavailability in both an agricultural soil with relatively low PAH concentrations, and MGP soils with relatively high PAH concentrations. The total concentrations of 12 PAHs (Σ PAH) in the MGP-A, -B, and agricultural soils were 178.21, 49.42, and 3.56 mg kg⁻¹, respectively (Table 1). Three-ring PAHs dominated (62%) the Σ PAH in the MGP-A soil, and phenanthrene accounted for 59% of the Σ PAH concentration. Four-ring PAHs were predominant in the MGP-B soil and agricultural soil (56% and 52% of the Σ PAH concentrations, respectively).

Table 1 PAH concentrations in the experimental soils (C_s)^a

Compound ^b	Rings	C_s (mg kg ⁻¹)		
		MGP-A	MGP-B	agricultural
phen	3	105.5±3.55	6.22±0.17	0.16±0.02
ant	3	5.38±0.21	2.91±0.10	0.03±0.05
flt	4	27.58±0.88	12.40±0.50	0.42±0.05
pyr	4	13.86±0.49	9.05±0.33	0.42±0.04
baa	4	6.38±1.36	3.88±0.54	0.42±0.03
chry	4	4.57±0.69	2.58±0.14	0.60±0.02
bbf	5	4.08±0.09	2.94±0.13	0.37±0.03
bkf	5	1.91±0.03	1.43±0.05	0.16±0.01
bap	5	1.77±0.05	1.89±0.09	0.26±0.02
daha	5	0.41±0.01	0.32±0.03	0.07±0.02
bghip	6	3.58±0.09	3.62±0.07	0.43±0.02
icdp	6	3.18±0.06	2.17±0.03	0.19±0.02
Σ PAH		178.21±6.76	49.42±1.82	3.56±0.27

a) Values are means ± SD of triplicate measurements;

b) 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

3.2 PAH bioavailability assessment using Tenax-TA extraction and SPME

The kinetics and fitting parameters of R^2 and k_{rap} for the industrial MGP-A, -B, and agricultural soils are listed in Table S1. Fitted values of the rapidly desorbing fractions (F_{rap}) of the two-site model for PAHs in the three soils were in the ranges of 0.18-0.54, 0.14-0.53, and 0.12-0.42, respectively (Table 2). The values of F_{rap} decreased as the PAH ring number increased;

the values for 5- and 6-ring PAHs were significantly lower than those for 3- and 4-ring PAHs. This finding is consistent with those of Richardson and Aitken (2011), who using the Tenax-TA extraction method on their untreated MGP soil, reported F_{rap} range from 0.13 to 0.53. However, our values were higher than those reported by Barnier et al. (2014) (0.25%-7.59%) for the assessment of the desorption kinetics of PAHs from aged industrial soils. Many reports have showed that bioavailability of organic pollutants in soil declines with time (Alexander, 2000; Bielska et al., 2014), and so the difference may be explained by the different aging time of the PAHs in our study cf. those of Barnier et al. (2014). In our study, the rapidly desorbing fractions of PAHs in the agricultural soil were lower than those in the MGP soils, indicating that the PAHs were more difficult to release from agricultural soil than from MGP soils. However, it was reported by Yang et al. (2013) that the F_{rap} values in agricultural soils were higher than those in low-PAH steel plant soils (0.02-0.27). The difference in the rapidly desorbing fractions of PAHs between the agricultural and industrial soils relates to the differences in soil texture, organic carbon content and aging histories. The agricultural soil had higher carbon content and less sandy soil than the MGP soils, which result in higher retention affinity of PAHs and lower rapidly desorbing fractions in the agricultural soil in our study.

Table 2 Rapidly desorbing fractions (F_{rap}) of PAHs in experimental soils

Ring	MGP-A	MGP-B	agricultural
3	0.46±0.03	0.53±0.01	0.42±0.03
4	0.54±0.02	0.53±0.02	0.26±0.02
5	0.37±0.03	0.3±0.01	0.16±0.01
6	0.18±0.02	0.14±0.01	0.12±0.02
ΣPAH	0.47±0.03	0.44±0.01	0.22±0.02

Freely dissolved PAH concentration in soil pore water (C_w) is related to the bioavailable portion of PAH in the soil (Cachada et al. 2014). By applying SPME fiber-water partition coefficients (K_f) and equation (2), the C_w values were calculated in our study. The K_f values were obtained from Ter Laak et al. (2006), as we used the same PDMS fiber. The C_w values for ΣPAH were 250, 24.7, and 5.7 $\mu\text{g L}^{-1}$ in the MGP-A, -B and agricultural soils, respectively (Table 3). For all the soil samples, 3- and 4-ring PAHs were predominant, ranging in concentrations from 1.44 to 208.94 $\mu\text{g L}^{-1}$, while 5- and 6-ring PAHs occurred with the lowest concentrations. This is

consistent with the results of Gomez-Eyles et al. (2012), who carried out an evaluation of PAH bioavailability in 10 field-contaminated soils using a number of extraction methods. The Σ PAH concentration in the MGP-A soil was almost four times higher than that in the MGP-B soil, but the C_w value of Σ PAH in the MGP-A soil was ten times higher than that in the MGP-B soil, indicating that PAH bioavailability was not only related to the soil bulk concentration, but also may be related to partitioning and sorption processes of PAHs in soils. In this study, some values for C_w in the agricultural soil were too low to detect. Harwood et al. (2013) proposed that SPME method is sometimes not sensitive enough to detect compounds occurring at very low environmental concentrations.

Table 3 Freely dissolved concentrations of PAH (C_w) in the experimental soil pore water

Ring	MGP-A	MGP-B	agricultural
3	208.94 $\pm$ 2.57	14.69 $\pm$ 0.59	4.19 $\pm$ 0.54
4	38.82 $\pm$ 1.51	9.84 $\pm$ 0.10	1.44 $\pm$ 0.16
5	1.04 $\pm$ 0.07	0.14 $\pm$ 0.007	0.05 $\pm$ 0.006
6	0.2 $\pm$ 0.02	0.03 $\pm$ 0.006	0.02 $\pm$ 0.004
Σ PAH	250 $\pm$ 4.80	24.70 $\pm$ 0.56	5.70 $\pm$ 0.63

3.3 PAH concentrations accumulated in earthworms

The concentration of Σ PAH accumulated by the earthworms (C_{worm}) in the agricultural soil was 31.8 mg kg lipid⁻¹ (Fig. 1). The earthworms accumulated a higher proportion of 4-ring PAHs (63%), even though the freely dissolved concentrations of 4-ring PAHs were lower than those of 3-ring PAHs. This observation is in line with previous findings on the bioaccumulation of PAHs by earthworms in soils (Gomez-Eyles et al. 2010, Parrish et al. 2006). C_{worm} values reported by Yang et al. (2013) in soil from the vicinity of the Meishan steel plant (Nanjing, China) are similar to the values we observed in the agricultural soil, as well as the C_s values.

Fig. 1 Concentrations of PAHs in the earthworms (C_{worm} mg kg lipid⁻¹) in the agricultural soil

None of the earthworms survived in the bioassay with the industrial soils (both MGP-A and -B), suggesting that earthworms might be not suitable for assessing the bioavailability of PAHs in such soils because of the toxicity associated with the high levels of PAHs. That said, some studies, for example, Arp et al. (2014) and Kreitinger et al. (2007), successfully measured the PAH bioaccumulation by earthworms in MGP soils with of 168-6760 mg kg⁻¹, which were much higher than those we measured in the MGP soils. Similarly, Kreitinger et al. (2007) reported that 75% of earthworms died in a MGP soil with a bulk PAH concentration of 521mg kg⁻¹, whereas all the earthworms exposed to another heavily PAH-contaminated soil (6760 mg kg⁻¹) survived. Consequently, acute toxicity to the earthworm, and thus actual environmental risk, may be not related to or accurately predicted by soil bulk PAH concentration. The low bioavailability and toxicity of PAHs in some of the MGP soils are likely due to the stronger sorption of PAHs to organic carbon and the extensive weathering that has occurred during the past 50 to 100 years (Kreitinger et al. 2007, Hawthorne & Grabanski 2000). The strong sorption of organic carbon controls the release of PAHs and may be responsible for the reduced bioavailability of PAHs in soils. Extensive weathering could remove the more mobile fraction of the hydrocarbons, resulting in an enrichment of tightly bound PAHs that are not readily available to earthworms. Our industrial soil samples were from an MGP site that had been abandoned for less than a decade, with the TOC contents being 1.3% and 1.6%. Kreitinger et al. (2007) sites, however, had been

closed for approximately 60 years, and had higher TOC contents ranging from 2.6% to 8.7%. This may also explain why bioavailable PAH concentrations in our MGP soils were higher than those in other studies. Higher bioavailability was also confirmed by the F_{rap} values we observed (14-54%) (Table 2), which were much higher than those in aged MGP soils from the center of France (0.25%-3.64%) (Barnier et al. 2014). In addition, the toxicity of PAHs in the MGP soils may be also related to the biodegradation metabolites present in those soils. Different bacteria are involved in the biodegradation of PAHs and may produce different metabolites (Bacosa et al. 2015a, Bacosa et al. 2015b). More bioavailable metabolites might increase the toxicity of the MGP soils in our study. Moreover, 3- and 4-ring PAHs were the predominant PAH compounds in the MGP soils, which were more closely associated with earthworm mortality in our study (Cvancarova et al. 2013).

3.4 Relationship between biodegradation and SPME

Biodegradation has frequently been applied to measure the bioavailability of contaminants (Cui et al. 2013, Janbandhu & Fulekar 2011). In this study, the F_{rap} values were similar in the two MGP soils, however the Σ PAH concentrations in the MGP-A soil and the corresponding C_w values were higher than those in the MGP-B soil. Therefore we only tested biodegradation, and changes of soil bulk concentrations and C_w of PAHs in the MGP-A soil. When inoculated with the *Mycobacteria* sp., 95% of the 3-ring PAH bulk concentration was removed from the soil (Fig. 2), with 77% reduction of the freely dissolved 3-ring PAH concentration in soil pore water. For *Mucor* sp., the relevant percentages were 93% and 77%, respectively. Generally, the changes in concentrations originated mainly from the 3- and 4-ring PAHs. It should be noted that percent changes in some high molecular weight (HMW) PAH concentrations were negative, an observation ascribed to an increase in their concentrations due to microbial activity. Soil microorganisms might produce biosurfactants that enhanced the bioavailability of HMW PAHs (Haritash & Kaushik 2009), or they may have active efflux mechanisms that transported the PAHs out of the cell (Bugg et al. 2000). The above mechanisms may play an important role in releasing these highly recalcitrant PAHs into the aqueous phase. C_w is a representative of the bioavailable fraction of hydrophobic organic contaminants (HOCs) (Josefsson et al. 2015, Muijs & Jonker 2011). The PAH reduction calculated on the concentration difference of PAHs in the soil pore

water were notably lower than the corresponding PAH degradation calculated on the basis of the soil bulk PAH concentrations both for *Mycobacteria* sp. and *Mucor* sp., indicating that the change in the environmental risk of PAHs following biodegradation was underestimated if only variation in C_w was considered.

Fig.2 The percent changes in PAH concentrations in soil and soil pore water following biodegradation by (a) *Mycobacteria* sp. and (b) *Mucor* sp. in the MGP-A soil

3.5 PAH bioaccumulation in diluted MGP-A soil after biodegradation

As noted earlier, none of the earthworms survived in the bioassay with MGP soils. There have been studies using diluted soils to investigate the bioavailability of PAHs to plants (Cofield et al. 2008) and the ecotoxicity of PAHs to earthworms (Cvancarova et al. 2013). In that context, we diluted both pre- and post-biodegradation MGP-A soil samples with uncontaminated soils into 2, 4, and 8 times. The earthworms in the pre-biodegradation diluted soils still died in the experiment, thus, only the PAHs accumulated by earthworms (100% survival) in the post-biodegradation diluted soils were considered (Table S2). The C_{worm} values in the diluted MGP-A soils were in the range of 6.57-12.74 mg kg⁻¹ ww (wet weight). The 4-ring PAHs were dominant PAHs (55.7-69.2%) in the post-biodegradation diluted MGP-A soils and ranged in concentrations from 3.69 mg kg⁻¹ to 8.26 mg kg⁻¹. Concentrations of 3-ring PAHs were lower than those of 4- and 5-ring PAHs. The lower amount of 3-ring PAH accumulation might be attributed to the relatively low residual amounts of PAHs following biodegradation and the high lipophilicity of 5- and 6-ring PAHs. It should be noted that the C_{worm} values did not decrease proportionally with the dilution ratio in the MGP-A soil, indicating that the use of diluted soils to investigate bioavailability of PAHs is not

always appropriate.

3.6 Applicability of Tenax extraction and SPME to assess bioaccumulation

Tenax-TA and SPME extractions target different components of the matrix. Tenax-TA extraction measures the PAHs in soils that are potentially bioaccessible, while SPME measures the PAHs readily available for the equilibrium partitioning process (Reichenberg & Mayer 2006). Previous studies have demonstrated the use of SPME to estimate the bioavailability of HOCs in laboratory-spiked sediments or in field sediments (Arp et al. 2009, Harwood et al. 2013). In our study, the concentrations of individual PAHs in earthworms were predicted using equation (3). The concentrations of dibenz(ah)anthracene and indeno(cd)pyrene were not available, as the two compounds were not detected in the SPME fibers (Table S3).

Linear correlations were calculated between the PAH concentrations measured in *E. fetida* (mg kg lipid⁻¹) and the bioavailable PAH concentrations predicted by Tenax-TA (mg kg OC⁻¹) and SPME (mg kg lipid⁻¹) in the agricultural soil. The regression equations for the two methods were $y=1.03x$ and $y=2.50x$ (Fig. 3), respectively. Rapidly desorbing concentrations extracted by Tenax-TA generally predicted PAH concentrations in earthworms, with a slope of 1.03 ($R^2=0.66$). Reid et al. (2000) theorized that slope of the regression line must be taken into consideration in order to provide sufficient information on the suitability of extraction methods to determine bioavailability, with a slope of 1 for the fitted line being ideal. Therefore, the Tenax-TA extraction generally gave a good prediction for PAH accumulation that was actually accumulated in the earthworms. This is similar to the previously reported application of the Tenax-TA method in PAH-contaminated sediments (van der Heijden & Jonker 2009) and soils (Yang et al. 2013). However, Gomez-Eyles et al. (2010) reported no clear correlations between PAH accumulation in earthworms and the rapidly desorbing fraction of PAHs in spiked soils. The difference in predictive capability between our study and that of Gomez-Eyles et al. (2010) might be due to differences in the soil samples and the applied earthworm bioassay approach. In this study the earthworms were exposed to 20 g of agricultural soils according to Tang et al. (1998), whereas in Gomez-Eyles et al. (2010), the earthworms were exposed to 250 g of spiked soils.

Fig.3 Relationships between PAH concentrations measured in *E. fetida* and (a) PAH concentrations extracted by Tenax-TA, and (b) concentrations in earthworms predicted by SPME in the agricultural soil

There was also a good correlation ($R^2=0.62$) between the measured and predicted PAH concentrations (by SPME) in *E. fetida* in the agricultural soil (Fig. 3b). Generally, the SPME method underestimated earthworm concentrations by a factor of 2.5 (Fig. 3b; regression equation was $y=2.50x$). Some studies on MGP soils have reported that SPME predictions of individual PAHs body residues in earthworms within a factor of 10 are satisfactory (Gomez-Eyles et al. 2012, Jonker et al. 2007). Considering that the results were related to a very complex sample set and the equation of $\log BCF = \log K_{ow}$ might introduce a certain degree of uncertainty, this method seemed appropriate for predicting PAH bioaccumulation in contaminated agricultural soil in our study. There are very few studies that have focused on testing SPME methods to assess bioavailability in soils with low PAH contamination (Cachada et al. 2014). Thus more detailed studies using a larger number of soils and sediments with low PAH contamination would be necessary to carry out and to confirm the applicability of the SPME method.

PAH bioavailability assessed by SPME and Tenax-TA extraction represents the chemical activity and bioaccessibility of PAHs, respectively (Cui et al., 2013). To our knowledge, this is the first study predicting the bioavailability of PAHs in historically contaminated agricultural soil to earthworms by SPME and Tenax-TA extraction in a comparative manner. The Tenax-TA method predicted PAH bioaccumulation by earthworms exposed to the agricultural soil more accurately

394 than that of SPME. Yang et al. (2013) also found that Tenax 6 h-extracted PAHs had strong
395 correlations with earthworm accumulated PAHs in industrial soils with PAH concentrations of
396 1.74-12.74 mg kg⁻¹. However, as different agricultural soils have different physicochemical
397 properties and contain different levels of PAHs, the extent of which the Tenax-TA method can be
398 used for prediction of PAH accumulation in earthworms requires further investigation, although
399 the results in the present study are very promising.

4 Conclusion

The results from this study revealed that the rapidly desorbing fractions of PAHs in the agricultural soil were lower than those in the two MGP soils, while there was no significant difference between the MGP-A and -B soils. Earthworms could not be used as an indicator of PAH bioavailability in the MGP because the high molecular weight PAHs dominate this matrix and cause toxicity to earthworms. Tenax-TA extraction and SPME target different components of the matrix. We observed good correlations between the PAH concentrations measured in *E. fetida* and the bioavailable PAH contents predicted by Tenax-TA and SPME in the agricultural soil. Both methods provide a cost-effective tool for risk assessment of the soil, which might be considered as a promising substitute for the bioassays. The Tenax-TA method predicted PAH bioaccumulation in earthworms in agricultural soil more accurately than those of SPME. The study adds to the body of work on the potential application of chemical and biological methods in evaluating the bioavailability of PAHs in industrial and agricultural soils.

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Reference

- Alexander, M., 2000. Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environmental Science & Technology* 34, 4259-4265.
- Arp, H.P.H., Breedveld, G.D., Cornelissen, G., 2009. Estimating the in situ Sediment-Porewater Distribution of PAHs and Chlorinated Aromatic Hydrocarbons in Anthropogenic Impacted Sediments. *Environmental Science & Technology* 43, 5576-5585.
- Arp, H.P.H., Lundstedt, S., Josefsson, S., Cornelissen, G., Enell, A., Allard, A.-S., Kleja, D.B., 2014. Native Oxy-PAHs, N-PACs, and PAHs in Historically Contaminated Soils from Sweden, Belgium, and France: Their Soil-Porewater Partitioning Behavior, Bioaccumulation in *Enchytraeus crypticus*, and Bioavailability. *Environmental Science & Technology* 48, 11187-11195.
- Bacosa, H.P., Erdner, D.L., Liu, Z., 2015a. Differentiating the roles of photooxidation and biodegradation in the weathering of Light Louisiana Sweet crude oil in surface water from the Deepwater Horizon site. *Marine Pollution Bulletin* 95, 265-272.
- Bacosa, H.P., Liu, Z.F., Erdner, D.L., 2015b. Natural Sunlight Shapes Crude Oil-Degrading Bacterial Communities in Northern Gulf of Mexico Surface Waters. *Frontiers in Microbiology* 6.
- Barnier, C., Ouvrard, S., Robin, C., Morel, J.L., 2014. Desorption kinetics of PAHs from aged industrial soils for availability assessment. *Science of the Total Environment* 470, 639-645.
- Bayat, J., Hashemi, S.H., Khoshbakht, K., Deihimfard, R., Shahbazi, A., Momeni-Vesalian, R., 2015. Monitoring of polycyclic aromatic hydrocarbons on agricultural lands surrounding Tehran oil refinery. *Environmental monitoring and assessment* 187, 4646-4646.
- Beck, A.J., Johnson, D.L., Jones, K.C., 1996. The form and bioavailability of non-ionic organic chemicals in sewage sludge-amended agricultural soils. *Science of the Total Environment* 185, 125-149.
- Bergknut, M., Sehlin, E., Lundstedt, S., Andersson, P.L., Haglund, P., Tysklind, M., 2007. Comparison of techniques for estimating PAH bioavailability: Uptake in *Eisenia fetida*, passive samplers and leaching using various solvents and additives. *Environmental Pollution* 145, 154-160.
- Bielska, L., Smidova, K., Hofman, J., 2014. Solid phase microextraction of organic pollutants from natural and artificial soils and comparison with bioaccumulation in earthworms.

448 Ecotoxicology and Environmental Safety 100, 44-52.

449 Bugg, T., Foght, J.M., Pickard, M.A., Gray, M.R., 2000. Uptake and active efflux of polycyclic
 450 aromatic hydrocarbons by *Pseudomonas fluorescens* LP6a. Applied and environmental
 451 microbiology 66, 5387-5392.

452 Cachada, A., Pereira, R., Ferreira da Silva, E., Duarte, A.C., 2014. The prediction of PAHs
 453 bioavailability in soils using chemical methods: State of the art and future challenges. Science
 454 of the Total Environment 472, 463-480.

455 Cofield, N., Banks, M.K., Schwab, A.P., 2008. Lability of polycyclic aromatic hydrocarbons in the
 456 rhizosphere. Chemosphere 70, 1644-1652.

457 Cornelissen, G., vanNoort, P.C.M., Govers, H.A.J., 1997. Desorption kinetics of chlorobenzenes,
 458 polycyclic aromatic hydrocarbons, and polychlorinated biphenyls: Sediment extraction with
 459 Tenax(R) and effects of contact time and solute hydrophobicity. Environmental Toxicology
 460 and Chemistry 16, 1351-1357.

461 Cui, X., Mayer, P., Gan, J., 2013. Methods to assess bioavailability of hydrophobic organic
 462 contaminants: Principles, operations, and limitations. Environmental Pollution 172, 223-234.

463 Cvcancarova, M., Kresinova, Z., Cajthaml, T., 2013. Influence of the bioaccessible fraction of
 464 polycyclic aromatic hydrocarbons on the ecotoxicity of historically contaminated soils.
 465 Journal of Hazardous Materials 254, 116-124.

466 Duan, Y., Shen, G., Tao, S., Hong, J., Chen, Y., Xue, M., Li, T., Su, S., Shen, H., Fu, X., Meng, Q.,
 467 Zhang, J., Zhang, B., Han, X., Song, K., 2015. Characteristics of polycyclic aromatic
 468 hydrocarbons in agricultural soils at a typical coke production base in Shanxi, China.
 469 Chemosphere 127, 64-69.

470 Gomez-Eyles, J.L., Collins, C.D., Hodson, M.E., 2010. Relative proportions of polycyclic aromatic
 471 hydrocarbons differ between accumulation bioassays and chemical methods to predict
 472 bioavailability. Environmental Pollution 158, 278-284.

473 Gomez-Eyles, J.L., Jonker, M.T.O., Hodson, M.E., Collins, C.D., 2012. Passive Samplers Provide a
 474 Better Prediction of PAH Bioaccumulation in Earthworms and Plant Roots than Exhaustive,
 475 Mild Solvent, and Cyclodextrin Extractions. Environmental Science & Technology 46,
 476 962-969.

477 Gong, X., Xu, X., Gong, Z., Li, X., Jia, C., Guo, M., Li, H., 2015. Remediation of PAH-contaminated
 478 soil at a gas manufacturing plant by a combined two-phase partition system washing and
 479 microbial degradation process. Environmental Science and Pollution Research 22,
 480 12001-12010.

481 Gong, Z., Wang, X., Tu, Y., Wu, J., Sun, Y., Li, P., 2010. Polycyclic aromatic hydrocarbon removal
 482 from contaminated soils using fatty acid methyl esters. Chemosphere 79, 138-143.

483 Guo, M., Gong, Z., Allinson, G., Tai, P., Miao, R., Li, X., Jia, C., Zhuang, J., 2016. Variations in the
 484 bioavailability of polycyclic aromatic hydrocarbons in industrial and agricultural soils after
 485 bioremediation. *Chemosphere* 144, 1513-1520.

486 Guo, M., Gong, Z., Miao, R., Su, D., Li, X., Jia, C., Zhuang, J., 2017. The influence of root exudates of
 487 maize and soybean on polycyclic aromatic hydrocarbons degradation and soil bacterial
 488 community structure. *Ecological Engineering* 99, 22–30.

489 Haritash, A.K., Kaushik, C.P., 2009. Biodegradation aspects of Polycyclic Aromatic Hydrocarbons
 490 (PAHs): A review. *Journal of Hazardous Materials* 169, 1-15.

491 Harwood, A.D., Landrum, P.F., Weston, D.P., Lydy, M.J., 2013. Using SPME fibers and Tenax to
 492 predict the bioavailability of pyrethroids and chlorpyrifos in field sediments. *Environmental*
 493 *Pollution* 173, 47-51.

494 Hawthorne, S.B., Grabanski, C.B., Miller, D.J., 2006. Measured partitioning coefficients for parent and
 495 alkyl polycyclic aromatic hydrocarbons in 114 historically contaminated sediments: Part 1.
 496 K-OC values. *Environmental Toxicology and Chemistry* 25, 2901-2911.

497 Hu, J., Aitken, M.D., 2012. Desorption of polycyclic aromatic hydrocarbons from field-contaminated
 498 soil to a two-dimensional hydrophobic surface before and after bioremediation. *Chemosphere*
 499 89, 542-547.

500 Janbandhu, A., Fulekar, M.H., 2011. Biodegradation of phenanthrene using adapted microbial
 501 consortium isolated from petrochemical contaminated environment. *Journal of Hazardous*
 502 *Materials* 187, 333-340.

503 Jonker, M.T.O., van der Heijden, S.A., Kreitinger, J.P., Hawthorne, S.B., 2007. Predicting PAH
 504 bioaccumulation and toxicity in earthworms exposed to manufactured gas plant soils with
 505 solid-phase microextraction. *Environmental Science & Technology* 41, 7472-7478.

506 Josefsson, S., Arp, H.P.H., Kleja, D.B., Enell, A., Lundstedt, S., 2015. Determination of
 507 polyoxymethylene (POM) - water partition coefficients for oxy-PAHs and PAHs.
 508 *Chemosphere* 119, 1268-1274.

509 Kreitinger, J.P., Quinones-Rivera, A., Neuhauser, E.F., Alexander, M., Hawthorne, S.B., 2007.
 510 Supercritical carbon dioxide extraction as a predictor of polycyclic aromatic hydrocarbon
 511 bioaccumulation and toxicity by earthworms in manufactured-gas plant site soils.
 512 *Environmental Toxicology and Chemistry* 26, 1809-1817.

513 Li, C., Cui, X.Y., Fan, Y.Y., Teng, Y., Nan, Z.R., Ma, L.Q., 2015. Tenax as sorption sink for in vitro
 514 bioaccessibility measurement of polycyclic aromatic hydrocarbons in soils. *Environmental*
 515 *Pollution* 196, 47-52.

516 Liu, M., Tian, S., Chen, P., Zhu, L., 2011. Predicting the bioavailability of sediment-associated
 517 polybrominated diphenyl ethers using a 45-d sequential Tenax extraction. *Chemosphere* 85,

518 424-431.

519 Lu, M., Yuan, D., Lin, Q., Ouyang, T., 2010. Assessment of the bioaccessibility of polycyclic aromatic
520 hydrocarbons in topsoils from different urban functional areas using an in vitro
521 gastrointestinal test. *Environmental Monitoring and Assessment* 166, 29-39.

522 Muijs, B., Jonker, M.T.O., 2011. Assessing the Bioavailability of Complex Petroleum Hydrocarbon
523 Mixtures in Sediments. *Environmental Science & Technology* 45, 3554-3561.

524 Oleszczuk, P., 2009. Application of three methods used for the evaluation of polycyclic aromatic
525 hydrocarbons (PAHs) bioaccessibility for sewage sludge composting. *Bioresour Technol* 100,
526 413-420.

527 Parrish, Z.D., White, J.C., Isleyen, M., Gent, M.P.N., Iannucci-Berger, W., Eitzer, B.D., Kelsey, J.W.,
528 Incorvia Mattina, M., 2006. Accumulation of weathered polycyclic aromatic hydrocarbons
529 (PAHs) by plant and earthworm species. *Chemosphere* 64, 609-618.

530 Reichenberg, F., Mayer, P., 2006. Two complementary sides of bioavailability: Accessibility and
531 chemical activity of organic contaminants in sediments and soils. *Environmental Toxicology*
532 *and Chemistry* 25, 1239-1245.

533 Reid, B.J., Jones, K.C., Semple, K.T., 2000. Bioavailability of persistent organic pollutants in soils and
534 sediments - a perspective on mechanisms, consequences and assessment. *Environmental*
535 *Pollution* 108, 103-112.

536 Rhodes, A.H., Carlin, A., Semple, K.T., 2008. Impact of black carbon in the extraction and
537 mineralization of phenanthrene in soil. *Environmental Science & Technology* 42, 740-745.

538 Spasojevic, J.M., Maletic, S.P., Roncevic, S.D., Radnovic, D.V., Cucak, D.I., Trickovic, J.S., Dalmacija,
539 B.D., 2015. Using chemical desorption of PAHs from sediment to model biodegradation
540 during bioavailability assessment. *Journal of Hazardous Materials* 283, 60-69.

541 Tang, J.X., Carroquino, M.J., Robertson, B.K., Alexander, M., 1998. Combined effect of sequestration
542 and bioremediation in reducing the bioavailability of polycyclic aromatic hydrocarbons in sod.
543 *Environmental Science & Technology* 32, 3586-3590.

544 Tao, Y., Zhang, S., Wang, Z., Christie, P., 2009. Predicting bioavailability of PAHs in
545 field-contaminated soils by passive sampling with triolein embedded cellulose acetate
546 membranes. *Environmental Pollution* 157, 545-551.

547 Ter Laak, T.L., Barendregt, A., Hermens, J.L.M., 2006. Freely dissolved pore water concentrations and
548 sorption coefficients of PAHs in spiked aged, and field-contaminated soils. *Environmental*
549 *Science & Technology* 40, 2184-2190.

550 Trimble, T.A., You, J., Lydy, M.J., 2008. Bioavailability of PCBs from field-collected sediments:
551 Application of Tenax extraction and matrix-SPME techniques. *Chemosphere* 71, 337-344.

552 van der Heijden, S.A., Jonker, M.T.O., 2009. PAH Bioavailability in Field Sediments: Comparing

553 Different Methods for Predicting in Situ Bioaccumulation. *Environmental Science &*
554 *Technology* 43, 3757-3763.

555 Van der Wal, L., Jager, T., Fleuren, R., Barendregt, A., Sinnige, T.L., Van Gestel, C.A.M., Hermens,
556 J.L.M., 2004. Solid-phase microextraction to predict bioavailability and accumulation of
557 organic micropollutants in terrestrial organisms after exposure to a field-contaminated soil.
558 *Environmental Science & Technology* 38, 4842-4848.

559 Vasseur, P., Bonnard, M., 2014. Ecogenotoxicology in earthworms: A review. *Curr. Zool.* 60, 255-272.

560 Wang, C., Zhu, L.Z., Zhang, C.L., 2015. A new speciation scheme of soil polycyclic aromatic
561 hydrocarbons for risk assessment. *Journal of Soils and Sediments* 15, 1139-1149.

562 Wang, S., Li, X., Liu, W., Li, P., Kong, L., Ren, W., Wu, H., Tu, Y., 2012. Degradation of pyrene by
563 immobilized microorganisms in saline-alkaline soil. *Journal of Environmental Sciences* 24,
564 1662-1669.

565 Wu, J., Gong, Z., Zheng, L., Yi, Y., Jin, J., Li, X., Li, P., 2010. Removal of high concentrations of
566 polycyclic aromatic hydrocarbons from contaminated soil by biodiesel. *Frontiers of*
567 *Environmental Science & Engineering in China* 4, 387-394.

568 Yang, X., Lv, Z., Bian, Y., Wang, F., Gu, C., Song, Y., Jiang, X., 2013. Predicting PAHs bioavailability
569 for earthworms by mild solvents and Tenax extraction. *Journal of Environmental Chemical*
570 *Engineering* 1, 768-776.

571 You, J., Harwood, A.D., Li, H.Z., Lydy, M.J., 2011. Chemical techniques for assessing bioavailability
572 of sediment-associated contaminants: SPME versus Tenax extraction. *Journal of*
573 *Environmental Monitoring* 13, 792-800.