Tenax as sorption sink for in vitro bioaccessibility measurement of polycyclic aromatic hydrocarbons in soils

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A B S T R A C T

Physiologically based in vitro methods have been developed to measure bioaccessibility of organic contaminants in soils. However, bioaccessibility of hydrophobic organic contaminants (HOCs) can be underestimated by in vitro tests if gastrointestinal (GI) solution fails to provide sufficient sorption sink for HOCs. To circumvent this drawback, Tenax was included in GI solution as sorption sink to trap mobilized HOCs and maintain the desorption gradient between soil and GI solution. Polycyclic aromatic hydrocarbons (PAHs) were selected as target HOCs, and physiologically based extraction test (PBET) was selected as the in vitro method. Inclusion of Tenax in GI solution increased bioaccessibility of PAHs in five spiked soils from 8.25–20.8% to 55.7–65.9% and the bioaccessibility of PAHs in a field contaminated soil from 3.70–6.92% to 16.3–31.0%. Our results demonstrated the effectiveness of Tenax as sorption sink to enhance PAH mobilization in bioaccessibility measurement in soils.

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1. Introduction

Assessment of health risk posed by contaminated soil may be overestimated without considering contaminant bioavailability (Dean and Ma, 2007; Juhasz et al., 2014). In vivo methods using animal models can effectively measure contaminant bioavailability in soils, which is the fraction available for uptake into circulation system in humans. However, ethical consideration and high cost associated with animal models make them unsuitable for large scale measurement. Consequently, various in vitro gastrointestinal (GI) extraction methods have been developed to measure contaminant bioaccessibility, which is usually defined as the fraction of contaminants released from soil into simulated GI solution, and hence available for uptake by humans (Rodriguez and Basta, 1999; Ruby et al., 2002; Van de Wiele et al., 2004).

A good correlation between in vitro and in vivo results needs to be established before in vitro tests become valid. However, acceptable correlation (i.e., \( r^2 > 0.60 \)) is not always possible for hydrophobic organic contaminants (HOCs), partially because of the underestimation of HOC bioaccessibility by in vitro tests (Pu et al., 2006; Smith et al., 2012). Due to the presence of lipid membrane of intestinal cells, intestinal sorption continuously removes HOCs from the digestive fluid and therefore maintains a concentration gradient for further desorption (Vasiluk et al., 2007; Wang et al., 2011). However, in vitro methods are usually operated under static conditions without considering dynamic processes of intestinal sorption, leading to the underestimation of HOCs bioavailability. In a recent study, correlation between in vitro bioaccessibility and in vivo bioavailability based on swine model was improved from \( r^2 = 0.03 \) to \( r^2 = 0.45 \) after C18 membrane was added to GI solution as sorption sink to simulate dynamic uptake process by intestinal cells (James et al., 2011). The results indicate that inclusion of sorption sink in GI solution may be a promising approach to optimize in vitro methods for better prediction of HOC bioavailability.

It has been reported that part of dissolved HOCs in GI solution can be resorbed onto assimilated soil, leading to underestimated bioaccessibility since only dissolved HOCs was quantified as bio-accessible (Tao et al., 2009, 2010). For example, deuterated polycyclic aromatic hydrocarbons (PAHs) were spiked into GI solution to characterize the sorption by assimilated soil and to quantify the underestimation in bioaccessibility. It was found that PAH bioaccessibility was 70% and significantly higher than 47% when PAH re-sorption onto assimilated soil was not counted (Tao et al., 2010). As an alternative of using radio-labeled compounds, the authors
proposed the inclusion of sorption sink in GI solution to trap dissolved HOCs and to prevent them from being resorbed onto soil (Tao et al., 2010).

Clearly, inclusion of a sorption sink, which can provide sufficient sorption capacity for HOCs and fast trap of dissolved HOCs to prevent re-sorption onto assimilated soil, is essential for successful application of in vitro methods to measure HOC bioaccessibility in soils. Tenax, which is porous polymer resin and originally used as column packing material, has been widely used to investigate desorption kinetics of HOCs due to its infinite sorption capacity (Pignatello, 1990) and rapid scavenging of HOCs from aqueous phase (van Noort et al., 2003). Therefore, it can be expected that Tenax may be an ideal sorption sink to optimize in vitro methods for HOC bioaccessibility measurement in soils. Tenax is usually mixed with sediment slurry to construct desorption kinetics, which can be described by two-phase (or three-phase) compartment model for rapid and slow (or very slow) desorption pools (Cornelissen et al., 1997, 1998). Many studies have shown that the rapidly desorbed HOCs extracted by Tenax can be used to predict bioavailability and/or toxicity of sediment-associated HOCs to benthic organisms (Cui et al., 2013; You et al., 2011). The novelty in the current work was to expand the application of Tenax to the area of human bioaccessibility research by coupling physiologically based in vitro methods with Tenax. In other words, GI solution in vitro methods simulates the mobilization of human digestive tract, and Tenax serves as sorption sink to mimic dynamic process of intestinal sorption.

In this study, we explored the feasibility of Tenax as sorption sink to mimic dynamic process of intestinal sorption in human digestive tract and to enhance the mobilization of HOCs from soil. The most studied in vitro method, i.e., physiologically based extraction test (PBET, Ruby et al., 2002; Gouliarmou et al., 2013), was selected as the in vitro method with PAHs as the target compounds. The objectives of this work were to: 1) test Tenax as a suitable sorption sink by determining PAH sorption kinetics and capacity from GI solution; 2) measure PAH bioaccessibility in five artificially contaminated soils with the addition of Tenax in GI solution; and 3) determine PAH bioaccessibility in a naturally contaminated soil with the addition of Tenax in GI solution.

2. Materials and methods

2.1. Chemicals

Pyrene (purity >99.0%) was obtained from Sigma–Aldrich (St. Louis, MO, USA), and stock solution was made in methanol at concentration of 50 mg/l. Mixture standard of PAHs, including 16 PAH congeners listed by U.S. Environmental Protection Agency (USEPA) as priority contaminants, was purchased from Aldrich Industrial Corporation (Shanghai, China) and stock solution was made in methanol at concentration of 100 mg/l for each PAH congener.

The gastric and intestinal solutions for PBET were prepared according to Gouliarmou et al. (2013). Tenax TA (60–80 mesh) was purchased from Sigma–Aldrich. Before use, Tenax TA beads were cleaned by 10 ml hexane:acetone (v/v 1:1) in a sonicator for 5 min for three times. Other chemicals or solvents used were of high performance liquid chromatography (HPLC) or analytical grade.

2.2. Soil samples

Both spiked and field contaminated soils were used in the current study. Five pristine soils used for spiking included soils from Shenyang of Liaoning Province (SY), Lanzhou of Gansu Province (LZ), Jiyuan of Henan Province (JY1 and JY2), and Huangshi of HuBei Province (HS) in China. Soil samples were air dried and sieved to <2 mm and <250 µm for characterization and bioaccessibility study. The properties of these samples are shown in Table 1.

To generate samples for bioaccessibility test, aliquots of 0.2 g (dry weight) of the five soils were spiked with 40 µl pyrene stock solution to give an initial concentration of 10 mg/kg. The spiked soil samples were left in fume hood overnight until the solvent was completely evaporated. After that, the spiked soil (i.e., 0.2 g) was subjected to bioaccessibility test as described in Section 2.2.2. Other chemicals or solvents used were further incubated for 4 h, and supernatant was then obtained after centrifugation.

2.3. Sorption kinetics and capacity of Tenax

Before being used as sorption sink for in vitro test, the sorption kinetics and capacity of Tenax need to be tested. Kinetic tests were performed for intestinal solution not for gastric solution, since the hypothesis is that Tenax can be used to simulate the uptake of HOCs by lipid membrane of intestinal cells. Briefly, an aliquot of 20 ml intestinal solution was preheated to 37 °C in water bath and spiked with PAHs at concentration of 10 µg/l for each PAH congener. Aliquots of Tenax (0.25 g) were weighed into 50 ml of glass tubes and added with PAH-spiked intestinal solution. Since Tenax has similar sorption capacity as OC in soil or sediment (Cornelissen et al., 1997), 0.25 g Tenax was selected, which was 5 times of OC content in intestinal solution (i.e., 2182 mg/l) to ensure that sufficient sorption capacity can be provided by Tenax for PAHs to maintain concentration gradient. The glass tubes were then shaken at 150 rpm in an incubator at 37 °C for 5, 10, 20, and 40 min, and 1, 2, and 4 h with duplicates. At each time interval, Tenax was collected by centrifugation and filtration using qualitative filter paper. Tenax trapped in filter paper was air-dried and extracted by sonication using 10 ml of acetone for three consecutive times (Cui et al., 2010). The extracts from the same sample were combined and evaporated to near dryness on a rotary evaporator. The condensed residue was reconstituted in 2 ml of methanol, filtered through 0.22 µm filters into 2 ml amber HPLC vials and stored at –20 °C until analysis.

Sorption kinetics of PAHs by Tenax were fitted using the following equation:

$$F_{eq} = \frac{C_t - C_{eq}}{C_{eq}}$$

where $C_t$ is PAH mass sorbed by Tenax at time t, $C_{eq}$ is the initial PAH mass added to intestinal solution, $F_{eq}$ represents the fraction of PAHs sorbed at equilibrium and k is the rate constant.

2.4. Inclusion of Tenax for bioaccessibility measurement of PAHs in soils

Bioaccessibility of PAHs was measured using PBET according to Ruby et al. (2002) and Gouliarmou et al. (2013) with slight modifications. Briefly, an aliquot of 20 ml gastric solution (pH = 2.5) was placed into 50 ml glass tube and added 0.2 g of spiked soils or field contaminated soil at solid:solution ratio of 1:100. The tubes were shaken at 150 rpm in an incubator at 37 °C for 1 h. After that, the solution was converted to intestinal solution by adjusting pH to 7 and adding 0.035 g bile salts and 0.01 g pancreatin, and Tenax was added to serve as sorption sink. Meanwhile, parallel treatments without Tenax were included for comparison. These glass tubes were further incubated for 4 h, and supernatant was then obtained after centrifugation at 3000 rpm for 5 min. For treatment without Tenax, PAHs in supernatant were extracted by liquid–liquid extraction. Briefly, an aliquot of 10 ml supernatant was extracted with 10 ml dichloromethane in a 150 ml separatory funnel for three times. All the extracts were dried by filtration through anhydrous sodium sulfate and combined in 150 ml flask bottles. The pooled extracts were evaporated to near dryness on a rotary evaporator and reconstituted in 2 ml of methanol, which was filtered through 0.22 µm filters into 2 ml amber HPLC vials and stored at –20 °C until analysis. For treatment with Tenax, Tenax beads in supernatant were harvested by filtration using filter paper and washed three times by deionized (DI) water to remove small soil particles stick to the surface. Tenax trapped in filter paper was air-dried for overnight and extracted in the same way described above.

Bioaccessibility of pyrene (spiked soils) or PAHs (field contaminated soil) was calculated by the following equation:

$$\text{Bioaccessibility} = \frac{\text{in vitro pyrene/PAH}}{\text{total pyrene/PAH}} \times 100$$

where in vitro pyrene/PAH is the pyrene/PAH mass in GI solution, and total pyrene/PAH is the total mass of pyrene/PAHs in soil <250 µm.

Table 1 Physicochemical properties of soils used for spiked samples.

<table>
<thead>
<tr>
<th>Soil</th>
<th>pH</th>
<th>TOC (%)</th>
<th>CEC (mol/kg)</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SY</td>
<td>6.1</td>
<td>0.7</td>
<td>7.1</td>
<td>25.0</td>
<td>69.7</td>
<td>5.3</td>
</tr>
<tr>
<td>LZ</td>
<td>6.0</td>
<td>0.9</td>
<td>4.7</td>
<td>31.3</td>
<td>62.2</td>
<td>5.5</td>
</tr>
<tr>
<td>JY1</td>
<td>7.4</td>
<td>1.3</td>
<td>7.2</td>
<td>16.8</td>
<td>75.7</td>
<td>7.5</td>
</tr>
<tr>
<td>JY2</td>
<td>7.0</td>
<td>3.2</td>
<td>7.3</td>
<td>17.2</td>
<td>75.4</td>
<td>7.4</td>
</tr>
<tr>
<td>HS</td>
<td>7.9</td>
<td>1.6</td>
<td>8.2</td>
<td>31.8</td>
<td>61.8</td>
<td>6.4</td>
</tr>
</tbody>
</table>
2.5. PAH analysis

Analysis of PAHs was conducted on HPLC (Waters 2695, Ireland) coupled with XBridge™ C18 column (5 μm, 4.6 × 250 mm). Column temperature was set at 30 °C and mobile phase was 80:20 methanol: water with a flow rate of 1.0 ml/min. In series fluorescence spectrophotometer wavelength was programmed according to elution time, including excitation/emission at 260/430 nm for PYR, FLT, and CHR, and 297/430 nm for BbF, BKF, BaP, and BPY.

2.6. Quality assurance/quality control

The recovery efficiencies for PAHs were determined by spiking samples with standards of PAHs. For the 7 PAHs investigated in the current work, 53.3–75.3% were recovered by liquid–liquid extraction from PBET intestinal solution, and recovery efficiencies were 79.0–93.1% for soil samples. Pentachloronitrobenzene was included as a surrogate to monitor the extraction and cleanup procedures, and the efficiencies were 79.0–93.1% for soil samples. Pentachloronitrobenzene was included as a surrogate to monitor the extraction and cleanup procedures, and the detection limits for PAHs on HPLC were 0.10–0.42 μg/l. Reagent and procedure blanks were included in all tests, and there were no detection of PAHs in the blank controls.

3. Results and discussion

3.1. Sorption kinetics and capacity of Tenax

To serve as an effective sorption sink to enhance desorption of PAHs from soil into GI solutions, the sorption kinetics of PAHs by Tenax should be fast and shorter than the time span for GI incubation. In addition, Tenax should have sufficient sorption capacity to trap PAHs dissolved in GI solution, inducing a concentration gradient between soil and GI solutions. In this study, the sorption kinetics and capacity of PAHs by Tenax were tested by spiking 10 μg/l PAHs into PBET intestinal solution.

The sorption kinetic curves and simulated parameters (Eq. (1)) are shown in Fig. 1 and Table 2. In PBET intestinal solution, PAH sorption kinetics by Tenax was rapid, and the duration to reach 99% of equilibrium (5099) was within 19 min for all treatments, which was much shorter than the incubation time of 4 h. The sorption kinetics became slow with the increase of PAH hydrophobicity. For example, the shortest equilibrium time (5099) was observed for pyrene (PYR) at 4.61 min, and the longest was for benzo(a)pyrene (BPY) at 18.4 min (Table 2). The inverse relationship between sorption rate and PAH hydrophobicity observed for Tenax was comparable to that for silicone rod, which was used as sorption sink in GI solution (Goularmou et al., 2013). In their study, the sorption kinetics of 6 PAH congeners (naphthalene, phenanthrene, anthraene, PYR, FLT, and BaP) by silicone in GI solution were investigated, and the longest 5099 was observed for the most hydrophobic congeners, i.e., BaP at 4.1 and 1.8 h in gastric and intestinal solutions. The slower sorption kinetics for PAHs with high KOW can be attributed to the relatively slower diffusion rates of more hydrophobic PAHs in GI solution. This is supported in a previous study, in which the diffusion rates of PAHs in gut digestive fluid from deposit-feeding polychaete decreased from 0.12 h⁻¹ for naphthalene to 0.01 h⁻¹ for BaP (Mayer et al., 2007).

In addition to rapid sorption kinetics, the high sorption capacity of Tenax for PAHs was also confirmed. At equilibrium, more than 87% of PAHs was sorbed by Tenax in all treatments (Table 2). The sorption capacity of Tenax as sorption sink was in agreement with previous report that a composite of silicone and activated carbon was used as diffusive sink to induce desorption of PAH from soils into gastric solution, and more than 90% of spiked PAHs in gastric solution were sorbed by the sorption sink after 1 h incubation (Collins et al., 2013). It may be concerned that the low spiking concentration (10 μg/l) here is not sufficient to demonstrate the sorption capacity of Tenax. However, in our preliminary test, a higher concentration (75 μg/l) of pyrene was spiked into the intestinal solution, and the sorption kinetics was investigated in the same way. It was found that 89 ± 2.9% of pyrene was trapped by Tenax when reaching equilibrium (Supporting Information, Figure S1), which was not significantly different from 87 ± 2.2% at spiking concentration of 10 μg/l (Table 2). This may demonstrate that Tenax can provide high sorption capacity, which is independent of the initial concentrations of contaminants in intestinal solution.

Sorption kinetic test showed that 0.25 g of Tenax provided fast sorption rate (<19 min) and high sorption capacity (>87%) for PAHs in intestinal solution, which ensured that the concentration gradient between the soil matrix and intestinal solution can be

![sorption kinetic curves of PAHs by Tenax in intestinal solution of physiologically based extraction test (PBET).](image)

Table 2

Simulated parameters (based on Eq. (1)) from sorption kinetic curves of PAHs in intestinal solution of physiologically based extraction test (PBET).

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>Log KOWx</th>
</tr>
</thead>
<tbody>
<tr>
<td>PYR</td>
<td>0.99</td>
<td>5.18</td>
</tr>
<tr>
<td>FLT</td>
<td>0.99</td>
<td>5.22</td>
</tr>
<tr>
<td>CHR</td>
<td>0.99</td>
<td>5.91</td>
</tr>
<tr>
<td>BbF</td>
<td>0.99</td>
<td>6.12</td>
</tr>
<tr>
<td>BKF</td>
<td>0.99</td>
<td>6.12</td>
</tr>
<tr>
<td>BaP</td>
<td>0.99</td>
<td>6.50</td>
</tr>
<tr>
<td>BPY</td>
<td>0.99</td>
<td>6.58</td>
</tr>
</tbody>
</table>

x Tang et al., 2006.
maintained for further desorption. Therefore, Tenax at 0.25 g was used as the sorption sink in later experiment.

### 3.2. Inclusion of Tenax for bioaccessibility measurement in spiked soils

In the present study, five soils spiked with pyrene were subject to PBET extraction with or without Tenax (Fig. 2). Pyrene bioaccessibility without Tenax were 8.25–20.8% (averaging at 14.6%) in the five soils. When added with 0.25 g Tenax, significantly more pyrene was mobilized into GI solution for all five soils. Pyrene bioaccessibility increased to 55.7–65.9% (averaging at 61.7%), ~4.2 folds higher than that without Tenax, which demonstrated the effectiveness of Tenax as sorption sink to enhance pyrene mobilization from soils into GI solution. However, spiked soils are different from field contaminated soils due to the lack of aging effect of PAHs as well as the co-existence of other contaminants. Therefore, the feasibility of Tenax as sorption sink needs to be tested with field contaminated soil.

To better understand the impact of soil property on bioaccessibility of PAHs, bioaccessibility of PAHs (both with and without Tenax) were correlated with OC and clay contents, which are often considered as the most important properties governing bioaccessibility of PAHs (Rostami and Juhasz, 2011). There was a general inverse relationship between bioaccessibility and OC as well as clay contents (Fig. 3) but correlation was insignificant ($r = 0.22–0.75$ and $p = 0.14–0.72$). The insignificant correlation in the current study may be due to the limited number of soil samples (only 5 soils). In addition, the compositions of soil OC can be different among the soils, which are also important for binding of HOCs in soils (Cui et al., 2013; Semple et al., 2013).

### 3.3. Inclusion of Tenax for bioaccessibility measurement in field contaminated soil

In addition to spiked soils, the inclusion of Tenax to measure PAH bioaccessibility was also applied to a field contaminated soil. The comparison between treatments with and without Tenax for PAHs is shown in Fig. 4. Inclusion of Tenax in PBET GI solution generally enhanced PAH bioaccessibility. Bioaccessibility of PAHs without Tenax was 3.70–6.92%, and increased to 16.3–31.0% when added with Tenax. When compared based on the average of all the PAH congeners, PAH bioaccessibility increased from 4.90% to 21.4%, i.e., 4.4 folds when Tenax was added into the PBET GI solution.

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**Fig. 2.** Pyrene bioaccessibility in five spiked soils (SY, LZ, JY1, JY2, and HS) using PBET with and without Tenax.

**Fig. 3.** Relationships among the soil organic carbon (OC) content, clay content, and pyrene bioaccessibility by PBET extraction with or without Tenax in five spiked soils.

**Fig. 4.** Bioaccessibility (%) of PAHs (pyrene (PYR), fluoranthene (FLT), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BKF), benzo(a)pyrene (BaP), and benzo(ghi)perylene (BPY)) in a field contaminated soil using PBET with and without Tenax.
4.4-fold increase in PAH bioaccessibility in the current study was comparable to Collins et al. (2013) where PAH bioaccessibility was increased by a factor of 1.3–3.1 when a composite of silicone and activated carbon was used as sorption sink. In a different study, PAH released from field contaminated soils into GI solution increased from 6.6 ± 4.1 ng to 0.29 ± 0.16 μg Bp equivalents when C18 membrane was included as sorption sink (James et al., 2011). If compared with mean values, about 44-fold increase of PAH mobilization was observed at the addition of C18 membrane. However, this does not mean C18 membrane is more effective than Tenax as sorption sink, because (1) the comparison based on the mean values (i.e., 6.6 ng vs 0.29 μg) was inappropriate due to the large variation associated with the mean values, and (2) direct comparison between James et al. (2011) and the current study was not doable, since PAH bioaccessibility in James et al. (2011) was expressed by BaP equivalents instead of PAH concentrations like this study. Nevertheless, the current result demonstrated that Tenax, similar to composite of silicone and activated carbon (Collins et al., 2013; Gouliarmou et al., 2013) and C18 membrane (James et al., 2011), can act as an effective sorption sink in in vitro methods to enhance PAH mobilization from contaminated soils.

The effect of Tenax on mobilization of pyrene was compared between contaminated soil and spiked soils with similar OC contents (i.e., 11% of field soil vs 0.9% and 1.3% for LZ and JY1 soils). The effect of Tenax was less pronounced for the contaminated soil than that for spiked soils. For instance, pyrene bioaccessibility increased 3.6-folds for the contaminated soil, while the increase for LZ and JY1 soils were 5.3- and 5.0-folds with Tenax. The less impact by Tenax in contaminated soil was mainly due to the aging effect of PAHs in the field. Tenax-assisted desorption of PAHs usually exhibits a biphasic behavior, including an initial phase of rapid desorption followed by a slow desorption (Cornelissen et al., 1997). The HOCs desorbed in the first 6–30 h can generally be considered from the rapid desorption pool (Oen et al., 2006; Yang et al., 2008). Prolonged contact time between HOCs and soil (i.e., aging effect) reduces the magnitude of both the rapidly desorbing pool and desorption rate (Cornelissen et al., 1998; Morrison et al., 2000). Pyrene trapped by Tenax in the current study can be expected to be from rapid desorption pool, because digestive incubation time span for PBET was 5 h. Consequently, pyrene collected from Tenax was less for field soil than that for spiked soil due to the aging effect, explaining the less pronounced impact of Tenax on pyrene bioaccessibility enhancement in the field contaminated soil compared with spiked soils.

The effect of Tenax on PAH bioaccessibility enhancement was also investigated based on the relationship between bioaccessibility ratios of with and without Tenax and Log $K_{ow}$. As shown in Fig. 5, the ratios, even though not strongly ($p = 0.25$ and $r = 0.5$), generally decreased with the increase of Log $K_{ow}$, indicating that the influence of Tenax was less pronounced for the more hydrophobic PAHs. On contrast, more pronounced effect of bile on improving bioaccessibility of PAHs with higher $K_{ow}$ has been reported by Tang et al. (2006) and Kang et al. (2013). Though both Tenax and bile help mobilization of PAH into GI solution, the rate limiting factor of the two may be different. For bile, PAH solubility in GI solution was increased by PAH incorporation into micelles formed by bile (Tang et al., 2006). For PAHs with lower $K_{ow}$ which have relatively higher solubility, the effect of bile will be offset to some extent by the higher solubility. On the other hand, Tenax as sorption sink to maintain PAH-free condition in GI solution allows PAHs to desorb from soil in proportion to its diffusion and binding limitations (You et al., 2007). In other words, when Tenax is included in GI solution, the rate limiting factor is the kinetics of PAH sorption by Tenax after PAH desorption from soil into GI solution as well as PAH desorption from soil. As indicated in Table 2, the sorption rate constant ($k$) decreased with the hydrophobicity of PAHs, explaining why the effect of Tenax was less pronounced for PAHs with high hydrophobicity. As for the desorption of PAHs from soil, more hydrophobic contaminants tend to be bound to soil organic carbon stronger than less hydrophobic ones through $\pi-\pi$ and hydrophobic interactions (Pignatello and Xing, 1996), leading to slower desorption rate and less rapid desorption pool of organic contaminants with high hydrophobicity (de la Cal et al., 2008). Consequently, less desorption in GI solution and therefore less pronounced effect of Tenax was found for more hydrophobic PAHs.

3.4. Risk assessment implications

In the current study, addition of Tenax as sorption sink in GI solution significantly increased the amount of PAHs mobilized from soils, suggesting that inclusion of sorption sink in in vitro method may be necessary to accurately estimate PAH bioaccessibility in contaminated soils. This is due to the hydrophobicity of PAHs and their strong binding with soils, and may be also applicable to other HOCs like organochlorine pesticides and brominated flame retardants in soils. Similar with the analytical advantage offered by previously reported sorption sink, e.g., C18 membrane in James et al. (2011) and silicon rod in Collins et al. (2013) and Gouliarmou et al. (2013), a simple back-extraction was also provided by Tenax without extra filtration or purification prior to analysis for PAHs.

It is possible that addition of sorption sink (i.e., Tenax) may create a concentration gradient more than the actual GI sorption. However, such a positive bias leads to more conservative bioaccessibility estimation, which is acceptable in term of risk assessment. However, it is still too early to conclude that inclusion of Tenax as sorption sink can improve the accuracy for bioaccessibility measurement, because the in vitro results need to be validated against in vivo animal models. However, there is some technical barrier to measure PAH in vivo bioavailability due to the complexity of metabolism, distribution and excretion of PAHs in animals. There are two pathways for PAHs to go after being absorbed by the intestinal epithelium, i.e., entering hepatic portal circulation or being transported to the liver (Jubas et al., 2014). Due to biliary excretion, some degraded PAHs in the hepatic portal system may not reach the circulation system, which will not be considered as bioavailable. On the other hand, some degraded...
PAHs, which enter the circulation system, won’t be counted as bioavailable if only the parent compounds are determined. Approaches using various biological endpoints, such as blood, urine, adipose tissue, or faecal, have been adopted to circumvent those constraints (Gron et al., 2007; James et al., 2011; Reeves et al., 2001), but all biological endpoints have limitations. However, it should be kept in mind that the difficulty to obtain accurate in vivo data is specific for PAHs, in vitro methods with inclusion of Tenax can be correlated with in vivo data for other HOCs, which is highly needed in the future investigations.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2014.09.016.

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