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

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ABSTRACT

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.

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 bioaccessible (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

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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 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 Aladdin 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 five soil samples are shown in Table 1.

Table 1
Physicochemical properties of soils used for spiked samples.

Soil	pH	TOC (%)	CEC (mol/kg)	Sand (%)	Silt (%)	Clay (%)
SY	6.1	0.7	7.1	25.0	69.7	5.3
LZ	6.0	0.9	4.7	31.3	62.2	6.5
JY1	7.4	1.3	7.2	16.8	75.7	7.5
JY2	7.0	3.2	7.3	17.2	75.4	7.4
HS	7.9	1.6	8.2	31.8	61.8	6.4

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 subject to bioaccessibility test as one treatment. The field contaminated soil was collected from PAH-contaminated agricultural land at Wuxi, Jiangsu, China. Organic carbon (OC) content and pH value of the soil were 1.11% and 6.4, respectively (Sun et al., 2013). Though concentrations of 16 USEPA priority PAHs were measured, only concentrations of 7 PAHs in both soil and GI solution were well above detection limits. In order to ensure the reliable analysis results, only the 7 PAHs were investigated in this study. Concentrations (dry weight) of the 7 PAHs in soil <250 μm were 609 μg/kg (pyrene, PYR), 818 μg/kg (fluoranthene, FLT), 282 μg/kg (chrysene, CHR), 491 μg/kg (benzo[b]fluoranthene, BbF), 219 μg/kg (benzo[k]fluoranthene, BKF), 391 μg/kg (benzo(α)pyrene, BaP), and 551 μg/kg (benzo(ghi)perylene, BPY).

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 (IKA®RV10, Germany). 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:

$$C_t = C_0 \cdot F_{eq}\left(1 - e^{-kt}\right) \quad (1)$$

Where C_t is PAH mass sorbed by Tenax at time t, C_0 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 as described above.

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

$$\text{Bioaccessibility}\ (\%) = \left[\frac{\text{in vitro pyrene/PAH}}{\text{total pyrene/PAH}}\right] \times 100 \quad (2)$$

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.