Introduction

Pyrene belongs to polycyclic aromatic hydrocarbons (PAHs) that are common environmental contaminants arising from a variety of sources, including fossil fuel combustion, oil spills and industrial processes. Pyrene and other PAHs are considered potential human carcinogens (Mahadevan et al., 2005). The European Union, Taiwan and the United States have identified them as priority pollutants.

The degree of subsurface PAH contamination is increased because of their adsorption on sediments and soils. When bioremediation is used to clean up PAH contamination in soils, availability of PAH in water-soluble forms is most advantageous because most microorganisms require high water activity for active metabolism (Maier et al., 2000). Therefore, contaminants that rapidly disappear during bioremediation are characterized as having adequate bioavailability and biodegradability. Among the several definitions of bioavailability adopted in different scientific fields, the definition that suits this study adequately is the one by Cuypers (2001), quoted below: "bioavailability is the ability of a contaminant to desorb to the aqueous phase, with the time frame of an experiment, under infinite dilution conditions."

Reliability of solid phase microextraction in estimating bioavailability of pyrene in soil

Abstract

Solid phase microextraction (SPME) coupled with gas chromatography was employed to estimate bioavailability of pyrene in soils with different properties of textures, organic matter contents (SOM) and aging periods. Experimental results indicated that biodegradation rates increased from 0.10 (sandy loam) to 0.15 (silty loam) µg g⁻¹ hr⁻¹. By contrast, biodegradation rate decreased from 0.10 (1.3% SOM) to 0.04 (7.6% SOM) µg g⁻¹ hr⁻¹. The amounts of pyrene biodegraded decreased 27% when SOM was modified from 1.3 to 7.6%, indicating that distributions of pyrene in soils at biodegradation end points were affected by the SOM. Sequestration as measured by sonication extraction had evidently occurred in aged soil samples. SPME measurements slightly overestimated the amount of pyrene degraded by indigenous and seeded microorganisms, in soils with the different properties (correlation coefficient, R² = 0.74). The present study demonstrates that the SPME method cannot replace biodegradation tests commonly used for predicting bioremediation efficacy.

Key words

SPME, Gas chromatography, Bioavailability, Pyrene, Soil
Bioavailability of organic contaminants in soils is evaluated using a number of chemical methods (Reid et al., 2000; Cuypers et al., 2002). Exhaustive extraction of contaminants by strong acids, chelating agents, and solvents is a common practice. Despite the efficiency of these extraction methods, certain limitations may still persist because these methods usually cause destruction, alteration and swelling, all of which adversely impact the soil structure. In addition, contaminant bioavailability is often overestimated because the exhaustively extracted amounts are markedly higher than those that can be biodegraded by microorganisms, i.e. a high bioavailable fraction may result.

To correct the overestimation of exhaustive extraction, alternative non-exhaustive (mild) extraction methods have emerged (Kelsey et al., 1997; Mayer et al., 2000; Parkerton et al., 2000; Reid et al., 2000; Cornelissen et al., 2001; Cuypers et al., 2002). Non-exhaustive extraction procedures thus allow a more appropriate evaluation of contaminant transfer along a biodegradation pathway (Alexander, 1995). Among these non-exhaustive procedures is solid phase microextraction (SPME) developed by Arthur and Pawliszyn (1990). The merits of SPME over conventional extraction procedures have been demonstrated (Pawliszyn, 1997). More recently, complex hydrocarbon mixtures of toluene, o-xylene, and 4 polycyclic aromatics were tested using SPME to estimate toxicity to rainbow trout (Parkerton et al., 2000). According to their results, the fractions extracted from aqueous samples by SPME correlated with the amounts that triggered acute toxicity. Additionally, the optimal conditions of SPME for extracting commercial diesel fuel from soil have been described (Cam and Gagni, 2001).

Although their use may be more appropriate than exhaustive extraction for contaminated soils, non-exhaustive methods may still inadequately mimic the processes inherent to bioavailability. A feasible means to mimicking bioavailability must be developed to correlate with uptake or degradation by microorganisms.

This study was to validate SPME for its ability to correlate with bioavailability to microorganisms capable of degrading pyrene in soil solutions. Various soil textures, organic matter contents (SOM) and aging periods were also investigated for their influences on the method.

**Materials and Methods**

**Preparation of soil samples**: The target pollutant was pyrene. Pyrene was added to soil in bottle reactors. The soils with varied properties in textures, SOM and aging with pyrene were evaluated with the extraction of pyrene using SPME. Evaluations were performed on total pyrene, extracted pyrene from aqueous solutions by SPME and residual pyrene that remained following bioremediation assays. The amounts extracted by SPME were correlated with the amounts degraded by indigenous and seeded microorganisms.

A paddy field soil matrix was collected from below the root zone (15-30 cm) in Chang Hua County, Taiwan. This soil was air-dried for 3 days (water content < 3%, w/w) and passed through a 0.42 mm mesh sieve to remove roots and other vascular materials and characterized as sandy loam with an organic carbon content (SOM) of 1.3% (w/w) and pH=7.7 (hereafter denote as the original soil).

The effects of soil properties on pyrene bioremediation/bioavailability were studied by amending the original soil. The amendment materials were a silt/clay mixture and a bagasse compost, for changing the soil texture and SOM, respectively. The silt/clay mixture was made by sieving the original soil with a 0.075 mm mesh sieve. Loam soil was then obtained by adding the silt/clay mixture to the original with a ratio of 1 to 4 based on dry weight, and a silty loam soil 2 to 3. The bagasse compost was sieved with a 0.42 mm mesh sieve and added to the original soil, resulting in the soils having a SOM of 5.2 and 7.6%. All differently prepared soil samples were then sterilized by 25 kGy γ-irradiation for 30 min. by using a 60Co source (China Biotech, Taichung, Taiwan) and storing them in dark at 4°C before use. For aging experiments, some of the sterile, original soil was spiked with pyrene (described below) and air-tight sealed and stored for 6, 10, and 14 months.

A stock solution of pyrene (Aldrich, purity > 98%), 5,000 mg l⁻¹, was made by dissolving 0.2 g pyrene in 40 ml acetone. The sterilized soil samples were spiked aseptically with the pyrene stock solution to a final concentration of 100 µg pyrene g⁻¹ soil. The pyrene-spiked soils were agitated on an end-over-end shaker for 24 hr to ensure thorough mixing, followed by vacuuming to let pyrene transfer into the soils and place it in a fume hood; acetone was then allowed to evaporate. Triplicate samples of the soils were measured for SPME and biodegradation by seeded and indigenous microorganisms.

**SPME procedures and GC analysis**: The direct SPME method used in this study was adopted and modified from Pawliszyn (1997). Portable field samplers with SPME fibers of 10 mm length with a 100 µm PDMS coating were purchased from Supelco (cat. no. 504823; Bellefonte, CA). The volume of the fiber coating was 0.612 µl (Supelco). After 36 hr of the soil spiking, the fiber was inserted (30 min.) into the soil bottle through a syringe needle that pierced the septum. Finally, the extraction was terminated by retracting the fiber through the septum. The fibers were immediately introduced into an injector of a gas chromatography (GC, Agilent 6890N) for thermal desorption. The fibers entered the injector through a needle that pierced the injector septum and stayed in the injector for 3 min. desorption. The GC was equipped with a flame ionization detector (FID) and a capillary column (DB-5, 30 m×0.32 mm ID×1.0 µm film thickness, J and W Scientific) was used with pure nitrogen as carrier gas at 50 psi. The temperatures of the injector and the detector were 270 and 300°C, respectively. The oven temperature program was initiated at 120°C, and increased to 300°C at a rate of 20°C min⁻¹, and held for 4 min. Retention times of 1-methyl-Nap (as internal standard) and pyrene were 2.56 and 10.87 min., respectively, and the method detection limit (MDL) was 1 µg l⁻¹ (S/N > 3).
SPME in estimating bioavailability of pyrene in soil

Bioremediation assays and sonication extraction: The bioavailable fractions of pyrene in all aged, fresh, and texture-/SOM-amended and unamended soil samples were determined by biodegradation tests using batch-wise assays conducted over a period of approx. 1200 hr. Either indigenous or seeded (pyrene-acclimated) microorganisms adjusted to $3 \times 10^6$ CFU g$^{-1}$ soil were added to all soil samples. The indigenous were obtained from the original soil following the method reported elsewhere (Nikiema et al., 2005). The seeded microorganisms, taken from a chemostat fed with pyrene, consisted of Pseudomonas rhodesiae, Rhodococcus ruber, and Serratia marcescens. The non-added served as the control.

Pyrene residues in bioassay samples were extracted by sonication (Misonix, sonicator 3000) following the method NIEA 167.00C provided by the Environmental Protection Administration of Taiwan (2002).

Results and Discussion

Effects of soil properties on pyrene degradation: The soil samples differed in textures, SOM and aging time were compared with respect to their effects on pyrene degradation by the indigenous or the seeded cultures. Fig. 1 and 2 respectively represent the pyrene degradation in the soils with various textures and SOM. With significant lag periods, pyrene was more slowly degraded in both the indigenous and seeded soil. This was also the case for the soils with more SOM (Fig. 2). Overall biodegradation rates of pyrene in the first stage (approx. 400 hr) slightly increased from 0.10 (sandy loam) to 0.15 (silty loam) µg g$^{-1}$ hr$^{-1}$ for the seeded. Zhang and Young (1997) indicated that Nap degradation is faster in soils containing more fines. Since the initial concentrations of microorganisms were the same, the faster degradation rate observed in this study can only be attributed to the higher specific surface area of the finer soil (Mott 1990) instead of the higher microbial concentration that prevailed in finer soil (Hall et al., 2005).

For both the indigenous (Fig. 2A) and seeded soils (Fig. 2B), pyrene degradation was slower when the SOM was higher; the biodegradation rate in the first stage decreased from 0.15 (sandy loam) to 0.03 (silty loam) µg g$^{-1}$ hr$^{-1}$ for the indigenous and 0.10 µg g$^{-1}$ hr$^{-1}$ for the seeded.
Fig. 4: Effects of aging on extractability of Pyrene in original soil

Fig. 5: Correlation between degraded Pyrene by indigenous (○) and seeded microorganisms (●) and aqueous Pyrene estimated by SPME. Dotted line represents a 1:1 slope. Error bars represent one standard deviation (SPME extraction, n = 3; biodegradation, n = 2–6)

(sandy loam) to 0.04 (silty loam) µg g⁻¹hr⁻¹ for the seeded. This finding suggests that more pyrene could have portioned into the soil with higher SOM.

The total amounts of the biodegraded pyrene were not significantly different in the soils with different textures for both the indigenous and the seeded (Fig. 1). With respect to SOM effects, however, the total amounts of the biodegraded pyrene in the soil with SOM of 7.6% decreased 26% (the indigenous) and 27% (the seeded) of the original (SOM 1.3%), indicating that distributions of pyrene in the soils at biodegradation end points were strongly affected by the SOM. The big differences in the initial concentrations (Fig. 2) further suggested that pyrene could have portioned into bagasse compost.

Fig. 3 illustrates the final distributions of pyrene in the seeded soils with different textures and SOM. Again, we can clearly see that the SOM had more adverse effects on the bioavailability to microbial degradation. The huge portion (56.1%) of the non-extractable pyrene in the soil with an SOM of 7.6% indicates that pyrene was stably retained in the soil. In line with this, although higher SOM results in lower bioavailability, leading to poor bioremediation efficacy, addition of natural organic sorbent like bagasse compost to contaminated soils may be advantageous in reducing risks of soil contamination (Tang et al., 2007, 2008).

Fig. 4 indicates that the sequestration of pyrene as measured by sonication extraction at time intervals (6, 10 and 14 months) significantly occurred. The extracted amounts showed statistical difference from each other. Notably, the SOM in the aging experiment was merely 1.3%. Therefore, we can infer that sequestration could occur in the soils with a higher SOM (Cornelissen et al., 1998; Nam et al., 1998; Chung and Alexander, 2002).

Estimation of pyrene bioavailability by SPME: A previous study has demonstrated that SPME is a promising method to estimate the bioremediation efficacy of Nap-contaminated soils with various properties (Liu et al., 2010). However, Fig. 5 reveals that the SPME measurements tended to overestimate the amount of pyrene degraded by indigenous and seeded microorganisms in soils with different properties. By using XAD-2, Lei et al. (2004) estimated PAH bioavailability to benthic organisms, indicating the extraction tended to overestimate the extent of the biodegradation of some higher ring compounds as observed. The authors concluded that the bioremediation of those higher ring PAHs is often limited by their resistance to degradation rather than their bioavailability. In the present study, the seeded microorganisms were taken from a chemostat fed with pyrene (pyrene-acclimated), consisting of Pseudomonas rhodesiae, Rhodococcus ruber, and Serratia marcescens that are known degraders of aromatic compounds. Therefore, this study agrees that pyrene might be bioavailable in terms of their accessibility to the aqueous phase but are not susceptible to biodegradation under the experimental conditions. It is very likely that some unknown microbial factors, such as lack of cometabolic substrates, limit pyrene degradation (Huesemann et al., 2004).

SPME is a more convenient method with a lower cost than XAD-2, but bioavailability of pyrene was slightly overestimated in this study. Experimental results obtained in this study clearly demonstrate that SPME can not replace traditional, time-consuming biodegradation tests for predicting bioremediation efficacy of high ring PAHs contaminated soil.

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References


