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Biomimetic accumulation of PAHs from soils by triolein-embedded cellulose acetate membranes (TECAMs) to estimate their bioavailability

Yuqiang Tao^a, Shuzhen Zhang^{a,*}, Zijian Wang^a, Runhui Ke^a,
Xiao-quan Shan^a, Peter Christie^b

^aState Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China

^bAgricultural and Environmental Science Department, Queen's University Belfast, Newforge Lane, Belfast BT9 5PX, UK

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ABSTRACT

A new passive sampling device (PSD), a triolein-embedded cellulose acetate membrane (TECAM), was used to biomimetically accumulate naphthalene, phenanthrene, pyrene and benzo[a]pyrene from ten spiked soils and a soil spiked with different concentrations of these polycyclic aromatic hydrocarbons (PAHs). TECAM exposure conditions were optimized. The quantities of PAHs accumulated by TECAMs were positively and linearly related to their concentrations in the soil. PAHs accumulated by TECAMs were negatively related to soil organic matter (SOM) and positively related to the dissolved organic carbon (DOC) according to the results obtained from ten spiked soils. Aging time (1–150 days) had a significant effect on accumulation of PAHs by TECAMs. A good linear relationship ($R^2 = 0.970\text{--}0.993$) was observed between TECAM-accumulated PAHs and PAHs accumulated by earthworms (*Eisenia andrei*). The data indicate that TECAM represents a useful surrogate to estimate the bioavailability of PAHs and perhaps other hydrophobic organic contaminants (HOCs) in soils.

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1. Introduction

Hydrophobic organic contaminants (HOCs) such as polycyclic aromatic hydrocarbons (PAHs) in soils are a major issue of concern. Bioavailability is an important factor in risk assessment and depends on soil characteristics such as pH, organic matter content and moisture, chemical properties of the contaminants and aging time of the contaminants in soil (Alexander, 2000).

Several approaches have been developed to estimate bioavailability of HOCs in soils. The assessment may be performed by direct exposure of various organisms to soils with subsequent determination of contaminant uptake or

degradation (Semple et al., 2003). However, biological assays are often time-consuming and expensive and lack the precision required for regulatory purposes (Liste and Alexander, 2002). Chemical extraction methods have been proposed and applied to measure the bioavailable fraction of HOCs in soils (Alexander, 1995; Kelsey et al., 1997; Tang et al., 2002). Harsh, vigorous extraction procedures may remove a large proportion of a contaminant from soils, resulting in an overestimation of bioavailability (Reid et al., 2000a; Tang et al., 2002). Some authors (Kelsey et al., 1997; Reid et al., 2000a,b; Liste and Alexander, 2002) have proposed the use of more gentle and selective extractants, such as ethanol, ethanol-water, methanol-water, *n*-butanol, hexane, cyclodextrin or

*Corresponding author. Tel.: +86 10 62849683; fax: +86 10 62923563.

E-mail address: szzhang@rcees.ac.cn (S. Zhang).

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organic acids, to predict bioavailability of HOCs in soils. However, it is unrealistic to expect to predict bioavailability of different HOCs using a range of sorbents under a wide range of conditions simply by using a single chemical extractant (Alexander, 2000; Reid et al., 2000a). Furthermore, chemical extraction methods are usually time-consuming, expensive and of relatively low precision.

Passive sampling devices (PSDs) such as semi-permeable membrane devices (SPMDs) (Huckins et al., 1990), solid-phase microextraction (SPME) (Conder et al., 2003; Van Der Wal et al., 2004) and C₁₈ sampling bags (Awata et al., 2000) or disks (Krauss and Wilcke, 2001), have been used to measure bioavailability of organic contaminants in soils and sediments. The triolein-containing SPMDs developed by Huckins et al. (1990) are commonly used for sampling HOCs in water (Huckins et al., 1999), air (Söderström et al., 2005) and sediments (Rantalainen et al., 2000). SPMDs have attracted great interest as potential biological surrogates to evaluate the availability of chemicals to organisms and have been successfully applied to estimate bioavailability of HOCs to aquatic organisms (e.g. fishes and mussels) (Verweij et al., 2004; Gourlay et al., 2005). However, their application in terrestrial environments (including soils) is relatively new and very limited applications of SPMDs have been reported to sample or monitor HOCs in soils (Rantalainen et al., 1998; Ahn et al., 2005). Although SPMEs have been more widely used to predict bioavailability of HOCs in soils than other PSDs, disadvantages have been pointed out related to their fiber stability (physical or chemical) and sensitivity (Hernandez et al., 2000). C₁₈ sampling bags (Awata et al., 2000) or disks (Krauss and Wilcke, 2001) have also been reported to measure bioavailability of organic contaminants in soil, but the sampling was conducted at 40 °C for 15 days. Moreover, since the volume of the disk and the corresponding volume of the hydrophobic C₁₈-material are much larger than that of SPMD or SPME fiber, when it is used in soils a high soil water holding capacity is required to avoid depletion, which exceeds the normal moisture content of field soil (Verhaar et al., 1995).

A new type of composite membrane, the triolein-embedded cellulose acetate membrane (TECAM) in which triolein drops are embedded in the matrix of cellulose acetate polymers, was recently developed by Wang and co-workers (Xu et al., 2005). TECAMs have been successfully used to sample HOCs in water and have shown a much shorter uptake equilibrium time than SPMDs made of low-density polyethylene membranes (Xu et al., 2005; Ke et al., 2006) because cellulose dialysis membranes have hydrophilic hydroxyl groups.

In the present study, TECAMs were used to biomimetically accumulate naphthalene (Nap), phenanthrene (Phe), pyrene (Pyr) and benzo[a]pyrene (BaP) from ten spiked soils and a spiked soil with different concentrations of these PAHs. TECAM exposure conditions were optimized. Relationships between the accumulation of PAHs by TECAMs and concentrations and aging time of the PAHs in the soil and soil properties were investigated. In addition, accumulation of PAHs by TECAMs and earthworms (*Eisenia andrei*) were compared to evaluate the possibility of using TECAM as a surrogate to estimate bioavailability of PAHs in soils.

2. Materials and methods

2.1. Chemicals, reagents, TECAMs and earthworms

Nap, Phe, Pyr and BaP of >99% purity were purchased from Acros Organics (New Jersey, USA) and used for identification and quantification. All solvents used, i.e. *n*-hexane, dichloromethane and methanol, were of HPLC grade.

TECAMs were prepared according to Xu et al. (2005). They were made of cellulose acetate (17.5 wt%, Eastman, USA), acetone (69 wt%), 1,4-dioxane (10 wt%), anhydrous magnesium perchlorate (2 wt%) and triolein (1.5 wt%, Sigma, USA). All the membranes were kept in distilled water before use.

The earthworm species used was *Eisenia andrei*. The individuals were of similar weight (fresh weight about 0.15–0.20 g) and size (about 4–5 cm) and had 79±6.1 wt% moisture content and a lipid content of 3.08±0.61% (mean±SD, *n* = 15) on a dry-weight basis. They were allowed to acclimatize to laboratory conditions for at least 2 weeks and were analyzed for PAH residues before use.

2.2. Soil preparation

Samples of the ten soils used in this study were collected from the top 20 cm of the soil profiles of agricultural fields in different provinces or cities in China. Each soil sample was air-dried, passed through a 2 mm sieve and sterilized by γ -irradiation from a ⁶⁰Co source. Soil organic matter (SOM) content was determined using the Walkley–Black method (Nelson and Sommers, 1982). Dissolved organic carbon (DOC) was determined with a total organic carbon analyzer (Phoenix, 8000, Tekmar-Dohrmann Co., USA) after shaking 1 g of soil in 30 ml deionized water for 24 h, centrifuging at 7000g for 20 min and filtering with a 0.45 µm cellulose nitrate membrane. Selected soil properties are listed in Table 1.

Beijing soil was spiked with Nap, Phe, Pyr and BaP in acetone to produce a series of initial concentrations (0.2, 0.5, 2.0, 5.0 and 10.0 µg g⁻¹), and the other nine soils were spiked with one concentration of 5 µg g⁻¹ only. Each spiked soil was mixed thoroughly to distribute the chemicals uniformly and to allow the solvent to evaporate and then stored in brown glass containers at room temperature for aging.

2.3. TECAM biomimetic accumulation

Spiked soil (10 g) was placed in a 100 ml beaker. A piece of TECAM (2 × 3 cm, triolein 4.84 wt% dry weight, 2.5 mg cm⁻² dry weight) was embedded in the soil and 3 ml deionized water was added dropwise to the beaker. The beaker was covered with polyethylene membrane to prevent moisture loss and then kept in the dark at room temperature. Three replicates were set up for each PAH concentration or each soil. TECAMs were collected from the beakers after 21 days. The schematic diagram of TECAM accumulation of PAHs from soil is shown in Fig. 1.

2.4. Accumulation of PAHs by earthworms

Three active earthworms acclimatized to laboratory conditions for at least 2 weeks were transferred to a 500 ml glass

Table 1 – Selected physicochemical properties of the soils and the concentrations of PAHs in soils after incubation for 3 months

Soil	Classification	Nap ($\mu\text{g g}^{-1}$)	Phe ($\mu\text{g g}^{-1}$)	Pyr ($\mu\text{g g}^{-1}$)	BaP ($\mu\text{g g}^{-1}$)	OM (%)	DOC ($\mu\text{g g}^{-1}$)	pH
Kunming	Ultisol	2.80	4.37	4.33	3.90	2.87	325.0	7.12
Yingtian	Ultisol	2.93	5.94	5.83	5.89	1.79	248.5	5.02
Qiqihaer	Mollisol	2.22	2.74	3.18	3.63	3.85	172.2	8.02
Wuhu	Alfisol	2.06	4.96	4.80	4.85	3.34	390.7	5.93
Lanzhou	Aridisol	2.49	3.79	4.53	4.83	2.13	102.4	7.51
Beijing	Alfisol	7.46	8.95	5.84	8.31	3.11	335.8	7.37
Chendu	Inceptisol	1.86	2.82	2.59	2.17	1.98	181.0	7.89
Tianjin	Alfisol	4.53	3.23	3.19	3.13	3.05	159.2	7.75
Daxing	Alfisol	2.55	2.93	2.45	2.58	2.56	129.2	7.88
Xiamen	Ultisol	3.11	4.12	3.82	4.16	2.10	423.2	4.96

Values represent means ($n = 3$).

Fig. 1 – Schematic diagram of TECAM accumulation of PAHs from the soil.

beaker containing a 300 g soil subsample. Deionized water (90 ml) was added dropwise to moisten the soil. The beakers were covered with moist filter paper and kept in the dark at 20–22 °C. Earthworms were recovered after 21 days.

2.5. Determination of PAHs in soil pore water

Soil (20 g) and 6 ml deionized water were added to a 50 ml glass centrifuge tube which was kept in the dark at 20–22 °C for 48 h. Pore water was collected by centrifuging the mixture at 4000g for 30 min and then filtering (0.45 μm pore size). Pore water (5 ml) was extracted with 5 ml *n*-hexane three times. The organic phase was then dehydrated with anhydrous sodium sulfate and evaporated under a gentle nitrogen stream, solvent-exchanged into methanol (1.00 ml) and stored prior to analysis.

2.6. Chemical analysis

Triplicate subsamples of each soil were collected and analyzed by the following steps. Each soil subsample (2 g) was thoroughly mixed with 6–7 g anhydrous sodium sulfate, loaded into a Soxhlet thimble and extracted with 100 ml *n*-hexane/dichloromethane (1:1 v/v) at 60 °C for 24 h. The

extract was reduced to about 5 ml with a rotary evaporator, purged to about 1–2 ml under a gentle nitrogen stream and then cleaned with a 10 g neutral alumina/anhydrous sodium sulfate composite column and eluted with 60 ml *n*-hexane/dichloromethane (4:1 v/v). The eluates were finally evaporated, solvent-exchanged into methanol (1.00 ml) and stored prior to analysis.

Earthworms were removed from the soils and allowed to depurate for 24 h to purge the gut contents on wet filter paper. They were then cleaned with deionized water to remove external soil particles, freeze dried, weighed and cut into small pieces that were mixed with 3–5 g anhydrous sodium sulfate and ground with a mortar and pestle to obtain homogeneous samples. Extraction and cleanup were the same as for soil as described above.

TECAM samples were rinsed with deionized water, wiped with clean filter paper, dialyzed in 20 ml *n*-hexane for 24 h and rinsed with 1 ml *n*-hexane three times. All the solutions were combined together. Dialysis solutions of TECAMs were evaporated under a gentle nitrogen stream, solvent-exchanged into methanol (1.00 ml) and stored prior to analysis. TECAMs were stable in *n*-hexane with negligible weight loss of triolein or cellulose acetate during dialysis, and therefore no cleanup of the samples was required.

PAHs in samples were analyzed by HPLC-FLD (Agilent 1200 Series). Chromatographic separation and resolution were best achieved by using a Supelcosil (Supelco, Bellefonte, CA) LC-PAH reverse-phase C_{18} column (4.6 $\times$ 150 mm, 5 μm particle size) specific for PAH analysis. Samples were injected with a 20 μl syringe, the injection volume was 20 μl , the mobile phase was methanol/water (0–11 min, from 80%/20% to 90%/10%; 11–18 min, 90%/10%) and the C_{18} column was kept at 25 °C. The excitation wavelength of Nap, Phe, Pyr and BaP was 260 nm. The emission wavelength of Nap and Phe was 350 nm and that of Pyr and BaP was 420 nm.

Results were obtained from three replicates in the case of TECAMs and soils and from five replicates in the case of earthworms. Solvent, TECAM, soil and earthworm controls were included. Extraction recoveries of Nap, Phe, Pyr and BaP in TECAM were determined by adding the standard solution of these PAHs at seven different concentrations to a solvent

mixture of acetone (69 wt%) and 1,4-dioxane (10 wt%) before TECAM preparation. The recoveries of Nap, Phe, Pyr and BaP were $75.2 \pm 2.1\%$, $83.7 \pm 1.9\%$, $89.4 \pm 2.7\%$ and $93.5 \pm 2.8\%$, respectively. Extraction recoveries of Nap, Phe, Pyr and BaP in soil were determined by adding standard solutions of PAHs at seven concentrations to the soil. The recoveries were $87.3 \pm 2.7\%$, $94.3 \pm 1.9\%$, $90.5 \pm 2.2\%$ and $87.3 \pm 1.8\%$.

3. Results and discussion

3.1. Optimization of TECAM exposure conditions

TECAMs accumulated the four PAHs in soil within 48 h (unpublished data), much more quickly than other PSDs composed of hydrophobic polymers or polyethylene films. The exposure time of 21 days was chosen in this study to maintain the same conditions as used for earthworms.

TECAMs of sizes 4, 6, 9, 12 and 16 cm² were used to investigate the optimum TECAM size for accumulation of PAHs. The accumulation of each PAH in TECAM was positively related to the size of the TECAM. However, the concentration of each PAH ($\mu\text{g g}^{-1}$ TECAM) was almost constant (Fig. 2A) irrespective of the TECAM size and 6 cm² TECAMs were used later in the study. The concentrations of Nap, Phe, Pyr and BaP in pore water were 87.8, 14.0, 5.17 and $1.27 \mu\text{g l}^{-1}$ and the volume of pore water was 3 ml (water-to-soil ratio 30%). The amounts of Nap, Phe, Pyr and BaP in pore water were therefore 263.3, 42.0, 15.5 and 3.81 ng, accounting for 0.45%, 0.06%, 0.03% and 0.03% of the total amount of Nap, Phe, Pyr and BaP in the soil. The amounts of Nap, Phe, Pyr and BaP accumulated in the 6 cm² TECAMs were 63.6, 104.1, 43.2 and 9.45 ng, accounting for 0.11%, 0.16%, 0.09% and 0.08% of the total amount of Nap, Phe, Pyr and BaP in the soil, values close to the fractions of PAHs in pore water. This suggests that the fraction of PAHs accumulated in TECAMs was the bioavailable fraction in the soil and therefore TECAMs have the potential to predict the bioavailability of PAHs in soils. In addition, only a small fraction of the total amount of PAHs in soil was present in pore water and transported to the membrane through aqueous pathways, and the amount of PAHs in the soil remained virtually unchanged after TECAMs were embedded in the soils. This suggests that TECAM sampling was virtually nondestructive and led to minimum disturbance of soils.

Five different water-to-soil ratios, namely 20%, 30%, 50%, 100% and 200%, were chosen to investigate the effect of soil moisture on the accumulation of PAHs by TECAM. The concentrations of Nap and Phe in TECAM increased as the ratio of water to soil increased from 20% to 200% (Fig. 2B). However, the concentrations of Pyr and BaP in TECAM did not change significantly. This may be due to differences in their aqueous solubility and ability to desorb from soil into pore water. The aqueous solubilities of Nap and Phe are 30.2 and 1.18 mg l^{-1} (Petty et al., 1994) and their $\log K_{ow}$ values are 3.33 and 4.57 (Schwarzenbach, et al., 2003), respectively. They are not difficult to desorb from soil into pore water. In contrast, Pyr and BaP have low water solubilities (0.135 and 0.0038 mg l^{-1}) and high hydrophobicities ($\log K_{ow} = 5.13$ and 6.13). They tend to interact strongly with SOM and are difficult

Fig. 2 – Relationship between PAH concentration in TECAMs and the size of TECAMs (A), and the ratio of water and soil (B).

to desorb from soil into pore water. Therefore, the dissolved amounts of Pyr and BaP in soil pore water increased slightly as the ratio of water to soil increased. A ratio of 30% was used in the later experiment because it is equivalent to a 60–70% of soil water holding capacity and matches with the field soil moisture content and the environmental conditions experienced by the earthworms.

3.2. Relationship between accumulation of PAHs by TECAMs and PAH concentrations in the soil

Beijing soil spiked with Nap, Phe, Pyr and BaP at initial concentrations of 0.2, 0.5, 2.0, 5.0 and $10.0 \mu\text{g g}^{-1}$ after incubation for three months was used in this part of the study. TECAMs were embedded in the soil for 21 days.

A strong linear relationship was found between the concentrations of PAHs accumulated by TECAMs (C_{TECAM}) and concentrations of PAHs in the soil (C_{soil}) (see Fig. 3). The slope, referred to as the apparent partitioning coefficient (K_{TECAM}) after 21 days, was 0.47, 2.84, 1.24 and 0.33 for Nap, Phe, Pyr and BaP, respectively. No linear relationship existed between $\log K_{\text{TECAM}}$ and $\log K_{\text{ow}}$ of these four PAHs, with the maximum being observed for Phe ($\log K_{\text{ow}} = 4.57$) (Fig. 4A). This result agrees well with previous reports. Several authors (Gobas et al., 1989; Nendza, 1991; Verhaar et al., 1999) have observed that at low and intermediate $\log K_{\text{ow}}$ there seems to be a linear relationship between $\log \text{BCF}$ (bioconcentration factor) and $\log K_{\text{ow}}$, while at high $\log K_{\text{ow}}$ deviations were seen, generally indicating that for these compounds BCF increases less than proportionally with increasing $\log K_{\text{ow}}$. However, a significant linear relationship ($R^2 = 0.998$, $P < 0.01$) was found between $\log K_{\text{TECAM-W}}$ (the partition coefficient of PAHs between TECAM and water) and $\log K_{\text{ow}}$ (Fig. 4B). The likely explanation for the decline in accumulation from soil by TECAMs for the PAHs with higher $\log K_{\text{ow}}$ is as follows. The uptake rate of HOCs to PSDs was controlled mainly by the membrane and aqueous boundary layer (Booij et al., 2003). Resistance posed by the aqueous boundary layer may have been reduced as a result of chemical desorption from the particles near the membrane surface. However, PAHs with high $\log K_{\text{ow}}$ values could interact strongly with soil organic matter (SOM) due to their high hydrophobicity, resulting in their strong sequestration in soil and resistance to desorption. Furthermore, nanopores or voids are abundant in the organic fraction of soils (Malekani et al., 1997; White et al.,

Fig. 4 – Correlations between K_{TECAM} and K_{ow} (A), and $K_{\text{TECAM-W}}$ and K_{ow} (B).

Fig. 3 – Relationship between PAH accumulation in TECAMs and the concentrations in the soil.

1998). PAHs with higher $\log K_{ow}$ usually have larger molecular volumes. If the molecules were entrapped in such pores or voids they would tend not to escape and would become nonbioavailable. PAHs with higher $\log K_{ow}$ may therefore show greater resistance posed by the aqueous boundary layer, resulting in lower accumulation in TECAMs.

3.3. Effect of soil characteristics on accumulation of PAHs by TECAMs

TECAMs were exposed to ten soils spiked with $5 \mu\text{g g}^{-1}$ Nap, Phe, Pyr and BaP after incubation for three months. Relationships between the concentrations of PAHs accumulated by TECAMs and the contents of SOM and dissolved organic carbon (DOC) were investigated. There was a negative correlation between PAHs in TECAMs and SOM (Fig. 5A). This trend would be expected because PAHs may interact with SOM or be entrapped into macromolecular structures, nanopores or voids of SOM. This fraction of PAHs is hard to desorb into soil pore water, resulting in lower equilibrium concentrations of PAHs in pore water. A positive correlation was observed between PAHs in TECAMs and DOC (Fig. 5B). Similar relationships were reported by Tao et al. (2006), when they analyzed the concentrations of PAHs accumulated by wheat (*Triticum aestivum* L.), SOM and DOC in soils. Kukkonen and Oikari (1991) provided very clear evidence of the enhancement of bioavailability of organic compounds. They found that ten natural water samples with DOC concentrations

below approximately 10 mg l^{-1} enhanced the uptake of naphthalene into *D. magna*. Muir et al. (1994) also found a marked enhancement of bioconcentration for the uptake of pyrethroids into rainbow trout. Haitzer et al. (1998) also reviewed enhancement of the bioavailability of organic compounds due to low concentrations of DOC ($<10 \text{ mg l}^{-1}$) in natural waters. However, the mechanism remained obscure. In the present study the DOC of the 10 soils varied from 102.4 to $423.2 \mu\text{g g}^{-1}$, but we also observed a positive correlation between PAHs in TECAMs and DOC. There is still no information on enhancement of bioavailability of organic compounds taken up by PSDs due to DOC in soil pore water. DOC may enhance the kinetics of desorption or transport of PAHs in soils, and the desorption of DOC-bound PAHs near the TECAM surface would reduce the resistance posed by the aqueous boundary layer, leading to an increase in uptake rate and an extra flux to TECAMs, resulting in an enhancement of bioavailability of PAH accumulation in TECAMs.

3.4. Effect of aging on accumulation of PAHs by TECAMs

Previous laboratory and field studies have shown that aging of chemicals in soil has led to a diminished bioavailability to microorganisms (Steinberg et al., 1987), earthworms (Morrisson et al., 2000) and plants (Führ and Mittelstaedt, 1980). A number of studies have investigated the aging of PAHs in soils or sediments by determining their extractability with solvents. However, there is no published information on

Fig. 5 – Relationship between PAHs accumulated by TECAMs and SOM and DOC of ten different soils.

the use of PSDs to estimate aging effects on bioavailability of HOCs in soils. To evaluate the effect of aging on Nap, Phe, Pyr and BaP accumulation in TECAMs, Beijing soil was spiked with $5 \mu\text{g g}^{-1}$ Nap, Phe, Pyr and BaP and aged for 1, 3, 15, 60, 90, 120 and 150 days. The concentrations of the PAHs in the soil changed less than 10% during this aging period. Accumulation of PAHs by TECAMs was plotted against aging time (Fig. 6) and this showed that aging had a significant effect on accumulation of PAHs by TECAMs. A biphasic accumulation pattern was observed for Nap, Phe and Pyr with an initial rapid decrease followed by a slower decline. In contrast, this pattern was not observed for BaP, and this may be attributed to its higher rate of sorption to the soil and stronger

interaction with SOM even during the very initial stage of the aging period due to its higher hydrophobicity. The observed pattern of accumulation of PAHs by TECAMs is consistent with the plot of the extractability and bioavailability of HOCs in soil under the influence of contact time demonstrated in the review by Semple et al. (2003). This result indicates the potential use of TECAM as a surrogate to estimate bioavailability of aged HOCs in soils in the future.

3.5. Comparison of PAHs accumulated by TECAMs and earthworms

A good linear relationship ($R^2 = 0.970\text{--}0.993$, $P < 0.01$) was found between PAHs accumulated by TECAMs and earthworms in soil spiked with five different concentrations of PAHs (Fig. 7). Van Der Wal et al. (2004) observed that concentrations of polychlorinated biphenyls (PCBs) and organic chlorinated pesticides (OCPs) in two earthworm species were linearly related to the concentrations in SPME fibers. Awata et al. (2000) also reported a good correlation between the maximum accumulations of dieldrin, endrin and DDT in C_{18} sampling bags and earthworms. We observed that the accumulation of Pyr and BaP in earthworms was higher than in TECAMs. This may have arisen from differences in the exposure routes of TECAMs and earthworms as well as the activities of earthworms in soil. TECAMs can only accumulate PAHs via passive diffusion, which depends mainly on the freely dissolved concentrations in soil pore water. The up-take by earthworms includes diffusion of the contaminants

Fig. 6 – Effect of aging on PAHs accumulated by TECAMs.

Fig. 7 – Relationship between PAHs accumulated by TECAMs and earthworms ($n = 5$).

through dermis and digestion of particle-associated contaminants in soil (Belfroid et al., 1996). The first route is similar to the uptake by TECAMs and freely dissolved PAHs in soil pore water are accumulated mainly by passive diffusion. The second route leads to the diffusion of the contaminants across the gastrointestinal tract and accumulation in the earthworm tissues (Krauss et al., 2000). Activities of earthworms such as creeping, digging holes and excretion may also result in local disturbance of soil and the dissolution of a fraction of particle-associated PAHs into soil pore water. However, TECAMs were gently embedded in the soils. Pyr and BaP have stronger interaction with SOM due to their higher hydrophobicity, leading to a low concentration in soil pore water and a lower bioavailability to TECAMs. On other hand, due to their higher hydrophobicity, Pyr and BaP tend to bind strongly to tissues, resulting in their higher accumulation in earthworms. Nevertheless, very good linear relationships existed between the accumulation of PAHs in TECAMs and earthworms, suggesting that TECAMs may be useful as a surrogate to estimate the bioavailability of PAHs in soils.

4. Conclusions

The present study has indicated that TECAMs can efficiently and quickly accumulate PAHs from spiked soils and that TECAMs are sensitive to the concentrations of PAHs in soils, to soil characteristics and to the aging time of the PAHs. Laboratory preparation of TECAMs is easy and the accumulation process is also simple and requires no cleanup step. We therefore conclude that TECAM represents a useful surrogate to estimate the bioavailability of PAHs and perhaps other HOCs in soils. However, further research will be required to test their applicability under field conditions or for other compounds.

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