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Fractionation of polycyclic aromatic hydrocarbon residues in soils

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ABSTRACT

Understanding the forms and availabilities of polycyclic aromatic hydrocarbons (PAHs) would have considerable benefits for their risk assessment, and is of crucial importance for food security and remediation strategies in contaminated sites. In this work, the forms of six PAHs (fluorene, phenanthrene, fluoranthene, pyrene, benzo[a]anthracene, and benzo[a]pyrene) in soils were separated into three fractions including a desorbing fraction, a non-desorbing fraction, and a bound residual fraction using a sequential extraction mass balance approach. The desorbing and non-desorbing fractions were extracted with hydroxypropyl-beta-cyclodextrin (HPCD) and dichloromethane:acetone (1:1, vol/vol), respectively. The desorbing and non-desorbing fractions always dominated the total PAH content in soils. The proportion of bound PAH residue in nonsterilized soils was small (<16%), and even smaller (4.5%) in sterilized soils. The concentrations of the desorbing fraction of PAHs as well as the percentage of this fraction to the total PAH content in soils clearly decreased in 0–16 weeks, which may be due to microbial biodegradation and its transfer to other fractions in soils. The concentrations of the non-desorbing PAH fractions increased in sterilized soils, while remaining nearly constant or decreasing to some extent in nonsterilized soils after 16 weeks. The proportion of non-desorbing PAH fractions significantly increased in 16 week-incubation, and this proportion was positively correlated with the molecular weights of the PAHs tested, indicating that larger PAHs are more likely to be present in non-desorbing fractions. The bound PAH residue tended to increase at first and decrease thereafter over the 0–16-week period, and microbes played an important role in the formation of bound residue.

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1. Introduction

Soil is considered to be one of the most important natural resources for human beings. However, organic pollutants occur frequently within the soil environment as a result of air deposition, sewage irrigation, and industrial accidents. This organic pollution triggered by human activities has been a long-term environmental problem in past decades [1–3]. Because of the health hazards of these organic contaminants, knowledge on their transport and fate in the soil environment is of crucial importance in dealing with contaminated sites.

Polycyclic aromatic hydrocarbons (PAHs) are by-products of the incomplete combustion or pyrolysis of organic materials. They are considered to be priority pollutants in the environment and are of major concern due to their recalcitrance and strong mutagenic/carcinogenic properties [4,5]. The hydrophobic characteristic and persistence of PAHs results in their accumulation and enrichment in soils [6]. PAHs are widespread and occur at high concentrations of hundreds of mg/kg in soils of many countries [7]. The contamination of PAHs in soil is a worldwide environmental problem.

When entering into soils, a significant proportion of the organic contaminants is not extractable, but is found bound to soil solids. These bound contaminant residues are less available for plant uptake. Researchers now realize that data on only the extractable or total concentrations of a given organic chemical may be of limited utility when assessing its environmental significance [8]. Instead, the form and availability of these contaminants in soil are the most important indices for risk assessment.

The forms of organic contaminants in soil environments have been reported in literatures [9–13]. Macleod and Semple [8] observed that the extractable fraction of pyrene decreased significantly, whereas the bound residue increased with its contact time in soil. Similar results were observed by other researchers [14,15]. However, the PAH concentrations tested in these studies were at their native concentrations in soils, which may be far lower than those at contaminated sites. In addition, only a very limited number of PAHs and soils have been investigated thus far, while the interactions between the forms of PAHs and the influences of soil properties as well as other environmental factors, such as microbial activity on PAH forms still remain unclear.

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Thus the aim of this study was to fractionate the forms of PAHs in soils. The influence of aging time and microbial activities on the forms of PAHs was also investigated. Results of this study will have considerable benefits for risk assessment, food security, and development of remediation strategies for contaminated sites.

2. Materials and methods

2.1. Reagents

Hydroxypropyl-beta-cyclodextrin (HPCD; >99% purity) was obtained from Qianhui Fine Chemical Co., Ltd (Zibo, China). The mild extraction solution consisted of 70 mmol/L HPCD and 0.05 g NaN_3 per mL in Milli-Q water. Fluorene (FLU), phenanthrene (PHE), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA), and benzo[a]pyrene (BaP) with a purity >98% were obtained from Aldrich Chemical Co. Some properties of these PAHs are listed in Table 1.

2.2. Soil treatments

Four typical zonal soils in China, all previously free of PAHs, were collected from the A (0–20 cm) horizon, air-dried, and sieved. Some soil properties are listed in Table 2. Soils were spiked with a solution of PAHs in acetone. After the acetone evaporated, the treated soils were progressively diluted with unspiked soils and sieved again several times to homogenize the soil samples [16,17]. The calculated final concentrations of FLU, FHE, FLT, PYR, BaA, and BaP in treated soils were 100, 100, 100, 100, 80, and, 50 mg/kg, respectively.

2.3. Experimental design

The forms of the PAHs in soils were examined using microcosms similar to those reported by Macleod and Semple [8]. Treated soils were packed into amber glass microcosms (each with 25 g soil). The microcosms were sealed with glass caps. Three replicates were examined for each treatment. NaN_3 solution (0.5%) was added to one-half of all microcosms in order to inhibit microbial growth. The soil water contents were adjusted to 20% of the soil water holding capacity. After incubation for 0, 2, 4, 8, 12, and 16 weeks in microcosms at 25 °C in the darkness, the soils were sampled, and the forms of the PAHs were determined.

2.4. Fractionation of PAH residues

A sequential extraction/chemical mass balance approach described by Sabate et al. [18] was used to fractionate the forms of PAHs in soils. PAHs in soil were separated into three fractions: a desorbing fraction, a non-desorbing fraction, and a bound residual fraction.

2.4.1. Desorbing fraction

A mild extraction technique to obtain the desorbing fraction of PAHs was adapted according to the methods described by Reid et al. [19] and Cuypers et al. [20]. Since excellent correlation was observed by these authors between the desorbing fraction and the biodegradable PAH fraction, this method could be used to estimate the bioavailable portion that would be degraded by microbes within a reasonable time.

Three grams of treated soil from each microcosm were placed in a 25-mL glass centrifuge tube, and 15 mL of the mild extraction solution were added. Tubes were closed with a Teflon-liner cap, shielded from light, and shaken horizontally at 150 rpm at 25 °C. At 60, 120, and 240 h, tubes were centrifuged for 25 min at 2000 rpm to separate soil from aqueous solution. The supernatant was collected, and fresh mild extraction solution was added. Tubes were then shaken and centrifuged again. The supernatant was liquid–liquid extracted three times using 10 mL of dichloromethane, and the extraction efficiency was tested. Organic phases were dehydrated by percolation through Na_2SO_4 anhydride and combined. The solvent was firstly concentrated by rotary evaporation, then evaporated under a gentle stream of N_2, and diluted with methanol to a final volume of 2 mL. After filtration through a 0.22-μm filter, PAHs were detected by high pressure liquid chromatography (HPLC).

2.4.2. Non-desorbing fraction

This fraction was obtained by exhaustive extraction following mild extraction. After 240 h of mild extraction for the desorbing fraction, the pellet (soil) was dried at 37 °C for 24 h. Dried soil was then placed in a 25-mL glass centrifuge tube, and 10 mL of a solution of dichloromethane:acetone (1:1, vol/vol) were added. Extractions were conducted four times in an ultrasonic bath for 10 min. Soil and solvent were separated by centrifugation for 25 min at 2000 rpm, and then treated as described above.

2.4.3. Bound residue extraction

Dried soil samples resulting from exhaustive extractions were extracted in order to obtain the bound residue fraction.

Table 1
Some properties of PAHs used in this study.

PAHs	Molecular weight (g/mol)	logK_{ow}	S_w (mg/l)[a]	K_H
Fluorene	166.22	4.18	1.9	3.18×10^{-3}
Phenanthrene	178.23	4.57	1.1	1.31×10^{-3}
Fluoranthene	202.26	5.22	0.26	4.20×10^{-4}
Pyrene	202.26	5.18	0.13	3.72×10^{-4}
Benzo[a]anthracene	228.30	5.91	0.011	2.35×10^{-4}
Benzo[a]pyrene	252.32	6.04	0.0038	1.86×10^{-5}

[a] At 25 °C.

Table 2
Soil properties of the tested soils.

Soil no.	Soil type	Location (Province of China)	pH value	Soil organic carbon content (g/kg)	Clay content (%)	Sand content (%)	Silt content (%)
Soil 1	Kandiudult	Jiangxi	4.56	9.97	36.8	40.7	22.5
Soil 2	Paleudult	Hubei	4.74	9.47	39.2	9.20	51.6
Soil 3	TypicPaleudalfs	Jiangsu	6.02	14.3	24.7	13.4	61.9
Soil 4	Eutrochrepts	Shandong	7.35	6.92	18.4	34.7	46.9

The extraction method was as described by Richnow et al. [21].

After exhaustive extraction, soil samples were placed in glass vials. A 10 mL solution of 2 mol/L NaOH was added to each vial. The vials were closed with Teflon-lined caps and then heat-treated at 100 °C for 2 h. The aqueous fraction was obtained by centrifugation at 2000 rpm for 25 min, acidified with 6 mol/L HCl to a pH < 2, and liquid–liquid extracted three times with 10 mL of dichloromethane. The samples were then treated as described above.

2.5. Chemical and statistical analysis

The PAH extracts of each fraction were analyzed by an HPLC (LC-20AT; Shimadzu) fitted with a UV detector and a 4.6 mm × 250-mm reverse phase C_{18} column using methanol as the mobile phase at a flow rate of 0.65 mL/min (40 °C). Aliquots (20 μL) of each sample were injected into the HPLC system by an autosampler.

All data were statistically processed using Microsoft Office Excel 2003 and SPSS 13.0. The PAH concentrations of each fraction were tested for their differences at a significance level of $p < 0.05$, unless otherwise indicated.

3. Results and discussion

3.1. Residues of PAHs in soils

Here, the PAHs in soils were fractionated into a desorbing fraction, a non-desorbing fraction, and a bound residual fraction. The extractable residue includes the desorbing and non-desorbing fractions. After extraction procedures (a) and (b), the remaining PAHs in the soil sample were defined as the non-extractable soil-bound residue.

Fig. 1 illustrates the total contents of the tested PAHs in soil 3 as a function of aging time from 0 to 16 weeks. The concentrations of the PAHs generally decreased with time. For instance, the concentrations of FLU, FHE, FLT, PYR, BaA, and BaP in nonsterilized soil 3 were reduced to 15.1, 10.9, 20.8, 23.8, 65.4, and 42.7 mg/kg in 16 weeks. However, their concentrations in corresponding sterilized soil 3 after 16 weeks were higher (61.1, 81.5, 84.1, 73.5, 83.4, and 52.3 mg/kg, respectively). Since the microcosms were sealed duration the incubation, the volatility of PAHs could be ignored. The sorption of PAHs to the glass wall of microcosm was observed to be negligible. The results indicated that microbial degradation may be the predominant contributor to the dissipation of PAHs in soil.

The dissipation ratios (D_{PAH}) of the six tested PAHs, i.e., the amount of PAH dissipated relative to the amount initially added to the soil, were calculated. D_{PAH} values after 16 weeks were 83.9%, 87.6%, 74.7%, 64.2%, 15.3%, and 18.7% for FLU, FHE, FLT, PYR, BaA, and BaP in soil 3, respectively. Clearly, the order of the D_{PAH} values of these compounds was closely negatively correlated with their molecular weights and number of benzene-rings, suggesting that PAHs with higher molecular weights and larger K_{ow} are more recalcitrant and difficult to be biodegraded in soil [18,22]. This was also supported by the slightly decrease of the residual concentrations of PAHs with higher molecular weights such as BaA and BaP in nonsterilized soils in 0–16 weeks (Fig. 1). Results of our work also suggest the importance of the role of microbes in the biodegradation of PAHs in the soil environment.

3.2. Desorbing fraction of PAHs

The desorbing fraction of organic chemicals in soil is the most bioavailable portion and is usually determined by extraction with an aqueous phase. However, for those hydrophobic organic chemicals such as PAHs with poor aqueous solubility and strong affinity toward soil organic matter, extraction of this fraction using an aqueous solution has great limitations, since only a small amount of these compounds exists in the aqueous phase, while a large labile and potentially bioavailable pool remains in the solid phase. To solve this problem, some authors [19,23,24] proposed to enhance the solubility of these chemicals with cyclodextrin solutions. It was observed that organic chemicals extracted with cyclodextrin solutions were strongly correlated with their bioavailable portions [19]. Therefore, we used cyclodextrin (HPCD) solutions in this study to obtain the desorbing fractions of PAHs in treated soils.

Fig. 2 displays the concentrations of the desorbing fraction of PAHs in soil 3 over 0–16 weeks. As shown, the concentrations of the desorbing fraction of PAHs clearly decreased after 16 weeks, and were only 11.8–67.0% of their initial concentrations of this fraction in nonsterilized soil 3 (Table 3). However, for different PAHs, the magnitude of decrease of this fraction varied greatly, and 85.3%, 88.2%, 78.8%, 69.1%, 33.0%, and 41.0% of the desorbing fractions of FLU, FHE, FLT, PYR, BaA, and BaP dissipated after 16 weeks. Clearly, this order was inversely correlated with the molecular weights and benzene-ring numbers of the tested PAHs. Concentrations of the desorbing PAH fraction were much higher at 16 weeks in sterilized soils versus nonsterilized treatments. On a whole, the concentrations of this fraction in sterilized soils decreased to some extend after 4 weeks, and then remained nearly constant in 8–16 week-incubation (Fig. 2). The former decrease after 4 weeks in this fraction in sterilized soils may be attributed to transfer of "easily desorbing sites" to "difficultly desorbing sites" and "irreversible sites" [25]. Since the desorbing fractions of organic compounds are the most bioavailable, the above results indicate that synergistic microbial degradation dominated the dissipation of desorbing fractions of PAHs in the soil environment.

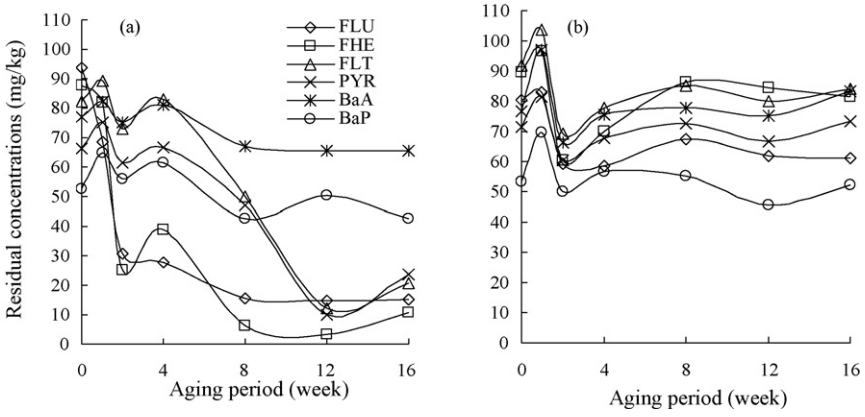

Fig. 1. Residual concentrations of PAHs in nonsterilized (a) and sterilized (b) soil 3 as a function of time.

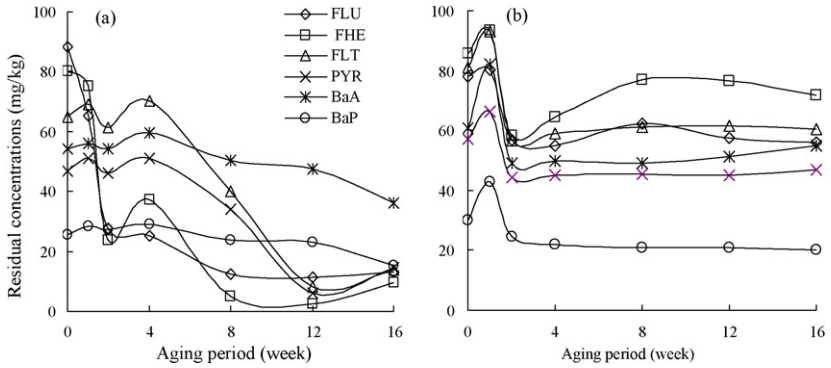

Fig. 2. Concentrations of the desorbing fraction of PAHs in nonsterilized (a) and sterilized (b) soil 3 as a function of time.

Table 3
The concentrations of the total and desorbing fraction of PAHs in soils.

PAHs	$C_{0\text{-total}}$ (mg/kg)	$C_{16\text{wk-total}}$ (mg/kg)	ΔC_{total} (mg/kg)	$C_{0\text{-HPCD}}$ (mg/kg)	$C_{16\text{wk-HPCD}}$ (mg/kg)	$\Delta C_{\text{total-HPCD}}$ (mg/kg)	$\Delta C_{\text{total-HPCD}}/\Delta C_{\text{total}}$ (%)
Fluorene	93.82	15.09	78.73	88.28	13.01	75.27	95.61
Phenanthrene	87.78	10.91	76.87	80.08	9.42	70.67	91.93
Fluoranthene	82.12	20.82	61.30	65.06	13.80	51.26	83.63
Pyrene	66.34	23.77	42.57	46.85	14.47	32.38	76.07
Benzo[a]anthracene	77.17	65.40	11.78	54.10	36.24	17.86	151.7
Benzo[a]pyrene	52.50	42.71	9.79	25.64	15.13	10.51	107.4

$C_{0\text{-total}}$ and $C_{16\text{wk-total}}$ were the concentrations of the total PAH contents at 0 and 16 weeks, respectively. $C_{0\text{-HPCD}}$ and $C_{16\text{wk-HPCD}}$ were the concentrations of the desorbing fraction of PAHs in soils at 0 and 16 weeks, respectively. ΔC_{total} and $\Delta C_{\text{total-HPCD}}$ were the dissipation of the total and desorbing fraction of PAHs at 0 and 16 weeks, respectively. $\Delta C_{\text{total}} = C_{0\text{-total}} - C_{16\text{wk-total}}$; $\Delta C_{\text{total-HPCD}} = C_{0\text{-HPCD}} - C_{16\text{wk-HPCD}}$.

Interestingly, as tabulated in Table 3, the dissipation amount of the desorbing fractions of the tested PAHs was about 76.1–152% of their total dissipation in soils (nonsterilized soil 3 as an example). This means that the desorbing fraction was most easily degraded, and that degradation of this fraction contributed predominantly to the total dissipation of PAHs in soils. However, not all of the decrease in the desorbing fractions of PAHs was ascribed to microbial biodegradation. In fact, some of this fraction could transfer to other fractions (such as non-desorbing and bound residual fractions). As seen in Table 3, the dissipation amount of the desorbing fraction of BaA and BaP was more than 100% (107.4% and 151.7%, respectively) of their total dissipation in soils. Thus, it is highly likely that parts of their desorbing fractions transferred to other forms in the soil environment.

In addition, the percentage of the desorbing fraction relative to the total contents of PAHs at specific time points was calculated and illustrated in Fig. 3. As seen, this percentage decreased from 94.1%, 91.2%, 79.2%, 70.6%, 70.1%, and 48.8% to 86.2%, 86.2%, 66.3%, 60.9%, 55.4%, and 35.4% after 16 weeks of aging in nonsterilized soil 3 for FLU, FHE, FLT, PYR, BaA, and BaP, respectively. However, this percentage was slightly higher in the sterilized control soils. As stated, the decrease in this percentage over the 0–16 weeks can also be ascribed to both microbial degradation of this fraction and its transfer into other fractions in the soil.

3.3. Non-desorbing fraction of PAHs

The non-desorbing fractions of the six PAHs in soil 3 as a function of time are given in Fig. 4. Concentrations of this fraction of PAHs with lower molecular weight generally decreased in nonsterilized soils over 0–16 weeks. For instance, the concentrations of the non-desorbing fractions of FLU, FHE, FLT, and PYR after a 16-week aging decreased from 4.74, 7.53, 16.6, and 19.0 mg/kg to 2.06, 1.49, 6.74, and 9.19 mg/kg, respectively. However, for BaA and BaP with five benzene-rings and high molecular weights, the concentrations of this fraction were almost constant over the time period, reflecting the recalcitrant nature of these compounds.

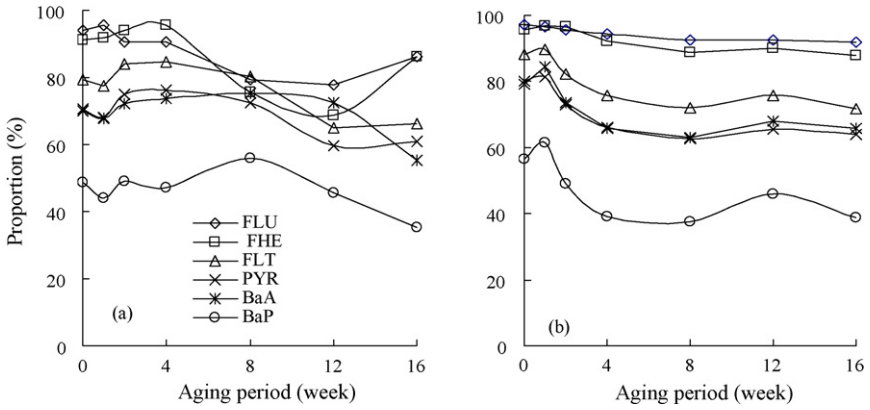

Fig. 3. The proportion of the desorbing fraction to the total contents of PAHs in nonsterilized (a) and sterilized (b) soil 3 as a function of time.

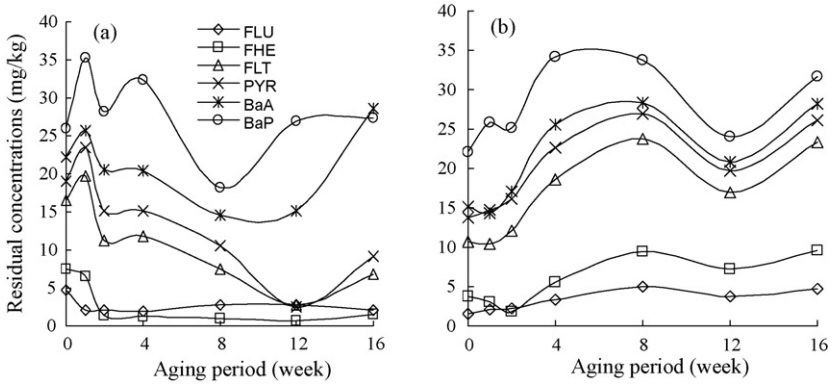

Fig. 4. Concentrations of the non-desorbing fraction of PAHs in nonsterilized (a) and sterilized (b) soil 3 as a function of time.

In sterilized control soils (Fig. 4b), the concentrations of the non-desorbing fraction of PAHs after 16 weeks increased to 4.73, 9.62, 23.4, 26.1, 28.2, and 31.7 mg/kg for FLU, FHE, FLT, PYR, BaA, and BaP, respectively. These changes were the opposite of those observed for the nonsterilized soils (Fig. 4a), indicating the major contribution of the microbial degradation to the dissipation of this fraction of PAHs in nonsterilized treatments. In addition, microbial activities were excluded in sterilized soils, and biodegradation of the desorbing fractions of PAHs could be negligible, while the desorbing fractions of PAHs may partially transfer into other fractions such as the non-desorbing fraction, resulting in an increase in the concentrations of non-desorbing fraction in sterilized control treatments.

The percentage of the non-desorbing fractions relative to their total contents at specific time points was calculated. As shown in Fig. 5, this percentage increased over 0–16 weeks irrespective of sterilized and nonsterilized soils, which was the opposite of the trend for the desorbing fractions of PAHs in these soils (Fig. 3). For instance, the percentage for FLU, FHE, FLT, PYR, BaA, and BaP in nonsterilized soils (Figure 5a) after 16 weeks increased from 5.05%, 8.57%, 20.2%, 28.6%, 28.9%, and 49.5% to 13.6%, 13.62%, 32.4%, 38.7%, 43.8%, and 64.0%, respectively. This percentage was higher for all of the tested PAHs in sterilized versus nonsterilized soils. In sterilized treatments, since microbial activities were excluded, the increase in this percentage could be ascribed to the transfer of the desorbing fraction. However, in nonsterilized soils, most of the desorbing PAH fractions were degraded (as described earlier), consequently resulting in the higher proportion of non-desorbing PAH residues after 16 weeks. In addition, this percentage was positively correlated with the molecular weights of the tested PAHs (Fig. 5b), indicating that the larger PAHs were more likely to be present in the non-desorbing fractions.

One notes that, as displayed in Figs. 3 and 5, the desorbing and non-desorbing fractions always dominated the total residue of PAHs, and more than 96% of the fluorene, 86% of the phenanthrene, 85% of the fluoranthene, 83% of the pyrene, 96% of the benzo[a]anthracene, and 98% of the benzo[a]pyrene existed as extractable fractions (including desorbing and non-desorbing fractions).

3.4. Bound PAH residue

Fig. 6 displays the concentrations of the bound PAH residue in soil 3 during the 0–16-week incubation. As shown, the concentrations of the PAH-bound residues were much lower than those of the desorbing and non-desorbing fractions (Figs. 2 and 4). The concentrations of bound PAH residue generally tended to increase first and decrease thereafter over 0–16 weeks. At early stages, the increase in this PAH fraction could result from the transfer of other PAH fractions as described above. The obvious decreases in the desorbing and non-desorbing PAH fractions over the 0–16 weeks as shown in Figs. 2(a) and 4(a) support this hypothesis. However, the bound PAH residue tended to decrease after 8–12 weeks. As reported by other investigators, the bound residues of organic chemicals may be conditionally released into the soil environment [26–28]. Here, since the desorbing fractions of PAHs were sharply reduced after 16 weeks primarily due to microbial degradation, the bound PAH residue may be released and become part of the desorbing and non-desorbing PAH fractions. On the other hand, the newly observed PAH-bound residues from the early stages are still unstable [29], and they may again change to other forms of PAHs. As seen in the sterilized control soils (Fig. 6b), the concentrations of the bound PAH residue obviously decreased after 12–16 weeks after the early stages of increase. However, during this period, the desorbing frac-

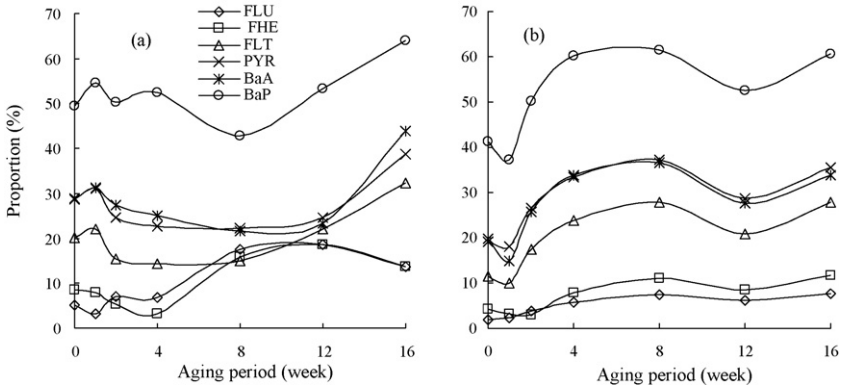

Fig. 5. The proportion of the non-desorbing fraction to the total contents of PAHs in nonsterilized (a) and sterilized (b) soil 3 as a function of time.

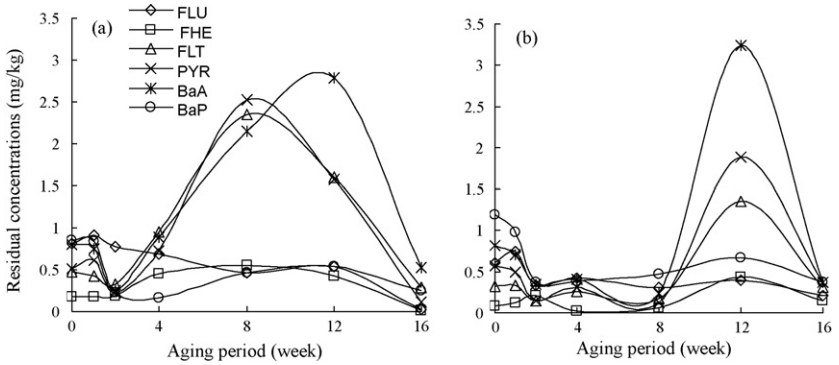

Fig. 6. Concentrations of the PAH-bound residue in nonsterilized (a) and sterilized (b) soil 3 as a function of time.

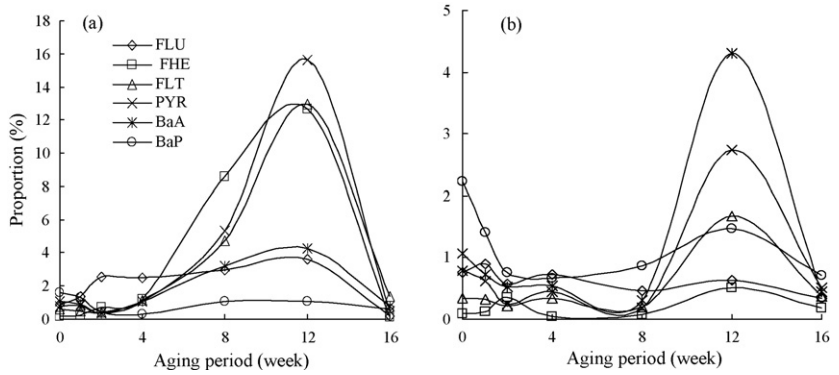

Fig. 7. The proportion of the bound residue of PAH to its total contents in nonsterilized (a) and sterilized (b) soil 3 as a function of time.

tion and the total contents of the PAHs remained nearly constant, while the non-desorbing PAH fractions increased, as exhibited in Figs. 1, 2b, and 4b. This indicated that the bound PAH residues may partially transfer into the non-desorbing fractions between 12 and 16 weeks.

The percentage of the bound PAH residue relative to the total contents of PAHs at specific time points was calculated and shown in Fig. 7. This percentage tended to first increase, and then decrease thereafter from 0 to 16 weeks irrespective of nonsterilized or sterilized control soils. Compared to the desorbing and non-desorbing PAH fractions, these percentages were much lower, less than 16% and 4.5% for the nonsterilized and sterilized soils, respectively. In addition, this percentage was higher in nonsterilized soils versus sterilized controls. The influence of microbial activities on aging and

Table 4
The concentrations of the desorbing fraction, non-desorbing fraction, and bound residue of PAHs in different soils (mg/kg).

PAHs	Forms	Soils	Aging time (week)		
			1	4	16
Fluorene (mg/kg)	Desorbing fraction	Soil 1	70.9 ± 6.98	30.6 ± 0.64	12.7 ± 0.65
		Soil 2	58.2 ± 3.69	16.4 ± 1.55	13.0 ± 1.17
		Soil 3	65.3 ± 4.93	25.3 ± 1.43	13.0 ± 4.27
		Soil 4	39.2 ± 2.65	16.5 ± 4.53	9.9 ± 0.53
	Non-desorbing fraction	Soil 1	1.43 ± 0.33	1.69 ± 0.33	1.19 ± 0.09
		Soil 2	1.69 ± 0.42	1.31 ± 0.22	1.29 ± 0.20
		Soil 3	2.13 ± 0.03	1.90 ± 0.16	2.06 ± 0.15
		Soil 4	0.94 ± 0.52	1.36 ± 0.22	0.94 ± 0.05
	Bound residue	Soil 1	0.82 ± 0.09	0.58 ± 0.08	0.18 ± 0.00
		Soil 2	0.14 ± 0.00	0.72 ± 0.05	0.63 ± 0.08
		Soil 3	0.91 ± 0.09	0.69 ± 0.04	0.03 ± 0.00
		Soil 4	0.57 ± 0.07	0.82 ± 0.07	0.23 ± 0.00
Fluoranthene (mg/kg)	Desorbing fraction	Soil 1	69.6 ± 3.44	68.7 ± 3.64	54.3 ± 2.42
		Soil 2	72.7 ± 5.94	67.8 ± 5.95	29.8 ± 1.78
		Soil 3	69.2 ± 6.94	70.1 ± 3.71	13.8 ± 1.72
		Soil 4	79.18 ± 784	47.8 ± 7.62	7.28 ± 0.55
	Non-desorbing fraction	Soil 1	10.7 ± 2.89	7.47 ± 0.85	16.5 ± 3.23
		Soil 2	11.2 ± 0.99	11.8 ± 0.96	12.4 ± 1.93
		Soil 3	13.5 ± 1.35	11.8 ± 0.99	6.74 ± 0.79
		Soil 4	3.90 ± 0.71	5.19 ± 0.35	2.30 ± 0.11
	Bound residue	Soil 1	0.30 ± 0.07	1.50 ± 0.91	0.94 ± 0.22
		Soil 2	0.34 ± 0.03	0.43 ± 0.07	0.21 ± 0.01
		Soil 3	0.43 ± 0.06	0.95 ± 0.08	0.29 ± 0.09
		Soil 4	0.44 ± 0.07	0.31 ± 0.01	0.07 ± 0.002

sequestration of hydrophobic organic chemicals in soil is an area of growing interest [30,31]. In this experiment, biological activity was shown to play an important role in the formation of bound PAH residue. This is similar to findings of previous studies demonstrating that the presence of microbes would lead to the formation of larger solvent non-extractable residues [8].

3.5. PAH forms in different soils

The forms of tested PAHs in different soils varied greatly. The forms of FLU and FLT as representative PAHs aging for 16 weeks in four typical zonal soils of China are listed in Table 4. The desorbing fraction was the largest portion of FLU and FLT in soils. The percentage of the desorbing fraction of FLU relative to its total contents in soils 1–4 were 96.9%, 96.9%, 95.5%, and 96.3% at 1 week, and 90.3%, 87.1%, 86.2%, and 89.5% after 16 weeks, respectively. Similarly, the amounts of desorbing fractions of FLT accounted for 77.5–94.8% and 66.3–75.7% of its total contents at 1 and 16 weeks for soils 1–4.

Compared with the desorbing fraction, the amounts of the non-desorbing fractions of FLU and FLT were lower in soils. The percentage of the non-desorbing fraction of FLU relative to its total content in soils 1–4 were 2.0–3.1% and 8.4–13.6% at 1 and 16-week, respectively; and this percentage of FLT was 4.7–22.1% and 23.0–32.4% at 1 and 16-week, larger than that of FLU. This was consistent with the result described previously that the larger PAHs were more likely to be present in the non-desorbing fractions.

The concentrations of the PAH-bound residue were much lower than those of the desorbing and non-desorbing fractions in soils 1–4. For example, the percentages of the bound residual fraction relative to the total contents of FLU and FLT in four soils after 16 week-aging were only 0.2–4.2% and 0.5–1.4%, respectively. Compared with the desorbing and non-desorbing fractions, the bound residue contributed a much smaller portion in all tested soils.

4. Conclusions

Using a sequential extraction mass balance approach, the PAHs in soils were fractionated into three fractions: a desorbing fraction, a non-desorbing fraction, and a bound residual fraction. More than 83% of each PAH tested was extractable, and the desorbing and non-desorbing fractions always dominated the total PAH contents in soils. The portions of bound PAH residue were very small (<16%), and even smaller (4.5%) in sterilized soils.

During the aging period of 0–16 weeks, the desorbing fraction of PAHs in tested soils clearly decreased, and microbial degradation contributed predominantly to the decrease in this fraction. The concentrations of the non-desorbing fraction of PAHs increased in sterilized soils, and remained nearly constant or decreased to some extent in nonsterilized soils. However, the percentage of this fraction relative to the total content of PAHs significantly increased in 0–16 weeks, and this percentage was correlated with the molecular weights of the tested PAHs, indicating that PAHs with larger molecular weights were more likely to be present in the non-desorbing fraction. The bound PAH residue tended to increase at first, and decrease thereafter over the 0–16 weeks, and microbes contributed to the formation of bound residues in soils.

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