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In this study, we have evaluated the extent to which organic matter contents in soils influence the accumulation of PAHs by the roots of wheat plants and have developed a rapid chemical method for determining the bioavailability of PAH. Four polycyclic aromatic hydrocarbons (PAHs), naphthalene, acenaphthylene, fluorene, and phenanthrene, were added to natural soil samples with different amounts of organic matter for pot experiments to evaluate apparent bioavailability of PAHs to wheat roots (Triticum aestivum L.). The extractabilities of PAHs in the soil were tested by a sequential extraction scheme using accelerated solvent extraction with water, n-hexane, and a mixture of dichloromethane and acetone as solvents. The water or n-hexane-extractable PAHs were positively correlated to dissolved organic matter (DOM) and negatively correlated to total organic matter (TOM), indicating mobilization and immobilization effects of DOM and TOM on soil PAHs, respectively. The apparent accumulation of PAHs by wheat roots was also positively and negatively correlated to DOM and TOM, respectively. As a result, there are positive correlations between the amounts of PAHs extracted by water or n-hexane and the quantities accumulated in plant roots, suggesting the feasibility of using water- or n-hexanes-extractable fractions as indicators of PAH availability to plants.

Introduction

Severe contamination of agricultural soils by polycyclic aromatic hydrocarbons (PAHs) occurs in many places in China mainly as a result of coal and biomass combustion (1). Because ingestion is the main source of human exposure to PAHs (2) and vegetables are basic ingredients for the Chinese diet (3), it is important to know how and to what extent PAHs are accumulated in vegetables produced in contaminated soils.

Previous studies have disclosed PAHs contamination in foods including vegetables (1, 4). Deposition from air may be the main pathway for accumulation of PAHs in vegetables. In some cases, direct relationships between soil and plant PAH concentrations were also observed, suggesting a pathway from contaminated soil to plant roots (5).

Plants can take up only a portion of persistent organic pollutants in soil. This is because the environmental significance of bound residue of PAHs depends strongly on its bioavailability (6). The bioavailable residue is the fraction of a compound in soils that can be taken up by plants and/or soil-dwelling animals, in contrast to the nonavailable fraction (6). Physicochemical properties of soils have been considered responsible for the retention of organic pollutants in soil matrices. Because of their partially hydrophobic nature and complex molecular configuration, there is a strong affinity of naturally occurring organic matter in soil to persistent organic pollutants. However, soluble and insoluble fractions of organic matter in soil may play totally opposite roles in mobilization and retention of the pollutants. Dissolved organic matter (DOM) can solubilize PAHs in forest soils, while insoluble soil organic matter, which is dominant in total organic matter (TOM), may immobilize PAHs (7, 8).

It is reasonable to assume that the fraction of bound organic pollutants that cannot be taken up by plant root may not be recovered from soil by certain mild chemical extraction. An extraction procedure that mimics and predicts bioavailability of pollutants would be preferable for assessing exposure. The feasibility of predicting the bioavailability of organic compounds to various organisms using selective chemical extractants has been assessed previously (9, 10). Although bioavailability is organism- and species-specific and a single chemical test is insufficient to precisely assess bioavailability accurately (11), extraction with nonexhaustive selective extractants that mimics the bioavailability of organic pollutants is useful to provide predictors of exposure. Among the techniques available for extraction of organic pollutants in soil, accelerated solvent extraction (ASE) is an automated system for the extraction of environmental analytes from solid matrices. This tool not only reduces sample preparation time and the amount of solvent required, but also maintains relatively constant extraction conditions. Besides, a sequential extraction scheme can be easily realized by using ASE since the entire extraction process is fully automated and programmable (12).

The objectives of this study were 2-fold: (1) to investigate the influence of organic matter content on the extractability and bioavailability of naphthalene (NAP), acenaphthylene (ACE), fluorene (FLU), and phenanthrene (PHE) in soil; and (2) to evaluate the feasibility of predicting the bioavailability of these PAH compounds to wheat uptake. A series of soil samples with different TOM and DOM contents were used, and a sequential ASE extraction scheme was applied. We tested the hypotheses that both extractability and bioavailability of PAHs in these soils depend on soil organic matter content and that a mild selective extractant may mimic the plant uptake from the soil. Clay content was also tested using the same procedure but revealed no influence on the bioavailability and is, therefore, not discussed in this paper.

Methodology

Reagents and Glassware. A mixture of PAH16 stock standard and a working standard solution was prepared (Chem. Service) with n-hexane. NAP, ACE, FLU, and PHE for soil spiking were from Fluka (> 99.9%). Other pesticide grade solvents included n-hexane (Scharlan, Spain), acetone (Te-dia), dichloromethane, and pentane (Dikma). Silica gel (Dikma, 80-100 mesh) was heated at 450 °C for 10 h, kept in
a sealed desiccator, and reactivated at 130 °C for 4 h immediately prior to use. Granular anhydrous sodium sulfate and quartz sand (analytical grade, Beijing Reagent, China) were heated at 650 °C in a furnace for 6 h prior to use and stored in the sealed desiccator. All glassware was cleaned in an ultrasonic cleaner (Kunshan KQ-500B, China) and heated at 350 °C for 12 h.

**Soil Samples.** Twenty soil samples were collected from a mountainside near the Shisanling Reservoir in Beijing. At each location, a 1–20 cm surface horizon was sampled. After air-drying at room temperature, samples were crushed to pass through a 20-mesh sieve. Contents of NAP, ACE, FLU, PHE, TOM, DOM, and pH of the samples were measured. TOM was determined using a TOC analyzer (Shimadzu 5000A, Japan), and DOM was determined using a multiple solid–water ratio method (23). Soil pH was measured using 10.0 g of air-dried soil suspended in 25 mL of deionized water with a glass electrode pH meter (F-13, Shanghai). Seven samples with relatively low levels of PAHs and different organic matter contents were selected. The sampling locations, PAH contents, and other soil properties are listed in Table 1.

Soil samples were artificially contaminated with NAP, ACE, FLU, and PHE. We prepared a stock solution of 25 mg/L of each PAH compound in acetonitrile. 10 mL of acetonitrile solution and 1 mL of stock solution were added to a blender, to which 500 g of air-dried soil samples were added as five additions with 100 g each time and blended for 20 s between the additions and finally for a full 5 min. The samples were stored in open containers in a fume hood for 24 h until all acetonitrile evaporated and subsequently aged in sealed glass bottles in dark at room temperature for 64 days. The nominal concentration of each individual PAH compound was 50.0 ng/g. The concentrations were measured prior to and immediately after the cultivation experiment, and the average values of the two measurements were applied in this study as soil concentrations.

**Wheat Cultivation.** Wheat (Triticum aestivum L.) seeds obtained from the Chinese Agricultural University were germinated in a culture dish by placing them on a filter paper soaked in a saturated solution of calcium sulfate at 20–25 °C. One hundred pre-germinated seedlings with seminal leaves were transplanted to each pot containing 200 g of PAHs-amended soil on 300 g of fine sand. The top and the bottom diameters of the pots were 12 and 10 cm, respectively, and the depth was 13 cm. Pots were cultivated in an environmentally controlled cultivation. At the beginning, distilled water was added to each pot and soil moisture was measured as 40%. Control pots without plants were immediately weighed. During the cultivation period, the pots were watered every other day with deionized water to 40% moisture and the amount of water used was based on the recorded weight of the control pot. The irrigation was conducted slowly to prevent any leaching from soil to the sand layer. The plants were grown under a cycle of 14-h days at 25 °C and 10-h nights at 20 °C. Three replicates were set up for each soil sample. The wheat was cultivated for 60 days. Both soil (0–15 cm) and plant samples were sampled and measured for NAP, ACE, FLU, and PHE.

**Sample Extraction.** A sequential extraction was conducted using 5-g samples in 34 mL stainless steel vessels with ASE (Dionex 300). The dead volume of each vessel was filled with quartz sand, and extractions were performed at 10 335 kPa in the following sequence of decreasing polarity: (1) extraction with distilled water at 90 °C; (2) extraction with n-hexane at 140 °C; and (3) extraction with a 1:1 mixture of dichloromethane and acetone at 140 °C.

Each step was conducted for a 5 min warm-up followed by 5-min static extraction. After each step of the extraction, the vessels were rinsed with 17 mL of the same carrier solvent and the extracted analytes were purged using pressurized nitrogen at 10 335 kPa. The final volume of the extract from each step was approximately 40 mL. The results of a preliminary experiment revealed that the total amounts of the PAHs extracted by the three steps were between 89% and 116% of those by a single extraction using a mixture of dichloromethane and acetone.

Wheat roots were washed with tap water, rinsed with deionized water, and oven dried at 60 °C. The dried masses were recorded, and the samples were ground using a stainless steel pulverizer. The pulverized root samples (0.6 g) were subjected to silica gel column cleanup. A glass column (300 mm length × 10 mm i.d.) was packed with 10 g of silica gel and about 20 mm length of anhydrous sodium sulfate. All elutions were carried out at 2 mL/min. The extracts were concentrated using the rotary evaporator before being transferred to the gel column using cyclohexane (2 × 2 mL). The column was eluted with 25 mL of n-hexane followed by 50 mL of a 3:2 mixture of pentane and dichloromethane at a rate of 2 mL/min. The eluate was concentrated to near dryness in the rotary evaporator, transferred with n-hexane, and brought to 1.0 mL by nitrogen blowdown. The cleanup procedure for the plant samples included sulfonation and silica gel column chromatography in sequence to remove lipids and other co-extractants. The extract was concentrated to a trace fraction and then quantitatively transferred to a

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**Table 1.** Sampling Locations, Baseline PAH Concentrations, pH, TOM, and DOM of the Soil Samples

<table>
<thead>
<tr>
<th>no.</th>
<th>location</th>
<th>land use</th>
<th>topography</th>
<th>TOM mgC/g</th>
<th>DOM mgC/Kg</th>
<th>pH</th>
<th>NAP ng/g</th>
<th>ACE ng/g</th>
<th>FLU ng/g</th>
<th>PHE ng/g</th>
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<tr>
<td>1</td>
<td>40°,17′,43″–116°,11′,49″</td>
<td>wasteland</td>
<td>hillside</td>
<td>1.3</td>
<td>31.0</td>
<td>6.83</td>
<td>1.00</td>
<td>0.70</td>
<td>0.32</td>
<td>0.89</td>
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<tr>
<td>2</td>
<td>40°,17′,18″–116°,14′,10″</td>
<td>pasture</td>
<td>hillside</td>
<td>5.3</td>
<td>25.0</td>
<td>6.70</td>
<td>1.90</td>
<td>0.88</td>
<td>1.11</td>
<td>1.42</td>
</tr>
<tr>
<td>3</td>
<td>40°,18′,05″–116°,11′,26″</td>
<td>wheat</td>
<td>hillside</td>
<td>6.9</td>
<td>17.2</td>
<td>6.87</td>
<td>1.95</td>
<td>0.32</td>
<td>0.80</td>
<td>1.10</td>
</tr>
<tr>
<td>4</td>
<td>40°,16′,12″–116°,13′,45″</td>
<td>orchard</td>
<td>level land</td>
<td>8.7</td>
<td>22.0</td>
<td>7.20</td>
<td>1.60</td>
<td>1.24</td>
<td>1.08</td>
<td>2.27</td>
</tr>
<tr>
<td>5</td>
<td>39°,59′,26″–116°,16′,31″</td>
<td>wood</td>
<td>level land</td>
<td>10.1</td>
<td>22.1</td>
<td>6.75</td>
<td>2.16</td>
<td>1.60</td>
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<td>1.87</td>
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<td>6</td>
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<td>alluvial flat</td>
<td>10.4</td>
<td>18.5</td>
<td>6.12</td>
<td>2.82</td>
<td>1.58</td>
<td>1.62</td>
<td>2.75</td>
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<tr>
<td>7</td>
<td>40°,16′,08″–116°,13′,41″</td>
<td>wasteland</td>
<td>hillside</td>
<td>12.0</td>
<td>13.1</td>
<td>6.25</td>
<td>1.96</td>
<td>1.34</td>
<td>1.81</td>
<td>2.39</td>
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</table>
after 64 days of aging and to contribute to this loss. Average concentrations of the two, including volatilization, degradation, and sequestration could be ACE, FLU, and PHE, respectively. A number of processes (and 9.9 to 2.7) during the cultivation period. The concentrations dropped from 50 ng/g quickly at the beginning and gradually leveled off during the Experiment.

Changes of PAH Concentrations in the Soils during the Experiment. Concentrations of PAHs in the soils decreased quickly at the beginning and gradually leveled off during the cultivation period. The concentrations dropped from 50 ng/g to 2.7 ± 0.42, 10.1 ± 1.66, 20.6 ± 2.70, and 25.6 ± 4.79 ng/g after 64 days of aging and to 2.1 ± 0.25, 3.8 ± 0.20, 8.7 ± 0.82, and 9.9 ± 0.90 ng/g by the end of the cultivation for NAP, ACE, FLU, and PHE, respectively. A number of processes including volatilization, degradation, and sequestration could contribute to this loss. Average concentrations of the two measurements before and after the cultivation were taken as the exposure concentrations. The experimental measurements included native PAHs, which could be less bioavailable than the spiked PAHs. However, they could not be distinguished in this study.

Effect of Soil Organic Matter on Extractability of Various Solvents for PAHs. Recoveries of individual PAHs as well as the sum of the four (PAH4) with water were negatively correlated to TOM and positively correlated to DOM (Figure 1). With a single exception of FLU, significant correlations exist at a level of 0.05 (critical r value is 0.707). For the seven soils studied, while DOM increased around 3 times from 13.1 to 31.0 mg/kg, the water-extractable PAH4 increased from 7.0 to 14.7 ng/g.

The influence of DOM on a wide range of hydrophobic organic chemicals has been suggested by several previous groups of investigators. For example, Wilcke indicated that PAHs in soil are mainly associated with organic matter and soot-like carbon and their mobility increases in the presence of DOM (14). Burche and Hirner studied the mobilization potential of PAHs, polychlorinated biphenyls (PCBs), and phenol derivatives in soil and revealed that leaching behavior of PAHs and PCBs depended on both concentration and quality of DOM in soil (15). On the other hand, TOM can retain PAHs in soil, resulting in the negative correlation (8).

Similar to the water-extractable fractions, the quantities of n-hexane-extracted PAHs decreased as TOM increased (Figure 2). With the exception of NAP, the negative correlations between the individual n-hexane-extracted PAH compounds and soil TOM were significant at 0.05 levels. This is consistent with results reported in the literature. Conte et al. found that recoveries of PAHs were higher for soil samples oxidized with hydrogen peroxide to remove organic matter than those for non-oxidized samples (8). The n-hexane recovered PAHs were part of those associated with the soil matrix. Soil organic matter played an important role of binding PAHs and preventing them from being extracted (8). Unlike the water-extractable fraction,
which linearly correlated with DOM, the correlation between the \( n \)-hexane-extractable PAHs and soil TOM was nonlinear. With an initial increase in TOM from 1.3 to 6.9 mg/g, PAHs extracted by \( n \)-hexane remained more or less constant. The recoveries of PAHs decreased sharply when soil TOM increased from 6.9 to 12.0 mg/g.

The relationship between the quantities of dichloromethane–acetone-extracted PAHs and TOM in soil is illustrated in Figure 3. Because this procedure is often applied to measure the total extractable PAHs in soil or sediment (16), it was used in this study as the last step to recover PAHs that remained after the first two extractions.

Because the water and \( n \)-hexane-extractable fractions were all negatively correlated with soil TOM, those extracted with the dichloromethane–acetone mixture were the remnants from the first two extractions. Therefore, the positive correlation shown in Figure 3 reflects the relationship between the extractability of the remnants and soil TOM. As indicated in the Methodology section, the amounts of PAHs extracted with a single extraction using dichloromethane–acetone were approximately equal to the sums of those extracted by the three steps in sequence. Figure 4 presents the sum of the three extractions. Even though the total recoveries seem to decrease slightly as soil TOM increased, the result of a one-way ANOVA ($\alpha = 0.05$) indicates no significant difference among the seven treatments. If the potential of sequestration is taken into consideration, it is expected that the extractability of PAHs would be reduced as soil TOM increased (17). Conceivably, the duration of the experiment was too brief to show such an effect.

The three fractions of extracted PAH* average 25.5%, 44.8%, and 29.7%, respectively. However, the amounts changed among the soil samples. For example, as soil TOM increased from 1.3 to 12.0 mg/g, the amount of PAH* extracted by dichloromethane–acetone increased from 14.0% to 48.2%, whereas the fraction extracted by water decreased from 33.2% to 18.6%.

**Effect of Organic Matter on Apparent Bioavailability of PAHs to Uptake by Wheat.** Because plant shoots often accumulate PAHs directly from air (14), only PAHs accumulated in wheat roots were used to assess the influence of soil organic matter on bioavailability. Among many physicochemical properties that can be used to characterize soil properties, the most significant ones that reduce the bioavailability of PAHs are quantity of organic carbon and their hydrophobicity (18). On the other hand, Wilcke has found that the association of PAHs with DOM enhances the mobility of PAHs (14). The positive correlation between PAHs in plant roots and DOM and the negative correlation between PAHs in plant roots and TOM shown in Figure 5 are consistent with those reported in the literature, suggesting that TOM and DOM act actively to either immobilize or mobilize PAHs in soil for plant root uptake. Although the correlations are significant at a level of 0.05, the negative correlation between the root concentration and soil TOM tends to be nonlinear, especially for FLU, PHE, and PAH* (Figure 5, bottom). As compared to those illustrated in Figure 2, it can be seen that the nonlinearity is similar to that between the \( n \)-hexane-
extractable PAHs and soil TOM; for example, the influence of TOM on the apparent bioavailability becomes stronger as TOM increases.

**Discussion**

**Relationship between the Extractability and the Apparent Bioavailability.** Based on the results presented in Figures 1, 2, and 5, water or $n$-hexane-extracted PAHs and wheat root accumulated PAHs are all negatively correlated to soil TOM, suggesting a positive correlation between the extractability (water, $n$-hexane, and water + $n$-hexane) and the apparent bioavailability of PAHs (Figure 6).

With two exceptions of water-extracted ACE and $n$-hexanes-extracted NAP, significant linear correlations are revealed between the water- or $n$-hexane-extracted and plant root accumulated PAHs. When the four PAHs are pooled together, the correlations are significant at levels of 0.016% ($r = 0.98$) and 0.059% ($r = 0.97$) for water and $n$-hexane fractions, respectively. In terms of the total PAH compounds studied, both extractants are satisfactory as a single chemical test to indicate the apparent bioavailability to root uptake. If the results of the two steps (water and $n$-hexane) are pooled together, the correlation coefficient between the extraction and accumulation increased to 0.99, which is significant at a level of 0.008%.

As indicated above, the correlations between the water or $n$-hexane-extracted PAHs and soil TOM and the correlation between the root-accumulated PAHs and soil TOM are not linear. However, the curvilinear patterns are similar with relatively sharp changes in the high TOM range. The linear correlation between the extractability and the apparent bioavailability shown in Figure 6, therefore, is a result of such similarity. According to these results, it appears that the bioavailability of PAHs to plant root uptake can be mimicked using a single ASE extraction with either water or $n$-hexane. In a similar experiment using humic substances amended soils, it was found that the $n$-hexane-extractable fraction of DDT is a good indicator of bioavailability to plant roots (19). It should be noted that quantitative results from this study could not be simply extrapolated to other plants or other chemicals, because the bioavailability of chemicals is species specific.

**Differences among the PAH Compounds.** The four PAH compounds studied are different in their extractability and bioavailability. If quantities of the water or $n$-hexane-extracted PAH fractions were plotted against their water solubility ($S_w$), both in log-scale, linear relationships are demonstrated (Figure 7). Moreover, the two regression lines are parallel to each other, indicating that the extractability of $n$-hexane is stronger than water and their dependences on water solubility are quantitatively comparable.

The bioavailability of PAHs partially depends on their hydrophilic nature as well as on their binding in soil. Therefore, it is expected that the partition of hydrophobic

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**FIGURE 6.** Relationship between the quantities of NAP, ACE, FLU, PHE, and PAH$_4$, accumulated in the plant roots and those extracted with water, $n$-hexane, and the sum of water and $n$-hexane (mean values of the two extractions at day 64 and day 124). Means (□) ± standard deviations (bar) are presented for both variables.

**FIGURE 7.** Amount of PAHs extracted with water or $n$-hexane (log-scaled) as functions of log-scaled water solubility ($S_w$).

**FIGURE 8.** Relationships between the ratios of root uptake over water-extracted PAHs and $K_{ow}$. Between the root-accumulated PAHs and soil TOM are not linear. However, the curvilinear patterns are similar with relatively sharp changes in the high TOM range. The linear correlation between the extractability and the apparent bioavailability shown in Figure 6, therefore, is a result of such similarity. According to these results, it appears that the bioavailability of PAHs to plant root uptake can be mimicked using a single ASE extraction with either water or $n$-hexane. In a similar experiment using humic substances amended soils, it was found that the $n$-hexane-extractable fraction of DDT is a good indicator of bioavailability to plant roots (19). It should be noted that quantitative results from this study could not be simply extrapolated to other plants or other chemicals, because the bioavailability of chemicals is species specific.
PAHs between water-based soil solution and plant roots can be described by the water–octanol partition coefficient ($K_{ow}$) to a certain extent. As shown in Figure 8, the calculated ratios for PAH accumulation in roots to the amount extracted with water are positively proportional to $K_{ow}$ (significant at level of 0.001), indicating that the PAH compounds with relatively higher $K_{ow}$ values have a stronger tendency of bioaccumulation.

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**Literature Cited**


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