

Transport and Fate of Polycyclic Aromatic Hydrocarbons in Soil-Plant System

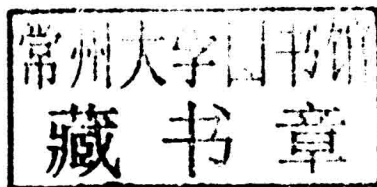
Yanzheng Gao
Juan Liu Fuxing Kang



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Preface

Anthropogenic soil contamination has become a worldwide environmental problem during the past decades. Natural and xenobiotic organic pollutants present in soils could be taken up by plants, which is a major pathway for toxic substances to enter food chain. Polycyclic aromatic hydrocarbons (PAHs) are a well-recognized group of organic contaminants that have received vast attention largely due to the concerns on their high toxicity and recalcitrance in soil environment. Hydrophobic characteristics and persistence of PAHs in the environment result in their accumulation and enrichment in soils. PAHs present in soils can be absorbed by plants. Given that plants serve as the foundation of human and animal food webs, daily consumption of PAH-contaminated food could potentially increase human and animal exposure to hazardous substances. Better understanding of transport and fate of PAHs in soil-plant system is therefore essential to protecting human and ecological health from exposure to contaminants.

In the past several years, the research theme of Research Group of Organic Contaminant Control and Soil Remediation, Nanjing Agricultural University has been focusing on the transport and fate of PAHs in soil-plant continuum. The research program received financial supports from National Science Foundation of China (41171193, 41171380, 21477056, 21077056, 40971137, 40701073, 20777036, 20507009), Science Foundation of Jiangsu Province (BK20130030, BK2009315, BK2007580, BK2006518), and Special Fund for Agro-scientific Research in Public Interest, China (201503107). The major findings are incorporated in this book.

The book composed of two parts, Part I Soil and Part II Plant. Part I focuses on the fractions, availability and rhizospheric distributions of PAHs in soil environments. We elucidated the partitioning process of PAHs among soil, water and plant roots. During plant growth, roots could actively or passively release a range of organic compounds referred to as root exudates. Low-molecular-weight organic acids (LMWOAs) occurring widely in soils are a group of natural products present in root exudates. In Part I, the impacts of root exudates and LMWOAs to sorption/desorption, release and availability of PAHs in soils were elucidated. Part II focuses on uptake, subcellular distributions, and metabolism of PAHs in plants. Microorganisms

associated with plants play a key role in PAH uptake by plants. Plant-arbuscular mycorrhizal fungi (AMF) symbioses are ubiquitous in the environment. We investigated the influence of AMF on plant uptake and accumulation of PAHs from soils, and evaluated the functions of AMF hyphae in the PAH uptake by plants. Endophytic bacteria form one of the microbial communities most closely associated with plants. Colonization of PAH-degrading endophytic bacteria provided a novel method for removal of PAHs within plants.

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Yanzheng Gao

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PART I SOIL

Chapter 1 The forms and availability of PAHs in soil

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 (Gao et al., 2009). This organic pollution triggered by human activities has been a long-term environmental problem in past decades (Führ and Mittelstaedt, 1980; Kipopoulou et al., 1999; Gao et al., 2007). 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.

As priority pollutants that are commonly found in the soil environment, polycyclic aromatic hydrocarbons (PAHs) are of major concern due to their recalcitrance and strong mutagenic/carcinogenic properties (Weber and Huang, 2003; Tang et al., 2007). The hydrophobic characteristic and persistence of PAHs result in their accumulation and enrichment in soils. PAHs are widespread and occur at high concentrations of hundreds of mg/kg in soils of many countries (Joner and Leyval, 2003; Ling et al., 2013). Contamination of soil with PAHs poses risks to human and ecosystem health.

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 (Ling et al., 2010). 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 (Macleod and Semple, 2003). Instead, the form and availability of these contaminants in soil are the most important indices for risk assessment.

1.1 The forms of PAHs in soil

The forms of organic contaminants in soil environments have been reported in literatures (Monteiro et al., 1999; Northcott and Jones, 2000; Loiseau and Barriuso, 2002; Lesan and Bhandari, 2004). Macleod and Semple (2003) 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 (Kohl and Rice, 1998; Kacker et al., 2002). 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. Recently, we fractionated the forms of parent PAH compounds in soils (Gao et al., 2009; Ling et al., 2010). The influence of aging time and microbial activities on the forms of PAHs was also investigated. Results of this work will have considerable benefits for risk assessment, food security, and development of remediation strategies for contaminated sites.

1.1.1 Fractionation methods of PAH residues in soil

A sequential extraction/chemical mass balance approach described by Sabat et al (2006) was used to fractionate the forms of parent PAH compounds in soils. PAHs in soil were separated into three fractions: a desorbing fraction, a non-desorbing fraction, and a bound residual fraction (Gao et al., 2009; Ling et al., 2010).

(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. (2000) and Cuypers et al. (2002). 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. Mild extraction solution consisted of 70 mmol/L hydroxypropyl- β -cyclodextrin (HPCD) and 0.05 g NaN₃ per mL in Milli-Q water. Tubes were closed with a Teflon-liner cap, shielded from light, and shaken horizontally at 150 r/min at 25°C. At 60 h, 120 h and 240 h, tubes were centrifuged for 25 min at 2000 r/min 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 Na₂SO₄ anhydride and combined. The solvent was firstly concentrated by rotary evaporation, then evaporated under a gentle stream of N₂, and diluted with methanol to a final volume of 2 mL. After filtration through a 0.22 μ m filter, PAHs were detected by high pressure liquid chromatography (HPLC).

(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 r/min, and then treated as described above.

(3) Bound residue extraction. Dried soil samples resulting from exhaustive extractions were extracted in order to obtain the bound residue fraction. The extraction method was as described by Richnow et al. (2000). 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 r/min 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.

1.1.2 Desorbing fraction of PAHs in soil

The desorbing fraction of a compound in soils has been shown to be the most bioavailable portion (Reid et al., 2000; Cuypers et al., 2002). In this study, we utilized water in combination with HPCD to extract the desorbing fractions of PAHs in a yellow-brown soil collected from Nanjing, Jiangsu. The soil type is a Typic Paleudalf, a typical zonal soil in East China, with a pH of 6.02, 14.3 g/kg soil organic carbon content (f_{oc}), 24.7% clay, 13.4% sand, and 61.9% silt. Test PAHs included fluorene (FLU), phenanthrene (PHE), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA), and benzo[a]pyrene (BaP). Cyclodextrins have a hydrophilic cavity but also contain a hydrophobic organic cavity within their molecular structure, which allows the formation of a water-soluble inclusion complex between the cyclodextrin molecule and low polarity organic compounds (Shieh and Hedges, 1996). Successful use of HPCD for estimation of the desorbing PAH fraction and correlation with the biodegradable fraction of organic compounds in a few soils and sediments have been reported (Reid et al., 2000; Cuypers et al., 2002).

The concentrations of the desorbing fraction of PAHs in soil over 0~16 week were given in Figure 1-1. 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 non-sterilized soil. However, the decrease magnitude of this fraction varied greatly for different PAHs, 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 PAHs 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 extent after 4 weeks, and then remained nearly constant in 8~16 week-incubation (Figure 1-1). 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” (Sun and Li, 2005). 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.

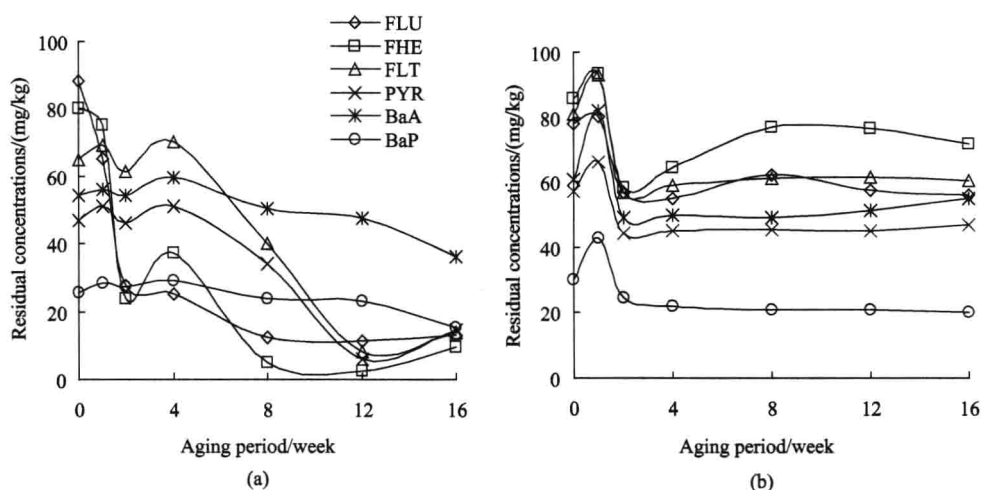


Figure 1-1 Concentrations of the desorbing fraction of PAHs in non-sterilized (a) and sterilized (b) soil as a function of time

Interestingly, as tabulated in Table 1-1, the dissipation amount of the desorbing fractions of the tested PAHs was about 76.1%~152% of their total dissipation in soils (non-sterilized soil 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

Table 1-1 The concentrations of the total and desorbing fraction of PAHs in soils

PAHs	$C_{0\text{-total}}$ /(mg/kg)	$C_{16\text{weeks-total}}$ /(mg/kg)	ΔC_{total} /(mg/kg)	$C_{0\text{-HPCD}}$ /(mg/kg)	$C_{16\text{weeks-HPCD}}$ /(mg/kg)	$\Delta C_{\text{total-HPCD}}$ /(mg/kg)	$\frac{\Delta C_{\text{total-HPCD}}}{\Delta C_{\text{total}}}$ /%
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
Fluoranthrene	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

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

fractions). As seen in Table 1-1, 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.

The percentage of the desorbing fraction relative to the total contents of PAHs at specific time points was calculated and illustrated in Figure 1-2. 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 non-sterilized 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 week can also be ascribed to both microbial degradation of this fraction and its transfer into other fractions in the soil.

1.1.3 Non-desorbing fraction of PAHs in soil

The non-desorbing fractions of the six PAHs in soil as a function of time are given in Figure 1-3. Concentrations of this fraction of PAHs with lower molecular weight generally decreased in non-sterilized soils over 0~16 week. For instance, the concentrations of the non-desorbing fractions of FLU, FHE, FLT, and PYR after a 16 weeks aging decreased from 4.74 mg/kg, 7.53 mg/kg, 16.6 mg/kg, and 19.0mg/kg to 2.06 mg/kg, 1.49 mg/kg, 6.74 mg/kg, and 9.19mg/kg, respectively. However, for