3658 *J. Agric. Food Chem.* **2006,** *54,* 3658–3662

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Tea Plant Uptake and Translocation of Polycyclic Aromatic Hydrocarbons from Water and around Air

DAOHUI LIN,[†] LIZHONG ZHU,*,[†] WEI HE,[‡] AND YOUYING TU[‡]

Department of Environmental Science, Zhejiang University, Hangzhou, China 310028, and
Department of Tea Science, Zhejiang University, Hangzhou, Zhejiang, China 310029

This study, which aimed to investigate the capacity of tea plant uptake and translocation of polycyclic aromatic hydrocarbons (PAHs), was divided into two sections. One was to study tea plant root uptake of PAHs from water and translocation to leaves. The other was to research tea plant leaf uptake of PAHs from air. It was observed that tea plant roots and leaves could strongly accumulate PAHs from around the environment. The capacity of tea plant uptake and translocation of PAHs were found to be closely relative to the physical−chemical properties of PAHs. With the increase of lgK_{ow} (octanol−water partition coefficient) of the PAHs, both root concentration factors and leaf concentration factors increased exponentially, while translocation factors from roots to leaves decreased exponentially.

KEYWORDS: **PAHs; tea; plant uptake; plant translocation**

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), a class of compounds that consist of two or more fused aromatic rings, are a well-known class of carcinogens found in some foods, and they have been intensively studied over the past few years (*1*, *2*). Gaseous and particle-bound PAHs can be transported over long distances before deposition and may accumulate in vegetation (*3*−*7*). This could indirectly cause human exposure to PAHs through food consumption and, thus, might pose a human health threat.

Next to water, tea is the most widely consumed beverage in the world. There is lots of scientific evidence indicating that tea consumption may have health-promoting properties, including reduction of cholesterol, depression of hypertension, antioxidation and antimicrobial effects, and protection against cardiovascular disease and cancer (*8*−*10*). However, residues of certain chemical contaminants in tea leaves, which may pose a health threat to tea drinkers, are occasionally detected. The main contaminants that have been investigated widely and intensively are heavy metals, fluoride, and pesticides (*11*−*17*). PAHs have been detected in some made teas around the world and can be released into tea water (*18*−*21*). Our previous research revealed that PAHs in a certain kind of made tea mainly came from the manufacturing process (*20*). However, PAHs were also detected in fresh tea leaves (*20*), indicating that PAHs could be accumulated by fresh tea leaves from around the environment.

During the past decades, there has been considerable interest in understanding plant uptake and accumulation of organic contaminants including PAHs (*3*−*5*, *22*−*30*). Organic contaminants may enter vegetation by partitioning from contaminated soil or water to the root and translocating in the vegetation by the xylem. Organic contaminants may also enter plants from the atmosphere by gas-phase and particle-phase deposition onto the waxy cuticle of the leaf or by uptake through the stomata, translocating by the phloem. These two pathways are a function of the physical−chemical properties of the pollutants, the environmental conditions, and the plant species. Most of hydrophilic contaminants with lgK_{ow} (octanol−water partition coefficient) < 4 can be accumulated by roots and be acropetally translocated within plant, while lipophilic organic contaminants with lgK_{ow} > 4 may strongly partition onto the epidermis of the roots and cannot be drawn into the inner roots and be translocated within the plant (*4*). The main pathway for lipophilic organic contaminants entering plant leaf is from the air to the leaf surface; however, the partition of these contaminants from the outer leaf to the inner leaf is slow and is rarely transported by the phloem since it is also water-based (*4*). However, there are a few exceptions. Hulster et al. observed that zucchini and pumpkins accumulated and translocated higher concentrations of PCDD/Fs from contaminated soils (*31*). PAHs are a certain class of semivolatile organic compounds with lgK_{ow} values ranging from about 3 to 7. Numerous studies showed that PAHs were accumulated in both vegetable roots and leaves (*3*−*5*, *25*, *30*). However, it is still under investigation whether PAHs can be translocated within plants.

Tea plants possess high leaf surface areas and high root densities. Some studies have revealed that metals and fluoride can be accumulated by tea roots and leaves and then be translocated within the tea plant (*32*−*35*). Organic pesticides can also be accumulated by tea leaves from around air (*36*−*38*). However, little information is available about whether

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* To whom correspondence should be addressed. Tel: 86-571-88273733. Fax: 86-571-88273693. E-mail: zlz@zju.edu.cn.
[†] Department of Environmental Science.
[‡] Department of Tea Science.

10.1021/jf052909c CCC: $33.50

organic contaminants including PAHs can be accumulated by tea roots or leaves and then be translocated within the tea plant.

The aim of this study was to investigate the tea plant uptake, accumulation, and translocation behaviors of PAHs from water and air, naphthalene (NA), acenaphthylene (ACY), fluorine (FL), phenanthrene (PHEN), and pyrene (PY) as representatives. The lgK_{ow} values of these five PAHs are as follows: NA, 3.36; ACY, 4.08; FL, 4.18; PHEN, 4.46; and PY, 5.3, respectively (*21*).

MATERIALS AND METHODS

Preparation of Tea Seedlings. A variety of tea plants, namely, Longjing 43 belonging to the small-leafed variety of *Camellia sinensis* L., were chosen for the present study. Longjing tea, which is mainly produced in Hangzhou City of Zhejiang Province in China, is one of the most famous green teas. Two year old Longjing 43 seedlings were obtained from the tea plantation of the Tea Research Institute of the Chinese Academy of Agricultural Sciences. After the seedlings were collected and thoroughly washed with water, the tea seedlings were allowed to grow in nutrient solutions for 1 week before they were used for the uptake study. The compositions of the nutrient solutions were as follows: 20 ppm $(NH_4)_2SO_4$, 10 ppm NH_4NO_3, 3.1 ppm NaH_2PO_4, 40 ppm K_2SO_4, 15 ppm $CaCl_2{\cdot}2H_2O$, 0.35 ppm $EDTA{\cdot}FeNa{\cdot}3H_2O$, 25 ppm $MgSO_4{\cdot}3H_2O$, 20 ppm $Al_2(SO_4)_3{\cdot}18H_2O$, 0.1 ppm $ZnSO_4{\cdot}7H_2O$, 0.1 ppm H_3BO_3, 0.025 ppm $CuSO_4{\cdot}5H_2O$, 1 ppm $MnSO_4{\cdot}H_2O$, and 0.05 ppm $Na_2MoO_4{\cdot}2H_2O$. All of these compositions were analytical grade with a purity of >98%.

Treatments with PAHs. The uniform tea seedlings selected for the uptake study were about 60 cm in height with about 15 leaves on each seedling. Six seedlings were loosely bundled together in the lower part of the stems with Teflon tape and were cultured in an aluminous barrel through a drilled hole on the cap. The open areas between the cap and the seedlings were sealed with a sponge. There was 8 L of nutrient solution in each aluminous barrel. The roots of the seedlings were immersed just below the surface of the solution. The experiment was divided into three groups, each of which was composed of three aluminous barrels with seedlings hydroponically cultured. Group 1 was set as a blank control without PAHs added into the solutions. Group 2 was set to study the root uptake of PAHs from the solutions. Five kinds of PAHs obtained from Aldrich Chemical with a purity of >98% were added into the solutions in group 2 with initial concentrations as follows: NA, 326 ppb; ACY, 847 ppb; FL, 139 ppb; PHEN, 802 ppb; and PY, 509 ppb larger than its solubility (about 120 ppb). Group 3 was set to study leaf uptake of PAHs from around the air. Three barrels with seedlings cultured in nutrient solutions without PAHs added were placed in a glass chamber with a length, breadth, and height of 1, 1, and 1 m, respectively. Certain amounts of the five PAH particles were placed on the bottom to increase the air PAH concentrations in the chamber. The experiment lasting for 10 days was carried out in a greenhouse with temperatures of about 25−30 °C in the daytime and 15−20 °C in the nighttime. The six barrels of groups 1 and 2 were randomly placed together in the greenhouse to avoid the difference of air PAH concentrations. At the end of the experiment, the solutions, the air inside and outside the chamber, and the tea seedlings sectioned into main roots, small roots, stems, and leaves were sampled for PAH analysis.

Extract and Analysis of PAHs. Air samples were taken with low noise small samplers (MP-15CF mini pump, Shibita, Japan) operated at 1.0 L/min equipped with a Whatman glass fiber filter (GFF, 25 mm, Whatman, England) collecting particle-bound PAHs and XAD-2 (2.5 g) retaining PAHs in the gaseous phase, respectively (*39*, *40*). PAH concentrations in the air were shown with the sum of concentrations in two phases. Air sampling programs were performed for 3 h just before the end of the experiment with samplers located inside and outside of the glass chamber at the height of the seedling leaves. In general, air samples were extracted by ultrasonication at 30 °C for 30 min with a 20 mL mixture of dichloromethane (DCM) and acetonitrile (3:2, v/v). Then, 10 mL of extraction raffinate with 30 μL of dimethyl sulfoxide (DMSO) was evaporated to dryness under a gentle flow of nitrogen gas at room temperature before 2 mL of acetonitrile was added (*39*, *40*).

The extract of PAHs from seedling samples was described elsewhere (*30*). A certain amount of seedling samples was ground, homogenized, and extracted by ultrasonication with a 1:1 (v/v) solution of acetone and hexane. The extracts were then decanted and collected. This process was repeated in triplicate. All extracts were combined and passed through a column packed with anhydrous Na_2SO_4 using 10 mL of a 1:1 (v/v) mixture of DCM and acetone. The extracts were evaporated to dryness by a rotary evaporator and then dissolved in 2 mL of hexane. One milliliter of the solution was filtered through a silica gel column and eluted with 10 mL of hexane and DCM (1:1, v/v) and then was evaporated to dryness. The residue was dissolved in 2 mL of acetonitrile for analysis. The water solution was diluted using methanol to obtain the final solution with 50% (v/v) methanol (*41*).

The analysis of PAHs was described elsewhere (*40*). All extracts of the treated samples of air, seedlings, and water solutions were filtered with a 0.22 μm minisart filter in a vial sealed with a PTFE-lined cap. Then, 15 μL extracts were injected by autosampler to be analyzed by a high-performance liquid chromatography (HPLC) system (Agilent 1100, United States) consisting of a quaternary pump, a PAH column (Agilent, C-18, φ4.6 mm × 250 mm), a UV detector, a data processor, and a system controller.

All solvents (methanol, DCM, acetonitrile, and hexane) used for sample preparation and analysis were HPLC grade from TEDIA Co. (United States). Chromatography silica gel (200−300 mesh) used for sample purification was purchased from Huadong Medical Corp. (China).

RESULTS AND DISCUSSION

Uptake of PAHs from Water by Tea Seedling Roots. The results of PAH analysis are shown in **Table 1**. It can be observed that root PAH contents in group 2 at the end of the experiment were obviously higher than those in groups 1 and 3, indicating that the tea plant roots can strongly accumulate PAHs from water. Root PAHs detected at the end of the experiment in group 1 mainly might come from the nutrient solutions, which contained certain amounts of PAHs at the beginning of the experiment (see **Table 1**). Root PAH concentrations in group 3 were higher than those in group 1, indicating that air PAHs that volatilized from the PAH particles placed on the bottom of the glass chamber might enter into the solutions and then accumulate in the tea roots. This can be illustrated by the fact that PAH concentrations in the solution of group 3 were higher than those in group 1 and the concentrations of NA, FL, and ACY were even higher than those in group 2. However, only the root concentration of NA among the five PAHs in group 3 was higher than that in group 2. This might be because NA possesses the largest volatility and solubility among the five PAHs and could be relatively easily translocated among the air, water, and tea roots.

Root concentration factors (RCFs), defined as the ratio of the PAH concentrations in root and in solution of group 2 at the end of the experiment (only in this group, all five of the PAHs could be detected in solutions), were calculated. PAH concentrations in small roots were higher than those in the main roots because small roots possess larger surfaces. The average RCFs of small roots were about eight times higher than those of the main roots in this study. It can be seen from **Figures 1** and **2** that for small roots or for main roots, the lgRCFs of the five PAHs were all proportional to their lgK_{ow} values with a correlative equation of lgRCFs $= 1.241\,\mathrm{lg}K_{ow} - 1.152$ and lgRCFs $= 0.791\,\mathrm{lg}K_{ow} + 0.354$, respectively. This indicated that RCFs exponentially increased with the increase of the lgK_{ow} values of the five PAHs.

Uptake of PAHs from around Air by Tea Leaves. The surface area of the above-ground parts of plant usually exceeds the surface area of the ground in which they grow. It was

Table 1. PAHs Concentrations of Water Solutions, Air, and Tea Seedlings at Both the Beginning and the End of the Experiment[a]

samples	NA	ACY	FL	PHEN	PY
		solution at the beginning (μg/L)			
groups 1 and 3	1.40	12.3	2.00	17.0	27.1
group 2	326	847	139	802	509
		solution at the end (μg/L)			
group 1	0.12 ± 0.02	ND	ND	ND	ND
group 2	0.15 ± 0.02	5.88 ± 1.13	1.00 ± 0.22	6.09 ± 1.21	53.8 ± 10.1
group 3	0.15 ± 0.02	11.8 ± 1.61	1.49 ± 0.28	1.07 ± 0.29	ND
		air at the end (μg/m^3)			
out of the glass chamber	0.580	0.120	0.060	0.280	ND
in the glass chamber	1040	956	341	50.8	0.630
		sections of tea seedlings at the end (μg/kg in dry weight)			
small roots in group 1	106 ± 10	172 ± 35	161 ± 20	540 ± 46	248 ± 47
small roots in group 2	230 ± 43	5730 ± 291	2360 ± 376	52600 ± 9980	68400 ± 11200
small roots in group 3	311 ± 56	4420 ± 866	994 ± 89	1280 ± 175	1310 ± 84
main roots in group 1	34.9 ± 6.9	17.8 ± 3.2	14.5 ± 1.9	99.7 ± 12.3	56.9 ± 8.1
main roots in group 2	168 ± 13	4270 ± 650	1750 ± 140	8220 ± 821	9040 ± 104
main roots in group 3	198 ± 36	1470 ± 198	281±45	175 ± 10	57.3 ± 9.8
leaves in group 1	169 ± 23	19.3 ± 2.1	14.5 ± 2.4	164 ± 32	44.8 ± 3.5
leaves in group 2	197 ± 19	39.1 ± 5.1	22.7 ± 4.5	172 ± 12	47.7 ± 3.1
leaves in group 3	13000 ± 1460	125000 ± 14100	53800 ± 5760	40300 ± 2560	3200 ± 644
stems in group 1	22.4 ± 2.4	28.7 ± 1.7	10.6 ± 2.0	47.3 ± 3.5	24.3 ± 4.3
stems in group 2	27.8 ± 0.7	49.7 ± 2.5	17.8 ± 1.4	48.3 ± 5.6	29.2 ± 2.3
stems in group 3	5030 ± 914	11200 ± 1340	5560 ± 675	5190 ± 601	536 ± 92

[a] Note: Group 1 was set as a black control without PAHs added into solutions. Group 2 was set to study the root uptake with PAHs added into solutions. Group 3 was set to study leaf uptake with PAH particles placed on the bottom of the glass chamber. ND, not detected.

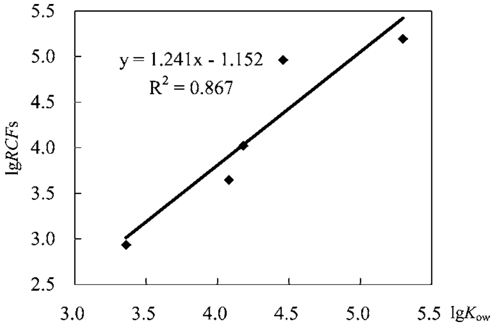

Figure 1. Correlation between small RCFs and lgK_{ow} of the PAHs.

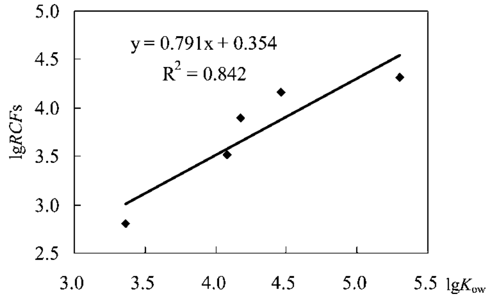

Figure 2. Correlation between main RCFs and lgK_{ow} of the PAHs.

estimated that the ratio of the leaf area to the ground area could be up to 20 (*36*). The leaves of the Longjing tea plant are thin and tender, and the surface area per unit weight of leaves is relatively larger than that of other crops (*36*). A layer of wax covers the surface of the fresh tea leaf, which may absorb PAHs from air, and the stomata of the fresh tea leaf may offer another pathway for PAHs to enter the leaf. To reveal the relationship between the PAH concentrations of tea leaves and the air around the leaves, the glass chamber was designed, inside of which air PAH concentrations were much higher than those outside, as shown in **Table 1**. The results showed that the leaf PAH concentrations inside the glass chamber were obviously higher than those outside, indicating that the fresh tea leaves could strongly accumulate the PAHs from around the air.

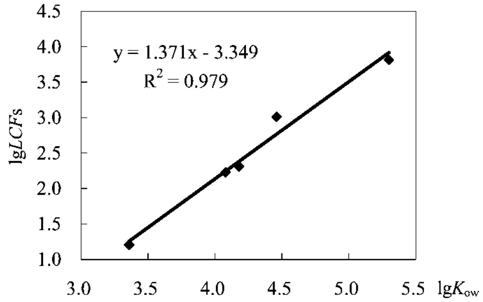

Figure 3. Correlation between LCFs and lgK_{ow} of the PAHs.

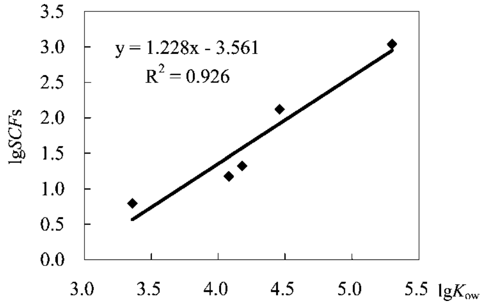

Figure 4. Correlation between SCFs and lgK_{ow} of the PAHs.

To demonstrate the capacity of the fresh tea leaves accumulation of the PAHs from air, leaf concentration factors (LCFs) and stem concentration factors (SCFs), defined as the ratios of leaf PAH concentrations to air PAH concentrations and stem PAH concentrations to air PAH concentrations, respectively, were obtained. The PAH concentrations in leaves were much higher than those in stems because the leaves possess a larger surface. The average LCFs of the PAHs were about seven times higher than the average SCFs in this study. It can be seen from **Figures 3** and **4** that the lgLCFs and lgSCFs of the five PAHs were all proportional to their lgK_{ow} values with correlative equations of lgLCFs $= 1.371\,\text{lg}K_{ow} - 3.349$ and lgSCFs =

Table 2. Ratio of the PAHs Translocated from Root to ∑PAHs in Leaf and the TF[a]

PAHs	NA	ACY	FL	PHEN	PY
R_t (%)	14.4	50.5	36.4	4.98	6.15
TF (%)	22.8	10.3	0.376	0.016	0.004

[a] Note: R_t is the ratio of the acropetal translocation of PAHs from root to ∑PAHs in leaf; TF is the ratio of the acropetal translocation of PAHs from root to ∑PAHs in root.

1.228 lgK_{ow} − 3.561, respectively. Although the lgLCFs values in groups 1 and 2 also increased with the increase of lgK_{ow} values of the PAHs, their relativities were lower than that in group 3, which might be due to the fact that the leaf PAH concentrations in groups 1 and 2 were much lower than those in group 3 and might be more easily influenced by the potential translocation of the PAHs from roots to leaves.

Tea Seedlings Translocation of PAHs. Although above-ground sections (including leaves and stems) apparently accumulated PAHs from the air, the concentrations of PAHs, especially of NA, ACY, and FL, in group 2 were still higher than those in group 1. This, in fact, suggested that the translocation of PAHs from roots to above-ground sections was also found, despite the uptake and accumulation from the air in above-ground sections. PAHs in leaves should be from two pathways, uptake from air and translocation from roots. Thus, leaf PAHs concentrations (C_L) can be calculated by the following equation: $C_L = C_a + C_t$, where C_a is PAH uptake from the air and C_t is PAH translocation from roots. So, the contribution of PAH translocation from roots to leaves to C_L (R_t) can be calculated by $R_t = C_t/C_L$. As for group 2, C_t should be the leaf PAH concentration margins between group 2 and group 1. Results (**Table 2**) showed that about 4.98−50.5% of PAHs in the leaves of group 2 was translocated from roots. It was obvious that acropetal translocation of PAHs from roots was closely related to the physical−chemical properties of PAHs. The R_t values of NA, ACY, and FL were much larger than those of PHEN and PY.

To demonstrate the capacity of the acropetal translocation of PAHs by tea seedlings from roots to leaves, a TF (*42*) was defined as the following equation: TF = C_t/C_R, where C_R is the PAH concentration in roots. As for group 2, C_R should be the root PAH concentration margins between group 2 and group 1. The TFs of the five PAHs are listed in **Table 2**.

To be taken up by tea roots and then translocated to leaves, PAHs first have to be absorbed on the root epidermis and then penetrate through the epidermis and be transported to the leaves by the xylem. Hydrophilic organic contaminants with lgK_{ow} < 1.5 may not be easily sorbed onto the root epidermis, and hydrophobic organic contaminants with lgK_{ow} > 4 will be extremely sorbed by the root epidermis, so they may have difficulty being taken up through the root pathway (*4*). The process of plant translocation of organic contaminants may be treated as a series of contaminant partitions between plant water and plant organic components (*43*). Contaminants with larger lgK_{ow} values will be more easily partitioned into the plant organic components, and their TFs will be lower. This study revealed that the TFs tended to decrease exponentially with the increase of lgK_{ow} values of PAHs. PHEN (lgK_{ow} = 4.46) and PY (lgK_{ow} = 5.3) almost could not be acropetally translocated within the tea seedlings because their TFs were lower than 0.016%.

Organic contaminants on the surface of plant leaves may be strongly bound into the waxy cuticle layer. Some can enter into the inner leaves and be translocated by the phloem. It was reported that PAHs in the leaves of some vegetation could not be translocated to the underground part (*30*). In this study, the leaf PAH concentrations in group 3 were much higher than those in group 2, while the root PAH concentrations in group 3 were lower than those in group 2. This indicated that the translocation of PAHs between tea leaves and tea roots was very limited. Although the root PAH concentrations in group 3 were obviously higher than those in group 1, in this study, it could not be concluded whether or not the increased root PAHs in group 3 were translocated from the leaf PAHs because they might come from the solutions in which the PAH concentrations were higher than those in the solution of group 1 at the end of the experiment.

ABBREVIATIONS USED

PAHs, polycyclic aromatic hydrocarbons; K_{ow}, octanol−water partition coefficient; NA, naphthalene; ACY, acenaphthylene; FL, fluorine; PHEN, phenanthrene; PY, pyrene; DCM, dichloromethane; DMSO, dimethyl sulfoxide; RCFs, root concentration factors; LCFs, leaf concentration factors; SCFs, stem concentration factors; TF, translocation factor.

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Received for review November 22, 2005. Revised manuscript received January 9, 2006. Accepted January 11, 2006. This project was jointly supported by the National Natural Science Foundation of China (20337010, 20507015), National Basic Research Program of China (2003CB415004), and Zhejiang Provincial Natural Science Foundation of China (M403041).

JF052909C