Uptake Pathways of Polycyclic Aromatic Hydrocarbons in White Clover

YANZHEN GAO † AND C.D COLLINS*‡
College of Resource and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, P.R. China, and Department of Soil Science, The University of Reading, Whiteknights, Reading, RG6 6DW, U.K.

Received March 3, 2009. Revised manuscript received June 18, 2009. Accepted June 25, 2009.

An understanding of the primary pathways of plant uptake of organic pollutants is important to enable the risks from crops grown on contaminated soils to be assessed. A series of experiments were undertaken to quantify the importance of the pathways of contamination and the subsequent transport within the plant using white clover plants grown in solution culture. Root uptake was primarily an absorption process, but a component of the contamination was a result of the transpiration flux to the shoot for higher solubility compounds. The root contamination can be easily predicted using a simple relationship with KOW, although if a composition model was used based on lipid content, a significant under prediction of the contamination was observed. Shoot uptake was driven by the transpiration stream flux which was related to the solubility of the individual PAH rather than the KOW. However, the experiment was over a short duration, 6 days, and models based on KOW may be better for crops grown in the field where the vegetation will approach equilibrium and transpiration cannot easily be measured. A significant fraction of the shoot contamination resulted from aerial deposition derived from volatilized PAH. This pathway was more significant for compounds approaching log KOW > 9 and log KOW < −3. The shoot uptake pathways need further investigation to enable them to be modeled separately. There was no evidence of significant systemic transport of the PAH, so transport outside the transpiration stream is likely to be limited.

Introduction

Plant uptake of organic pollutants is important when considering the transfer of pollutants from soils into the foodchain. Understanding the predominant pathways for plant uptake is therefore essential to protect human and ecological health when exposure to contaminated soils occurs. Dry deposition from the atmosphere (1−4), and transfer from the soil to root and shoot (5−8) have both been identified as significant pathways of crop contamination with organic pollutants. A number of models have been developed to predict plant uptake of organic pollutants (9−11); these vary in complexity from simple regression models (11) to those describing plant physiological processes (9). When gathering data to calibrate and validate plant uptake models it is essential that all significant pathways are measured. However, there are few studies where all potential plant uptake pathways have been isolated and their contribution to plant contamination quantified. For example, many papers simply compare a bioconcentration factor (BCF) for the plant material based on a soil concentration.

Welsch-Pausch et al. (12) investigated the main pathways of ryegrass contamination by dioxins and furans and reported that dry deposition was the most significant. Little root uptake and subsequent translocation of these compounds would be expected because they are tightly bound to soil as a consequence of their high n-octanol–water partition coefficients (KOW) (13). Volatilization and subsequent dry deposition was a significant pathway for chlorobenzenes (14) with higher deposition for those compounds with a larger n-octanol-air partition coefficients KOW; similar findings have been reported for PCBs (15). Root uptake and transport in the transpiration stream was found to be the dominant pathway for the plant accumulation of pyrene and phenanthrene from soil and this was correlated to the KOW of the pollutant (5). The latter pathway has been investigated by a number of workers and the transport from the root to the shoot is governed by a bell shaped relationship with a maximum log KOW ≈ 2 (16−18). Plant composition is frequently a parameter within plant uptake models, with retention of organic pollutants being calculated from the lipid content of the plant and the KOW of the chemical under investigation (9, 10). When the maximum potential uptake by plant material was calculated using a composition partition model good agreement was reported for lindane and chlorinated solvents (19).

Polycyclic aromatic hydrocarbons (PAHs) are frequently detected, toxic, industrial pollutants that may potentially accumulate in plant material and subsequently contaminate food chains (5, 20). They have a range of physicochemical properties which will result in potentially different contamination pathways for individual PAH accumulation by the plant (Table 1). For example Cousins and Mackay (21) suggested that uptake from the atmosphere is the important pathway for chemicals with log KOW > 6 and a log KOW > −6 (KOW = dimensionless air−water partition coefficient), and uptake from the soil with subsequent translocation to the leaves is important for chemicals with log KOW < 2.5 and log KOW < −1. The aim of the current study was to investigate the primary pathways of accumulation of PAHs in white clover (Trifolium repens).

Materials and Methods

All chemicals were of analytical grade and supplied by Sigma Aldrich, UK. Plants were sown in acid washed sand in a glasshouse with supplementary lighting to provide a 16 h day, with a mean day and night temperatures 20/15 °C. Fourteen days after germination plants were transferred to

<table>
<thead>
<tr>
<th>PAHs</th>
<th>logKOW</th>
<th>Sw (mg/L)</th>
<th>logKOW</th>
<th>logKOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>naphthalene</td>
<td>3.37</td>
<td>31.0</td>
<td>−1.75</td>
<td>5.13</td>
</tr>
<tr>
<td>acenaphthene</td>
<td>3.92</td>
<td>3.8</td>
<td>−2.31</td>
<td>6.39</td>
</tr>
<tr>
<td>fluorene</td>
<td>4.18</td>
<td>1.9</td>
<td>−2.50</td>
<td>6.68</td>
</tr>
<tr>
<td>phenanthrene</td>
<td>4.57</td>
<td>1.1</td>
<td>−2.88</td>
<td>7.45</td>
</tr>
<tr>
<td>fluoranthene</td>
<td>5.22</td>
<td>0.26</td>
<td>−3.38</td>
<td>8.60</td>
</tr>
<tr>
<td>pyrene</td>
<td>5.18</td>
<td>0.13</td>
<td>−3.43</td>
<td>8.61</td>
</tr>
</tbody>
</table>
For shoot material an additional clean up step was added.

acetone-rinsed 200 mL borosilicate glass jars, containing 1/4 strength Hoagland’s solution. The treatments imposed were designed to quantify the pathways of PAH uptake. Treatment A, passive root uptake; Treatment B, active root uptake and transpiration; Treatment C, influence of transpiration on root and shoot uptake; Treatment D, phloem mobility; Treatment E, volatilization and shoot deposition (Figure 1). Each solution was spiked with a mixture of the six PAHs: naphthalene (Nap), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), fluoranthene (Fluan), and pyrene (Pyr). The spiking solution was prepared by adding each PAH at its solubility limit (solv) (taken from Table 1) directly to the Hoagland’s solution. This was then passed through a 0.2 um filter to remove any undissolved PAH. Treatments A and B, and C were also used to investigate the impact of growth solution concentration and time, respectively, on the uptake dynamics of PAHs in white clover. Solution concentrations used in the treatments are given in the Supporting Information (SI). There were three replicates for each treatment. Control plants were grown under the same environmental conditions in a greenhouse without a source of PAH. After 6 days treated plants began to show signs of stress so the experiment was stopped, this was corroborated by reduced transpiration in contaminated plants (see SI). The plants were harvested washed with deionized water, dried with tissue paper, frozen, and then freeze-dried. This prevented any volatilization losses and/or further metabolism of compounds. Lipid content of plant shoots and roots was determined by extraction of freeze-dried plant material in a 1:1 (v/v) solution of acetone and hexane. The mixture was ultrasonicated for 1 h, the solvent was then decanted onto a preweighed dried pan and allowed to evaporate for 24 h, and the process was then repeated. The pan was weighed and the percent lipid content was calculated.

**PAH Analysis.** Growth solutions (15 mL) were extracted into (10 mL) of a 1:1 acetone-hexane mixture and placed on an end-over-end shaker for 30 min. 2 mL of the supernatant was then decanted into a vial for GC analysis. Root material was ground with a pestle and mortar, 0.5 g ultrasonicated for 1 h in a 22 mL vial with 10 mL of DCM. The samples were then passed through a 0.2 μm filter, blown down with nitrogen and resuspended in 2 mL DCM before PAH analysis. For shoot material an additional clean up step was added whereby 7 mL of the extract was added to a 2 g column of deactivated silica gel and a further 1 mL of DCM was then added. The eluate was then blown down with nitrogen and resuspended in 2 mL of DCM. All analysis were undertaken on an Agilent 5890 GC FID with splitless injection using an 30 m SPB5 column, film thickness 0.25 μm, diameter 0.32 mm. The temperature program was; initial 45 °C, ramp 15 °C min⁻¹ to 320 °C and held for 10 min. The injector and detector temperatures were 220 and 250 °C, respectively. Reagent and procedural blanks were analyzed simultaneously and external standards were included in each GC run. The calibration curves for the individual PAH covered a range 0.1→500 μg mL⁻¹ with regression coefficients of 0.999→0.996. Compound recoveries were calculated from spiked samples with standards at concentrations of 1000 μg g⁻¹ for roots and 5 μg g⁻¹ for shoots, values ranged from 84.5 to 100.6% for roots and 67.3 to 80.3% for shoots. Pyrene data are not reported for the shoots as an unknown compound coeluted with this PAH preventing accurate quantification. Data are expressed as a root concentration factor (RCF) where RCF = root concentration (mg g⁻¹)/solution concentration (mg mL⁻¹), shoot concentration factor (SCF) where SCF = shoot concentration (mg g⁻¹)/solution concentration (mg mL⁻¹) and as a transfer factor (TF) where TF = shoot concentration (mg g⁻¹)/ root concentration (mg g⁻¹).

All statistical analysis was undertaken using STATISTICA (v7, Statsoft Corporation, Tulsa, OK). Data were checked for normality, transformations undertaken if necessary or non-parametric statistics used where original and transformed data were not normal. Statistical analyses were used are multiple ANOVA followed by a Duncan’s multiple range test and generalized linear modeling. Model fitting was undertaken using the nonlinear estimation function within STATISTICA.

**Results and Discussion**

**Root Uptake.** The accumulation of PAH in roots increased with increasing $K_{OW}$ of the compound Nap < Ace < Flu < Phe < Fluan < Pyr ($p < 0.01$). The model of Ryan (7) which describes the root uptake of organic chemicals from solution:

$$\text{RCF} = 10^{0.77\log K_{OW} - 1.52} + 0.82$$

has been used widely in plant uptake models for risk assessments (22, 23), underestimated the root accumulation observed here. When the same function

$$\text{RCF} = 10^{ab\log K_{OW} - b} + c$$

was used to fit the data the constants $b$ and $c$ were not significant ($p > 0.05$), with $a = 0.74$, this accounted for 65% of the variation in the data. However use of this model would result in a negative RCF for those compounds with $\log K_{OW} < 3.8$, so $c$ was set to 5.5 the mean value for the $\Delta_{ACTIVE}$ root systems with $\log K_{OW} < 3.8$ in these experiments.
This alteration did not affect the value of $a$, or the proportion of variance the fitted function accounted for. Within the treatments there were significant differences in the RCF between B and $A_{\text{READ}}$ for all PAHs ($p < 0.01$), there was a higher root accumulation of naphthalene in B c.f. $A_{\text{ACTIVE}}$ ($p < 0.05$), but not for other PAHs ($p > 0.05$) (Figure 2). There were no differences in RCFs between those plants with shoots grown in the light compared to those with shoots in the dark (Treatment C). These findings indicate that an active root is required to accumulate PAH, it is not solely a sorption process as indicated by many workers, and that a transpiration stream flux enhances root uptake for more water-soluble compounds such as naphthalene. There was no phloem mobility of PAHs (Treatment D) as none were recovered from the half of root growing in the clean solution. This would support the paradigm of phloem mobile compounds needing a log $K_{\text{OW}} < 0$ or an acid dissociation constant $> -6$ and log $K_{\text{OW}} < 3$ (24).

Translocation from Root to Shoot. Shoot accumulation of PAH also increased with increasing $K_{\text{OW}}$. Nap $<$ Ace $<$ Flu $<$ Phe $<$ Fluan ($p < 0.05$). The TF showed the reverse trend Nap $>$ Ace $>$ Flu $>$ Phe $>$ Fluan ($p < 0.05$) demonstrating the importance of water solubility in the transfer of PAH from the root to the shoot. Others have expressed this transfer as the transpiration stream concentration factor which is also seen to decline with increasing log $K_{\text{OW}}$ beyond a value of 2 (16, 17). As observed for the RCF there was no difference in SCF between $C_{\text{LIGHT}}$ and $C_{\text{DARK}}$ indicating that over the relatively short duration of this experiment shading did not significantly affect the accumulation of PAH in the shoot, or more likely it was ineffective in reducing the transpiration flux. Future experiments may require a longer period to reveal differences as a consequence of increased transpiration, as over the 6 day experimental period equilibrium had not been obtained for any of the PAHs as demonstrated by the increase in the TF with time (Figure 3). In similar studies with ryegrass, shoot concentrations of hexachlorobenzene (log $K_{\text{OW}} = 5.5$), tetrachloroethylene (log $K_{\text{OW}} = 3.38$), and trichloroethylene (log $K_{\text{OW}} = 2.53$) attained equilibrium after $>200$, 80, and 60 h respectively (19). The longer equilibrium times for higher $K_{\text{OW}}$ compounds have been attributed to the kinetic limits on the uptake of these compounds, related to their low water solubility and hence depleted concentration in the transpiration stream relative to the root (9).

For both $D_{\text{POLL}}$ and $E_{\text{CLEAN}}$, there was significant accumulation in the shoot compared to clean treatments ($p < 0.001$) (Figure 4), but $D_{\text{CLEAN}}$ and $E_{\text{CLEAN}}$, treatments had higher shoot concentrations than plants grown in a clean environment. When the two pathways of contamination were separated root to shoot via transpiration was dominant for Nap, Ace, Flu, and Phen and dry deposition from the surrounding atmosphere dominated for Fluan. The potential significance of dry deposition following volatilisation has been previously reported (25–27). There was no significant correlation with chemical properties in our study, but chemicals with log $K_{\text{OW}} > 9$ and log $K_{\text{OW}} < -3$ have been proposed to have a significant proportion of their contamination via the soil air plant pathway, while others have stated that chemicals with $K_{\text{OW}} > -4$ may are predominantly deposited via this route (7). Fluan ($\log K_{\text{OW}} > 8.6$ and log $K_{\text{OW}} < -3.38$) is the closest to these physicochemical properties. The high contribution of direct aerial deposition from volatilization to plant contamination is noteworthy and supports the proposal that this is an important pathway for the accumulation of organic chemicals by vegetation from polluted soils (25, 26). This pathway is often ignored in studies used to parametrize models for the uptake of organic chemicals by crops.

The accumulation in the shoot was driven by the transpiration stream flux for all PAHs ($r^2 > 0.65$), the gradient calculated from the fitted linear functions for each individual PAH correlated with the water solubility of the individual chemicals ($r^2=0.998$, $p < 0.001$), but not their log $K_{\text{OW}}$ ($r^2 = 0.48$, $p > 0.1$). This finding arose because the plant material had not attained equilibrium with the contaminants over the short time scale of the study (Figure 3). Where equilibrium or near equilibrium conditions exist the relation with log $K_{\text{OW}}$ is likely to be improved.

Influence of Time and Concentration. The RCF declined with time for Nap, did not alter for Ace and Flu, and increased throughout the experimental period for Phe, Fluan, and Pyr but increased at a slower rate from 2 days. The increasing time to equilibrium with increasing $K_{\text{OW}}$ and declining accumulation rate has been observed previously (19). The SCF increased more rapidly with time for those PAH with lower $K_{\text{OW}}$. These findings would be supported by transpiration stream concentration factor relationships reported by others where transport to the shoot declines with log $K_{\text{OW}} > 3$ (16–18). The increasing SCF over time results from the larger volume of water transpired with time which drives the flux of chemical into the shoot. The combination of these processes resulted in an increasing TF over the duration of the experiment, with higher TFS for those PAH with higher $S_{\text{V}}$ and lower $K_{\text{OW}}$ (Figure 3).

The RCF increased with increasing concentration for all PAHs (Figure 5). With the exception of naphthalene all the PAHs exhibited an asymptote at the higher concentrations. Partition coefficients of the individual PAH to plant
material ($K_{pl}$), were calculated from a lipid composition model

$$C_{eq} = C_{sol}(f_{lip}K_{ow})$$  \hspace{1cm} (3)

$$K_{pl} = C_{eq}/C_{sol}$$  \hspace{1cm} (4)

where $C_{eq}$ = equilibrium concentration of PAH in the plant, $C_{sol}$ = solution concentration of PAH, $f_{lip}$ = fraction of plant lipid (0.055 for shoot and 0.028 for root on a dry weight basis). $K_{pl}$ was also calculated using a Freundlich model, and a sorption model from fits to the linear portion of the data (Figure 5 and Table 2). The three models exhibited some deviation, with the lipid model predicting higher accumulation of naphthalene, but lower accumulation for the remaining PAH compared to the sorption and Freundlich models. The Freundlich model predicted very high $K_{pl}$ for pyrene. The potential reasons for this are the lower $K_{ow}$ compounds are more likely to be subject to metabolism within the plant; second deviations between octanol and plant lipids, other workers have found triolein to be a better surrogate (28); and third the lipid extraction was not exhaustive, a multiphase extraction has been proposed to extract all lipid components from plant tissue (28).

**FIGURE 3.** Alteration in the transfer factor from root to shoot of PAH with time (N.B. log scale on y-axis, error bars ± 1 sd).

**FIGURE 4.** Difference in shoot concentration ($C_{pl}$) for mean sum of PAH concentration between clean and polluted treatments. D-POLL plants grown with half roots in contaminated solution and shoot in contaminated air, D-CLEAN plants grown with roots in clean solution and shoot contaminated air, E-POLL plants grown with roots in contaminated solution and shoots contaminated air, E-CLEAN plants grown with roots in clean solution but shoots in contaminated air, CONTROL plants grown in clean solution and air (error bars ± 1 sd).

**FIGURE 5.** Influence of solution concentration ($C_{sol}$) on root concentration ($C_{pl}$) of (a) naphthalene, (b) phenanthrene, and (c) pyrene.
TABLE 2. Calculated Partition ($K_{pl}$) of PAH from Solution to Plant Components Using a Lipid Composition Model and Freundlich Fit to Experimental Data and a Sorption Model from the Linear Portion of the Data

<table>
<thead>
<tr>
<th></th>
<th>Lipid composition model</th>
<th>Freundlich model</th>
<th>Sorption model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>65.6 232 423</td>
<td>19.4 277 650</td>
<td>1 111 552</td>
</tr>
<tr>
<td>Shoot</td>
<td>165 582 1059</td>
<td>0.09 1.02 4.04</td>
<td>7.18 51.3</td>
</tr>
</tbody>
</table>

Increasing the concentration of the PAH reduced the transfer from the root to the shoot for all PAHs. This was a consequence of reduced transpiration, which declined as the PAH concentration of the spiked solution increased ($r^2 = 0.58$, $p < 0.001$), the Freundlich model could not therefore be used to calculate the $K_{pl}$. Using the SCF from across all treatments, which was equivalent to the lowest concentration used in treatment B, to derive a $K_{pl}$ a large divergence can be seen with the lipid model. The main reason for this is that the shoots had not attained equilibrium as discussed previously. In addition the toxic action of the PAH may reduce shoot uptake, whereas metabolism of the compounds after uptake will also decrease shoot concentrations.

Overall Comments on Pathways and Uptake Kinetics.

The contamination of roots by PAHs is mainly driven by sorption, but the transpiration stream flux is important for more water-soluble compounds such as Nap. The subsequent movement of PAH from the root to the shoot is driven by the transpiration stream flux, and is therefore greater for more water-soluble compounds. However, there is also significant deposition of PAHs from the air. The latter may arise from volatilization from the contaminated matrix as well as background concentrations; the former pathway is frequently ignored in model parametrization studies and is potentially important for those compounds within the boundaries $\log K_{oc} > 9$ and $\log K_{ow} < -3$. Across a relatively limited range of PAH concentrations there was significant toxic effects which reduced their shoot accumulation as a consequence of reduced transpiration (e.g., Fluor 0.02–0.64 μg mL$^{-1}$), it is important future studies use concentrations at the low end of this range to prevent such negative effects influencing the calculations of potential accumulation.

Over the course of a growing season the root and the shoot will approach equilibrium, therefore for the purpose of exposure modeling it is proposed that an equilibrium approach is adopted. This will be slightly conservative, but this is appropriate for risk assessment. When modeling plant uptake of nonionic organic pollutants the use of a simplified version of the Ryan model (7) is proposed for root crops, whereas shoot concentrations can be calculated from a combined approach using aerial deposition and root to shoot transfer. Models that isolate these two processes need to be developed.

Acknowledgments

We thank the Royal Society for the provision of a BP China fellowship to Y.G.

Supporting Information Available

Details of the solution concentrations used in the experiments and the reduction in plant transpiration with increasing PAH concentration in the growth solution. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited


(19) Allard, A. S.; Malmberg, M.; Neilson, A. H.; Remberger, M. Accumulation of polycyclic aromatic hydrocarbons from creosote-contaminated soil in selected plants and the oligochoete.


(25) Harrad, S.; Ren, I. Z.; Hazrati, S. Chiral signatures of PCBs 95 and 149 in indoor air, grass, duplicate diets and human faeces. *Chemosphere* 2006, 63 (8), 1368–1376.

