

The Absorption and Translocation of Polychlorinated Biphenyl Congeners by *Cucurbita pepo* ssp *pepo*

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ABSTRACT: The mobility of polychlorinated biphenyl (PCB) congeners within *Cucurbita pepo* ssp *pepo* cv. Howden (pumpkin), a PCB phytoextracting plant, was investigated through a comparison of field-weathered soil, root, shoot, and xylem sap congener profiles. This is the first study to show the presence of PCBs in xylem sap (range: 0.03–0.18 $\mu\text{g}\cdot\text{mL}^{-1}$), confirming that PCB translocation throughout the plant occurs via this medium. A comparison of soil ($5.2 \pm 2.5 \mu\text{g}\cdot\text{g}^{-1}$), root ($27.1 \pm 2.1 \mu\text{g}\cdot\text{g}^{-1}$), shoot (range: $1.9 \pm 0.5 \mu\text{g}\cdot\text{g}^{-1}$ – $8.2 \pm 1.4 \mu\text{g}\cdot\text{g}^{-1}$), and xylem sap ($0.09 \pm 0.04 \mu\text{g}\cdot\text{g}^{-1}$) samples showed significant differences in congener profiles, with lower chlorinated congeners (predominately trichlorinated ones) found within xylem sap in higher amounts than higher chlorinated congeners. The total PCB concentrations of xylem sap samples collected at various lengths along the primary plant shoot were not significantly different from each other, while those of primary shoot tissue samples significantly decreased (two-sample *t* test, $p = 0.01$) as the distance from the plant base increased. PCA analysis of individual congeners in the roots, shoots and xylem sap indicated that movement of the PCB congeners in the plant was affected by the number of chlorines in the molecule, and hence possibly $\log K_{ow}$ and molecular weight, but not by planarity.



INTRODUCTION

Polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs) that were widely used for industrial purposes until the 1970s when they were banned in North America due to their toxicity. The health effects and environmental properties of PCB molecules are influenced by the degree and configuration of the chlorine atoms. From a total of 209 PCB congeners, 68 can assume a planar configuration. The monitoring of these congeners is particularly important due to their potential to cause cancer, and noncancer effects, including immunotoxicity, endocrine effects, developmental toxicity, and chloracne.¹

The bioavailability of POPs to flora and soil microorganisms was for many years assumed to be negligible as a result of their physicochemical properties such as low water solubility.² However a number of specific plant species that have the ability to mobilize POPs^{3,4} have been identified. These include specific members of the *Cucurbitaceae* family, which have been demonstrated to take up and transport soil-bound POPs, including dichlorodiphenyldichloroethylene (DDE),⁵ chlordane,⁶ PCDDs, PCDFs,⁷ and PCBs.⁸ Phytoextraction is defined as the mobilization of contaminants from the soil, followed by absorption into the roots, and subsequent translocation into above-ground plant tissues. Mechanisms for this process remain largely unknown but as POPs do not occur naturally in the environment, it is likely that they accumulate in plants opportunistically, potentially by pathways evolved for acquisition of soil-derived nutrients.⁹

POPs enter vascular tissue by penetrating into the roots of plants. They first adsorb to root epidermal surfaces¹⁰ and then diffuse into the root core. The thickness of waxes on the root epidermal surface can determine the capacity of adsorbing extremely

hydrophobic molecules.⁷ Movement into the vascular tissue may be limited by the conformation of the contaminant and by transmembrane proteins.^{11–13} It has recently been shown that protein-like materials in the xylem sap of cucurbits increase the water solubility of dieldrin and may aid in the translocation of hydrophobic organic molecules.¹⁴

Following passage through a cell membrane, contaminants may be carried with water and solutes upward from the root to aerial plant parts through the xylem, driven by transpiration and root pressure. In xylem sap, contaminants can also diffuse laterally into adjacent plant tissues and become concentrated.¹⁵ This can occur as a result of equilibration of the aqueous phase in the plant shoots with the contaminant in the xylem, or as a result of sorption onto lipophilic solids.¹⁶ Chlordane, dieldrin, dichlorodiphenyltrichloroethane (DDT) and its metabolites, and polycyclic aromatic hydrocarbons (PAHs, including: phenanthrene, anthracene, fluorene, and pyrene) (all POPs) have previously been identified in the xylem saps of *C. pepo* species.³ It is therefore expected that PCBs translocate into shoot tissues through the xylem sap; however, PCBs have not been previously identified in this medium.

The aim of the present study is to increase the understanding of PCB transport in *Cucurbita pepo* by analyzing the PCB congener profiles in soil, root, shoot, and xylem sap collected from plants grown in a weathered, Aroclor 1248-contaminated soil. The effects of properties such as $\log K_{ow}$ will be examined and where possible

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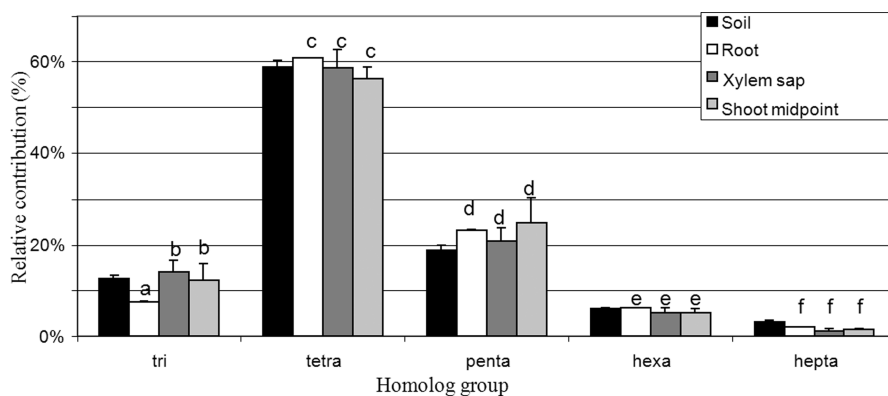


Figure 1. Mean relative contributions (%) of congener homologue groups in soil, root, xylem sap, and shoot midpoint samples. Di, octa, and nona homologue groups are excluded as they contribute <1% the total concentration. Error bars reflect one standard deviation. Sample groups that share a letter were not found to be statistically different from each other in a post hoc Tukey multiple comparison test of root, xylem sap, and shoot midpoint samples.

extrapolated to remediation outcomes for other POPs. The movement of the planar congeners, which includes the dioxin like congeners, will be looked at specifically as they can potentially impact health and environmental quality.

MATERIALS AND METHODS

Site Description. This study took place at a Brownfield site contaminated by a former chemical company in Lindsay, ON, Canada. PCB-containing oil was inadvertently released onto the soil at various locations across the site during 30 years of industrial operation. A 12 × 12 m phytoremediation plot was established by researchers at the Royal Military College of Canada (RMC) in Kingston, ON in the spring of 2006. The soil at this site, contaminated with Aroclor 1248, is predominately clay, with 4.3% total organic carbon. Prior to planting each year, the contaminated plot was homogenized using a gas powered rotary tiller, and amended with fertilizer (140 g·m⁻², 6:12:12 C-I-L Tomato Food) to encourage plant growth. For this study, *Cucurbita pepo* ssp *pepo* cv. Howden plants were grown on the plot from June 10th to September 1, 2009.

Sample Collection. After 83 days of growth, xylem sap was collected from nine plants. For each plant, sap was collected at one of three different shoot locations along the primary stem: (i) at a distance of 5 cm from the base ($n = 2$), (ii) at the intersection of the main shoot and secondary shoot (21 ± 4 cm from the base) ($n = 4$), and (iii) at a distance of 100 cm from the base ($n = 3$). Past 100 cm, the volume of sap collected was insufficient for analysis. Xylem sap samples were collected within 30–90 min of sunrise. To encourage increased flow rates for sufficient volume collection, the plants were well watered at the base, before and during collection.

Pumpkin shoots were cut with methanol cleaned shears, and fluids were allowed to seep out of the plant for three minutes. During this time phloem vessels are sealed via callose secretions, and contamination of xylem sap by phloem sap can be avoided.¹⁷ The detopped plant portion still connected to the roots was then thoroughly washed with distilled water and blotted dry with a Kimwipe to remove any excess phloem sap. Through protein sequencing and immunoblotting, it has been shown that preparing a detopped pumpkin (*Cucurbita maxima* Duch. cv. gelber Zentner) in this manner rids the plant of any significant levels of phloem contamination.¹⁸ Over a time period of approximately

120 min, xylem sap was manually collected using glass pipettes as it flowed from the pumpkin plants remaining in the ground that had been severed. Xylem sap from each of the nine plants was pipetted into separate glass vials chilling on ice in a cooler. When a sufficient volume of xylem sap was obtained (>2 mL), the vials were sealed and transported to the Analytical Services Unit at Queen's University in Kingston, ON where they were refrigerated at 4 °C until time of analysis.

Plant shoot samples were taken from all pumpkin plants that had been tapped for xylem sap. One shoot sample measuring 10 cm in length was collected from the midpoint of each plant and another was taken from each plant directly above and below the point from which xylem sap was collected. Roots from each of the plants were collected, finely chopped with methanol cleaned shears, and homogenized to create one composite sample which was analyzed in duplicate. Both shoot and root samples were washed with water to remove soil particles and patted dry. Composite soil samples were also collected at a depth of 0–20 cm from random locations on the contaminated plot at this time ($n = 3$). All samples were stored in individual Whirlpak bags and stored at 4 °C until time of analysis.

Analytical Procedures. *PCB Congeners in Soil and Plant Samples.* The analytical methods used for soil and plant samples were based on the methods described in Zeeb et al.⁸ Prior to analysis, all soil samples were subsampled for the determination of wet/dry ratio. The samples were accurately weighed (10 g wet mass (wm)) and spiked with the surrogate standards ¹³C-labeled IUPAC congeners 101 and 194 (EC9605-RS, Wellington Laboratories), and decachlorobiphenyl (DCBP). Ottawa sand (40 g) and sodium sulfate (20 g) were mixed with the samples. The samples were extracted in a Soxhlet apparatus for 4 h at 4–6 cycles·h⁻¹ using 250 mL of dichloromethane. The extract was then concentrated by rotoevaporation and the solvent was exchanged for hexane. The concentrated extract was then applied to a Florisil column for cleanup. The column was rinsed with hexane and the eluant containing the PCBs diluted to 10.0 mL.

Plant samples were finely chopped with methanol cleaned metal scissors, and their wet masses were recorded. Samples were dried for a minimum of 12 h in a vented oven set to 25 °C, and their dry mass was then recorded. Approximately 1 g of dry plant sample was then ground with sodium sulfate and Ottawa sand using a ceramic mortar and pestle, and then extracted as described above.

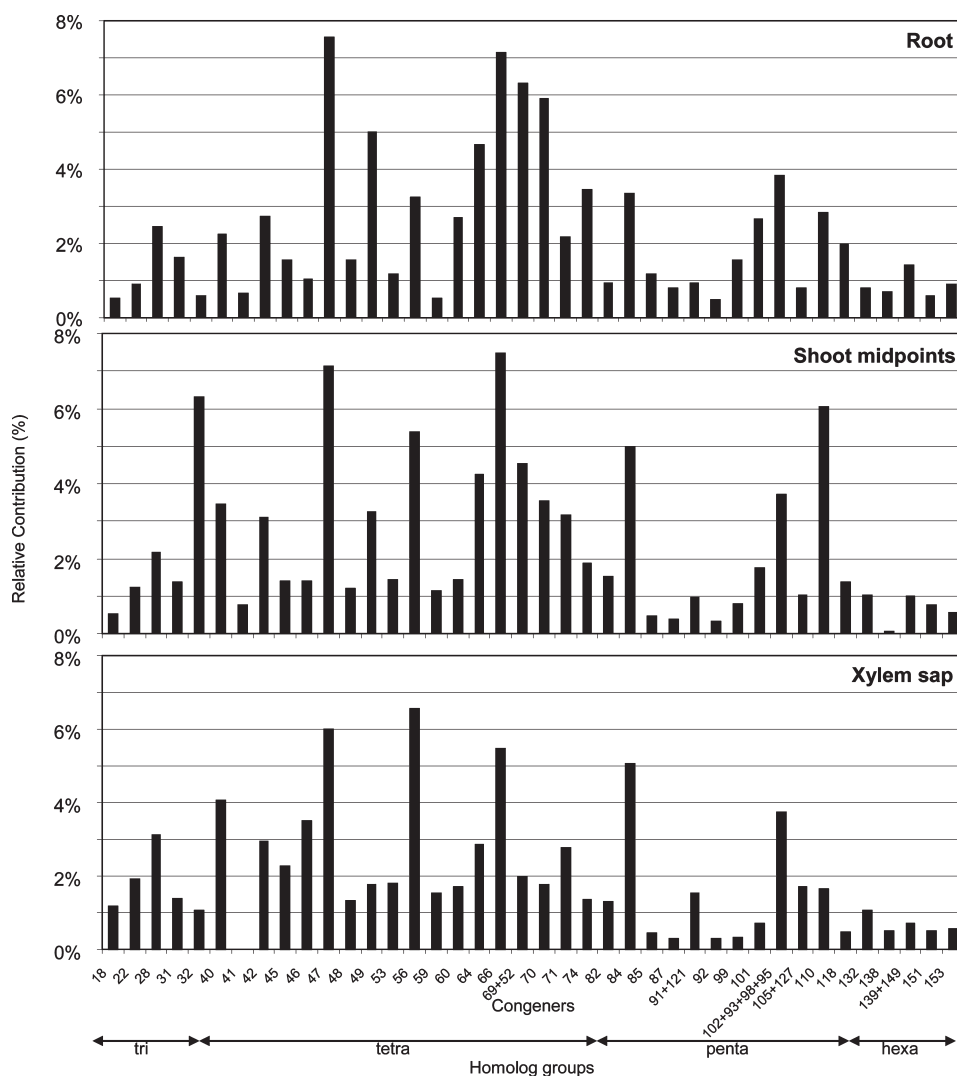


Figure 2. Mean relative contributions (%) of the 40 most prevalent congeners in plant root tissue compared to shoot midpoint tissue and xylem sap, with corresponding homologue group.

PCB Congeners in Xylem Sap Samples. Xylem sap samples were extracted in a separatory funnel and spiked with the surrogate standard EC9605-SS (Wellington Laboratories) (a solution/mixture of eight ^{13}C -labeled IUPAC congeners in isooctane, nos. 28, 52, 118, 153, 180, 202, and 209). Dichloromethane was then added to the funnel and the separatory funnel was shaken for two minutes. After settling, the dichloromethane phase, which contained the PCBs, was filtered through a filter paper and sodium sulfate into a round-bottom flask. This was repeated two more times for a total of three extractions. The dichloromethane was concentrated to a volume of 0.2–0.5 mL through rotoevaporation and the solvent was exchanged for hexane.

Soil, plant, and xylem sap samples were analyzed for congeners by gas chromatography (GC) with tandem mass spectrometry (MS/MS), using a Varian 3800 gas chromatograph with a Varian 1177 injector, SGE Forte capillary column (60 m, 0.25 mm i.d. \times 0.25 μm film thickness), equipped with a Varian 4000 MS/MS detector, with Varian 8400 auto sampler, and Varian MS Workstation V.6.6 software. Detection limits were 0.01 $\mu\text{g}\cdot\text{g}^{-1}$ for soil and plant samples, and 0.5 $\text{ng}\cdot\text{mL}^{-1}$ for xylem sap samples.

Quality Assurance/Quality Control (QA/QC). For every nine plant or soil samples extracted by Soxhlet and subsequently prepared, one analytical blank (Ottawa sand and sodium sulfate), one control sample (a blank sample spiked with a known amount of CEN PCB congener mix-1 (IUPAC nos. 18, 28, 31, 44, 52, 101, 118, 138, 149, 153, 180, and 194 in heptane)), and one analytical duplicate were also Soxhlet extracted and processed. All analytical blanks had PCB concentrations below the detection limits and control samples averaged 108% of the expected value. For every two xylem sap samples extracted, one analytical blank, and one control sample spiked with CEN were also extracted. All analytical blanks had PCB concentrations below the detection limits and control samples averaged 97% of the expected value.

Statistical Analyses. The soil, plant, and xylem sap congener signatures were examined using principal components analysis (PCA). To standardize the samples for PCA, all congeners were normalized to congener 153, a recalcitrant molecule that was a major component of technical PCB formulations,¹⁹ and therefore found in relatively large quantities in all samples. Congeners that were detected in greater than 50% of samples, within each

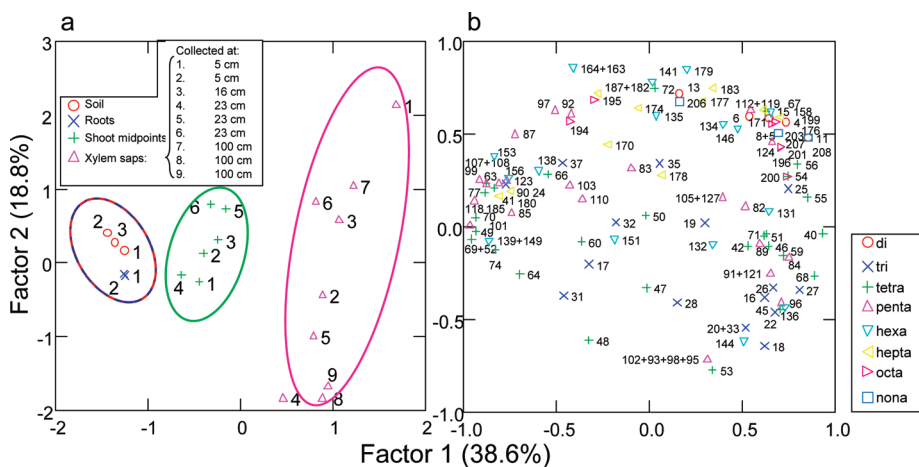


Figure 3. (a) Principal components scores of soil, pumpkin plant roots, shoot midpoints, and xylem saps. (b) Principal components loadings of congener compositions for samples shown in (a).

sample group, were carried forward for further statistical analysis. This included a total of 77 congeners in the soil samples, 64 in the plant samples, and 70 in the xylem sap samples. Prior to PCA analysis, each congener was converted to a relative contribution (%) of the total number of congeners. Relative contributions were normalized by subtracting the mean and dividing by the standard deviation. One-way ANOVA (at a significance level of 0.05) was used to assess the statistical difference between PCB congener contributions (%) in various sample groups, and post hoc multiple comparison testing was performed using the Tukey method (at a significance level of 0.05). Two-sample *t* tests (at a significance level of 0.05) were also used to assess the statistical significance of differences between PCB congener contributions (%) in various sample groups. All statistical analysis was performed using SYSTAT 13.

RESULTS AND DISCUSSION

Concentrations. Soil samples collected at the time of xylem sap collection were found to have a mean total PCB concentration of $5.2 \pm 2.5 \mu\text{g} \cdot \text{g}^{-1}$ ($n = 3$). The plant root composite sample had an average PCB concentration of $27.1 \pm 2.1 \mu\text{g} \cdot \text{g}^{-1}$ ($n = 2$), approximately five times greater than the PCB concentration of the soil, consistent with previous results from PCB phytoextraction studies using pumpkins.^{20,21} Other hydrophobic contaminants, including PAHs, PCDDs, and PCDFs, have also been found to accumulate significantly in plant roots.¹⁵

The nine xylem sap samples had total PCB concentrations ranging from 0.03 to $0.18 \mu\text{g} \cdot \text{mL}^{-1}$. The concentrations in the xylem saps collected from 5 cm, 21 ± 4 cm, and 100 cm were not statistically different from each other (one-factor ANOVA, $F(2,6) = 0.16, p = 0.86$), averaging $0.11 \pm 0.10 \mu\text{g} \cdot \text{mL}^{-1}$ ($n = 2$), $0.08 \pm 0.01 \mu\text{g} \cdot \text{mL}^{-1}$ ($n = 4$), and $0.09 \pm 0.03 \mu\text{g} \cdot \text{mL}^{-1}$ ($n = 3$), respectively. These concentrations are at the upper limit of Aroclor 1248 water solubility values, which range from $0.05 \mu\text{g} \cdot \text{mL}^{-1}$ ²² to $0.10 \mu\text{g} \cdot \text{mL}^{-1}$.²³

Shoot samples from the plant midpoint, and from the xylem sap extraction points were analyzed from six of the plants from which xylem sap was collected. The mean PCB concentration was $8.2 \pm 1.4 \mu\text{g} \cdot \text{g}^{-1}$ ($n = 2$) at the shoot base, $5.7 \pm 0.9 \mu\text{g} \cdot \text{g}^{-1}$ ($n = 3$) at the junction of the main and secondary shoots (an average distance of 21 ± 4 cm from the base), $2.2 \mu\text{g} \cdot \text{g}^{-1}$ ($n = 1$) at 100 cm from the base, and $1.9 \pm 0.5 \mu\text{g} \cdot \text{g}^{-1}$ ($n = 6$) at

the shoot midpoints (170 ± 2.1 cm from the plant base). As the distance from the plant base increased, the PCB concentration decreased significantly (two-sample *t* test, $p = 0.01$). This result corresponds to previous findings,²⁰ and was expected. It is interesting to note that in contrast, no significant differences were seen in the concentrations of xylem sap samples collected at different locations along the plant shoot.

Congener Profiles. The presence of PCBs in xylem sap confirms the translocation of PCB congeners to aerial plant tissues within *C. pepo* via this medium. Homologue contributions (Figure 1) and the 40 most prevalent individual congeners (Figure 2) were examined to look for differences in the congener profiles of the different plant parts. The congener pattern is dominated by tetra and penta chlorinated biphenyls, characteristic of Aroclor 1248, suggesting that the relative concentration of a PCB congener in the soil is what primarily drives relative absorption and subsequent translocation of that congener in the plant. The distribution of the tri, tetra, penta, hexa, and hepta homologue groups within the plant (including root, xylem sap, and shoot midpoint samples) shows some interesting trends (Figure 1). Specifically the portion of the trichlorobiphenyls in the xylem sap ($14.0 \pm 2.6\%$ $n = 9$) is significantly greater than in the plant roots ($7.5 \pm 0.2\%$ $n = 2$) (one-factor ANOVA, $F(2,14) = 3.96, p = 0.04$). Conversely, the penta ($23.2 \pm 0.1\%$ $n = 2$), hexa ($6.3 \pm 0.0\%$ $n = 2$), and hepta ($2.0 \pm 0.0\%$ $n = 2$) homologue groups, are significantly higher in the roots as compared to the xylem sap ($20.8 \pm 2.8\%$, $5.0 \pm 1.4\%$, and $1.2 \pm 0.5\%$, respectively, $n = 9$) ($p < 0.029$). The above differences may be due to the increased solubility of the lower chlorinated trichlorobiphenyls in the xylem and/or reflect the easier movement of the smaller less chlorinated trichlorobiphenyls. A similar trend (Figure 1) is observed when soil and roots are compared, with significantly more highly chlorinated penta, hexa and hepta chlorobiphenyls (two-sample *t* test, $p = 0.016$), and significantly less trichlorobiphenyls (two-sample *t* test, $p = 0.009$) proportionately in the roots than in the soils.

A comparison of congener profiles using principal components analysis (PCA) produced two principal components (PC) that explained 57.4% of the total variance in the data. A plot of sample scores produced distinct groupings of the four sample types (Figure 3a). Soil and root samples are grouped in close proximity to one another toward, indicating that they have similar congener profiles. This confirms that pumpkins have the ability

to sorb the majority of Aroclor 1248.^{24,25} Xylem sap samples grouped distinctly separate from the other samples (Figure 3a), while shoot samples are located midway between the soil/root and xylem sap samples.

The low end of PC1 on the congener loadings plot (Figure 3b), corresponding to soil and root samples, contains predominately tetra- and pentachlorinated congeners, with some hexa- and heptachlorinated congeners. While congeners from all homologue groups were present in xylem sap to some degree, the majority of congeners were tri-, tetra-, chlorinated. This finding confirms that lower chlorinated congeners are more likely to be found in xylem sap than in other plant tissues. There are no discernible differences between the congener profiles of xylem sap samples collected from the various shoot locations. We have previously shown evidence for differences in shoot congener profiles with distance from the roots however only for shoot samples at distances exceeding one m.²⁰

To reach the xylem, chemicals taken into the plant roots must penetrate the plant epidermis, cortex, endodermis, and pericycle.¹⁵ At the endodermis, all materials must pass through at least one cell membrane. The mechanism for the transport of the hydrophobic compounds from roots to shoots is still unclear. Researchers have suggested transport is aided by protein-like materials.^{14,26} Our PCB congener data indicates that the number of chlorines and hence possibly the log K_{ow} or molecule size affects the movement through the plant. Specifically, for the congeners in this study; the trichlorinated biphenyls had log K_{ow} values ranging from 5.02 to 5.83 whereas the penta-, hexa-, and hepta-chlorinated biphenyls ranged from 5.71 to 7.36.¹ The tetra-chlorinated biphenyls, for which no statistical differences in distribution were found, have intermediate values ranging from 5.21 to 6.36. Similarly, molecular weight may play a role with trichlorinated biphenyls at 257.5 and penta-, hexa-, and hepta-chlorinated biphenyls at 326.4, 360.9, and 395.3, respectively. Hence, congeners which have lower log K_{ow} values and lower molecular weights may have a greater ability to pass through the cell membrane into the plant vascular tissue. In comparison to PCB congeners, pyrene diel-drin, chlordane, and DDT, all of which have previously been identified in xylem sap, have log K_{ow} values of 4.88, 5.2, 5.8, and 6.91 and molecular weights of 202.3, 380.0, 409.8, and 354.5, respectively.²⁷ DDT is particularly interesting in that some studies have shown that specific varieties *C. pepo* can translocate this POP effectively²⁸ despite it having a relatively high log K_{ow} and molecular weight, particularly when compared with the trichlorobiphenyls. Hence, while we can conclude that within PCB congeners the number of chlorines, and therefore log K_{ow} and molecular weight, clearly affect the phytoextraction of POPs, the overall mechanism of uptake depends on additional factors which still need to be elucidated before a comprehensive model can be proposed.

Planarity. Planar PCB congeners were investigated to determine if this molecular configuration resulted in altered congener mobility from soil, into and throughout plants. It has been suggested the congener absorption and translocation within plants is affected by planarity²⁵ and that one chlorine at the 2- or 2'- (*ortho*) position in nonplanar PCB congeners may promote the dissociation of PCBs from soil matrices by organic acids. Furthermore planar congeners, particularly dioxin-like congeners, are of particular interest since they have been associated with negative health effects.²⁹

The soil, root, shoot, and xylem sap samples contained 32 planar congeners, including five dioxin-like congeners. Planar

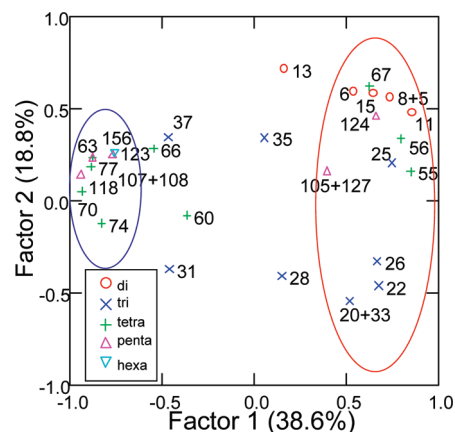


Figure 4. Principal components loadings of planar congeners for samples shown in Figure 3a. The congeners circled in blue correspond most strongly to soil and root samples, while congeners circled in red correspond most strongly to xylem sap samples.

congeners comprised $39.3 \pm 0.3\%$ ($n = 3$) of the total soil concentration, $32.1 \pm 0.1\%$ ($n = 2$) of the total root concentration, $27.6 \pm 4.2\%$ ($n = 6$) of the total shoot midpoint concentration, and $31.5 \pm 4.5\%$ ($n = 9$) of the total xylem sap concentration. A one-way ANOVA showed significant differences exist between the concentrations of planar congeners within the groups ($F(3,16) = 5.9$, $p = 0.007$). The root, shoot, and xylem sap samples all contained fewer planar congeners than the soil samples, supporting the supposition²⁵ that nonplanar congeners are more likely to become dissociated from the soil matrix than planar congeners. Hence phytoextraction with *Cucurbita pepo* ssp. *pepo* may preferentially leave the dioxin-like PCB congeners in the soil. Although this is undesirable for remediation, from the broader perspective of PCBs in the environment, this result suggests that the PCBs with the most negative health effects are less likely to enter the food chain.

Planar congeners, present in the initial PCA (Figure 3) of soil, root, shoot, and xylem sap samples, were further investigated. An approximately equal division of these congeners correlate highly with xylem sap (circled in red, far right) and with root and soil (circled in blue, far left) samples (Figure 4). This result suggests that congener planarity does not promote entry from soil or roots into xylem sap. The planar congeners that correlate most with the xylem sap are lower chlorinated congeners (di, tri, and tetra), while those that correlate with the root and soil samples are slightly more chlorinated (tetra, penta, and hexa).

In this first study showing the presence of PCB congeners in xylem sap, our analysis suggests that degree of chlorination (and related characteristics such as log K_{ow} and molecule size), has a greater influence on translocation than conformation. Thus any mechanistic model of transport for hydrophobic persistent organic pollutants within plants should incorporate log K_{ow} and/or molecule size. By furthering our understanding of this translocation process, improvements in the phytoextraction of POPs may ultimately be achieved.

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