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Predictive Relationships for Uptake of Organic Contaminants by Hybrid Poplar Trees

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Twelve organic compounds commonly found at hazardous waste sites were studied for uptake by hybrid poplar trees. The vegetative uptake of many of these compounds has not previously been demonstrated for plant species being utilized for phytoremediation, such as hybrid poplar trees. Experiments were conducted hydroponically utilizing ^{14}C-labeled compounds to ascertain translocation and fate. Predictive relationships for the translocation and partitioning to plant tissues were developed from the experimental data. Translocation and partitioning relationships based on compounds' octanol−water partitioning coefficients (log K_{ow}) produced the best results, but the relationships did not allow for fully accurate prediction of each contaminant's fate. Translocation and subsequent transpiration of volatile organic compounds (VOCs) from the leaves to the atmosphere was shown to be a significant pathway. As full-scale phytoremediation systems are deliberated, the pathways investigated here should be considered in terms of a contaminant removal mechanism and potential contamination of the vegetation.

Introduction

Prior to recent interest in phytoremediation, research on plant−organic chemical interactions focused upon herbicide fate, herbicide toxicity, and crop contamination. Over the past decade, phytoremediation has evolved from an interesting concept into an innovative technology on the cusp of full-scale implementation. Field-scale phytoremediation studies are currently being conducted for a number of compounds utilizing a variety of plants (*1*). However, the plant/chemical interaction is not well understood for many persistent organic contaminants, particularly volatile organic compounds (VOCs), and for plants useful in phytoremediation. In this paper, predictive relationships are developed for a number of common organic contaminants with hybrid poplar trees in hydroponic solution.

Briggs et al. (*2*) reported the first predictive relationships for the uptake of a compound as a function of the compound's physical−chemical properties. For barley and herbicide chemicals, root uptake from water was related to the logarithm of the compound's octanol−water partition coefficient, log K_{ow}. Briggs et al. (*2*) related the log K_{ow} of the organic compounds to the transpiration stream concentration factor, TSCF (*3*). The TSCF is calculated as the concentration in the transpiration stream divided by the bulk solution concentration in contact with the root tissues. TSCF values were determined for organic compounds and reach a maximum of 0.8 at a corresponding log K_{ow} of 1.8. The interaction of the compound and the root surface is a determining process in compound translocation, for the chemical must pass the symplast of the endodermis in order to be translocated from the roots (*4*). Compounds exhibiting lower hydrophobicity, log $K_{ow} < 1.8$, will apparently not pass through the lipid membranes associated with the epidermal layers of the roots. However, more hydrophobic compounds, log $K_{ow} > 1.8$, can enter the root tissues but do not enter the xylem for translocation from the roots to the shoots and leaves. These compounds become bound in the mucigel associated with the root surface and to the lipid membranes of the root's epidermis (*5*). In summation, the study concluded that binding or exclusion at the root interface decreases translocation and leads to lower TSCF values at log K_{ow} values greater or less than 1.8.

Studies have been conducted that support the developed predictive relationship for the uptake of organic compounds. However, they generally have not investigated priority pollutants or VOCs that may be targeted for phytoremediation nor investigated plant species with distinct phytoremediation potential, such as hybrid poplar trees (*6*). Historically, studies of plant chemical interaction have investigated agricultural chemicals (*3, 4, 7−9*) or investigated the interaction of contaminants with crop species (*10−18*). One recent investigation revealed that trichloroethylene (TCE) can be translocated by hybrid poplar trees (*19*). TCE can also be transpired from the leaves to the surrounding atmosphere as TCE or can be transformed to aerobic metabolites in the leaf tissues (*19*). The results were a qualitative indication of TCE volatilization from the leaves, as opposed to a quantitative measurement. Prior to this work, it had been reported that TCE uptake was insignificant (*10*) or that TCE was not translocated (*16*). Vegetation-facilitated volatilization of VOCs to the atmosphere is perhaps the least investigated fate pathway that may occur in phytoremediation treatment of sites contaminated with VOCs.

Other work has focused upon the interaction of vegetation with compounds such as nitrobenzene, dinitrobenzene, bromacil, and phenol (*13, 14, 20*). Dissimilarity in translocation biochemistry among different plant species also has a significant influence on contaminant uptake. McFarlane et al. (*21*) found great disparity among eight plant species studied for uptake of nitrobenzene. The leaves of lettuce plants accumulated 80% of the assimilated label. In contrast, translocation to the leaves for soybeans was less than 10%. Transformation of the original contaminant also varies among plant−soil systems. It was also found that 80% of the nitrobenzene assimilated by honeysuckle was converted to polar metabolites in hydroponic studies, while the corresponding fraction in lettuce leaves was only 12% (*21*). Whether these transformations were microbially mediated prior to uptake or occurred within the plant was not established. In summation, previous studies have shown that there is variability in organic contaminant transport in higher plants, with respect to specific compound properties and plant species characteristics. This study investigates 12 organic compounds of interest at hazardous waste sites. The compounds include a variety of volatile, semivolatile, and nonvolatile compounds spanning a wide range of physical and chemical properties (Table 1).

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TABLE 1. Selected Physical Chemical Properties of Investigated Compounds[a]

compound	log K_{ow}	solubility $-\log C_w^{sat}$ at 25 °C (mol/L)	Henry's const K_H', at 25 °C, 1 atm (dimensionless)	vapor pressure $-\log P°$ at 25 °C (atm)
RDX[b]	0.87	4.57		
aniline[e]	0.90	0.41	2.2×10^{-5}	2.89
phenol	1.45	0.20	$>1.0 \times 10^{-5}$	3.59[d]
nitrobenzene	1.83	1.77	0.0025[c]	3.68
benzene	2.13	1.64	0.2250	0.90
TCE	2.33	2.04	0.4370	1.01
atrazine	2.69	3.81	1×10^{-7} [c]	9.40[c]
toluene	2.69	2.25	0.2760	1.42
ethylbenzene	3.15	2.80	0.3240	1.90
m-xylene	3.20	2.77	0.2520	1.98[d]
1,2,4-trichlorobenzene	4.25	3.65	0.1130	3.21
pentachlorophenol[f]	5.04	4.27	1.5×10^{-4} [c]	6.75[c]

[a] Note: Source is Schwarzenbach et al. (*32*) unless otherwise noted. [b] Research demolition explosive. [c] Source: Schnoor (*33*). [d] Source: Howard (*34*). [e] Aniline was in the protonated form, $pK_a = 4.63$. [f] Pentachlorophenol was in the anion form, $pK_a = 4.75$.

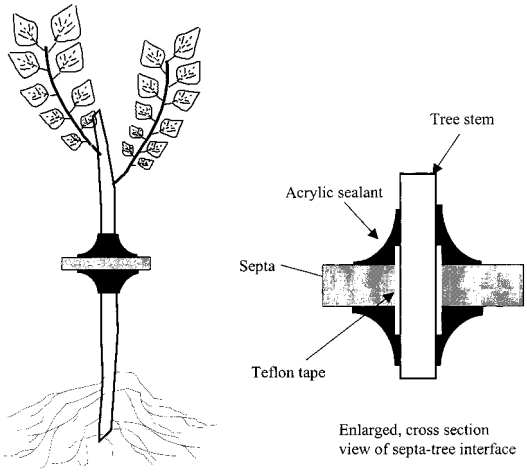

FIGURE 1. Schematics of cuttings prepared for uptake experiment.

Materials and Methods

Specific compounds investigated include the BTEX chemical suite consisting of benzene, toluene, ethylbenzene, and *m*-xylene; substituted benzene compounds such as nitrobenzene, 1,2,4-trichlorobenzene (TCB), aniline, phenol, and pentachlorophenol (PCP); and other problem contaminants such as atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine), RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine), and TCE.

All uptake studies were performed utilizing whole plants. Cuttings, male clones from adult Imperial Carolina hybrid poplars *(Populus deltoides* × *nigra*, DN34), were selected for uniform size, approximately 35 cm long by 6 mm diameter. The use of cuttings is the routine method used for the propagation of hybrid poplars. This method offers the advantage of using clones in experiments, thus avoiding genetic variability. The utilization of cuttings is possible due to preformed root initials possessed by the poplars and is the preferred method used for hybrid poplar propagation by the pulp and paper industry. In addition, using this method in the lab mimics the field application of hybrid poplars in phytoremediation efforts (*22*). The cuttings were wrapped with a 1-cm wide layer of Teflon tape approximately 10 cm from the base of the cutting. The cutting was then affixed with a predrilled screw cap and predrilled Teflon-lined septum that fit snugly over the Teflon tape (Figure 1). Cuttings were next allowed to root hydroponically for approximately 2 weeks in half-strength modified Hoagland's nutrient solution (*23*). The interface of the septum and cutting was sealed with acrylic sealant. The rooted and sealed

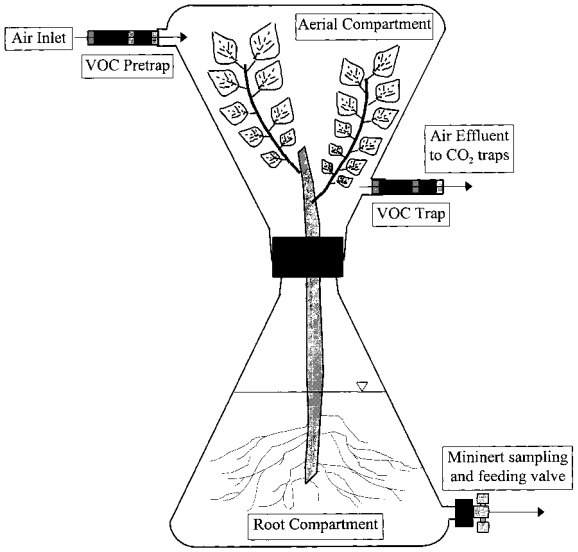

FIGURE 2. Reactor schematic.

cuttings were individually planted into 270-mL modified screw-top culture flasks containing modified Hoagland's inorganic nutrient media, pH 6.0−6.5 (Figure 2). All contaminants exist in their neutral state except PCP, which dissociates and also exists as pentachlorophenolate (*24*). Blank controls, with no poplar cutting, and root/stem controls were also prepared for each experiment. Root/stem controls were prepared as described for planted reactors with the exception that the above septa portion of the cutting was excised 1 day prior to placement into the sealed reactors. The cut stem was not treated in any way.

Just prior to the contaminant addition, a modified 1-L Erlenmeyer flask was sealed over the aerial or above septa portion of the poplar cutting. The air effluent port of the aerial compartment was fitted with an activated carbon trap (Orbo tube 32 large, Supelco) designed to collect VOCs. A schematic of the assembled reactor design is shown in Figure 2.

When these hydroponic reactors were fully sealed, the compound to be studied was added to the system. To allow for the quantification of tissue-related fractions of the added contaminant, each reactor received a mixture of ^{14}C-radiolabeled (hot) compound and nonlabeled (cold) compound. The specific activities and suppliers are presented elsewhere (*23*). Contaminants were each added as a saturated aqueous solution with the target concentration of approximately 50 ppm (mg/L) and an equilibrium headspace

TABLE 2. Mass Balance Data for Uptake Studies[a]

compound (*n*)	exposure medium (liquid and headspace)	transpiration stream (orbo tubes)	roots (oxidation)	bottom stem (oxidation)	upper stem (oxidation)	leaves (oxidation)	reactor components (extraction)	total ^{14}C recovery
aniline (9)	48.1 ± 19.4	ND[b]	10.2 ± 8.0	16.9 ± 3.6	3.8 ± 1.9	11.4 ± 8.2	ND	88.6 ± 6.8
phenol (6)	63.6 ± 13.2	ND	5.8 ± 2.0	9.6 ± 3.7	2.2 ± 1.3	2.4 ± 2.0	ND	83.5 ± 13.2
nitrobenzene (6)	66.7 ± 3.7	0.8 ± 0.55	0.25 ± 0.09	15.6 ± 2.6	2.9 ± 0.8	0.3 ± 0.12	7.9 ± 1.6	94.6 ± 2.9
benzene (3)	66.8 ± 4.9	18.1 ± 2.5	0.11 ± 0.07	1.1 ± 0.9	0.39 ± 0.20	0.09 ± 0.03	7.0 ± 0.8	93.6 ± 0.6
TCE (4)	47.0 ± 7.9	21.4 ± 5.3	0.66 ± 0.22	2.0 ± 0.9	0.44 ± 0.25	0.30 ± 0.11	10.8 ± 3.6	82.9 ± 8.1
atrazine (6)	45.0 ± 8.3	ND	8.7 ± 2.5	8.6 ± 2.7	2.4 ± 0.4	33.6 ± 5.4	ND	98.1 ± 6.6
toluene (3)	50.9 ± 1.3	10.1 ± 3.3	0.35 ± 0.18	7.1 ± 0.9	1.7 ± 1.3	0.52 ± 0.26	13.7 ± 1.0	84.3 ± 3.5
ethylbenzene (6)	39.2 ± 5.9	10.4 ± 4.6	1.2 ± 0.4	6.4 ± 0.7	0.98 ± 0.51	1.6 ± 1.5	24.9 ± 2.3	85.2 ± 2.4
m-xylene (4)	40.6 ± 3.0	9.2 ± 1.1	3.0 ± 1.0	8.2 ± 1.1	1.6 ± 0.4	0.61 ± 0.32	17.5 ± 1.8	80.7 ± 3.4
1,2,4-TCB (4)	9.4 ± 1.6	ND	2.1 ± 3.1	15.0 ± 2.8	0.10 ± 0.07	0.14 ± 0.10	67.9 ± 2.5	94.6 ± 6.8
PCP (7)	66.4 ± 14.7	ND	8.1 ± 4.9	19.2 ± 13.8	0.60 ± 0.64	1.10 ± 0.69	ND	95.4 ± 5.2

[a] Values represent the percent recovery of the ^{14}C added at time = 0. [b] ND, not dectected.

concentration for volatile compounds, as calculated using published Henry's law constants listed in Table 1. The exact concentration of the saturated solutions was determined analytically. Atrazine was added at a concentration of 0.27 ppm due to the low solubility and phytotoxicity of atrazine. TCB and PCP were added as a concentrated methanol solution with target concentrations of 10 and 4.6 ppm, respectively, due to low aqueous solubilities. The total aqueous volume in the reactors was 200 mL.

Radiolabeled (^{14}C) compounds were added as a concentrated methanol solution. The cosolvency effect was determined to be negligible. Research by Munz and Roberts (*25*) has shown that the Henry's law constant, octanol−water partition coefficient, and aqueous solubility were not affected by solvent concentrations below a solvent mole fraction of 5×10^{-3} using methanol and 2-propanol. Solvent concentrations used to deliver the compounds to the hydroponic reactors in these experiments were at least 1 order of magnitude less than concentrations determined to cause a cosolvency effect. Methanol is known to be toxic to plants, but no toxic effects were seen at the low levels utilized in this study.

The sealed and dosed reactors were placed in the growth chamber constructed in a walk-in fume hood. The growth chamber was maintained at 25 ± 3 °C. Light was supplied by sets of eight Vita-Lite 40-W fluorescent tubes. Photon flux at the leaf surface inside the reactor was measured at 290−310 μEinsteins m^{-2} s^{-1} in the photoactive range, λ_{PAR} = 400−700 nm. The photoperiod was set at 16 h/d. Airflow through the aerial compartment of the reactor was maintained at 0.8−1.1 L/min utilizing a vacuum pump. Transpiration of water was gravimeterically monitored at 6-h to 2-d intervals.

The activated carbon traps used to capture any transpired (volatilized) compounds in the effluent air were changed at 6-h to 2-d intervals. For analysis, the activated carbon from the trap was transferred to a 5-mL vial. The carbon was extracted with a 2.0-mL aliquot of carbon disulfide (ACS Certified, Fisher). Tests showed the efficiency of collection for the traps and extraction to be greater than 94% for ^{14}C-labeled TCE and benzene. The CS_2 extractant was analyzed in parallel via liquid scintillation counting (LSC) techniques and gas chromatography (GC). LSC analysis of the traps was conducted by placing 50-μL aliquots of the carbon disulfide (CS_2) into 20-mL glass scintillation vials containing 20 mL of Scintiverse I scintillation cocktail (Fisher). Samples were then counted in the Beckman 6000IC liquid scintillation counter. Detailed counting methods are presented elsewhere (*23*). If ^{14}C activity was detected in the CS_2, a 0.8-mL aliquot of the CS_2 extractant was placed into a 2-mL crimp-top autosampler vial for GC analysis. Duplicate 2.0-μL samples were injected into an HP 5890 GC. All TCE and TCB analysis was carried out via an electron capture detector (ECD), and all other compounds were analyzed via a flame ionization detector (FID) (*23*). Peak identification for each compound was accomplished by comparing peak retention times with standards. External standards were used to generate five-point standard curves, pre- and post-analysis, for each sample set analyzed.

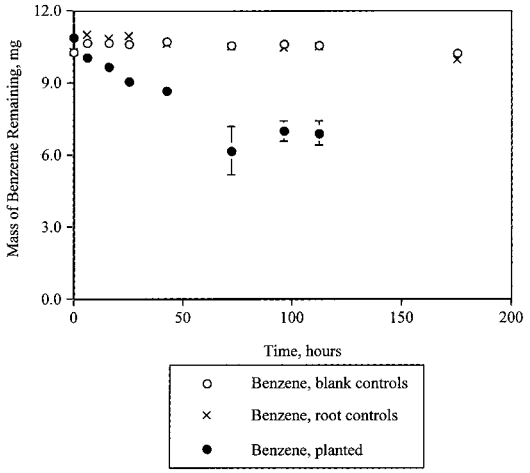

FIGURE 3. Mass of benzene remaining in the root compartment of the bioreactors as determined by GC analysis. Impulse input of chemical at *t* = 0 h to hydroponic system. Error bars represent ± 1 SD, *n* = 3.

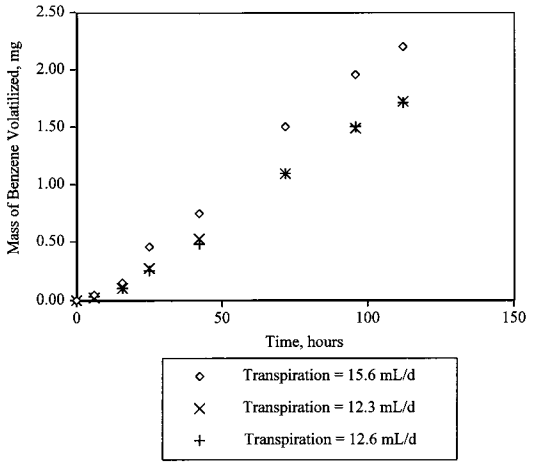

FIGURE 4. Volatilization of benzene from poplar leaves as determined by GC analysis. Impulse input of chemical at *t* = 0 h to hydroponic system. Error bars represent ± 1 SD.

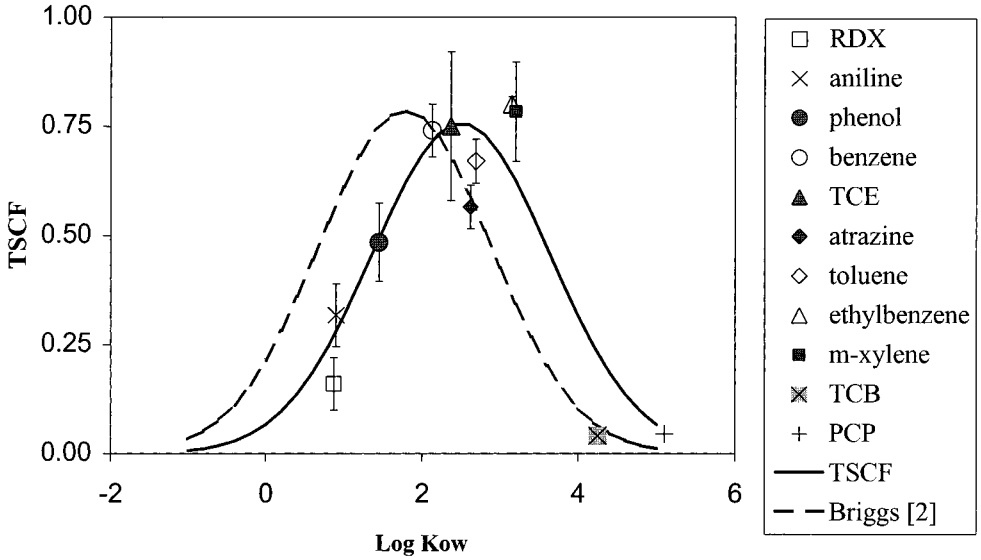

FIGURE 5. Experimentally determined TSCF values plotted as a function of log K_{ow}. The plotted lines represent relationships developed from the experimentally collected data using a nonlinear regression and least squares minimization method:

$$\log \text{TSCF} = 0.756 \exp\{-(\log K_{ow} - 2.50)^2/2.58\}$$

and by Briggs et al. (*2*):

$$\log \text{TSCF} = 0.784 \exp\{-(\log K_{ow} - 1.78)^2/2.44\}$$

Error bars represent ± 1 SD.

At 6-h to 2-d intervals, the aqueous concentration of the compound in each reactor was analyzed. Parallel sampling was again conducted via LSC and either GC or HPLC. Samples of the hydroponic solution, 100 μL, were collected by gas-tight syringe from each reactor and were injected into 20 mL of Scintiverse and counted on the LSC. For volatile compounds, a separate 100-μL sample was injected into a 20-mL headspace autosampler vial that was capped with a Teflon-lined septa. Sample size was 10 μL for TCE. The vials were then placed in a HP 19395A headspace autosampler. Vials were equilibrated at 37 °C for 30 min, and 100-μL headspace samples were injected into the GC. For the nonvolatile and semivolatile compounds, HPLC analysis was conducted utilizing a Gilson 305/307 dual-pump gradient system. Compounds were detected with a Spectra Physics Spectra 100 variable wavelength detector and a Packard Radio-Chromatographic A500 detector in series. Analytes were separated via reverse-phase columns. The retention times of the analytes were determined using both nonlabeled and ^{14}C-labeled standards.

Experiments ranged from 88 to 180 h. At the termination of each experiment, aqueous solutions were sampled as described above, and the reactors were sacrificed for further analysis. The biomass was separated into roots, leaves, and stems and weighed promptly. To minimize loss of volatiles, biomass samples (<1.0 g) were directly combusted by biological oxidation (BO) to CO_2, which was trapped and analyzed via LSC. This procedure, referred to as BO-LSC, is detailed elsewhere (*23*). To close mass balances, the sealant used to seal the tree−septa union was also extracted for ^{14}C activity. All of the sealant was placed into 20 mL of methanol and shaken on a shaker table at 120 rpm for 48 h. This virtually liquefied the acrylic sealant. From the sealant extract, 50-μL samples were injected into 20 mL of Scintisafe Econo F cocktail (Fisher) and were analyzed via LSC.

Another predictive relationship determined was the root concentration factor, RCF. The RCF is the concentration sorbed to the root tissues divided by the aqueous concentration (*26*). To determine the RCF values, uncontaminated fresh roots were harvested from poplar cuttings actively growing in hydroponic solution in the laboratory incubator. Harvested root biomass was blotted dry and placed in 21-mL crimp top vials filled with 20 mL of deionized H_2O. The vials were dosed with a mixture of cold and labeled compound in the same method as the uptake experiments. Each experiment was conducted on 4−5 vials with roots, and two controls were dosed at the same concentrations but containing no root tissue.

Reactors were sealed and placed in the dark at 25 °C. Vials were tested daily by taking 100-μL samples via a 100-μL gas-tight syringe and performing subsequent LSC analysis. Sampling was continued at 12−24-h intervals until equilibrium was assured. At the conclusion of each experiment, the vials were analyzed via LSC and either GC or HPLC to confirm that ^{14}C activity was present as the parent compound. The root tissues were removed from the vials, immediately blotted dry, weighed, and analyzed via BO-LSC to determine total ^{14}C levels sorbed.

Results and Discussion

The vigorously growing poplar cuttings showed no visible signs of toxicity or depressed transpiration rates at the concentrations tested in these short-term experiments. However, these short-term experiments should not be interpreted as conclusive toxicity tests or representative of long-term exposure. It is thought that long term exposure to these levels could result in toxicity. A limited number of reactors either did not fully seal at the tree−septum interface or the tree−septa seal was compromised during the rigors of sampling throughout the experiment. Compromised seals were detected visually or as a sudden evolution of the VOCs in the aerial compartment. Data obtained from reactors that were compromised were excluded. Mass balances (total recovery of ^{14}C label) in whole plant uptake studies were typically over 85%. The lowest recovery was for *m*-xylene, 81% (Table 2).

The mass associated with the root compartment of the reactor is shown in Figure 3 for benzene. The data for benzene are representative of results seen for the other volatile compounds. Results are plotted as mass of the VOCs in the bottom portion of the reactors and not as concentration because the aqueous volume in the reactors decreased as

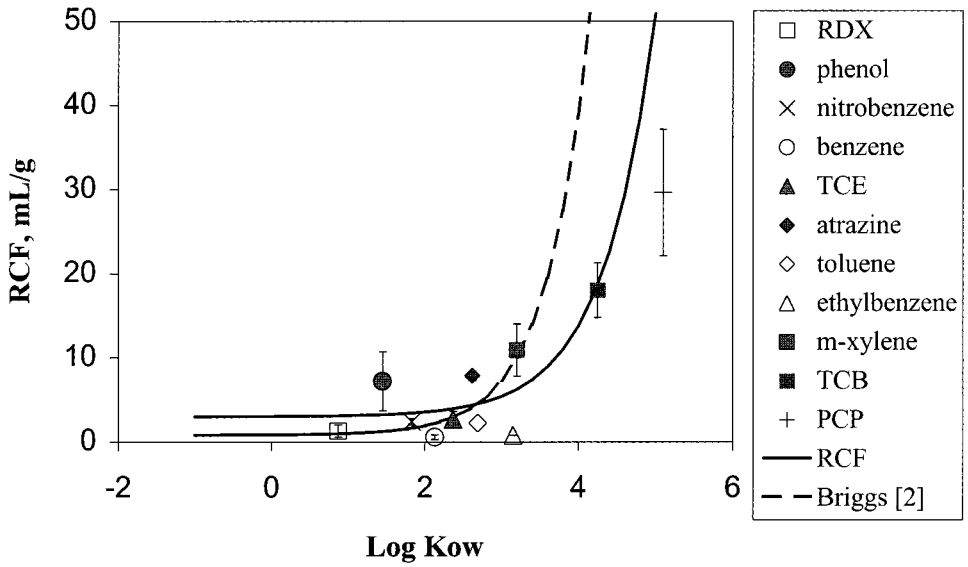

FIGURE 6. Experimentally determined RCF values. Root tissue weights were determined as fresh weight. The plotted lines represent relationships developed from the experimental data using nonlinear regression and least squares minimization method:

$$\log (RCF - 3.0) = 0.65 \log K_{ow} - 1.57$$

and by Briggs et al. (*2*):

$$\log (RCF - 0.82) = 0.77 \log K_{ow} - 1.52$$

Error bars represent ± 1 SD.

transpiration by the cuttings removed the solution. The mass also included the gaseous phase VOCs in the headspace of the root compartment.

Results in Figure 3 are similar to those of the volatile compounds toluene, ethylbenzene, *m*-xylene, and TCE. For the more hydrophobic contaminant TCB, the aqueous mass showed an initial decrease in the planted reactors and rooted controls due to sorption. TCB mass remained essentially constant after initial sorption to the plant tissue. For all VOCs other than TCB, the planted reactors showed a gradual depletion of the contaminants over time. Planted cuttings removed the other VOCs from the hydroponic reactors by uptake and transpiration. Analysis of the activated carbon traps from the aerial compartment revealed that large fractions of certain VOCs were transpired from the leaves to the surrounding air. Data on benzene transpiration are displayed in Figure 4. Benzene was readily taken up, translocated to the leaves, and transpired from the leaves to the aerial compartment of the reactors. Toluene, ethylbenzene, *m*-xylene, and TCE were also readily transpired from the leaf tissues. The relative volatilization of each VOC can be seen in Table 2. The compounds were detected in the aerial compartments in as little as 24 h for benzene. However, toluene, ethylbenzene, *m*-xylene, and TCE all took longer to appear in the aerial compartment, taking up to 50 h to transpire a mass similar to benzene. TCB was not detected in the aerial compartment. In Figure 4, a relationship between the volume of water transpired and the mass of the benzene transpired is evident. The data points represent individual reactors, and the transpiration rates were the average daily rates for each cutting during the entire experiment. This experiment with benzene was conducted for 112 h. The mass of benzene transpired was related to the water volume transpired. This was a consistent finding for VOCs other than TCB.

There were trace portions of the semivolatile compound nitrobenzene collected from the effluent from the aerial compartment, but they were not significantly different from zero. There was no measurable ^{14}C activity in the activated carbon traps of the aerial compartment for the semivolatile compounds aniline and pentachlorophenol or for the nonvolatile compounds atrazine, RDX, and phenol, thus showing that there was no volatilization from the leaves. At experiment initiation, mass of the contaminants in the root compartment decreased rapidly due to sorption. The mass in solution of the rooted controls and blank reactors stabilized rapidly after sorption occurred and remained constant throughout the experiment. The mass of atrazine, phenol, nitrobenzene, aniline, and RDX in the root compartment of the planted reactors each decreased over time with atrazine being the most rapidly depleted. The depletion from the root compartment was due to uptake and translocation. Oxidation of the plant tissues revealed that the ^{14}C-label had been sequestered in the leaf and upper stem tissues. The aqueous mass of PCP in the planted reactors remained constant after the initial sorption period, indicating that it was not translocated. PCP was not detected at concentrations significantly different from zero in the upper plant sections or in the transpiration stream. It should also be considered that PCP also exists as pentachlorophenolate at the pH range used in this study.

The form of the contaminants sequestered in the plant tissues was not delineated in this experiment. However, in other studies, hybrid poplars have been shown to metabolize atrazine in the leaf tissues to hydroxylated, dealkylated products (*27*). Phytodegradation of other contaminants such as TCE by hybrid poplars (*19*) and TNT by aquatic species (*28*) has been recently proven in whole-plant and cell culture studies.

Mass remaining in the planted reactors was also dependent upon transpiration rates for these non- and semivolatile compounds. For aniline, the reactor with a transpiration rate of 17.4 mL/d had 6.7 mg of aniline remaining after 88 h. With higher transpiration rates, aniline was depleted more rapidly. In the reactor transpiring 41.9 mL/d, the aniline remaining in the reactor after 88 h was only 1.35 mg, less than 15% of the initial mass.

Transpiration Stream Concentration Factor. Oxidation of the poplar biomass revealed the uptake of ^{14}C and storage in the tissues after the exposure of the root tissues to the ^{14}C-labeled compounds. The uptake data were utilized to determine the TSCF for the investigated compounds. The

TSCF is defined as the concentration of the analyte in the transpiration stream within the plant divided by the concentration in the bulk solution from which uptake is occurring, eq 1. TSCF can be utilized in phytoremediation studies to represent how readily compounds are taken up and translocated by a plant species. Uptake of a solute, either contaminant or nutrient, can be estimated as the TSCF multiplied by the volume of water transpired and concentration in the surrounding aqueous solution, eq 2. TSCF values were determined from data collected for the aqueous concentration, volumetric water transpiration, and total uptake. Data were entered into a finite difference model, which utilized eq 3. For eq 3, the linear average of the bulk solution concentration is assumed to be the concentration from time = $t1$ to time = $t2$. This assumption remains valid as long as the compound of interest is not transformed in the bulk solution, which defies the definition of TSCF. The TSCF values were determined utilizing eq 3:

$$\text{TSCF} = C_{\text{transpiration stream}}/C_{\text{bulk solution}} \quad (1)$$

$$\text{uptake} = \text{TSCF} \times \text{Trans} \times C_{\text{bulk solution}} \quad (2)$$

$$\text{uptake}_{t1-t2} = \text{TSCF} \times \text{Trans}_{(t1-t2)} \times (C_{\text{bulk solution, } t1} + C_{\text{bulk solution, } t2})/2 \quad (3)$$

where uptake is the mass translocated into cutting, mg/d; TSCF is the transpiration stream concentration factor, dimensionless; Trans is the water transpired, mL/d; $C_{\text{transpiration stream}}$ is the concentration in transpiration stream, mg/L; $C_{\text{bulk solution}}$ is the concentration in bulk solution, mg/L; $t1$ = time 1; $t2$ = time 2; uptake_{t1-t2} is the uptake from $t1$ to $t2$.

Total uptake was measured as the sum of ^{14}C activity found to be translocated and stored in plant tissues plus the ^{14}C captured in the exhausted airstream for VOCs. The translocated ^{14}C activity does not include sorbed activity associated with the roots or the submerged stem. To exclude any sorption, ^{14}C activity associated with root biomass was not included in the uptake calculations, and the concentration in the upper stem was assumed to be the average concentration of the entire stem. The resulting TSCF values were averaged for all reactors used in each experiment.

TSCF values were plotted as a function of the compound's log K_{ow}, molecular weight, and aqueous solubility. The TSCF vs log K_{ow} plot was the only relationship to reveal a good fit, Figure 5. This plot also includes the TSCF of RDX, which was determined by a co-worker (*29*). The equation in Figure *5* fits the experimental data well with the exception of the TSCF determined for nitrobenzene. Nitrobenzene was not readily translocated throughout the plant. This resulted in a low TSCF value. Research has revealed similar results for 2,4,6-trinitrotoluene (TNT) (*30*). Experiments with TNT revealed that the compound was not translocated to leaf tissues in poplar cuttings, but it did accumulate in the lower stem and roots. The log K_{ow} values of nitrobenzene and TNT, 1.83 and 1.90, respectively, indicate that they should be easily translocated. Nonetheless, these compounds were not translocated to the expected levels. The TSCF for nitrobenzene was determined to be 0.30 in this study, and Thompson et al. (*30*) found a TSCF of 0.46 for TNT. The nitro groups are a common substituent shared by these compounds and may be a factor in the root exclusion of nitrobenzene. Reduction of the nitro groups to amino groups at the root may lead to covalent bonding and immobilization (*6*). Nitrobenzene has also been shown to cause the suppression of root elongation at levels similar to the 50 ppm concentrations used here (*20*). In light of the potential transformation or toxicity, which would void the TSCF relationship, the TSCF for nitrobenzene was not included in Figure 5.

Compounds such as TCB and PCP, which are hydrophobic, tended to sorb to the root tissues and were occluded from entering the translocation stream. Compounds such as aniline, RDX, and phenol, which are less hydrophobic, apparently did not pass through the organic membranes, such as the endodermis, associated with the roots. Such trans-membrane movement is necessary in order for translocation to occur. Differences between the relationship developed here and those previously developed may be a consequence of the plant species used and the compounds investigated. Differences in plant species resulted in widely disparate translocation in previous research (*2, 21*).

In Figure 5, these values are compared to a relationship reported in the literature by Briggs et al. for uptake of *O*-methylcarbamoyloximes and substituted phenylureas by barley (*2*). A new relationship was developed by altering the coefficients in the equation to attain the best fit to the data, as determined by a least squares method. The relationship is presented. The purpose of this relationship is not to present an alternative relationship to that of Briggs et al. or to refute previous work but to offer a relationship developed for common contaminants and plant species that are common in phytoremediation applications. The TSCF of compounds, other than nitrobenzene, follow the derived relationship. This relationship had a similar shape to that taken from the literature and for transport of cinmethylin and related compounds in a pressure chamber with soybeans (*31*). Uptake experiments presented here suggest that the pinnacle of the curve be located at a slightly greater log K_{ow} value, 2.50, than Briggs et al. (*2*), although the difference may be arbitrary. There are deviations between the data and the developed relationship. The deviations encountered in this study are similar to that of previous studies concerning organic compound translocation.

Root Concentration Factor. Root concentration factors (RCF) are reported in Figure 6. The data show a general increase for RCF values with increasing log K_{ow} values. The relationship determined in similar experiments with barley roots and herbicide-related compounds is also displayed (*2*). The expected trend of greater sorption for more hydrophobic compounds is shown for this experiment. Lower sorption for hydrophobic compounds, such as TCB, was found than previously shown in the literature (*2*). This appears to be an effect of the plant species utilized and individual contaminants investigated.

We have developed a systematic data set and predictive relationships for common organic contaminants at waste sites. This research revealed that log K_{ow} can be used to estimate the fate of common organic contaminants in phytoremediation with hybrid poplar trees. However, this relationship should be utilized only as an estimating tool and not as a definitive predictor of organic contaminate uptake or as a replacement for uptake and fate studies when the uptake of organic contaminants poses a potential ecological or human health threat. The relationship overestimated the uptake of nitrobenzene. This research and other research with TNT suggest that nitro substituents may be transformed and sorbed at the root surface. This work also reveals that specific VOCs such as the BTEX compounds and TCE, which are amenable to phytoremediation, can be transpired to the surrounding atmosphere after translocation to leaves of the hybrid poplar trees. The limiting factors are the exclusion or binding and metabolism that occur at the roots. These results are for short-term hydroponic experiments in the absence of soil sorption processes. The results should not be utilized as toxicological considerations, and

discretion should be taken when extrapolating this work to field situations.

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Literature Cited

(1) U.S. EPA. *A Citizen's Guide to Phytoremediation*; EPA 542-F-96-014; U.S. EPA: Cincinnati, OH, 1996.
(2) Briggs, G. G.; Bromilow, R. H.; Evans, A. A. *Pestic. Sci.* **1982**, *13*, 495.
(3) Shone, M. G. T.; Wood, A. V. *J. Exp. Bot.* **1972**, *23*, 141.
(4) Trapp, S.; Mc Farlane, C.; Matthies, M. *Environ. Toxicol. Chem.* **1994**, *13*, 413.
(5) Clarkson, D. T. Root Structure and Sites of Ion Uptake. In *Plant Roots the Hidden Half*; Waisel, Y., Eshel, A., Kafkafi, U., Eds.; Marcel-Dekker, Inc.: New York, 1991; 415.
(6) Schnoor, J. L.; Licht, L. A.; McCutcheon, S. C.; Wolfe, N. L.; Carriera, L. H. *Environ. Sci. Technol.* **1995**, *29*, 318A.
(7) Walker, A. *Pestic. Sci*, **1972**, *3*, 139−148.
(8) Briggs, G. G.; Rigitano, R. L. O.; Bromilow, R. H. *Pestic. Sci.* **1987**, *19*, 101.
(9) Behrendt, H.; Bruggemann, R. *Chemosphere* **1993**, *27*, 2325.
(10) Anderson, T. A.; Walton, B. T. *Environ. Toxicol. Chem.* **1995**, *14*, 2041.
(11) Edwards, N. T.; Ross-Todd, B. M.; Garver, E. G. *Environ. Exp. Bot.* **1982**, *22*, 349.
(12) Edwards, N. T. *Environ. Toxicol. Chem.* **1986**, *5*, 659.
(13) McFarlane, J. C.; Pfleeger, T.; Fletcher, J. *J. Environ. Qual.* **1987**, *16*, 372.
(14) McFarlane, J. C.; Nolt, C.; Wickliff, C.; Pfleeger, T.; Shimabuku, R.; McDowell, M. *J. Environ. Toxicol. Chem.* **1987**, *6*, 847.
(15) Schroll, R.; Scheunert, I. *Chemosphere* **1993**, *26*, 1631.
(16) Schroll, R.; et al. *Chemosphere* **1994**, *28*, 297.
(17) Topp, E.; Scheunert, I.; Attar, A.; Korte, F. *Ecotoxicol. Environ. Saf.* **1986**, *11*, 219.
(18) Wild, S. R.; Jones, K. C. *J. Environ. Qual.* **1992**, *21*, 217.
(19) Newman, L.A.; et al. *Environ. Sci. Technol.* **1997**, *31*, 1062.
(20) Fletcher, J. S.; McFarlane, J. C.; Pfleeger, T.; Wickliff, C. *Chemosphere* **1990**, *20*, 513.
(21) McFarlane, J. C.; Pfleeger, T.; Fletcher, J. S. *J. Environ. Toxicol. Chem.* **1990**, *9*, 513.
(22) Licht, L. A. Ecolotree, Personal communication, 1997.
(23) Burken, J. G. Ph.D. Dissertation, University of Iowa, 1996.
(24) Arcand, Y.; Hawari, J.; Guiot, S. R. *Water Res.* **1995**, *29*, 131.
(25) Munz, C.; Roberts, P. V. *Environ. Sci. Technol.* **1986**, *20*, 830.
(26) Shone, M. G. T.; Wood, A. V. *J. Exp. Bot.* **1974**, *25*, 390.
(27) Burken, J. G.; Schnoor, J. L. *Environ. Sci. Technol.* **1997**, *31*, 1399.
(28) Hughes, J. B.; Shanks, J.; Vanderford, M.; Lauritzen, J.; Bhadra, R. *Environ. Sci. Technol.* **1997**, *31*, 266.
(29) Thompson, P. L. Ph.D. Dissertation, The University of Iowa, 1997.
(30) Thompson, P. L.; Ramer, L. A.; Schnoor, J. L. *Environ. Sci. Technol.* **1998**, *32*, 975.
(31) Hsu, F. C.; Marxmiller, R. L.; Yang, A. Y. *Plant Physiol.* **1990**, *93*, 1573.
(32) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry;* John Wiley & Sons: New York, 1993; p 681.
(33) Schnoor, J. L. *Environmental Modeling Fate and Transport of Pollutants in Water, Air, and Soil;* Schnoor, J. L., Zehnder, A. J. B., Eds.; Wiley-Interscience: New York, 1996; p 682.
(34) Howard, P. H. Solvents. In *Handbook of Environmental Fate & Exposure Data;* Howard, P. H., Ed.; Lewis Publishers: New York, 1991.

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