Direct Measurement of VOC Diffusivities in Tree Tissues: Impacts on Tree-Based Phytoremediation and Plant Contamination

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TCE Diffusion to the Atmosphere in Phytoremediation Applications

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The phytoremediation of trichloroethylene (TCE) and other chlorinated compounds has been studied over the past decade, and full-scale systems are in place. The results regarding TCE fates and removal pathways are inconclusive and conflicting, particularly the results regarding volatilization to the atmosphere. Research presented here demonstrates that TCE is taken up by trees and volatilized to the atmosphere. TCE diffusion along the transpiration pathway is shown to be the primary process for TCE volatilization, although volatilization can occur from both stems and leaves. Two concurrent processes influence the eventual fate: transport with transpiration stream through xylem tissues and diffusion from transpiration stream to atmosphere. TCE diffusion flux invariably decreased with height for trees planted in soil or grown hydroponically. In both laboratory experiments and field sampling, TCE concentrations in the transpiration stream (e.g., xylem tissues) decreased with elevation. In field samples, TCE concentrations also decreased in the radial direction, providing fundamental evidence for diffusion. The TCE concentrations in tissues responded linearly to the exposure concentrations at the roots, while TCE diffusion from tree stems was influenced by concentration and transpiration rates.

Introduction

Phytoremediation is of great interest because of its economic, aesthetic, and environmental benefits. Since its advent, phytoremediation has been studied as a way to treat metals, radionuclides, and organic compounds (*1*, *2*). Among them, trichloroethylene (TCE) is an extensively studied compound because of its widespread contamination and recalcitrant nature of the extensive groundwater plumes that exist (*2*−*4*).

TCE was observed to be taken up by plants in both lab-scale experiments and field-site-scale studies (*3*−*6*). When organic contaminants are taken up by plants, determining the fate of parent compounds and metabolites is of great importance. Following uptake, organic chemicals may have variable fates. The organic chemicals may bind to the wood tissues, be degraded to form metabolites, or be transferred to the atmosphere (*1*−*6*).

Enzymatic transformations are possible, and an axenic poplar cell culture experiment exhibited conclusively that poplar cells are capable of transforming and mineralizing TCE without the involvement of microbial metabolism (*6*). The metabolites of TCE in cell cultures include trichloroethanol, trichloroacetic acid, and dichloroacetic acid, which was the most predominant. Chloral hydrate was also found at levels below method detection limits. Chloral hydrate is a product of TCE oxidation by cytochrome P-450 oxygenase and the precursor of trichloroethanol and trichloroacetic acid in mammalian systems (*7*). However, the degradation of TCE and storage of metabolites in plant tissues are minor as compared to the total mass of TCE taken up by plants as shown in ^{14}C-labeled TCE experiments (*3*, *5*, *6*, *8*, *9*).

Transfer to the atmosphere is also possible after uptake by plants. Mass distribution and volatilization of 11 different organic compounds ranging from nonvolatile to volatile following uptake by hybrid poplar trees was investigated, and volatilization was shown to correlate with vapor pressure (V_p) (*8*). Compounds with a $V_p > 0.01$ atm were volatilized, with higher vapor pressures relating to greater volatilization. Volatilization was much lower for compounds with a $V_p < 0.01$ atm (*8*). When all published data on uptake and volatilization of TCE is considered, findings are not entirely consistent as some research showed minimal to nearly zero aboveground transpiration of TCE (*6*, *9*).

Uptake and transpiration of TCE is more uncertain in field settings. In a test site dosed with TCE for 2 yr, the transpiration from leaf tissues was highly variable, ranging from undetectable to about 1.6 μg of TCE leaf^{-1} h^{-1}, while over 99% of the added TCE was removed (*6*). Transpiration of TCE from leaves in the same test accounted for less than 9% of the added TCE in the first 2 yr, and transpiration of TCE was not detected after year 2 (*6*). In another study at Carswell AFB, TCE was not present in the leaf tissue even though the groundwater at the site is as high as 930 ppb (*10*). At Aberdeen Proving Grounds (APG) in Maryland, a plume with concentration up to 260 ppm total VOCs was treated with a poplar tree phytoremediation system in 1996. The phytoremediation system showed effectiveness in containing the plume, but limited TCE ranging from 14 to 210 ppbv was detected in the off gas from the leaf tissues in the 1997 growing season (*11*) and later measurements. These results indicated that uptake and transpiration of TCE to the atmosphere from leaf tissues was not substantial.

Recent findings provide new insight to uptake of VOCs. Investigation of tree core samples from Savannah River site in South Carolina showed that trees do uptake TCE. Tree cores analysis revealed TCE concentrations in tree tissues related to groundwater contaminants as determined by standard groundwater sampling (*4*). The correlation of TCE levels in transpiration stream to the TCE concentration in groundwater was confirmed recently in lab-scale research (*12*). The work done at the Savannah River site also indicated decreasing TCE concentrations along the transpiration path (i.e., with height up the trunk), and a hypothesis was posed indicating diffusive loss through the stem (*4*). Similar decreases have been detected in the lab, diffusion was suggested, and diffusion coefficients were proposed (*13*). The purpose of the study was to directly measure TCE diffusion from stems to the atmosphere and evaluate if diffusion could account for the TCE removal and be a dominant process in phytoremediation systems.

Materials and Methods

Soil Experiment. Wide-mouth glass bottles (1000 mL) fitted with Teflon-lined lids were used in the experiment. Uniform silica sand (200 g) was placed on the bottom of the bottles and leveled in order to distribute the feeding evenly. Potting soil (250 g) was then placed on top of the sand, Figure 1.

Hybrid poplar whips (*P. deltoides* × *P. nigra*, clone DN 34) were cut to an approximate length of 30 cm and inserted through holes in the lid of each reactor. A Teflon feeding

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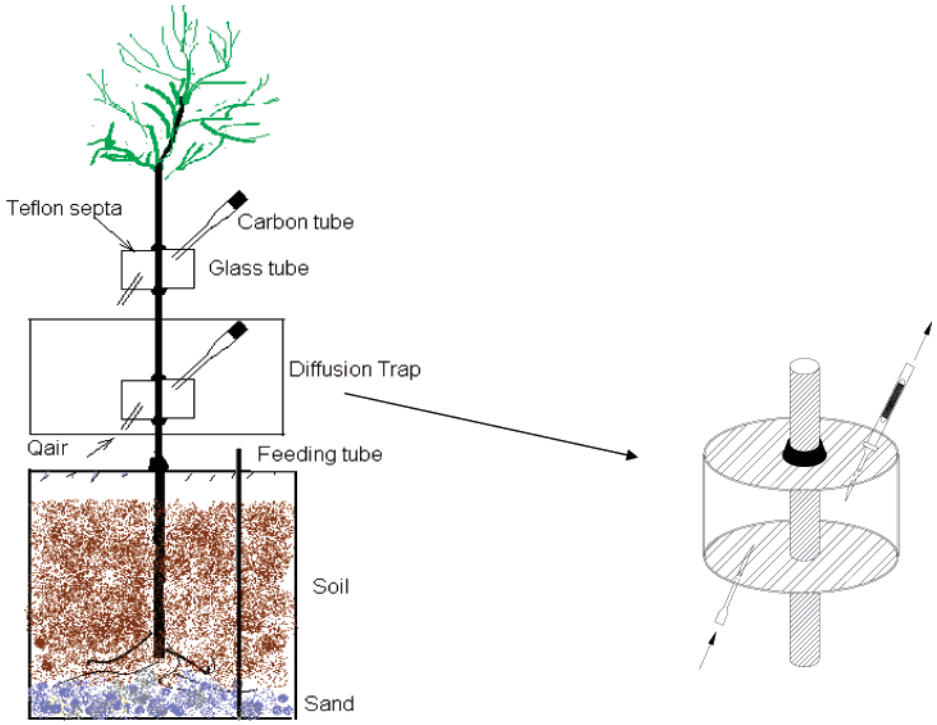

FIGURE 1. Experimental arrangement for soil experiments with detailed schematic of diffusion trap.

tube was inserted into another hole in the lid and placed such that the plants would be fed from the bottom to mimic field conditions and avoid direct volatilization. The reactors were wrapped with aluminum foil to prevent algal growth. The dry reactors with the plants were weighed and recorded.

The reactors were watered with 200 mL of tap water in the beginning to a target point of 80% field saturation, weighed, and recorded again. Reactors were placed in a walk-in fume hood fitted with a set of 10 40-W fluorescent bulbs (Verilux) with an intensity at the leaf surface of 85−90 μmol s^{-1} m^{-2}, in the range of photosynthetically active radiation (PAR = λ 400−700 nm). Light intensity was measured with a Quantum meter (Apogee Instruments). Lights were on for a 16-h photoperiod per day. The temperature in the laboratory was between 20 and 24 °C.

The plants were watered weekly to maintain the soil moisture. When cuttings showed signs of sprouting leaves, two 1 in. i.d. × 1 in. long glass tubes were put around the tree cuttings. A Teflon septum was put in each end of the tubes and sealed to the cutting and the glass tube with Teflon tape and acrylic caulk. Tubes were placed at 1 and 4 in. above the lid (Figure 1). The sealed tubes are termed "diffusion traps". The Teflon feeding tubes and the cuttings were sealed at the lid interface to seal the reactors. Two syringe needles were inserted through the septa for each diffusion trap. One needle was connected to an activated carbon tube (Orbo tube-#32 large, Supelco). Each activated carbon tube was attached to a flow meter and vacuum system. Airflow was set at 0.1 L/min through the diffusion traps during the entire experiment. The setup was configured as shown in Figure 1.

Reactors were watered with tap water every 3 d. For watering, reactors were weighed, transpiration was recorded, and transpired water was replaced. After transpiration rates stabilized at about 3 weeks, clean tap water was replaced with TCE solutions of 50, 220, 550, and 820 ppm for watering events. Duplicates were made for each feeding concentration. The reactors were weighed, and the activated carbon tubes at both heights were changed at each watering (e.g., dosing) event. After 28−32 d, TCE solutions were replaced with deionized water, and watering was continued until day 52. Background samples were collected by pulling ambient air

TABLE 1. Composition of Modified Hoagland's Solution

Macronutrients, mg/L (ppm)					
KH_2PO_4	$Ca(NO_3)_2$	$CaSO_4$	KNO_3	$MgSO_4$	K_2SO_4
208	161	289	137	469	161

Micronutrients, mg/L (ppm)					
Cl	B	Mn	Zn	Cu	Mo
1.77	0.27	0.11	0.131	0.032	0.05

of the fume hood through the activated carbon tubes at the same rate, 0.1 L/min. After the experiment was terminated, the poplar sections above the septa were cut into 2.5 in. sections and put into vials (20 mL). The vials were closed immediately with a Teflon rubber septum and sealed with crimp top seal. The new growth stems and leaves were also sampled similarly. All biomass samples placed in the vials (20 mL) were allowed to equilibrate with the headspace for at least 48 h. Headspace samples from 0.1 to 1.0 mL were directly injected into a HP5890 gas chromatograph. Compounds were quantified by comparison to five-point standard curves. The concentrations in the dry biomass were calculated from the headspace concentrations measured via GC. The relationships were described elsewhere (*12*).

Hydroponic Experiment. Cuttings of about 30 cm long and 1 cm in diameter rooted in one-quarter strength modified Hoagland's solution. The components of the modified Hoagland's solutions are listed in Table 1. Each cutting was fitted with a Teflon septa and screw cap during the rooting process as described elsewhere (*3*). The same light was supplied as used for soil experiment. After 2 weeks, the cuttings together with the sealed cap were transferred to flasks (250 mL) filled with one-quarter strength Hoagland's solution dosed with a known concentration of TCE. The interface of the septum and cutting was sealed with caulk soon after the transfer. TCE concentrations were 2, 5, 20, and 50 ppm in the hydroponic solutions. Three replicates were made for each concentration.

Three diffusion traps were placed around the cuttings along the height of stems at 2, 4, and 6 in. above the septum.

Due to limited apparatus, the trees dosed at 50 ppm did not receive diffusion traps. TCE concentrations in the reactors were analyzed every 12 h in the first 2 d. The concentrations in the reactors remained within 5% of the initial concentrations. After day 2, the solution was replaced every 24 h until the end of the experiment to maintain consistent concentrations in the reactors. The experiment was terminated after 10 d. The poplar cuttings were cut into small sections (2.5 in.), put into vials (20 mL), and subsequently analyzed via headspace sampling as described above for soil experiments.

Activated Carbon Analysis. The activated carbon in each Orbo tube was placed into a vial (4 mL) and extracted with pure carbon disulfide (2 mL, ACS Certified). The vials were sealed immediately with Teflon-lined rubber septa and caps and put onto an orbital shaker for 24 h. The extraction efficiency of TCE sorbed to the activated carbon by CS_2 was previously determined to be greater than 94% (*3*). Following the extraction, 1 mL of supernate liquid was transferred to a vial (2 mL) with a pipet. The vials were sealed with Teflon-lined rubber septa and crimp top seal. Liquid samples (2 μL) were injected into GC using a HP 6890 series injector and autosampler.

Field Studies. Core samples were collected at the Aberdeen Proving Ground J-Field site, Edgewood, MD, as previously described (*11*). Tree cores were taken and analyzed from five-year-old poplars. The poplars are growing over a plume of 1,1,2,2-tetrachloroethane (TeCA) and TCE. This site is well-characterized and has been an active phytoremediation project since 1996 (*11*, *14*). Tree 55 was felled and cored at roughly 1-m intervals. Tree numbers used herein are initial tree identifiers and have no significant meaning other than to identify which trees were analyzed and track the associated data. Following the collection of the tree cores, the root system of the tree was exposed using a backhoe to trench next to the trunk of the tree. Core samples of the central root were collected just below the ground surface, and sections of smaller roots were collected using a pruning shears down to a depth of 2 m. All samples were immediately put into headspace vials (20 mL) and capped.

Samples were collected from additional trees in a radial coordinate. Cores were collected from trees 169 and 63; each was approximately 12 cm in diameter. Three cores were collected within 2 in. of each other from each tree. Each core was sectioned immediately into three approximately equal lengths of 2.0 cm. The radial samples correspond to sections taken 0−2, 2−4, and 4−6 cm from the center of the sapwood to the outer layer of the cambium. The term "radial coordinate" refers to the polar coordinates used, with 0 being the center of the trunk. Each section was designated as inner, middle, or outer, respectively. The three inner samples were combined in one vial for each tree, and the same was performed for the middle and outer sections. The inner and middle cores were entirely xylem tissue while outer cores also included the phloem and cambium tissues. All collected cores were transported back to Engineering Research Center (ERC) laboratories at the University of Missouri—Rolla for analysis.

Results and Discussion

TCE was collected in all diffusion traps for trees exposed to TCE. Lower diffusion traps invariably collected greater TCE mass than upper diffusion traps for each tree. In the soil experiment, the diffusion rate increased over time during the first 8 d of dosing period. The 8 d is hypothesized to be the period needed to reach an equilibrium level of sorption with the soil and tree tissues. Following day 8, the diffusion rates stabilized. TCE diffusion from the stems versus time is shown in Figure 2. The slope of the curve from day 8 to day 28 indicates the diffusion rate in milligrams of TCE per day into the 1-in. diffusion trap. The diffusion rate declined after

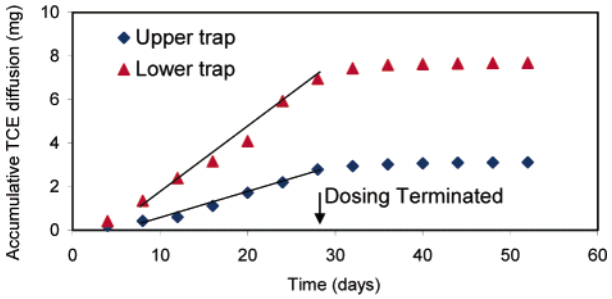

FIGURE 2. TCE accumulation in the upper ($H = 4$ in.) and lower ($H = 1$ in.) diffusion traps over the experimental period for poplars grown in soil. The reactor was dosed with 550 ppm TCE until day 28 when clean water replaced TCE-contaminated water. Slope from day 8 to day 28 represents the diffusion rate for each trap. Rates of all reactors are listed in Table 2.

TABLE 2. Diffusion Rate for Day 8 to Day 28 for TCE Collected from Diffusion Traps and Total Mass of Diffusion over Entire Soil Experiment[a]

TCE dose concn (ppm)	diffusion rate (mg/d in.) day 8−28		total mass TCE (mg/in.) entire experiment	
	upper trap	lower trap	upper trap	lower trap
50	0.026	0.045	0.97	1.90
200	0.046	0.069	1.52	2.41
550	0.109	0.287	3.71	7.69
820	0.226	0.419	6.48	12.43

[a] Averages are shown for two reactors at each dose concentration.

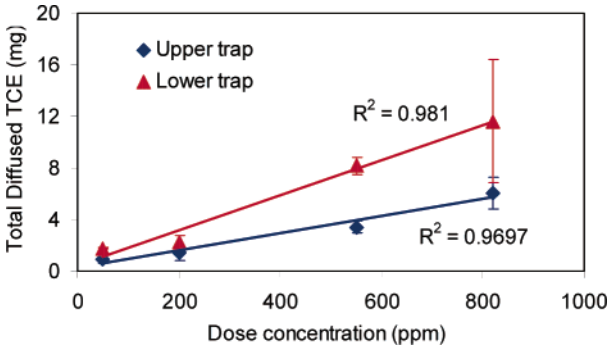

FIGURE 3. Total TCE diffusion for soil experiments plotted vs the dose concentration. Error bars represent the maximum and minimum values ($n = 2$).

dosing ceased on day 28 and decreased to zero (below method detection limits) at approximately day 36. TCE diffused from the stems for up to 8 d after the reactors were dosed with DI water, indicating the lingering impacts following the groundwater change. This period is thought to be the time to desorb and flush TCE from the soil and tissues. Accumulation curves over time were similar to Figure 2 for all reactors. Background sampling (Orbo Tubes) of air in the fume hood did not reveal measurable TCE, proving that the TCE mass collected from the diffusion traps was from the stem and not the ambient air in the fume hood.

The diffusion rate and the total amount of TCE collected from diffusion traps correlate with the dosing concentration linearly for the soil experiment (Table 2). The correlation curve between the diffused mass and the dosing concentration is shown in Figure 3. There was significant variation in TCE diffusion at the concentration of 820 ppm. Phytotoxicity may impact the high dose concentrations (*15*). One tree receiving 820 ppm TCE in the feedwater (820-A) exhibited acute toxicity signs such as yellowing leaves at day 24, and subsequently water transpiration decreased after day 28 as

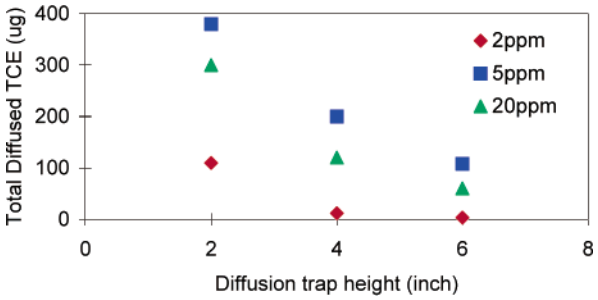

FIGURE 4. Total mass of TCE captured in diffusion traps as a function of elevation for hydroponic experiment. Average transpiration rates were 6.2, 15.6, and 5.3 mL/d, respectively, for the 2, 5, and 20 ppm dosed reactors ($n = 3$).

compared with deionized water controls and the other tree dosed with 820 ppm (820-B). No toxic impacts were detected before day 24. Tree 820-B showed no toxic signs whatsoever, and water usage prior to day 24 was not different between the two individuals. The cause for the difference is considered to be the variability of individual tree cuttings and growth patterns, which can directly impact the TCE uptake. Even though the two individual trees received 820 ppm TCE and nearly the same TCE mass was delivered to the root zone, the TCE diffusion was greater for 820-A at all sampling events. Over the 28 d of exposure, TCE diffusion into the lower trap was 14.8 mg for tree 820-A versus 8.3 mg for 820-B. One hypothesis is that the roots of 820-A utilized water closer to the feed tube and that no dilution or dissipation of the 820 ppm took place prior to root uptake. This hypothesis follows that higher TCE uptake caused both greater diffusion and toxicity. The reason for the variation in diffusion and uptake for the 820 ppm dose concentration remains undetermined, and all data are included in Figure 3.

A decrease in TCE diffusion with elevation was also observed in hydroponic experiments (Figure 4). The decline in diffusion rate was not linear, with an exponential decrease in diffusion with elevation. Transpiration was not uniform among the cuttings used in the experiment. For the randomly selected cuttings, the lowest transpiration rate was observed for the 20 ppm reactor (averaging 5.3 mL/d) while the reactor exposed to 5 ppm was the highest (averaging 15.6 mL/d). The cutting dosed at 2 ppm transpired 6.2 mL/d. Less TCE was accumulated in the traps from 20 ppm reactor than from 5 ppm reactor (Figure 4). The greater H_2O transpiration rate for the 5 ppm reactor was the cause for the increased TCE diffusion. The linkage between plant uptake of contaminants and transpiration of water was also observed previously (*6*, *8*). In sampling TCE concentrations in the living tissue at the experiment termination, TCE levels in the transpiration stream and the dry biomass declined with height along the stem in all cases. TCE was detected in the leaf tissues for all concentrations greater than 2 ppm. The TCE concentration in the transpiration stream and dry biomass also correlate to the dosing concentration of TCE (Figure 5). The tissue concentrations were not directly related to transpiration rates, whereas diffusion rates were directly related as discussed above.

Diffusion from the trunks of mature trees is also indicated from data collected at the APG J-Field site, and diffusion of TCE and TeCA has been detected, although exact rates were not quantified (*14*). TCE concentrations in the transpiration stream and dry biomass decreased with height up the trunk for samples from APG (Figure 6). The trend was also observed below ground with deeper root samples containing more contaminant. Samples collected from the APG site exhibited the declining concentration of TCE in the radial direction for the trunks, an indication that TCE volatilizes to the atmosphere via diffusion. The declining concentration of TCE in the radial direction in a sample taken from APG is shown in Figure 7a. The phenomenon was also observed for TeCA in the same trees (Figure 7b). The data provide evidence of a radial concentration gradient, providing fundamental evidence that diffusion occurs. Diffusion of VOCs into wood,

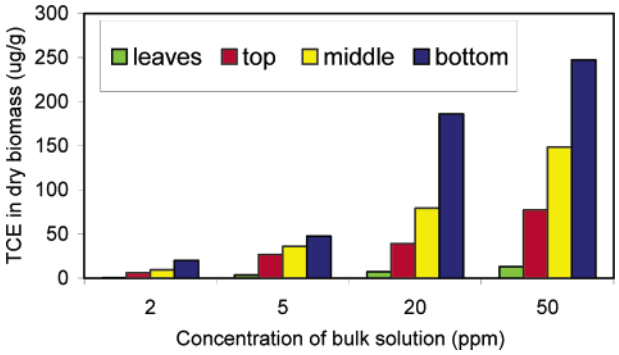

FIGURE 5. TCE concentrations (μg of TCE/g dry biomass) vs TCE feed solution concentrations. Bottom, middle, and top refer to equal length sections of the poplar stem, starting at the interface with the septum.

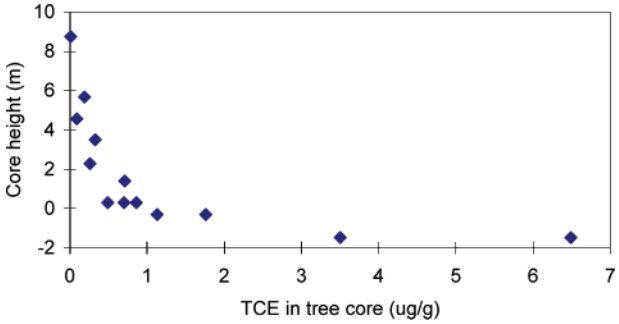

FIGURE 6. TCE concentration in cores of tree 55 vs the height of tree stem. Zero height refers to ground elevation, and samples with negative heights were taken from roots.

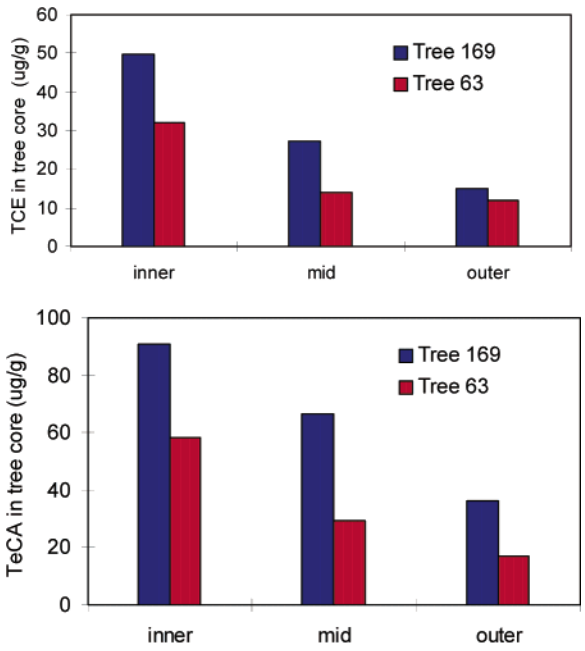

FIGURE 7. TCE and TeCA distribution in tree cores collected at Aberdeen Proving Ground, J-Field Site. Inner, mid, and outer refer to core sections from 0−2, 2−4, and 4−6 cm from the center of the sapwood to the interface of the cambium and the ambient atmosphere.

not living tissue, has been studied and found to occur rapidly (*13*, *16*).

Accumulation of TCE in biomass was not significant. Concentrations in the woody tissues were low at the termination of the hydroponic studies and accounted for less than 1% of the total TCE removed from the reactors, even though dosing was maintained right to the moment of tissue analysis. In the soil experiments, the final TCE mass in the tree tissues was less than 0.05% of the mass removed from the soil. While levels were low, TCE did transport throughout the cuttings. TCE was detected in leaves for all concentrations greater than 2 ppm in the hydroponic laboratory studies. This study did not utilize ^{14}C-labeled compounds, and analysis was not done for metabolites that may have been present.

TCE was rarely detected in the leaf tissues taken from the APG site, even though VOC levels in the groundwater were well over 200 ppm. TCE was detected in the leaf tissues in laboratory experiments for exposure concentrations greater than 2 ppm. Two hypotheses are posed for this difference in TCE levels in leaf tissues when comparing the field and laboratory findings. Hypothesis 1 is based upon the relative dominance of two concurrent processes. One process is diffusion in the radial direction as stated, and the other is the translocation with the transpiration stream in xylem tissues. Whether TCE can translocate to the leaves depends on the comparative rates of the two processes. The transport time to the leaves is a function of the tree height divided by the translocation velocity. The velocity can be estimated from the amount of water transpired by plants and the cross section of the tree stem. This results in transport time being a direct function of height and water transpiration and inverse function of stem diameter. The diffusion process is considered to be following Fick's first law. The diffusion time scale is a direct function of the diffusion path length, in this case the radial distance from the transpiration flow (xylem) to the atmosphere, which corresponds to the radius of the stem. A comparison of the lab and field plants showed that the height of trees sampled in the field is over 50 times greater than the laboratory-scale trees while the radius of trees in the field is roughly 10 times of that of cuttings used in the lab. In addition, the xylem flow in mature hardwood trees is heavily weighted toward the distal or outer regions of the xylem, and the outer 4 cm dominates the sap flux density (*17*), effectively making the diffusion path shorter. This comparison indicates that the diffusion process should be more dominant in the field, relative to the laboratory, when considering fates: diffusion to atmosphere versus transport to the leaves. This hypothesis is supported by the observed results presented in Figures 6 and 7. Efforts are ongoing to generate a comprehensive model for the transport and diffusion processes. Hypothesis 2 is that an artifact of the laboratory arrangement impacts TCE transport based upon the confined nature of the reactors (Figure 1). As vapor-phase TCE is confined in the headspace, diffusion from the stem below the septum is limited. In fact, diffusion into the stem is possible. Therefore the concentration decline in subsurface stem and roots observed in the field (Figure 6) is not expected in the laboratory arrangement as diffusion from the subsurface tissues is likely eliminated. The confined TCE vapor phase may increase TCE concentration in the stem below the barrier provided by the septum of the reactor. While the reactors' artifact may alter the TCE concentration at and below the septum, it does not alter the processes above the septum in anyway. This hypothesis shares a scientific basis with hypothesis 1 in that diffusion can occur below ground, thereby increasing the likelihood of VOC diffusion out of xylem tissues before reaching the leaves in a field setting. This hypothesis has bearing in that the higher concentrations used in the lab and confined nature of the reactor could cause a yet undiscovered physiological response, increasing diffusion. While some laboratory concentrations were higher (820 and 550 ppm in soil experiments), some concentrations at the APG site were higher (260 ppm total Cl VOCs) than the majority of exposure concentrations in the laboratory. No supporting evidence was discovered to support this hypothesis, but no hypotheses were ruled out.

Care must be taken regarding interpreting field samples and extrapolation of laboratory results to field settings without identifying inherent differences. For large trees, roots from different radial directions of the tree can collect water with highly variable contaminant concentrations, and thus different contaminant profiles for different sides of the same tree can be observed. In addition, there can be large variations and heterogeneities in the xylem flow for large trees, depending on the arrangement of tracheids and vessels of different species. Five different patterns of transpiration movement in xylems have been identified (*18*). The extrapolation is further complicated by xylem flow being impacted by injuries of plants, canopy closure, and even incident light direction. Additionally, as is clearly shown in this work, relative fates can be quite different given variable scales of processes (ratio of height to stem diameter) that are inherently different in lab and field settings.

This study confirmed the decrease of TCE concentration in the transpiration stream with height both in the field and in the laboratory. The concentration decline results from TCE diffusion along the transpiration pathway to the atmosphere. The TCE mass diffusion rates and the TCE levels in the xylem tissues and transpiration stream correspond linearly to the concentration of feed solution, although transpiration rates also impacted diffusion rates. This diffusion hypothesis is supported at the mechanistic level by presence of a radial concentration gradient. The collection of TCE as it diffuses from the stem substantiates the theory.

TCE accumulation in tissues was minor as compared to the total mass delivered to laboratory reactors, which agrees with previous findings (*9*, *19*); however, complete mass balances were not attempted. TCE is taken up by plants and volatilized to the atmosphere. TCE volatilization from stems via diffusion to the atmosphere constitutes a major fate of TCE after uptake by plants and is an important mechanism for TCE removal in phytoremediation applications.

Acknowledgments

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