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Biophysical Mechanisms of Trichloroethene Uptake and Loss in Baldcypress Growing in Shallow Contaminated Groundwater

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Wetland vegetation may be useful in the remediation of shallow contaminated aquifers. Mesocosm experiments were conducted to describe the regulatory mechanisms affecting trichloroethene (TCE) removal rates from groundwater by flood-adapted wetland trees at a contaminated site. TCE flux through baldcypress [*Taxodium distichum* (L.) Rich] seedlings grown in glass-carboys decreased from day to night and from August to December. The diel fluctuation coincided with changes in leaf-level physiology, as the daytime flux was significantly correlated with net photosynthesis but not with respiration at night. A decrease in seedling water use from summer to winter explained the large seasonal difference in TCE flux. A simple model that simulates gas-phase diffusion through aerenchyma tested the importance of diffusion of TCE vapor from roots to the stem. The modeled diffusive flux was within 64% of the observed value during the winter but could only explain 8% of the summer flux. Seedling water use was a good estimator of flux during the summer. Hence, evapotranspiration (ET) in the summer may serve as a good predictor for the potential of TCE removal by baldcypress trees, while diffusive flux may better approximate potential contaminant loss in the winter.

Introduction

Phytoremediation has received considerable attention as a mechanism of groundwater contaminant removal owing to its relative cost effectiveness, low maintenance, and superior aesthetics compared to more traditional industrial methods of waste remediation. Plants have been shown to affect contaminant removal in three primary ways: (1) enhancement of microbially mediated contaminant degradation (bioremediation) by supplementing the soil with factors that are limiting to the growth of soil bacteria (e.g., labile organic compounds and/or oxygen); (2) intra- or extracellular enzyme catalysis of contaminants within the rhizosphere and/or shoot tissue; and (3) mass transport through root and shoot systems accompanied by adsorption to the tissue and/or volatilization to the atmosphere (1–4). Past studies have concentrated on the effect that vegetation has on the biochemical mechanisms

of contaminant removal through the study of microbial and plant-based metabolic/enzymatic reactions. In comparison, the more biophysical mechanisms of remediation have received less attention.

The significance of biophysical mass transport as a phytoremediation mechanism is dependent upon the physiochemical properties of the targeted contaminant. Certain volatile organic contaminants (VOCs) that have the appropriate *n*-octanol water partition coefficient, such as TCE [$\log K_{ow} = 2.53$ (5)], for example, can traverse root cell membranes and be transported through the plant water conduction system during transpiration (6, 7).

Another biophysical mechanism for mass transport that may be important for VOCs is diffusive transport through the air space of root and shoot tissue. The same pathway that transports atmospheric oxygen to root systems in flood-adapted wetland species also can act as a conduit for transport of soil gases to the atmosphere (8). As a mechanism of methane transport, for example, flux through wetland plants can account for up to 95% of total gas emission in anaerobic, waterlogged sediment (9).

The root systems of flood-adapted woody plants such as baldcypress [*Taxodium distichum* (L.) Rich], tupelo (*Nyssa aquatica* L.), and willow (*Salix nigra* Marsh.) are aerated by gas exchange through the stomata and stem lenticels (10). Pertinent to VOC removal, flood-adapted species have greater root porosity, and thus, greater capacities to aerate their rhizosphere than do flood-intolerant species (11). This implies that a diffusion-based mechanism of contaminant removal may have greater importance in flood-adapted wetland species relative to other VOC removal mechanisms.

With this in mind, wetland trees may prove useful for the remediation of shallow contaminated aquifers. The potential for contaminant transport through flood-adapted trees was investigated previously at a flood plain site near the TNX area of the Savannah River Site, SC (12). The investigation showed that chlorinated ethenes were present in the trunks of baldcypress trees growing above shallow groundwater containing the same contaminants (12). Our objective in this earlier study was to measure the temporal and spatial distribution of contaminants within and among trees at the site (12). In this paper, we present data that describe the biophysical mechanisms affecting contaminant removal rates from groundwater. We hypothesized that trees removed contaminants by mass transport during transpiration and/or by gas-phase diffusion through aeration tissue. To test the relative importance of these pathways, we conducted trichloroethene (TCE) gas flux and loss experiments seasonally on baldcypress seedlings grown in glass-carboy mesocosms.

Materials and Methods

Forty-five bareroot baldcypress seedlings were planted in both 5 gallon and 2.8 gallon glass-carboy mesocosms (Figure 1) that were kept outside under natural light. Seedlings were planted in a gravel/sand substrate, and the carboy water level was maintained above the substrate surface throughout the experiment. Nutrients were added monthly to the carboy water to approximate 1/4 strength Hoagland's solution (13). By the end of the 1997 growing season, the seedlings had reached an average height of 1.5 m and average stem diameter of 2.5 cm.

Transpiration (Water Use). Constant-head or Mariotte bottles were attached to the carboys to maintain a constant water level and to measure seedling water use (14) (see Figure 1). The rate of water flow from the Mariotte bottle to the

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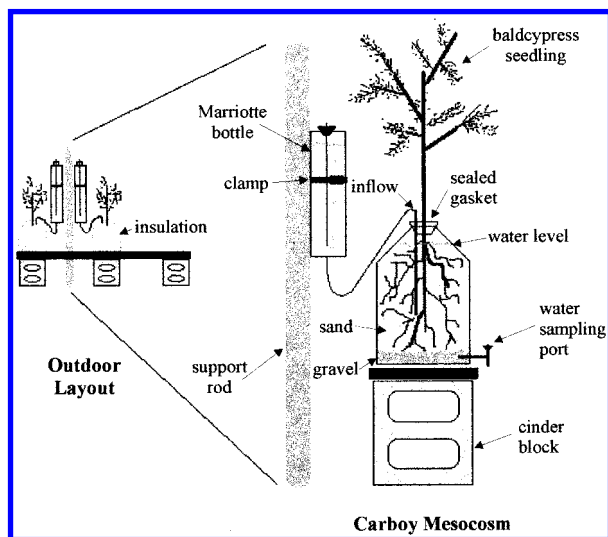


FIGURE 1. Schematic of the experimental carboy mesocosm with baldcypress seedling and layout at the outdoor facility.

carboy is driven by the change in hydrostatic pressure in the carboy. In this manner, water from the Mariotte bottle exactly replaces water lost from the carboy by transpiration. Seedling water use was monitored throughout the experiment from June to December.

Through-Plant TCE Flux. TCE flux experiments were conducted from August to December. The September flux measurements were excluded due to hardware malfunction. Flux experiments ($n = 6$ seedlings/month) were initiated by the addition of 200–300 μL of pure liquid TCE to small and large carboys, respectively, resulting in dissolved concentrations that ranged 60–100 μM . The space between the neck of the carboy and the trunk of the seedling was sealed with closed cell neoprene and silicone glue. Controls (four replicates) consisted of carboys with substrate, but in place of the seedling, a wooden dowel rod was sealed within the opening at the top of the carboy.

Through-plant TCE flux was determined by a static chamber technique. Over each mesocosm, we sealed an airtight, clear polycarbonate (Lexan) chamber (80 L). A sensor head mounted on the top of the chamber monitored photosynthetically active radiation and chamber temperature. Two 12-volt fans mixed the chamber headspace by drawing air from the top and blowing it across a radiator at the bottom, through which chilled water was circulated, to maintain ambient temperature. TCE was measured by drawing a sample of chamber air into a 100 μL glass syringe followed by immediate injection into the GC (Photovac 10Splus with a photoionization detection system) on site. The chamber air was sampled every 10 min for a 30 min time series. The flux was computed from the slope of the linear regression of TCE concentration vs time. Sorption of TCE onto the chamber walls was assumed to be negligible. Typically, TCE flux through each of the dosed seedlings was measured four separate times: twice during the day and following night, on consecutive days.

To correlate contaminant transport with plant physiology, seedling CO_2 exchange and TCE flux were measured concurrently. CO_2 gas concentration was quantified by pumping a fraction of the chamber headspace through an infrared gas analyzer (Licor 6200 portable photosynthesis system, Lincoln, Nebraska) in a closed system. The analyzer logged CO_2 concentration every 3 s over a 4 min sampling interval during the day between 1200 and 1600 h, and at night between 2000 and 0300 h, depending on the season. The CO_2 flux was computed by determining the slope of CO_2 concentration vs time.

TCE Loss from Carboy Water. Experiments designed to determine the loss rate of TCE from the water/root/substrate solution (referred to as carboy water) were conducted on separate mesocosms in the winter (November to February) and summer (June to August) on live seedlings. TCE loss from carboy water in mesocosms containing dead, intact seedlings was determined in the summer as well. In these experiments, TCE-saturated water was added to previously drained carboys. Controls (five replicates) consisted of carboys with the severed stem of a seedling sealed within the neck of the carboy. The stem sections were 15 cm long and were placed above the water surface in the carboy. Approximately 8 cm protruded out of the carboy exposed to ambient conditions.

TCE concentration in the carboy water was sampled weekly (summer) to biweekly (winter) by collecting water from the outflow valve at the base of the carboy with a glass syringe. A total of 0.5 mL of water was injected into a 10 mL headspace vial, sealed immediately with a Teflon-faced butyl stopper, and crimped. Samples were stored at 5 $^{\circ}\text{C}$ until the experiment was completed. All samples from a given experiment were analyzed on the same date by GC analysis of the headspace gas. TCE concentration in the sampled water was back-calculated using Henry's law and a partition coefficient of 0.007 $\text{m}^3 \text{ atm mol}^{-1}$ (15). Loss rate constants (m) were computed for each experimental series by fitting an exponential model ($C = be^{-mt}$) to the observed concentrations (C), where b is the intercept and t is time.

TCE in Plant Tissue. To determine the distribution of contaminant in plant tissue, samples of stems and roots were collected. In the seedlings, samples were collected following the experiments on TCE loss from carboy water. Roots were washed with tap water to remove sand, sealed in serum vials, heated overnight at 40 $^{\circ}\text{C}$, and analyzed the following day for TCE by GC. These were compared with samples of stem tissue collected from the base of the seedlings and of wood collected from the trunk of a mature baldcypress tree growing within the chlorinated solvent contaminant plume at the field site from July to January 1997–98. The shoot tissue was collected with an incremental corer.

Data Analysis. Statistics were performed with the SAS software package (SAS Institute Inc., Cary, NC). Analysis of variance tests were performed (PROC GLM) on log-normalized values to evaluate differences in diel and monthly measurements of TCE flux and CO_2 exchange. Unless otherwise noted, data summaries give the arithmetic mean and 95% confidence interval. Means with overlapping error bars indicate no significant difference at $p = 0.05$ level.

Results and Discussion

Field Measurements. Temporal differences may be important to consider when the potential for phytoremediation is assessed during a cost analysis of a contaminant removal project. A simple test for temporal differences in TCE removal at the field site was conducted by collecting tree cores at the base of a mature baldcypress tree growing within the chlorinated solvent plume. There was a significant decrease in the TCE contained in these cores from summer to winter (data not shown). Possible reasons for the observed seasonal decline of TCE in the mature tree include a corresponding summer to winter decrease in the contaminant concentration in the shallow groundwater, a decrease in the rate of uptake of contaminated groundwater, or increase in the rate of contaminant loss from the woody tissue. The results of the seedling experiments that follow help to clarify which mechanism is responsible for seasonal variations in TCE concentration.

Mesocosm Experiments. The mesocosm experiments showed a strong trend in both the diel ($p = 0.0757$) and monthly ($p = 0.0022$) measurements of TCE flux from the

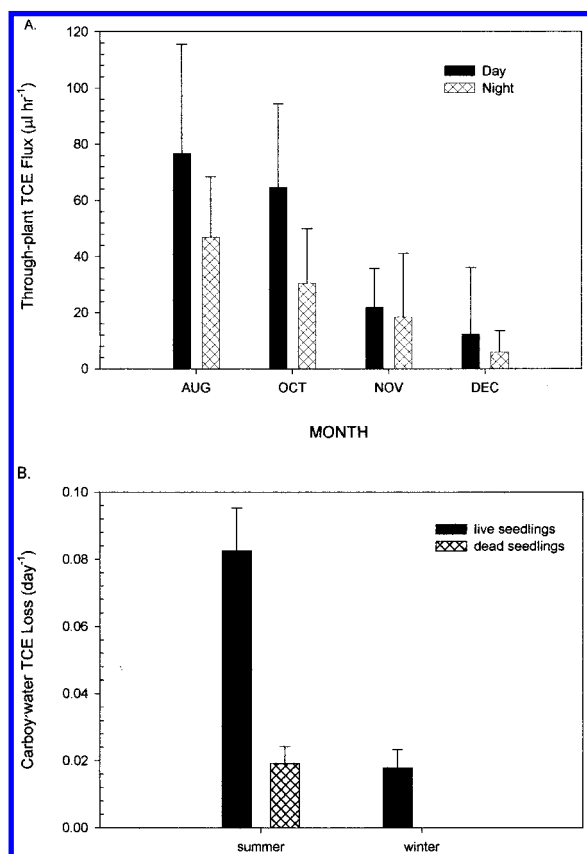


FIGURE 2. TCE removal from the rooting zone of baldcypress seedlings. (A) TCE flux ($\mu\text{L h}^{-1}$) through the aboveground portion of the plant. (B) Mean fractional TCE loss from carboy water during the summer and winter. Means for the parameter estimate (m) were computed from individual determinations of loss rate after fitting an exponential model to each time series ($C = be^{-mt}$).

aboveground portion of baldcypress seedlings (Figure 2a). Through-plant TCE fluxes were significantly greater in the summer and early fall (August and October) compared to the early winter months, and there was a tendency toward higher fluxes during the day. These data provide direct evidence for TCE transport from contaminated water in the rooting zone to the atmosphere through the plant.

Independent measurements of TCE loss from carboy water corroborate the summer to winter difference in removal rate with a greater loss rate in the summer experiment (Figure 2b). There was no significant TCE loss from control carboys, measured both with the static chamber and by monitoring carboy water TCE concentrations over a 40-day period. Using a mass balance approach, the estimated through-plant TCE flux based on the loss rates measured from carboy water (Figure 2b) agreed reasonably well with the observed flux (static chamber flux, Table 1), suggesting that the majority of contaminant removal occurred by mass transport to the atmosphere rather than by biodegradation.

TCE Uptake and Loss as a Function of Water Use. Diel and seasonal variation in plant water conduction can explain the dynamics of TCE removal from the mesocosms. TCE dissolved in water flowing through the symplast of roots can be transported across the endodermis that separates the root cortex from the stele, and, thus, loaded into the network of xylem cells during transpiration (2). In this way, TCE in groundwater becomes part of the soil-plant-atmosphere continuum. A recent study confirmed this mode of transport for TCE in hybrid poplar (4).

The rate of transpiration is largely controlled by the opening and closing of leaf stomata that respond to a gradient

of water potential between the soil and the atmosphere (see ref 16 for review). TCE dissolved in the xylem water can vaporize along with liquid water at the surfaces of leaf mesophyll cells and freely diffuse through the stomata while they are open during the day. At night, when the stomata are closed to conserve water, vapor losses across the leaf surface are much slower because the waxy cuticle of the leaf epidermis hampers diffusion. The close link between TCE transport in trees and rate of water transport strongly suggest that stomatal activity is a probable control for the diel change in contaminant flux.

Although we did not measure stomatal conductance directly, measurements of CO_2 exchange were made concurrently with the TCE flux determinations. The net uptake of CO_2 (net photosynthesis) by leaves during the day was positively correlated with TCE flux normalized for carboy TCE concentrations (Figure 3a). However, there was no relationship between CO_2 production (respiration) and TCE flux at night (Figure 3b). This supports a model of stomatal control of TCE vapor flux.

Specific water use, or average water use normalized for seedling cross-sectional area, declined over the course of the experiment from June to December (Figure 4). The decline in water use coincides with the large decrease in through-plant TCE flux from summer to winter (Figure 2a).

Through-plant TCE flux was estimated from measurements of water use by assuming that all contaminant transport occurred in the bulk flow of water through the plant. This estimate agreed with TCE flux determined by static chamber measurements in August (Table 1), indicating that transpirative contaminant transport was a significant TCE removal mechanism. Water use during the winter, however, was near zero, and we also measured significant loss from dead seedlings in the summer (Figure 2b). The lack of correlation between water use data and TCE flux during decreased transpiration in the winter implies that trees do not need to be conducting significant amounts of water to remove TCE from groundwater.

Diffusive Transport. To explain TCE removal under conditions of near-zero transpiration, the potential for gas-phase diffusion through internal air spaces in the roots and stems was tested. Previous investigations have shown that such a transport mechanism exists for oxygen and methane in wetland plant species. Wetland plants enhance root aeration by a network of air spaces in the roots and shoots, called aerenchyma tissue (17). In woody species oxygen enters the plant through stem lenticels and diffuses along a concentration gradient downward to roots (18). Buttress formation in baldcypress is thought to be a mechanism for increasing the surface area for aeration through lenticels concentrated at the base of the trunk (18). This pathway for internal aeration also can serve as a mode of transport for soil gases to the atmosphere that is faster than diffusion through a saturated soil column (19). Hence, the plant conduit for soil gas emission could be an important factor in the removal rate of VOCs in contact with the rhizosphere. The observed decrease in tissue TCE from roots to shoots in the seedlings (Table 1) and contaminant loss under conditions of near-zero transpiration is consistent with diffusion-based transport.

Internal aeration in baldcypress seedlings was simulated using a simple model of oxygen diffusion in roots (10), modified for TCE (Table 2) (1) to test if model-simulated diffusion was consistent with direct measurements of contaminant flux and (2) to verify that the diel and seasonal differences in TCE flux through the seedlings could not be explained by temperature control of both liquid-gas partitioning and the diffusion constant. Models of gas diffusion in herbaceous species take into account both axial and radial transport along the shoot (e.g., ref 20), but the mechanisms

TABLE 1. Comparison among Observed, Estimated, and Modeled TCE Removal by Baldcypress Seedlings^a

measurement series	condition	TCE concentration				through-plant TCE flux ($\mu\text{L}\cdot\text{h}^{-1}$)							
		initial carboy (μM)	final carboy (μM)	roots (nmol g^{-1})	stem (nmol g^{-1})	mean temp ($^{\circ}\text{C}$)	measured water use ($\text{cm}^3\text{ h}^{-1}$)	simulated carboy temp ($^{\circ}\text{C}$)	determined by static chamber	estimated from [TCE] decline in carboy water	estimated from measured water use	calculated from diffusion model	
		Through-Plant Flux Experiments											
August-97	day	79				34	30	26.9	76.63 (17.19)		37.78	56.54	6.48
	night	83				18	24.6	25.1	46.96 (8.79)		39.60	48.62	5.76
October-97	day	59				22	7.6	17.9	64.66 (13.48)		28.13	10.70	2.59
	night	61				13	7.2	17	30.51 (8.42)		29.04	10.37	2.50
November-97	day	63				16	3.8	12.5	21.87 (6.21)		7.54	5.76	1.63
	night	70				8	3.9	11.6	18.35 (7.17)		8.35	6.58	1.97
December-97	day	87				21	0.47	18.5	12.26 (8.59)		10.42	0.96	4.03
	night	91				14	0.34	17.6	5.99 (2.38)		10.75	0.72	3.84
Water Loss Experiments													
summer-dead-98		77	44	11.8 (4.8)	7.5 (3.0)	0					8.78	0.00	6.24
summer-live-98		80	13	2.7 (2.4)	0.7 (0.6)								
winter-live-97/98		60	32	10.0 (1.0)	4.4 (2.1)								

^a Root and stem tissue TCE is based on grams fresh weight. Numbers in parentheses are the standard error of the mean.

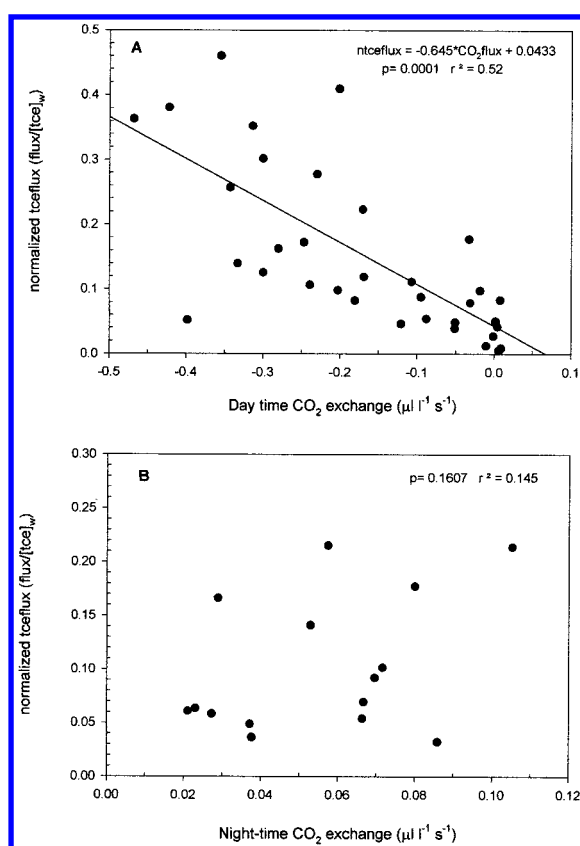


FIGURE 3. Correlation between CO_2 exchange (negative values represent net CO_2 uptake) and TCE flux from seedlings ($\text{nL}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$) normalized for carboy TCE concentration (μM). (A) Measurements made in the light. (B) Night time observations.

for diffusion in woody plants are ill described. Our model makes the simplifying assumptions that diffusion from roots to shoots is controlled by the resistance imparted by a tortuous path along root cells, a fractional porosity of the root system (10), and the stem cross-sectional area. Table 1 contains the results of the modeled diffusive flux compared to the measured static chamber through-plant flux and the estimates of TCE through-plant flux, calculated from the TCE loss rate from carboy water (Figure 2b) and the average monthly water use data (Figure 4).

The model-simulated diffusive flux could account for 8–64% of the observed flux as ambient temperatures

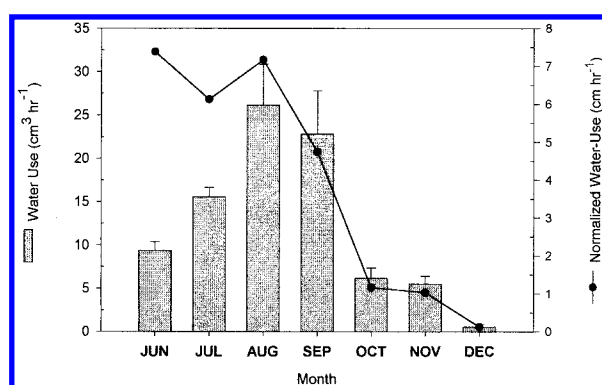


FIGURE 4. Average monthly water use by seedlings as measured by Mariotte bottles. The normalized water use takes into account seedling growth by taking the average monthly water use divided by the mean stem basal area of all healthy seedlings.

decreased from August to December. The modeled day/night difference in TCE flux that could be attributed to the effect of temperature on gas partitioning and the diffusion coefficient could not explain the observed diel variation (Table 1). This is further evidence that the diel variation in summer TCE flux is a function of a change in transpiration rate, regulated by leaf-level physiology.

The modeled diffusion of TCE from roots to the stem in the summer approximated the flux estimated from the loss of TCE from carboys with dead, intact seedlings (Table 1). The close agreement between these estimates is probably due to the fact that the model assumes that gas diffusion occurs across the entire cross-sectional area of the stem. This assumption is more realistic for dead seedlings that lack a living endodermis, since dead cells within the stele would increase the area for gas diffusion. The results from the modeling exercise imply that diffusion occurs regardless of the physiological state of the plant, and that under conditions of near-zero transpiration, a simple diffusion model can provide a reasonably accurate approximation of contaminant transport through the seedlings.

Implications for Contaminant Loss at the Field Site. Results from this study (Table 1) indicate that the measured TCE flux through seedlings during the summer could be approximated by the product of the transpiration rate and TCE concentration in the rooting zone, while diffusion served as a reasonable predictor of contaminant transport during periods of dormancy. Significant uncertainties are involved in scaling up from seedlings to mature trees. However, if

TABLE 2. Diffusion Model for TCE Flux through Internal Air Spaces

- i. Diffusion (
- D_f
-) of TCE (
- $\mu\text{mol s}^{-1}$
-) from roots to the atmosphere:

$$D_f = \frac{1}{r_d} (C_r - C_a)$$

 C_r = TCE concentration in the gas phase of roots C_a = TCE concentration in the atmosphere

- ii.
- r_d
- = The non-metabolic resistance of roots (11):

$$r_d = \frac{L}{TED_{\text{corr}}A}$$

L = diffusive path length;

30 cm

T = tortuosity factor (23);

0.75

E = fractional porosity of root segment (24);

0.15

A = stem cross-sectional area;

4 cm²

- iii.
- D_{corr}
- = vapor diffusivity corrected for temperature:

$$D_{\text{corr}} = D \left(\frac{T_A}{293} \right)^{1.75}$$

 D = TCE vapor diffusion coefficient^ψ;0.096 cm² s⁻¹ T_A = ambient temperature (°K)

- iv. Gas phase TCE in roots (
- C_r
-)*:

$$C_r = \frac{TCE_w}{(1/k_h) \times RT_s}$$

 TCE_w = TCE concentration in Carboy water

R = universal gas constant

- v.
- k_h
- = Henry's law constant adjusted for temperature (15):

$$k_h = e^{-5594 \left(\frac{1}{T_s} \right) + 14.244}$$

- vi.
- T_s
- = sinusoidal approximation of carboy substrate temperature (25):

$$T_s = \bar{T}_A + \Delta T_A e^{\frac{-z}{d}} \cos \left(\frac{2\pi}{p} - \frac{z}{d} \right)$$

 \bar{T}_A = ambient temperature (°C)

Z = depth;

30 cm

t = time (hrs)

- vii. d = damping depth:

$$d = \left(\frac{pK}{\pi H_c} \right)^{1/2}$$

P = period;

24 hrs

 K = thermal conductivity coefficient for wet soil (26);2160 J hr⁻¹ m⁻¹ °C⁻¹ H_c = volumetric heat capacity for a moist sandy loam (26);2 × 10⁶ J m⁻³ °C⁻¹

^ψ Four orders of magnitude greater than the solution diffusivity (27) as suggested (28). * We have assumed that the resistance to TCE flux into the gas space of roots is negligible.

TCE loss in the mature forest is proportional to transpiration rate, as in the mesocosm experiments, then the potential annual removal of TCE by flood plain trees can be conservatively estimated using the lowest reported value for evapotranspiration (ET) (76 cm year⁻¹) of forests within the vicinity of the contaminated area (21). Assuming that the median (1800 nmol L⁻¹) of the range of measured TCE concentrations in groundwater below the contaminated flood plain (12) is representative of average rhizospheric values, an estimate for potential TCE flux through trees, based on annual ET, would be 13.5 mol of TCE ha⁻¹ year⁻¹. An hourly estimate of TCE flux through trees based on weekly average potential ET values reported for July in an area dominated by evergreen pine trees near the field site [0.023 cm h⁻¹ (22)] would be 4.1 mmol of TCE ha⁻¹ h⁻¹.

There was reasonable agreement between the model-simulated diffusive flux of TCE and the measured TCE flux through seedlings under conditions of near-zero transpiration. To gain an impression for the potential importance of diffusive transport of TCE through trees at the field site throughout the year, we adjusted the diffusion model (Table 2) for seedlings to mature trees based on a diffusive path length of 3 m, the distance from the rooting zone (1.5 m) to the top of the buttress on trees growing at the field site, and a cross-sectional area equivalent to the average basal area per hectare (28 m² ha⁻¹) reported for a typical southeastern flood plain forest (17). The modeled result was a potential hourly flux that decreased from 0.032 to 0.019 mmol of TCE ha⁻¹ h⁻¹ as temperatures decreased from summer to winter. Therefore, potential TCE flux due to diffusion is 2 orders of magnitude lower than the estimated flux due to transpiration in the summertime. Integrated over the year, diffusion could account for only 1% of the potential contaminant removal. Hence, our data imply that ET-based flux of TCE-contaminated groundwater from the rooting zone through trees is

the major pathway for contaminant removal and will be important in the field if ET represents a significant proportion of the water budget at a site.

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