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Eucalyptus urograndis and *Pinus taeda* enhance removal of chlorobenzene and benzene in sand culture: A greenhouse study

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ABSTRACT

Contamination of soils and groundwater by chlorobenzene and benzene is a common problem at industrial sites worldwide. Since chemical remediation techniques are rarely completely effective, remnants of these contaminants often persist at levels that can still influence ecosystem health. We evaluated the potential of *Pinus taeda* and *Eucalyptus urograndis* to accelerate the removal of these compounds from sand/water systems using a completely randomized block greenhouse experiment with a no-plant control. At 2-day intervals, we added a solution containing both chlorobenzene and benzene with the same concentration of 50 mg L^{-1} (25 mg pot^{-1}), and we monitored leachate concentrations daily. The planted treatments showed greater decrease of contaminants over time. In the absence of plants, the contaminant mass decreased 50–60% during each 2-day cycle; whereas, in the planted treatments the contaminant mass decreased 91–98%. At the end of the experiment the plant roots, leaves, and the sand-substrate each contained less than 1 mg kg^{-1} of contaminants, which is ~1% of the total contaminant mass added. In addition, we observed no tree mortality even at concentrations exceeding the aqueous solubility limit of both compounds. Our results suggest both trees are good candidates for remediating chlorobenzene and benzene in soils and groundwater.

KEYWORDS

Organic contaminants; phytoremediation; volatilization

Introduction

Benzene-based compounds (*e.g.*, chlorobenzene and benzene) are used widely as precursors for synthesis of pesticides and industrial chemicals, and are common soil and groundwater pollutants from industrial spills (Ellis and Rivett 2007). When present in water bodies at high concentrations, *i.e.*, exceeding 2 or 0.1 mg L^{-1} for chlorobenzene and benzene, respectively, these pollutants pose risks to human and animal health (US EPA 2000, 2013) and thus, many approaches exist for removing them from environmental systems. The most commonly applied technologies (*e.g.*, soil excavation, washing/burning, or groundwater pump-and-treat) capitalize on the high vapor pressures of these volatile organic compounds (VOC) (Pilon-Smits 2005). These remediation options can be expensive and difficult to implement (Seeger *et al.* 2011) leading many to propose alternative methods using plants (*e.g.*, phytoremediation) to accelerate volatilization and potentially promote degradation of these and similar VOCs either within the plant itself or in the plant rhizosphere (Arthur *et al.* 2005; Graziani *et al.* 2016). Phytoremediation projects often cost only 25–40% of the total cost of conventional remediation techniques (Green and Hoffnagle 2004) and can either be used alone, or in conjunction with other remediation methods (*e.g., in situ* chemical oxidation and/or reduction) to remediate contaminant residuals (*e.g.*, as a polishing process).

Plants may enhance chlorobenzene (CB) and benzene (BZ) remediation by promoting degradation in the rhizosphere (rhizodegradation), or by absorbing and either volatilizing the compounds (phytovolatilization), or by degrading them within the plant (phytodegradation) (Yifru and Nzengung 2008). Phytoremediation of CB and BZ has been documented in studies using, for instance, hybrid poplars (*Populus deltoides*) (Burken and Schnoor 1998), willows trees (*Salix* sp.) (Nzengung 2005), *Phragmites australis* (Braeckevelt *et al.* 2008), and *Juncus effusus* (Braeckevelt *et al.* 2011). However, no data exist on the potential for phytoremediation of CB and BZ using plant species that thrive at lower latitude (subtropical to tropical) environments, principally *Eucalyptus* sp. and/or *Pinus* sp. These two species are the most traditional and commonly used trees for forest plantations in tropical and subtropical regions, and *Pinus* sp. in temperate and boreal plantations (FAO 2000). These two globally widespread tree species can survive and grow rapidly on a variety of sites (Lamprecht 1990), including compacted and nutrient-limited sites.

A key factor in successful phytoremediation of VOCs is selecting plants that can survive exposure to the pollutants (ITRC 2009) and also have high root mass and evapotranspiration (ET) rates (Schnoor 1997). The tree species (poplar and willow) used previously for phytoremediation of CB and BZ have evapotranspiration varying between 0.6 and 3.8 mm day^{-1} (Guidi *et al.* 2008). Meanwhile, in a (sub)tropical environment, evapotranspiration demands are often higher and thus, phytoremediation could be more feasible. Evapotranspiration rates of mature *Eucalyptus* sp. and *Pinus*

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sp. are between 3.0 and 10.0 mm day^{-1} (Dye 1987; Facco 2004; Sacramento Neto 2001), and 1.5 and 6.5 mm day^{-1} (Samuelson *et al.* 2008; Gonzalez-Benecke and Martin 2010), respectively. If these plant species can survive exposure to VOCs, then they are likely good candidates for phytoremediation in (sub)tropical environments.

Many contaminated industrial sites have disturbed soil and subsoils (Yong 2000). Grading during development, continual trafficking by heavy industrial equipment, and paving tend to create biogeochemical conditions that differ substantially from natural soils. These industrial sites can also be partially covered by impermeable asphalt and concrete surfaces (*i.e.*, hardscape) that restrict natural water and gas exchange. Complete removal of this hardscape may be impractical both from a cost standpoint and because it may mobilize contaminants (Hall and Odle 2004). In these situations, the role of plants and their ability to extend their root systems takes on greater significance.

Therefore, we conducted research experiments structured around two questions: (1) Can *Eucalyptus sp.* and *Pinus sp.* survive when exposed to high concentrations of benzene-based pollutants? (2) Can they enhance removal of chlorobenzene and benzene from contaminated soils? We addressed the first question by exposing only *Pinus taeda* seedlings to a wide range of chlorobenzene/benzene concentrations for 30 days. We addressed the second question by repeatedly exposing *Eucalyptus urograndis* and *P. taeda* (and a no-plant control) to chlorobenzene and benzene, and monitoring the loss of these contaminants in a randomized block designed greenhouse experiment. To isolate the effects of planted trees on contaminant remediation, we planted the trees into a sand matrix with low organic matter and limited sorption capacity.

Materials and methods

Experiment 1: Survival of P. taeda exposed to chlorobenzene and benzene

This feasibility study helped us design a more complete greenhouse study (Experiment 2), and to determine an upper bound for contaminant toxicity on the seedlings. We used only pine seedlings for this initial study. *P. taeda* seedlings (provided by Georgia Pacific Inc., Albany, Georgia) were potted in small cones of PVC (20 cm high by 4.5 cm in diameter), containing an unfertilized commercial soil mix (Fafard 3M) and placed in a fume hood with two rows of fluorescent light (Figure S1). The plants were arranged in a randomized block design with four-replicates of five concentration treatments based on nominal concentrations present in groundwater at a known contaminated site in southern Brazil, corresponding to 100 mg L^{-1} for chlorobenzene (CB) and 50 mg L^{-1} for benzene (BZ), which is the treatment 1 × *T* (Table 1). The five treatments (0 or tap-water control, 0.1 × *T*, 1 × *T*, 5 × *T*, and 40 × *T*) were irrigated every 2 days for 30 days with 50 mL of each solution or tap water (Table 1). The 40 × *T* treatment exceeded the compounds solubility (500 and 1,800 mg L^{-1} for chlorobenzene and benzene, respectively) and, thus, the irrigated solution for this treatment comprised an emulsified dissolved and non-aqueous phase.

To assess the impact of the contaminant treatments we measured seedling height weekly and monitored the plants for changes in physiology and signs of mortality daily. The relative growth height was compared using a one-way ANOVA test performed in SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

Experiment 2: Phytoremediation of VOCs in greenhouse

Experimental design and plant establishment

The phytoremediation experiment was conducted in a greenhouse at the University of Georgia (Whitehall Forest, Athens, GA, USA), with temperature maintained at 25 ± 3°C and relative humidity at 70 ± 5%, from August to December of 2012. We chose *E. urograndis* and *P. taeda* as candidate trees with high growth rates that can thrive in tropical and subtropical (eucalyptus) or subtropical and temperate (pine) regions. *E. urograndis* seedlings were obtained from Arborgen Inc. (Summerville, South Carolina) and *P. taeda* seedlings from Georgia Pacific Inc. (Albany, Georgia). Seedlings were placed in a randomized block design replicated in four complete blocks to account for any variations within greenhouse conditions (Hammer and Douglas 1997). The dosing solution was an aqueous mix containing both chlorobenzene and benzene at 50 mg L^{-1} each (we just used one concentration). Treatments were a factorial combination of three plant conditions (eucalyptus, pine, and no plant) and two contaminant conditions (contaminant solution or water); thus, there were six treatments (three with contaminated solution and three no-dosed controls) for a total of 24 pots ($n = 4$ reps).

The seedlings were planted in 40-cm-tall (15 cm I.D.) PVC columns filled with washed sand. Each column was capped at the bottom and linked through a bulkhead to an outlet valve and a clear PVC water head control pipe (1.25 cm I.D.) set at 32 cm below of the surface of the sand (Figures 1 and 2). The control pipe (or loading port) was plugged with a stopper covered by aluminum foil to minimize volatilization of the VOC compounds. This design simulated the conditions where plant roots would be in contact with contaminants at a field site.

Table 1. Treatments and concentrations used to test survivorship of *Pinus taeda*. Seedlings exposed to both chlorobenzene and benzene in the root zone.

Compound	Concentrations in different reactors					Solubility in water at 20°C	Density
	Control	0.1 × *T*	1 × *T*	5 × *T*	40 × *T*		
	mg L^{-1}					mg L^{-1}	g mL^{-1}
Chlorobenzene	0	10	100	500	1,000[†]	500	1.11
Benzene	0	5	50	250	2,000	1,800	0.88

[†]Concentration is 10 times the nominal concentration (1 × *T*) instead of 40×.

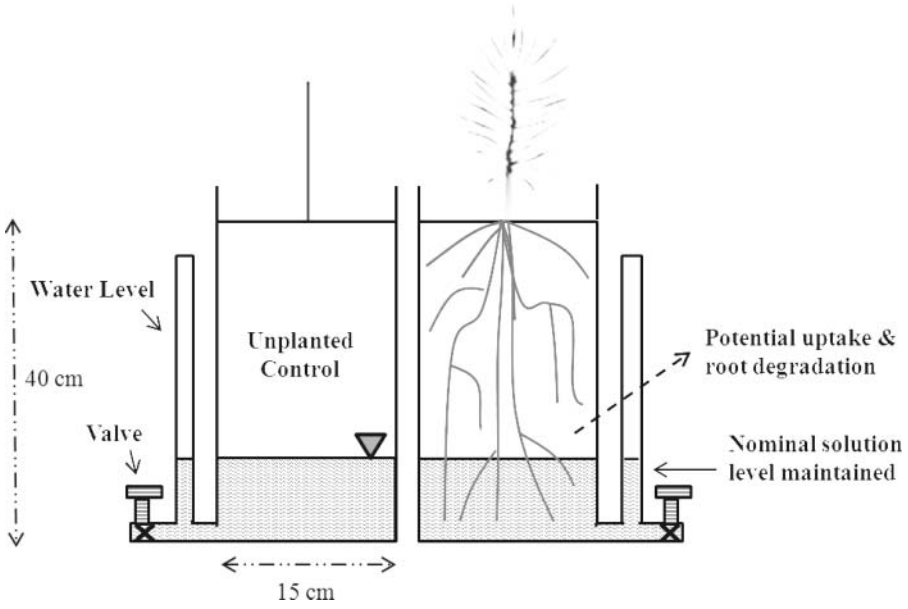

Figure 1. Schematic of unplanted (control) and planted pots in the greenhouse (sand substrate).

Thus, the contaminant solution moved up into the root zone through a combination of capillary rise and (evapo) transpiration.

The pots containing planted and unplanted treatments were all fertilized with Hoagland complete media solution (Plant Media Inc., Dublin, OH) containing N, P, K, Ca, Mg, S, B, Fe, Mn, Zn, Cu, and Mo (Hoagland and Arnon 1950; McCullough *et al.* 2015). Each week, 100 mL of a solution containing 1.6 g of Hoagland's solid media in 1 L of water was applied from the top of the pots. Throughout the establishment period and during the experiment, seedling height (H) and diameter (D) were measured bi-weekly from the soil surface to the apical bud and at the root collar, respectively. Growth response expressed by the stem volume index (SVI) was calculated from the change in diameter squared times height (D^2H).

Contaminant application and sample collection

After a 4-week-establishment period, we began dosing the pots with 50 mg L^{-1} of both chlorobenzene and benzene through the loading port. Seven doses were applied to all pots over 7 weeks (once per week), in order to acclimate the microbial community and plants to the presence of the contaminants in the irrigation water. We then began experimental monitoring as described below through four additional dosing events at 2-day intervals.

Before each dosing event, we drained the contaminant solution completely from the pot through the outlet valve. The contaminant solution was then added through the loading port until the solution reached a constant level of 20 cm below the sand surface (12 cm in head of contaminant solution). Immediately after dosing, we collected a 20-mL sample of the solution through the outlet valve (sampling port) and froze it immediately in a Qorpak® glass bottle until analysis (see below). The recorded water level was checked every 3–8 hours throughout the experiment and tap water was added to maintain similar water levels across all treatments, accounting for any variation in evapotranspiration. These water additions were used to calculate evapotranspiration rates from each pot. At 24 and 48 hours after the dosing, we collected additional 20-mL samples through the sampling port. After 48 hours, we drained the solution from each pot and began the next dosing event within 1 hour. This was repeated similarly through four cycles. In the last (fourth) cycle, we continued collecting samples every

Figure 2. Greenhouse experimental setup containing eucalyptus (blue bands), pine (green bands), and no-plant controls (white striped bands) in the greenhouse.

24 hours up to 120 hours without draining the solution. Evapotranspiration rates were recorded from changes in the water level observed in the clear external PVC pipe (measured with a ruler), which was hydraulically connected to the main pot (Figure 1).

Contaminant analysis

All aqueous samples were sealed with minimum headspace and stored frozen at −20°C. Immediately after thawing the samples, they were unsealed and a 5-mL aliquot was rapidly collected to minimize any VOC losses. The 5-mL aqueous samples were then subjected to a liquid–liquid extraction with 5 mL of hexane and 1 mL of saturated NaCl, and then shaken for 5 minutes in a 25-mL separatory funnel (Wennersten *et al.* 2004; Pratt and Stevens 1992). The NaCl was added to break any emulsion and to concentrate the analytes in the organic phase through the salting out effect (Eganhouse and Calder 1976). After shaking, the hexane phase was collected in a separate 30-mL glass bottle, and the aqueous layer was re-extracted with an additional 5 mL of hexane (no additional NaCl) for 5 minutes. Both hexane extracts were pooled together for analysis. This procedure yielded an extraction recovery of 78–89% established during initial method development and confirmed through surrogates for each batch analyzed. We obtained the method detection limit by repeated measurements of low compound concentration in the sample matrix. The detection limit for both chlorobenzene and benzene was 0.05 ± 0.01 mg L^{-1}.

The hexane extracts were loaded in an AS 2000 autosampler from where 1.0 μL was withdrawn from each sample and injected into a Thermo Finnigan Trace gas chromatograph (GC) connected to a Polaris Q ion trap mass spectrometer (MS). A Restek Rtx-VRX column (60 m × 0.32 mm × 1.4 μm thickness), recommended in EPA method 8260-B for VOC analysis (US EPA 1996a), was used to separate chlorobenzene and benzene prior to quantifying the analytes on the mass spectrometer (MS). The GC conditions were as follows: Inlet temp of 180°C; oven gradient program: initial 40°C (1 minute), 10°C min^{-1} to 220°C (0.5 minute), then 20°C min^{-1} to 240°C. The MS interface temperature was maintained at 240°C. The carrier gas (Helium) was set for a constant flow of 1.0 mL min^{-1}. In the chromatograms, the retention time (RT) was 11.5 minutes for benzene and 15.5 minutes for chlorobenzene.

Chlorobenzene was analyzed by running the instrument in single ion monitoring (SIM) mode (US EPA 1996a) while benzene was analyzed in ion trap MS^2 mode, which approximates analysis via tandem mass spectrometer (Busch *et al.* 1990). Samples were quantified from a standard curve obtained by injecting standards of chlorobenzene and benzene dissolved in hexane from 0.1 to 100 mg L^{-1}. Fresh standards were prepared twice a week. The concentration of the compounds was quantified using Polaris Xcalibur software (ver. 1.3). For each sample, the mass of chlorobenzene or benzene was calculated by multiplying the analyte concentration (obtained from the GC-MS) by the volume of solution in the planted or unplanted pot.

Post-experiment analysis of sand, plant roots, and foliage

At the end of the greenhouse experiment, we collected leaf tissue as well as sand and roots from the top 10 cm, and from 20 to 30 cm of depth in the pots. All materials were placed in plastic bags, quickly frozen until analysis for chlorobenzene and benzene was performed.

Frozen samples were placed in a 60-mL Qorpak glass bottle with either 20 g of root, 10 g of sand or 2 g of leaf tissue combined with 30, 30, or 25 mL of hexane, respectively. Roots were cut and frozen immediately after cutting. Leaves were grounded in liquid nitrogen. Root, sand, and leaf samples were extracted into hexane using ultrasonic extraction method of Dunnivant and Elzerman (1988) and Ozcan *et al.* (2009) for 30 minutes and then stored in a 2-mL vial until analysis. The leaf samples required an additional cleaning step where they were processed through a Florisil cartridge, according to US EPA Method 3620C (US EPA 2007). The recovery following this clean-up step was 93% for both analytes. All extracts were analyzed by GC-MS as described above. Results were expressed as compound mass per mass of solid phase material (*i.e.*, μg/g of sand, root, or foliage).

Calculated removal and statistical analysis

To estimate the extent of CB and BZ removal due to the presence of the plants (Table 2), the pollutant concentrations of the no-plant controls were subtracted from those in the planted treatments on the second day of measurement for the first three applications and on day 2 and 5 for the fourth application. Similar to Experiment #1, we used an ANOVA (glm, SAS version 9.2) repeated measures to test for treatment differences in contaminant removal following each dosing event and to test for differences in relative growth and evapotranspiration.

Table 2. Mass removal of contaminants attributed to trees (eucalyptus or pine), as calculated from the difference in total loss between unplanted controls and planted treatments, for the four applications in the greenhouse study (Experiment 2).

		First application (second day)	Second application (second day)	Third application (second day)	Fourth application (second day)	Fourth application (fifth day)
Compound	Plant	Phytoremediation effect† (mg pot^{-1})				
Chlorobenzene	Eucalyptus	$9.7 \pm 1.5^{**}$	$8.9 \pm 1.7^{**}$	$6.5 \pm 1.9^{**}$	$4.5 \pm 2.2^{**}$	$2.3 \pm 0.5^{*}$
	Pine	$6.9 \pm 2.7^{**}$	$6.8 \pm 2.9^{**}$	$4.7 \pm 2.5^{**}$	$2.7 \pm 2.0^{*}$	$1.3 \pm 1.5^{\S}$
Benzene	Eucalyptus	$4.6 \pm 1.1^{**}$	$4.2 \pm 0.8^{**}$	$3.6 \pm 1.4^{**}$	$2.2 \pm 1.3^{*}$	$1.4 \pm 0.5^{*}$
	Pine	$3.1 \pm 1.6^{**}$	$3.0 \pm 0.8^{**}$	$2.8 \pm 1.6^{**}$	$2.2 \pm 1.3^{*}$	$1.0 \pm 0.8^{*}$

†Phytoremediation effect = mass difference between unplanted controls and planted treatments.
*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
§Not significant.

Results and discussion

Experiment 1: Survival of P. taeda exposed to chlorobenzene and benzene

None of the pre-rooted *P. taeda* seedlings exhibited any signs of stress or mortality (Figure S2) during 30 days of dosing with CB and BZ, despite one treatment with concentrations exceeding the aqueous solubility of both compounds (Table 1). We recorded an average height increase of 2.0 ± 0.8 cm across all treatments, which was similar across treatments ($p = 0.92$). Furthermore, we observed root growth in all treatments, but we did not quantify root mass or length changes.

Based on these data, we surmise that chlorobenzene and benzene are unlikely to cause significant toxicity to pre-rooted *P. taeda* if used in phytoremediation projects, since seedlings are typically more sensitive to pollutants than the mature plants (Anderson *et al.* 1975). We suspect *E. urograndis* would exhibit similar survivorship if exposed to these contaminant levels. Our observations agree with those by Ferro *et al.* (1999) who observed a lack of phytotoxic effects when poplar trees were exposed to a VOC mixture of aromatic compounds, chlorinated aliphatics, and alcohols, with concentrations as high as 169 mg L^{-1}. The limitations for using *E. urograndis* and *P. taeda* trees in the remediation of VOCs is thus not likely to be toxicity, but their effectiveness at enhancing contaminant removal.

Experiment 2: Phytoremediation of VOCs in greenhouse

Plant growth dynamics

Similar to our first experiment, we observed no visual or physiological differences among the planted-treatments and planted-controls (Figures S3 and S4), suggesting that *E. urograndis* and *P. taeda* can survive and grow in contact with high concentrations of these contaminants. Throughout the greenhouse experiment, there were no significant differences in the height (p-value > 0.45), diameter (p-value > 0.48), or stem volume index (p-value > 0.37) of either eucalyptus or pine trees growing with or without chlorobenzene and benzene application (Figure S5). Similarly, the evapotranspiration rates for the treatments with and without both compounds were statistically the same (p-value > 0.35): 455 ± 72 mL day^{-1} $plant^{-1}$ for *E. urograndis* and 278 ± 59 mL day^{-1} $plant^{-1}$ for *P. taeda*. However, eucalyptus did exhibit a higher growth rate (Figure S5) and a higher transpiration rate (p-value < 0.01) than pine. Evaporation rates for the no-plant controls were 12 ± 8 mL day^{-1} pot^{-1}.

Compound removal

The total mass of chlorobenzene and benzene removed from the planted pots (7–10 mg day^{-1}) was higher than in the non-planted controls (3–6 mg day^{-1}) (Figures 3 and 4), with similar decreases observed in contaminant concentration (*i.e.*, mg L^{-1}) (Figures S6 and S7). Across all applications, the fractional mass removal (in mg day^{-1}) was similar for CB and BZ during the first 2 days following dosing (Figures 3 and 4). Following the fourth application, which was monitored for 5 days after dosing, near complete compound removal was observed for all treatments including the no-plant controls.

In total, 91–98% of the applied CB and 84–97% of the applied BZ was removed in the pots planted with trees (Figures 3 and 4), corresponding to a mass removal of 20 ± 0.3 to 1.1 ± 0.8 mg pot^{-1} for CB, and 10 ± 0.2 to 0.9 ± 0.6 mg pot^{-1} for BZ. In contrast, only 39–64% of the applied chlorobenzene (decrease of 20 ± 0.3 to 9.6 ± 2.3 mg pot^{-1}) and 38–63% of the applied benzene (decrease of 10 ± 0.2 to 4.9 ± 1.2 mg pot^{-1}) was removed in unplanted control plots. Thus, contaminant mass removal attributed to the trees (mass in unplanted pots minus mass in the planted pots) was 1.3–10 mg for CB and 1.0–4.6 mg for BZ (Table 2). Essentially, *E. urograndis* and *P. taeda* doubled the removal of contaminants compared to unplanted controls. Eucalyptus with a higher evapotranspiration rate than the pine, removed more contaminant mass than pine, although this difference was not statistically significant (p-value > 0.12).

Both chlorobenzene and benzene are highly volatile compounds, with standard vapor pressures of 12 and 95.2 mm Hg at 25°C (Howard 1991), and half-lives of 0.3–12 and 0.5–10 days in soil (Howard 1991; Salgado and Marona 2004), for CB and BZ, respectively. The open water volatilization rates for the nutrient/contaminant solution are 4.8 × 10^{-5} and 4.1 × 10^{-5} mg cm^{-2} s^{-1} for CB and BZ, respectively (calculations in Supporting Information, Section 2) (Hemond and Fechner-Levy

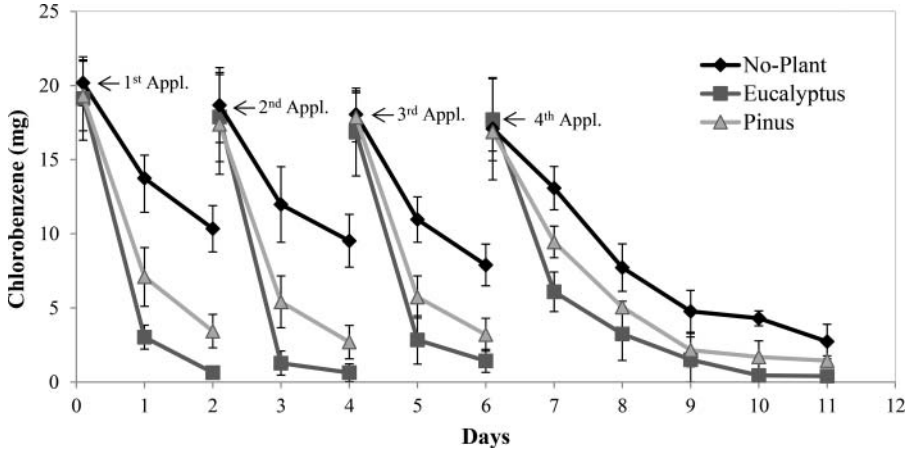

Figure 3. Loss of chlorobenzene under eucalyptus, pine, and no-plant controls following four 50 mg L^{-1} applications (Appl.) of chlorobenzene in the greenhouse experiment. Error bars are ±1 S.D. for $n = 4$ reps per treatment.

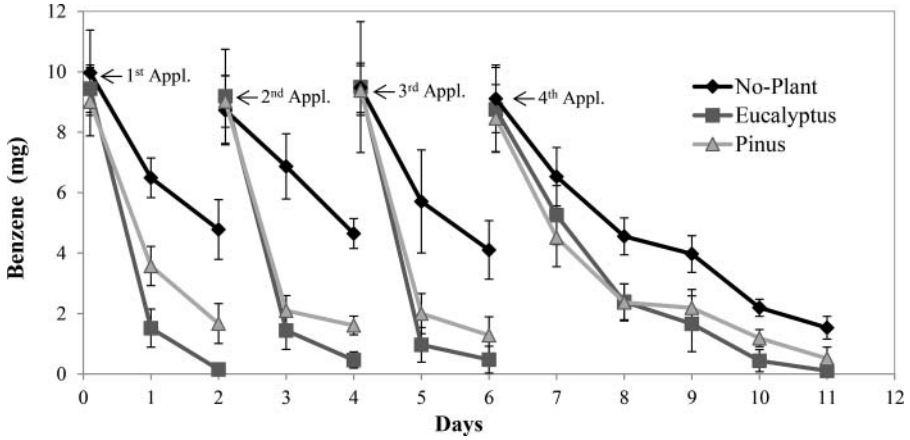

Figure 4. Loss of benzene under eucalyptus, pine, and no-plant controls following four 50 mg L^{-1} applications (Appl.) of benzene in the greenhouse experiment. Error bars are ±1 S.D. for $n = 4$ reps per treatment.

2000; Schwarzenbach *et al.* 2003; US EPA 1996b). Normalized to the surface area of our pots (706 cm^2), the potential volatilization flux is thus 122 and 103 mg $hour^{-1}$ for CB and BZ, respectively, which is much higher than the rates we observed (Figures 3 and 4). The top of each pot was open to the atmosphere and the soil matrix was washed sand, both of which should facilitate volatilization and minimize sorption. Evidently, physical impediments between the solution and the atmosphere (sand particles, pore tortuosity, etc.) play a role in attenuating losses due to volatilization. In this regard, the higher loss of contaminants associated with the planted treatments may derive from the active transpiration and evaporative losses contributed by the plant.

Potential mechanisms

Evapotranspiration (ET) is correlated to the removal of VOCs from soil and water during phytoremediation. We observed in our experiment that the high ET rates accounted for a greater fraction of CB and BZ removal by both plants. Therefore, the difference in removal of either CB or BZ (Figures 3 and 4) can be attributed to the transpiration rate of the plant (Figures S8 and S9). By normalizing the compound mass by concentration, and plot with the evapotranspiration rate, we established that eucalyptus with the highest ET showed a higher contaminant removal than pine (intermediate ET), and higher than the no-plant control, which has the lowest ET and lowest contaminant removal (Figures S and S9).

To evaluate the role of phytovolatilization in our experiment, we used a model proposed by Burken and Schnoor (1998) for plant uptake of VOCs: $U = \text{TSCF} \times T \times C$, where U is the uptake rate of the contaminant, mg day^{-1}; TSCF is the transpiration stream concentration factor, dimensionless; T is the transpiration rate of vegetation, L day^{-1}; and C is the compound concentration, mg L^{-1}. Assuming that log K_{ow} for chlorobenzene is 2.98, and log K_{ow} for benzene is 2.13, the TSCF for chlorobenzene is 0.68 and for benzene is 0.71 (Pilon-Smits 2005; US EPA 1996b). Using values of T and C from our greenhouse experiment, the model predicts 93% removal of CB in the eucalyptus treatment (Table 3), which is in agreement with our observed 91–98% removal well. However, the model overestimates removal of BZ in the eucalyptus treatment (30% more) and underestimates removal of both CB and BZ (43% and 20% less, respectively) in the pine treatment (Table 3). The underestimation of CB and BZ by the ET-based model may suggest a larger contribution of rhizodegradation in the pine treatments. Based on this model, the rhizodegradation was even more prominent in the CB than BZ treatment for pine, perhaps suggesting the root exudates were used by root zone microorganisms in the co-metabolism and anaerobic dechlorination of CB to benzene before degrading the benzene-ring structure (Prytula and Pavlostathis 1996). Nonetheless, this model does suggest phytovolatilization is a key mechanism of CB and BZ removal in our greenhouse experiment.

In addition to phytovolatilization, previous studies have ascribed CB removal through rhizodegradation and/or microbial degradation (Imfeld *et al.* 2009; Gomez-Hermosillo *et al.* 2006). For instance, Braeckevelt *et al.* (2011) found the common rush (*Juncus effusus*) in planted fixed-bed reactors decreased chlorobenzene by 99% of its initial 16.6 mg L^{-1}

Table 3. Predicted plant uptake of VOCs in the greenhouse experiment and model parameters.

Plant	Compound	TSCF‡	Initial concentration, C	Transpiration, T	Uptake, U†	Predicted removal§
			mg L^{-1}	L × (2 days)$^{-1}$	mg × (2 days)$^{-1}$	%
Eucalyptus	CB	0.68	30	0.455 × 2	18.56	93
Eucalyptus	BZ	0.71	20	0.455 × 2	12.92	129
Pine	CB	0.68	30	0.278 × 2	11.34	57
Pine	BZ	0.71	20	0.278 × 2	7.90	79

†Predictions based on uptake model on Burken and Schnoor (1998) with uptake (U) = TSCF × T × C.
‡TSCF, transpiration stream concentration factor, dimensionless (Pilon-Smits 2005).
§Values of U divided by initial mass added to each pot, 20 mg for CB and 10 mg for BZ (see Figures 3 and 4).
CB, chlorobenzene; BZ, benzene

concentration, but that injection of easily degraded organic matter decreased removal rates by diverting microbial activity away from dechlorination. Benzene remediation has also been ascribed to a variety of other processes including plant uptake, phytodegradation, and microbial degradation (Nzengung 2005; Burken and Schnoor 1998). According to Seeger *et al.* (2011), in a planted gravel filter/plant root mat experiment, the monitored benzene decreased 81–99% of its initial concentration, due to microbial degradation and plant uptake.

We did not find any accumulated chlorobenzene and benzene in the sand, plant roots, or plant leaves. Based on our detection limit of 1 μg of compound per g of material (sand, root, or leaf), we estimate no more than 2.1 $\pm$ 0.6 mg of the contaminants remained in each pot at the end of the experiment, which comprises <2% of the total CB or BZ added to each pot (~200 mg each). Therefore, we conclude the plants did not accumulate appreciable quantities of BZ or CB in their tissues. This is consistent with prior work that confirmed plant uptake, translocation and transpiration using ^{14}C labeled BZ and CB, but found minimal net accumulation in plant tissues (Burken and Schnoor 1998; Gomez-Hermosillo *et al.* 2006).

Conclusion

Our greenhouse experiment demonstrates *E. urograndis* and *P. taeda* trees can enhance the removal of chlorobenzene and benzene from sand and water relative to unplanted controls. This enhanced removal appears most closely tied to plant transpiration, suggesting that trees with higher transpiration rates are likely to yield higher rates of VOC removal via phytovolatilization. We also found that these plants could grow for over 5 months in sand pore waters containing high concentrations of chlorobenzene and benzene without showing any observable evidence of phytotoxicity. We conclude that phytoremediation of chlorobenzene and benzene contaminated sites is a feasible and cost-effective technology for regions where *E. urograndis* and/or *P. taeda* are able to grow.

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