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Uptake and Biotransformation of Trichloroethylene by Hybrid Poplars

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Poplar trees were found to be capable of taking up trichloroethylene (TCE) and degrading it to several known metabolic products: trichloroethanol, trichloroacetic acid, and dichloracetic acid. Poplars were also shown to transpire TCE in measurable amounts. To eliminate the possibility that the degradation we observed was produced solely by rhizosphere organisms, axenic poplar tumor cell cultures were tested; the cultures produced the same intermediate metabolic products. When dosed with [^{14}C]TCE, cell cultures also produced low levels of radiolabeled carbon dioxide and a labeled insoluble residue. These results show that significant TCE uptake and biotransformation occurs in poplar, which demonstrates the potential for the use of poplars for in situ remediation of TCE.

Introduction

Trichloroethylene (TCE) is a major contaminant of the soil and groundwater in the United States. It has been used in many ways since the nineteenth century, ranging from an anesthetic by the medical community (*1*), a degreaser by industry and the military, to a solvent by the dry cleaning industry, and in variety of ways by the general public. Widespread use and indiscriminate disposal makes TCE one of the most common pollutants listed by the U.S. Environmental Protection Agency. Recent studies indicating possible carcinogenic activity by TCE (*2*) make immediate remediation of TCE-contaminated sites a priority. The task of removing TCE from the environment is enormous, with estimates of the time for removal of TCE using current engineering technology, particularly pump-and-treat procedures, ranging from decades to centuries (*3*).

Existing methods for remediation of TCE-contaminated aquifers include pumping groundwater to the surface where it may be stripped to the atmosphere, sorbed onto activated carbon, or destroyed chemically or by microbial attack. *In situ* methods involve stimulation of anaerobic and/or aerobic microbial activities in the aquifer or installation of chemically reactive zones. Unfortunately, some of the more reactive microbial systems require the application of potentially toxic "inducer" compounds. These technologies all require extended time commitments along with considerable commitment of manpower and financial resources.

Recently, the use of plants for environmental cleanup (phytoremediation) has attracted considerable interest due to its potentially lower cost and suitability for applications that require longevity and low maintenance. Other factors that favor plants for remediation purposes are their roles in co-metabolic processes and their enrichment of rhizospheric organisms that may be involved in degradation processes (*2, 4*). This increases the potential for the use of enhanced numbers or transgenicly improved endosymbiots in waste remediation. An additional consideration is aesthetics; the public is more likely to react favorably to a grove of trees when the alternative is the industrial appearance of conventional remediation technologies.

Not all contaminated aquifers will be suitable sites for phytoremediation. Aquifers that are under urban areas may not have the available area to allow a sufficient number of trees to be planted or land costs may be prohibitive. Aquifers that are overlaid with an impermeable stratum will preclude access to the contaminated groundwater by the roots. Some contaminated aquifers may just be too deep for plant roots to reach and penetrate. However, according to John Wilson of the Kerr Labs, U.S. EPA (personal communication), approximately 80% of the polluted groundwaters are within 20 m of the surface, which means that a significant number of sites are accessible to plant root systems.

Previous work has indicated that TCE is oxidized more readily in the rhizospheres of some plants, such as pine or legumes, as compared to unvegetated soil but that mineralization of TCE was limited (*4, 5*). Uptake of TCE by plant roots was correlated with water uptake, but total uptake was small and translocation insignificant (*4*). Others have reported that plants do not take up TCE from the soil but rather respire TCE volatilized from the soil surface (*3*).

This study was undertaken to determine if a different plant, such as hybrid poplar, would be capable of metabolizing TCE. This paper presents the recent results of a study of the potential for phytoremediation of TCE using hybrid poplar. Both the uptake and the oxidation of TCE in poplar tissue culture and whole plants were examined.

Poplars were the plants of choice for several reasons. Much is already known about the physiology and culture of poplar trees. Several different cultivars as well as sterile cultures of poplar cells were available for study. Additionally, poplars have been used by many groups in field demonstrations of phytoremediation projects (*5, 6*). On these sites, poplars were chosen because of their rapid growth and development of extensive root systems. The hybrid poplar clones used in this study can grow at the rate of 3 m or more per year and at five years of age obtain a diameter of 13 cm or more at the height of 1 m from ground level. The transpiration rate of a 5-year-old tree varies between 100 and 200 L of water per day, depending on conditions (R. F. Stettler, College of Forest Resouces, University of Washington, Seattle, WA: personal communication), and poplar roots will rapidly elongate toward an aquifer. Because poplars are amenable to transformation (*7*), enhancement of natural degradation pathways may be possible.

Data are presented from trials involving whole plants grown under greenhouse conditions as well as tumor cell cultures. Hybrid poplar plants were resistant to the toxic effects of TCE at the doses tested and demonstrated the ability

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to transpire and degrade TCE. These findings show that hybrid poplars are in fact capable of degradation of TCE and support the feasibility of remediation of TCE-contaminated sites using hybrid poplars.

Methods

Axenic cell cultures (tumor cells derived from the *Populus trichocarpa* × *P. deltoides* clone [H11-11]) were studied to determine if poplar cells were capable of metabolizing TCE in the absence of the of soil and mycorrhizal flora. The cell line was generated via transformation with *Agrobacterium tumerefaciens* A281. These cells were used because of their rapid growth and ease of culture and handling. Cells were tested for their ability to remain viable in sealed flasks. Viablity counts began dropping after 8 d when air exchange over the cells was eliminated. Cells were also tested for sensitivity to TCE and were found be capable of growth in 260 ppm TCE without loss of vitality when compared to non-dosed cells grown under the same conditions.

Cells were grown to 60% of the volume of the growth medium as determined after settling for 1 h. The cells were subcultured by diluting by 50% with Murashige and Skoog basal salt medium (MS medium) (*8*) without hormones, and 500-mL aliquots were placed in 1-L three-necked flasks. Cells were dosed either with 50 mL of MS medium saturated with TCE (saturation is approximately 0.8 g/L media as calculated by GC/MS) or 50 mL of MS medium only and the flasks sealed with rubber stoppers coated with Teflon sheets. The cells were incubated with shaking and illumination under two 40-W fluorescent bulbs with a quanta reading of 30 μm s^{-1} m^2 for 3 (batch 1) or 5 d (batch 2) at room temperature. At the end of the test, the cultures were plated on LB agar and incubated for 5 d to ensure that there were no contaminating bacterial or fungal cells in the culture. The cells were separated from the media by centrifugation for 20 min at 5000*g*. The cells and the media were stored at −20 °C. Using the pathways for mammalian hepatic microsomal transfromation of TCE (*9*) as a guide, we looked for TCE, chloral hydrate, di- and trichloroacetic acid, and trichloroethanol in the samples as these were the most likely metabolites that would be present in high enough concentrations to be detectable by our methods of analysis.

Two grams of the pelleted cells was mixed with 2 mL of 1 N H_2SO_4/10% NaCl and vortexed for 30 s. Ten milliliters of methyl *tert*-butyl ether (MTBE) was added, and the mixture was vortexed for 2 min and then centrifuged for 5 min. Seven milliliters of the MTBE extract was drawn off and dried over 2 g of Na_2SO_4 for 2 h at room temperature. One milliliter of extract was mixed with 10 μL of an internal standard (25 mg/mL dibromochloropropane [DBCP] in MTBE) and analyzed on a *Hewlett Packard 5880 GC with an electron capture* detector set at 325 °C and a N_2 gas flow of 45 mL/min.

To test for the presence of di- and trichloroacetic acid, 2 g of the cell pellet was extracted twice with 7 mL of 0.25 N NaOH, vortexed for 5 min, and centrifuged for 5 min. The supernatants were combined and acidified to less than pH 1 with 50% H_2SO_4 and then quantitatively transferred to a centrifuge tube containing 2 g of Na_2SO_4. Three milliliters of MTBE was added, and the tube was vortexed for 2 min and centrifuged for 10 min. Two milliliters of the MTBE extract was mixed with 250 μL of saturated diazomethane in MTBE. The mixture was held at 4 °C for 15 min and at room temperature for 15 min. Twenty milligrams of silica gel was added to adsorb excess diazomethane. One milliliter portions of the supernatant were mixed with 10 μL of DBCP internal standard and analyzed on a GC/MS with identical GC conditions as described above.

The axenic cell cultures were also tested for the formation of radiolabeled carbon dioxide produced by the mineralization of TCE. The cells were grown to 60% of the volume of the growth medium and were subcultured by diluting 50% with MS medium without hormones to a final volume of 500 mL. The cells were placed in a 1-L three-necked round-bottom flask and dosed to 1% by volume with MS medium saturated with TCE and 0.9 μCi of [^{14}C]TCE (the TCE was uniformly labeled; the experimental dose resulted in a specific activity of 30 μCi/mmol TCE or 2 × 10^6 cpm) to act as a tracer. Duplicate cultures were incubated for 4 d with shaking and illumination. All connections were composed of either glass or Teflon to decrease adsorption and evaporation of the TCE. A carbon tube consisting of a 2-g primary portion and a 0.5-g secondary portion was attached to the third neck of the flask with Teflon tubing. An impinger containing 50 mL of 1 N NaOH was connected after the carbon tube to trap carbon dioxide. A Teflon stopcock was positioned between the flask arm and the carbon trap to prevent loss of the TCE during the incubation period. A second Teflon stopcock was placed on the first neck of the flask to allow entrance of air at the end of the incubation period. At the end of the incubation period, the stopcocks were opened, and a vacuum was applied to the outlet end of the trapping train for 1 h to remove all volatized compounds from the flask. The level of radioactivity in the two traps was determined as well as the amount of TCE remaining in the supernatant and the cells. Media only and kill controls were also run. Killed controls were autoclaved for 30 min at 121 °C and 15 psi. Both were handled in the same fashion as live cells.

The amount of radioactivity in the NaOH was determined by direct counting in Hionicfluor scintillation cocktail in a Beckman LS 7000 scintillation counter. The carbon from the two portions of the tube was extracted with 3 mL of toluene for 0.5 h. The toluene was counted under the same conditions as the NaOH. If there were no detectable counts in the secondary carbon trap, then all counts in NaOH were assumed to be due to carbon dioxide.

The radioactivity in the sodium hydroxide trap was confirmed as carbon dioxide by precipitating the contents of the trap with 1 M barium chloride until the formation of precipitate stopped. The resulting suspension was centrifuged, and the pellet was transferred to a clean impinger that was connected to a second impinger containing 1 N sodium hydroxide. A vacuum was applied, and 2 mL of 6 N hydrochloric acid was added to the barium precipitate pellet to release the carbon dioxide. The vacuum was maintained for 2 h.

To ensure that there was no degradation of TCE or other compounds to carbonate if trapped in the sodium hydroxide a control experiment was run. A 3 mM sample of TCE containing 2.89 × 10^5 counts was added to 15 mL of 1 N sodium hydroxide through a cap with a Teflon septum. After 2 d the mixture was brought to 40 mM sodium carbonate (which served as a carrier), and 1 M barium chloride was added until no additional precipitate was formed. If there was no degradation of the TCE or metabolites to carbonate all of the labeled material would be found in solution. The mixture was centrifuged, and the pellet was solubilized with 6 M hydrochloric acid. The resulting gas was bubbled through an impinger containing sodium hydroxide for 3 h. The amount of radioactivity in both the hydrochloric acid and the sodium hydroxide was determined along with the supernatant from the original precipitation.

Separate samples of the cells exposed to radioactive TCE were treated to extract radioactive components that were not detected by gas chromatography. The cell suspension was allowed to settle, and the amount of radioactivity in the supernatant was determined. The cells were extracted four times with 20 mL of 2 N H_2SO_4, and the four supernatants were combined. The remaining cell pellet was also extracted four times with 100% methanol and then 100% MTBE. Each set of the four combined supernatants was also counted. The remaining cell pellet was oxidized for 2 min in a Packard

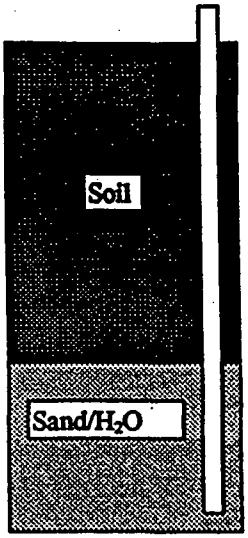

FIGURE 1. Diagram of plant pipe. Water or TCE-containing water was added directly to the sand layer through the inner watering tube. Water was applied to the plants on top of the soil as needed. The soil layer was 60 cm tall, and the sand/water layer was 30 cm tall for a total height of 90 cm. The diameter of the chamber was 20.5 cm.

TABLE 1. Amounts of TCE and Metabolites Found in Supernatant and Axenic Cells Exposed to TCE[a]

	TCE	chloral hydrate	trichloro-ethanol	dichloro-acetic acid	trichloro-acetic acid
control 1					
pellet	ND40	ND40	ND40	ND10	ND10
supernatant	ND40	ND40	ND40	ND10	ND10
control 2					
pellet	ND40	ND40	ND40	ND10	ND10
supernatant	ND40	ND40	ND40	ND10	ND10
exposed, batch 1					
pellet	ND40	ND40	60	12000	ND10
supernatant	2000	ND40	760	1600	ND10
exposed, batch 2					
pellet	ND40	ND40	80	39000	130
supernatant	ND40	ND40	110	3800	30

[a] Amounts given are ng of TCE or metabolite per g of sample. ND, not detected at stated limit.

Model 307 oxidizer, and the released carbon dioxide was trapped in 1 N NaOH and counted.

Initial whole plant experiments involved growing 12 poplar plants (two clones from *P. trichocarpa* × *P. deltoides* crosses [H11-11 and 50-189] and one clone of *P. trichocarpa* × *P. maximowiczii* [282-190]) in PVC pipe chambers. The chambers were 1.0 m tall with an internal diameter of 20.5 cm. A watering tube, 1.1 m long with an inside diameter of 1.5 cm, was inserted to the bottom of each chamber prior to filling. The chambers were filled to a depth of 30 cm with medium sand, followed by 60 cm of Sultan series silt loam (Figure 1). Dormant 1-year-old stem cuttings 30 cm long were placed in each chamber. Nine days after planting, two plants of each clone were dosed through the watering tube with water containing approximately 50 ppm TCE; the other six plants received water without TCE. Plants were watered to the 30-cm mark on a biweekly basis, either with water or the TCE-containing water as designated. Soil surfaces were watered with pure water as needed.

After 20 and 31 weeks, the plants were tested to determine if TCE was transpired. Individual leaves were enclosed loosely in polyethylene bags and partially closed at the petiole so as to allow for free entrance of air. The atmosphere of each bag was sampled for 0.5 h using a portable SKC air sampling pump equipped with a charcoal filter (SKC West, Fullerton, CA). The bag had a volume of 3 L, and the pumping rate was 90 mL/min. The charcoal was extracted with pentane, and the amount of absorbed TCE was determined by gas chromatography with electron capture detection (*10*).

After 8 months, the plants were harvested and examined for morphological differences, and detailed measurements were obtained. Samples of leaves, stem, and upper, middle and lower roots were analyzed for TCE and possible metabolites. Plant tissue samples were cooled on dry ice for 10

TABLE 2. Recovery of Radiolabeled TCE and Metabolites[a]

	cells		
	batch 1	batch 2	media only
% recovered in			
carbon traps	82	92	83
supernatant	6.1	2.2	11
pellet			
acid extractable	3.6	3.7	
non-extractable	0.1	0.2	
CO_2 trap	1.5	1.5	0

[a] Traps leading off culture vessels contained activated carbon for the capture of volatile organic compounds and sodium hydroxide for the capture of carbon dioxide. Cell pellets were extracted four times with 2 N H_2SO_4 and then four times with methanol. The remaining pellet was oxidized, and labeled carbon dioxide was trapped in sodium hydroxide. Numbers shown are percentages of total amount applied, which was 2 × 10^6 cpm. Background counts were subtracted, and numbers were corrected for quenching before calculating percentage.

FIGURE 2. Plants on the right were dosed with TCE; plants on the left were the control.

TABLE 3. TCE Treated and Control Plant Growth Data

clone[a]	dosed	height	av stem diameter (mm)	leaf area (cm^2)	no. of leaves	root wt (g)	length (cm) of fine roots	length (cm) of coarse roots
282-190	no			5996	112			
282-190	no	182	14.5	5624	56	24	7318	1501
282-190	yes	119	15.8	4200	42	16	2252	1204
H11-11	no	180	15.7	9695	90			
H11-11	no	204	18.8	16537	138			
H11-11	yes	176	18.0	8505	65			
H11-11	yes	155	17.1	6907	122			
50-189	no	197	14.5	7705	72			
50-189	no	201	16.2	9969	78	156	46969	13193
50-189	yes	184	12.9	5889	60			
50-189	yes	186	13.7	7546	63	82	9785	6558

[a] H11-11 and 50-189 are *P. trichocarpa* × *P. deltoides* hybrids, and 282-190 is a *P. trichocarpa* × *P. maximowiczii* hybrid.

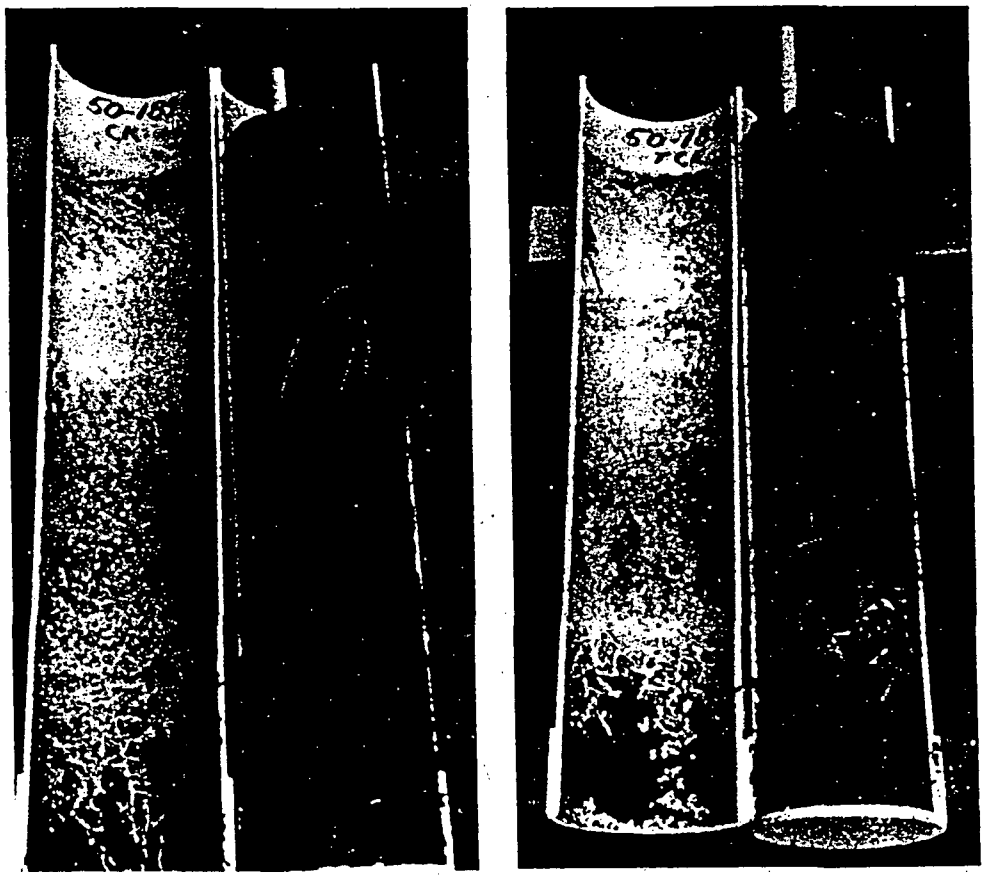

FIGURE 3. Photo of roots of control vs exposed plants. The control plant (on the left) has fine roots extending well into the sand layer The TCE-dosed plant (on the right) shows a dramatic evidence of lack of root growth into the saturated zone of the sand.

min and then ground with an equal volume of dry ice. After being warmed to room temperature, they were treated the same as the cell cultures, with the following cleanup steps added: after drying over Na_2SO_4, 5 mL of the MTBE extract was mixed with 50 μL of the internal standard. A silica gel SPE cartridge was conditioned with MTBE, and 4 mL of the sample extract was pulled through the cartridge with the final 2 mL collected for analysis.

Results

The results of the axenic poplar cell culture experiments show conclusively that poplar cells are capable of transforming and mineralizing TCE without the involvement of microbial metabolism. In cell cultures, TCE was converted to trichloroethanol and di- and trichloroacetic acid (Table 1). Dichloroacetic acid was most prevalent, exceeding 10 ppm in the cell pellets. No detectable levels of chloral hydrate were found.

Plant cell cultures exposed to radiolabeled TCE oxidized approximately 1–2% of the applied TCE to carbon dioxide within 4 d, as confirmed by barium precipitation (Table 2). Additionally, after extracting the cells with both sulfuric acid and methanol, a portion of the radioactivity was found to be incorporated into an insoluble residue (Table 2). Extracted killed control cells were found to have accumulated an equivalent amount of bound radioactivity in the insoluble residue as the live cells (data not shown). This is most likely due to abiotic binding of the TCE to the cell wall or cell membrane components. The exact means of this incorporation is currently under study. The finding of these metabolites and incorporated radioactivity are important, as the incorporation of TCE within the plant structure or conversion of TCE to catabolites via plant metabolism is a more desirable fate than transpiration into the atmosphere. We have found that the amount of incorporation varied depending on the length of exposure. However, in both labeled TCE and non

TABLE 4. Amounts of TCE and Metabolites Found in Plant Tissues[a]

clone[b]	tissue	dosed with TCE	TCE	chloral hydrate	trichloethanol	dichloroacetic acid	trichloroacetic acid
H11-11	leaves	no	ND40	ND40	ND40	ND10	ND10
	stems		ND40	ND40	ND40	ND10	ND10
50-189	leaves	no	ND40	ND40	ND40	ND10	25
	stems		ND40	ND40	ND40	ND10	ND10
50-189	leaves	no	ND40	ND40	ND40	ND10	ND10
	stems		15	ND40	ND40	ND10	ND10
H11-11	leaves	yes	13	ND40	180	ND10	1100
	stems		770	ND40	140	ND10	31
50-189	leaves	yes	49	ND40	19	180	7200
	stems		1900	ND40	170	ND10	22
50-189	leaves	yes	27	ND40	24	ND10	2100
	stems		1300	ND40	125	ND10	100
H11-11	roots	yes					
	upper		13	ND40	200	320	44
	middle		150	ND40	110	25	21
	lower		640	ND40	31	270	44

[a] Plants were watered with either 50 ppm TCE or water as designated and grown under greenhouse conditions. Amounts given are ng of TCE or metabolite per g of sample. ND, not seen at stated detection limit. [b] Both clones used in this study were *Populus trichocarpa* × *P. deltoides*.

labeled TCE experiments that ran for 4 d, there was a similar ratio between amount dosed and amount metabolized to acid-extractable metabolites. Plants treated with TCE in the greenhouse experiment appeared to grow normally, but measurements showed them to be slightly smaller than the control trees. Trees exposed to TCE for 8 months only grew to 85% of the height of the control trees (Figure 2 and Table 3). Measurement of the quantities of both fine and coarse roots showed that the major difference in the plants was in the amount of fine roots formed by the plants. While the roots of the control plants extended well into the sand layer at the bottom of the pots, the fine root structures of the exposed plants did not (Figure 3 and Table 3). Thus, the major physiological change in the plants when exposed to 50 ppm TCE is in those portions of the plant in direct contact with the compound. However, there was sufficient root growth in the soil to support the growth of the crown.

Analysis for chlorinated compounds in the plants was performed to determine if the plants were able to metabolize TCE. Due to the open nature of this experimental system, it was not possible to conduct a true mass balance to account for all of the TCE applied to the system.

Chlorinated compounds were undetectable in the stems or leaves of the three control plants, with the exception of low levels of TCE in one of the stem samples and trichloroacetic acid in one of the leaf samples (Table 4). This could be due to the uptake of transpired TCE from nearby plants. In exposed plants, significant amounts of TCE were detected in the stems of the plants, but the level in the leaves was minimal. Levels of trichloroethanol were roughly equal to TCE levels in leaf samples, while the level of trichlorethanol in stems was much lower than that of TCE. Trichloroacetic acid was detected in the leaves and stems, with leaf concentrations of trichloroacetic acid exceeding 1 ppm. There was no detectable chloral hydrate, and only one leaf sample had any detectable levels of dichloroacetic acid.

Root samples were divided into upper, middle, and lower sections and analyzed separately. Root tissue contained TCE as expected as well as trichloroethanol dichloroacetic acid and low levels of trichloroacetic acid. No chloral hydrate was detected in the root. The observed metabolic conversion of TCE to trichloroethanol occurred in the lowest root tissue, and trichloroethanol and TCE were transported through the stem to the leaves where they were probably converted to other compounds, most likely trichloroacetic acid.

The lack of chloral hydrate in any plant or plant cell culture sample suggests either that poplars do not use this compound as a metabolic intermediate or that we were unable to detect it. This could be due to a short half-life of chloral hydrate

TABLE 5. Transpiration of TCE[a]

clone[b]	plant no.	LPI no.[c]	dosed	(μg) TCE
H11-11	6	21	yes	0.448
H11-11	6	10	yes	0.053
H11-11	8	20	yes	0.541
50-189	12	27	yes	0.811
50-189	12	10	yes	0.360
289-19	2	21	yes	0.088
289-19	2	10	yes	0.223
H11-11	7	27	no	0.085

[a] Micrograms of TCE obtained from leaf bags during a 0.5 h test period. [b] H11-11 and 50-189 are *P. trichocarpa* × *P. deltoides* hybrids, and 282-190 is a *P. trichocarpa* × *P. maximowiczii* hybrid. [c] Leaf Plastichron Index Number, counting from the top of the tree, the first leaf to exceed 2.0 cm in length is considered LPI no. 0; the second leaf down is LPI no. 1, etc.

in this system. In mammalian systems, trichloroethanol and trichloroacetic acid are produced from chloral hydrate, which itself is a product of TCE oxidation by cytochrome P-450 oxygenase (*11*), so the latter hypothesis appears more likely. The formation of dichloroacetic acid follows a different pathway: it is generally thought to be formed by the decomposition of trichloroethylene epoxide produced during oxygenase attack on TCE.

The amounts of TCE collected from bags enclosing individual leaves show that the plants are capable of transpiring TCE (Table 5). However, these experiments were not corrected for TCE losses due to due to adsorption of the TCE to the bags or loss through volitilization. Also, we were unable to measure water transpiration rates for each leaf prior to and immediately after placement in the bag. Thus, these results should be viewed as a qualitative indication of TCE transpiration by poplar trees and not as quantitative measurements. Additional studies are currently underway in mass balance chambers under more controlled conditions to quantify these findings.

The results of these experiments indicate that phytoremediation using hybrid poplar trees may be useful for removing TCE from the environment. As shown in the cell culture studies, the H11-11 poplar hybrid has the ability to mineralize TCE to carbon dioxide. These cells were also able to incorporate ^{14}C from the [^{14}C]TCE into insoluble products possibly cell wall constituents. Young H11-11 trees were able to transpire TCE taken up from the soil and to oxidize the TCE to expected metabolites.

Field trials are currently underway using the H11-11 hybrid clone at a highly monitored site. We are tracking TCE removal

from the system by monitoring the concentration of TCE in the effluent water that has passed through a stand of trees by looking for metabolites in plant tissues and by studying transpiration of TCE by the trees using a variety of analytical methods. Identification of metabolites in these plants, grown under field conditions, shows that the processes seen in the lab are also occurring in the field.

This is the first paper that shows conclusively that plants are capable of the types of degradation of toxic compounds that have formerly been seen primarily with microorganisms. These types of metabolic processes may indeed be specific to certain plants, just as certain bacteria are capable of degrading different compounds. Detailed studies of the capabilities of different plants species, or even ecotypes or varieties within a species, will help in making the proper selection of plants for the remediation of a contaminated site. Not all contaminated aquifers will be suitable sites for remediation using poplar, just as not all sites are suitable for any one type of remediation effort. But selection of the proper trees for the site, as determined by studies like these, will enhance the likelihood of a successful remediation effort.

In conclusion, the results presented here show that hybrid poplars are capable of both TCE transpiration and metabolizing TCE, and they validate the potential for using these plants as an attractive alternative to existing remediation methods.

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