Metabolic response of native Southeastern trees to Trichloroethylene

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ABSTRACT

Phytoremediation of trichloroethylene (TCE) from contaminated groundwater has been performed using fast-growing tree species that maintain a high water demand. Tree species with these characteristics make excellent candidates for phytoremediation applications due to their ability to take up large amounts of groundwater, and therefore contaminant. Several metabolites of TCE have been identified in the tissue of poplars including trichloroethanol (TCEOH) and di- and trichloroacetic acids (DCAA, TCAA). The presence of these metabolites indicates that TCE degradation is taking place through natural metabolism of exogenous compounds in the plant system. However, it is important to expand the range of plants that can be utilized in varying areas of the country for phytoremediation. By screening native tree species for the ability to take up and degrade TCE we hoped to identify phytoremediation candidates suitable for the Southeast. This study was a greenhouse based project that simulates the effects of groundwater TCE on the plant system. In this study, we examined native tree and plant species of the Southeast by studying their interaction with TCE. Tissue analysis by gas chromatography revealed the presence of TCE, TCEOH, DCAA, and TCAA in various samples. While overall growth did not appear to be affected by TCE treatment, some species possess more potential for phytoremediation field applications.

INTRODUCTION

Phytoremediation has been a rapidly growing field of environmental remediation for nearly fifteen years. Phytoremediation can be used as a remediation technique on many types of contaminated soils, waters, and sediments, and may be applied for remediation of contaminants ranging from heavy metals to polyaromatic hydrocarbons (PAH's) (1). The species and variety of plant used on a phytoremediation field plot will depend greatly on the location of the plot. The best results may be obtained by using plants that are indigenous to the area to which they will be applied. Non-native species may also perform well, however, it is not recommended to use invasive species that may take over the native habitat of other plants. For remediation of groundwater contaminants, deep-rooting, fast-growing plants are best. Plants with these characteristics usually also have the capacity to take up and transpire large quantities of water. For example, hybrid poplars are one of the few species that have performed especially well in a phytoremediation plot (2). Establishing tree species appropriate for phytoremediation can be accomplished by using small-scale greenhouse screening experiments (3). In this study, we examined three species of trees native to the southeastern United States which possess the appropriate characteristics for phytoremediation candidates. Sycamore (*Plantanus* L.), sweetgum (*liquidambar styraciflua* L.), eastern cottonwood (*Populus deltoides*), willow (*Salix* L.), and tobacco (*Nicotiana tabacum* var.Burley) were all examined for their ability to degrade the halogenated hydrocarbon, trichloroethylene (TCE) (4). Analytical examination of TCE degradation in trees has revealed several metabolites of TCE including trichloroethanol (TCEOH), dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA). Degradation of TCE in plants is believed to occur along an oxidative, enzymatic pathway that may be similar to that known for several mammals, including humans, and several microorganisms in the aerobic degradation portions of the pathway (5). In order to determine the ability of Sycamore, Sweetgum, eastern cottonwood willow (*Salix*), and tobacco (burley) to degrade TCE, a controlled greenhouse study was performed. All five tree species were exposed to two different concentrations of TCE at 50 and 150 ppm to investigate for metabolite production and inhibition of growth. Tissue was analyzed for TCE and TCEOH, DCAA, and TCAA including roots, stems, and leaves. In addition, transpiration samples were taken from willow trees and tobacco plants.

MATERIALS AND METHODS

Tree species

Sycamore (*Plantanus* L.)

Sweetgum (*Liquidambar styraciflua* L.)

Cottonwood (*Populus deltoides* Bartr.)

Tobacco (*Nicotiana tabacum* var.Burley)

Willow (*Salix*)

**Sycamore and Sweetgum donated by Westvaco Farms, SC.*

**Willow cuttings were generously donated by Dr. Timothy Volk, SUNY,Albany.*

**Tobacco seeds were donated by Dr. Chris Barton*

Experimental Design

Five replicates of each tree species were used for each experimental group for sycamore, sweetgum, and cottonwood. Replicates were increased to 8 for tobacco and willow. Trees were grown in glass beakers containing a bottom layer of pea gravel, followed by native soil obtained from the Savannah River site for sycamore, sweetgum and cottonwood. Topsoil was used to grow tobacco and willow. Both types of soil were supplemented with weekly all-purpose fertilizer.A glass rod was inserted into each beaker for the purposes of bottom-watering.

Each tree was watered as needed according to soil moisture measurements taken with a soil moisture meter for estimation. Control groups were watered with tap water containing no added TCE, low dose experimental groups were watered with tap water with a TCE concentration of 50 ppm TCE, and high dose experimental groups were watered with tap water with a concentration of 150 ppm TCE.

All tissue samples were collected on the same day. Tissue was weighed and flash frozen on liquid nitrogen and stored at -80ºC until extraction.

Air samples were collected by vacuum pump (1L/min) into air sampling bags attached to Tenex absorption tubes.

Analyses

-TCE and TCEOH were analyzed by an acidified/MTBE extraction.

-Di- and Tri- chloro acetic acids were extracted from root and stem tissue using a hot methanol extraction.

-Air samples were analyzed by automated thermal desorption.

-Samples were sealed in 2 ml vials with 2 µl of ethylene dibromide (internal standard) for analyses by gas chromatography (GC) with electron capture detection.

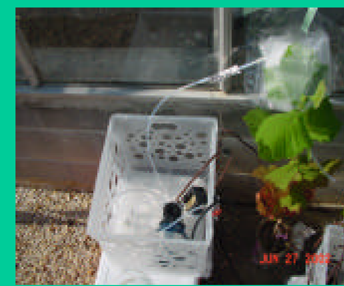


Figure 4. TCE transpiration from willow and tobacco

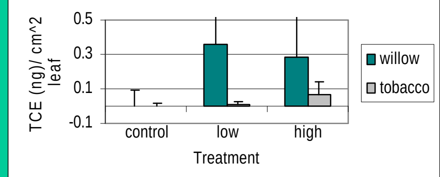


Table 1-Average fresh weights (g) of various tissue sampled

	Treatment	sycamore	cottonwood	sweetgum	willow	tobacco
Leaf	control	24	18	18	14	74
	low	30	21	21	15	52
	high	29	24	24	11	49
Stem	control	12	6	6	19	17
	low	17	7	8	17	14
	high	17	11	10	15	11
Root	control	39	19	16	122	134
	low	49	35	37	101	87
	high	68	47	20	47	70

Table 2-Average water uptake (ml) per plant for 2 months

Treatment	sycamore	cottonwood	sweetgum	willow	tobacco
control	5340	4800	4040	3063	4525
low	5925	5000	4400	3144	4281
high	6200	5200	4560	2831	3981

Figure5. Average Height at 3 months

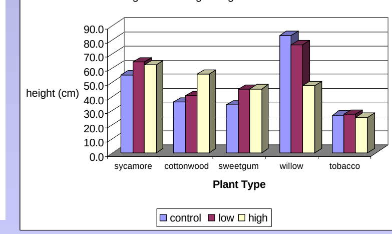


Table 3 - Average Leaf Surface Area (cm^2) per Plant

Treatment	sycamore	cottonwood	sweetgum	willow	tobacco
control	1464	641	658	1187	2338
low	2115	953	1134	1938	1773
high	1778	917	1318	1484	1651

RESULTS

TCE Distribution

TCE was observed in all tissues in sycamore, sweetgum, and cottonwood at concentrations ranging from low ppb ranges to low ppm ranges. Higher concentrations were observed in the roots while the lowest concentrations were observed in the leaves. This trend exemplifies the route of TCE uptake from the soil through the plant tissue. TCE was observed only at low levels in the roots and stems of willow and tobacco.

Metabolite Distribution

TCEOH was observed only in the tissue of the roots and stems in sycamore and cottonwood with the highest concentrations observed in cottonwood (Figures 1). TCEOH, DCAA, and TCAA are products of aerobic metabolism and would therefore not be expected in the soil unless aerobic microbial degradation were taking place. Concentrations of TCEOH are usually so low in the leaves due to volatilization that they can be difficult to detect. Endogenous DCAA was observed in all tissues of all five plant types. DCAA did not appear to increase when plants were dosed with TCE (Figure 2). Endogenous TCAA levels greatly increased in all plants when dosed with TCE, specifically in the leaves (Figure 3). Overall, the highest levels of both DCAA and TCAA were observed in the leaves.

Air Sampling

Air samples taken from willow trees and tobacco plants revealed that willow trees were transpiring a greater amount of TCE per square centimeter than tobacco (Figure 4). Air samples collected from sycamore, sweetgum and cottonwood have not been processed.

Growth Parameters

Average fresh weights of all trees were recorded and compared (Table 1). For each type of plant observed, the average total amount of water per treatment for an individual plant is reported (Table 2).

Average heights of each type of plant and treatment were recorded during and at the end of the experiment (Figure 5). The average total leaf surface area was estimated for each type of plant and treatment (Table 3).

CONCLUSIONS AND FUTURE PLANS

Hybrid cottonwood trees are commonly used for phytoremediation applications because of their water uptake potential and the ability to degrade TCE. The cottonwood and sycamore trees in this study both exhibit the ability to degrade TCE into TCEOH, as well as high TCAA. Sweetgum and willow trees produce trichloroacetic acid in the presence of TCE even though no detectable levels of TCEOH are observed. The volatility of TCEOH may account for this decrease. Although tobacco appears to transport and transpire TCE, the levels of metabolites observed are insignificant compared to the trees observed in this study.

All data collected and observed in these two studies will be statistically analyzed and compared. Phytoremediation of TCE relies not only on a plant's ability to degrade TCE, but also the ability of a the plant or tree to survive in the field. We have shown that native southeastern plants are capable of degrading TCE and may be used for phytoremediation field applications

Figure 1. TCEOH in roots and stems of Sycamore and Cottonwood

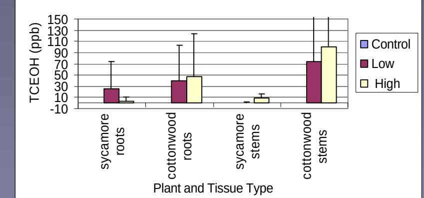


Figure 2. DCAA in leaves

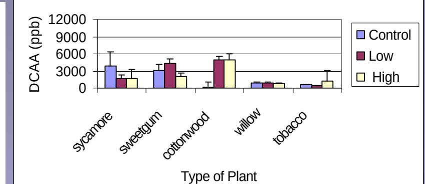
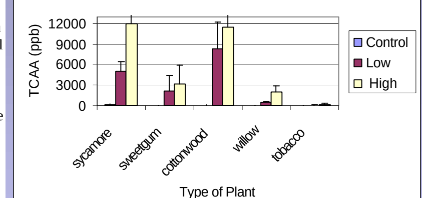


Figure 3. TCAA in leaves



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