Determining an Optimum Tree Species for the Phytoremediation of Cumene and 4-Cumylphenol in Groundwater[1]

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Abstract

Cumene and 4-cumylphenol are the primary constituents of concern in a groundwater plume at a chemical facility located in Plaquemine, Louisiana. Phytoremediation, a method that uses plants to remove contaminants from water and soil, was posed as a solution to removing the constituents of concern and creating hydraulic control of the plume. Five tree species, eastern redcedar, bald cypress, black willow, eastern cottonwood, and water oak, were chosen as potential remediators. Eastern redcedar and water oak did not tolerate the saline, contaminated water. Bald cypress, black willow, and eastern cottonwood trees were irrigated with deionized water, deionized water containing 0.5 $mg \cdot L^{-1}$ (0.5 ppm) cumene and 1 $mg \cdot L^{-1}$ (1 ppm) 4-cumylphenol as the low concentration, or deionized water containing 1 $mg \cdot L^{-1}$ (1 ppm) cumene and 4 $mg \cdot L^{-1}$ (4 ppm) 4-cumylphenol referred to as the high concentration. Both bald cypress and black willow were the best tree species for remediation of the groundwater as they were able to sequester the constituents of concern in their lower root tissue. Mean concentration of 4-cumylphenol detected in bald cypress roots at the end of the study were 1.72 $mg \cdot kg^{-1}$ in the low concentration water treatment and 1.50 $mg \cdot kg^{-1}$ in the high concentration water treatment. Mean concentration of 4-cumylphenol detected in black willow roots at the end of the study were 16.58 $mg \cdot kg^{-1}$ in the low concentration water treatment and 25.65 $mg \cdot kg^{-1}$ in the high concentration water treatment. Bald cypress was ultimately chosen for full scale planting in the fall of 2008.

Index words: black willow, eastern cottonwood, bald cypress, water contamination, water treatment.

Species used in this study: black willow (*Salix nigra Marsh*.); eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.); bald cypress (*Taxodium distichum* (L.) L.C. Rich.); eastern redcedar (*Juniperus virginiana* L.); water oak (*Quercus nigra* L.).

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Significance to the Horticulture Industry

During periods of an economic upturn, landscaping residential and commercial areas is popular. But in economic downtimes, funds for new landscaping may be limited. Alternative uses for landscape plants aside from aesthetics would help nurserymen continue to prosper in economic downturns. Contamination of water supplies can be a concern with chemical manufacturing. Phytoremediation is a method used to remove or sequester contamination through the use of plant materials. Establishing tolerance thresholds of landscape plants to individual chemicals would not only enable chemical manufactures to develop phytoremediation options, but also add a new sector of clients to the horticulture industry. Bald cypress and black willow were determined to be the best tree species in this study at removing cumene and 4-cumylphenol from contaminated water. Chemicals of concern should be individually tested with tree species to determine which species, if any, remediate chemicals in groundwater.

Introduction

Environmental hazards from chemical manufacturing have been associated with negative health effects on residents located in close proximity to chemical facilities (Cristaldi et al. 1991, Barbosa et al 1995, Valberg et al. 1997, Talmage et al. 1999). Therefore, as the number of oil-refining and chemical-processing plants along the southern portion of the Mississippi River have risen from 126 in 1962 to 196 in 2002 (Colten 2006), the potential impact of these facilities on local residents has also risen.

One method used to mitigate negative impacts of chemical spills is phytoremediation, which uses living plants for the treatment of contaminated groundwater, sediment, sludge and soil (Mirck et al. 2005). Plants absorb, and in some cases degrade, hazardous organic substances through processes such as uptake, accumulation, metabolism, and microbial transformation (Shimp et al. 1993). Phytoremediation potentially has fewer environmental drawbacks as compared to more traditional remediation methods such as soil removal, onsite mechanical treatment, and soil venting and sparging. Sparging is a method by which pressurized air is injected into contaminated water (generally water contaminated by volatile organic compounds). Injecting the air can help the contaminants transform into a vapor state where biodegradation more easily occurs or the vaporized contaminates are vacuumed from the water source. Methods that strip the contaminated area remove harmful chemicals but also remove microorganisms. Microorganisms are a valuable part of the soil system and if possible should be left in the remediated site to aid with proper growth of future vegetation during and after removal of contaminants (Siddiqui 2004). In many instances, the costs associated with phytoremediation are equal to or less expensive than traditional remediation methods. Schnoor et al. (1995) estimated that phytoremediation generally costs between 10 to 50% of the total costs for other traditional remediation methods.

Although phytoremediation can be an economical and effective stabilization and remediation method, its effectiveness is dependent upon the plant's ability to stabilize or remediate the contaminant of concern. For this experiment, the contaminant of concern was a combination of cumene (isopropyl benzene), α, α-dimethylbenzyl alcohol (α, α-DMBA), total phenols and 4-cumylphenol. The contami-

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nants were present at 0.483, 0.0416, 0.071, and 4.40 mg·L^{-1}, respectively, for cumene, α, α-dimethylbenzyl alcohol, total phenols, and 4-cumylphenol in a groundwater plume located anywhere from 3 to 6 m (10 to 20 ft) below the ground surface (Fontenot 2009). These chemicals had been in the water since the 1970s and thought to have been remediated once. Originally the chemicals were put into a holding pond on the property's site. The pond water was pumped and treated and soil was remediated by removal. The pond was then backfilled with uncontaminated soil and monitoring wells were installed. However some of the ground water was still testing positive for cumene and 4-cumylphenol. So an alternative remediation plan had to be implemented. Cumene and 4-cumylphenol were the only contaminants in excess of regulatory standards (0.066 mg·L^{-1} (0.066 ppm) for cumene and 0.180 mg·L^{-1} (0.180 ppm) for 4-cumylphenol) at the site (personal communication with Swain Munson, Principal Consultant for Professional Service Industries Inc., Baton Rouge, LA).

Information regarding the potential metabolites for cumene and 4-cumylphenol under aerobic conditions in soil is thought to follow the subsequent metabolic pathways. There are two hypothesized metabolic pathways for cumene: 1) cumene will metabolize into α, α-DMBA then into α-methylstyrene and water, and 2) cumene will metabolize into acetophenone and methanol (Fontenot 2009). Methanol will then break down into formaldehyde followed by formic acid and finally into carbon dioxide and water. Metabolites of 4-cumylphenol breakdown are believed to be α-methylstyrene and phenol in aerobic conditions.

Phenol was the primary contaminant at the site (Fontenot 2009). Contaminants investigated during bench scale and field pilot studies for the soil remediation for this project site performed in the late 1980s, and phenol was documented to rapidly break down (mineralize) to harmless byproducts in aerobic soil conditions with no plants present (Georgia-Pacific Corporation, 1987 report, Hazardous Waste Closure Plan for the North/South Organic Pond at the Georgia Gulf Corporation. Volume 2). All of the other contaminants, including cumene, α-methylstyrene, acetophenone and 4-cumylphenol, were also documented to mineralize during these previous pilot studies. They remain in the well water because the chemicals are in anaerobic conditions at 3.1 to 6.7 m (10 to 20 ft) below ground.

According to the Interstate Technology Regulatory Council's (ITRC) decision tree, a tool developed to guide users to different suitable technologies to remediate contaminated sites, translocation of α, α-DMBA in plants will occur as it's octanol water partition coefficient (log K_{ow}) is 1.95, whereas, cumene and 4-cumlyphenol will not pass the root membrane since their log K_{ow} values are 3.66 and 4.12 respectively (Schenectady International 2006). Burken and Schnoor (1999) confirmed phenol will be absorbed by plants and that it remains primarily in the roots and bottom portion of the stem. The octanol water partition coefficient assists in determining the applicable portions of the tree to sample when completing final tissue samples for contaminants and their metabolites. Those contaminants with higher log K_{ow}'s (3.0 and above), if absorbed in or on a plants roots, will not likely leave the root tissue, whereas contaminants that are within the 1.0 to 3.0 log K_{ow} range may pass into the roots and move through the xylem up into the leaves, with the extent specific to each contaminant. Based on the chemical make-up of cumene and 4-cumylphenol and their proposed translocation patterns, it is not likely that these two constituents of concern will move further than onto or immediately in the tree's roots. Rather cumene and 4-cumyphenol will adhere to the outside of the trees roots and begin to degrade because the roots will provide aerobic conditions.

The proposed methods of phytoremediation for evaluation in this study included rhizodegradation, phytostabilization and hydraulic control combined with monitored natural attenuation due to contaminant properties at this location. The objectives of this research were to evaluate tree species for survival and growth when subjected to irrigation water contaminated with cumene and 4-cumylphenol, to determine water usage for each tree species tested, and to determine if cumene or 4-cumylphenol were sequestered or metabolized in the tree's tissue.

Materials and Methods

Greenhouse, hydroponic design, and data collection. Studies to determine if select tree species would remediate cumene and 4-cumylphenol occurred over a period of 2 years. The pilot study occurred between June 2006 to March 2007 and the second study occurred from October 2007 to July 2008. Both studies ran for 9 months. Results from the pilot study are not presented in this paper.

A greenhouse 10 by 20 m (30 by 60 ft) was constructed at the chemical facility to house a hydroponic system that prevented precipitation from diluting contaminant water used to irrigate trees. Greenhouse environmental conditions, including relative humidity, temperature, and insect and disease incidence were continuously monitored throughout the experiments. Temperatures in the greenhouse had an average high and low of 36.0 C (97 F) and 8.4 C (47 F) with relative humidity between 65 and 71%. Two studies were conducted concurrently in the greenhouse between October 2007 and July 2008. The studies were a phytoremediation study and a water use by tree species study.

According to the USDA hardiness zones, Louisiana environmental conditions (Zone 9) were within the acceptable range for suitable tree growth (United States Department of Agriculture, Agriculture Research Service 2014). Select tree species were established in plastic injection mold pots (2.7 L, 1 trade gal); filled with FAFARD Red Bag Mix #2 (Hummert International, Earth City, MO) and amended with 4.53 kg of Osmocote Plus12-14 month (Scotts-Sierra Horticultural Products Company, Marysville, OH). Rubber aprons 929 cm^2 (144 in^2) were fitted to the outside of each pot to prevent evaporation between pot seams and the hydroponic trough. Plants were purchased as bare root seedlings ranging between 30.5 to 61 cm (12 and 24 in) in height at the start of the study. Selection of tree species was based on root structure of the trees, preliminary experiments, and scientific literature concerning phytoremediation characteristics.

Trees in both the water usage study and phytoremediation capabilities study were evaluated for growth and quality by assessing tree height (cm), caliper (mm), and leaf color determined visually each month. Foliage was rated on a scale from 1 to 6, 1 representing no green color (only yellow or brown foliage, or no leaves), 3 representing a lighter green color or mottled yellow and brown foliage, and 6 representing healthy dark green foliage. As trees entered a dormant period they were given a visual rating of 1. Tree heights were measured from the soil line in the container to the top of the

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tallest growing point. Caliper was measured even with the top of the container.

Water usage by tree species trial. The water usage portion of the greenhouse used a complete randomized design with 15 troughs, with five trees in each trough. Each trough only included one tree species, with three troughs per species. Five tree species were studied for water usage: bald cypress [*Taxodium distichum* (L.) L.C. Rich.], black willow (*Salix nigra Marsh*.), eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.), eastern redcedar (*Juniperus virginiana* L.) and water oak (*Quercus nigra L*.). Eastern redcedar and water oak were included in the water use portion of the research but failed to survive contact with the contaminated saline water source in the pilot study (data not shown) and thus were not included in the phytoremediation capability trial. Deionized water was filled in each trough to a designated level and measured again at the end of the week to determine the number of gallons used per week. When temperatures increased and trees began using more water, water level was checked biweekly to ensure troughs did not run out of water.

Phytoremediation capability trial. The phytoremediation experiment was arranged as a complete randomized block design, divided into three blocks, eight troughs per block and three trees per trough, one tree from each of the tested species (bald cypress, black willow, and eastern cottonwood). There were a total of twenty-four troughs, eight troughs per water treatment. The three water contaminant treatments in the phytoremediation portion of the study included deionized water, deionized water containing 0.5 mg·L^{-1} (0.5 ppm) cumene and 1 mg·L^{-1} (1 ppm) 4-cumylphenol and deionized water containing 1 mg·L^{-1} (1 ppm) cumene and 4 mg·L^{-1} (4 ppm) 4-cumylphenol referred to as control, low contamination and high contamination, respectively. Deionized water was obtained at the chemical facility and spiked with chemical grade contaminants to achieve the desired concentrations. The low and high contaminant concentrations represent expected exposure levels to trees used for phytoremediation. It was assumed that trees would use water from the contaminated zone 3 to 6 m (10 to 20 ft) below ground) and from the top 0.9 m (3 ft) of soil, which should not be contaminated as it comes from rainwater. Only in periods of extreme drought should trees use water from the contaminated zone only.

Feeder tanks 189 L (50 gal) were positioned on elevated tables at the northern side of the greenhouse. Tanks were filled with contaminant water treatments and connected to troughs by 2.54 cm (1 in) polyvinyl chloride pipe. One trough from each water contaminant treatment was fitted with a sub-port to sample water throughout the study. All troughs were fitted with a flush saddle and drained via a 2.54 cm (1 in) hard polyvinyl chloride pipe to a sub-tank. Water from the sub tank was periodically pumped and disposed with other contaminated water at the facility.

Monthly input and discharge water samples were collected weekly from each water treatment. Vials with a capacity of 40 mL (1.4 fl oz) containing 2 mL dichloromethane (DCM) were transported to the greenhouse. Three vials were filled from each of the three water contaminant treatments. Water samples were collected from designated troughs at the beginning of the week and end of the week. Vials were stored on ice, sonicated and transported to the LSU-RCAT lab. One mL of DCM was extracted from each water sample and placed into a 2 mL crimp top vial with the addition of a 1.0 µL of internal standard. Water samples were analyzed in the Louisiana State University Environmental Sciences Response and Chemical Assessment Team Laboratory (LSU R-CAT Lab) using a modified EPA method 8270 (U.S. Environmental Protection Agency 2007). Each week the troughs were filled with water treatments to a designated level. At the end of the week, the water level was measured and recorded with the remaining trough water discarded. Troughs were filled to the original designated level. In warmer months, the frequency for measuring and replacing water treatments was increased to twice a week. Initial and final plant tissue samples (roots only) were collected and tested for the presence of contaminants. Plant tissue analysis was conducted by Pace Analytical Lab (Minneapolis, MN) using EPA method 8260 for cumene (U.S. Environmental Protection Agency 1996) and EPA method 8270 for 4-cumylphenol (U.S. Environmental Protection Agency 2007).

After nine months, four root sample replicates (each at 100 g fresh weight) of each tree species in each water treatment were sent to Pace Analytical Lab for analysis of contaminants and their metabolites. A total of twelve initial root samples (collected from seedlings that never entered the hydroponic system) and thirty-six final root samples were sent for analysis. Soil was carefully washed away from the roots using deionized water. The roots were then weighed and wrapped in foil. Pace Analytical analyzed the tissue samples using EPA method 8260 (U.S. Environmental Protection Agency 1996) for cumene and EPA method 8270 for 4-cumylphenol. Both mass of the sample and moisture content play a role in determining the detection limit of compounds. Because the actual mass of tissue used and moisture content of each sample was slightly different, there was a different method detection limit for each sample submitted.

Soil extraction method. Initial and final media samples were collected and tested for the presence of the contaminants at the LSU-RCAT Laboratory. Four soil samples from each tree species in each water treatment (n = 36) were collected at the conclusion of the 304 d greenhouse experiment. Final soil samples were analyzed to account for a portion of the mass of chemicals introduced to the hydroponic system. Soil samples were weighed and recorded. The samples were placed in 250 mL beakers and mixed with anhydrous sodium sulfate to remove additional water from the sample. DCM was added to the beaker, covering the mixture, and spiked with 1 mL surrogate standard to account for percent recovery. After extraction, recovery rates were all above 86 percent. The beakers were then placed in a sonic dismembrator for ten-minutes. After sonication, the DCM was poured through a funnel of anhydrous sodium sulfate and collected. The procedure was conducted three times with extraction solutions combined. The combined extraction solutions were condensed using a rotary evaporator. A gentle stream of nitrogen was used to further condense samples to 1 mL which were then placed into a 2 mL gas chromatography/mass spectrometry (GC/MS) crimp top vial. Ten µL of internal standard was added to the vial and then analyzed using a GC/MS for cumene and 4-cumylphenol using the modified EPA method 8270 as described in the cumene and 4-cumylphenol analysis section.

Four composite soil samples collected from the media prior to filling containers were analyzed for contaminants

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prior to the initiation of the experiment. Cumene was not detected, but 4-cumylphenol was present at concentrations below practical quantitation level (PQL). Method detection limits (MDLs) are established using a pure calibration standard. The practical quantitation levels (PQLs) are then determined based on the MDL for each analyte in the calibration standard. PQLs provide the expected concentration for each analyte that can be reliably achieved within specific limits of precision and accuracy. Analytes can be detected but at a decreased certainty if below the PQL

Sludge extraction methods. The bottoms of each trough were covered in 'sludge', a combination of decomposing roots, settled media and water, which was also analyzed for contaminants at the conclusion of the study at the LSU-RCAT Laboratory. The weight of each sludge sample was measured in grams, the sample was put into a 200 mL beaker, and anhydrous sodium sulfate was added to further remove any moisture in the sample. DCM was then poured into the 200 mL beaker to cover the sample and anhydrous sodium sulfate mixture. One mL of surrogate standard was then added to the mixture to later validate the efficiency of the extraction. The beaker containing the sample was then placed in a sonic dismembrator (Fisher Scientific FS14H Fisher Scientific, Hampton, NH) for ten minutes. While the sample was on the sonic dismembrator, number 2 Whatman 15.0 cm No. 40 ashless filter papers (Fisher Scientific, Hampton, NH) were folded in half and placed in a glass funnel to act as a filter. The funnel was placed on a rotary evaporator flask and anhydrous sodium sulfate was placed inside the filter paper lined funnel. The DCM from the sample in the 200 mL beaker was then poured into the funnel over the rotary evaporation flask. This was carefully completed so that the actual sludge sample did not get into the funnel, only the DCM. Additional DCM was then added to the funnel and added to the beaker containing the sludge sample and placed on the sonic dismembrator for an additional 10 minutes. The DCM from the sample was again poured into the funnel. This process was repeated a total of 3 times. Rotary evaporation and a gentle stream of nitrogen were used to condense the sample to a final volume of 1 mL. The 1 mL sample was then placed into a 2 mL crimp top amber vial along with 10 mL of internal standard and capped. Samples were run on the GC/MS using the methods as described in the analysis of cumene and 4-cumylphenol section.

Cumene and 4-cumylphenol analyses. Compounds from the soil and sludge extractions were separated using GC/MS system (5890 GC) coupled with a Hewlett Packard 5972 MSD (Hewlett Packard Palo Alto, CA). The GC was configured with a 30 m by 0.25 mm ID by 0.25 µm film 5% diphenyl/95% dimethyl polysiloxane high resolution capillary column. Samples were auto-injected into the GC at 250 C using a high temperature, low thermal-bleed septa. The initial column temperature was 40 C for 4 minutes and subsequently increased to 280 C at 6 C/minute before being held for 3 min. The sample run time was 47 min per sample. The interface to the MS is maintained at 280 C. Ultra high purity (UHP) helium was the carrier gas. The mass spectrometer (MS) was operated in Selective Ion Monitoring (SIM) mode to maximize the detection of cumene and 4-cumylphenol. The MS was operated so that selective ions for each acquisition window were scanned at a rate >1.8 scans/sec with a dwell time of 75 ms. At the initiation of each analysis period and every 12 h the MS was calibrated using perfluorotributylamine (PFTBA), an internal instrument standard. Prior to analyses of collected samples, a calibration standard was analyzed. This standard operating procedure ensured quality assurance/quality control of the instrument conditions prior to sample analysis. Spectral data was processed by Chemstation™ Software (Agilent, Santa Clara, CA) using a customized data processing macro developed by LSU-RCAT.

The analysis method was conducted on each sample and results in raw integration data transferred to a database spreadsheet program for quantitative analysis. A macro printout was also generated and contained the extracted ion chromatography data in addition to raw integration data. Analyte concentrations were calculated based on the internal standard using integrated peak areas under the curve. An internal standard mixture, composed of naphthalene-d_8, acenaphthene-d_{10}, chrysene-d_{12}, and perylene-d_{12} (at concentrations of 10 ng·uL^{-1} (10 ppb)) was spiked into sample extracts just prior to analysis. The concentrations of specific target analytes were determined by a 5-point calibration and internal standard method.

Statistical analysis. Tree height, caliper and visual rating averages and significances were determined using the PROC Mixed 1998 SAS program accompanied by Saxton's Macro (SAS Institute Inc., Cary, NC). Differences reported are of Least Squares Means. A PROC Mixed SAS program paired with Saxton's Macro was also used to statistically analyze water use by tree species data. Statistical analysis was not performed between species for the visual ratings as the species had different growth rates. Statistical analysis was not performed on the contaminant concentration in plant tissue.

Results and Discussion

Water use by tree species in non-contaminated water troughs. Based on measurements throughout the entire study, a daily water use by species average was calculated (Table 1). Black willow followed by eastern cottonwood had the greatest water uptake rates compared to eastern red cedar, bald cypress and water oak trees. No differences in water use rates were observed between bald cypress and water oak.

Water uptake data was also collected from the trees exposed to contaminated water treatments for bald cypress, black willow and eastern cottonwood trees. Trees growing in deionized water used 1.74 L·day^{-1} (0.46 gal·day^{-1}) compared to1.58 L·day^{-1} (0.41 gal·day^{-1}) in the low water concentration and 1.38 L·day^{-1} (0.36 gal·day^{-1}) for trees growing in the high

Table 1. Daily average of deionized water (liters) used by tree species over a nine month period in a greenhouse trial between October 2007 to July 2008.

Species	Average daily water use (L)^z
Black willow	1.28a
Eastern cottonwood	0.95b
Eastern redcedar	0.64c
Bald cypress	0.53cd
Water oak	0.34d

^{z}Values in columns with different letters are statistically significant at $p \leq 0.0001$ using a PROC Mixed SAS program paired with Saxton's Macro.

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Table 2. **Average daily water used for individual troughs containing one of each of the tree species bald cypress, black willow and eastern cottonwood by water treatment, deionized, low contaminant and high contaminant water treatments between October 2007 to July 2008[z].**

Water treatment	Average daily water use (L)[y]
Deionized water	1.74a
Low contaminant	1.58ab
High contaminant	1.38b

[z]Each trough contained one water treatment, Low Contaminant (deionized water containing 0.5 $mg \cdot L^{-1}$ (0.5 ppm) cumene and 1 $mg \cdot L^{-1}$ (1 ppm) 4-cumylphenol) or High Contaminant (deionized water containing 1 $mg \cdot L^{-1}$ (1 ppm) cumene and 4 $mg \cdot L^{-1}$ (4 ppm) 4-cumylphenol). Each trough contained one of each of the three tree species: bald cypress (*Taxodium distichum*), eastern cottonwood (*Populus deltoides*) and black willow (*Salix nigra*).

[y]Numbers with different letters are statistically significant at $p \leq 0.006$ using a PROC Mixed SAS program paired with Saxton's Macro.

water concentration (Table 2). Each trough had one of each of the three tree species growing in it therefore water uptake could not be separated by tree species. Trees in the deionized water (control water) treatment on average used more water than trees growing in the high water concentration $p \leq 0.006$ (Table 2). There were no statistical differences in water uptake between the deionized water and low water concentration and between the low and high water concentrations. The different levels of contaminated water were originally designed to depict periods of time when trees would be using all or portions of the water they need for survival from the contaminated groundwater. But in reality the deionized water treatment reflects trees not exposed to contaminated water, the low water concentration reflects trees exposed to a lower dose, and the high water concentration rate reflects exposure to a higher dose of contact with contaminated water.

Plant growth. Tree height was not significantly affected by contaminant levels for bald cypress or eastern cottonwood trees (Table 3). Three of the eight bald cypress trees growing in the high contaminated water treatment died prior to the end of the experiment. There was not a clear prognosis on why these 3 trees died. There were no apparent insect or disease symptoms to these trees. The troughs that housed these three trees did not leak water nor did they ever run dry. Presence of contaminants cumene and 4-cumylphenol could explain the death but is not very clear as the other 5 trees remained actively growing and healthy throughout the trial. The three trees that died were included in final data collection of height and calipers. The tallest black willow trees grew in the high contaminated water treatments followed by the low contaminated water treatment and the shortest black willow grew in the deionized water treatments. These results suggest that the black willow is the most tolerant tree species of those tested in the contaminated water treatments.

Trunk diameters increased in all tree species from start to end of the study regardless of water contaminant treatments. There were no significant differences in caliper measurements at the end of the study within species between water treatments, indicating that the presence or lack of chemicals in the water do not affect trunk diameter (Table 3).

Leaf color in eastern cottonwood trees in all contaminant treatments and in bald cypress at the high contaminant treatment significantly declined compared to the trees growing in the deionized water treatment as determined visually at the end of the study (Table 3). The decrease in eastern cottonwood rating scores at the end of the study as compared to their visual ratings at the start of the study were based on pale foliage in all three water treatments and not mortality.

All black willow trees survived the study with excellent visual ratings in all water treatments, indicating the ability of this species to survive contact with, and thrive in the presence of, the contaminated water. One eastern cottonwood tree in the low water concentration and one eastern cottonwood tree in the high treatment died in May, again there were no apparent insect or disease symptoms so presence of contamination is a potential factor in the death of these trees. However, the remaining 7 trees in each water treatment were healthy

Table 3. **Average percent change from October 2007 to June 2008 in height (m), caliper (mm), and visual rate (Scale 1–6) of bald cypress, black willow and cottonwood, trees growing in contaminated water treatments[z].**

	% Height increase[y] (meters)			% Caliper increase[x] (mm)			% Change in visual rating[w] (using visual rate scale 1-6)		
Water treatment[v]	Bald cypress	Black willow	Eastern cottonwood	Bald cypress	Black willow	Eastern cottonwood	Bald cypress	Black willow	Eastern cottonwood
Deionized	62.8a	78.1a	68.2a	78.0a	74.3a	67.2a	2.3ab	0.0a	–32.4abc
Low concentration	53.5a	81.6b	71.7a	72.2a	75.8a	66.9a	5.3a	0.0a	–41.8bc
High concentration	57.6a	80.0b	70.9a	73.0a	75.9a	70.2a	–41.7d	0.0a	–47.8c
Significance	NS	*	NS	NS	NS	NS	*	NS	NS

[z]Percent change in height, caliper and visual rate values with different letters within columns are significantly different at $p \leq 0.05$ using a PROC Mixed SAS program paired with Saxton's Macro.

[y]Initial average height measurements: bald cypress 0.35 DI, 0.40 Low, and 0.36 High: black willow 0.82 DI, 0.76 Low, and 0.83 High: eastern cottonwood 0.35 DI, 0.34 Low, and 0.32 High.

[x]Initial average caliper measurements: bald cypress2.8 DI, 3.2 Low, and 3.1 High: black willow 5.6 DI, 5.3 Low, and 5.1 High: eastern cottonwood 4.3 DI, 4.7 Low, and 3.6 High.

[w]Initial average visual rating measurements: bald cypress 5.6 DI, 5.5 Low, and 5.5 High: black willow 6.0 DI, 6.0 Low, and 6.0 High: eastern cottonwood 5.9 DI, 5.9 Low, and 6.0 High. Visual scale 1 = no leaves or all brown leaves; 3 = half brown half green leaves; 6 = all green leaves.

[v]Deionized (deionized water only), Low (deionized water containing 0.5 $mg \cdot L^{-1}$ (0.5 ppm) cumene and 1 $mg \cdot L^{-1}$ (1 ppm) 4-cumylphenol) or High (deionized water containing 1 $mg \cdot L^{-1}$ (1 ppm) cumene and 4 $mg \cdot L^{-1}$ (4 ppm) 4-cumylphenol). % change in height, caliper and visual rating.

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through the end of the trial. The three bald cypress and two eastern cottonwood trees that died did so in the last 2 months of the experiment. These trees may have declined because of the small containers they were planted in, lack of fertilization, poor quality seedlings or contact with contaminants, since the greenhouse did reach temperatures far above 21 C (70 F) environmental conditions were not considered a factor. Eastern cottonwood trees at the end of the study did not significantly differ in their visual ratings between water treatments. However, eastern cottonweed tress in all three treatments declined significantly in their visual ratings from the start to the end of the study. This species may be a poor selection because it may not be suited to a Louisiana climate inside a greenhouse structure or may not be suited for growth in hydroponic systems.

All black willow water treatment combinations and bald cypress growing in deionized water and low contaminate concentrations showed no deleterious effects to their foliage during the course of the 304 d experiment (Table 3). The visual ratings suggest that the black willow tree is the optimal species for full scale planting over a contaminated plume with bald cypress as a secondary choice for remediation.

Target compounds and their potential metabolites — plant tissue samples. During the pilot study (June 2006 to March 2007), contaminants and their metabolites were not detected within tree shoot tissues (data not shown). Therefore, the presence of the contaminants and metabolites were only collected from root tissue samples in the second year (October 2007 to July 2008) of the study. Contaminants and metabolites were not detected in the initial root samples for any of the three tree species analyzed at 0 d. At the conclusion of the 304 d experiment, 4-cumylphenol was detected in root tissues for all three tree species growing in the low and high contamination water treatments. Black willow, bald cypress and eastern cottonwood trees contained an average of 16.6, 1.7, and 1.4 $mg \cdot kg^{-1}$ 4-cumylphenol in the low contaminant water treatment and 25.6, 1.5, and 3.8 $mg \cdot kg^{-1}$ 4-cumylphenol in the high contaminant water treatments, respectively (data not shown). A metabolite of cumene, α – DMBA, was detected only in black willow and eastern cottonwood root tissues exposed to the high contaminant water treatment at 0.364 and 0.627 $mg \cdot kg^{-1}$, respectively (data not shown). Although roots were also analyzed for the presence of cumene, acetophenone, α-methyl styrene and phenol, these compounds were not detected (data not shown). Results suggest that the black willow is the best candidate for sequestration of cumene and 4-cumylphenol when grown in contaminated water.

Semi-volatile organic compounds (SVOCs) and volatile organic compounds (VOCs) considered in this study as tentatively identified compounds (TICs) are possible metabolites of the parent compounds, cumene and 4-cumylphenol. Because the parent compound 4-cumylphenol was present in root tissues for all three-tree species evaluated, initial TICs were compared to final TIC results. Compounds that were present in initial and final samples were excluded because they are most likely natural-occurring constituents within the root tissues. Comparison of TIC constituents were also compared between species per water contaminant treatments. Compounds that were present in both contaminated and non-contaminated water samples were excluded from further analysis. Two VOC TICs were identified in root tissue of black willow trees growing in the low concentration water treatment, pentane 0.03 $mg \cdot kg^{-1}$ and 1-octen-3-ol 0.21 $mg \cdot kg^{-1}$ (data not shown). Two VOC TICs were identified in the final low concentration water treatment in eastern cottonwood root samples; they were acetone 0.07 $mg \cdot kg^{-1}$ and 1-octen-3-ol 0.34 $mg \cdot kg^{-1}$. Two VOC TICs were identified in the high concentration water treatment bald cypress root samples; they were α – pinene 0.08 $mg \cdot kg^{-1}$ and 1,4-pentadiene,2,3,3-trimethyl- 0.02 $mg \cdot kg^{-1}$ (data not shown). There were no new VOC TICs found in eastern cottonwood roots growing in the final high concentration water treatment or found in the bald cypress roots growing in the low concentration water treatment.

Several SVOC TICs were identified in the final root tissue samples that were not identified in the initial root tissue samples or in the final deionized water samples. Of these, several were disregarded because their molecular weights were greater than 4-cumylphenol and they had more complex structures than the original parent compounds. The SVOC TICs listed have met the same two criteria that the VOC TICs met. They were not identified in the deionized water treatment and they were not identified in the initial tissue samples. Vanillin, (synonym is 4-hydroxy-3-methoxy-benzaldehyde) 0.8 $mg \cdot kg^{-1}$ was identified in a low concentration water treatment in one bald cypress root sample (data not shown). Vanillin is common in plant–based food materials and could be a natural component of plants. Propenoic acid, 3-phenyl- at concentrations of 1.5 and 1.1 $mg \cdot kg^{-1}$ were identified in two of the four eastern cottonwood root samples collected in the low concentration water treatment and one of the four eastern cottonwood root samples collected from the high concentration water treatment at a concentration of 1.3 $mg \cdot kg^{-1}$. In one of the four eastern cottonwood root samples from the high concentration water treatment, c9H-xanthene at a concentration of 2.1 $mg \cdot kg^{-1}$ was identified. Vanillin (benzaldehyde, 4-hydroxy-3-methoxy) at a concentration of 0.8 $mg \cdot kg^{-1}$ was identified in a bald cypress root sample in the high concentration water treatment. The final SVOC TIC identified was 2-cyclohexen-1-one at a concentration of 11 $mg \cdot kg^{-1}$ identified in a black willow root sample from the high concentration water treatment (data not shown). The SVOCs, similar to the VOCs, amounted to less than 1% of the total mass of parent compound that was added to the hydroponic system, and are not considered to be significant metabolites of the parent compounds. Some of the parent compounds were sequestered in the root tissue of the trees, but metabolites were not found. The contaminants are either being absorbed by the tree roots quickly, metabolized into non-detectable analytes or the total concentration of parent compound is not being taken up by the trees roots.

Soil samples. The PACE reporting limit for 4-cumylphenol in soil is 0.33 $mg \cdot kg^{-1}$. The contaminants were not detected for any of the soil samples in quantities above practical concentration levels (data not shown). Therefore, the contaminants cumene and 4-cumylpheonol are not sequestered in the media surrounding tree roots.

Sludge samples. Sludge in this study is defined as the remaining soil, plant debris and water in the bottom of the hydroponic troughs at the conclusion of the experiment. Cumene and 4-cumylphenol were not present in the non-contaminated treatment for any tree species. However, trees

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exposed to the low contaminant water treatments had cumene concentrations between 0.4 and 2.0 $\mu g \cdot mL^{-1}$ but no detectable 4-cumylphenol concentrations (data not shown). In the high contaminant treatment, sludge contaminants ranged from 0.00 to 1.50 μg cumene$\cdot mL^{-1}$ with no detection of 4-cumylphenol. The total mass of cumene present in sludge samples accounted for < 0.5% of the total mass of cumene injected into the hydroponic system over the course of the greenhouse experiment.

Both bald cypress and black willow tree species have phytoremediation capabilities after exposure to cumene and 4-cumylphenol-contaminated water. The bald cypress caliper and height measurements in the low and high concentration water treatments were not significantly different from caliper and height measurements of bald cypress growing in the deionized water. The average leaf rating of bald cypress trees in the high concentration water treatment was lower than bald cypress trees growing in the low concentration and deionized water treatments at the end of the study. The purpose of the high water treatment was to test the trees tolerance at the highest recorded water contamination rate. In a field situation, the trees would not likely be exposed to these high levels of contamination as they would use a combination of fresh rain water and contaminated plume water. The black willow was the only tree with a visual leaf rating of 6.0 (dark green and healthy) at the end of the study. Eastern cottonwood and bald cypress trees maintained a 4 to 4.5 and 4 to 5.8 visual ratings, respectively, at the end of the study. In addition to plant quality, the concentration of contaminants and TICs detected in final plant tissue was an important factor in selecting the tree species for full-scale implementation of phytoremediation. Four-cumylphenol was detected in the roots (growing in low concentration and high concentration water contamination) of all three tree species. Black willow trees had the highest concentrations of 4-cumylphenol detected. Black willows were larger in stature and took up more water than other tested species. There were no significant TICs found in the tree tissue.

Although black willow proved to be the most suitable candidate for phytoremediation of both cumene and 4-cumylphenol, bald cypress species was ultimately chosen as the candidate for full scale planting and phytoremediation at the closed and capped surface impoundment located at the chemical plant facilities due to its tolerance to salt water, a deep root system, and low maintenance requirements (Fontenot 2009). While black willow was acceptable for phytoremediation of the contaminants, bald cypress was the tree species that is most compatible with the chemical facility security requirements, which included the ability to see a fence line, potential decrease in debris from storms, and ability to prune the trees. The SVOC and VOC TICs detected in the roots of trees used in this study amounted to less than 1% of the total mass of parent compound that was added to the hydroponic system, Therefore it is difficult to determine how rapidly and in what quantity the trees are absorbing and metabolizing the chemicals. However, some absorption is probably occurring because there were no chemicals of concern found in the soil surrounding the tree roots. Additionally, no parent materials or their metabolites were detected in the tree shoots or in the discharge water each time troughs were drained.

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