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Benzene Toxicity and Removal in La[illegible]tory Phytoremediation Studies

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Abstract: Hybrid poplar cuttings were shown to impact the fate and transport of subsurface benzene, while toxicity to the poplars was not observed. Laboratory experiments investigated the toxicity response of poplar cuttings to benzene exposure, contaminant distribution in plant tissues, contaminant degradation in the soil profile, and contaminant volatilization from the soil and plant tissues. Two separate studies were conducted to evaluate these parameters. The first study examined the toxicity of benzene to hybrid poplar cuttings in batch reactors. Poplar cuttings were exposed to various concentrations of benzene contaminated water in two different types of soil. Transpiration rates were measured as an indicator of acute toxicity. No acute toxicity was noted for dose concentrations up to 1,000 ppm. The second study evaluated benzene fate and transport. Live poplar cuttings and excised controls were planted in flow-through reactors and supplied with an influent benzene stream to mimic plume conditions. The presence of live poplar cuttings enhanced benzene degradation and decreased the effluent mass of benzene. A small amount of benzene was also volatilized from the plant tissues, providing evidence of plant-enhanced volatilization. Causes for the higher degradation rates appeared to be greater microbial populations of benzene degraders and a more oxygen-rich environment. The higher redox potential observed may be an artifact of the laboratory reactor design. The results obtained in this research combined with previous studies provide evidence that phytoremediation has the potential for effective, efficient, and environmentally friendly application at sites highly contaminated with benzene and potentially for other sites contaminated with biodegradable organics or volatile organic compounds.

INTRODUCTION

The most common organic ground-water contaminants in the United States are trichloroethylene, tetrachloroethylene, benzene, toluene, and xylene (Baird 1995). Ground-water contamination due to benzene is typically associated with the leakage of refined petroleum products from underground storage tanks (Suthersan 1997) but can also be related to various manufacturing processes. According to the Toxics Release Inventory published by the Agency for Toxic Substances and Disease Registry of the U.S. Environmental Protection Agency, releases of benzene to water and land totaled over 2×10^6 lb between 1987 and 1992. In 1989 the EPA placed a maximum contaminant level of 5 parts/billion for ground-water contamination levels of benzene.

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Phytoremediation, the use of plants to remove contaminants from subsurface water and soil systems, is a relatively new technology developed in the past two decades as an alternative to more expensive remediation technologies, such as pump-and-treat, air sparging, reactive walls, and stabilization technologies. Various species of grasses have been shown to increase the degradation of numerous petroleum-based organics such as anthracene, pyrene, and other polycyclic aromatic hydrocarbons (Schwab and Banks 1994; Reilley et al. 1996; Wetzel et al. 1996). There are a number of studies that have investigated organic contaminant fate and toxicity in plants (Briggs et al. 1982; Bromilow and Chamberlain 1995; Trapp and McFarlane 1995; Burken and Schnoor 1998; Schneidemann et al. 1998; Thompson et al. 1998; Trapp 2000; Dietz and Schnoor 2001). Work with benzene indicated significant uptake and volatilization of benzene from the leaves of poplar trees grown hydroponically but show little apparent degradation (Burken and Schnoor 1999). In this study by Burken and Schnoor (1999) the majority of benzene taken up from the hydroponic solution was volatilized. Uptake and fate of benzene using poplar trees grown in soil was much different as little was volatilized and the results were quite variable (Burken 1996). In research with alfalfa plants and soil, soil volatilization was the dominant removal mechanism in a small laboratory study (Ferro et al. 1997). The same study concluded that the plant biomass did not accumulate benzene at measurable levels and that the alfalfa plants did not increase mineralization. Ferro et al. (1999) conducted two field-scale studies measuring the phytotoxicity of a volatile organic compound mixture to poplar trees. These studies provided no conclusive results

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indicating any plant toxicity from exposure to the volatile organic compound mixture.

Recent research has generated a general understanding of fate, transport, and toxicity processes of petroleum-based organics but has also generated many questions concerning the effectiveness and practicality of the use of phytoremediation for such contaminants. There has been much conjecture regarding the phytoremediation of many organics based upon limited studies with selected compounds.

In the research presented in this paper, hybrid poplar trees were tested in whole-plant laboratory-scale studies to investigate the potential to effectively remediate benzene in ground water and soil. The objectives of the following studies were to

- Assess the toxicity levels of various concentrations of benzene to poplar cuttings.
- Investigate the degradation of benzene in the rhizosphere.
- Determine the distribution of benzene and its metabolites in the plant tissues.
- Delineate the amount of benzene volatilized from plant tissues compared to the benzene volatilized from the soil.

The studies presented here provide specific data relating to the phytoremediation of benzene contaminated soil and ground water and will add to the overall knowledge base relating to phytoremediation of organics.

METHODS AND MATERIALS

The hybrid poplar *Populus deltoides* × *Populus nigra* (DN-34), also known as Imperial Carolina, is used for all the studies detailed herein. This established hybrid grows well in the midwestern United States and is disease resistant.

Toxicity

All toxicity studies conducted and reviewed for the completion of this research used the following arrangement. Plant growth reactors were 1-L clear, wide-mouth glass bottles (I-Chem, Fisher Science) fitted with Teflon-lined lids. Uniform silica sand, 150 g, was placed in the bottom of the jars and leveled. On top of the sand, soil was placed in accordance with the specifications set forth for each toxicity study.

DN-34 hybrid poplar whips were cut to an approximate length of 8 in. They were then allowed to begin sprouting in a bed of sand watered with 1/4 strength Hoagland's solution (Table 1). Once the whips exhibited evidence of sprouting, they were rinsed clean with deionized water and inserted into the soil through holes in the caps of the reactors. A Teflon feeding tube was inserted through a hole in the lid of each reactor and placed so that the plants would be watered from the bottom, to mimic field conditions and limit direct volatilization. The reactors were wrapped in foil to prevent algal growth. Latex caulk was applied at the lid interface around the feeding tubes and the whips to seal the reactor. The dry reactors with the plants were weighed and recorded. These reactors were configured as shown in Fig. 1.

TABLE 1. Hoagland's Nutrient Solution

Ingredient	Concentration (mg/L)
KH_2PO_4	208
$Ca(NO_3)_2$	161
$CaSO_4$	289
KNO_3	137
$MgSO_4$	469
K_2SO_4	161

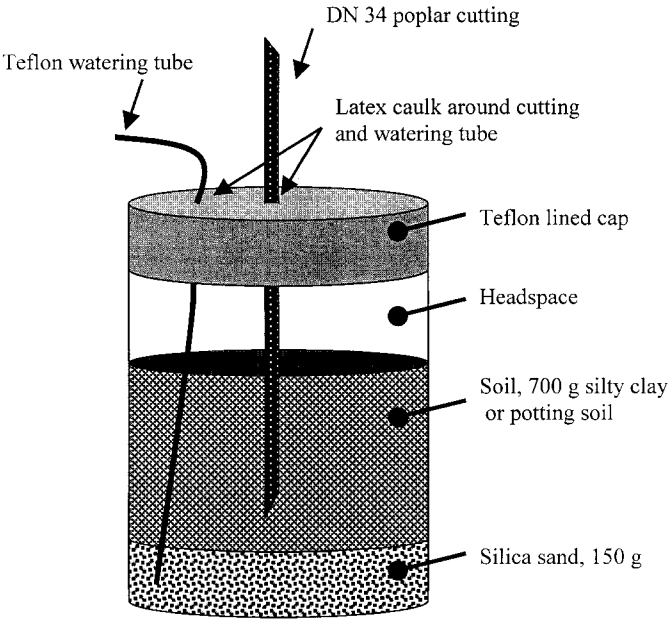

FIG. 1. Schematic of Laboratory Setup for Benzene Toxicity Studies

Water was added to reach a soil moisture of 80% of field capacity. The reactors were placed in a fume hood fitted at 20 and 24°C with a set of eight 40-W fluorescent bulbs (Verilux, full spectrum natural light) with an approximate intensity of 1,580 lx, on a 16-h photoperiod. At the time of reactor assembly, the poplar cuttings were watered with 1/4 strength Hoagland's solution (Table 1), but after this initial nutrient supplement to the cuttings in the reactors they were watered only with deionized (DI) water for 3 weeks before being exposed to benzene-contaminated water. This growth time in the reactors was permitted to allow for further sprouting of the whips. A stock solution of DI water saturated with benzene was placed in dark brown glass bottles and stored on a rotating mixer table at low speed. The stock solution was used for watering the poplar cuttings.

Study 1A

The soil specified for this study was silty-clay native Missouri soil (see Table 2 for soil properties). The soil

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TABLE 2. Soil Properties of Silty-Clay Used in Benzene Toxicity Tests

Constituent	Measurement and units
Phosphorous (Bray)	23 ppm
Potassium, K	100 ppm
Zinc (Zn)	0.9 ppm
Sodium (Na)	9 ppm
Sulfur (S)	19 ppm
Boron (B)	1 ppm
Copper (Cu)	1.1 ppm
Manganese (Mn)	215 ppm
Iron (Fe)	132 ppm
Organic matter	2.4%
pH	5.0
Buffer index	6.4
Calcium (Ca^{2+})	900 ppm
Magnesium (Mg^{2+})	280 ppm

Note: Laboratory analysis performed by Minnesota Valley Testing Laboratories, Inc., Nevada, Iowa.

TABLE 3. Soil Properties of Potting Mix Used in Toxicity Tests[a]

Constituent	Measurement and units
Available phosphate (P_2O_5)	0.14%
Potassium oxide (K_2O)	0.14%
Iron (Fe)	0.1%
Zinc (Zn)	0.05 ppm
Cobalt	28.05 ppm
Cadmium	0.1 ppm
Arsenic	0.1 ppm
Mercury	0.001 ppm
Molybdenum	0.1 ppm
Nickel	0.1 ppm
Lead	0.1 ppm
Selenium	0.5 ppm

[a]From Swiss Farm Products, Inc. (1999). Adapted from the Washington State Department of Agriculture, Fertilizer Product Information Webpage.

was sieved to remove coarse material and was then homogenized. Soil, 700 g, was placed into the reactors on top of the silica sand, and the reactors were assembled as described previously.

A final crop of 18 uniform, viable poplar cuttings was used for the study. These cuttings were sorted into six groups with three plants each. Group A was designated the control group. Groups B through F were dosed with 1, 5, 10, 50, and 100 ppm benzene, respectively, at each watering event.

Each time the plants were watered, a predetermined amount of saturated benzene solution was mixed with DI water in a beaker and immediately transferred via syringe into the Teflon feeding tubes. The reactors were weighed both before and after each watering event to measure transpiration and verify the amount of benzene solution added gravimetrically. Increased plant mass due to growth was negligible (<5 g/cutting) over the course of the experiments. Cuttings were watered at average intervals of 3–5 days.

After 17 days of showing no toxic effects from the benzene, the dose concentrations for groups B and C were increased from 1 and 5 ppm to 500 and 1,000 ppm, respectively. The study ran for a total of 115 days, including 17 days for the initial dosing arrangement and 98 days for the modified dosing arrangement.

Study 1B

The experimental setup was identical to Study 1A with the exceptions that follow. The soil used was a commercial potting soil (Swiss Farms Products, Inc.) (see Table 3 for soil properties). A final crop of 13 poplars was used. These were sorted into three groups with three plants each, plus one extra. Group D was designated the control group. Group B was dosed with 100 ppm, Group C with 500 ppm, and Group A with 1,000 ppm. The study ran for a total of 122 days. One extra plant was dosed with benzene saturated DI water (approximately 1,800 ppm) for the final 51 days. The commencement of dosing was staggered for the poplar cuttings in Study 1B because a uniform crop of viable poplars was not established at the initiation of the experiment. Thus, the study was initiated with a uniform crop of nine poplars comprising groups B, C, and D as described previously. Dosing continued for 122 days. Group A was established and dosing began on day 44 and continued for 78 days. One lone reactor, 1E, was dosed with benzene saturated DI water beginning on day 81 and continued for 41 days. All cuttings were approximately the same age and size when dosing began. The dosing method was changed so that a predetermined amount of stock solution and DI water were mixed directly within the syringe, minimizing possible volatilization of benzene from the beaker during mixing.

Fate and Transport

Study 1C

After the plants were given a final feeding of benzene-contaminated DI water, the growth lights remained on for 24 h prior to termination. At the end of the 24-h period, each reactor was weighed and disassembled. Each plant was separated into leaves, new stem growth, and the original plant stem. Soil cores were also taken from each reactor, using a 0.3-in.-diameter hollow push tube. All samples were weighed and immediately transferred to 20-mL crimp-seal vials. After reactors were disassembled, the sample vials were allowed to equalize for a minimum of 48 h before analysis. All samples were then analyzed via gas chromatography (GC) to determine the gas-phase concentration of benzene (X. Ma, personal communication, 2000).

Study 2

One-liter screw top modified Erlenmeyer flasks were filled with 300 g of uniform silica sand and 800 g of the silty-clay soil (Table 2). Hybrid poplar whips were inserted through holes in Teflon-lined lids and planted in the flasks. An inverted 1-L Erlenmeyer flask was placed over the protruding stem of the poplar whip and sealed in place above the lower flask to create an enclosed system. The flasks were modified to accommodate influent and

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effluent airstreams in both the upper and lower chambers and influent and effluent aqueous streams through the bottom of the soil compartment, thus creating a continuous-feed system. A schematic of the arrangement is shown in Fig. 2.

The experiment was comprised of eight reactors. Reactors 1 through 6 contained live poplar cuttings, and reactors 7 and 8 contained the roots and stem of hybrid poplars, the foliar portion being excised prior to benzene introduction. The reactors were then supplied with an influent stream of 25 mg/L of benzene to mimic plume conditions, at a flow rate of 100 mL/day for the duration of the experiment. Airflow through the aerial chamber of the reactors was regulated at 2 L/min and through the bottom soil compartment of the reactors was regulated at 0.1–0.5 L/min. Reactors were kept in a growth room at 20–30°C with a 1,000-W mercury halide light (Compact Metalarc, Sylvania) on a 16-h photoperiod. Light intensity was measured at >210 μmol/m^2-s (photoactive range) at the leaf surface in the aerial compartment.

Effluent collection bottles and activated carbon traps were used to monitor aqueous benzene leaving the enclosed system. The effluent was analyzed directly at the effluent port via GC, 2-μL liquid injection. The effluent was collected daily in 250-mL sample bottles to determine effluent volumetric flow. Activated carbon traps (Supelco), 1 g, were placed as shown in Fig. 2 to collect organics in the off-gases of both the top aerial chamber and the bottom soil compartment. These traps were changed at 24–48-h intervals during the first stages of the experiment and at greater intervals as the project continued. It became evident that the traps were not being saturated with benzene and gas-phase concentrations were steady. After collection of the organics in the off-gases, the activated carbon in each trap was placed in 4-mL vials. The activated carbon was extracted by adding 2-mL carbon disulfide to each vial and placing sealed vials on an orbital shaker table for 24 h. Samples of the extract, 2 μL, were analyzed via GC using a liquid autosampler.

Over a 3-day period, beginning on day 36, ^{14}C-labeled benzene was used to evaluate contaminant fate in plant tissues, soil, and in the off-gases. Once per day, 5 μCi of uniformly labeled benzene was injected into the influent line at the reactor inlet. Sodium hydroxide (NaOH) traps were added to the air effluent line in series after the activated carbon traps to capture CO_2, which would not be adsorbed by the activated carbon. The traps used a fritted-glass diffuser to pass the effluent airstream through 150 mL of 1 N NaOH. Aliquots, 200 μL, of the NaOH were analyzed via liquid scintillation counting (LSC) methods. During exposure of the system to ^{14}C benzene, aqueous effluent samples were tested via LSC techniques in addition to the GC analysis. Aqueous effluent and NaOH traps were monitored for 2 days after the use of the ^{14}C benzene was halted.

Following termination of the study, the reactors were dismantled, and the poplar tissues were directly analyzed for benzene and/or benzene metabolites, as ^{14}C. Plant tissues from each reactor were separated into leaves, stems, and roots; weighed; and analyzed using biological oxidation and LSC. Random soil cores, three to six per reactor, were taken and analyzed via biological oxidation and LSC. Detailed methodology for determining ^{14}C in tissue and soil via biological oxidation and LSC appear in Burken (1996).

In addition to analyzing for ^{14}C content at the close of the study, soil cores were analyzed for microbial populations. A series of five soil cores per reactor were collected and analyzed for both total heterotrophs and benzene degraders. Microbial analysis was done on soil core samples

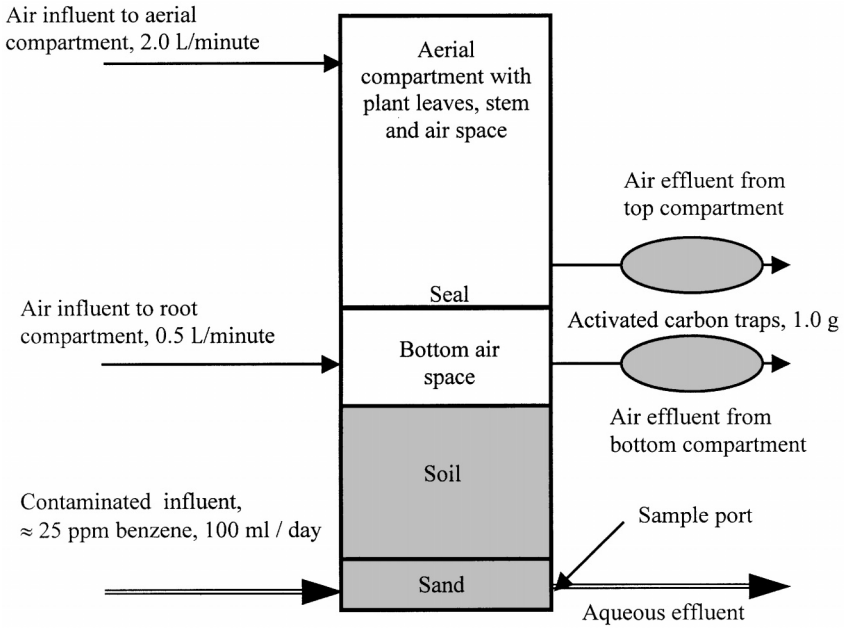

FIG. 2. Schematic of Laboratory Setup for Study 2: Benzene Fate and Transport

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weighing approximately 1 g. Soil samples were removed from each reactor using a push-tube. Each soil sample was then weighed and added to a 150-mL Erlenmeyer flask containing 10 mL of phosphate buffer solution (100 μM, pH 7). The flasks were agitated on a shaker table for 15 min and subsequently allowed to settle. Serial dilutions were performed on 1-mL aliquots of each sample solution down to a low value of 10^{-10}. A 1-mL aliquot of each serial dilution was then placed on each of two types of 60-mm-diameter agar plates to allow for colony growth. One set of plates contained nonselective media and promoted total heterotroph growth. The other set of plates was prepared with only minimal salts media and 2 μg/mL of benzene as the sole carbon source. The inoculated plates were incubated for 48 h at 30°C, and all colonies visible were counted manually.

Near the end of the experiment, the pH and oxidation-reduction potential of the effluent solution were tested directly at the effluent port before appreciable change could occur. Approximately 30 mL of effluent were collected from each of three reactors with live cuttings (reactors 2, 4, and 6) and one reactor with an excised poplar cutting (reactor 8). Data were collected using an oxidation-reduction potential probe (Orion, Model 967800) and a pH probe and meter (Hanna, model 9025).

DISCUSSION OF RESULTS

There was no evidence of acute short-term toxicity in the hybrid poplar cuttings at the close of Studies 1A and 1B, as shown by the stable transpiration rates of the poplar cuttings in Figs. 3–6. The control groups, Group A in Study 1A and Group D in Study 1B, were anticipated to show the highest transpiration rate throughout the studies, and the plants dosed with various concentrations of benzene were expected to exhibit increasingly lower transpiration respective to benzene concentration. Plants being dosed with toxic levels of benzene were also expected to exhibit chlorosis (yellowing) and leaf drop (Ferro et al. 1999). These results were unexpected as earlier screen tests done hydroponically indicated rapid toxicity at concentrations below 500 ppm (unpublished observations).

There are several possibilities for this difference in apparent toxicity between hydroponic studies and soil studies. The type of soils used may impact the exposure of benzene to the plant roots, because of potential sorption of benzene to the soil. However, over the period of this study it was expected that the sorptive capacity of the soil would have reached equilibrium. The hypothesis that equilibrium was reached is supported by data from Study 2 showing steady effluent concentrations. Microbial degradation could act as a buffer to toxicity at the high mass-loading rates in both experiments, acting to degrade the benzene prior to contacting the poplar roots, as opposed to the hydroponic observations. Regardless of mechanism, poplars were able to withstand benzene feed concentrations of up to 1,000 ppm and continue growing for 78 days or more in the limited light and space associated with the laboratory study.

Study 1B was initially offset, and the study was terminated earlier than initially planned, for all polar cuttings began setting buds and going into senescence for undetermined reasons. Table 4 shows how this offsets the data. Onset of senescence was determined not to be a result of benzene exposure, as the control group exhibited the same behavior to the same extent as the dosed groups. When the cuttings began senescing, the transpiration rate for all

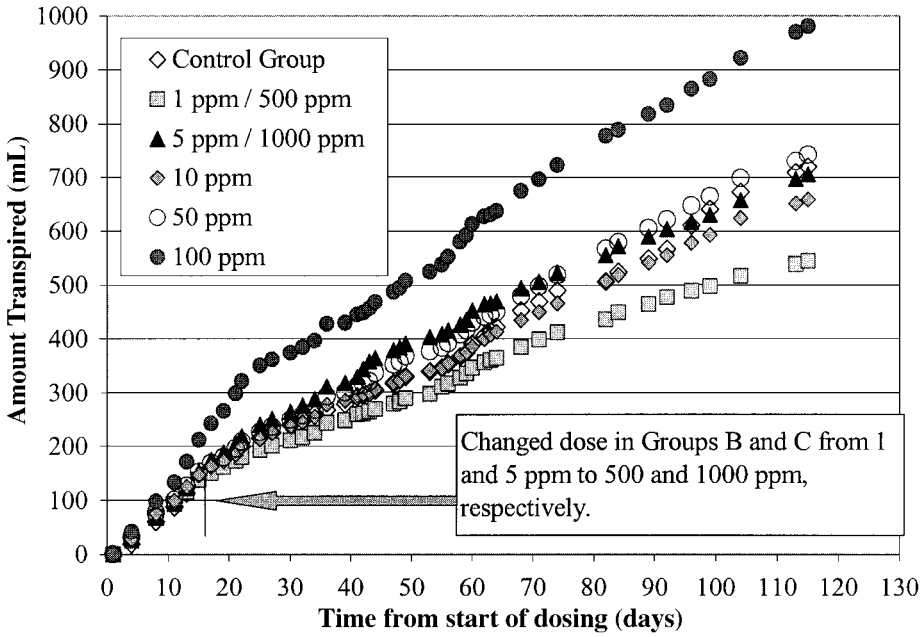

FIG. 3. Study 1A: Average (n = 3) Cumulative Transpiration of DN-34 Poplar Cuttings Planted in Silty Clay Soil and Dosed with Benzene-Contaminated DI Water; Length of Study is 115 Days; Dose Change as Noted, 17 Days into the Study; Dose Specified Is Feed Water Concentration (See Fig. 4 for Statistical Analysis)

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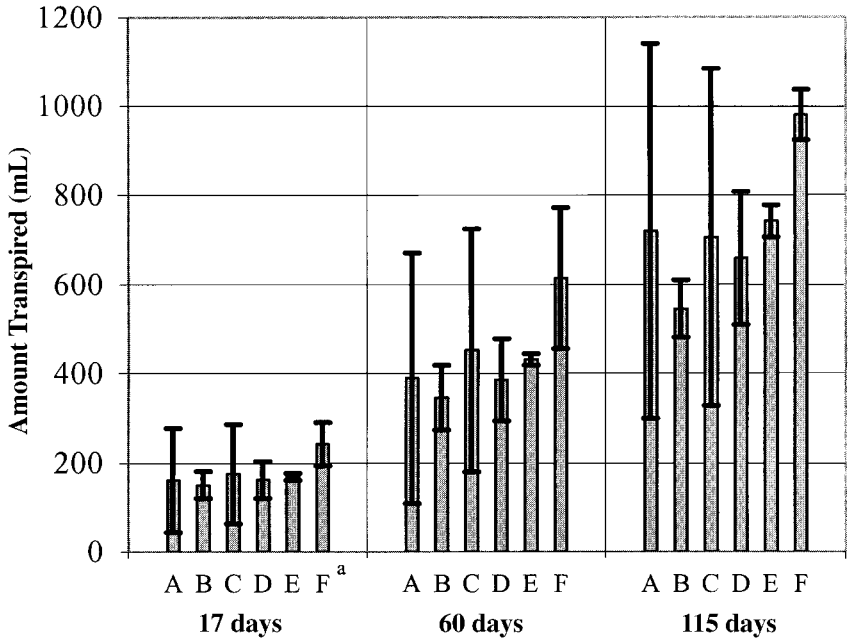
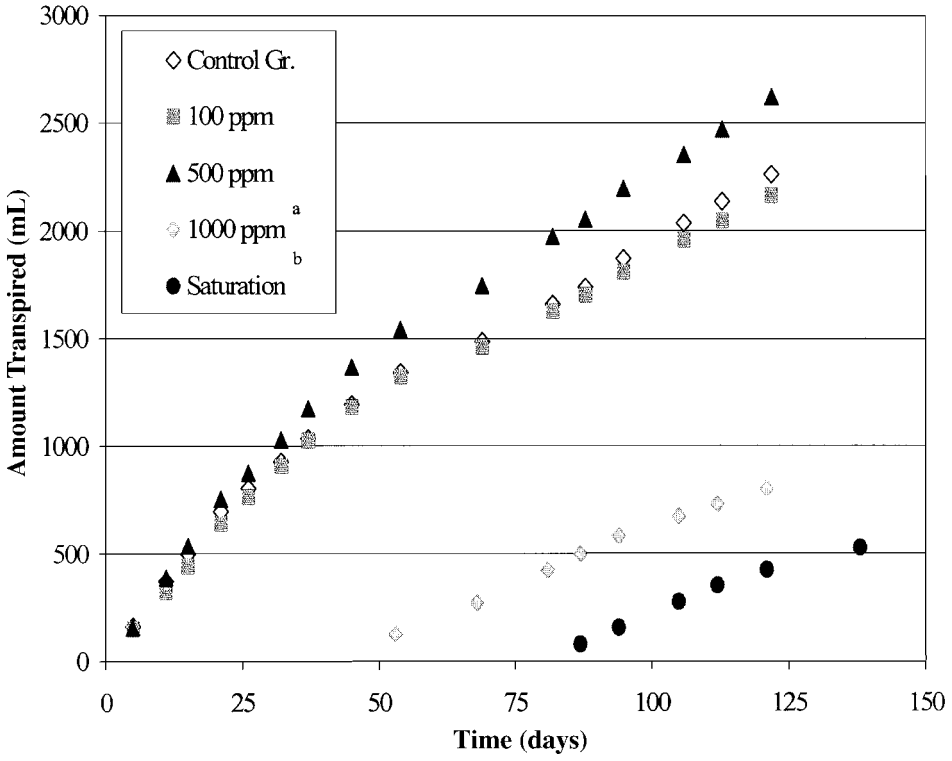


[a] Group, dose: Group A - no benzene dose; Group B - 1 ppm from 1 to 17 days, 500 ppm from 18 to 115 days; Group C - 5 ppm from 1 to 17 days, 1000 ppm from 18 to 115 days; Group D - 10 ppm; Group E - 50 ppm; Group F - 100 ppm.

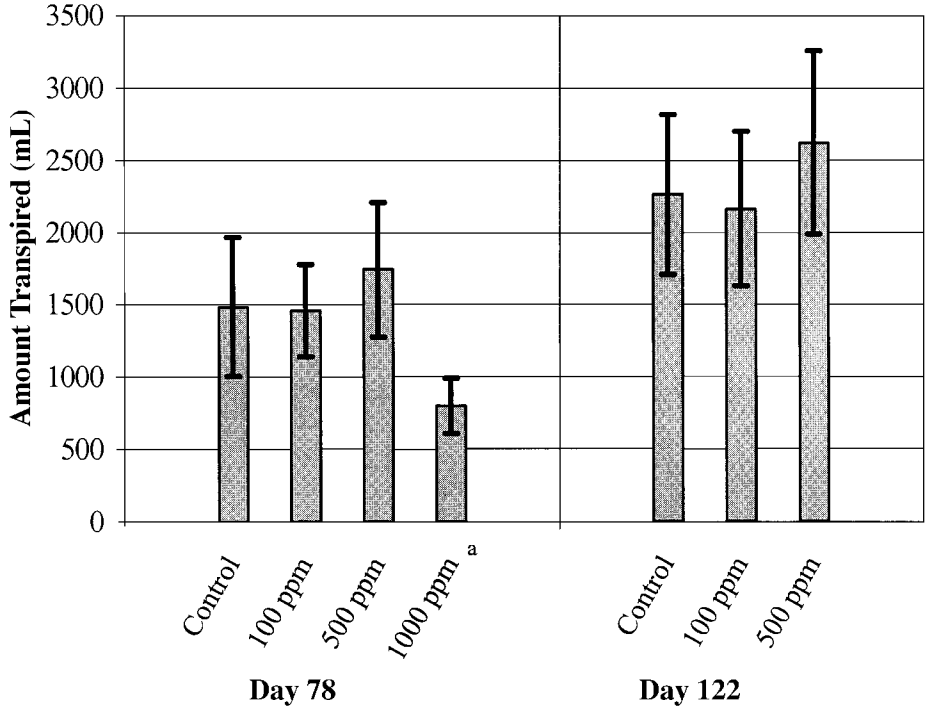
FIG. 4. Study 1A: Average (n = 3) Cumulative Transpiration Data Taken from Fig. 3 at Days 17, 60, and 115, Plotted with 90% Confidence Interval



[a] begin dosing on day 44.

[b] begin dosing on day 81, only one individual.

FIG. 5. Study 1B: Average (n = 3) Cumulative Transpiration of DN-34 Poplar Cuttings Planted in Potting Soil Dosed with Benzene-Contaminated DI Water; Length of Study Was 122 Days; Difficulty Encountered in Establishing Enough Live Cuttings for All Desired Dose Concentrations to Be Evaluated. Thus, Some Cuttings Were Established at Later Dates; Dose Specified Is Feed Water Concentration (See Fig. 6 for Statistical Analysis)



[a] Group, dose: Group D - no benzene dose; Group B - 100 ppm; Group C - 500 ppm; Group A - 1000 ppm.

FIG. 6. Study 1B: Average (n = 3) Cumulative Transpiration Data Taken from Fig. 5 at Days 78 and 122, Plotted With 90% Confidence Interval

groups declined. Toxicity was determined by comparing the slopes of the lines for each group, or the transpiration rate. Accounting for both senescence and staggered start date of dosing, the transpiration rates of the different groups were not significantly different. No statistical value can be gleaned from the data gathered on plant 1E, except to note that the plant did not die within the 51 days.

Benzene found in the headspace of plant tissue samples from Study 1C conclusively shows that benzene is being taken up into the plant (Fig. 7). Thus, lack of acute toxicity is not due to root exclusion or complete degradation in the soil. It must be noted that these values do not represent total benzene mass in the plant tissue samples, but rather the benzene mass that partitions to the headspace of the sample vials. Presented values are conservative and are not intended to be taken quantitatively.

In Study 2, documenting fate and transport, the presence of the live poplar cuttings enhanced the degradation rate of the benzene, and dramatically decreased the aqueous effluent mass of benzene (Fig. 8). Total input of benzene over the course of the experiment was 137 mg to each reactor. The aqueous effluent from reactors with excised poplar cuttings contained an average of 42 mg of benzene over the course of the experiment, whereas reactors with live cuttings only had an average total of 10 mg in their aqueous effluent during the experiment. The majority of that mass was detected in the first 8 days of the study (Fig. 8).

Benzene was also volatilized from the plant tissues, providing evidence to the extent of plant-enhanced volatilization that has not been previously documented in soil systems. Activated carbon traps collected a total of 2–5 mg of benzene from the aerial compartment per live cutting over the course of the experiment. However, this accounted for only 1–4% of the influent benzene, showing that volatilization from plant tissues was appreciable, but not a major pathway for contaminant fate. These observed removal pathways were a result of live cuttings. Reactors with excised cuttings did not exhibit the removal seen in the reactors with live cuttings.

The soil profile maintained higher benzene degradation rates with the live poplar cuttings present. Mineralization, measured as $^{14}CO_2$, in reactors with live cuttings accounted for up to 18% of the applied ^{14}C-labeled benzene in one reactor to a low of 3% in another reactor (Table

TABLE 4. Planting Schedule and Dosing Arrangement for Study 1B: Benzene Toxicity in Potting Soil

Benzene concentration (ppm)	Planted May 19/ dosing begins June 26	Planted June 23/ dosing begins Aug. 9
Control	1D, 2D, 3D	
100	1B, 2B, 3B	
500	1C, 2C, 3C	
1,000		1A, 2A, 3A

Note: Individual reactors are outlined in each column, corresponding to when reactors were planted and benzene dose concentration was received.

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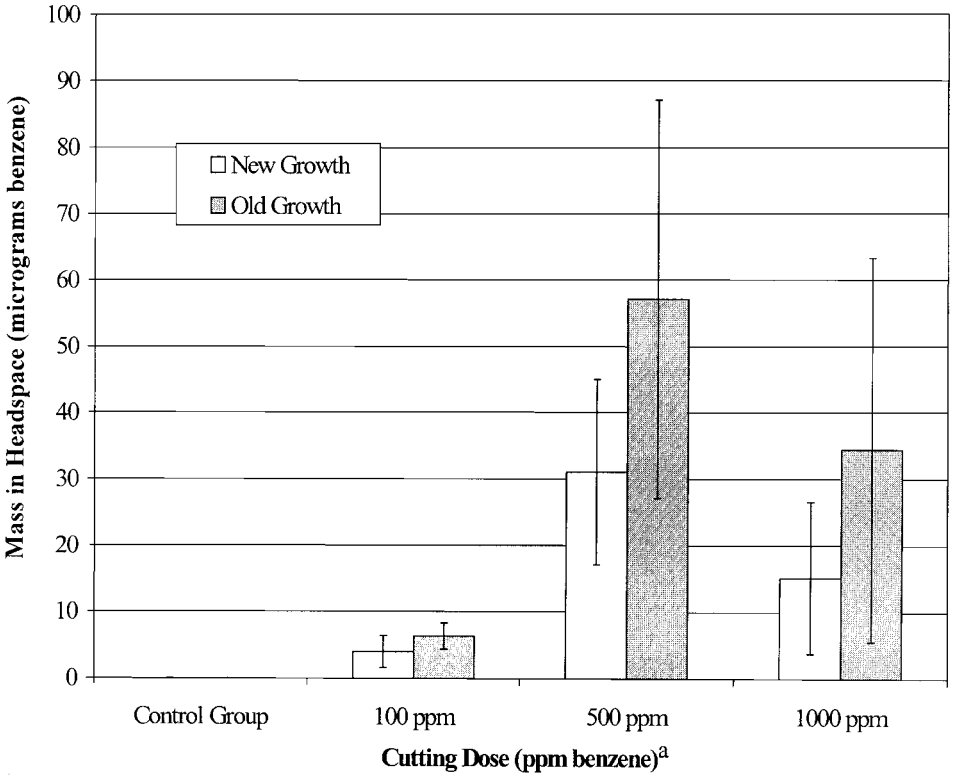

[a] Group, dose: Group D - no benzene dose; Group B - 100 ppm; Group C - 500 ppm; Group A - 1000 ppm.

FIG. 7. Study 1C: Headspace Mass of Benzene Found in Plant Tissues at Close of Study 1B, Benzene Toxicity with Potting Soil; Values Have Not Been Normalized to Account for Total Benzene Mass in Plant Tissue (Mass Remaining Sorbed Is Not Known); Dose Specified Is Feed Water Concentration; Error Bars Represent Standard Deviation, $n = 3$

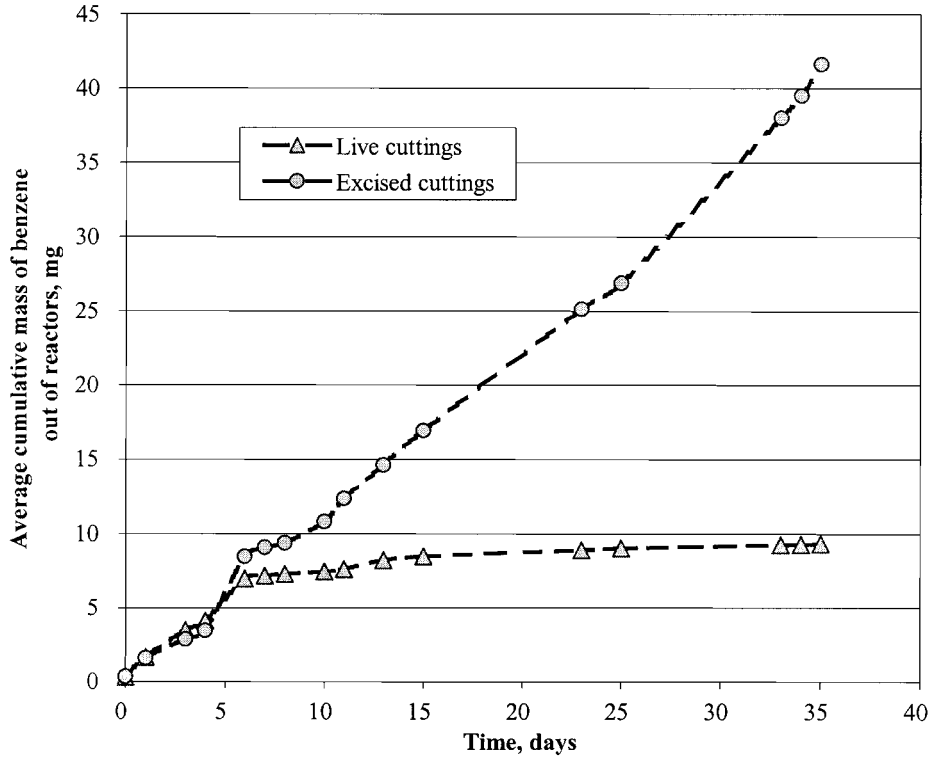

FIG. 8. Study 2: Average Cumulative Mass of Benzene Collected in Effluent of Flow-Through Reactors ($n = 6$ for Reactors with Live Cuttings, $n = 2$ for Reactors with Excised Cuttings); Sampling Was Done on Aqueous Effluent Port of Reactor (Fig. 2) Prior to Collection Bottle

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5). Reactors with excised cuttings did not develop measurable amounts of $^{14}CO_2$ over the course of the experiment. The relatively short period of time that the poplar cuttings were exposed to ^{14}C-labeled benzene (3 days) may cause the variability noted in $^{14}CO_2$ production and the variability in mass balance calculations. The hypothesis is that $^{14}CO_2$ produced in the degradation did not adequately diffuse from the soil in all reactors to be captured in the NaOH traps scrubbing the effluent of the headspace. This could explain the disparity observed in mineralization and in mass balance calculations. Complete mass balance closure was not a direct objective of this study. The use of ^{14}C was limited to only a few days, and only small soil samples could be analyzed using the existing oxidation techniques available. Far more extensive sampling protocols would be needed to track mineralization and incorporation into soil organic matter over an extended study.

Analysis of the microbial population in the reactor soil shows that, for both benzene degraders and total heterotrophs, the reactors with live cuttings contained more total heterotrophs and benzene degraders per gram of soil than the reactors with the excised cuttings. Table 6 shows a summary of the microbial counts for all reactors. This indicates that plant exudates from the roots of live trees may increase the bacterial population in the rhizosphere. Increased microbial populations are commonly observed in the rhizosphere, although increased populations are not always capable of contaminant degradation. In this work the live cuttings did show increased degrader populations. Along with greater microbial populations and higher benzene degradation rates, reactors with live poplar cuttings exhibited higher redox potential in the effluent, and presumably in the soil, than reactors with excised cuttings. The redox conditions in the reactors with live cuttings indicate that conditions were aerobic to anoxic. The redox potential values are listed in Table 7. The higher redox conditions observed with the presence of the poplar cuttings are amenable to benzene metabolism and therefore could be directly responsible for the greater numbers of benzene degraders in the reactors with live cuttings.

The high redox potential and increased microbial population in the reactors with live cuttings could be an indirect effect of the laboratory setup. Active transpiration of water from the soil and thus increased diffusion of air into the soil from the headspace of the root compartment is likely what caused a more oxic environment around the roots of the live poplars. There is no evidence that the poplars directly supplied oxygen or alternative electron acceptors.

The precipitation of iron was observed in the effluent lines and collection bottles of reactors 7 and 8, which contained the excised cuttings. Iron precipitation indicates that iron reduction was occurring in these reactors and that redox conditions were indeed lower throughout the soil profile. The effluent collection bottles for reactors 7 and 8 quickly became lined with iron deposits, whereas none of the six effluent collection bottles for reactors 1 through 6 contained any observable deposits. Ferric iron (Fe^{3+}) in the reactor soil was likely biologically reduced to ferrous iron (Fe^{2+}), which is highly soluble. The soluble iron (Fe^{2+}) was then transported out of the saturated reactor soil and into the oxygen-rich environment of the effluent collection bottles, where it was oxidized and precipitated. It should be noted that there was no iron addition with the feed water. This is suggestive evidence that redox potentials below zero existed (Norris et al. 1994) in the reactors with excised cuttings, which is even lower than the measured values listed in Table 7 for the effluent.

According to the results for the biological oxidation and LSC, accumulation of ^{14}C in the plant tissues of the poplar cuttings was negligible. Results for biomass analysis indicated values were not significantly different from zero, 0.3% ± 0.7%. Table 5 outlines the fate of benzene at the termination of the experiment. Additionally, there were no

TABLE 5. Fate of Benzene Added to Reactors in Study 2: Benzene Fate and Transport

Fate of benzene	Reactors with live cuttings (n = 6) (%)	Reactors with excised cutting (n = 2) (%)
Effluent, aqueous[a]	6.9	30.0
Retained in soil[b]	25.0	41.6
Volatilized from soil[a]	0.26	0.4
Mineralized in soil[b]	3 to 18[c]	0.1
Volatilized from plant tissues[a]	2.3	Not applicable
Retained plant tissues[b]	0.3 ±0.7	Not applicable
Total recovered	up to ~53	~77

Note: Values represent data collected and analyzed via GC throughout the study, as well as ^{14}C data collected at the end of the study. Values represent the average of the reactors for a given treatment, except where noted.

[a]Average of measured values, n = 6.

[b]Measured as ^{14}C via biological oxidation and/or LSC techniques, no chemical confirmation allowed. Note that ^{14}C levels were monitored in reactors 2, 4, 6, and 8. It follows for averaged data values, n = 3 for reactors with live cuttings and n = 1 for reactors with excised cuttings.

[c]Range of values observed.

TABLE 6. Microbial Count for Continuous Flow Reactors in Study 2: Benzene Fate and Transport

Microorganism	Soil	Live cutting	Excised cutting
Total heterographs per gram soil	Silty clay	10,000–160,000	100–400
	Sand	400–20,000	<100
Benzene degraders per gram soil	Silty clay	20,000–100,000	100–200
	Sand	400–3,000	None observed

TABLE 7. Redox and pH Measurements for Aqueous Effluent from Reactors in Study 2: Benzene Fate and Transport

Reactor	Redox potential (mV)	pH
2 (live cutting)	276 ± 45	6.3 + 0.06
4 (live cutting)	183 ± 34	6.4 ± 0.14
6 (live cutting)	150 ± 43	6.3 ± 0.08
8 (excised cutting)	67.3 ± 8.7	6.7 ± 0.04

Note: Triplicate measurements ± one standard deviation.

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detectable benzene levels in the leaves of the poplars used in Toxicity Study 1B. These results are consistent with earlier work that showed no accumulation in plant tissues (Ferro 1997).

CONCLUSIONS AND RECOMMENDATIONS

The fate and transport study revealed that the presence of live poplar cuttings did alter the fate of benzene in the laboratory-scale system. Results indicate that uptake of benzene and water did occur, but at much lower rates than had been observed in previous hydroponic systems. The lower uptake could be caused by the difference in exposure to benzene at the root surface when the exposure is in the vadose zone as compared to hydroponic studies with similar concentrations. Lower concentrations could be caused by sorption, volatilization to soil pore space, and microbial degradation.

Redox conditions noted in the study, which were affected by the presence of the cuttings, may have partially been an artifact of the laboratory setup. The reactor soil was not able to drain as freely as could occur under field conditions, and, consequently, the reactors with excised poplar cuttings were significantly more saturated than the reactors with live cuttings, lending to the anaerobic conditions noted in reactors 7 and 8. It is not understood, however, how the laboratory results presented here translate to what is expected under field conditions. One recent field study shows that the presence of hybrid poplars, and thus rhizosphere microorganisms, increased the concentration of organic acids in the soil profile enough to cause a resultant decrease in the redox potential of the planted area (Jones et al. 1999). On the other hand, the ability of poplar trees to lower the level of the water table, and thus increase available oxygen to the root zone, is well established. Transpiration rates have been measured at 4.8 mm/day in a poplar stand (Hinckley et al. 1994).

Degradation and mineralization were significant pathways for benzene removal in the reactors with live cuttings. Higher populations of total heterotrophs and benzene degrader were detected, indicating that the presence of the live poplar cuttings along with the benzene stimulated the degraders.

Overall, these results, combined with previous hydroponic studies and related field studies provide evidence that phytoremediation has the potential to provide effective, low cost treatment for biodegradable compounds and potentially for other VOCs. It also shows that for benzene the end result is environmentally acceptable, since minimal benzene residues remain in the plant tissues, thus reducing the possibility of introduction of the contaminant into the food chain.

Acute toxicity was not observed at concentrations to 1,000 ppm in these laboratory tests. Toxicity of shock loads and toxic effects on the sprouting process should also be considered when applying phytoremediation technologies in the field.

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