01 Jan 2000

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## Recommended Citation

C. Ross et al., "Degradation and Uptake of Benzene in Laboratory Phytoremediation Studies," *Environmental and Pipeline Engineering 2000*, pp. 476 - 482, American Society of Civil Engineers, Jan 2000.

The definitive version is available at https://doi.org/10.1061/40507(282)52

# Degradation and Uptake of Benzene in Laboratory Phytoremediation Studies

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*Abstract*

In whole-plant laboratory studies, hybrid poplar trees were shown to impact a variety of fate and transport mechanisms for benzene. Laboratory experiments investigated the distribution of the contaminant in the plant tissues, degradation in the soil profile, and volatilization from both the soil and leaf tissues. A new testing system was developed that allowed for rapid testing that is more field-representative than earlier studies. Whole plants were utilized in a reactor design that included both a saturated and a vadose zone. The continuous feed reactors were supplied with a steady influent benzene stream to mimic plume conditions. The presence of the poplar trees enhanced the degradation rate of the benzene, and dramatically decreased the effluent mass of benzene. Benzene was also volatilized from the leaf tissues, providing evidence to the extent of plant enhanced volatilization that has not been previously documented. The observed degradation and removal pathways were a result of active/live trees. Reactors with killed controls did not exhibit the removal seen in the live tree replicates. The soil profile maintained higher degradation rates with the trees present. Causes for the higher degradation rates appeared to be larger microbial populations of benzene degraders and preferable redox conditions in the presence of the poplars. These results combined with previous hydroponic and related field scale studies to provide evidence that phytoremediation has potential for effective, efficient, and environmentally friendly application at benzene contaminated sites, and potentially other contaminated sites with biodegradable organics or VOCs.

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*Introduction*

There are several previous studies which indicate a need for further research in the area of benzene toxicity as well as fate and transport. Ferro *et al.* (Ferro, Kennedy et al., 1999) conducted two field scale studies measuring the phytotoxicity of poplar trees to a volatile organic compound (VOC) mixture. These studies provided no conclusive results indicating any plant toxicity from exposure to the VOC mixture. There appear to be no current studies focusing upon the specific toxicity of benzene. Other 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). The majority of the benzene taken up from the hydroponic solution was volatilized. Uptake and volatilization of benzene from 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 the 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. 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; Scheidemann, et al., 1998). These research efforts have generated a general understanding of fate, transport, and toxicity processes, but they have also brought to light many exceptions to the current understanding. There has been much conjecture regarding the phytoremediation of many organics based upon a few studies with selected compounds. Until research is conducted on specific contaminant/plant interactions, these speculations are not adequately founded.

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

1) investigate the degradation of benzene in the rhizosphere,
2) determine the distribution of benzene and its metabolites in the plant tissues,
3) elucidate volatilization from the soil and leaf tissues, and
4) determine the toxicity level of the trees to different concentrations of benzene.

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

*Methods and Materials*

Toxicity Test Hybrid poplar trees of type DN-34 were used in all of the following studies. The DN-34 hybrid poplar trees have been shown to impact several fate and transport mechanisms for benzene in earlier studies and poplar trees are widely applied in phytoremediation projects. The first experiment to determine the toxicity level of

benzene for poplar trees was performed using 1000 mL clear wide mouth glass bottles (I-Chem, Fisher Science) that were fitted with Teflon lined lids. Uniform dry sand, 150 g, was placed in the bottom of the jars and leveled. A Teflon feeding tube was placed into the bottom of the reactor. On top of the sand, 700 g of dry, uniform clay-silt native Missouri soil was placed in the jars. This soil was run through several sieves to remove course material and then homogenized. DN-34 hybrid poplar whips were cut to an approximate length of 8". They were then allowed to begin sprouting in a bed of sand watered with ¼ strength Hoagland's solution. Once the whips showed evidence of sprouting, they were rinsed clean with deionized water and inserted through holes in the Teflon lined caps into the reactors. The reactors were wrapped in foil to prevent algae growth. The feeding tubes were placed so that the plants would be fed from the bottom, to mimic field conditions and limit direct volatilization. The dry reactors with the plants were weighed and recorded. These reactors were configured as shown in Figure 1.

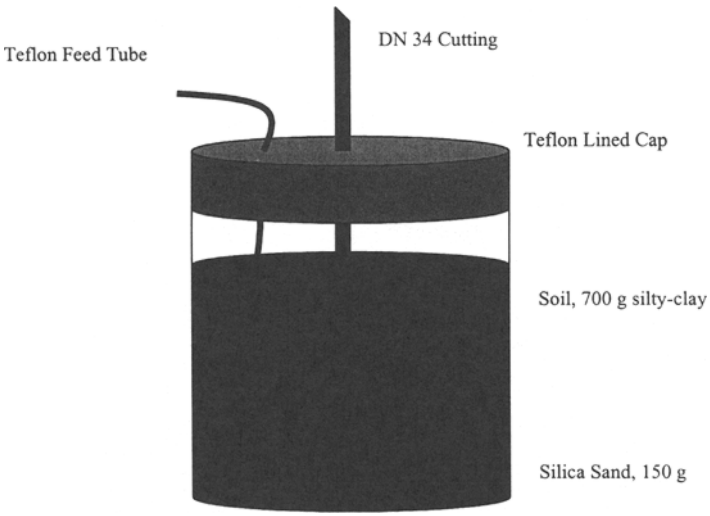


**Figure 1**. Reactor Setup for Toxicity Test

The saturation point of the soil in the reactors was determined so that the plants could be watered to a target point of 80% saturation. The reactors were kept under a 40 watt fluorescent light source on a sixteen hour photo period. The temperature in the laboratory was between 20 and 24° C. The plants were watered with tap water for several weeks before being exposed to benzene contaminated water. This was done to allow further sprouting of the whips so that uniform individuals could be used in the study. After several weeks of growth, 18 reactors with uniform, viable poplars were used for the experiment. These 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, respectively.

The method of dosing the plants with benzene was accomplished by keeping a stock solution of deionized water saturated with benzene and placed in dark brown glass bottles on a rotating mixing table at low speed. A specified amount of stock solution was then be mixed with deionized water at feeding time and immediately transferred to the reactors via syringe into the teflon feeding tubes. The plants were weighed both before and after each watering event.

After several weeks of showing no visible effects from the benzene, the dosages for groups B and C were increased from 1 and 5 ppm to 500 and 1000 ppm, respectively. All the plants were dosed at their respective levels for several months before the experiment was completed.

Fate and Transport A new testing system was developed that allowed for rapid, field representative testing. Whole plants were utilized in a reactor design that included both a saturated and a vadose zone. A schematic of the arrangement is shown in Figure 2. The reactors were kept in a windowless room with a mercury halide light source on a

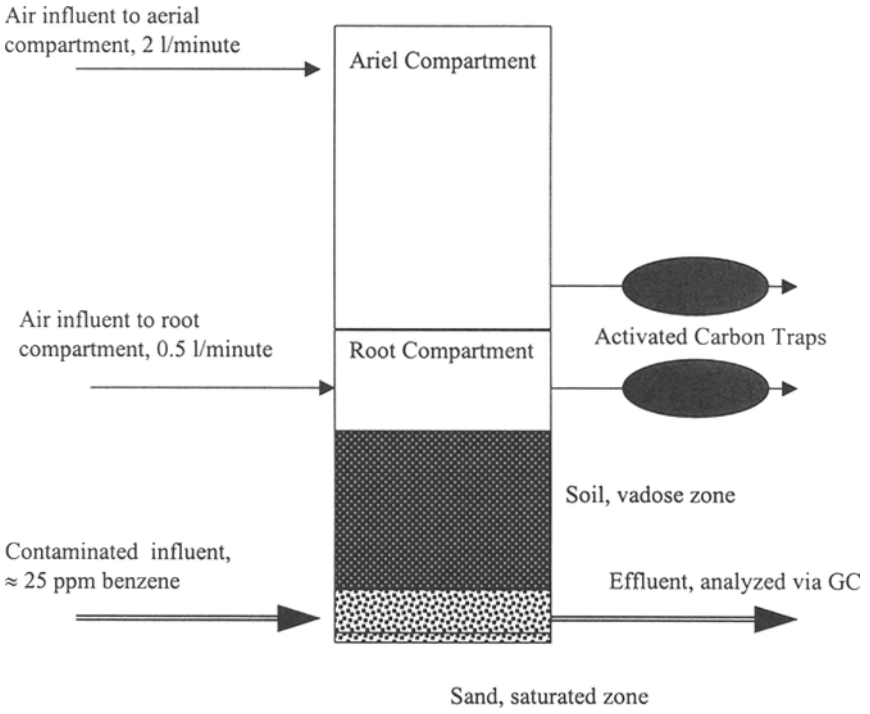


**Figure 2**. Schematic of fate and transport experimental arrangement.

sixteen hour photo period. Light intensity was measured at >210 μmol/$m^2$-sec at the leaf surface in the aerial compartment. The temperature in the room was between 20 and 30° C. Air flow through the aerial compartment of the reactors was regulated at 2 liters per minute and through the bottom of the reactors at 0.1 to 0.5 liters per minute.

There were eight reactors, six of which contained live DN-34 hybrid poplars and two which contained the non-growing roots and stem of DN-34 hybrid poplars. The continuous feed reactors were supplied with a steady influent stream of 25 mg/L benzene to mimic plume conditions. This pump was run at a constant rate of 100 mL/day for the duration of the experiment. The effluent was collected daily in sample bottles and analyzed via gas chromatography (GC) to determine benzene concentration. Activated carbon traps, 1 g, were placed as shown in Figure 2 to collect off-gases. These traps were changed at 24 to 48 hour intervals for the duration of the experiment. The activated carbon in these traps was extracted with carbon disulfide, 2 mL, for 24 hours on an orbit shaker table. Samples of the extract, 2 μL, were injected to the GC utilizing a liquid autosampler. Over a three-day period near the close of the experiment, $^{14}C$ labeled benzene was utilized to evaluate fate. Once per day, 5 μCi of uniformly labeled benzene was added to the influent line at the inlet. Traps were added to the air effluent ports after the activated carbon traps to capture and $CO_2$ generated. The traps used a fritted glass diffuser to pass the air stream through 150 mL of 1 N NaOH. Aliquots, 200 μL, of the NaOH were analyzed via liquid scintillation counting (LSC) methods. Effluent samples were then tested via techniques in addition to the GC analysis. Liquid effluent and NaOH traps were monitored for two days after the use of the $^{14}C$ benzene was halted. Following termination of the experiment, the reactors were dismantled and the poplar tissues were directly analyzed. A series of five soil cores were also collected and analyzed. Method for tissue and soil analysis appear elsewhere (Burken and Schnoor, 1997).

**Results and Discussion**

There was no evidence of benzene toxicity at the close of the toxicity testing. This was unexpected as earlier screen tests done hydroponically indicated rapid toxicity at concentrations below 500 ppm (data not shown). There are several possibilities for this differences between hydroponic and soil tests. The type of clay/silt soil used in the experiment would certainly impact the exposure of benzene to the plant roots. However, over the long period of this study it was expected that any sorption properties of the soil would quickly be saturated. Microbial degradation of the benzene was suspected for the lack of toxicity at the high mass loading rates. It is possible that while groups B and C were dosed with 1 ppm and 5 ppm, a population of benzene degraders was given a chance to proliferate, thus building up a resistance to the higher doses that were given to the trees later in the experiment. Regardless of the reason, the plants were able to withstand very high benzene concentrations, up to 1000 ppm, and continue growing for months in the study.

In the fate and transport study, the presence of the poplar trees enhanced the degradation rate of the benzene, and dramatically decreased the effluent mass of benzene. Total input of benzene over the experiment was 137 mg. The effluent from reactors void

of live poplars contained 42 mg of benzene over the course of the experiment, whereas planted reactors only had a total of 10 mg in their aqueous effluent during the experiment. The majority of that mass was detected in the first 8 days. Benzene was also volatilized from the leaf 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 to 5 mg of benzene per plant over the course of the experiment, only 1 to 4 percent of the influent benzene. The observed removal pathways were a result of active/live trees. Reactors with killed controls did not exhibit the removal seen in the live tree replicates. The soil profile maintained higher degradation rates with the trees present. Mineralization accounted for up to 18% of the applied $^{14}C$ in one planted reactor to a low of 3 % in another planted reactor. Unplanted reactors did not evolve measurable amounts of $^{14}CO_2$ over the course of the experiment. Causes for the higher degradation rates were shown to be greater microbial populations of benzene degraders and a higher redox potential in the presence of the poplars. Accumulation of $^{14}C$ in the plant tissues was negligible. Results for biomass analysis were not significantly different from zero, $0.3\% \pm 0.7\%$.

*Conclusions and Recommendations*

Due of the unique results obtained in the toxicity test, it is recommended that further testing be done to firmly establish the toxicity level of the DN-34 hybrid poplar tree. Toxicity to shock loads, and toxic effects on the sprouting process are to be evaluated. Repeat studies should be run using the same type of soil and immediate dosing of higher levels without the initial lower concentrations to observe how the acclimation of benzene degraders might have affected the toxicity levels. Tests should also be done using different soils to establish the soil type as a factor in benzene toxicity. Testing could also be performed on reactors with sterilized soil and the results compared to the other studies to determine the overall effect of the microbial population in the soil.

Fate and transport testing showed that the poplar cutting greatly altered the fate of benzene in the laboratory-scale system. Results indicate that uptake and transpiration did occur, but at much lower rates than had been observed in previous hydroponic systems. The lower uptake could be cause by the difference in exposure at the root surface when the exposure is in the vadose zone. Mineralization was a major pathway for benzene removal in planted systems. Higher benzene degrader populations were also detected, indicating that the presence of the plants, in concert with the benzene concentrations stimulated the degraders. Redox conditions were also altered by the presence of the poplars. It is noted that the increased redox potential reading may have partially been an artifact of the experiment arrangement. Soils in the glass flasks were not able to freely drain, and the planted reactor were not as saturated as the unplanted reactors. The impact of poplar trees on the water table is well established with transpiration rates measured at 4.8 mm/day in a poplar stand (Hinckley, et al., 1994), however it is not understood how the laboratory results presented here translate to what is expected in the field. This work strengthens the base of knowledge regarding phytoremediation.

Overall, these results combined with previous hydroponic studies and related field studies provide evidence that phytoremediation can provide effective, low cost treatment for biodegradable compounds and potentially for other VOCs, and that for benzene the end result is environmentally friendly, as minimal benzene residues remain in the plant tissues.

*References*

Briggs, G. G., R. H. Bromilow, et al. (1982). "Relationships between Lipophicity and Root Uptake and Translocation of Non-ionized Chemicals by Barley." *Pestic. Sci.* 13: 495-504.

Bromilow, R. H. and K. Chamberlain (1995). "Principles Governing Uptake and Transport of Chemicals". Plant Contamination: Modeling and Simulation of Organic Chemical Processes. S. Trapp and J. C. McFarlane. Boca Raton, Fl., CRC Press Inc.: 38-68.

Burken, J. G. (1996). "Uptake and Fate of Organic Contaminants by Hybrid Poplar Trees". Civil and Environmental Engineering, University of Iowa. Iowa City.

Burken, J. G. and J. L. Schnoor (1997). "Uptake and Metabolism of Atrazine by Hybrid Poplar Trees." *Environ. Sci. Technol.* 31(5): 1399-1406.

Burken, J. G. and J. L. Schnoor (1998). "Predictive Relationships for Uptake of Organic Contaminants by Hybrid Poplar Trees." *Environ. Sci. Technol.* 32(21): 3379-3385.

Burken, J. G. and J. L. Schnoor (1999). "Distribution and volatilization of organic contaminants following uptake by hybrid poplar trees." *Intl. J. Phytoremediation* 1(2): 139-152.

Ferro, A. M., J. Kennedy, et al. (1997). "Fate of Benzene in Soils Planted with Alfalfa: Uptake, Volatilization and Degradation". *Phytoremediation of soil and water contaminants*. E. Kruger, T. Anderson and J. Coats. Washington, D.C., American Chemical Society: 223-237.

Ferro, A. M., J. Kennedy, et al. (1999). "Toxicity assessment of volatile organic Compounds in poplar trees." *Internat, J. Phytoremediation* 1(1): 9-17.

Hinckley, T. M., J. R. Brooks, et al. (1994). "Water flux in a hybrid poplar stand." *Tree Physiology* 14: 1005-1018.

Reilley, K. A., M. K. Banks, et al. (1996). "Organic Chemicals in the Environment: Dissipation of Polycyclic Aromatic Hydroarbons in the Rhizosphere." *J. Environ. Qual.* 25(March-April): 212-219.

Scheidemann, P., A. Klunk, et al. (1998). "Species Dependent Uptake and Tolerance of Nitroaromatic Compounds by Higher Plants." *J. Plant Physiol.* 152: 242-247.

Schwab, A. P. and M. K. Banks (1994). "Biologically Mediated Dissipation of Polyaromatic Hydrocarbons in the Root Zone". *Bioremediation through Rhizosphere Technology*. T. A. Anderson and J. R. Coats. Washington, D.C., American Chemical Society: 132-141.

Trapp, S. and J. C. McFarlane (1995). *Plant Contamination: Modeling and Simulation of Organic Chemical Processes*. Boca Raton, Fl., CRC Press Inc.

Wetzel, S. C., M. K. Banks, et al. (1996). "Rhizosphere efffects on the degradation of pyrene and anthracene in soil". *ASCE Conf. Proceedings*. Orlando, Fl: 605-613.