Phytoremediation of 1,4-Dioxane by Hybrid Poplar Trees

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ABSTRACT: 1,4-Dioxane (dioxane), a suspected carcinogen, is a persistent environmental pollutant that is difficult to remove from contaminated sites. This work investigated the feasibility of vegetative uptake as a site remediation alternative. In hydroponic studies, hybrid poplar cuttings (*Populus deltoides* × *nigra*, DN 34, Imperial Carolina) removed 23 mg/L dioxane rapidly. Within 9 days, a removal of 54.0 ± 19.0% was achieved. This removal corresponded to a transpiration stream concentration factor of 0.72 ± 0.07. Poplars also effectively remediated a dioxane-spiked soil (10 mg/kg). Only 18.8 ± 7.9% of the initial dioxane spike remained in planted soil after 15 days, compared with 72.0 ± 7.7% remaining in sterilized, unplanted soil. In both hydroponic and soil experiments, 76 to 83% of the dioxane taken up by poplars was transpired from leaf surfaces to the atmosphere, where it can be readily dispersed and photodegraded. These results suggest that phytoremediation is a viable alternative to remove dioxane from contaminated sites and should be considered for other hydrophilic contaminants. *Water Environ. Res.*, **72,** 313 (2000).

KEYWORDS: phytoremediation, 1,4-dioxane, hybrid poplars, transpiration stream concentration factor, rhizosphere, transpiration, mineralization.

1,4-Dioxane (dioxane), a suspected carcinogen, is widely used as a stabilizer for chlorinated solvents and as a solvent in paper and textile processing, paints, lacquers, and cosmetics. Dioxane is a cyclic ether (Figure 1) that exists as a liquid at room temperature, is fully miscible with water, and is highly mobile in soil (Table 1). In laboratory experiments, drinking water containing 10 000 mg/L dioxane was found to induce liver and nasal cancer in rats and mice (Klaassen et al., 1986). The U.S. Environmental Protection Agency (U.S. EPA) has established a drinking water dioxane concentration of 3 μg/L for a 10^{-6} cancer risk factor. Dioxane poses a threat to surface water and groundwater supplies because of its potential toxicity, extensive use (between 4.76×10^6 and 8.30×10^6 kg [10.5 and 18.3 mil. lb] were produced in the United States in 1990 [U.S. EPA, 1995]), high solubility and mobility, and resistance to biodegradation.

Conventional biological, physical, and chemical processes for treating dioxane-contaminated soil and water have been either ineffective or relatively expensive. Indigenous microorganisms typically cannot degrade this recalcitrant compound (Fincher and Payne, 1962; Francis et al., 1980; and Howard, 1990). Although a few studies have shown some biological degradation of dioxane (Bernhardt and Diekmann, 1991; Burback and Perry, 1993; and Roy et al., 1994), only one study has reported unequivocal evidence of dioxane mineralization and sustained utilization as a sole carbon and energy source by a pure culture (Parales et al., 1994). Degradation in the other studies was most likely caused by cometabolism, which is inefficient and limited by enzyme production and the availability of cofactors. Because of its low Henry's law constant (H = 0.000 2 mg/L air/mg/L water at 25 °C) and moderate vapor pressure (5.05 kPa [38.0 mm Hg] at 25 °C), dioxane is not readily volatilized by air stripping. Carbon adsorption is also ineffective at removing this hydrophilic compound from water (Adams et al., 1994). Effective treatment methods include UV radiation, distillation, and chlorination. However, chemical and heating costs can make these alternatives prohibitively expensive for site remediation.

Phytoremediation is the use of vegetation for remediating contaminated soil and groundwater. Economic, aesthetic, and environmental benefits support the use of vegetation for remediation. Phytoremediation typically costs only 10 to 50% as much as traditional remediation techniques, such as soil excavation (Bing, 1996; Erickson, 1997; Schnoor, 1997; and Watanabe, 1997), and is more aesthetically pleasing. Briggs et al. (1982) established a relationship to predict the efficiency of vegetative uptake as a function of the organic contaminant hydrophobicity, as given by the octanol/water partitioning coefficient (K_{ow}). Uptake efficiency was quantified in terms of the transpiration stream concentration factor (TSCF), which is defined as the pollutant concentration in the transpiration stream (within the plant) divided by the aqueous concentration in solution. A TSCF of 1.0 indicates that the chemical moves freely with water into the plant, whereas a TSCF of 0.0 indicates no tendency for chemical uptake.

Briggs et al. (1982) tested barley uptake of *o*-methylcarbamoyloximes (insecticides) and substituted phenylureas (herbicide analogs) that covered a wide range of K_{ow} values. These results are plotted along with the TSCF data obtained by Burken and Schnoor (1998), Thompson (1997), and the present study using hybrid poplars (Figure 2). As Figure 2 indicates, the predictive accuracy of these uptake models is uncertain. However, several trends do exist. Extremely lipophilic compounds (log $K_{ow} > 4.0$) had a TSCF of approximately zero. These compounds were so strongly bound to the roots that they were not further translocated to other plant tissues (Trapp et al., 1994).

On the other hand, hydrophilic compounds (log $K_{ow} < 0$), such as dioxane, presumably have low TSCF values because they do not readily bind with root membranes (to establish favorable concentration gradients), and their uptake is not assisted by transport proteins. The predicted uptake potential of dioxane is low, with TSCF values of only 0.14 (based on Briggs' correlation) and 0.03 (based on Burken's correlation). Other researchers (Topp et al., 1986) have suggested that molecular weight is a substance property more suitable for predicting chemical uptake by plants. Nevertheless, a plot of TSCF versus molecular weight (figure not shown) for the 13 TSCF values listed in Table 2 showed poor correlation (R^2 = 0.46 for correlation coefficient R).

Hybrid poplars are typically used in phytoremediation applica-

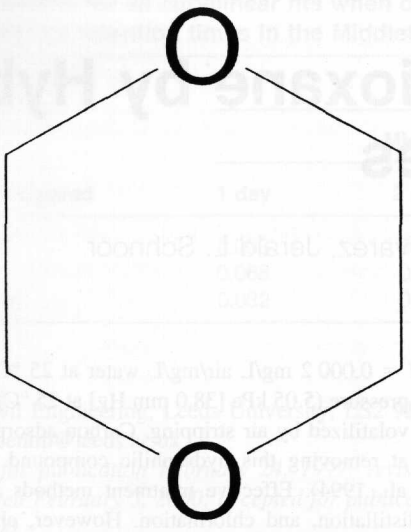

Figure 1—Chemical structure of 1,4-dioxane.

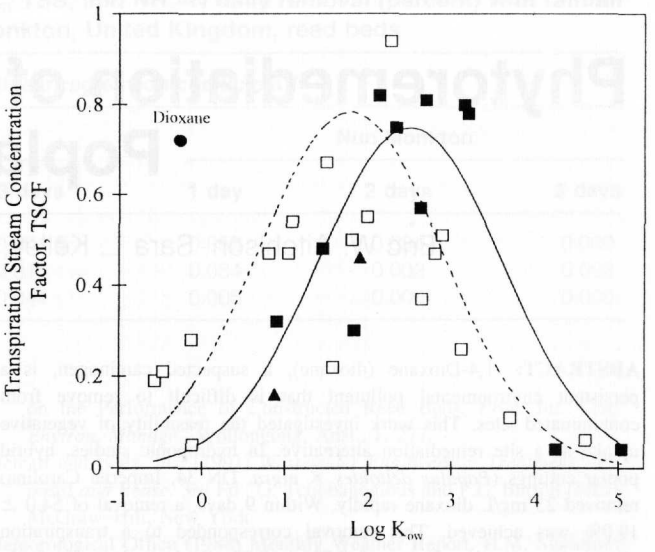

Figure 2—Efficiency of vegetative uptake of organic contaminants as a function of hydrophobicity. Figure is adapted from Briggs et al. (1982) and Burken and Schnoor (1998). Note that for Briggs et al. (1982): TSCF = 0.784 exp $-[(\log K_{ow} - 1.78)^2/2.44]$ and for Burken (1998): TSCF = 0.75 exp $-[(\log K_{ow} - 2.5)^2/2.44]$.

tions because they are easily propagated, develop deep root systems, exhibit high water uptake rates, and are tolerant of high concentrations of organics. Wichman (1990) observed no toxicity to hybrid poplars at 60 mg/L each of benzene, carbon tetrachloride, *m*-dichlorobenzene, *m*-xylene, toluene, and trichloroethylene, for a combined organic loading of 360 mg/L. Numerous laboratory and field studies have evaluated the effectiveness of hybrid poplar trees in remediating a variety of organic contaminants (Table 2). Nevertheless, the ability of poplars to remediate a chemical as hydrophilic as dioxane has not been studied.

The goal of this research was to determine the feasibility and efficacy of vegetative remediation at sites contaminated with 1,4-dioxane. This paper addresses the capacity of hybrid poplars for uptake and translocation of 1,4-dioxane, including the potential for volatilization of dioxane through leaf tissue.

Experimental Design and Methods

Two experiments were conducted to assess uptake and translocation of dioxane by hybrid poplars: (1) dioxane uptake from hydroponic solution and (2) dioxane uptake and mineralization from contaminated soil.

Techniques and Equipment Used in Both Experiments. Cuttings (200 mm [8 in.]), identical male clones from adult Imperial Carolina hybrid poplar trees (*Populus deltoides* × *nigra*, DN34), were used for both experiments. A predrilled screw cap and Teflon-lined septum (Weaton Scientific, Millville, New Jersey) were fit snugly around each of the cuttings. Rubber sealant (General Electric window and door, 100% silicone, Schenectady, New York) was applied to seal the screw caps and septums to the cuttings. The cuttings were grown in hydroponic solution using half-strength modified Hoagland's inorganic nutrient solution (Burken, 1993). After approximately 3 weeks, cuttings had developed 8 to 12 leaves and an appropriately dense root system for conducting the experiments.

Cuttings were planted in modified 278-mL glass screw top flasks (Corning, Corning, New York). Mininert sampling valves (Supelco, Bellefonte, Pennsylvania) were screwed onto the two sampling ports, and the flasks were wrapped in aluminum foil to prevent algae growth and dioxane photolysis. The predrilled screw caps were screwed to the flasks, with the septums helping to create

Table 1—Dioxane physical and chemical properties.

Property	Value	Reference
Synonyms	1,4-dioxane, dioxane, *p*-dioxane, diethylene dioxide, glycol ethylene ether	NIOSH (1977)
Molecular formula	$C_4H_8O_2$	Howard (1990)
Molecular weight	88.10	Howard (1990)
Boiling point	101.1 °C at 100 kPa	Howard (1990)
Melting point	11.80 °C	Howard (1990)
Water solubility	Miscible	Lange (1967)
Vapor pressure	5.05 kPa at 25 °C	Boublik et al. (1984)
Henry's Law constant	4.94×10^{-4} kPa-m^3/mol at 25 °C	Hine and Mookerjee (1975)
Log K_{ow}[a]	−0.27	Hansch and Leo (1985)

[a] Octanol/water partitioning coefficient.

Table 2—Phytoremediation studies using hybrid poplar trees.

Chemical	Molecular weight	Log K_{ow}	Description of study	Results	Reference
Aniline	93.1	0.90	Laboratory hydroponic experiment	TSCF = 0.32, mass distributed throughout plant	Burken and Schnoor (1998)
Atrazine	215.7	2.69	Small field plot study, Amana, Iowa	10–20% of atrazine applied was taken up	Paterson and Schnoor (1992)
Atrazine	215.7	2.69	Laboratory silica sand media and silt–loam soil experiments	Uptake = 92% of mass applied to sand, 20% to soil; primarily accumulated as metabolites in leaves	Burken (1993)
Atrazine	215.7	2.69	Laboratory hydroponic experiment	TSCF = 0.57, 60% of mass in plant tissue found in leaves	Burken and Schnoor (1998)
Benzene	78.1	2.13	Laboratory hydroponic experiment	TSCF = 0.82, 91% of mass taken up was transpired	Burken and Schnoor (1998)
Ethylbenzene	106.2	3.15	Laboratory hydroponic experiment	TSCF = 0.80, mass taken up was readily transpired	Burken and Schnoor (1998)
Nitrobenzene	123.1	1.83	Laboratory hydroponic experiment	TSCF = 0.82, 95% of mass in plant tissue found in the stem	Burken and Schnoor (1998)
Pentachlorophenol	266.3	5.04	Laboratory hydroponic experiment	TSCF = 0.04, 80% of mass in plant tissue found in the stem	Burken and Schnoor (1998)
Phenol	94.1	1.45	Laboratory hydroponic experiment	TSCF = 0.48, mass distributed throughout plant	Burken and Schnoor (1998)
Royal demolition explosive (RDX)	222.0	0.87	Laboratory hydroponic experiment	TSCF = 0.16, 60% of mass taken up found in leaves	Thompson (1997)
Toluene	92.1	2.69	Laboratory hydroponic experiment	TSCF = 0.81, mass taken up was readily transpired	Burken and Schnoor (1998)
Trichlorobenzene	181.5	4.25	Laboratory hydroponic experiment	TSCF = 0.04, 90% of mass taken up was found in the stem	Burken and Schnoor (1998)
Trichloroethylene	131.4	2.33	Laboratory potted plants and field site	Successful uptake and metabolism in plant tissue, some trichloroethylene transpired	Newman et al. (1997)
Trichloroethylene	131.4	2.33	Laboratory hydroponic experiment	Very slight uptake	Bugbee (1998)
Trichloroethylene	131.4	2.33	Laboratory hydroponic experiment	TSCF = 0.75, successful uptake and metabolism in plant tissue, 70% of trichloroethylene taken up was transpired	Burken and Schnoor (1998)
2,4,6-trinitrotoluene (TNT)	227.0	1.90	Laboratory hydroponic experiment	TSCF = 0.46, 75% of mass taken up found in roots	Thompson et al. (1998)
m-xylene	106.2	3.20	Laboratory hydroponic experiment	TSCF = 0.78, mass taken up was readily transpired	Burken and Schnoor (1998)
Several volatile organic compounds			Small field plot study, Amana, Iowa	Deep rooted poplars decreased migration; contaminant concentration in planted soil less than unplanted soil	Wichman (1990)

a reliable seal. Modified 1-L Erlenmeyer flasks, with the perimeters of the open ends sealed with the rubber sealant, were inverted over the cuttings and fit snugly around the screw cap to create the reactor aerial compartment. Parafilm tape was used to seal this joint. The inlet and outlet ports of the Erlenmeyer flasks were fitted with activated carbon traps (Orbo tube 32 large, Supelco) to capture dioxane transpired from the leaves.

The reactors were placed in a plant growth chamber constructed in a walk-in fume hood. The temperature in the growth chamber varied between 24 and 29 °C, and the light intensity in the photosynthetic active range (400 to 700 nm; Hopkins, 1995) varied between 110 to 165 μmol/m^2·s (measured with a LI-COR [Lincoln, Nebraska] LI-189 photometer). The photoperiod lasted for 16 hours per day. Hoses connected to a central pump withdrew headspace in the Erlenmeyer flasks at a rate of approximately 1 to 2 L/min. A schematic of the reactor design is shown in Figure 3.

Both pure ("cold") dioxane and radiolabeled ^{14}C-dioxane were used in the experiments. The radiolabel (Moravek Biochemical [Brea, California], purity > 98%) was used as a tracer to follow the fate and transport of dioxane, but it was not useful to differentiate between dioxane and potential transformation products. Dioxane was spiked at

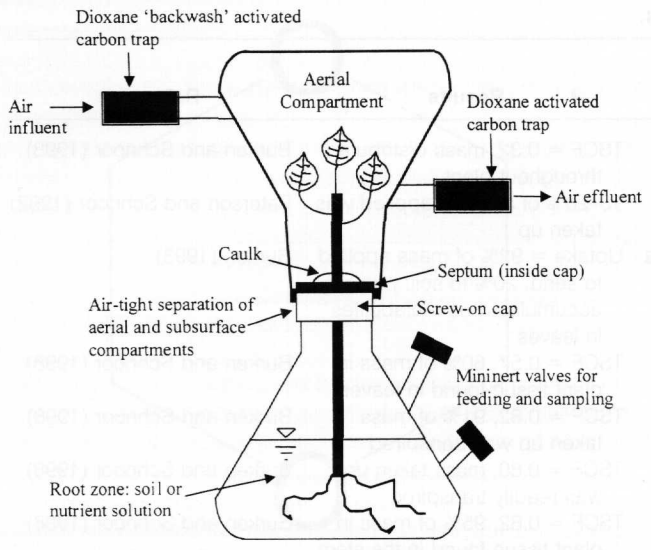

Figure 3—Schematic of the reactor design used for the hydroponic and contaminated soil experiments.

a mass ratio of 420:1 (pure:radiolabeled) for the hydroponic experiment and 130:1 for the contaminated soil experiment.

After termination of the hydroponic and contaminated soil experiments, cuttings were separated into their various components: roots, stem, petioles, and leaves. A biological material oxidizer (RJ Harvey Instrument Corporation [Hillsdale, New Jersey] OX-600) was used to combust this organic matter to CO_2. Approximately 1-g samples of each of the plant components were oxidized. For the contaminated soil experiment, soil was poured out and homogenized, and three to four samples (approximately 1 g each) were oxidized. Oxidation converted any ^{14}C-dioxane present in the sand, soil, or plant tissue to $^{14}CO_2$, which was then captured in 15 mL of scintillation cocktail (RJ Harvey Instrument Corporation). Ten milliliters of the cocktail was placed in a glass scintillation vial and assayed for $^{14}CO_2$ in a Beckman (Fullerton, California) 6000IC liquid scintillation counter (LSC). Soil samples were also dried for 24 hours in a 100 °C oven to determine final moisture content.

To determine ^{14}C-dioxane present in aqueous solutions (hydroponic solution, solvents, and sodium hydroxide [NaOH] traps), samples were injected to 15 mL of Scintiverse I scintillation cocktail (Fisher Scientific, Pittsburgh, Pennsylvania) or 15 mL of Ultima Gold scintillation cocktail (Packard, Berkshire, United Kingdom). The radioactivity in the vials was then counted on the LSC.

A Hewlett Packard (Palo Alto, California) 5890A gas chromatograph (GC) with a dual electron capture detector and flame ionization detector was used to determine dioxane concentrations in hydroponic solutions and solvents. Separation was accomplished with a J&W Scientific (Folsom, California) DB-Wax capillary column (300 mm in length, 0.53 mm inner diameter). Aqueous samples (1 mL) were injected to 2 mL GC sampling vials, and the vial tops were crimped to ensure a reliable seal. The vials were run on the GC using a Hewlett Packard autosampler.

Activated carbon traps (Orbo tube 32 large, Supelco) were used to capture dioxane transpired from leaves. To analyze the trapped dioxane, activated carbon was poured into 4-mL glass vials and submerged in 2 mL of acetone for 24 to 48 hours. Acetone samples were taken (100 μL), injected to 15-mL Scintiverse cocktail, and counted on the LSC. In addition, 1 mL samples were taken and placed in GC sampling vials for analysis.

Dioxane Uptake from Hydroponic Solution. Seven hydroponically grown cuttings were transferred to 278-mL flasks containing 200 mL of half-strength Hoagland's solution. For two of the seven cuttings, the tops were cut off just above the caps to create excised tree controls. These reactors did not evapotranspire significantly and served as controls to test for leaks from the reactors (rubber sealant integrity) and dioxane sorption to roots. After the reactors were completely sealed, they were spiked with a combination of cold dioxane and ^{14}C-dioxane (5.0 μCi per reactor) to achieve a total aqueous concentration of 22.7 mg/L dioxane. An unplanted control was also spiked to 22.7 mg/L to check for dioxane sorption to glass and leakage from reactors.

Daily sampling and maintenance were performed for the duration of the experiment. Reactors were weighed daily to determine the amount of solution transpired by the cuttings. The experiment was terminated after 9 days (217 hours), with the exception of one reactor that exhibited the highest transpiration rate. For this reactor, the hydroponic solution was nearly depleted (43 mL remaining) after only 7.5 days.

Dioxane Uptake and Mineralization from Contaminated Soil. Soil was obtained from the perimeter of a wastewater treatment lagoon located near Salisbury, North Carolina. Soil samples had been previously analyzed for 112 organic compounds and metals, and the soil was analyzed at this time for agronomic properties. The soil had a pH of 7.5 and an organic matter content of 0.9%, and it contained numerous organic contaminants (including dioxane at concentrations of 21.0 ± 32.3 mg/kg and biphenyl ether at 120.4 ± 140.8 mg/kg) and heavy metals. After hydroponic growth, 15 rooted cuttings were transferred to prepared 278-mL flasks. Between 180 and 200 g of air-dried soil was added to each reactor, and deionized water was added to achieve 80% of field capacity. The field capacity, which is the soil water-holding ca-

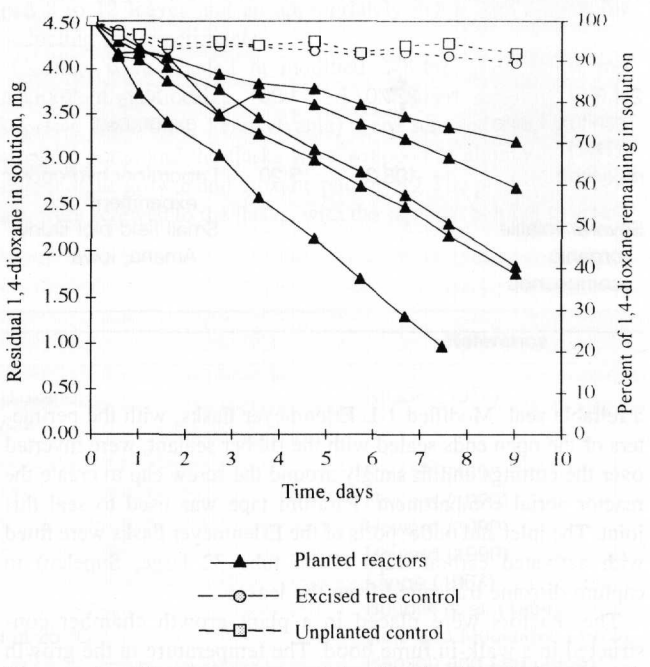

Figure 4—Dioxane removal in the hydroponic experiment. The initial dioxane mass was 4.5 mg (23 mg/L).

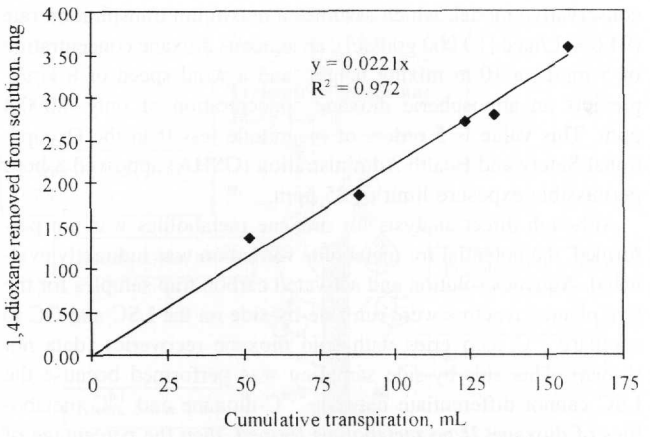

Figure 5—Increase in dioxane removal from hydroponic solution with higher cumulative transpiration.

pacity after gravity drain, was estimated by saturating the Salisbury soil, allowing it to drain, and drying it for 24 hours at 100 °C to determine soil moisture content. The 15 reactors were weighed and placed in the plant growth chamber for a 3- to 5-day acclimation period.

At the end of the acclimation period, the reactors were modified and spiked with dioxane. For 4 of the 15 cuttings, the tops were cut off just above the caps to create excised tree controls. Modified 1-L Erlenmeyer flasks were placed over the tops of the cuttings. Cold dioxane and ^{14}C-dioxane (7.0 μCi per reactor) were mixed with feed water and added through the top sampling port of the bottom flask. The total dioxane spike equaled 10 mg dioxane/kg air-dried soil. The reactors were placed in the plant growth chamber. Air withdrawn from the Erlenmeyer flasks passed through two activated carbon traps in series (to capture transpired dioxane) before bubbling through 15 to 20 mL of 1 M NaOH (to capture $^{14}CO_2$ emitted from the leaves). The reactors were watered approximately every 4 days using a gastight syringe pump system. As water was added through the bottom sampling port at a rate of 3.0 mL/min, headspace gas was removed through the top port at the same rate. The headspace gas was drawn into 5 mL of 1 M NaOH to capture $^{14}CO_2$ generated from dioxane mineralization. After headspace removal, the top sampling port was briefly loosened to allow air to enter the soil compartment (and hence prevent anaerobic conditions).

Four unplanted controls were also spiked at the same time as the 15 planted reactors. For three of the four controls, 3.4 mL of 10 000 mg/L mercuric chloride was mixed with dioxane and feed water before addition to the soil. Mercuric chloride was added to poison microorganisms and thus sterilize the soil.

Sampling and maintenance were performed approximately every 3 days for the duration of the experiment. Reactors were weighed to determine the amount of solution transpired by the cuttings, and deionized water was added as needed to replenish soil moisture to approximately 80% of field capacity. The experiment was terminated 14 to 18 days after spiking. For each reactor, headspace gas was pumped out the top sampling port and drawn through an activated carbon trap and a 15-mL 1 M NaOH trap. This final flushing was performed to ensure effective capture of $^{14}CO_2$. The cuttings were removed from the soil and separated into their various components for combustion in the biological oxidizer.

Results and Discussion

Dioxane Uptake from Hydroponic Solution. Dioxane was removed from the hydroponic solution rapidly (Figure 4). For the five planted reactors, 54.0 ± 19.0% of the initial dioxane spike (range of 30 to 78%) was removed from solution within 9 days. Dioxane was removed at constant rates from the planted reactors, as shown by a linear decrease in dioxane mass over time (Figure 4). Figure 5 reveals that a strong linear relationship also existed between dioxane removal and total transpiration (dioxane removed [mg] = 22.1 × water transpired [L], R^2 = 0.97).

One excised tree control and one unplanted control were used to assess the effect of dioxane sorption to roots, adsorption to glass, microbial degradation, and leaks from the reactors. The excised tree control removed 10.4% and the unplanted control removed 8.1% of the initial dioxane mass in solution. All of the removal took place within the first 48 hours of the experiment. Thus, this removal probably resulted from sorption to glass and roots. These results suggest that leaks and microbial degradation of dioxane in the reactors were minimal.

Dioxane translocation and fate pathways are summarized in Figure 6 and in Table 3. The radiolabel recoveries ranged between 84.7 and 94.3%. The high recoveries indicate that the primary fate pathways for dioxane were identified. Most of the plant-associated

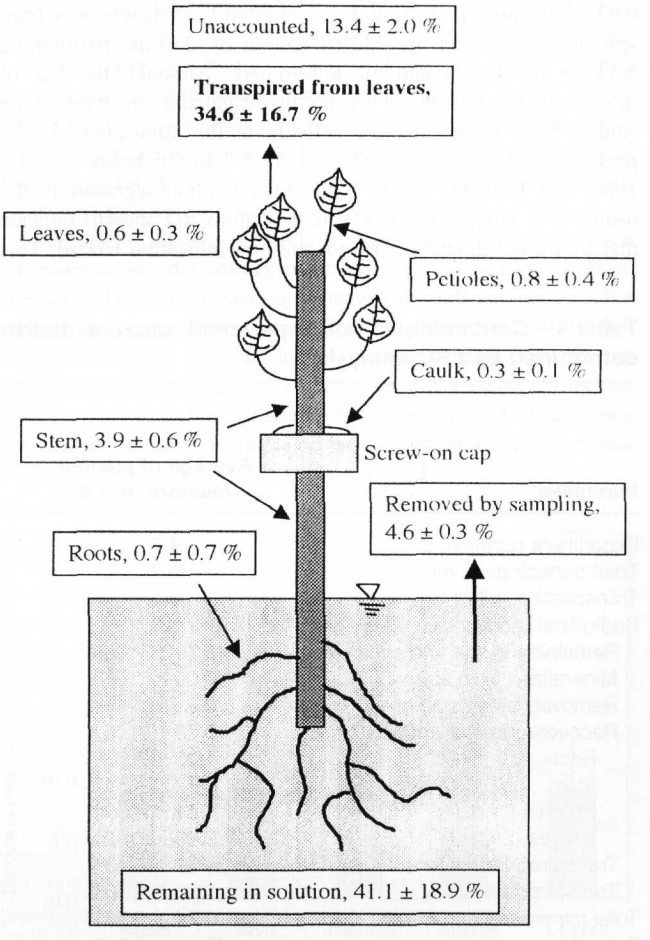

Figure 6—Fate of ^{14}C label in planted reactors (hydroponic experiment) as a percentage of the total ^{14}C-dioxane spike (5 μCi). Values represent the mean ± one standard deviation (n = 5).

Table 3—Distribution of plant-associated ^{14}C label at the end of the experiments.

Plant component	Hydroponic[a] experiment	Contaminated soil[b] experiment
Roots	10.8 ± 8.6%[c]	10.4 ± 4.6%
Stem	65.8 ± 14.4%	54.3 ± 8.8%
Petioles	12.9 ± 4.1%	13.2 ± 2.5%
Leaves	10.5 ± 3.3%	22.1 ± 6.1%

[a] $n = 5$.
[b] $n = 6$.
[c] Mean ± one standard deviation.

mass was recovered from the stem (Table 3). Based on the ease with which cuttings volatilized dioxane, it is likely that the ^{14}C recovered from plant tissue by combustion was primarily entrained in xylem water flow and not substantially incorporated to plant tissue. A large portion (83.6 ± 4.8%) of the ^{14}C taken up by full cuttings was lost to the atmosphere by volatilization from leaf surfaces.

A linear relationship was found between volatilized dioxane (recovered from activated carbon traps) and total transpiration (dioxane transpired [mg] = 14.7 × total transpiration [L], R^2 = 0.93), indicating that the dioxane emitted from leaves was transpiration-driven and not caused simply by dioxane partitioning between the leaves and the atmosphere. Although the fate of dioxane in the atmosphere was not investigated in this work, other studies have shown that its half-life in the atmosphere in the presence of hydroxyl radicals is only 6.7 to 9.6 hours (GEMS, 1986, and Howard, 1990). Even without photodegradation, the results of a simple box model (calculations not shown) indicate that transpired dioxane does not pose an inhalation hazard. This conservative model, which assumes a maximum transpiration rate (94 000 L/ha·d [10 000 gpd/ac]), an aqueous dioxane concentration of 5 mg/L, a 10-m mixing height, and a wind speed of 8 km/h, predicts an atmospheric dioxane concentration of only 0.000 4 ppm. This value is 5 orders of magnitude less than the Occupational Safety and Health Administration (OSHA) approved 8-hour permissible exposure limit of 25 ppm.

Although direct analysis for dioxane metabolites was not performed, the potential for metabolite formation was indirectly evaluated. Aqueous solution and activated carbon trap samples for the five planted reactors were run side-by-side on the LSC and GC to compare ^{14}C recoveries with cold dioxane recoveries (data not shown). This side-by-side sampling was performed because the LSC cannot differentiate between ^{14}C-dioxane and ^{14}C metabolites of dioxane. If no metabolites formed, then the percentage of total ^{14}C label recovered in a sample should equal the percentage of total dioxane recovered in a sample. For the aqueous solution samples, the cold dioxane recoveries were 18.2 ± 2.6% greater than the ^{14}C label recoveries. Thus, cold dioxane could account for all of the radiolabel, which suggests that no dioxane degradation occurred in solution. On the other hand, the activated carbon radiolabel recoveries were greater (12.8 ± 7.8%) than the cold dioxane recoveries. It is uncertain if this discrepancy resulted from experimental error or if dioxane was transformed inside the plant, leading to the transpiration of radiolabeled metabolites.

The TSCF was estimated for dioxane uptake by hybrid poplars. For each of the five planted reactors, the aqueous dioxane concentrations were calculated as an average of the initial and final concentrations (as determined by LSC analysis). The dioxane concentration in xylem water was estimated by dividing the total dioxane mass that passed the roots (mass in stem, petioles, leaves, and transpired from leaves) by the total volume of water transpired. This method assumes that (1) most of the ^{14}C recovered

Table 4—Contaminated soil experiment: dioxane distribution recovery at the end of the experiment (15 to 18 days), as determined by LSC analysis.[a]

Parameter	Average of planted reactors, $n = 6$	Average of excised tree controls, $n = 4$	Average of unplanted sterile soil controls, $n = 3$	Unplanted, $n = 1$
Experiment duration, d	14.7	16.0	15.3	18.0
Total transpiration, mL	59.9	3.6	1.0	
Transpiration, mL/d	4.1	0.2		
Radiolabel recoveries				
Remaining in soil and sand	18.79 ± 7.85%	54.42 ± 7.58%	72.02 ± 7.70%	69.68%
Mineralized from soil	0.09 ± 0.05%	0.15 ± 0.15%		
Removed by air suction	0.35 ± 0.41%	0.02 ± 0.01%		
Recovered from plant tissue	10.78 ± 2.80%	3.44 ± 1.63%		
Roots	1.20 ± 0.63%	0.13 ± 0.10%		
Stem	5.77 ± 1.54%	3.31 ± 1.57%		
Petioles	1.41 ± 0.39%			
Leaves	2.40 ± 1.02%			
Transpired as dioxane	34.32 ± 6.43%	3.50 ± 0.90%		
Transpired as $^{14}CO_2$	0.66 ± 0.40%	1.96 ± 1.54%		
Total mineralization (2, 6)	0.74 ± 0.38%	2.03 ± 1.64%		
Total dioxane taken up or mineralized (2, 4, 5, 6)	45.83 ± 8.61%	8.97 ± 3.52%		
Total recovery of ^{14}C (1–6)	65.00 ± 5.95%	63.46 ± 10.58%	72.02 ± 7.70%	69.68%

[a] Results presented as the percent of total ^{14}C-dioxane spike (7 μCi) added to reactors at $t = 0$.

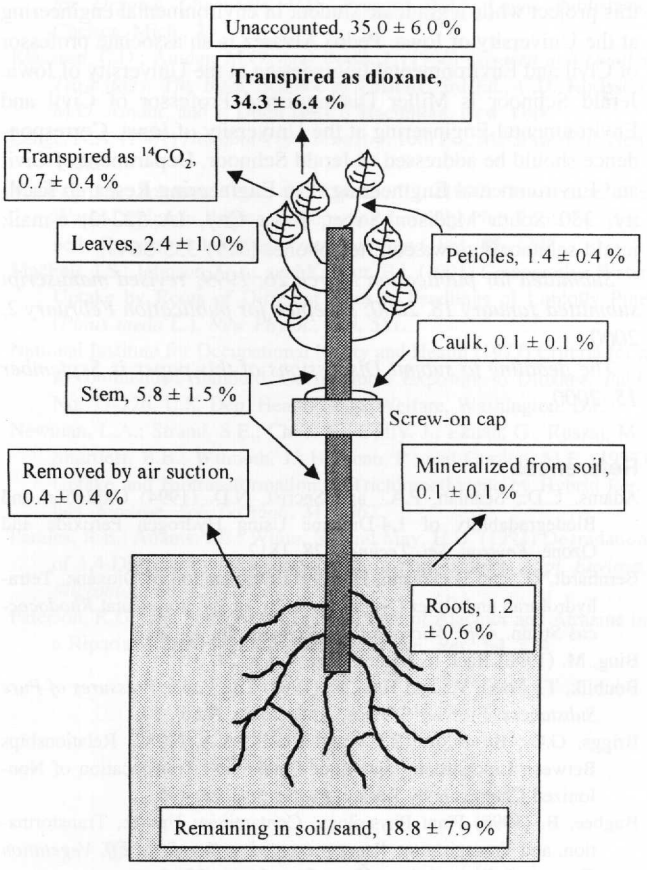

Figure 7—Fate of ^{14}C label in planted reactors (contaminated soil experiment) as a percentage of the total ^{14}C-dioxane spike (7 μCi). Values represent the mean ± one standard deviation (n = 6).

from plant tissue was entrained in xylem flow and had not been incorporated to plant tissue, (2) dioxane found in roots was sorbed to root exterior tissue and had not passed root membranes, and (3) a negligible volume of the water taken up was used for plant synthesis. This method is a conservative means for determining TSCF because it assumes that the unaccounted radiolabel portion (13.3 ± 2.0%) was not taken up. A TSCF of 0.72 ± 0.07 was obtained, indicating that these hybrid poplars had a high propensity to take up dioxane from aqueous solution.

As discussed previously, this measured TSCF is significantly greater than predicted by uptake models that are based on the contaminant K_{ow} value (Figure 2). This discrepancy may be caused by several factors. A primary factor could be that the widely accepted organic uptake theory, which maintains that organic compounds must bind with and pass through semi-permeable cell membranes, is inaccurate. Numerous studies (Chung and Kramer, 1975; Haussling et al., 1988; and MacFall et al., 1990 and 1991) have shown that significant water absorption can occur through suberized tissues and areas of lateral root emergence (where the emerging root has punctured the endodermis). Therefore, nondiscriminating pathways exist for water and chemical entrance to plants, which supports the notion of efficient plant uptake of hydrophilic compounds. Hydrogen bonding of dioxane with water is also feasible because oxygen has a greater electron affinity than carbon, which enhances its electrostatic attraction for water-bound hydrogen. It is possible that efficient uptake is achieved by a combination of the relatively low molecular weight of dioxane, its complete miscibility in water, and hydrogen bonding with water.

Dioxane Uptake and Mineralization from Contaminated Soil. Of the 11 full cuttings (healthy, growing cuttings with roots and leaves) originally planted, five died within the first week. These five cuttings were not assessed for dioxane distribution. Death was preceded by wilting of leaves, appearance of brown spots on leaf tips, browning of leaf perimeters, and low transpiration rates. Four of the surviving six full cuttings ("planted reactors") exhibited similar symptoms by the end of the 14- to 18-day experiment. It is not known which chemical(s) in the lagoon perimeter soil were toxic to the poplar cuttings, but dioxane toxicity is unlikely because a similar spike had no adverse affects in the hydroponic study.

Average ^{14}C-label distributions and recoveries at the end of the experiment for all reactors are provided in Table 4. Average ^{14}C label distributions for the six planted reactors are also displayed in Figure 7. Planted reactors readily removed dioxane from soil and transpired most of it to the atmosphere. After 2 weeks, 45.8 ± 8.6% of the initial ^{14}C-dioxane spike was found in plant tissue, mineralized to $^{14}CO_2$ in the soil zone or transpired from leaves as either ^{14}C-dioxane or $^{14}CO_2$. Of this total portion removed, 76.5 ± 3.9% was transpired through the leaves. Only 18.8 ± 7.9% of the initial dioxane spike remained in the planted soil after 15 days, compared to 72.0 ± 7.7% remaining in sterilized unplanted soil.

Dioxane mineralization from soil was assayed for the planted reactors and excised tree controls. Only 0.74 ± 0.38% of the radiolabel was recovered as $^{14}CO_2$ in planted reactors and 2.0 ± 1.6% in excised tree controls, indicating that mineralization was minimal. These results agree with the previously discussed recalcitrance of dioxane to microbial degradation.

The concentrations of radiolabel recovered from plant tissue expressed as mg dioxane/kg plant tissue are shown in Figure 8. The radiolabel concentrated most heavily in roots and petioles, but most of the mass was recovered from the stem (Table 3). Radiolabel distributions in plant materials were similar between the hydroponic and contaminated soil experiments. One exception was the difference in radiolabel distributions for leaves. It is unknown why leaves in the contaminated soil experiment had a greater

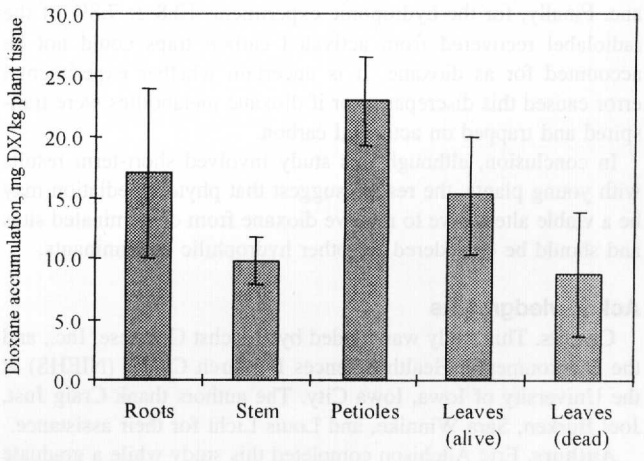

Figure 8—Dioxane concentrations in plant components (fresh weight basis) at the end of the soil experiment. Error bars represent ± one standard deviation (n = 6).

percentage of the total plant-associated radiolabel than those in the hydroponic experiment.

A correlation was found between the amount of radiolabel recovered from plant tissue and the water content of plant tissue (data not shown, $R^2 = 0.89$). The equation for this relationship is as follows: % of total plant-associated ^{14}C in a particular plant component = 0.926 × % of total plant-associated water in a particular plant component. This correlation suggests that most of the radiolabel recovered from plant tissue was water associated (in the transpiration stream) and not accumulating in plant tissue.

Conclusions

This study showed that hybrid poplar trees (*Populus deltoides* × *nigra*, DN34, Imperial Carolina) were effective at removing dioxane from water and soil in laboratory experiments. Specific conclusions that can be drawn from this research are as follows.

Hybrid poplars can efficiently take up dioxane from aqueous solution. In this study, poplar cuttings removed dioxane rapidly from a 23 mg/L solution. Cuttings removed 54.0 ± 19.0% of the initial dioxane mass in solution within 9 days via uptake and translocation. This removal corresponded to a TSCF of 0.72 ± 0.07.

Hybrid poplars can be effective at removing dioxane from contaminated soil. Poplar cuttings readily removed dioxane from a lagoon perimeter soil containing 0.9% organic matter and numerous organic and metal contaminants. Only 18.8 ± 7.9% of the initial dioxane spike remained in planted soil after 15 days, compared to 72.0 ± 7.7% remaining in sterilized unplanted soil.

The primary removal mechanism for dioxane was vegetative uptake through the roots. Of the dioxane taken up by poplar cuttings, most (76 to 83%) was volatilized to the atmosphere by transpiration from leaf surfaces. A box model for "worst-case" conditions caused by dioxane transpiration indicates that the expected concentrations of atmospheric dioxane and its potential byproducts pose little threat to human health.

Poplar cuttings did not exhibit visible toxic effects when subjected to aqueous concentrations of 23 mg/L dioxane. Dioxane mineralization or transformation to metabolites was not observed in aqueous solution. Mineralization of dioxane from soil was minimal, with only 0.74 ± 0.38% of the radiolabel recovered as $^{14}CO_2$ in planted reactors and 2.0 ± 1.6% in excised tree controls. These results reiterate the widely accepted recalcitrance of dioxane. Finally, for the hydroponic experiment, 12.8 ± 7.8% of the radiolabel recovered from activated carbon traps could not be accounted for as dioxane. It is uncertain whether experimental error caused this discrepancy or if dioxane metabolites were transpired and trapped on activated carbon.

In conclusion, although this study involved short-term results with young plants, the results suggest that phytoremediation may be a viable alternative to remove dioxane from contaminated sites and should be considered for other hydrophilic contaminants.

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