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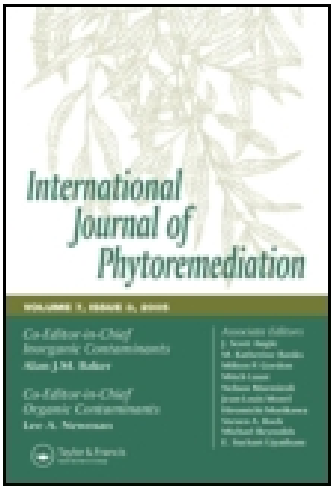

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Phytoremediation at contaminated sites is often complicated by the presence of more than one chemical. However, the effects of common co-contaminants such as ethylene glycol on the phytoremediation of other chemicals, e.g., 1,4-dioxane, is not well understood. Field studies with DN34 poplar trees revealed a 28% decline in growth rate in response to 10 g/L ethylene glycol in the groundwater, thus indicating a significant and deleterious effect on tree viability, and likely, the plants' utility for phytoremediation. Thorough investigations using* Arabidopsis thaliana*, with its small size and rapid life cycle, indicated significant growth reduction at 10 g/L and complete inhibition of germination at 40 g/L ethylene glycol. Ethylene glycol was almost as severe a stressor as the well characterized osmotic inhibitor, sorbitol. Watering potted trees with 10 g/L ethylene glycol reduced their growth by more than 50%, and similar results were observed in hydroponically grown poplar and willow trees. Under hydroponic conditions, 60 g/L ethylene glycol inhibited the phytovolatilization of 1,4-dioxane by more than 80%, and all trees evapo-transpired 1,4-dioxane less efficiently than water. In fact, this efficiency differed between trees and the difference became more pronounced in the presence of ethylene glycol.

KEY WORDS: osmotic effect, transpiration, poplar, willow, *Arabidopsis*, volatile chemicals, co-contaminants

INTRODUCTION

Many industrial sites are contaminated with a variety of chemicals that may include hydrocarbons (Alkorta and Garbisu 2001; Cross et al. 2006), heavy metals (Jarup 2003; Singh et al. 2003), chlorinated solvents (Jones, Kommalapati, and Constant 2001), and pesticides (Susarla, Medina, and McCutcheon 2002). More efficient ways to improve clean-up processes are being actively sought to meet increasingly stringent regulations governing permissible levels in the environment. Phytoremediation is a viable technology that assists in the clean-up of impacted areas through the use of plants that remove contaminants from soils and sediments, and ground and surface waters (Dzantor 2007; Pilon-Smits 2005; Pulford and Watson 2003; Susarla, Medina, and McCutcheon 2002). This technology has been shown to be effective at removing organic chemicals, lowering chlorinated benzenes and absorbing excess nutrients. Phytovolatilization and hydraulic control, also called phytopumping, are

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two phytoremediation processes that exploit the same physiological phenomena in plants (Alvarez and Illman 2006). These both involve removal of water from the soil by roots and its transfer through the plant vascular system to the leaves, the overall process involved is termed the transpiration stream. This process is driven by evaporative water loss from leaf surfaces. Phytopumping employs the transpiration stream as a pump to draw large volumes of contaminated water, thereby preventing the spread of chemicals beyond root systems. When plants have sufficiently large root systems this removal can effectively stop the spread of contaminated groundwater (Susarla, Medina, and McCutcheon 2002). Phytovolatilization also relies on the transpiration stream, but in addition to preventing the further spread of chemicals by slowing or stopping the flow of ground water, the chemicals of concern are also drawn through the transpiration stream and released into the atmosphere from leaves (Macek, Macková, and Kás, 2000). Tree species of *Populus* (poplar) and *Salix* (willow) are often used for these applications in the field because of their effective phytoremediation properties (Pulford and Watson 2003), i.e., they are able to take up large quantities of water with their extensive root systems, and they are generally very resilient to environmental conditions (Schnoor et al. 1995; Trapp and Karlson 2001).

Two commonly found chemicals in contaminated groundwater are the cyclic ether, 1,4-dioxane (dioxane), and the alcohol, ethylene glycol (EG). Dioxane has been used both as an industrial solvent and a solvent stabilizer, particularly for chlorinated solvents (Zenker, Borden, and Barlaz 2003; Tanabe et al. 2006). Furthermore, it has also been found to be a byproduct of chemical processes involving EG (Popoola 1991), and as such, is a common co-contaminant with it. Ethylene glycol is an alcohol that is used as an automotive anti-freeze and an airplane deicer as well as a common component used in the synthesis of plastics (Kim et al. 2001; Pillard and DuFresne 1999).

Dioxane is miscible in water and because it adsorbs very weakly to substrate particles, it travels through groundwater more quickly than most other chemicals. This makes it an ideal environmental indicator for how extensively contamination can spread from a source (Stickney et al. 2003; Tanabe et al. 2006) as well as signifying that, when present, it should be a priority for clean-up. Dioxane is considered a probable carcinogen (Stickney et al. 2003) and its drinking water limit has been set at 3 μg/L by the U.S. Environmental Protection Agency (EPA) (Aitchison et al. 2000). Traditional mechanisms of remediation for dioxane include air stripping, chemical treatment, and carbon adsorption, all of which are generally ineffective (Aitchison et al. 2000; Zenker, Borden, and Barlaz 2003). Processes that enhance oxidation and biodegradation have shown promise although they are both relatively labor intensive and expensive. (Adams, Scanlan, and Secrist 1994; Zenker, Borden, and Barlaz 2003). Alternatively, studies have examined the removal of dioxane by trees. Hybrid poplars have been shown to remove 54% of the dioxane from hydroponic solutions in the laboratory, of which up to 83% was released into the air (Aitchison et al. 2000). When dioxane is released into the atmosphere it reacts with hydroxyl radicals and is broken down with a half-life of 6–10 h (Howard 1990).

Ethylene glycol can severely affect the kidneys, nervous system, lungs, and heart, and the U.S. Agency for Toxic Substances and Disease Registry recommends that lifetime exposure rate by ingestion not exceed 14 ppm (ATSDR 2007a). It has been found in at least 37 of 1,689 National Priorities List sites identified by the U.S. EPA (ATSDR 2007b), where it can be present at concentrations well above 10 g/L (U.S. EPA 2002). Ethylene glycol is water miscible with a density of 1.1 kg/L and Henry's Law Constant of 8.05E-9 atm-m3/ mol. This chemical can be degraded aerobically at concentrations of up to 100 g/ L by indigenous microbes in soils that contain adequately high moisture contents (McVicker, Duffy, and

Stout 1998). Although degradation can occur under anaerobic conditions (Klotzbücher et al. 2007), it is severely impeded by the presence of clay in the substrate (McVicker, Duffy, and Stout 1998). Germination and growth studies have shown that EG has serious toxic effects on plants (Kim et al. 2001; Pillard and DuFresne 1999), and as a consequence, would be expected to lessen the efficiency of phytoremediation. However, its effect on phytopumping and phytovolatilization, per se, has not been assessed.

Therefore, the present work was undertaken to examine the effect of EG on growth and transpiration in plants. In addition, its effect was compared to that of the known general osmotic stressor, sorbitol (Ahmad, Larher, and Stewart 1979; Chen and Murata 2002; Lambers, Chapin III, and Pons 2008; Mikolajczyk et al. 2000). Osmotic pressure prevents normal water uptake and consequently alters water balance and nutrient distribution within plants. *Arabidopsis thaliana* was used as a model plant species to investigate the toxicity of this chemical and to provide a basis of comparison to this chemical's effects on hybrid poplars and willows.

MATERIALS AND METHODS

Tree Varieties

Hybrid poplars, DN34 and NM1, and hybrid willow, S301, were obtained from LandSaga Biogeographical Inc. (New Hamburg, ON, Canada). The trees were planted in ten inch pots and acclimated to greenhouse conditions supplemented with lighting provided by metal halide lamps for 16 h per day at 22°C. The pots were placed in trays and watered from the bottom every 2 days. Trees were prepared for experiments according to the following technique. Semi-woody sections from the LandSaga stock trees were cut into 15–20 cm lengths and their leaves removed. Then holes of 2–3 mm were drilled at a distance of 2.5 cm from the bottom of each of the tree cuttings. Toothpicks that had been pre-soaked for 12 h in a solution of indole-3-butyric acid (4000 mg/L in ethanol), a rooting hormone, and air dried were inserted into the holes (Struve and Blazich 1982). The cuttings were then planted to develop roots in 5 cm diameter pots containing a 1:1 mixture of Miracle-Gro® Premium Potting Mix (0.14–0.14–0.14) and coarse sand (Unimin 2075 from Emmett, ID), and watered as required.

Effect of Ethylene Glycol on Plant Growth

To investigate the effect of EG on whole plants grown in liquid culture, the model plant species, *Arabidopsis thaliana* (type Columbia), was propagated according to the method of Hetu, Tremblay, and Lefebvre (2005). Surface sterilized seeds were placed on 2.5 cm × 2.5 cm stainless steel screens of type 304 woven wire mesh (Ferrier Wire Goods, Toronto, Canada) on half strength MS nutrient agar (0.7%) plates containing 10 g/L sucrose. The seeds were placed in the dark for 3 days at 4°C to synchronize subsequent germination and then transferred to 16:8 h day:night periods using fluorescent lighting (150 μmol quanta photosynthetically active radiation $m^{-2}s^{-1}$) at 22°C. After 7 days, the anchored seedlings were aseptically transferred to 125 mL Erlenmeyer flasks containing 10 mL of half strength MS nutrient solution supplemented with 10 g/L sucrose, and these were placed onto a rotary shaker (75 rpm) under the same day to night regime as above. After 3 days the nutrient solution was increased to a volume of 15 mL and sucrose was increased to 30 g/L to enhance

root growth. The plants were then grown for an additional 7 days, with the nutrients and sucrose replenished at 3 and 6 days. Then the sucrose was replaced with 0–120 g/L EG and the plants were harvested 6 days later to determine their dry weights.

Arabidopsis thaliana (type Columbia) seedlings were also grown on agar plates containing sugar-free MS nutrient agar (0.7%) buffered to pH 5.8 with 25 mg/L MES [2-(N-morpholino) ethanesulfonic acid]. Concentrations of EG within the agar ranged from 10-90 g/L. Seeds were sown in replicates in a straight-line approximately 2 cm from the top edge of the plates. The plates were placed in the dark for 3 days at 4°C to synchronize subsequent germination prior to being set vertically under fluorescent lighting (16 h days) for 20 days at 22°C or until the plants' roots reached the bottom edge of the plates. Leaf number, foliar rosette diameter and root length were measured every second day after germination. Similar studies were also performed using sorbitol as an osmotic stressor. Comparisons between osmotic effects of ethylene glycol and sorbitol must take into account that the molar weight of sorbitol is approximately 2.94 times that of ethylene glycol.

Hydroponically Grown Trees

Poplars, DN34 and NM1, and willows, S301, were grown hydroponically in the presence of both EG and dioxane. Trees were placed into enclosed systems for seven days to assess their transpiration and the amount of dioxane they removed from the solution bathing the roots. To perform these investigations, six-week-old rooted tree cuttings were gently separated from their sandy soil mixture and placed into 500 mL polyethylene bottles containing dH_2O. These bottles were placed into deep trays containing 5 cm of water. Each tray contained a mist maker (Keri Electronic Co., Ltd., China) and was covered with clear plastic wrap for 12 days under greenhouse lighting conditions. Misting was set at 10 h per day for the first 4 days followed by decreases of one hour each day between days 5 and 12 at which point no further misting was applied. This prevented leaf wilt while enabling the cuttings to adjust to hydroponic conditions. Seven days after being placed into the bottles, the dH_2O was replaced by MS basal nutrient solution (Murashige and Skoog 1962).

On day 13, the trees were placed in enclosed systems constructed from 500 mL polyethylene bottles used as the root containing chambers over which 1L polyethylene bottles were employed as shoot chambers. The bottles were modified to include openings through which to supply treatment solutions and air flow. Roots and shoots were partitioned separately into each bottle connected at the lids. The tree trunks were wrapped in plastic film and inserted through holes in the lids followed by sealing with aquarium grade rubber silicone caulking. Masking tape was affixed to the outside of the lids to ensure that the bottles were securely fastened together and Parafilm® M (Fisher Scientific Co.) was wrapped over the masking tape to provide air-tight seals. Applying aluminum foil to the outside of the root chamber prevented algal growth. Trees were supplied with MS nutrient solution supplemented with 36 mg/ L dioxane, and either 0, 10, or 60 g/L EG. Charcoal tubes (ORBO 32 Large, Sigma-Aldrich), used to recover transpired dioxane, were connected between the upper valve of the 1 L bottles and plastic tubing attached to a vacuum pump drawing air at a rate of 1.5 L/min. Every day the tree chambers were disconnected from their pumps, weighed, and replenished with nutrient treatment solution back to the starting weights. Controls were trees with their leaves removed.

Field Studies

Trees were located in a clay-loam soil over a groundwater plume 1–2 m below grade that contained up to 100 g/L ethylene glycol and 5 mg/L 1,4-dioxane. Growth of field grown trees was determined by measuring increases in stem girth followed by conversion into percent incremental increase in stem cross-sectional areas. This was performed at five set distances from the ground level.

1,4-Dioxane Determination. The amount of dioxane in the charcoal tube traps was determined according to the National Institute for Occupational Safety and Health (NIOSH) Method 1602 (Schlecht and O'Connor, 2003). The first bed of charcoal in the tube was desorbed using 2 mL of carbon disulfide. Five μL of this mixture was run through a gas chromatograph using a flame ionization device to determine the amount of dioxane present (Analytical Services Unit, Queen's University, Kingston, Canada).

Statistical Analyses. Statistical comparisons were performed using either student t-tests or one-way ANOVAs with mean comparisons tested using the Tukey-Kramer HSD. All analyses were performed using JMP 6.0.2 (SAS Institute Inc., Canada).

RESULTS AND DISCUSSION

Plant Growth Studies

When DN34 poplars were grown at a contaminated field site that exposed their roots to 10 g/L EG, the increase in stem biomass over a one year period was only 156.5 $\pm$ 4% by comparison with 217 $\pm$ 16% for control trees (means and S.E., n = 4). These estimates were derived from measurements of stem diameter at five separate distances above the level of the soil. One percent EG in the groundwater stunted tree growth by 28%. Furthermore, over the same period on the same site, growth of *Populus balsamifera* in 100 g/L EG was only 35 $\pm$ 4% compared to 159 $\pm$ 7% in 10 g/L EG (mean and S.E., n = 4).

Effect of Ethylene Glycol and Sorbitol on *Arabidopsis thaliana*

In addition to biomass production, testing of the toxic effects of chemicals on plants also commonly employs measuring delays in the timing of seed germination and decreases in the size of shoots and roots (Klaine, Lewis, and Knuteson 2003). Other than shoot biomass estimates as given above for the poplars, these measurements can be very difficult to determine in trees such as hybrid poplar and willow that do not form seeds readily, if at all, and have multiple branching habits. Therefore, these growth parameters were investigated in some detail using the plant that is commonly used as a model experimental species, *Arabidopsis thaliana*. Although other studies on the effect of EG on plants have been performed (Kim et al. 2001; Pillard and DuFresne 1999), these are limited in scope and more extensive investigations were performed with Arabidopsis.

Studies using the axenic method of Hetu, Tremblay, and Lefebvre (2005) indicated that growth of the plant shoots were more sensitive to EG than the roots. The shoots showed a decline in growth of 42% at 60 g/L EG (ANOVA, $P < 0.05$, n = 6), whereas the roots did not show significant growth differences even at 150 g/L EG. However, in order to grow adequate amounts of root material by this liquid medium culturing method, plants were supplemented with sucrose for 7 days prior to exposure to EG. As a consequence, elevated carbohydrate content of the roots could have imparted tolerance to EG. It, therefore, became

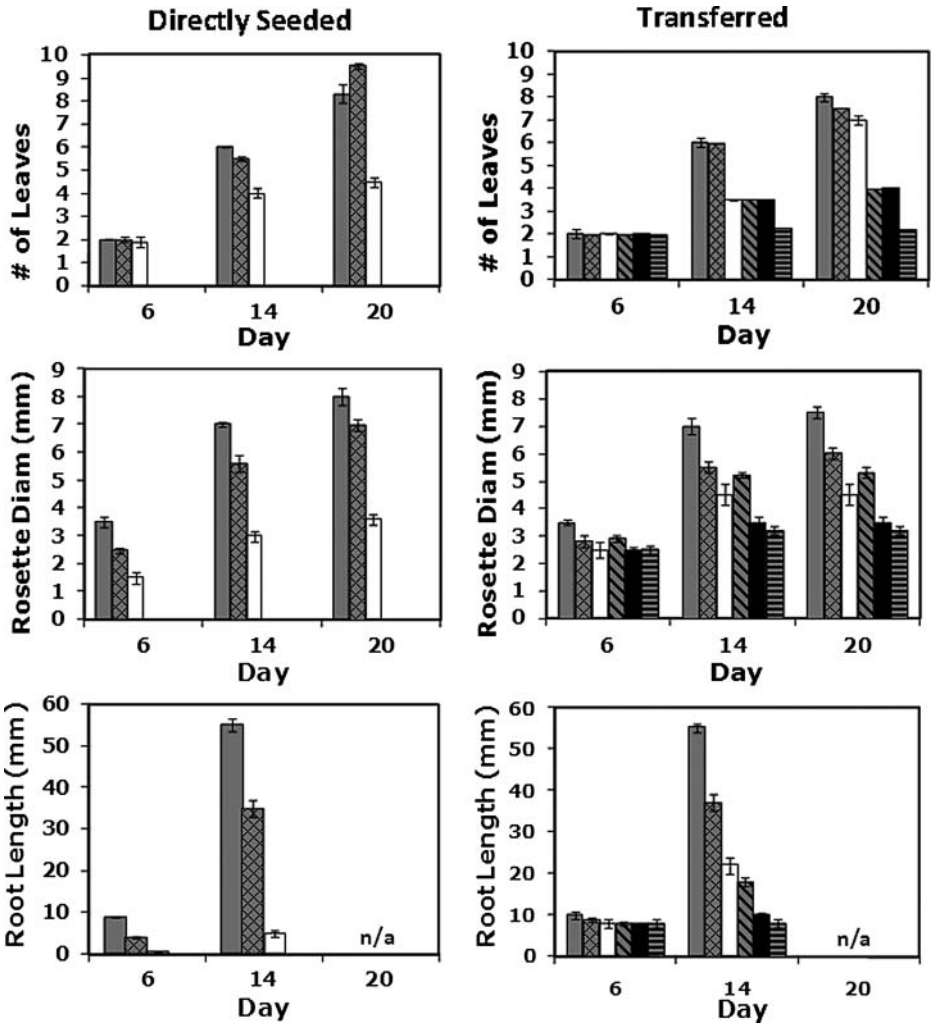

Figure 1 *Arabidopsis thaliana* placed on plates containing a range of ethylene glycol concentrations as either seeds (Directly Seeded) or after 6 days post germination on ethylene glycol-free plates (Transferred). Grey, no EG; cross hatches, 10 g/L; white, 30 g/L; descending hatches, 40 g/L; black, 60 g/L; horizontal hatches, 90 g/L ethylene glycol. Means and standard errors (n = 4).

necessary to abandon this growth method and perform these studies using sugar-free semi-solid agar medium.

Analysis of the effects of EG on the germination and growth of Arabidopsis was performed on agar medium in vertically oriented square Petri plates by placing the seeds on the agar 15 mm from the top of the plates. Plants were started from seed on EG containing nutrient agar (Figure 1, Directly seeded). Those placed on plates containing 40 g/L EG or higher did not germinate. By day 14 Arabidopsis leaf number of plants grown in 30 g/L EG decreased on average by 45% and 42% by comparison with the control and those grown at 10 g/L EG (ANOVA, $P < 0.05$, $n = 19$), respectively. This trend continued through to

day 20. In addition, at as early as 6 days on 30 g/L EG, the rosette diameter was 49% smaller than in the controls (ANOVA, $P < 0.05$, $n = 19$) indicating substantial reductions in shoot biomass. The effect was even more severe on the root system as EG elicited a 54% decline in growth at as little as 10 g/L (ANOVA, $P < 0.05$, $n = 19$). So this compound has a profound and deleterious effect on the growth of Arabidopsis plants and the root system is particularly sensitive. The findings of these direct exposure studies corroborate other studies involving the germination rates of radish (Kim et al. 2001) and the growth of lettuce and ryegrass (Pillard and DuFresne 1999). For example, radish seeds could not germinate at 50 g/ L EG (Kim et al. 2001). Similarly, with respect to glycols in general, the roots of lettuce and ryegrass were much more sensitive than shoots (Pillard and DuFresne 1999). Combined with our results using Arabidopsis, this is conclusive evidence that direct exposure to EG has a strong inhibitory effect on plant taxa as diverse as Poaceae (ryegrass), Asteraceae (lettuce), and Brassicaceae (Arabidopsis and radish). Therefore, EG at these levels would be expected to affect all higher plants, including the phytoremediation relevant Salicaceae (poplars and willows).

For purposes of phytoremediation, the enlisted plants are commonly first established in contaminant free soil prior to transplantation into polluted sites. In addition, the short root systems of seedlings or transplanted plants would not normally encounter pollutants until their roots grow and penetrate further into the contaminated substrate. Therefore, the effect of EG on already established plants was investigated. To do so *Arabidopsis thaliana* seeds were germinated and grown in EG-free medium for 6 days prior to transfer into media containing 10 to 90 g/ L EG (Figure 1, Transferred). Differences in leaf number, rosette diameter, and root length were clearly apparent 8 days after transfer. Those exposed to 30 g/L EG had one third fewer leaves than the control (ANOVA, $P < 0.05$, $n = 26$). Similarly, rosette diameters were reduced by an average of 38% (ANOVA, $P < 0.05$, $n = 26$). Here again, roots were more severely affected than shoots. For example, plants transferred to 10 g/ L EG had 31% shorter roots on average than controls at 8 days (ANOVA, $P < 0.05$, $n = 26$), even though their shoots were not affected significantly. Interestingly, although *A. thaliana* seeds could not germinate at 40 g/ L, transferred plants could grow in concentrations of up to and including 90 g/ L EG, albeit at a reduced rate; i.e., 70 and 50% rosette diameter, and 30 and 13% root length at 40 and 90 g/L, respectively. This supports the notion that transplanting established potted plants is a viable option for implementing phytoremediation at sites containing high levels of EG.

Even though the major stress caused by EG on plants was presumed to be osmotic in nature, possible complications through metabolic interference by this compound might also be implicated. For comparative reasons, sorbitol was employed as a known osmotic stressor.

Arabidopsis thaliana seeds were grown on plates containing various levels of sorbitol equivalent to the concentrations used for EG. Germination was prevented at 117.6 g/L sorbitol (Figure 2). Plants grown on plates containing 88.2 g/L sorbitol produced virtually no leaves (ANOVA, $P < 0.05$, $n = 18$) and had 95% shorter roots ($P < 0.05$, $n = 18$) after six days of growth. The plants also had 36% smaller rosette diameters by the sixth day of growth compared to the controls ($P < 0.05$, $n = 18$). On the other hand, plants grown in normal conditions for eight days and transferred to 88.2 g/L sorbitol for 6 days had 33% fewer leaves ($P < 0.05$, $n = 30$), 40% reduced rosette diameter ($P < 0.05$, $n = 30$), and 76% shorter roots ($P < 0.05$, $n = 30$). These reductions are similar in nature and even more severe than those seen when plants were transferred to EG containing plates

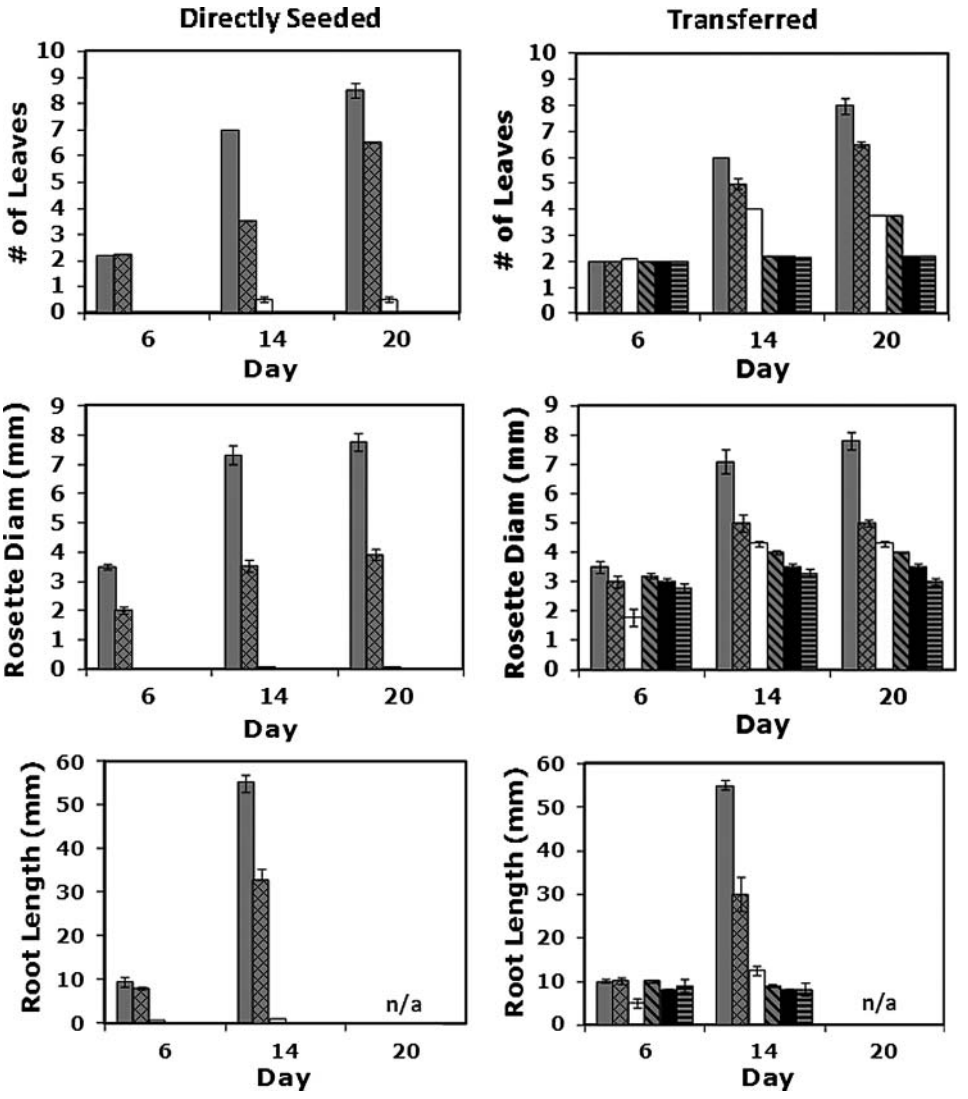

Figure 2 *Arabidopsis thaliana* placed on plates containing a range of sorbitol concentrations as either seeds (Directly Seeded) or after 6 days post germination on EG-free plates (Transferred). Grey, no sorbitol; cross hatches, 29.4 g/L; white, 88.2 g/L; descending hatches, 117.6 g/L; black, 176.4 g/L; horizontal hatches, 264.6 g/L sorbitol. These sorbitol concentrations are the same molar equivalents of the respective ethylene glycol concentrations in Figure 1. Means and standard errors (n = 4).

after growing for six days on normal MS nutrient plates. Considering that the anatomical alterations caused by both EG and sorbitol were very similar, the stress caused by EG to the plant tissue is deemed to be the result of a general osmotic stress and not because of any direct metabolic interference by EG.

Why EG did not have as severe an effect as the known osmotic inhibitor, sorbitol, may be because of the partial water-like properties of EG, itself (Huot et al. 1988; Matsugami et al. 2006). Nevertheless, the harmful effects of this common contaminant are severe.

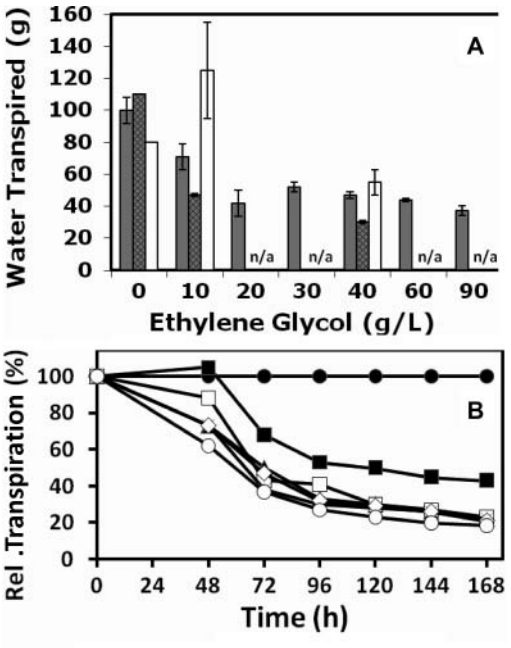

Figure 3 Transpiration from poplars DN34 and NM1, and willow S301 when planted in soil and subjected to varying ethylene glycol concentrations for 7 days. Grey, DN34; hatched, NM1; white, S301. A) Cumulative mean transpired. B) Relative mean transpiration by poplar variety DN34. ● , 0; ■, 10; ▲, 20; △, 30; □, 40; ◇, 60 and ○ , 90 g/L ethylene glycol. Means and standard errors (n = 4).

Effect of Ethylene Glycol on Transpiration from Trees

Trees can be employed as effective agents of phytoremediation in sites that contain water soluble contaminating chemicals. Their transpiration processes can volatilize large quantities of water into the atmosphere, thereby slowing down or even completely halting the flow of contaminated groundwater. Furthermore, because measuring transpiration rates is a non-destructive means of assessing plant health (Davis et al. 1974; Yeo and Flowers 1986), any reduction could be attributed to the effect of chemicals present in the substrate (Trapp et al. 2000). Because poplar and willow trees are commonly employed for phytoremediation purposes, the effect of EG on their rates of transpiration was investigated.

Poplar varieties, DN34 and NM1, and the willow, S301, were subjected to a range of EG concentrations and the amount of water transpired was determined after 7 days of treatment (Figure 3a). Increasing the concentration of EG significantly lowered transpiration. For example, at 20 g/L EG, DN34 exhibited a 49% decrease in transpired water by comparison to controls (ANOVA, $P < 0.05$, $n = 19$). Furthermore, some degree of leaf abscission was seen at all concentrations above 40 g/L. These effects occurred within 3 days in DN34 (Figure 3b) at which time the control trees transpired 55–62% more water than trees treated at or above 30 g/L EG (ANOVA, $P < 0.05$, $n = 19$). By day 4, the EG treated trees were using 42–79% less water than the control trees (ANOVA, $P < 0.05$, $n = 19$). Studies with other chemical contaminants have revealed similar reductions in transpiration; i.e., up to 50% for sunflower in excess fertilizer, detergent and oil (Gadallah 1996), poplar in 2,4,6-trinitrotoluene (Thompson et al. 1998) and willow in cyanide (Larsen, Ucisik,

and Trapp 2005), 3,5-dichlorophenol (Trapp et al. 2000), diesel and gasoline (Trapp et al. 2001), tributyltin, (Trapp, Ciucani, and Sismilich 2004), and phenol (Ucisik and Trapp 2006), however the chemicals in these studies are more likely to disturb metabolic processes than to have osmotic effects. In fact, EG may be the only common environmental contaminant to directly affect water availability in plants, although lipophilic compounds can compromise membrane integrity (Doucette et al. 2003).

Phytovolatilization of 1,4-Dioxane in the Presence of Ethylene Glycol

Aitchison and colleagues suggested some time ago (Aitchison et al. 2000) that phytovolatilization is a viable alternative for removal of dioxane and other hydrophilic compounds from contaminated sites. In fact, laboratory and greenhouse studies have indicated that large amounts of dioxane (Aitchison et al. 2000; Ouyang 2002) and methyl tertiary-butyl ether (Arnold, Parfitt, and Kaltreider 2007; Winnike-McMillan et al. 2003) can be removed by this process. Furthermore, even hydrophobic compounds such as trichloroethylene (Doucette et al. 2003; Orchard et al. 2000) can be readily phytovolatilized (Burken and Ma 2006). However, xenobiotic chemicals are usually present in contaminated field sites in mixtures of polyaromatic hydrocarbons (Huang et al. 2004), petroleum hydrocarbons (McCutcheon and Schnoor 2003), polychlorinated biphenyls (Olson, Reardon, and Pilon-Smits 2003), and BTEX hydrocarbons (Weishaar, Tsao, and Burken 2009) that could complicate phytoremediation efforts. Ethylene glycol is employed as an antifreeze and a feedstock for polymer production. Therefore, because 1,4-dioxane is a common impurity in antifreeze (U.S. EPA 2009a) and a byproduct of polymer synthesis (Popoola 1991), it is to be expected that they would co-contaminate many of the U.S. EPA National Priority List sites (ATSDR 2007b). Furthermore, there is a growing awareness of the widespread presence of 1,4-dioxane as analytical procedures have become more sensitive (U.S. EPA 2009b).

Therefore, hydroponic laboratory experiments using DN34 and NM1 poplars, and S301 willows were undertaken to determine the influence of EG on phytovolatilization of dioxane.

Concentrations of EG used in these laboratory studies emulated those of a field site that is currently under investigated. At least one U.S. EPA National Priority List site has similar concentrations of EG (U.S. EPA 2002) and contains substantial quantities of 1,4-dioxane (U.S. EPA 2006). The amount of water transpired by the trees was severely affected by increasing the EG treatment level (Figure 4a). Transpiration was lowered on average by 36% and 92% (ANOVA, $P < 0.05$, $n = 18$; $P < 0.05$, $n = 9$) in 10 g/L and 60 g/L EG, respectively, for all trees. Consequently, their ability to remove dioxane was also expected to decrease with increasing EG levels as is shown on a per tree basis in Figure 4b.

The efficiency of dioxane removal from solution was determined by dividing the amount of phytovolatilized dioxane by that which would volatilize if dioxane entered and passed through the trees in a manner identical to water. If it did the dioxane removal efficiency would be 100%. Interestingly, the efficiency of dioxane removal at 60 g/L EG was lowest for DN34 and highest for the willow, S301, with NM1 intermediate between the two (Figure 4c) and these differences became more pronounced at higher EG levels. Results indicated that DN34 at the 60 g/L EG released only 16% and 22% of the dioxane volatilized by controls and the 10 g/L EG treated plants (ANOVA, $P < 0.05$, $n = 18$), respectively. The effect of EG on NM1 and S301, in this regard, was significantly less (ANOVA, $P < 0.05$, $n = 18$ and 9 respectively for 10 and 60 g/L EG). Under the conditions of these experiments

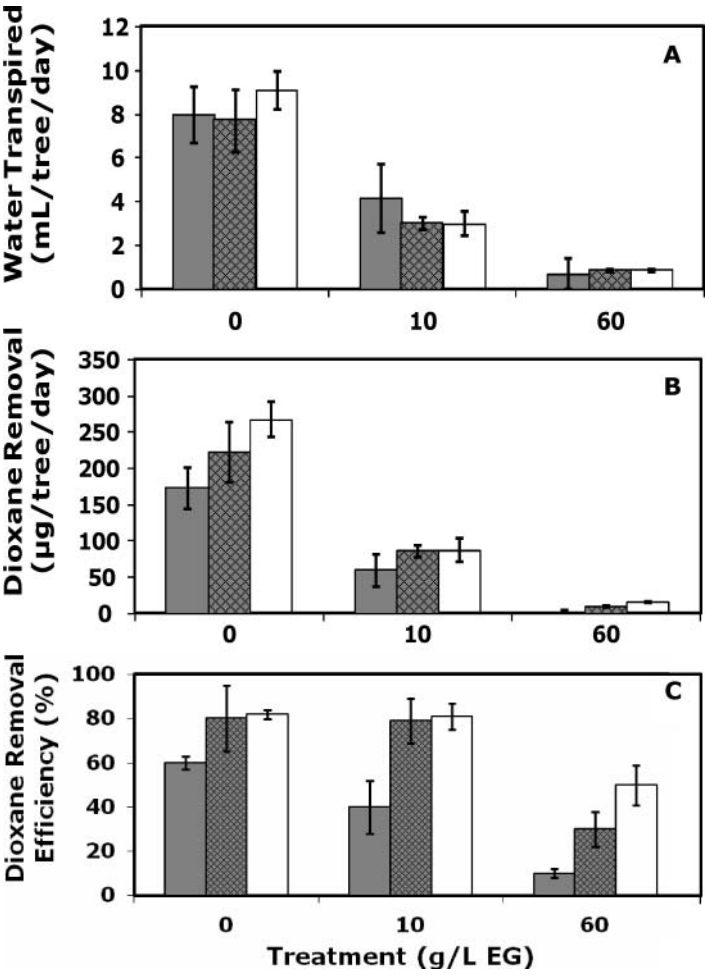

Figure 4 Total water transpired and dioxane removed by poplars (grey, DN34; hatched, NM1) and willow (white, S301) across hydroponic treatments with varying amounts of ethylene glycol after 7 days of growth. (a) Water transpired by the three tree varieties in 7 days. (b) Dioxane removed by the tree varieties. (c) Efficiency of dioxane removal by trees. Volatilized dioxane divided by amount of dioxane removed from the root solution if dioxane transpired in a similar manner to water. Means and standard errors (n = 4).

where transpiration was not different between the types of trees, it appears that NM1 and especially S301 out performed DN34 in their ability to phytovolatilize dioxane.

CONCLUSIONS

There are major deleterious effects of EG on transpiration and phytovolatilization of dioxane. These appear to stem mainly from an osmotic effect, although not as extreme as the effect of sorbitol in direct comparisons using *Arabidopsis* seedlings. Of note was that phytovolatilization of dioxane from trees is not solely dependent on the transpiration stream for water, i.e., dioxane is volatilized at a disproportionately lower rate to the effect of increasing EG concentrations on water transpiration. This could mean that dioxane

entry into roots is hindered by association with EG that might pose a serious drawback when applying phytoremediation at contaminated sites. Our preliminary efforts in the field indicate that only very low amounts of dioxane are volatilized by DN34 growing over a chemical plume containing EG (data not presented). However, further studies are required to assess how much of this can be attributed to the effect of EG, biodegradation of dioxane (Kelley et al. 2001), relative uptake of surface versus ground water (Clinton et al. 2004), or limited oxygen availability to roots closely associated with the chemical plume. It is also important to point out that even though all tree types investigated in the laboratory were severely affected by EG, NM1, and, in particular the willow S301, had better removal efficiencies than DN34. Although this study concentrated on dioxane in the presence of EG, it emphasizes the importance of plant choice for phytovolatilization treatments of chemicals at sites containing mixtures of chemical contaminants.

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