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Uptake and Transformation of Trichloroethylene by Hybrid Poplar: Laboratory Studies

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UPTAKE AND TRANSFORMATION OF TRICHLOROETHYLENE BY HYBRID POPLAR: LABORATORY STUDIES

by

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ABSTRACT

Uptake and Transformation of Trichloroethylene

by Hybrid Poplar: Laboratory Studies

by

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Trichloroethylene (TCE) was widely used as an industrial solvent and degreasing agent for most of the twentieth century. It is now a widespread groundwater contaminant. Phytoremediation may be a cost-effective cleanup method for TCE-contaminated soils and groundwater. Studies of environmental TCE fate are complicated by its volatility. The literature reports both significant and insignificant plant uptake of TCE. Conflicting findings may be due to differences in exposure level, conditions, and duration of the studies, or to experimental artifacts from laboratory systems.

This research quantified plant uptake and volatilization of TCE using a unique laboratory system. Hybrid poplar trees were exposed to 1 or 10 ppm TCE over a 43-d period. [^{14}C]TCE was added to four high-flow, aerated, hydroponic plant growth chamber systems designed to provide high mass recoveries, an optimal plant environment, and complete separation between foliar and root uptake.

Transpiration stream concentration factors (TSCFs) for TCE, calculated from total [^{14}C]TCE in shoot tissues plus phytovolatilized ^{14}C, were 0.11 for two 1 ppm treatments and 0.15 for a 10 ppm treatment with roughly 25% attributed to phytovolatilization. Though extending study duration from 26 to 43 d resulted in accumulation of more mass of ^{14}C in plant tissues, it had no effect on TSCF. These TSCF values are much lower than other published experimental values and values predicted by a theoretical relationship between TSCF and octanol-water partition coefficient. The TCE metabolites trichloroethanol (TCEt), trichloroacetic acid (TCAA), and dichloroacetic acid (DCAA) were identified in plant tissues of the 10-mg/L treatment.

Hybrid poplar uptake of TCAA and TCEt was quantified using a simpler aerated hydroponic system. TSCF values were calculated based on extractable parent compound in shoot tissues. TSCF for TCEt was < 0.01. Presence of TCAA in hydroponic solution and in leaf and root tissues indicated transformation of TCEt to TCAA. TSCF for TCAA was < 0.03 and decreased with increasing exposure concentration. TSCF also decreased under oxygen-limited root-zone conditions. Presence of DCAA in leaf and root tissues indicated transformation of TCAA to DCAA. Transformation of parent compound, coupled with low extractability, may contribute to low TSCFs.

(150 pages)

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INTRODUCTION

Overview

The remediation of organic pollutants is a perpetual and expensive problem. Over the next 30 years, government agencies and private industry in the United States alone will spend $6 billion per year to remediate contaminated soils and groundwater (Cunningham et al., 1996). Development of new, cost-effective methods for remediation is underway. Plant-based bioremediation, or phytoremediation, is a technology in its infancy when compared with other bioremediation strategies. Defined as "the use of green plants and their associated microbiota . . . to remove, contain or render harmless environmental contaminants (Cunningham et al., 1996)," phytoremediation utilizes the natural ability of plants to remediate polluted sites.

The ability of plants to affect the chemical, physical, and biological processes that occur in their immediate vicinities has long been recognized. Throughout its life cycle, a plant can significantly alter the soil that surrounds it, especially that in the rhizosphere. The rhizosphere consists of the root surfaces and adjacent soil (Marschner, 1995). Erosion caused by wind and rain is minimized by the presence of plants, while water flow in plant/soil systems is influenced by plant transpiration. Regulation of water flow may limit the migration of soil and water contaminants and may lead to the sorption of surface leachate (Schnoor et al., 1995). Additionally, plant root systems increase rhizosphere microbial activity through the addition of organic carbon from root exudates (Rovira and Davey, 1971; Marschner, 1995; McFarlane, 1995) and increase soil oxygen availability by removing soil water in transpiration.

The goal of phytoremediation is to remove or contain the contaminant or to alter its chemical and physical nature so that it is no longer a threat to human health and the environment. Mechanisms of phytoremediation include: 1) direct plant uptake, accumulation, and/or metabolism of contaminants; 2) stimulation of rhizosphere microbial populations by the release of root exudates and plant enzymes; 3) suppression of contaminant migration through hydraulic control; and 4) translocation of volatile or semivolatile organic compounds through the plant and subsequent volatilization from the leaf surface.

One target of this emerging technology is trichloroethylene (TCE). A chlorinated, volatile, organic hydrocarbon, TCE is among the most prevalent and recalcitrant groundwater contaminants in the United States and appears on the Environmental Protection Agency's list of priority pollutants. Because it is resistant to combustion and explosion, TCE was widely used as an industrial degreaser and solvent for most of the twentieth century. The extensive use and chemical stability of TCE have led to widespread groundwater and soil contamination.

The literature has reported both significant and nonsignificant uptake of chlorinated solvents. Recent studies (Burken, 1996; Newman et al., 1997) describe significant uptake followed by transpiration (phytovolatilization) of TCE by hybrid poplar. Others (Schroll et al., 1994; Schnabel et al., 1997; Orchard et al., 2000b) have reported little TCE uptake.

Trichloroethanol (TCEt) and trichloroacetic acid (TCAA) have been identified as products of mammalian TCE metabolism (Nelson et al., 1993) and have recently been recognized to be plant metabolites of TCE as well (Newman et al., 1997; Doucette et al.,

1998). The uptake of these TCE metabolites has been explored to an even lesser extent than that of TCE itself. Metabolism of TCE in the rhizosphere, on root surfaces, or within the plant can lead to the accumulation of metabolites in aboveground plant tissues. The combination of TCE uptake studies with studies on the uptake of prevalent TCE metabolites should lead to an understanding of the mobility of these compounds in plants as well as where in the plant and to what extent metabolism takes place.

TCE and other organic compounds that do not occur naturally and are believed to be resistant to environmental degradation are termed "xenobiotics." The octanol/water partition coefficient (K_{ow}) has been used to predict xenobiotic absorption by animal cells (Lien, 1985; McCarty et al., 1985). Plant uptake of xenobiotics may also be related to K_{ow}. Generally this property is expressed as its logarithm (log K_{ow}), and log K_{ow} values for xenobiotics range from about –0.5 (more hydrophilic) to 5 (more lipophilic).

Briggs et al. (1982) and Hsu et al. (1990) suggested similar relationships to predict the efficiency with which chemicals penetrate plant roots and move through xylem based upon octanol/water partition coefficient. These relationships are both supported and refuted in the literature. Topp et al. (1986) studied the uptake of 16 different organic chemicals and concluded that plant uptake is more closely linked to molecular weight than to octanol/water partition coefficient. However, the molecular weights of the chemicals studied are strongly correlated to their log K_{ow} values, and no compounds with log K_{ow} values < 2.6 were examined. McFarlane et al. (1987) showed that soybean uptake of phenol versus bromacil was different in spite of their "similar" log K_{ow} values of 1.49 and 2.02, respectively. While phenol appeared to be almost entirely

immobilized in the roots of soybean plants, bromacil was taken up rapidly and accumulated in the leaves.

Root accumulation of xenobiotics may be followed by translocation to the foliar portion of the plant. Once a compound has penetrated the root plasma membrane, translocation to the shoot via the xylem may be most efficient for compounds with intermediate polarity (Topp et al., 1986; Bromilow and Chamberlain, 1995). In soil, lipophilic compounds may partition to soil organic matter, making them less available for plant uptake. Thus, in soil, polar compounds are more bioavailable (Bromilow and Chamberlain, 1995).

In general, the literature assumes that the rate of plant uptake of any chemical remains constant over time and across a broad and non-phytotoxic range of chemical concentrations. This may only be true once equilibrium has been reached between the chemical compound and plant tissues.

Objectives

This research sought to measure the extent of uptake of TCE and two of its reported metabolites by hybrid poplar (Imperial Carolina, DN34, *Populus deltiodes x nigra*). The nonvolatile TCE metabolites TCEt and TCAA were studied in aerated hydroponic containers. Due to its volatility, TCE was studied in a flow-through, dual vacuum, sealed hydroponic chamber system using a mixture of radiolabeled and non-labeled compound. Specific objectives were to 1) quantify uptake and translocation of TCE, TCEt, and TCAA by hybrid poplar; 2) better understand where and to what extent

the metabolism of these compounds takes place in planted systems; and 3) determine the stability of these compounds in hybrid poplar after leaf and stem removal.

Hypotheses

Hypothesis 1: Uptake of TCE and its metabolites TCEt and TCAA by hybrid poplar will be related to the octanol/water partition coefficients of the compounds.

Hypothesis 2: Uptake of TCE metabolites by oxygen stressed roots will be increased relative to aerobic, nonstressed roots.

Hypothesis 3: Uptake of TCE metabolites by hybrid poplar will increase with increasing root-zone concentration.

Hypothesis 4: Uptake of TCE by hybrid poplar will increase over time.

Hypothesis 5: Hybrid poplar previously exposed to TCE will not desorb TCE or its metabolites into the root zone when TCE exposure is eliminated.

LITERATURE REVIEW

Degradation of TCE

Degradation plays an important role in phytoremediation. Because organic compounds commonly consist of reduced forms of carbon, degradation by oxidation is often energetically favorable. Because TCE is highly halogenated (chlorinated), it is relatively oxidized and may undergo reduction (Vogel et al., 1987). Reduction of TCE under anaerobic conditions is followed by dechlorination reactions (Figure 1). However, incomplete anaerobic metabolism of TCE may result in the accumulation of hazardous transformation products such as dichloroethylene or vinyl chloride (Vogel and McCarty, 1985; Hyman et al., 1995).

TCE can be degraded cometabolically under aerobic conditions. Cometabolism is defined as "the fortuitous biotransformation of a non-growth-supporting compound by a microorganism" (Hyman et al., 1995, p. 1480). Nonspecific enzymes or cofactors catalyze the biotransformations, while a growth-supporting substrate is simultaneously metabolized (Hyman et al., 1995). The microorganism producing the cometabolic enzyme gains no apparent benefit. Aerobic TCE cometabolism (Figure 2) can be catalyzed by oxygenase enzymes that use either methane or an alkene as their primary substrate.

The variety of oxygenases capable of TCE cometabolism includes methane monooxygenase (MMO), ammonia monooxygenase (AMO), toluene monooxygenase (TMO), toluene dioxygenase (TDO), and propane monooxygenase (PMO) (Harker and Kim, 1990; Vanelli et al., 1990). Plants may support this mechanism by transferring

exudates to anaerobic sites, thereby stimulating methanogens to produce methane. The methane in turn stimulates the cometabolism of TCE by aerobic methanotrophs via the MMO enzyme. Products of these reactions include chloral, dichloroacetic acid, trichloroacetic acid (TCAA), trichloroethanol (TCEt), and ultimately, CO_2. To date, bacteria that use phenol, propylene, cumene, or isoprene as substrates (Hyman et al., 1995) have also accomplished oxidation of TCE.

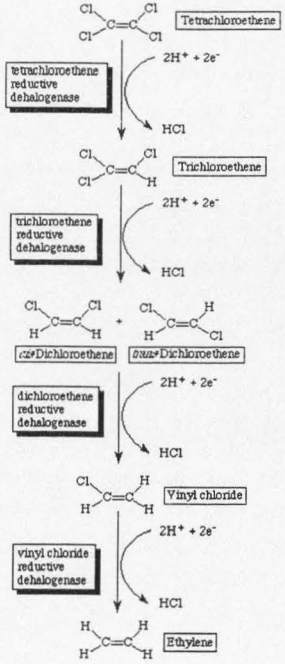


Fig. 1. TCE reductive dechlorination pathway (Ellis, 1997).

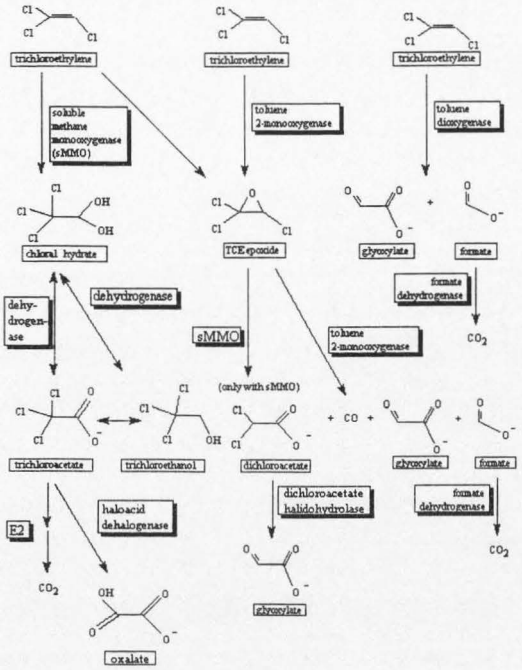


Fig. 2. Aerobic TCE degradation pathways (Oh, 1997).

Enhanced Degradation of Organic Compounds in the Rhizosphere

Because of its importance to crop productivity, research on microbial transformations in the rhizosphere has been concerned mainly with agricultural chemicals such as pesticides and fertilizers. Compared to bulk soil, microbial activity in the rhizosphere is increased by an order of magnitude or more. Increased microbial

populations in the rhizosphere are a direct effect of the release of root exudates, the source of organic carbon and substrate for microbial growth. Root exudates are comprised of both high molecular weight (HMW) and low molecular weight (LMW) compounds. Mucilage and ectoenzymes are the most significant of the HMW exudates, while LMW exudates are comprised of organic acids, sugars, phenolics, and amino acids (Marschner, 1995). Microbial populations in the rhizosphere may be found at a magnitude of 10^9 cells per gram of soil or root, but the only sites of relatively high activity are the root tips or sites of lateral root emergence where pulses of carbon are frequent (Crowley et al., 1997). Jordahl et al. (1997) found that poplars grown in a plant incubator released 0.25% ± 0.18% of biomass produced as soluble exudates and that the concentration of microorganisms in the rhizosphere was 3 to 5 times greater than that in the bulk soil. Due to the great density, diversity, and activity of microorganisms in the rhizosphere, enhanced degradation of contaminants may take place.

The rhizosphere may have little or no effect on degradation of xenobiotics if degrader organisms can grow independently using the contaminant as a substrate (Crowley et al., 1997). No microorganism that can grow on TCE as a lone carbon energy source has been isolated. However, enhanced populations of microorganisms were found to degrade TCE in the rhizospheres of multiple plant species growing on contaminated sites (Walton and Anderson, 1990).

Because quantities of organic carbon may be increased in the rhizosphere relative to bulk soil, the adsorption of lipophilic compounds to organic matter may result in decreased bioavailability of the compound. However, in a review of a range of conflicting results, Hurle and Walker (1980) concluded that the degradation of a chemical

is not always impeded by adsorption, nor does adsorption always lead to increased rates of loss.

Physiology of Plant Uptake

Plants are dynamic organisms with the ability to survive in environments with limited resources. Plants must concentrate and store available resources in order to live. The water that makes up between 70% and 90% of a typical plant delivers these resources to the plant root, shoot, and fruit. Consequently, plants have the potential to accumulate anthropogenic chemicals in conjunction with the acquisition of nutrients (McFarlane, 1995).

In general, as much of a plant grows below ground as above. In addition to providing anchorage and storage of energy-rich molecules, plant roots undergo physical and chemical reactions with the soil. Radial growth, development of secondary cell walls, layering of suberin, and deposition of stored materials all play a role in the development pattern of root systems (McFarlane, 1995). The surface area of roots of any pattern is dramatically increased by the growth of root hairs. These single-celled extensions of epidermal cells afford close contact with the soil, are short-lived, and are not observed in all species (McFarlane, 1995).

The movement of solutes across the root cortex and toward the stele occurs by two parallel pathways. The first is the apoplastic pathway, whereby solutes and water travel between cells in the intercellular spaces. The other pathway, the symplastic pathway, is characterized by penetration of the plasma membrane and movement by cell-to-cell transport through the plasmodesmata (Bromilow and Chamberlain, 1995;

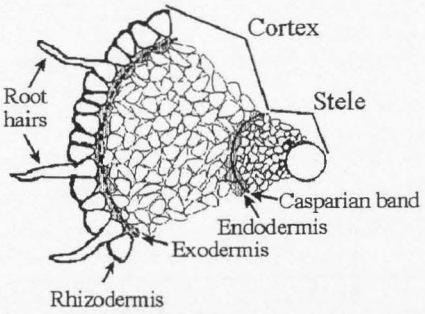


Fig. 3. Schematic drawing of root cross-section (adapted from Marschner, 1995). The hydrophobic Casparian band acts as a barrier against passive movement of solutes into the xylem.

Marschner, 1995). Compounds moving in the apoplastic pathway are stopped at the endodermis, the innermost layer of the cortex (Figure 3). Within this layer the Casparian band, constructed of hydrophobic incrustations called suberin, acts as a barrier against passive movement of solutes into the stele (Marschner, 1995).

Compounds can travel between cells throughout the cortex, but "uptake" does not occur until the compound moves into the living, symplastic part of plant cells. Because there are many intercellular spaces in roots, roots can accumulate high levels of xenobiotics without actually taking up any chemical.

The transport of solutes across membranes can be an active or a passive process. Active uptake occurs against the gradient of potential energy and must be linked to an energy-consuming mechanism (Marschner, 1995). With the possible exception of the hormone-like chemicals 2,4-D, no evidence exists for active uptake of any xenobiotics (McFarlane, 1995). Rather, uptake of anthropogenic compounds occurs passively. This

"downhill" transport occurs across aqueous pores or sometimes with the aid of carriers. Passive uptake results from accumulation of solutes on the outside of the membrane creating a concentration gradient, thereby causing the solute to diffuse from the higher, outside concentration to the lower, inside concentration (Marschner, 1995).

Once inside the root, contaminants have the potential for translocation to other parts of the plant via the vascular tissues, the xylem and the phloem. When stomata are open and a plant transpires, a continuous water potential gradient is created throughout the plant. In the xylem, a pressure potential is created and water moves upward from the roots to the shoots through the tracheary elements by mass flow. Mass flow in the phloem is typically from shoots to root tips. Phloem movement of water and solutes through the sieve elements depends on a gradient of osmotic pressure, which induces mass flow (Salisbury and Ross, 1994).

Uptake of Nonionized Organic Chemicals by Plant Roots

Plant uptake can vary with temperature, organic matter and water content of the soil, and plant characteristics such as type of root system and lipid content (Paterson and Mackay, 1994). Physico-chemical properties that might determine uptake of xenobiotics include water solubility (S), vapor pressure, Henry's Law constant (K_H), and lipophilicity or hydrophobicity, given by the logarithm of the chemical's octanol-water partitioning coefficient (log K_{ow}) (Simonich and Hites, 1995). Chemicals in the soil water phase may reach the root surface by mass water flow, penetrate the root, enter the xylem, and be transported in the transpiration stream. Once in the transpiration stream, compounds may

react with or partition into plant tissues, degrade, or escape by gaseous diffusion through stomates in leaves followed by movement to the atmosphere.

Russell and Shorrocks (1959) defined the transpiration stream concentration factor (TSCF) to express the relationship between the concentration of the compound in the transpiration stream (xylem) to the exposure concentration:

$$\text{TSCF} = \frac{\mu\text{g compound / mL } H_2O \text{ transpired}}{\mu\text{g compound / mL ambient solution}} . \qquad [1]$$

Similarly, the Root Concentration Factor (RCF), defined by Shone and Wood (1974) as:

$$\text{RCF} = \frac{\mu\text{g compound / } \mu\text{g fresh wt. roots}}{\mu\text{g compound / mL ambient solution}} \qquad [2]$$

was formulated to express the extent to which a compound is concentrated in the roots. A TSCF of 1.0 indicates unrestricted passive uptake of the compound into the plant. TSCFs lower than 1.0 indicate exclusion of the compound by the plant, while TSCFs greater than 1.0 infer active uptake.

Xenobiotic compounds that are highly water soluble ($\log K_{ow} < 0.5$) are not easily transported through the lipid bilayer of the root membrane (Briggs et al., 1982). Compounds that are lipid soluble tend to concentrate the lipid bilayer (Paterson and Mackay, 1994).

Briggs et al. (1982) suggest that for a given plant in a given set of environmental conditions, root uptake of a nonionized compound depends on the compound's octanol-water partition coefficient (K_{ow}). The authors used 10-day-old barley plants to study the

uptake of two groups of organic compounds with log K_{ow} values ranging from -0.57 to 4.6. One group represented systemic insecticides (*O*-methylcarbamoyloximes) while the other group consisted of inactive analogs of herbicides (substituted phenylureas). Eighteen nonionized chemicals were tested.

Groups of six plants were transferred to vessels containing nutrient solution spiked with the designated ^{14}C-labeled chemical. Plants were held in an aluminum plate by drilled rubber bungs. The plate rested on a 6.5 cm-diameter crystallizing dish with darkened sides. Following a 24- or 48-h uptake period, plants were harvested, weighed, and analyzed.

The RCF was determined for each of these chemicals (Figure 4). These data suggest that in barley, nonionized chemicals with greater lipophilicities bind to root tissues more readily. A regression line was fitted to the data and the equation:

$$\text{Log (RCF-0.82)} = 0.77 \log K_{ow} - 1.52 \qquad [3]$$

was formulated to express the relationship between RCF and log K_{ow}.

The authors found that TSCF values increased with log K_{ow} up to a maximum of 0.8 at log K_{ow} = 1.8 (Figure 5). However, TSCF decreased as log K_{ow} increased for compounds with log $K_{ow} > 1.8$. Compounds with a log $K_{ow} > 4.5$ yielded a TSCF close to zero. A bell-shaped Gaussian curve was "fitted to the data for illustrative purposes" (Briggs et al., 1982) and the equation:

$$\text{TSCF} = 0.784 \exp -[(\log K_{ow} - 1.78)^2/2.44] \qquad [4]$$

was determined to relate log K_{ow} and TSCF.

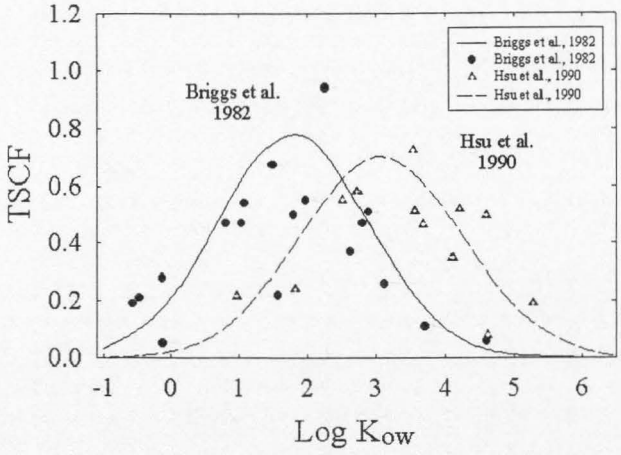


Fig. 5. Data and curves generated relating TSCF to log K_{ow} (Briggs et al., 1982; Hsu et al., 1990). Curves represent a theoretical relationship between TSCF and log K_{ow}. Each data point was generated for an individual chemical compound. Differences between curves may be due to differing plant parameters and differences in data collection methods.

readily enter roots, with a TSCF of around 0.8. Data collected and curves generated by Briggs et al. (1982) and Hsu et al. (1990) are shown in Figure 5.

Topp et al. (1986) examined the correlation between uptake of ^{14}C-labeled organic chemicals and K_{ow}. Using barley and cress in 7-day soil studies, they determined that root adsorption of an assortment of both pesticide and non-pesticide chemicals from the soil was correlated with K_{ow} but that translocation to shoots was more efficient for chemicals with intermediate solubilities. Compounds with molecular weights less than 300 g/mol had more rapid uptake. The authors concluded that translocation to plant shoots was better correlated with molecular weight than with log K_{ow} values. However,

the molecular weights of the chemicals studied are strongly correlated to their log K_{ow} values, and no compounds with log K_{ow} values < 2.6 were examined. TSCF values were not reported.

McFarlane et al. (1987) studied the influence of transpiration rate on the uptake of bromacil, nitrobenzene, and phenol by soybean plants. The three compounds, which have similar log K_{ow} values, had different uptake rates. While ^{14}C associated with bromacil was distributed throughout the plant, the majority of the radiolabel provided as phenol and nitrobenzene remained associated with the roots. The authors speculate that metabolism of phenol and nitrobenzene by the soybean roots resulted in immobile compounds. TSCF values were not reported. They concluded that although octanol/water partitioning coefficients are useful in predicting the fate of organic chemicals in animals and correlate with root binding and plant uptake for many pesticides, they "may not be equally useful in describing uptake and binding of nonpesticide chemicals in plants" (McFarlane et al., 1987, p. 372).

Uptake of Ionized Chemicals by Plant Roots

Plant uptake of weak acids by roots is a pH-dependent process that increases as the pH of the solution surrounding the roots decreases (Bromilow and Chamberlain, 1995). This characteristic is attributed to the diffusion of non-dissociated molecules across the root membrane. Once across the membrane, dissociation of the weak acid occurs in the cytoplasm (pH 7.3), resulting in the "ion-trap effect" (Briggs et al., 1987). Briggs et al. (1987) examined uptake and translocation of weak organic acids by barley.

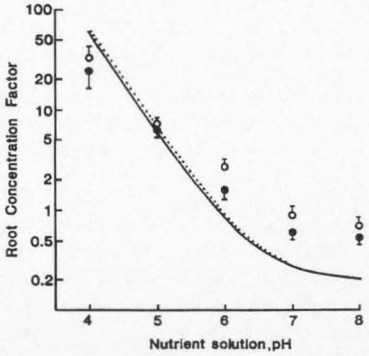


Fig. 6. Relationship between RCF and pH (Briggs et al., 1987). Open circles and dashed line represent 2,4-D. Closed circles and solid line represent 3,5-D. Points are measured data while lines represent the ion trap effect theory. The effect is attributed to diffusion of non-dissociated molecules across the root membrane. Once across the membrane, dissociation of the weak acid occurs in the cytoplasm, resulting in the "ion-trap effect" (Briggs et al., 1987).

Procedures were similar to those described above (Briggs et al., 1982) but the chemicals tested included indol-3-ylacetic, 2,4- and 3,5-dichlorophenoxyacetic (2,4D and 3,5-D), benzoic, and naphthylacetic acids. The data, along with a curve for each data set representing the RCF values predicted by the ion-trap mechanism, are shown in Figure 6.

Ionized weak acids tend to accumulate in areas of high pH. Thus, the xylem and cell vacuoles with pHs of about 5.0 and 5.5, respectively, would be less likely destinations for weak acids than the phloem (pH 7.5), and cytoplasm (pH 7.0). As a result, weak acids are much more likely than nonionized chemicals to move in the phloem sap to areas of new growth (Bromilow and Chamberlain, 1995).

Uptake of Volatile Organic Compounds

Xenobiotics with high Henry's Law constants are categorized as volatile organic compounds or VOCs. VOCs can volatilize from the soil surface and bind to leaf surfaces through foliar sorption. Foliar sorption involves gas-phase and particle-phase deposition of the xenobiotic onto the waxy cuticle of plant leaves. Uptake can occur through either the cuticle or the stomata and can potentially result in downward translocation of the contaminant via the phloem (Simonich and Hites, 1995). Consequently, root versus foliar uptake pathways for VOCs must be quantified separately.

Using a variety of agricultural plants, Schroll et al. (1994) quantified uptake of eight chemicals with a wide variation in physico-chemical and structural properties. Results indicated that for the VOCs TCE and chlorobenzene, total uptake was dominated by foliar uptake, which was followed by downward transport to the roots.

Root sorption of VOCs is probable for VOCs with high lipophilicities. Translocation to the shoot, however, is unlikely, except in the case of compounds with intermediate log K_{ow} values (Bromilow and Chamberlain, 1995). Burken (1996) found that VOCs with log K_{ow} values between 2.13 and 3.15 readily entered roots and were translocated to shoots where they were subsequently volatilized.

Other Influences on Root Uptake

As mentioned previously, the uptake of xenobiotics is also governed by environmental conditions. Climatic conditions determine plant transpiration rates, which in turn control rates of water movement to the root surface and in the xylem. Soil type

also plays a role in the availability of xenobiotics. Organic chemicals may bind on the soil solid phase when organic matter contents are high. This decreases the leaching potential but also decreases bioavailability of the compounds (Bromilow and Chamberlain, 1995). Plant species vary in lipid content and anatomy, so uptake of organic contaminants should be species-dependent as well. Finally, the fraction of plant water needs that is met by contaminated groundwater (versus surface water) plays a major role in the volume of contaminated groundwater, and therefore the mass of contaminant, processed by the plant (Orchard et al., 2000b).

Hybrid Poplar for Phytoremediation

The genus *Populus* includes poplars, cottonwoods, and aspens and has a wide geographical distribution ranging from southern Alaska into Central America (Gordon et al., 1997). In addition to the 30 or so species of *Populus* distributed throughout the Northern hemisphere, *Populus* spp. can cross within the genus to produce a great number of hybrids (Dickmann and Stuart, 1983). Poplars have been crossed by growers in controlled breeding to maximize growth rates, hardiness, and yield.

Poplars are phreatophytic plants, capable of extending their roots to the capillary fringe to draw water from the zone of saturation when surface water is unavailable (Robinson, 1958). A community of these trees can cause a depression in the water table significant enough to induce a hydraulic barrier to contaminant transport. For example, Gordon et al. (1997) reported a water table drawdown of 140 cm/year by a stand of 5-year-old trees planted at a density of 1,750 trees/ha in the warm, arid conditions of eastern Washington state.

Recent research on the phytoremediation of TCE has used hybrid poplar. Although hybrid poplars have the ability to grow deep roots, root biomass decreases with increasing depth as with other plants. Precipitation, soil bulk density, shallow bedrock, horizontally stratified layers of shale or clay, permafrost, and the depth to groundwater limit depth of rooting (Shimp et al., 1993; Canadell et al., 1996). In the soil, contaminant distribution is based on the age, source, and nature of the spill or release as well as the soil type and rainfall and temperature patterns (Cunningham et al., 1996). In order for a contaminant to be influenced by plants or their associated microbiota, the compound must be in or near the vicinity of plant roots. Contaminants will only accumulate at the root surface if leaching away by mass flow is avoided (Bell, 1992).

Trichloroethylene

Trichloroethylene (TCE) is a colorless, non-flammable, volatile liquid with a characteristic etheral odor that has commonly been used as an industrial and dry-cleaning solvent, degreaser, and fumigant (Merck Index, 1989). These uses of TCE have contributed extensively to its occurrence at waste sites and as a contaminant of groundwater. Physicochemical properties of TCE are shown in Table 1.

TCE is a VOC and a dense non-aqueous phase liquid (DNAPL), so it presents a challenge for bioremediation. Its volatility leads to substantial partitioning and upward migration, predominantly by vertical gaseous phase diffusion in the unsaturated zone (Narayanan et al., 1995). TCE tends to enter the soil in a relatively small area, then diffuse through the unsaturated zone (vadose zone) above the water table, and then down through the aquifer (Cunningham et al., 1996). Consequently, the contaminated zone is

Table 1. Physicochemical properties of TCE (Howard and Meylan, 1997).

Property	Value
Molecular formula	C_2HCl_3
Molecular weight	131.4 g mol^{-1}
Specific gravity	1.47 g mL^{-1}
Boiling point	86.7 °C
Vapor pressure	69.0 mm Hg at 25 °C
Aqueous solubility	3.139 mg L^{-1} at 25 °C
Log octanol/water partition coefficient (K_{ow})	2.42
Henry's Law constant	9.85 x 10^{-3} atm m^3/mol at 25 °C

narrow at the top and increases in magnitude as it goes deeper into the soil profile.

TCE is only slightly soluble in water. Its hydrophobic nature causes it to partition into soils and sediments, both from the liquid and vapor phases. Peterson et al. (1988) found sorption of TCE to synthetic soil to increase with decreasing water content. These observations are consistent with the concept that water competes with organic compounds for sorption sites on soil, substantially influencing its transport within the vadose zone (Fares et al., 1995).

Trichloroacetic Acid and Trichloroethanol

Trichloroacetic acid (TCAA, sometimes referred to as TCA) is a chlorinated aliphatic acid used as a preemergence herbicide to control grasses. Though widely used in the 1950's and 60's, TCAA is no longer sold or used in the United States (Thomson, 1990). Still, TCAA is present in soils and groundwater throughout the U.S. as a breakdown product of short-chain hydrocarbons such as dry cleaning agents and degreasers (Sutinen et al., 1995).

TCAA is water soluble and nonvolatile (Table 2). TCAA degrades in soil but is quite stable in plants and in animals (Crafts, 1964; Ashton and Crafts, 1973). TCAA changes the character of leaf surface waxes and can therefore possibly increase transpiration in TCAA-treated plants (Kiermayer, 1964; Ashton and Crafts, 1973). TCAA has been reported to cause growth inhibition and a decrease in the size of cell nuclei in the meristems of several plant species (Avato et al., 1984). Other studies have shown TCAA to cause yellowing of leaves and formative effects (Sutinen et al., 1995; Crafts, 1964). Additionally, TCAA probably interferes with production of pantothenic acid, a precursor to coenzyme A, which is required for several essential biochemical reactions in higher plants and in microorganisms (Ashton and Crafts, 1973). Evidence also exists for incorporation of the carbon atoms of the herbicide the into cellular constituents of TCAA degraders (Smith, 1988).

Table 2. Physicochemical properties of TCAA and TCEt (Howard and Meylan, 1997).

Property	TCAA	TCEt
Molecular formula	$C_2HCl_3O_2$	$C_2H_3Cl_3O$
Molecular weight	163.4 g mol^{-1}	149.4 g mol^{-1}
Melting point	57.58 °C	18 °C
Boiling point	196-197 °C	151-153 °C
Vapor pressure	$4.54x10^{-9}$ mm Hg at 25 °C	1.08 mm Hg at 25 °C
Aqueous solubility (S)	$1.3x10^{6}$ mg L^{-1} at 25 °C	$1.5x10^{4}$ mg L^{-1} at 25 °C
Log K_{ow}[a]	1.33	1.42
K_H[b]	$2.39x10^{-8}$ atm m^3/mol at 25 °C	$1.56x10^{-7}$ atm m^3/mol at 25 °C

[a]Octanol/water partition coefficient

[b]Henry's Law constant

Schroll et al. (1994) studied the uptake of TCAA by barley and oats. Radiolabeled compound was used and root uptake and foliar uptake (presumably of $^{14}CO_2$ resulting from mineralization of TCAA in the rhizosphere) were quantified separately. Uptake was observed by both roots and leaves and transport of the radiolabel within the plant occurred in both the root-to-shoot and shoot-to-root directions. Only "small portions" of the parent compound were found in plant tissues (Schroll et al., 1994).

Trichloroethanol (TCEt) is slightly less soluble and more volatile than TCAA (Table 2). Although TCEt does not have a commercial use, it is a product of human TCE metabolism. Breakdown of TCE in the human body by cytochrome P450 results in the formation of TCAA and TCEt (Bernauer et al., 1996). Both of these compounds are detectable in the blood and urine of individuals exposed to TCE (Bernauer et al., 1996; Yoshida et al., 1996).

Related Laboratory Studies with TCE

In whole plant experiments Anderson and Walton (1995) found that $^{14}CO_2$ production from radiolabeled TCE in vegetated soils was greater than that in unvegetated soils. Soils used for the study were collected from nonvegetated areas of a TCE-contaminated site. The production of $^{14}CO_2$ in vegetated soils occurred primarily in the first 3 d. Mass recoveries of greater than 70% were reported and tissue concentrations ranged from 1% to 21% of the total label recovered. No attempt was made to identify metabolites in plant tissues. When normalized to transpiration, root concentrations for all experiments were similar. In addition, the amount of ^{14}C on the charcoal traps increased

with increasing evapotranspiration, indicating a dependence of contaminant uptake on evapotranspiration.

These results suggest that enhanced mineralization of ^{14}C-TCE may be possible without exposure to the plant, but prolonged exposure to the microbial population may be required. Tests to determine the influence of previous plant exposure to TCE on enhanced TCE degradation as well as the potential for leguminous species to enhance TCE degradation were inconclusive. Factors which may influence microbial mineralization are suggested to include: structure or surface area of the root system, the selective influence of root exudates, or the type of root associations present in the system.

The fate and transport of TCE and 1,1,1 trichloroethane (TCA) in a chamber with alfalfa plants was studied at Kansas State University by Narayanan et al. (1995). The laboratory chamber used for the investigation consisted of two identical U-shaped channels, each 10 cm wide, 1.8 m in flow length, and 35 cm deep. Channels were packed with silty sand soil and were enclosed by a glass and aluminum cover.

While groundwater concentrations of TCA remained constant over the entire length of the channel, TCE concentrations dropped approximately 27% from the inlet to the outlet. CO_2 was not monitored in the system, but based upon chloride analyses the authors estimated that about 17% of the applied TCE was mineralized.
Rates of accumulation of TCE and TCA in the headspace remained constant even after the aboveground portions of the plants were harvested, indicating that volatilization from the soil surface may have been a more significant fate pathway than transpiration from the plant.

TCE and TCA were accounted for in the outflow groundwater, outflow gas-phase, and parent material transformed in the groundwater. Daily mass balances showed that approximately 40% of the applied TCE and TCA could not be accounted for. Controls were not provided because "several others have demonstrated . . . that enhanced biodegradation occurs in vegetated soil environments relative to nonvegetated soils" (Narayanan et al., 1995, p. 2438).

Research at the University of Iowa (Burken, 1996) explored the fate of a variety of organic contaminants. Both volatile and nonvolatile chemicals were examined including benzene, toluene, ethylbenzene, *m*-xylene, TCE, and 1,2,4-TCB. Experiments were carried out in planted hydroponic systems. Each plant chamber was constructed using a modified 1-L flask (upper chamber) sealed to a modified 270-mL screw top culture flask (lower chamber) (Figure 7). An Orbo® tube was connected to the aerial portion of the chamber to trap VOCs. Air was pulled through the upper chamber at approximately 1 L/min. Each reactor was spiked with a mixture of radiolabeled and unradiolabeled compound to an aqueous phase concentration of approximately 50 mg/L. Experiments ran for 8 d. Analyses were conducted by liquid scintillation counting, gas chromatography, or combustion of plant tissue, depending on the sample type.

Blank and root controls with reasonably constant aqueous concentrations were included, but it was not clear that they were set up exactly like the dosed chambers. For the TCE study, mass balance was reported at 83%. Foliar plant tissue contained ~ 3% of

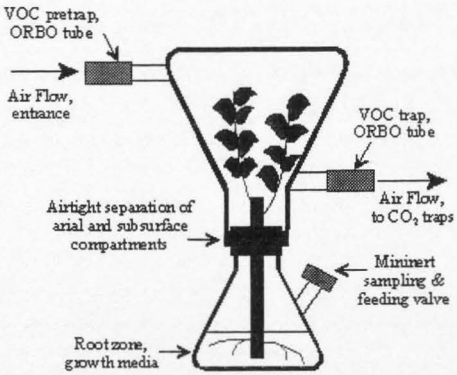


Fig. 7. Chamber used in studies by Burken (1996). Foliar chamber consists of a 1-L flask, root zone chamber a 270-mL flask. Airflow through the foliar chamber was 1 L/min with a static root zone. Target root-zone exposure concentration was 50 mg/L.

the applied label while volatilized compound and that sorbed to the reactor and components accounted for 21% and 11%, respectively. Results for all VOCs tested show a tight correlation between water transpiration and contaminant transpiration. Data for TCE include a single, average transpiration rate of 10 mL/d. Attempts were not made in plant tissue analyses to distinguish between parent compound and metabolites.

Schnabel et al. (1997) studied the uptake and transformation of TCE by three edible garden vegetables. Tomatoes, carrots, and spinach were used to represent edible fruit, root, and leaf crops, respectively. Vegetables were grown in glazed ceramic pots enclosed in modified aquariums. Although air was continuously pulled through the bioreactors, only three to six chamber air exchanges occurred daily (calculated to be 0.24 L/min at the minimum flow for the smaller chambers and 1.7 L/min at the maximum flow

for the larger chambers). Two TCE treatment levels, 560 μg/L and 140 μg/L, were applied in this study. Four of each plant type were fed the higher concentration and four the lower concentration. Three sterilized and three nonsterilized controls, all unplanted and treated with the higher TCE concentration, were included to evaluate plant effects on soil sorption and microbial degradation of TCE. Mass recoveries ranged from 45% to 73% of the applied ^{14}C label. The authors presume that most of the unrecovered label leaked from the system.

The authors conclude that microbial mineralization of TCE was not a significant fate pathway in the bioreactors. Because radiolabel was found by combustion of plant tissues, yet no readily extractable TCE or metabolites were detectable, the authors conclude that compounds associated with the radiolabel were covalently bound to the plant tissue (Schnabel et al., 1997). The health effects of TCE bound residue are not known, but the authors propose that "the observed sequestration of TCE into bound residue suggests the potential for plants to enhance the cleanup of TCE contaminated sites" (Schnabel et al., 1997, p. 823).

At the University of Washington, experiments by Newman et al. (1997) with axenic poplar cell cultures showed transformation and mineralization of TCE independent of microbial metabolism. Products of TCE degradation in these experiments were trichloroethanol (TCEt), trichloroacetic acid (TCAA), and dichloroacetic acid (DCAA). Chloral hydrate was not detected and it was hypothesized that in plant systems, its short half-life lends to difficulties in its isolation. Approximately 1-2% of the applied ^{14}C-TCE was mineralized to $^{14}CO_2$ in 4 d. A portion of the radiolabel was bound to the tissues as an insoluble residue.

Newman et al. (1997) also conducted whole plant experiments with 12 hybrid poplar trees (four each of three distinct clones) in chambers constructed of 1.0-m tall PVC pipe with an inside diameter of 20.5 cm. A tube 1.1-m long was inserted to the bottom of each chamber for watering. Six (two of each clone) were dosed via the watering tube with water containing 50 mg/L TCE while the other six plants remained undosed controls. During the study, plants were watered biweekly through the watering tube and soil surfaces were watered with pure water as needed (Newman et al., 1997).

After 20 and 31 weeks, individual leaves were loosely enclosed (to allow free entrance of air) in polyethylene bags to determine if TCE was transpired. It was suggested that in this study, TCE was "transpired" at a rate of 1.0 μg of TCE per leaf per h (Gordon et al., 1997). Because phytovolatilization of TCE was not correlated to water transpiration due to an inability to measure water transpiration rates during sampling of the leaves, "these results should be viewed as a qualitative indication of TCE transpiration by poplar trees and not as a quantitative measurement" (Newman et al., 1997, p. 1066).

After eight months, poplars were harvested and tissues were analyzed for TCE and metabolites. Two controls showed possible contamination, attributed to foliar uptake of TCE transpired by neighboring dosed plants. Results for dosed plants show higher TCE levels in roots and in stems than in leaves. TCEt and TCAA were detected in leaves and in stems to varying degrees while only one leaf sample had detectable DCAA. Root tissue contained TCE as well as TCEt and DCAA and low levels of TCAA. A mass balance was not possible in this system due to its open nature. Though the work by Newman et al. (1997) with plant cell cultures shows that hybrid poplars are capable of

TCE degradation, it is not possible to determine from data presented in this study whether TCE uptake and metabolism by hybrid poplars is significant toward TCE remediation.

Additional whole plant studies conducted at the University of Washington (Gordon et al., 1997) employed glass bioreactors followed by a series of traps in an attempt to obtain a mass balance for TCE in hybrid poplar trees. Bioreactors constructed of glass, aluminum foil, and inert inorganic materials were utilized. Rooted cuttings were transferred to vermiculite in the bioreactors and allowed to acclimate for one day prior to dosing. A glass plate sealed with silicon dental filler separated the root (soil) chamber from the chamber containing the stem and leaves. The headspace was continuously aerated while the root zone was aerated for 1 h each day. Flow rates were not specified. Furthermore, the concentration of TCE in the root zone is not given and cannot be inferred from the information given (1-4 x 10^6 cpm ^{14}C-TCE and 220 μg unlabelled TCE).

Within the first 2 d, most of the recovered radiolabel was found in the headspace methoxyethanol (organic) traps (averaging 8.6%) and the soil organic traps (averaging 6.5%). After 10 d, results show that about 14% of the total label applied was trapped in the headspace organic traps, compared to only 3% in the soil organic traps. Approximately 3% and 1% of the TCE was metabolized to CO_2 in the headspace and root zones, respectively. Based on these results, it was concluded that hybrid poplars in this system transpired and oxidized significant amounts of TCE. It does not appear that control experiments were performed and a mass balance was not supplied.

Newman et al. (1999) conducted studies in constructed, polyethylene-lined cells 1.5 m deep by 3.0 m wide by 5.7 m long. Cells contained a coarse sand layer overlaid

with silty clay loam soil. TCE was added to the sand layer via the cell's water inlet. Planted, TCE-dosed treatments were run, as well as unplanted, TCE-dosed treatments and planted, non-dosed treatments. All treatments received the same volume of water via the inlet over the course of the 3-year study. Additional water was supplied by surface irrigation and natural rain events. During the study, transpiration gas samples were taken 1) by loosely enclosing leaves in a Teflon bag and trapping air exiting the bag on an activated carbon trap, and 2) by using open-path Fourier transform infrared (OP-FTIR) spectroscopy to measure the ambient TCE concentration in the tree canopy air. Soil degradation of TCE in both planted and unplanted treatments was evidenced by a significant increase in chloride ion in soil samples. The authors suggest that the TCE was taken up by the plants and metabolized and that the leftover chloride was exuded back into the soil from the roots. TCE and its metabolites TCAA, TCEt, and DCAA were found in plant tissues. The authors concluded that 99% of the TCE added to the planted cells was removed. Although this was an artificial system, the results suggest that trees may have a significant impact on the remediation of shallow TCE comtaminated groundwater.

Fourier transform infrared (FT-IR) spectrometry was used by Davis et al. (1998) to measure gas phase concentrations of TCE and several other volatile organics in a chamber enclosing plants that had their roots exposed to contaminated hydroponic solution. A ratio corresponding to the TSCF was derived from the water use rate, gas flow rate, the initial solution concentration, and the steady-state gas phase concentration above the plants. Measurements of plant tissue were not attempted. Reported TSCF

values for poplar and saltcedar plants exposed to an initial TCE solution of 131 mg/L ranged from 0.1 to 0.58.

TCE Uptake Studies Conducted at Utah State University

Four identical, high-flow, sealed growth chamber system experiments have been developed for studies on plant uptake of VOCs (Orchard et al., 2000a) at Utah State University. Three studies were run with four chambers in each for a total of 12 individual chamber studies (Orchard et al., 2000b). The first two sets of chambers were dosed at 1 mg/L TCE to replicate conditions observed at a field site where the uptake of TCE by mature vegetation was being evaluated (Doucette et al., 1998). The first four chambers were each planted with a rooted hybrid poplar cutting. Three chambers were dosed at root zone concentrations of 1 mg/L while the fourth remained undosed as a control. Due to the sensitivity of the continuous dosing system, actual root zone concentrations averaged 0.6 mg/L. The trial ran for 12 d and results showed limited plant uptake of TCE with average shoot tissue concentrations ranging from 2.2 to 3.7 mg/kg. TCE was detected in 4 of 32 foliar VOC trap samples, and added ~0.1 to the TSCF for those chambers. Mass balances for the three dosed chambers ranged from 92% to 94%. Because results using this system were different than those found using simpler systems (Newman et al., 1997; Burken and Schnoor, 1998; Gordon et al., 1998), hypotheses were formulated regarding possible sources for the discrepancies.

The second study tested the hypothesis that static root zones in simple systems could cause anaerobic stress, whereby plant roots are exposed to an oxygen-depleted

environment, similar to that of flooded conditions. Because oxygen has a low solubility in water and because the rate of diffusion of oxygen in water is 10,000 times slower than in air, the delivery rate of oxygen to roots in flooded soils is low (Nilsen and Orcutt, 1996). In addition, anaerobic conditions support a unique host of bacteria that can affect nutrient relations in the soil. Anaerobic bacteria utilize molecules other than oxygen as electron acceptors to acquire energy through oxidation-reduction reactions. As continued activity of anaerobic bacteria causes redox potential to decrease, NO_3^- availability decreases followed by the reduction and unavailability of iron, sulfur, and manganese to plants (Nilsen and Orcutt, 1996). Root stress can also lead to increased exudation of carbon at the root surface (Barber and Gunn, 1974; Smucker, 1984; Haller and Stolp, 1985). Trolldenier and Hecht-Buccholz (1984) attributed considerably higher microbial populations in the root zone of oxygen-stressed plants grown in hydroponic culture to increased carbon exudation from roots.

Each of the four chambers in the second study was dosed at 1 mg/L with an actual average concentration of 0.9 mg/L for 11 d. Two chambers were each planted with a hydroponically rooted hybrid poplar cutting while the other two held stainless steel rods to replicate the seal used in planted systems without the actual plant matrix. One each of the planted and unplanted chambers was aerated while the other two chambers were bubbled with N_2 gas to create oxygen-reduced conditions in the root zone. The researchers expected to see increased accumulation of TCE in the roots of oxygen-stressed plants as root membranes became compromised due to anaerobic stress. However, results show uptake similar to that observed in the first study and no difference in the TSCFs for plants exposed to aerobic versus anaerobic root zone conditions.

Because studies that report uptake in the literature have been run at TCE concentrations in the range of 50 to 200 mg/L (Burken, 1996; Gordon et al., 1997; Newman et al., 1997; Burken and Schnoor, 1998; Davis et al., 1998), a second hypothesis was formulated regarding the effect of root-zone TCE concentration on plant uptake. Because studies in this system had all been run at 1 mg/L, it was possible that either root exposure to TCE was not high enough for measurable accumulation and uptake to take place or that plant stress caused by higher TCE concentrations may have increased membrane permeability and plant uptake. It is also possible that the plant's metabolic capacity was overwhelmed at higher concentrations, allowing TCE to be translocated and phytovolatilized.

To test this hypothesis, the third set of four chambers was run at higher concentrations. Two planted chambers were dosed at approximately 10 mg/L TCE while two others were dosed at approximately 70 mg/L TCE. One each of the 10-mg/L and 70-mg/L chambers ran for the usual 12-d period. The remaining two chambers, planted with younger and smaller trees to accommodate an extended growth period, ran for 26 d. Results from this trial showed that for a given concentration, TSCFs in plants exposed to TCE for 26 d did not differ from those exposed for only 12 d. TSCFs for the 10-mg/L treatments were slightly higher (average 0.24) than those for all prior 1-mg/L treatments (average 0.12), indicating a possible concentration-dependence. TSCFs for the 70-mg/L treatments differed widely. Because the tree planted in the 26-d chamber started much younger and smaller than that in the 12-d chamber, it was likely more susceptible to toxic effects. The TSCF for this chamber was only 0.02, while the TSCF for the 12-d chamber (0.22) was similar to those of the 10-mg/L chambers. ^{14}C was detected in 9 of 48 foliar

VOC trap samples, contributing ~.02 to the TSCFs of the 10-mg/L treatments. ^{14}C in the foliar VOC traps of the 70-mg/L, 12-d chamber contributed 0.21 to the TSCF of 0.22. This suggests that the high TCE concentration may have overwhelmed and even shut down the hybrid poplar's metabolic capacity. The observation of lower levels of TCE metabolites in the shoot tissues from this chamber as compared to the 10 mg/L chambers further supports this hypothesis.

TSCF values reported for all nine planted chambers were 10 to 25% of those reported by Burken and Schnoor (1998) and were also much lower than those predicted by theoretical relationships between plant uptake and log K_{ow} (Orchard et al., 2000b). The authors suggested that plant age and size as well as gas-phase transfer of TCE through stem cuttings are factors that may contribute to discrepancies in results among studies on plant uptake of TCE.

EFFECT OF LONG-TERM EXPOSURE ON THE UPTAKE AND TRANSLOCATION OF TCE BY HYBRID POPLAR

Introduction

Trichloroethylene (TCE), a suspected carcinogen, was widely used as an industrial solvent and degreasing agent for most of the twentieth century. It is now a widespread groundwater contaminant. Phytoremediation utilizes plants to remediate or stabilize contaminated sites and may be a cost-effective method for cleanup of TCE-contaminated soils and groundwater. Studies of the environmental fate of TCE are complicated by its volatility. The literature reports both significant and insignificant plant uptake and phytovolatilization of TCE (Walton and Anderson, 1990; Schroll et al., 1994; Anderson and Walton, 1995; Narayanan et al., 1995; Gordon et al., 1997; Newman et al., 1997; Schnabel et al., 1997; Burken and Schnoor, 1998; Davis et al., 1998; Newman et al., 1999; Orchard et al., 2000b). These conflicting findings may be due to differences in exposure level, conditions, and duration of the studies, or to experimental artifacts from laboratory systems.

Walton and Anderson (1990) carried out headspace analyses on soils collected from a TCE-contaminated field site. The authors observed higher rates of aerobic cometabolism in rhizosphere soil as compared to soil from an unplanted area of the site.

In a subsequent study using soil from a TCE-contaminated site and laboratory-grown soybean plants, Anderson and Walton (1995) found statistically greater mineralization of [^{14}C]TCE in vegetated soil treatments as compared to nonvegetated and

sterilized soil treatments. Radiolabel in the plant tissues, including roots, ranged from 1 to 21% of the total ^{14}C added to each system and was correltated to the amount of water transpired by the plant. Reported mass recoveries were greater than 70%.

The uptake of TCE by carrot and radish plants was studied by Schroll et al. (1994). Plants were grown in closed, aerated growth chambers that allowed the researchers to distinguish between root and foliar TCE uptake. The authors reported that foliar TCE uptake predominated and that it appeared that TCE moved in both the root to shoot and shoot to root directions within the plant.

Narayanan et al. (1995) investigated the ability of alfalfa plants to enhance TCE removal from spiked groundwater. Aqueous samples were taken from U-shaped, flow-through growth chambers packed with sandy silt loam soil. Decreasing TCE concentrations from the chamber inlet to the outlet and elevated chloride levels near the channel inlet were attributed to the aerobic biodegradation of TCE. The alfalfa plant tops were removed and after a 2-month period, similar degradation was observed. This suggests that living plants had little impact on TCE removal.

Gordon et al. (1997) related preliminary results from a study designed to separate root, stem, and crown uptake of TCE. [^{14}C]TCE was added to the root zone of a bioreactor. After 7 d, the authors detected 0.8% of the applied ^{14}C in the transpiration gas while a "questionable trace" was converted to $^{14}CO_2$ (Gordon et al., 1997). No mass recovery was reported.

Poplar trees were grown by Newman et al. (1997) in PVC columns containing sand and silt loam soil. Non-control plants were watered with 50 mg/L TCE. Transpiration gases were collected from leaves loosely enclosed in polyethylene bags.

TCE was detected in bags enclosing leaves from both TCE-dosed plants and control plants. After 8 months, TCE, trichloroethanol (TCEt), and trichloroacetic acid (TCAA) were found in the leaves, stems, and roots of the dosed plants. Dichloroacetic acid (DCAA) was found in the roots of all dosed plants. TCE and TCAA were detected in one of the control plants, and this was attributed to foliar uptake of transpired TCE. No mass balance was attempted.

TCE uptake and translocation in carrots, tomatoes, and spinach were studied by Schnabel et al. (1997) to evaluate to potential for TCE-contamination of the food chain. Plants were grown in glazed ceramic pots within closed, aerated growth chambers. Mass recoveries of [^{14}C]TCE ranged form 45 to 73%, with 1 to 2% found in plant tissues.

Burken and Schnoor (1998) examined the uptake of TCE by hybrid poplar plants grown in an enclosed hydroponic chamber system. Air was pulled through the foliar portion of the chamber and was periodically analyzed for TCE. TCE was detected in the air stream less than 48 h after dosing. Mass recovery was approximately 83% with 21% from TCE volatilization and 3 and 11% in plant tissue and sorbed to reactor components, respectively. A transpiration stream concentration factor (TSCF) of 0.75 was reported.

Fourier transform infrared (FT-IR) spectrometry was used by Davis et al. (1998) to measure the concentration of TCE in the gas phase in a chamber enclosing plants exposed to TCE-contaminated water. A ratio corresponding to the TSCF was derived from the water use rate, the gas flow rate, the initial solution concentration, and the steady-state gas phase concentration above the plants. The initial solution TCE concentration was 131 mg/L. Plant tissue measurements were not made. Reported TCSFs for TCE for saltcedar and poplar plants ranged from 0.1 to 0.58.

Newman et al. (1999) conducted studies in constructed, polyethylene-lined cells 1.5 m deep by 3.0 m wide by 5.7 m long, containing a coarse sand layer overlaid with silty clay loam soil. Planted, TCE-dosed treatments were run, as well as unplanted, TCE-dosed treatments and planted, non-dosed treatments. During the study, transpiration gas samples were taken 1) by loosely enclosing leaves in a Teflon bag and trapping air exiting the bag on an activated carbon trap, and 2) by using open-path Fourier transform infrared (OP-FTIR) spectroscopy to measure the ambient TCE concentration in the tree canopy air. Soil degradation of TCE in both planted and unplanted treatments was evidenced by a significant increase in chloride ion in soil samples. The authors suggest that the TCE was taken up by the plants and metabolized and that the leftover chloride was exuded back into the soil from the roots. TCE and its metabolites TCAA, TCEt and DCAA were found in plant tissues. The authors concluded that 99% of the TCE added to the planted cells was removed.

As illustrated in the above literature review, findings from laboratory studies designed to evaluate the fate of TCE in planted systems have been varied and inconclusive. This is especially true for TCE uptake and volatilization. Conflicting findings may be attributed to differences in exposure level, conditions, and duration of the studies, or to experimental artifacts from laboratory systems.

Four high-flow, dual vacuum growth chamber systems were constructed for use in phytoremediation studies involving volatile organic compounds. These growth chamber systems were utilized to determine the effects of long-term exposure on the uptake of radiolabeled TCE by hybrid poplar.

The hypothesis tested was that TCE uptake observed by poplar cuttings exposed to 1 mg/L or 10 mg/L TCE over an extended period (43 d) would be similar to uptake by poplars exposed to 1-mg/L and 10-mg/L treatments over a 12-d period. The "dosed" period in this study spanned 43 d. Upon completion of the dosed period, a stability study was initiated where one of the 1-mg/L-treated plants was allowed to continue growing in a TCE-free solution for three additional weeks. The hypothesis tested was that when the concentration gradient was reversed, TCE and/or its metabolites in the plant tissue would not come back into the bulk root zone solution. This is ultimately dependent upon where in the roots the ^{14}C is stored. Soluble ^{14}C-compounds in cell vacuoles could easily come back into solution when root turnover (cell death) occurs and cells disintegrate. However, ^{14}C that is bound to or incorporated into cell walls will not easily come back into solution, even in the event of cell death.

Materials and Methods

Growth Chamber Design

Experiments were conducted in four plant growth chamber systems constructed largely of glass traps, Teflon tubing, and Swagelok® type 316 stainless steel (SS) unions to maximize inertness. All were assembled in a walk-in plant growth chamber located at the Utah State University Crop Physiology Laboratory, Logan, Utah. The systems were utilized in three studies on the fate of TCE in hybrid poplar (Orchard et al., 2000b). Substantial modifications were made after these first three studies to improve trapping in the foliar portion of the system. Alterations in the airflow design were made. The large foliar airflow (5-10 L/min) was subsampled so that 50-100 mL/min passed through solid

charcoal traps. A third root-zone organic trap was also added to each system to improve mass balance.

Each of the four chamber systems was set up according to the schematic in Figure 8. The actual plant growth chambers have three sections each: a foliar chamber, a root-zone chamber, and a root/foliar seal. Each foliar chamber is constructed of a 21" length of 4" diameter glass tubing with a 71/60-mm, male, ground-glass (gg) joint at the bottom. Root-zone chambers are each 21" long and 3" in diameter and hold approximately 2 L of hydroponic solution. The top of each root-zone chamber consists of a 71/60-mm, female, gg joint. The root/foliar seal is constructed with one male (bottom) and one female (top) 71/60-mm, gg joint for connection to the foliar and root chambers. A molded glass cover seals off most of the root-zone environment while a small gg joint (19/22-mm) is centered in the cover to accommodate the stem of the plant (Figure 9). Encasing the poplar cutting with rope caulk (Frost King, Thermwell Products, Paterson, NJ) completes the seal. Rope caulk provides a flexible, gas-tight, nontoxic seal. Other sealants were tested, but did not provide an effective seal (Teflon tape) or solubilized the cutting's outer cambium layer (latex and oil-based silicone sealants).

In addition to the physical seal, a pressure differential of approximately –25 cm of water column (-0.36 psi) isolated the root and foliar chambers. The pressure differential guarantees that any compromise in the physical seal will not result in volatilization of TCE from the root zone to the upper chamber, mistakenly inferring plant uptake, translocation, and volatilization. Any leak in the root/foliar seal would be immediately evidenced by a complete loss of root-zone flow.

Gaseous TCE quickly reaches an equilibrium concentration in the headspace

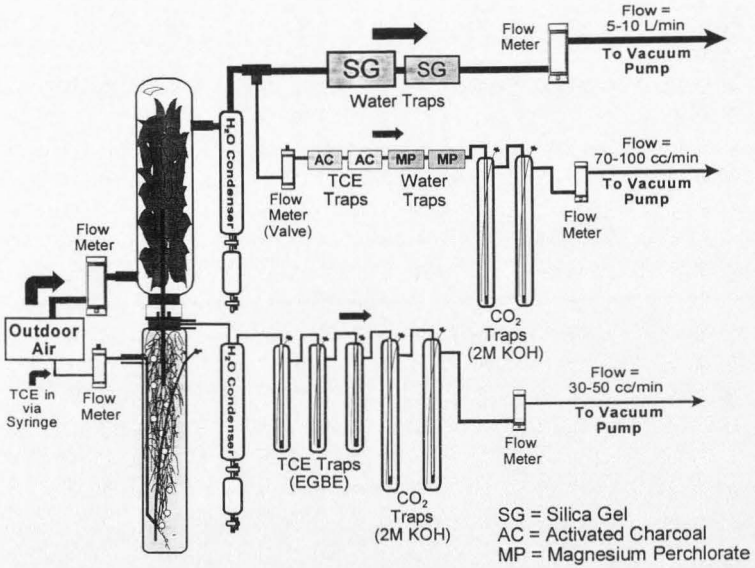


Fig. 8. Schematic of one complete chamber system. The system is designed to provide a natural plant environment, continuous root zone aeration, complete root/shoot separation, high mass recovery, and the ability to quantify phytovolatilized VOCs and mineralization to CO_2.

below the root/foliar seal. This high headspace TCE concentration creates a concentration gradient for diffusion into the exposed stem. This gradient drives the potential for "biological leaks" resulting from volatilization of TCE through the network of air-filled spaces in the woody stem of the poplar. However, the pressure differential induced by this growth chamber system hinders gaseous diffusion of TCE upward through the woody stem. Because TCE is not at a high concentration immediately below the soil surface in the field, elimination of "biological leaks" should more closely mimic field conditions.

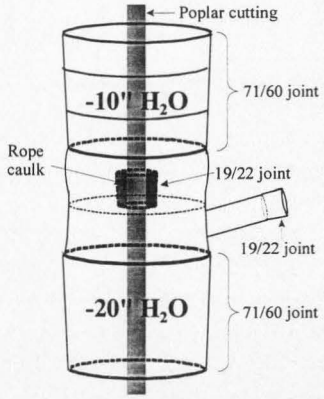


Fig. 9. Collar used to connect foliar and root-zone chambers. Both a physical seal of glass and rope caulk and a pressure differential of –10" (-25 cm) of water separate the root and foliar chambers.

In previous studies, some plant leaves turned a reddish color (Appendix F). Because increasing nutrient concentrations and better controlling root-zone solution pH did not seem to help, it was hypothesized that cold root zones, caused by close proximity to the recirculating cooling system components, contributed to this problem. To alleviate the cooling effect, a wall of 1-1/2" thick blue polyurethane foam was positioned between the four root zones and the cooled traps. The wall was painted black to keep the root zone as dark as possible. As an additional precaution, electric heating tape was laid along the floor of the dark box containing the root zones. This kept the root zones in the range of 20 to 22°C.

Following the plant growth chamber, the root-associated section of each system includes one water condenser, three liquid organic traps, and two liquid CO_2 traps. Root-zone organic traps hold 550 mL ethylene glycol monobutyl ether (EGBE). Root-zone

CO_2 traps hold 450 mL 2 M KOH. Root-zone organic and root-zone CO_2 traps are constructed of 1-1/2" diameter glass tubing topped with two 3/8" o.d. stems on either side of the female component of a 24/40-mm gg joint (Figure 10). One stem accommodates a compression fitting for use in connecting the traps, while the other stem houses the sampling needle. The male component of the 24/40-mm gg joint is narrowed to a 3/8" tube at the top of the joint to accommodate compression fittings, while a narrow glass tube is attached at the bottom and is inserted down through the 1-1/2" diameter trap. A regular capacity, medium-porosity gas dispersion tube (Model # CG-220-01, ChemGlass, Vineland, NJ) is attached to the bottom of the narrow tube so that the entire tube reaches the bottom of the trap. All liquid traps (root-zone organic, root-zone CO_2, and foliar CO_2) and water condensers are cooled to $< 5^{\circ}C$ with a recirculating, chilled water cooling system to minimize volatilization of the trapping solution and maximize drying of the air stream.

The foliar section of the system consists of a water condenser followed by a 3/8", SS Swagelok® tee. The foliar flow is split at this tee at 24-h intervals. When the flow is not split, the entire foliar air stream flows through three large (2" x 36") silica gel traps constructed of PVC pipe. These large silica gel traps hold approximately 600 g of indicating silica gel (Fisher Scientific, Pittsburgh, PA). When the flow is split, a small fraction (about 1/100) of the foliar air stream is diverted through two activated carbon traps (3/8" x 7" glass tube, 2.6 g coconut charcoal, 20/40 mesh, Supelco, Bellefonte, PA), two magnesium perchlorate dessicant traps (1" x 4" nylon tube, 10 g $MgClO_4$, Fisher Scientific, Pittsburgh, PA), and two liquid CO_2 traps. Liquid foliar CO_2 traps are

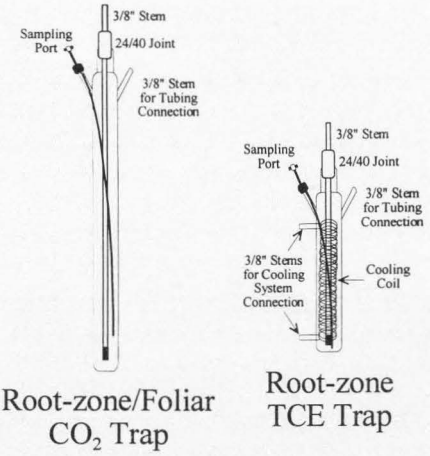


Fig. 10. Diagram of the two types of liquid traps.

identical in construction and type and volume of trapping solution to the root-zone CO_2 traps described above (Figure 10).

Connections from the plant chamber to the organic traps are made with SS Swagelok® unions (Crawford Fitting, Solon, OH) and 1/4" or 3/8" o.d. Teflon (PTFE) tubing (Fisher Scientific, Pittsburgh, PA). Subsequent connections between CO_2 traps are made with 3/8" o.d. nylon or high-density polyethylene (HDPE) fittings (Consolidated Plastics, Twinsburg, OH) and HDPE tubing.

Sampling ports are constructed of an appropriate length of SS tubing welded through the center of a SS Swagelok® cap. A 6", 14-gauge laboratory pipetting needle with leur-lock tip (Fisher Scientific, Pittsburgh, PA) is welded to the SS tubing above the cap. A Mininert® syringe valve (Fisher Scientific, Pittsburgh, PA) is attached to the leur-lock end of the pipetting needle to provide a gas-tight seal.

The four chamber systems were assembled within a Percival Scientific (Model PGW-132, Boone, IA) walk-in growth chamber with variable temperature and light controls. Experiments were carried out with a 16-h light period at ambient temperatures of approximately 22°C during the light period and 17°C at night.

Experimental Design

Chamber designations and treatments are shown in Table 3. Three planted chamber systems (Chambers A, B and D) were set up for the long-term experiment. Cuttings were rooted hydroponically in a greenhouse for 1 week, selected for uniformity in whip size and root growth, and placed in the growth chambers. Addition of TCE began one week after transplanting. At this time, each of the three plants had established several root initials and two or three roots at least 5 cm in length. Buds ranged from enlarged, green and breaking to broken with 2-3 nearly fully expanded leaves. A fourth, unplanted chamber (Chamber C) was also set up.

Steady root-zone concentrations were maintained by continuously adding a mixture of radiolabeled and non-labeled TCE (Appendix H) via the inlet air stream using a programmable syringe pump. Air was drawn through a 1/16" diameter SS needle to the bottom of the root-zone water column to ensure complete mixing. The specific activities

Table 3. Chambers and treatments.

Chamber	A	B	C	D
Type	Planted	Planted	Glass Rod	Planted
Duration	43 + 23 d	43 d	10 d	43 d
Exposure Concentration	1.15 mg/L	0.92 mg/L	1.72 mg/L	9.82 mg/L
Comments	Stability Study		Poisoned	

of the dosing solutions for the 1-mg/L (1.76×10^{-2} mCi $mmol^{-1}$) and the 10-mg/L (5.63×10^{-3} mCi $mmol^{-1}$) treatments were different by an order of magnitude. The 1-mg/L treatments were infused with the hot/cold TCE mixture at 0.35 μL per h while the 10-mg/L treatment was infused at 3.5 μL per h. Sampling and analysis of each root zone solution occurred daily and the injection rate of TCE was adjusted accordingly.

A time-weighted average root zone concentration of 1.0 ± 0.2 mg/L was maintained in Chambers A and B. Chamber D was dosed at 10 mg/L with a time-weighted average concentration of 9.8 mg/L. Chamber C was simultaneously "planted" with a glass rod in root-zone solution poisoned with 8 mM sodium azide. Although not a sterilizing agent, azide is an effective microbial inhibitor. This poisoned control chamber ran for only 10 d with a time-weighted average "root-zone" concentration of 1.7 mg/L. Results from Chamber C were compared to those of Orchard (1998), who saw some mineralization of [^{14}C]TCE to $^{14}CO_2$ (~1%) in both unplanted and planted treatments run for 10 d in this system. This experiment was useful toward determining whether mineralization observed by Orchard et al. (2000b) was due to root zone microbial activity or was just an artifact of the system.

Trapping Solutions

Liquid organic traps containing ethylene glycol monomethyl ether (EGME) were used in the initial system trial (Orchard, 1998) to trap volatile organics. Measurable amounts of EGME were lost from the traps, even when cooled to < 5°C. This prompted the addition of ethylene glycol monobutyl ether (EGBE). An 80% EGME/20% EGBE mixture was used in two subsequent trials. Following the third study, 100% EGBE was

tested and showed comparable trapping with minimal volatility. 100% EGBE was then used as the organic trapping solution for this study.

A 2.0-N solution of potassium hydroxide (KOH) was used in all CO_2 traps. At the flow rates used in this system, foaming of KOH solutions can be a problem. In the first three studies conducted in these growth chamber systems, traps were filled with 900 mL of 2.0 N KOH. An empty trap inserted in line after the CO_2 traps collected overflow due to foaming to protect the vacuum pump. To improve CO_2 detection limits in this study, all CO_2 traps were filled with approximately 450 mL trapping solution. Capacity of these traps, conservatively estimated using flow rates of 100 cc/min, is 180 d. Due to the reduced trapping volume and spatial constraints with the revised system, overflow traps were eliminated. However, significant foaming of a few CO_2 traps led to loss of trapping solution and damage to flow meters. Overflow traps are recommended for use in future studies. Analysis for $^{14}CO_2$ is accomplished using a barium chloride precipitation/re-evolution procedure (Appendices D and E).

Subsampling Scheme

Tests were conducted in the laboratory to determine the most feasible trapping scheme for the diverted low flow (Appendices A and B). Construction of traps identical to those used in the root zone was cost prohibitive as well as space prohibitive, so a test was conducted to determine the efficiency of running low flows through a set of three of the original, large, liquid traps. Use of a third trap proved important as almost 2% of the radiolabel spike was present in the third trap after only 10 d. Trapping efficiencies over 10 d were satisfactory for this experiment. However, the probability of poor detection

limits resulting from the use of a larger volume of trapping solution led to the notion of using dry traps and subsampling.

The exact fraction of flow that was subsampled had to be quantified in order to obtain an accurate mass balance. It was presumed that the use of dry traps and a second vacuum pump would stabilize flows, leading to a more accurate measure of flow through the traps and therefore a more accurate measure of the ratio of flows. Additionally, the use of dry traps could improve detection limits for foliar TCE.

The new, split-flow foliar trapping scheme was as follows (Figure 8). After passing through the foliar chamber at a flow rate of 5-10 L/min, air passed through a water condenser where humidity was brought from ~ 80% to ~ 30%. After exiting the water condenser, the flow was split using a Swagelok® 3/8" insert tee. Most of the air was directed through two large-capacity silica gel traps to scrub any remaining water from the air. As the plant grew and transpired larger quantities of water, construction of even larger silica gel traps and addition of a third silica gel trap became necessary. The diverted low flow (50-100 cc/min) passed through a flow meter followed by two activated carbon (Grade CT, Alltech, Deerfield, IL) traps to capture organic compounds. It then passed through two solid magnesium perchlorate dessicant traps to capture water and finally through two liquid, 2.0 N potassium hydroxide (KOH) traps to capture CO_2 before reaching the vacuum pump. Magnesium perchlorate was preferred over silica gel in the subsampling scheme because it has a much lower affinity for CO_2. Silica gel was used in the large traps because it is reusable and, therefore, much less expensive.

Rotometers were used to measure airflow rates. Gravimetric comparison of the mass of water collected by the silica gel and magnesium perchlorate traps was also used

to measure the flow distribution. Logistically, this split-flow trapping scheme could only be carried through on an every-second-day basis. An enormous amount of silica gel (~ 6 kg) was required to fill one chamber's set of large dessicant traps. A minimum of 16 h in an 80°C oven was required to completely dry the silica gel between trapping events. The collective packing, elution, sampling, and counting of the charcoal traps was the most time-consuming aspect of keeping the system running. Extra manpower, as well as the purchase of double the silica gel, activated carbon, and glass trap tubes would be required for a constant subsample. Split-flow traps were in line during every other 24-h period.

Foliar Sampling

Silica gel and magnesium perchlorate dessicant traps were weighed before insertion into the system and immediately following removal from the system. The difference of the initial trap mass subtracted from the remaining final mass was the mass of water trapped.

In order to collect kinetic data while at the same time maintaining low detection limits, dry charcoal organic traps replaced liquid traps in the foliar trapping design. These traps were exchanged at 24-h intervals and each used trap was eluted. The eluted volume was then counted by LSC to determine total ^{14}C trapped (Appendix A). Either methylene chloride or xylene was used to elute radiolabeled compounds from the activated carbon. In method development, methylene chloride gave greater elution efficiencies than carbon disulfide. Throughout the experiment, however, elution efficiency was consistently around 80%. Toward the end of the study, both xylene and pentane were tested as alternative solvents. One week before termination of the study,

methylene chloride was replaced by xylene, with elution efficiencies of 98%. The elution procedure for each charcoal trap began by transferring the activated carbon from the trap to a 20-mL VOA. Methylene chloride or xylene was then added through a Teflon-lined septa, with the VOA lid slightly loosened, until all headspace had been filled with solvent. Lids were tightened and VOAs were tumbled at 5 rpm for a minimum of 4 h. Following tumbling, triplicate 5-mL samples were taken and added to 15 mL of Ready Gel® scintillation cocktail for direct liquid scintillation counting (LSC).

Each set of charcoal traps packed for a given day was accompanied by a "trip blank" charcoal trap. The trip blank was handled identically to charcoal traps used in the chambers and was eluted and analyzed at the same time as the chamber system traps. In addition, a charcoal blank and a solvent blank were run. The charcoal blank consisted of 2.6 g charcoal (the mass in one trap) added to a VOA and eluted. The solvent blank consisted of a VOA filled with whatever solvent was being used for elution. These blanks were prepared, tumbled and sampled each time a set of traps was eluted.

Sampling Procedures

Stainless steel sampling needles equipped with Mininert® syringe valves were inserted through the tops to the bottoms of the root zone TCE and CO_2 traps, as well as the foliar CO_2 traps. This enabled sampling throughout the study without interruption of airflow. All samples were taken in triplicate for LSC analysis. Once the system was set up, a complete set of triplicate samples was taken from the liquid traps of each of the four chamber systems. The initial samples determined background LSC counts. These background counts were subtracted from sample counts throughout the study. One

sampling syringe was dedicated to each of the four chamber systems and syringes were rinsed three times with methanol between traps. Syringe rinses were saved and analyzed by LSC.

CO_2 traps were sampled only at the end of the study. Any $^{14}CO_2$ trapped was precipitated from solution with barium chloride ($BaCl_2 \cdot 2H_2O$), re-evolved with 10% v/v hydrochloric acid (HCl), trapped in 20 mL of a solution described by Abbot et al. (1992) consisting of 50% Ready Gel®, 40% methanol, and 10% monoethanolamine (MEA), and counted directly by LSC (Appendix C). Method detection limits (MDLs) were calculated based on 10 matrix spikes using radiolabeled bicarbonate. The use of a smaller volume of trapping solution lowered MDLs. However, only 1/120 to 1/133 of the total air leaving the chamber during sampling was trapped in foliar CO_2 traps. This increased the MDLs for those traps by a multiplier of 120 to 133.

Throughout the study, triplicate samples were taken from each root zone solution on a daily basis. Root zone organic traps were sampled after the first 24 h and every three to four d thereafter. One trap in each of the four systems was replaced with a fresh trap after the first two weeks and then weekly in order to maintain trapping efficiency throughout the study. Rather than replacing the entire set of three traps, only one fresh trap was added at each replacement. The old #1 trap was removed, the #2 and #3 traps became the new #1 and #2 traps, respectively, and the fresh trap became the new #3 trap. This trap "rotation" let each trap accumulate greater ^{14}C compound concentrations before being replaced. This led to improvement of both detection limits and mass balance recovery. All samples taken, their frequency and volume, and other parameters measured are listed in Table 4. The date and time were recorded for all samples.

Tissue Harvest

Upon termination of the dosed period, plants were removed from one of the 1-mg/L chambers (Chamber B) and from the 10-mg/L chamber (Chamber D). Root zone solution volumes were recorded and solutions were saved in screw-top Nalgene® bottles and stored at 4°C. Plants were separated into old leaf, new leaf, stem, upper whip, lower whip, and root components. Each plant component was weighed and subsequently stored at 4°C in screw-top glass jars with Teflon-lined lids until time of analysis. The glass rod (Chamber C) was rinsed with methanol, the rinsate was sampled, and radiolabel present was determined by LSC. All chamber system components were thoroughly rinsed with methanol. Tubing rinses were kept separate from chamber rinses. The volumes of the combined tubing rinsates and the combined chamber rinsates were each recorded and each rinsate was sampled in triplicate for LSC analysis.

Stability Study

Meanwhile, the foliar chamber was removed from the 1 mg/L plant remaining in the growth chamber (Chamber A). After 8 d, all leaves except those formed since removal of the foliar chamber were removed from the cutting. "Old" leaves were separated from "new" leaves. Leaves were analyzed by combustion for total radiolabel and by extraction for TCE and metabolites. Seven d later, the cutting was excised from the root portion. The harvested new leaf tissues and stem tissues were analyzed by combustion for total radiolabel and by extraction for TCE and metabolites. After eight more d, roots were harvested and analyzed by combustion for radiolabel and by extraction for TCE and metabolites. Root-zone solution samples were taken throughout

Table 4. Samples collected for TCE studies.

Sample location	Frequency	Volume	Other parameters measured
Root-zone solutions	Daily	3 mL	Final column volume
Root-zone water condensers	As H_2O is collected	5 mL	Total water collected
Foliar water condensers	As H_2O is collected	5 mL	Total water collected
Root-zone organic traps	Every 7 d	2 mL	Final trap volume
Foliar organic traps	Every other day	Whole trap	Initial and final trap mass
Root-zone CO_2 traps	End of experiment	10 mL	Final trap volume
Foliar CO_2 traps	End of experiment	10 mL	Final trap volume
Silica gel/magnesium perchlorate water traps	Every other day	Whole trap	Initial and final trap mass
Syringe rinses	After each trap is sampled	5 mL	None
Chamber rinses	End of experiment	5 mL	Total volume of methanol rinsate
Tubing rinses	End of experiment	5 mL	Total volume of methanol rinsate

to determine whether TCE or related compounds were discharged from the roots back into solution.

Tissue Analysis

Combustion/LSC analysis for ^{14}C was conducted on all plant tissues. Entire leaf and root tissue samples were crushed under liquid nitrogen with a mortar and pestle, while a coffee grinder was used to macerate the woody stems. Crushed tissue was subsampled in triplicate for dry weight analysis while 1-2 g portions of each tissue sample were placed in a combustion boat. Tissues were combusted at 900°C by a R.J. Harvey (Hillsdale, NJ) biological oxidizer, model OX-600. $^{14}CO_2$ evolved from the

combusted samples was trapped in the solution described by Abbot et al. (1992) consisting of 50% Ready Gel®, 40% methanol, and 10% MEA. Direct analysis of the trapping solution by LSC followed to determine total ^{14}C in each sample. All scintillation counting was done with a Beckman LS 1701 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, CA). Data calculation parameters were set as follows: counting time, 15 min; H# monitoring, on; sample repeats, 1; data calculation, DPMSL; replicates, 1; RCM, yes; and % error, 2.00.

When enough tissue was available, extraction followed by GC analyses for TCE and metabolites were conducted. Extraction procedures developed at the Utah Water Research Lab were carried out (Doucette et al., 1998) to identify and quantify TCE and its metabolites in both hydroponic solution and plant tissues. A series of aqueous extractions of each tissue type for each plant was carried out in duplicate (Figure 11). Crushed tissue of known weight was agitated in a Teflon centrifuge tube for 10 min with 15 mL of a 0.25 N NaOH solution on a reciprocating table shaker. Following agitation, samples were centrifuged at 10,000 rpm for 10 min. The supernatant was removed and retained in a disposable, polypropylene centrifuge tube. This aqueous extraction was repeated two additional times and the supernatant was collected each time. The combined supernatant was acidified to pH < 1 with 50% H_2SO_4 and subsequently extracted three times by shaking with 7 mL methyl tert-butyl ether (MTBE) for 5 minutes. Shaking with MTBE was followed by centrifugation for 5 minutes at 2500 rpm. Combined extracts were brought to 25 mL with MTBE and 2 g anhydrous sodium sulfate was added to remove residual water. Extracts were then analyzed by direct injection GC/ECD for TCEt and DCEt. Prior to determination of TCAA and DCAA, the extracted

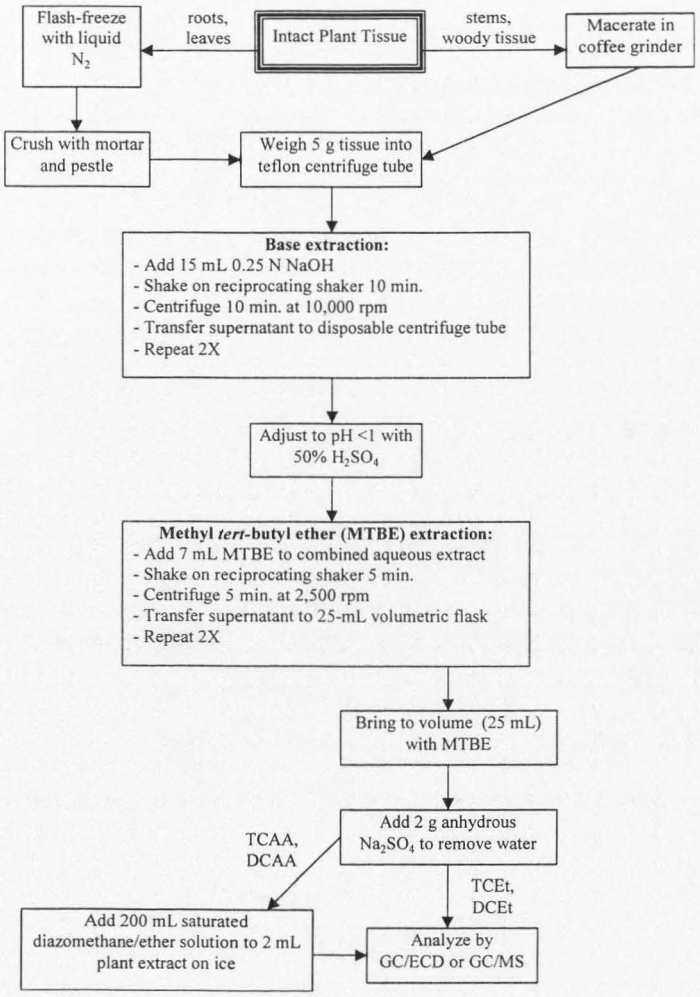


Fig. 11. Flow chart depicting extraction procedure for TCAA, DCAA, TCEt and DCEt. Procedures were developed at the Utah Water Research Laboratory (Doucette et al., 1998).

acid was methylated by addition of 200 µL of saturated diazomethane solution to 2 mL of extract. The resulting methyl ester derivative content was determined by direct injection GC/ECD. These plant extracts were analyzed with a Shimadzu GC14 gas chromatograph equipped with an ECD and DB-VRX capillary column (2.5 µm film thickness, 0.45 mm ID x 75 mm) (J&W Scientific, Folsom, CA). Samples were introduced to the GC either by thermal desorption or direct injection. Column and makeup flows were set at 8 and 40 mL/min nitrogen, respectively. Column oven temperature was 90°C isothermal for 6 min followed by a 20°C/min increase to 180°C. The detector and injection port temperatures were 300 °C and 210°C, respectively. Retention times for TCEt, TCAA, and DCAA under these conditions were 3.0, 5.2, and 3.2 min, respectively. Periodically, and whenever baseline drift was observed, the column was baked out at 240°C between samples. Another 2-mL aliquot of the extract was added directly to 18 mL of Ready Gel® scintillation cocktail and analyzed by LSC.

A purge and trap, GC method adapted from SW-846, Methods 5030 and 8010B was used to analyze for tissue-associated TCE. Samples were purged using a Dynatech Automated Purge and Trap system followed by a Shimadzu GC-14A gas chromatograph equipped with an electron capture detector (ECD) and a 75 m x 0.45 mm DB-VRX (2.5-mm film thickness) capillary column (J&W Scientific, Folsom, CA). Helium was used as the purge gas with a purge time of 11 min and a dry purge time of 2 min. Desorption and transfer line temperatures were set at 180 and 170°C, respectively. Column and makeup flows were set at 8 and 40 mL/min nitrogen, respectively. Column oven temperature was 60°C isothermal for 10 min, 5°C/min to 70°C, followed by a 40°C/min increase to 230°C

the purge gas with a purge time of 11 min and a dry purge time of 2 min. Desorption and transfer line temperatures were set at 180 and 170°C, respectively. Column and makeup flows were set at 8 and 40 mL/min nitrogen, respectively. Column oven temperature was 60°C isothermal for 10 min, 5°C/min to 70°C, followed by a 40°C/min increase to 230°C and held for 1 min. The retention time for TCE at these conditions was 11.8 min. The detector and injection port temperatures were 300 and 210°C, respectively. A method detection limit (MDL) of 6 ppt was determined for the purge and trap GC/ECD procedure based on the results of matrix spikes in accordance with Title 40, Code of Federal Regulations, Part 136 (40 CFR 136).

Results

Mass Recovery

Total recovery of ^{14}C in each chamber ranged from 93 to 99% (Table 5). Due to the flow-through nature of the system, the majority of the radiolabel was collected in the root zone organic traps. This was expected and is commensurate with results from previous studies using these systems (Orchard et al., 2000b).

Plant Health

Plant transpiration was determined from the amount of water added to the root zone over the course of the study, corrected for samples taken and for evaporation. Evaporation (determined from the unplanted control and in agreement with that observed by Orchard et al. (2000b)) was < 1% of the total evapotranspiration. On average, plants in this study transpired 105-115 mL/d. Average transpiration over the first 9 d was 15-20

mL/d. Both cumulative daily transpiration and plant height increased exponentially throughout the study, indicating healthy plant growth (Figures 12 and 13). There were no visible signs of TCE toxicity throughout the study for any of the treatments.

^{14}C Analysis

Analysis by combustion gives the total ^{14}C in plant tissues, including parent

Table 5. ^{14}C distribution summary.

Chamber	A	B	C	D
Exposure concentration	1.15 mg/L	0.92 mg/L	1.72 mg/L	9.82 mg/L
Exposure duration	43 d	43 d	10 d	43 d
Total ^{14}C dose	60.91 mCi	60.91 mCi	14.22 mCi	206.2 mCi
	%			
Root-zone solution	0.09	0.34	3.96	0.42
Root-zone organic traps	90.5	94.6	92.6	94.6
Foliar organic traps	0.03	0.03	ND[a] (<0.02)	0.03
Root-zone CO_2 traps	0.44	1.55	0.07	2.00
Foliar CO_2 traps	ND (<0.19)	ND (<0.30)	ND (<0.02)	ND (<0.04)
Plant tissue by combustion				
New leaves[b]	0.018	0.020	NA[c]	0.030
New new leaves[d]	0.006	NA	NA	NA
Old leaves[e]	0.039	0.040	NA	0.050
Stems	0.034	0.030	NA	0.030
Upper whip	0.008	0.005	NA	0.010
Lower whip	0.020	0.020	NA	0.030
Roots	0.288	0.400	NA	0.430
Apparatus rinsates	1.20	1.22	1.11	1.12
Total ^{14}C recovery	92.68	98.25	97.74	98.75

[a]Non-detect

[b]Includes young leaves at first or only leaf removal

[c]Includes all leaves formed after first leaf removal on stability study plant

[d]Not applicable

[e]Includes largest and most mature leaves at first or only leaf removal

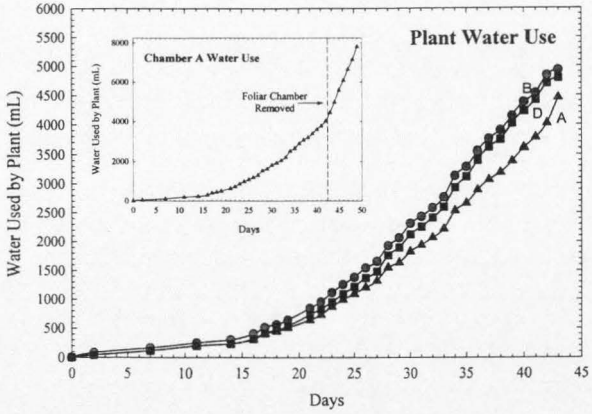


Fig. 12. Cumulative transpiration by each of the three plants. Transpiration rate of the plant in Chamber A increased after the foliar chamber was removed at the start of the stability study.

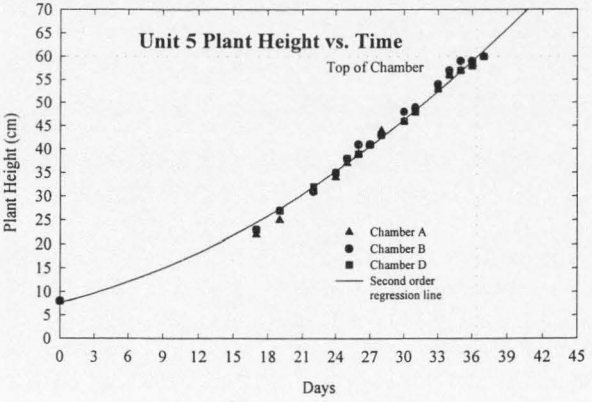


Fig. 13. Plant height throughout the study.

compound, metabolites, and any ^{14}C that has been bound to or incorporated into the plant tissue. The quantity of ^{14}C is converted to TCE mass equivalents based on the specific activity of the dosing solution and the ratio of hot to cold compound. The total TCE-equivalent mass in the shoot tissues of both of the 1-mg/L treatments was identical (0.41 and 0.39 mg for chambers A and B, respectively). However, distribution of the radiolabel among shoot tissues of each plant differed (Tables 6 and 7). This is to be expected as the plant in Chamber A remained in the growth chamber in TCE-free solution for three additional weeks. During this time, translocation of any mobile ^{14}C within the plant would have occurred. In addition, growth of the plant resulted in dilution of tissue concentrations. The total TCE-equivalent mass in the shoots of the 10-mg/L treatment was 5.45 mg, roughly 10 times that in the 1-mg/L treatments.

Foliar tissue concentrations by combustion (mg TCE mass equivalents per kg dry tissue mass) ranged from 6 to 21 mg/kg for the 1 mg/L treatments and from 90 to 420 mg/kg for the 10 mg/L treatment. Old leaves were analyzed separately from new leaves. In all cases, the concentration in the old leaves exceeded that in the new leaves, suggesting mobility of the radiolabel within the plant. Leaf concentrations were higher than stem concentrations. Orchard et al. (2000b) found the same to be true in 26-d treatments, but the opposite was true in shorter-term (12-d) treatments. Root tissue concentrations in this study, not corrected for sorption, were 256 and 484 mg/kg for the 1-mg/L treatments and 5180 mg/kg for the 10-mg/L treatment. These concentrations are higher than those reported by Orchard et al. (2000b).

Table 6. Data summary.

Chamber	A[a]	B	D
Exposure concentration	1.15 mg/L	0.92 mg/L	9.82 mg/L
Exposure duration	43 d[b]	43 d	43 d
New leaves (mg/kg)[c]	6.5 ± 0.6	16.9 ± 1.0	261 ± 23
Dry mass (g)	7.77	5.65	5.58
New new leaves (mg/kg)	6.0 ± 0.1	-	-
Dry mass (g)	4.69	-	-
Old leaves (mg/kg)	15.0 ± 2.5	27.7 ± 0.9	420 ± 84
Dry mass (g)	11.91	5.75	5.95
Upper whip (mg/kg)	10.2 ± 0.6	5.34 ± 0.3	90.6 ± 5.6
Dry mass (g)	3.52	3.83	2.83
Stems (mg/kg)	17.3 ± 7.1	21.1 ± 3.8	251 ± 38
Dry mass (g)	6.59	5.52	4.96
Avg. shoot concentration (mg/kg)	11.8 ± 2.4	18.9 ± 1.6	282 ± 43
Shoot dry mass (g)	34.48	20.75	19.32
Total ^{14}C in shoots (TCE equivalent mg)	0.41	0.39	5.45
^{14}C TCE phytovolatilized (mg)[d]	0.16	0.12	1.65
^{14}C CO_2 transpired (mg)	ND[e] (<1.47)	ND (<1.71)	ND (<4.92)
Transpiration (L)	4.45	4.93	4.79
TSCF[f]	0.11	0.11	0.15
Lower whip (mg/kg)	20.5 ± 8.6	17.1 ± 1.9	289 ± 53.5
Dry mass (g)	5.54	6.51	4.63
Roots (mg/kg)	256.5 ± 54.1	484 ± 35.5	5180 ± 534
Dry mass (g)	6.41	3.85	4.08
Root fresh mass (g)	128.1	83.7	88.7
RCF	1.42	2.02	2.15

[a]Stability study chamber

[b]66 days including stability study

[c]TCE equivalent concentration

[d]100% trapping efficiency assumed

[e]Non-detect

[f] $TSCF = \frac{\text{(Mass of chemical in shoot, mg) + Mass of chemical phytovolatilized, mg)}}{\text{(Volume water transpired, L) * (Root-zone solution concentration, mg/L)}}$

Table 7. Distribution of plant-associated radiolabel. Shoot-associated phytovolatilized TCE and CO_2 are included.

Chamber	A[a]	B	D
Exposure concentration	1.15 mg L^{-1}	0.92 mg L^{-1}	9.82 mg L^{-1}
Exposure duration	43 d	43 d	43 d
	%		
Foliar TCE traps	6.7	4.7	5.6
Foliar CO_2 traps	0.0	0.0	0.0
Plant tissue			
New leaves	2.2	3.8	4.9
New new leaves	1.2	NA[b]	NA
Old leaves	7.7	6.4	8.5
Stems	4.9	4.7	4.2
Upper whip	1.5	0.8	0.9
Lower whip	4.9	4.5	4.5
Roots	70.9	75.0	71.4
Total	100.0	100.0	100.0

[a]Stability study plant

[b]Not applicable

GC Analysis

Extraction of plant tissues followed by LSC showed that the total extractable label (includes TCAA, DCAA, and TCEt) in plant tissues was a small fraction (2 to 4% in roots and stems, 4 to 15% in leaves) of the total radiolabel as determined by combustion. This suggests that most of the radiolabel in the plant was present as bound residue (Table 8). Because it is impossible to rigorously quantify extraction efficiencies using these procedures, metabolite results should be regarded more qualitatively than quantitatively.

Table 8. Tissue analysis results. Extractable radiolabel in plant tissues accounts for 2 to 18% of the total radiolabel by combustion with the lowest extractable fraction in the roots and the highest extractable fraction in the old leaves of all three plants. The TCE metabolites TCAA, DCAA, and TCEt were most prevalent in the 10 mg/L treatment and in the old leaves of the 1 mg/L treatments.

Chamber	Tissue ^{14}C Conc. Combusiton, LSC	Metabolite Conc. Extract, LSC	Tissue TCE Conc. Purge and Trap	Tissue TCAA Conc. Extract, GC/ECD	Tissue DCAA Conc. Extract, GC/ECD	Tissue TCEt Conc. Extract, GC/ECD
	mg kg^{-1}	mg kg^{-1} TCE Equivalents				
A (1.15 mg L^{-1})						
New Leaves	6.52 ± 0.58	1.17 ± 0.19	0.38 ± 0.29	ND (<0.005)	.815 ± .178	ND (<0.01)
New New Leaves	6.03 ± 0.01	0.60 ± 0.10	0.04 ± 0.01	1.10 ± .559	6.81 ± 3.49[a]	6.69 ± 1.21[a]
Old Leaves	15.0 ± 2.5	2.52 ± 0.07	ND to 0.08	0.417 ± 0.0437	0.238 ± 0.0448	ND (<0.01)
Stems	17.3 ± 7.1	0.81 ± 0.08	0.03 ± 0.00	ND (<0.005)	2.56	[b]
Roots	267 ± 54	6.06 ± 0.36	ND to 0.12	[b]	[b]	[b]
B (0.92 mg L^{-1})						
New Leaves	19.4 ± 1.2	0.86 ± 0.38	0.08 ± 0.02	ND (<0.005)	ND (<0.02)	ND (<0.01)
Old Leaves	27.7 ± 0.9	1.59 ± 0.08	0.02 ± 0.01	ND - 0.140	ND (<0.02)	ND - 0.0796
Stems	21.1 ± 3.8	0.85 ± 0.13	ND (<0.02)	ND	ND (<0.02)	ND (<0.01)
Roots	484 ± 36	13.2 ± 2.4	4.15 ± 1.45	ND	ND (<0.02)	0.0991
D (9.82 mg L^{-1})						
New Leaves	261 ± 23	28.9 ± 3.0	ND (<0.01)	3.83 ± 0.863	ND (<0.02)	ND (<0.01)
Old Leaves	420 ± 84	66.4 ± 1.8	0.14 ± 0.01	12.2 ± 2.64	0.396 ± .055	0.595 ± 0.0022
Stems	251 ± 38	13.6	3.41 ± 1.06	0.592	0.447	ND (<0.01)
Roots	5178 ± 534	195 ± 20	60.7 ± 4.71	0.144	12.2	6.19 ± 1.53

[a]Bad data. Numbers are unusually high and exceed total radiolabel numbers.

[b]Noise in chromatograms interfered with peak quantification

TCAA, DCAA, and TCEt were detected by GC/ECD in the tissues of the 10-mg/L treatment. Average method detection limits (MDLs) in mg/kg dry weight for plant tissues were 0.075 for TCAA, 0.298 for DCAA, and 0.149 for TCEt. TCAA and DCAA were detected in the foliar tissues of the stability study plant (Chamber A, 1-mg/L treatment). Though his detection limits were higher, Orchard et al. (2000b) did not observe these metabolites in any 1-mg/L treatments. Metabolite concentrations in the 10-mg/L treatment (Chamber D) were generally higher than those observed in the 10-mg/L treatments of Orchard et al. (2000b), but in the same range. Metabolite concentrations in Chamber D were also in the range of those reported by Newman et al. (1997), who reported concentrations ranging from non-detect to 0.2 mg/kg for TCEt, 0.32 mg/kg DCAA, and 7.2 mg/kg for TCAA. In a more recent paper, Newman et al. (1999) found TCAA at 0.2 mg/kg and DCAA at 0.25 mg/kg in leaves of soil-grown poplars exposed to TCE for 3-5 months. These concentrations are much lower than those observed in the 10-mg/L treatment of this study (Chamber D) but are in the range of those in the 1-mg/L treatment.

Root-Zone TCE Traps

Due to the flow-through design of the system, most of the [^{14}C]TCE volatilized from the root-zone solution, and root zone organic traps captured over 90% of the applied label from each chamber. Rotation within each set of three organic traps confounds determination of overall carryover from trap to trap. However, samples taken on day 5, before the initial trap rotation, show volatilization of between 5% and 7% of the trapping solution resulting in carryover from trap to trap.

Foliar TCE Traps

Spikes of [^{14}C]TCE onto foliar organic traps were performed to verify that traps had ample capacity for TCE. These tests showed no breakthrough of ^{14}C from the initial trap to the backup trap in a 24-h period at airflows representative of the system. These tests verify that the traps had ample capacity. In one analysis (Appendix C), backup trap counts were considered to be background counts. Each day's counts for each chamber were subtracted from the initial trap counts for that chamber on that day. For the 1-mg/L treatments, counts never reached more than 30 dpm above the detection limit. The detection limit of 8.52 dpm for the foliar TCE traps was determined from a series of matrix spikes following the protocol described in EPA's SW-846 (USEPA, 1996). "Hits" in the 1-mg/L treatment traps were sporadic.

For the 10-mg/L treatment (Chamber D), counts just barely above the detectable limit were recorded on days 1 and 7, but no additional hits were recorded until day 21. Counts well above the detection limit (40-80 dpm) were seen in the first trap, while the second trap's counts remained below detectable limits from day 21 until the end of the study.

Traps from days 39 and 41 were somehow contaminated and data for all chambers on those days was lost. Contamination was evidenced by unusually high counts in both the initial and backup traps, as well as trip blanks. Fortunately, at this point in the study, counts in each chamber's traps were fairly stable from day to day. For each of those days, the average dpm on each chamber's traps from days 21 to 37 was assumed.

TSCF

The transpiration stream concentration factor (TSCF) is defined as the concentration of the compound in the transpiration stream divided by the bulk solution concentration. If the chemical is metabolized over the course of the experiment, the apparent TSCF should be corrected for the degradation rate of the compound, if known. In this study, TSCF was calculated by dividing the TCE equivalent mass in shoot tissues, plus the mass of TCE volatilized, by total water transpired. This quotient was then divided by the time-weighted average root-zone solution concentration. TSCF values reported here were calculated assuming that all ^{14}C measured in the shoots was TCE. Subtraction of extractable metabolites determined by LSC would lower the TSCF of Chambers A and B and D by < 0.01 and Chamber D by < 0.02.

Calculated TSCFs for the 1-mg/L treatments were both 0.11, while the TSCF for the 10 mg/L treatment was slightly higher, at 0.15 (Table 6). These values are similar to those obtained by Orchard et al. (2000b), who calculated an average TSCF of 0.12 for five replicate 1-mg/L treatments and an average TSCF of 0.20 for two 10-mg/L treatments. The analytical variability associated with TSCFs calculated in this study is estimated at $\pm < 0.02$ TSCF units. This estimate is based on the measured variability in the root zone concentration and the analytical variability within triplicate samples of foilar traps. Phytovolatilized TCE represented 27, 24, and 28% of the total TSCF for chambers A, B, and D, respectively. This fraction is similar to the 29 and 30% observed in the 12- and 26-day, 10-mg/L treatments of Orchard et al. (2000b) when foliar $^{14}CO_2$ is included. Based on these comparisons, extended study duration did not affect TSCF.

RCF

The root-associated portion of the original cutting ("lower whip") was analyzed separately from the roots, enabling the determination of a root concentration factor (RCF). This was not done in the previous studies by Orchard (1998). The RCF is the concentration in the roots divided by the aqueous solution concentration and measures partitioning of the compound to lipophilic components of the root solids. RCFs ranged from 1.42 to 2.15 (Table 6). These values correlate well to RCF values obtained for TCE (1.15 to 2.30) in sorption analyses with hybrid poplar roots (Utah Water Research Lab, Logan, UT, unpublished data). These numbers are roughly in agreement with a relationship between log K_{ow} and RCF developed by Briggs et al. (1982) for lipophilic compounds. Using Equation (1) from their paper and a log K_{ow} for TCE of 2.42, the expected RCF is 2.20. Equation (1) is a linear, partitioning relationship and does not take into account any uptake of the compound. The close correlation between the RCFs determined in this study and Equation (1) suggests that the partitioning process accounts for most of the root-associated TCE. Equation (2) in Briggs et al. (1982) takes into account a small uptake factor that is most prevalent for polar, ionizable compounds. When this equation is applied, again using 2.42 for the log K_{ow} of TCE, the expected RCF is increased to 3.02. This further suggests that partitioning is the dominant process and that uptake of the compound accounts for only a tiny fraction of the RCF.

TCE, as determined by purge and trap analysis, was detected in the plant tissues of all planted treatments (Table 8). The time between the crushing of frozen tissue and its addition to a VOA containing methanol was minimized. However, it is possible that volatilization of TCE from the tissue occurred in the interim. Newman et al. (1997)

reported hybrid poplar tissue concentrations ranging from non-detect to 1.9 mg/kg. In another study, Newman et al. (1999) observed TCE concentrations in foliar tissue ranging from non-detect to 0.1 mg/kg after 3-5 months of TCE exposure. TCE was detected in leaves at higher levels only after 2 years of exposure.

Root-Zone CO_2 Traps

Counts (dpm) were detected in the root-zone CO_2 traps of all three planted chambers (Table 9). These counts, as a percentage of the total radiolabel added, are similar to those seen by Orchard (1998). Counts were also detected in the first root-zone CO_2 trap of the poisoned control, Chamber C (equivalent to 0.025 mg CO_2). These counts were substantially reduced compared to Chambers A, B, and D. Because no bacteria capable of growing on a TCE-based medium (see next section) were found in the hydroponic solution of the poisoned chamber, it is probable that these counts are the result of carryover from root-zone organic traps. Orchard (1998) also saw counts in the root-zone CO_2 traps of unplanted control chambers using the same system (0.7 and 1.2% of the total radiolabel added). Comparatively reduced counts in the study described here may be a result of the addition of a third root-zone organic trap. Carryover of volatilized organic trapping solution may have contributed to the observed counts. A carryover test was conducted in the laboratory to test this hypothesis. A flask containing EGBE (organic trapping solution) was spiked with [^{14}C]TCE. The EGBE/TCE solution was pipetted into another flask containing 2M KOH (CO_2 trapping solution) until saturation, as indicated by the formation of a thin layer of EGBE above the KOH solution. The solution was then thoroughly mixed for 30 min and poured into a separatory funnel.

Table 9. CO_2 trap results. ^{14}C was not detected in any foliar CO_2 traps. ^{14}C (up to 2% of the total dose) in root-zone CO_2 traps suggests TCE mineralization in the rhizosphere. 0.07% of the total radiolabel was found in root-zone CO_2 traps of the poisoned control chamber, suggesting carryover from the root-zone organic traps.

Treatment	Trap	dpm/10 mL	Trap Vol (mL)	mg/trap	MDL[a] (mg/trap)	ND[b]	% of Total Dose	%
1 ppm	A Top 1	41.35 ± 11.66	455	0.60	0.85	X	0.132	0.13
66 d	A Top 2	-2.56 ± 2.83	340	0.01	0.62	X	0.002	
stability	A RZ 1	15891.61 ± 69.92	180	0.96	2.73E-03		0.212	
study	A RZ 2,1	7779.36 ± 121.46	285	0.75	4.32E-03		0.164	0.43
	A RZ 2,2	10442.49 ± 153.70	75	0.00	1.14E-03		0.058	
	B Top 1	73.98 ± 39.76	685	2.27	1.38	X	-0.024	0.00
1 ppm	B Top 2	0.72 ± 13.24	165	0.01	0.33	X	0.001	
43 d	B RZ 1	41004.90 ± 161.79	395	5.46	5.99E-03		1.198	1.55
	B RZ 2	17205.38 ± 256.28	275	1.59	5.69E-03		0.350	
1 ppm	C Top 1	-3.91 ± 7.76	440	0.00	0.01	X	-0.001	0.00
10 d	C Top 2	-9.58 ± 3.53	440	0.00	0.01	X	-0.001	
unplanted	C RZ 1	545.72 ± 30.03	415	0.08	6.29E-03		0.072	0.07
poisoned	C RZ 2	-7.32 ± 2.55	440	0.00	6.67E-03	X	-0.001	
	D Top 1	16.11 ± 8.72	295	0.568	1.86	X	0.012	0.01
10 ppm	D Top 2	-9.34 ± 4.07	460	0.00	3.06	X	0.000	
43 d	D RZ 1	217554.74 ± 4018.84	270	61.78	8.51E-05		1.283	2.00
	D RZ 2	93527.21 ± 551.15	350	34.43	1.10E-04		0.715	

[a]Method detection limit

[b]Non-detect

After 12 h, the lower KOH layer was separated from the EBGE layer and precipitated using the CO_2 precipitation procedure described previously. Results showed 9.3 ± 0.7% carryover of ^{14}C. However, xylene extraction of the root-zone CO_2 trapping solution from Chamber C showed no extractable counts, indicating that the radiolabel present was not TCE.

Microbial Enumeration

Because trace mineralization (0.07% of the total dose) was evidenced by the presence of radiolabel in the root zone CO_2 traps of the unplanted, poisoned control chamber, microbial analysis was conducted on the each chamber's root zone solution. Bacteria capable of growing on a minimal agar and TCE-based medium (Table 10) were

Table 10. Minimal growth medium with TCE.

Component	Value	Component	Value
Noble Agar, g	15	$(NH_4)_2HPO_3$, g	8
$NaHPO_4 \cdot 12H_2O$, g	2.5	KH_2PO_4, g	2
$MgSO_4 \cdot 7H_2O$, g	0.5	Yeast Extract, mg	100
$CaCl_2 \cdot 2H_2O$, mg	60	$FeSO_4 \cdot 7H_2O$, mg	30
$MnCl_2 \cdot 4H_2O$, μg	60	$CuSO_4 \cdot 5H_2O$, μg	15
Biotin, μg	12	Trichloroethylene, μL	7

enumerated in the nutrient solutions of each of the four chambers. A 10-fold serial dilution of each chamber's nutrient solution followed by culture gave counts of presumptive TCE degraders. Samples of the nutrient solutions from chambers B and D were diluted excessively, resulting in loss of data for those chambers. Presumptive TCE degraders were counted at 6.6×10^4 CFU/mL in nutrient solution from Chamber A (unpoisoned, planted, dosed). Counts from the poisoned control, Chamber C, showed no ($< 1 \times 10^1$ CFU/mL) viable bacteria. These results strongly suggest that counts (dpm) in the root zone CO_2 traps of the unplanted, poisoned control chamber were not a result of TCE mineralization and were, in fact, associated with some system artifact.

Foliar CO_2 Traps

Foliar CO_2 traps, sampled only at the end of the study, did not contain detectable levels of ^{14}C (Table 9). Initial analysis of one of the traps from Chamber B showed detectable counts, but subsequent analyses did not, possibly indicating the gradual volatilization of radiolabel from the trapping solution over time. Subsequent analyses of root zone CO_2 traps resulted in counts identical to the original analysis.

UPTAKE OF TRICHLOROETHANOL (TCEt) AND TRICHLOROACETIC ACID (TCAA) BY HYBRID POPLAR

Introduction

These studies had four aims: 1) to determine the extent of uptake of TCEt and TCAA by plants in a stress-free, hydroponic, aerobic environment as well as in an anaerobic environment; 2) to ascertain whether TCEt and TCAA are toxic to plants and at what levels; 3) to learn whether TCEt and TCAA are further metabolized one to the other, either in the root zone or within plant tissues; and 4) to help understand where transformations of TCE and its metabolites take place in planted systems. The TCEt study was conducted first followed by the TCAA study. Both studies were essentially identical with few exceptions. Any differences between the two studies are described.

Materials and Methods

Plant Propagation and Transplanting

Hybrid poplar (*Populus deltiodes x nigra*, DN34) cuttings 25 cm in length and approximately 2 cm in diameter were rooted hydroponically in a nutrient solution. After development of roots and once leaf growth had begun (approximately 2 weeks), 40 of the largest cuttings were each transplanted to individual, brown, 2-L, high-density polyethylene (HDPE) Nalgene® bottles filled nutrient solution.

Single-Bottle Hydroponics

Forty-eight HDPE bottles (40 planted, 8 unplanted) were arranged on a greenhouse bench. Air manifolds were constructed using lengths of 2" PVC pipe hooked to a diaphragm pump. Holes 1/8" in diameter were drilled along each of the two lengths of PVC, and a 36" length of 1/8" black air tubing was inserted into each hole. Air manifolds were placed such that each bottle received an air tube from the manifold. Bottles were filled with 2000 mL of a complete and appropriately dilute nutrient solution (pH = 5.6) (Table 11). Each rooted hybrid poplar cutting was positioned snugly in the center of a 2" diameter, closed-cell foam sleeve and each resulting "cap" was then placed in the 2" diameter mouth of each bottle. Nutrient solution levels were checked and bottles were refilled on a daily basis for one week prior to addition of TCEt or TCAA in order for plants to overcome any transplant shock. Nutrient solution pH tends to rise over time, so pH was monitored and adjusted when necessary (above 7) with 0.1 M nitric acid.

Table 11. Nutrient solution used in single-bottle studies.

Salt	Stock solution	mL/100 L	Final concentration
KNO_3	2.0 M	50	1 mM
KH_2PO_4	0.5 M	100	0.5 mM
KH_2PO_4	0.25 M	200	0.5 mM
K_2SiO_3	0.1 M	100	0.1 mM
K_2SO_4	0.5 M	0	0 mM
$Fe(NO_3)_3$	50 mM	10	5 μM
EDDHA	100 mM	40	40 μM
$MnCl_2$	60 mM	10	6 μM
$ZnCl_2$	20 mM	30	6 μM
H_3BO_3	20 mM	300	60 μM
$CuSO_4$	20 mM	10	2 μM
Na_2MoO_4	0.6 mM	15	0.09 μM

Ammonium nitrate (0.1 M) was also used to help replenish nitrogen and to stabilize pH between 4 and 7. This pH range is optimum for nutrient availability and plant growth and is representative of rhizosphere soils.

Treatments

Each of these two studies involved twelve treatments (ten planted and two unplanted) with four replicates each (Table 12). The two unplanted treatments varied only in concentration. The planted treatments varied in both concentration and in oxygen status of the root zone. Root-zone oxygen (aeration) status can dictate the consortia of microorganisms present in the rhizosphere. Anaerobic conditions support a unique host of bacteria that can affect nutrient relations in the soil. Anaerobic bacteria utilize molecules other than oxygen as electron acceptors to acquire energy through oxidation-reduction reactions. As continued activity of anaerobic bacteria causes redox potential to decrease, NO_3^- availability decreases followed by the reduction and unavailability of iron, sulfur, and manganese to plants (Nilsen and Orcutt, 1996). Aeration status may also have a profound effect on plant growth and may lead to increased exudation of carbon at the root surface (Barber and Gunn, 1974; Smucker, 1984; Haller and Stolp, 1985). Trolldenier and Hecht-Buccholz (1984) attributed considerably higher microbial populations in the root zone of oxygen-stressed plants grown in hydroponic culture to increased carbon exudation from roots.

For the TCEt study, two ages (one week apart) of plants were available, so planted bottles were numbered such that each treatment had one older and three younger plants. Plants of uniform age were used for the TCAA study, but the largest plants were

Table 12. Treatments for TCAA and TCEt single-bottle studies.

Treatment #	Aeration status	Dose
		mg L^{-1}
1	Aerobic	0
2	Anaerobic[a]	0
3	Mixed[b]	0
4	Aerobic	1
5	Anaerobic	1
6	Mixed	1
7	Aerobic	10
8	Anaerobic	10
9	Mixed	10
10	Aerobic	100
11 (unplanted)	Aerobic	1
12 (unplanted)	Aerobic	10

[a]Nitrogen gas bubbled into root zone
[b]Mixed: 3 d aerobic (air), 3 d anaerobic (nitrogen), repeat

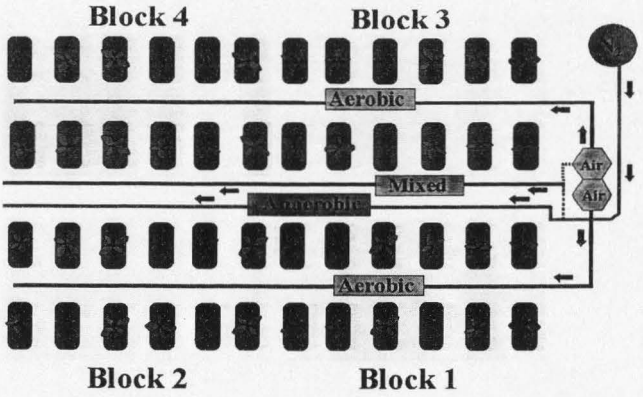
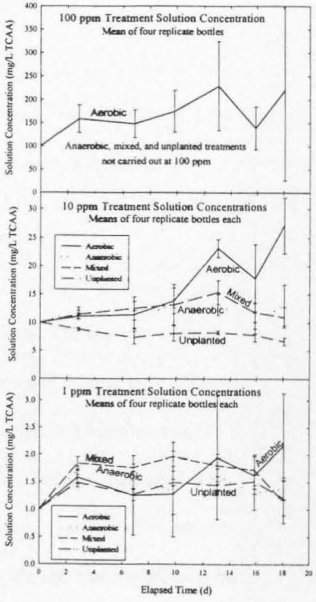


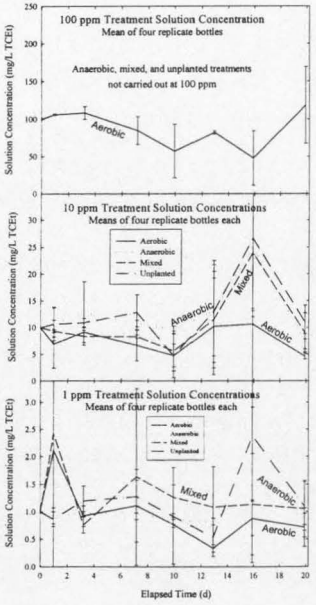
Fig. 14. Experimental setup for single-bottle studies. Each of four randomized, complete blocks conmtains one bottle for each of 12 treatments (ten planted and two unplanted). Nitrogen gas was bubbled into the root zones of the anaerobic treatments. Mixed treatments switched between anaerobic and aerobic root zones every three d.

put in one block to mimic the block of older plants in the TCEt study. In both studies the 48 bottles were spread across the bench in a randomized, complete block design to avoid any bias generated by uneven light distribution in the greenhouse (Figure 14).

Dosing

Stock solutions of 1000 mg/L of both TCEt and TCAA were prepared by either dilution of a concentrated solution with distilled, deionized water (TCEt) or dissolution of a crystalline solid (TCAA) in distilled, deionized water. Calculated quantities of chemical for initial dosing and for subsequent spiking at every other watering event were drawn from these stock solutions. Care was taken when adding the solution to avoid dripping the chemical directly onto plant roots. The initial dosing was designated as day 0. Each day, bottles were refilled with nutrient solution and the volume added to each bottle was recorded. A constant root-zone exposure concentration is desirable when determining TSCF values. We were unable to analyze hydroponic solutions rapidly enough to calculate the quantity of chemical lost between sampling events. Therefore, on every second day, additional compound (TCEt or TCAA) was added such that any water lost from each bottle was replenished at the original dosage concentration. Time-weighted average solution concentrations at the end of each study were generally within 25% of the intended dose. However, the time-weighted average for the 100 mg/L TCAA dose was elevated, at 172 mg/L. Average solution concentrations over time are shown in Figure 15. Note that one of the four replicate bottles of each treatment was harvested after day 16, and in some cases caused the average solution concentration to drop.





TCEt Study **TCAA Study**

Fig. 15. Solution concentrations over time.

Aeration

Bottles were aerated by bubbling either atmospheric air or nitrogen through individual 1/8" o.d. tubes connected to a manifold. A diaphragm pump and a nitrogen (N_2) gas tank were used to pump air and N_2, respectively. Bottles in the anaerobic treatments were switched to having N_2 bubbled into their root zones approximately 4 h prior to dosing. Bottles in the mixed treatment remained hooked to air for the first 3 d and were switched to N_2 for the next three d. This aeration pattern was repeated for the duration of each study.

Sampling

Blank root-zone solution samples were taken from each treatment prior to dosing on day 0. On day 1 of both studies, samples were taken from each of the 48 bottles. Samples of 20 mL were taken throughout the TCEt study. However, 20 mL was excessive and sample volumes were changed to 3 mL for the TCAA study. In both studies, syringes equipped with pipetting needles were used for sampling, each designated for an individual dose level (0, 1, 10, and 100 mg L^{-1}). Between sample sets, each syringe was rinsed once in methanol and twice in deionized water. Samples were taken every third day.

Harvesting

Prior to harvesting, a final sample of root zone solution was collected from each bottle. Because one block of plants in the TCEt study was older and larger, it was harvested one week earlier than the other three blocks. Plants in the TCAA study were all the same age, but the block containing the biggest plants was harvested one week

earlier in order to provide continuity between studies. At each harvesting event, plants were carefully removed from their bottles and the volume of solution remaining in the bottles was measured.

Upon removal, a photo was taken of each whole plant. Plants were subsequently separated into root, stem, and leaf components and fresh weight of each tissue component was obtained. In both studies, "roots" consisted of the underwater portion of the cutting and all roots, while "stems" consisted of the above-water portion of the cutting and the young branches. The "leaves" component included petioles and leaves as well as meristems. Leaf area was not determined in the TCEt study, but was measured in the TCAA study with a LI-COR leaf area meter (Lincoln, NE).

Solution and Tissue Analysis

Tissue was stored at 4°C until time of analysis. A known weight (~5 g) of crushed plant tissue was combined with 15 mL of a 0.1 N sulfuric acid/10% NaCl solution in a 50-mL Teflon centrifuge tube. Centrifuge tubes were shaken for 10 min on a reciprocating shaker, followed by 10 min of centrifugation at 10,000 rpm. The supernatant was separated from the plant tissue and deposited into a disposable polyethylene centrifuge tube. This extraction was repeated two additional times. The combined aqueous extracts were then further extracted three times by shaking with 7 mL volumes of MTBE for 5 min. Phase separation was accelerated by centrifugation for 5 min at 5000 rpm. The MTBE extracts were combined and brought to 25 mL volume. Any residual water was removed with 2 g anhydrous sodium sulfate and the extracts were analyzed for TCEt and DCEt by direct injection GC/ECD. To analyze for TCAA and

DCAA, a 1-mL aliquot of the dried extract was derivitized with diazomethane and analyzed. All plant and hydroponic solution extracts were analyzed with a Shimadzu GC14 gas chromatograph equipped with an ECD and DB-VRX capillary column (2.5 μm film thickness, 0.45 mm ID x 75 mm) (J&W Scientific, Folsom, CA). Samples were introduced to the GC by direct injection. Column and makeup flows were set at 8 and 40 mL/min nitrogen, respectively. Column oven temperature was 90°C isothermal for 6 min followed by a 20°C/min increase to 180°C. The detector and injection port temperatures were 300 °C and 210°C, respectively. Retention times for TCEt, TCAA, and DCAA under these conditions were 3.0, 5.2, and 3.2 min, respectively. Periodically, and whenever baseline drift was observed, the column was baked out at 240°C between samples. Method detection limits (MDLs) were determined based on 10 matrix spikes (Hayhurst, 1998; US EPA, 1996) and are listed in Table 13.

Table 13. Method detection limits for single-bottle studies. Tissue MDLs are expressed as the average MDL in mg/kg dry tissue.

	TCEt Study			TCAA Study		
	Leaves	**Stems**	**Roots**	**Leaves**	**Stems**	**Roots**
TCAA	**0.904**	**1.85**	**0.904**	**0.097**	**0.059**	**0.071**
DCAA	**NA[a]**	**NA**	**NA**	**0.291**	**0.178**	**0.212**
TCEt	**0.281**	**0.316**	**0.649**	**0.194**	**0.141**	**0.119**

[a]**Not analyzed**

Results

Growth Effects

TCEt Study: Increasing TCEt exposure concentration did not significantly decrease poplar growth. However, aeration status had a dramatic effect (Figure 16). Root growth was dramatically reduced. In some cases, roots became brown in color and slimy in texture. Overall growth was also dramatically affected. This was evidenced by plant dry weight, which was greatest in aerobic treatments, greatly decreased in mixed aeration treatments, and least in anaerobic treatments where plant dry weight was only 25% of aerobic plant dry weight (Figure 17).

TCAA Study: Tissue dry weights were similar across the 1-, 10-, and 100-mg/L TCAA treatments, but were slightly lower in 0-mg/L (control) treatments, suggesting growth *stimulation* by TCAA (Figure 17). Visual effects of TCAA, including chlorosis and yellowing of leaves as well as some formative effects, were observed in the 10-mg/L

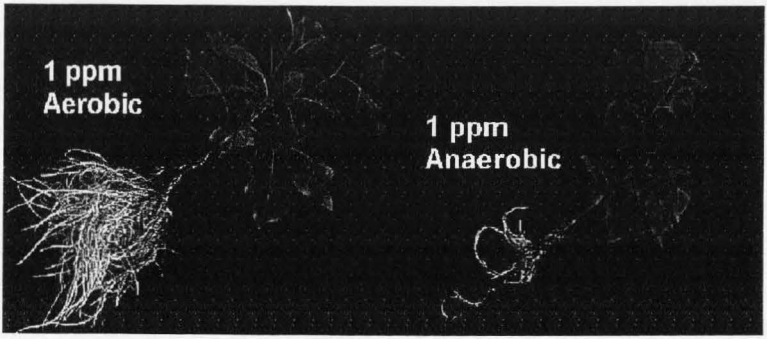
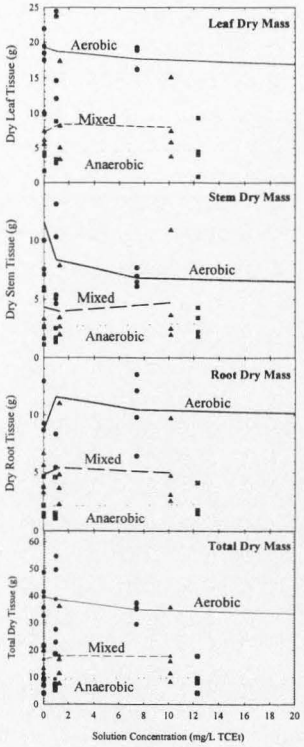
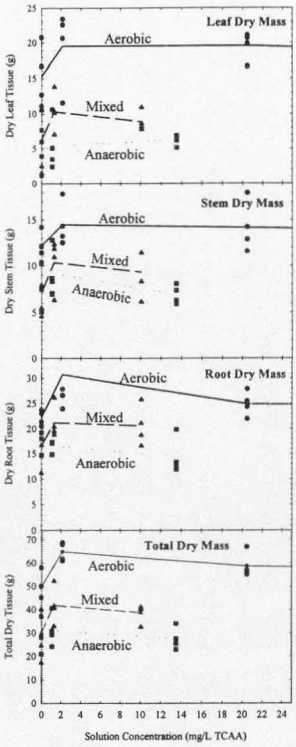


Fig. 16. Aeration effects on poplars exposed to TCEt. Similar effects were observed in the TCAA study. Roots in anaerobic treatments were often brown and fragile. Roots in aerobic treatments were white and strong.





TCEt Study **TCAA Study**

Fig. 17. Plant tissue dry weights.

treatments and to a greater extent in the 100-mg/L treatments. In grasses, TCAA inhibits the formation of a normal cuticle and can therefore possibly increase transpiration in TCAA-treated plants (Kiermayer, 1964; Ashton and Crafts, 1973). Effects on transpiration of hybrid poplar trees, as indicated by plant water use, were not apparent in this study (see graphs in Appendix G). Anaerobicity had similar effects to those observed in the TCEt study.

Tissue Analysis Results

TCEt Study: Tissue data show transformation of TCEt to TCAA. TCAA was found in leaf tissues at all exposure levels in the aerobic and mixed aeration treatments (3% to 23% by weight of total TCEt + TCAA). In stem tissues TCAA was detected only in the 10-mg/L and 100-mg/L treatments (0.3% to 2%), regardless of aeration status.

TCAA Study: Tissue data show transformation of TCAA to DCAA. DCAA was detected in ALL leaf samples (4% to 17% by weight of total TCAA + DCAA), but in only the stem samples of aerobic treatments (3% to 7%).

Transpiration Stream Concentration Factor (TSCF)

TCEt Study: Average (n = 4) transpiration stream concentration factors (TSCFs) for TCEt were low, ranging from 0.004 to 0.007. Trends in TSCF data across TCEt exposure levels and aeration status are not apparent (Figure 18). Extensive metabolism of TCEt in the root zone, evidenced by the presence of TCAA in hydroponic samples and root and leaf tissues, may have contributed to low TSCF numbers. This transformation was less prominent in anaerobic treatments, contrary to the idea that increased root exudation resulting from root stress would result in greater microbial activity.

TCAA Study: Average TSCFs (n = 4) for TCAA are generally higher and are much more dependent on treatment effect (Figure 18). They range from 0.003 to 0.034. TSCF values for the aerobic treatments were highest, followed by the mixed aeration and finally the anaerobic treatments, suggesting inhibition of uptake under hypoxic conditions. A similar trend was apparent across exposure levels, with the highest average TSCF values at the lowest dose level, then decreasing with increasing exposure concentration. DCAA was detected in the hydroponic solutions of only two (100 mg/L aerobic, 10 mg/L mixed) of 48 bottles where DCAA accounted for >0.01% by weight of the total TCAA + DCAA. These data indicate that most of the transformation of TCAA to DCAA took place within plant tissues.

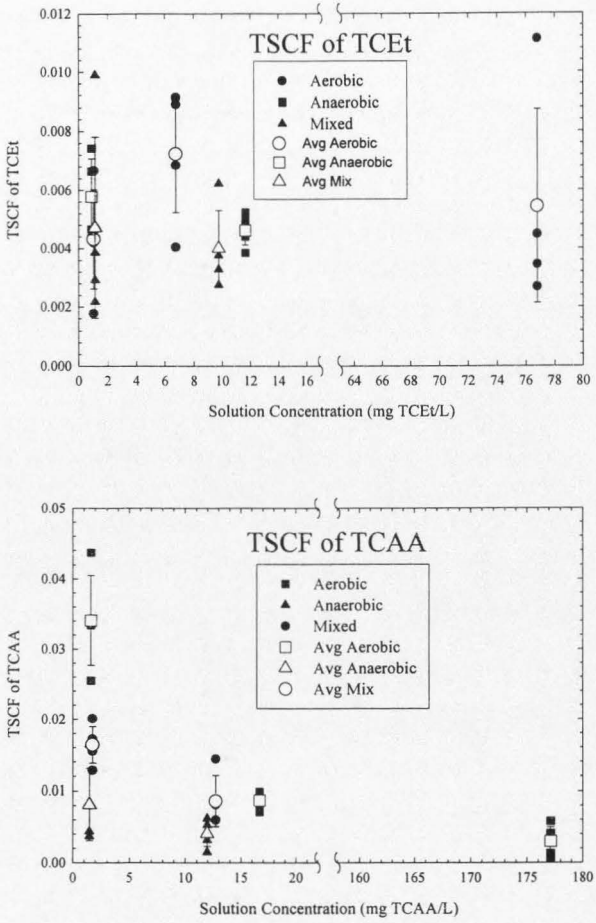


Fig. 18. Transpiration stream concentration factors of TCEt and TCAA, calculated from extractable parent compound in each of the two studies.

DISCUSSION

TSCF Comparison

Calculated TSCFs for TCE were greater than those of both TCEt and TCAA. This is in agreement with the relationships between TSCF and log K_{ow} suggested by Briggs et al. (1982) and Hsu et al. (1990). However, direct comparison of these TSCF values is difficult. TSCF for the TCE study is calculated based on total radiolabel in plant tissues as well as phytovolatilized radiolabel while calculations of TSCF for both TCEt and TCAA include only *extractable* parent compound in plant tissues. Degradation of TCE followed by incorporation of metabolites into cellular constituents can mistakenly infer the presence of TCE in plant tissues as determined by combustion and LSC. In contrast, degradation of TCEt and TCAA followed by incorporation of metabolites into cellular constituents results in the loss of extractable C related to the parent compound. TSCFs calculated for the TCEt and TCAA studies should, therefore, be expected to be lower relative to radiolabeled TCE, whether or not uptake was comparatively lower.

TSCF Components

The transpiration stream concentration factor for TCE can be broken down into tissue and volatilized components. Comparison of these individual components is useful in the determination of differences between treatments and between studies. Values were derived from information given in Burken, 1996 ("the thesis") and Burken and Schnoor, 1998 ("the paper"). Because the values necessary for this calculation are not given, they must be back-calculated from the available data. TCE distribution in the paper is

reported as a percentage of the total added. From the thesis, 11.7 mg TCE was added. Using the percentages given in Table 2 of the paper, this breaks down to 2.50 mg volatilized, 0.035 mg in leaves, and 0.051 mg in the upper stem. Burken and Schnoor also include the bottom stem concentration in the calculation of TSCF, but this must be corrected for sorption. From the thesis, this nonsorbed concentration should be identical to the concentration of the upper stem. Assuming the stem was split in half between the upper and lower components, the lower stem would have 0.051 mg TCE associated with it as well. Thus, the total mass of TCE in the transpiration stream would be 2.504 mg volatilized and (0.035 + 0.051 + 0.051) 0.137 mg associated with plant tissue. This comes out to a total of 2.641 mg TCE associated with the TSCF. A TSCF of 0.75 is reported in the paper. Of the total TSCF-associated TCE, 94.8% is attributed to volatilization. Therefore, (0.75 * 0.948) 0.71 is the TSCF component from volatilization. TCE in tissues accounts for the remaining 0.04.

In the study described in this thesis, the tissue component of the TSCF (0.11 - 0.16) ranged from 0.08 to 0.12, while the volatilized component of the TSCF ranged from 0.03 to 0.05. In each case, the volatilized component accounts for 25 to 28% of the total TSCF. Direct comparison of these numbers to those generated by Burken and Schnoor shows that: 1) tissue concentrations in this study are two to three times greater than Burken and Schnoor's, and 2) the volatilized component of the TSCF in this study is much (14 to 24 times) smaller.

The smaller tissue TSCF component observed by Burken and Schnoor may be due to toxic effects resulting from the combined use of small plants and high TCE

concentrations. This is supported by Orchard et al. (2000b) where exposure to 70 mg/L TCE was toxic to young plants, resulting in reduced TCE uptake.

Table 14 shows TSCF components from Burken and Schnoor (1998), Orchard et al. (2000b), and this study. TSCF values are broken down into tissue and phytovolatilized components. Any foliar-related $[^{14}C]CO_2$ detected by these studies should be included in TSCF calculations. Foliar-related $[^{14}C]CO_2$ was not detected in this study, nor by Burken and Schnoor (1998). In two cases, Orchard et al. (2000b) reported a tiny amount of radiolabel in foliar CO_2 traps. This component of the TSCF is excluded from Table 14.

These calculations show that the single largest difference between the TSCF calculated by Burken and Schnoor and those generated by this study and those of Orchard et al. (2000b) is the phytovolatilized component of the TSCF. This probably results from

Table 14. TSCF values from three studies, broken down into tissue and phytovolatilized components.

Study	Dose level	^{14}C in Shoots	$[^{14}C]TCE$ Phytovolatilized
	$mg\ L^{-1}$		
Orchard et al., 2000b	1	0.04	0.10
Orchard et al., 2000b	1	0.06	0.12
Orchard et al., 2000b	1	0.05	0.03
Orchard et al., 2000b	1	0.10	0.00
Orchard et al., 2000b	1	0.09	0.00
This study	1	0.08	0.03
This study	1	0.09	0.03
Orchard et al., 2000b	10	0.15	0.02
Orchard et al., 2000b	10	0.14	0.02
This study	10	0.12	0.04
Burken and Schnoor, 1998	52	0.04	0.71
Orchard et al., 2000b	70	0.01	0.21
Orchard et al., 2000b	70	0.02	0.00

system differences, specifically the presence or absence of a pressure gradient between the root and shoot compartments of each system.

In the system of Burken and Schnoor, air was pulled through the foliar compartment while the root compartment remained static. Though chamber pressures were not measured, it is possible that a negative pressure was generated by the airflow through the top. It is also possible that water removal from the root compartment through transpiration could have generated a negative pressure in the root zone. Small leaks in the root/foliar seal would equalize either or both of these pressures, if present. It is possible that the ^{14}C captured from the exhaust air stream was volatilized from the root zone through leaks in the root/shoot seal, and out of the chamber.

Barring any leaks though the root/shoot seal, another possible explanation exists for the notably large volatile component of the TSCF observed by Burken and Schnoor. The transfer of organic compounds from the root zone to aboveground plant parts is typically thought of in terms of translocation by solution flow through the xylem. For volatile compounds such as TCE, root-to-shoot gas phase transfer of TCE through air-filled spaces in roots and stems is another potential pathway. In systems where TCE concentrations are high in the headspace of the root zone, as is the case in both our studies and that of Burken and Schnoor, the gradient for gaseous diffusion of TCE through air-filled spaces is magnified. In our system, the negative pressure in the root zone would have, at the very least, resulted in the restriction of upward gaseous transfer of TCE through air-filled spaces. Conceivably, the negative pressure actually resulted in a tiny mass flow of air downward through the stem, completely preventing gas phase TCE transfer through the stem to the shoot chamber.

It is possible to test these hypotheses using either or both of the aforementioned systems. The two-flask system of Burken and Schnoor should be replicated at USU. Assuming the results obtained are similar to Burken and Schnoor's, further tests can be carried out to test the seal. A pressure gauge attached to the root zone should show increasing negative pressure as water is removed through transpiration. Stable or increasing, then decreasing pressure readings would indicate leaks. In the absence of leaks, the volatilization of TCE through air-filled spaces in plant tissues should be investigated. If this is, in fact, a significant fate process, placing a pressure differential comparable to that generated by our system on the root and shoot compartments should result in greatly reduced volatilization through plant tissues.

Our system can be tested by dosing only the foliar compartment with TCE. If no leaks are present, as evidenced by continuous bubbling in the root zone, any TCE found in the root zone can be attributed to mass flow by gaseous diffusion through the stem. Airflow through the foliar chamber should be kept to a minimum to keep the gaseous TCE concentration in the chamber sufficiently high. Potentially, foliar deposition of TCE followed by downward translocation through the phloem would result in radiolabel increases in the root zone. However, the stability study conducted in this system showed no detectable increases in root-zone radiolabel due to exudation from the plant, even when the roots were saturated with labeled compound at the initiation of the study.

If gas-phase transfer accounts for most or all of the difference between the Burken and Schnoor TSCF and ours, the question becomes, "which is more realistic?" In the field, gas transfer by diffusion decreases exponentially with distance. Therefore, the likelihood of significant TCE transfer by diffusion through roots over a distance of more

than 50 to 100 cm is small, even in highly porous tissues (Justin and Armstrong, 1987). Furthermore, gas phase transfer of TCE through the soil profile would be retarded by sorption of the compound to soil organic matter. The probability of significant concentrations of gaseous TCE even reaching the plant is low. Thus, a laboratory system that minimizes gaseous diffusion of TCE through stems should more realistically represent phytoremediation potential in the field.

Newman et al. (1999) conducted studies in constructed, polyethylene-lined cells 1.5 m deep by 3.0 m wide by 5.7 m long. Cells contained a coarse sand layer overlaid with silty clay loam soil. TCE was added to the sand layer via the cell's water inlet. Planted, TCE-dosed treatments were run, as well as unplanted, TCE-dosed treatments and planted, non-dosed treatments. All treatments received the same volume of water via the inlet over the course of the 3-year study. Additional water was supplied by surface irrigation and natural rain events. During the study, transpiration gas samples were taken 1) by loosely enclosing leaves in a Teflon bag and trapping air exiting the bag on an activated carbon trap, and 2) by using open-path Fourier transform infrared (OP-FTIR) spectroscopy to measure the ambient TCE concentration in the tree canopy air. Soil degradation of TCE in both planted and unplanted treatments was evidenced by a significant increase in chloride ion in soil samples. The authors suggest that the TCE was taken up by the plants and metabolized and that the leftover chloride was exuded back into the soil from the roots. TCE and its metabolites TCAA, TCEt, and DCAA were found in plant tissues. The authors concluded that 99% of the TCE added to the planted cells was removed. Although this was an artificial system, the results suggest that trees

may have a significant impact on the remediation of shallow TCE contaminated groundwater.

Other Related Laboratory Studies

The study by Burken (1996) described in the Literature Review used the apparatus in Figure 7 to examine uptake of both volatile and nonvolatile compounds including BTEX (benzene, toluene, ethylbenzene, and *m*-xylene), and 1,2,4-trichlorobenzene (TCB) over an 8-d period. All compounds were radiolabeled. In less than 24 h, BTEX were detected in the aerial compartments of the plant reactors, indicating unhindered translocation to the leaves and volatilization of these compounds. The data evidences a tight correlation between the volume of water transpired and the mass of contaminant volatilized for all BTEX experiments.

Experiments conducted with TCB were inconclusive, as most of the applied TCB (nearly 70%) was bound to the acrylic sealant separating the root and foliar chambers. Burken (1996) suggests that ample TCB remained in solution, even after sorption to the sealant occurred, for uptake to take place. However, the remaining TCB (with the highest log K_{ow} of 4.25) was found associated with plant tissues in the root zones of the reactors. None of the compounds tested accumulated in leaf tissues. The percent of applied radiolabel volatilized was shown to decrease as the negative log of vapor pressure values for the compounds increased from 1 to 3. Relationships between volatilization, hydrophobicity, and vapor pressure were independent of root zone concentration and total mass translocated. Burken (1996) notes that the overall percentage of the total applied

label volatilized would likely increase with time, but that the experiments conducted in this study were carried out over similar time periods.

A study conducted by Chard et al. (unpublished data, 1997) evidenced uptake of TCAA by hybrid poplar. Hybrid poplar (DN34) whips were cut to 14" length, rooted in hydroponic solution, and transferred to six 30-L tubs that held three poplars each. Lids were constructed of 2"-thick sheet foam insulation. Three treatment levels were chosen for this study and tubs were spiked with TCAA to bring the concentrations of two tubs of each to 0, 3, or 9 mg/L. Poplars were allowed to grow in the spiked treatment for 19 d. After 19 d, no treatment effects were apparent, so tubs were spiked again and TCAA concentrations were reestablished. Following the second spiking, TCAA was added with each watering such that tubs were replenished at TCAA concentrations corresponding to initial treatment levels. After another 23 d the study was terminated and root, young leaf, and old leaf tissue samples were analyzed for their TCAA concentrations.

Visual observations indicated that hybrid poplar growth was not significantly affected by TCAA at any of the concentrations to which they were exposed. Actual final tub concentrations were much higher than intended, with the "9-mg/L" treatments averaging 38 mg/L and the "3-mg/L" treatments averaging 11 mg/L. Transpiration was measured as the amount of water lost from the tubs. Evaporation from the tubs was assumed to be negligible. TSCFs for TCAA in this study were low and averaged 0.005 for the 3-mg/L treatment and 0.003 for the 9-mg/L treatment. Stem tissue was not analyzed in this study and its inclusion would likely have increased TSCF values.

To date, all USU studies have been conducted hydroponically. A proposed bioreactor for studies of TCE fate in a plant/soil system may be found in Appendix I.

IMPLICATIONS FOR PHYTOREMEDIATION

Plant uptake, metabolism, and volatilization were identified as mechanisms of TCE removal from contaminated groundwater. The role and relative significance of each these fate processes in TCE phytoremediation are uncertain. This is typified by the difference between TSCF values measured here and others reported in the literature is considered. The following simplified illustration highlights the critical variables involved in estimating TCE uptake by plants on a field scale.

Annual TCE uptake from a shallow aquifer per unit area per year can be estimated by:

$$\text{Mass of TCE removed by plant uptake} = (\text{TSCF})(C_{TCE})(T)(f)$$

where TSCF is assumed to be a constant, C_{TCE} is the average groundwater concentration of TCE (mg/L), T is the cumulative volume of water transpired per unit area per year (L/m^2-yr), and f is the fraction of plant water needs met by contaminated groundwater. This expression assumes that C_{TCE} is constant. A more accurate calculation would incorporate the reduction in C_{TCE} occurring over time as a function of physical, chemical, or biological processes as well as possible changes in TSCF with exposure concentration.

Transpiration rates in the field vary widely depending on soil water availability and evaporative demand. Potential transpiration rates, calculated from pan evaporation rates, are used to schedule irrigation of crop plants. Transpiration rates can be 10 L/m^2-d on hot days in well-watered soils in dry climates. Annual transpiration rates can be as high as 1800 L/m^2-yr in hot desert climates such as Arizona, and as low as 200 L/m^2-yr in

cool, moist environments like Alaska (Camp et al., 1996). In the summer, even well-watered crops can fail to attain their potential transpiration rate during periods of high evaporative demand due to partial stomatal closure. During winter months, deciduous trees drop their leaves and evergreen trees have low transpiration rates because they are dormant and often snow-covered or wet. When phreatophytic plants are forced to use groundwater, they typically do not achieve the high transpiration rates that occur with vegetation that uses surface water (Camp et al., 1996). Thus, the actual annual transpiration rate is usually below the potential rate. The cumulative annual transpiration rate is more useful in long-term phytoremediation calculations. Depending on the climate, 200 to 1400 L/m^2-yr probably represents a reasonable range of values for annual transpiration.

The fraction of plant water needs met by groundwater is difficult to measure and is poorly characterized. Groundwater use tends to decrease as the availability of surface water increases (Nilsen and Orcutt, 1996). Additional studies using stable isotope techniques (Nilsen and Orcutt, 1996) are necessary to determine a reasonable range of values for this parameter. Until such data are available, a range of groundwater use fractions from 0.1 to 0.5 (10 to 50% of plant water needs met by groundwater) is probably realistic for climates with more than 40 cm of precipitation per year.

Using a groundwater concentration of 1 mg/L, an average TSCF value of 0.12 [measured for the 1 mg/L treatments in Orchard et al. (2000b) and in this study], and the high and low estimates for transpiration rate and fraction of groundwater used, yearly plant uptake values ranging from 2.4 to 84 mg TCE/m^2-yr can be calculated using the annual uptake equation. As additional information regarding transpiration rates and the

fraction of groundwater used by plants becomes available, the estimated range can be narrowed. Using the TSCF value of 0.75 reported by Burken and Schnoor (1998) would increase the upper range value to 525 mg TCE/m^2-yr. Calculations of this type should be used at each potential site to determine if plant uptake might be a significant removal mechanism.

Important to note is that although the mass of TCE removed by plant uptake is small, other mechanisms may play an important role in phytoremediation of TCE contaminated sites. Plants transpire water. Through transpiration, plants can move contaminated water into the unsaturated zone by mass flow. In this zone, TCE may be degraded aerobically by soil microorganisms. Saturated microsites within the unsaturated zone may allow anaerobic degradation of aerobic degradation products to take place. Sorption of TCE to soil organic matter in the unsaturated zone may be construed as a stabilization mechanism. At the very least, the hydraulic effect that plants have may retard the migration of contaminated water off-site and into municipal drinking water sources.

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APPENDICES

Appendix A

Charcoal Trap Elution

STANDARD OPERATING PROCEDURE
Solvent Elution of Charcoal Traps

WARNING: Those who are exposed to methylene chloride (dichloromethane) are at increased risk of developing cancer, adverse effects on the heart, central nervous system and liver, and skin or eye irritation. Exposure may occur through inhalation, by absorption through the skin, or through contact with the skin (from OSHA Regulations, Standards – 29 CFR – 1910.105). Viton or PVA gloves are recommended for use when handling methylene chloride. Nitrile gloves are recommended when handling xylene. Always wear safety glasses and a lab coat. Perform all procedures under a properly functioning hood.

Procedures:

1. Remove charcoal traps from sealed dessicant containers.
2. For each trap, remove end caps and empty the charcoal trap into the corresponding VOA, being careful not to lose any charcoal in the process. Be sure that there is no charcoal on the outside of the VOA and screw the cap (with Teflon-lined septum) on.
3. Weigh out 2.5 g of fresh charcoal and add it to a VOA labeled "CS" (charcoal spike).
4. Weigh out 2.5 g of fresh charcoal and add it to a VOA labeled "CB" (charcoal blank).
5. Weigh all VOAs and record each one's mass in the column labeled "VOA + Charcoal Mass." The VOAs labeled "MB" (method blank) and "MS" (method spike) will be empty. Weigh them and record their mass (empty but with cap) in the same column.
6. Place all VOAs under a hood. For each VOA with charcoal:
   a. Loosen the cap.
   b. Fill a 5 mL leur-lock syringe with solvent (xylene or methylene chloride) and screw on a syringe tip.
   c. Puncture the VOA's septum with the syringe tip and inject the contents of the syringe.
   d. Screw the cap back on tightly.
   e. Remove the leur-lock tip from the syringe.
7. When each VOA containing charcoal has had 5 mL solvent injected, remove the caps one by one. Rinse each glass trap tube with solvent into the corresponding VOA. Carefully fill the VOA the rest of the way with solvent until the headspace is minimized (to 1-2 mm below the top of the cap threads) and re-cap the VOA *tightly*.

8. Remove the caps from the empty VOAs labeled "MB" and "MS" and fill each one to the top, minimizing headspace as described in step 6.
9. Inject an appropriate volume of ^{14}C-labeled TCE into the VOA labeled "CS" and the VOA labeled "MS." DO NOT rinse the syringe between spikes.
10. After spiking, rinse the spiking syringe a minimum of three times with methanol.
11. Puncture the septum of the VOA labeled "MB" one time with a clean syringe. At this point, each septum should have one and only one puncture hole.
12. Weigh each VOA again and record each mass in the column labeled "VOA + Solvent Mass."
13. Be sure each cap is screwed on tightly and place VOAs into the tumbler.
14. Begin tumbling at setting "4" and record the time on the datasheet.
15. Tumble 2-5 h and record the time when tumbling is ended.
16. Pull triplicate, 5-mL samples from each VOA and add each sample to a labeled Maxi vial containing 15-mL scintillation cocktail.
17. Analyze by LSC.
18. Clean the charcoal:
    a. Pour any remaining solvent into a liquid radioactive waste container. Be careful not to lose any charcoal in the process.
    b. Rinse charcoal once with clean solvent and twice with methanol, pouring rinsate into a liquid radioactive waste container.
    c. Empty charcoal into a pan and let dry under a hood.
    d. Cover pan with aluminum foil to avoid particulate contamination while baking.
    e. Bake charcoal at 180°C for 24 h.
    f. Empty charcoal into an airtight container and store in dessicator.

Appendix B

Charcoal Trap Tests

Charcoal Trapping and Elution Efficiency Tests

Coconut charcoal (Supelco, Inc., Bellefonte, PA) was chosen as the trapping medium to trap [^{14}C]TCE and related organic compounds in the foliar portion of the trapping scheme. Because charcoal trapping efficiencies should be similar to those of Tenax®, trap size was designed based upon the Tenax® trap size used by Hayhurst (1998).

Carbon disulfide (CS_2) is commonly used to elute organic compounds from charcoal. This approach was attempted for use in elution of radiolabeled TCE off of charcoal traps. Charcoal traps were each constructed using a 16.5 cm-long piece of 9 mm o.d., 7 mm i.d. glass tubing. A small glass wool plug was inserted at one end and 2.6 ± 0.05 g coconut charcoal were added. Charcoal was settled using a vortex test tube mixer to ensure uniformity of packing before another glass wool plug was inserted to hold the charcoal in place. Spike tests were conducted by spiking [^{14}C]TCE directly onto a charcoal trap while a 50 cc/min vacuum was pulled on the opposite end to ensure that any volatilized TCE would pass through the trap. The vacuum remained in place for five minutes following the spike.

Initial spikes of CS_2 into Ready Gel® scintillation cocktail (Beckman Instruments, Inc., Fullerton, CA) showed that CS_2 causes problems with LSC. Burken (1996) noted significant quench problems when volumes greater than 50 μL CS_2 were used. When 1, 2, 3, 4, 5, and 6 mL CS_2 were each added to individual maxi scintillation vials with Ready Gel® (20 mL total volume), we found the opposite to be true. Quench was absent and the instrument gave an output of "H# ABORT: COUNT RATE TOO LOW." We attempted to overcome this problem with the addition of deionized water. 5 mL of deionized water were added to the scintillation cocktail with 3 mL CS_2, but the same result ensued. The H# was then turned off and the samples recounted. With the H# off, the instrument gave readings of 32.40-41.13 cpm for the same samples. However, the manufacturer does not recommend use of the LSC without the H#. When 50 μL CS_2 were spiked into Ready Gel®, background counts were close to that of the scintillation cocktail alone, around 60 dpm. It was then determined that 50 μL samples would be

used. Because the sample volume would be so small, mini scintillation vials (7 mL total volume) were chosen for use in order to minimize both the dilution of the sample and the use of scintillation cocktail. Background counts of 50 µL CS_2 in mini vials averaged 56 dpm.

To test elution efficiencies, charcoal from each spiked trap was emptied into a 20-mL VOA. Care was taken to completely remove all charcoal from the glass wool. Three initial elutions were performed with 6, 8, and 10 mL CS_2 to determine the minimum quantity of solvent necessary to completely elute the TCE. In each case, CS_2 was added directly to the VOA containing charcoal from one trap. Lids were replaced and tightened, and VOAs were shaken for a minimum of two h. Triplicate 50 µL samples were pulled from each VOA, added to 20 mL scintillation cocktail, and counted by LSC. Recoveries for the 10, 8 and 6 mL elutions were 132%, 118%, and 110%, respectively. These high recoveries raised concern that some CS_2 was volatilizing and either filling the headspace in the VOA or escaping the VOA completely, probably through the Teflon-lined septum. Loss of CS_2 would result in a concentration of TCE and recoveries greater than 100%. One proposed solution to this problem was to fill the headspace in the VOA. Another proposed solution was to try another solvent, specifically methylene chloride. Both possibilities were tested. Six charcoal traps were packed and spiked with [^{14}C]TCE in the same manner as described previously. Each trap was emptied into a 20-mL VOA. Three VOAs were filled (minimizing headspace) with CS_2 and three with methylene chloride. All VOAs were shaken for 2.5 h and triplicate 50 µL samples were pulled from each. The third of each set of triplicate samples was spiked with additional [^{14}C]TCE and all samples were counted by LSC. Recoveries for the three traps eluted with CS_2 were much lower than expected, at 27.5, 43.4, and 40.7%. Recoveries of the additional spikes were also low, at 46.1, 41.2, and 69.2%. Recoveries for the three traps eluted with methylene chloride were 85.4%, 85.2, and 80.8%. Recoveries of the additional spikes were 94.7, 89.1, and 98.2%.

At this point, methylene chloride was the solvent of choice. Because so much solvent is required to fill the headspace of each VOA, larger sample volumes were desirable. A matrix test was conducted and 5-mL samples of methylene chloride caused

very little, if any quench.

Spike tests were carried out to determine both the trapping efficiency of the charcoal traps and the efficiency of the elution procedure. Attempts at application of TCE to the traps in gaseous form did not result in good recoveries, not because of poor trapping efficiency, but because of poor delivery. In order to overcome the problem of poor delivery, a thermal desorber was set up such that a TCE spike could be injected and transferred onto a charcoal trap in gaseous form at a flow rate similar to what the trap would encounter in the growth chamber system. The thermal desorber did not work properly. Chris Pajak fixed it and got ~90% recovery from two spikes, but no further spikes were attempted as the study was already underway.

During method development, [^{14}C]TCE recoveries from manually spiked charcoal traps eluted with methylene chloride were consistently ~90%. However, once the growth chamber systems were up and running, recoveries from trap spikes dropped and were consistently ~80%. Toward the end of the study, an elution test was run using two other solvents, pentane and xylene. Results of this test indicated that while elution with pentane was far less efficient (54% recovery) than elution with methylene chloride, elution with xylene was actually more efficient, with recoveries near 99%. All remaining charcoal traps (three sets) were eluted with xylene.

Appendix C

Charcoal Trap Data

Charcoal Trap Data

Background counts of about 100 dpm were measured in the daily trip blanks. These counts were subtracted from the counts measured in the treatment traps. The data are thus reported either as "dpm above background trapped in 24 h," or as mg TCE equivalents. TCE equivalent mass is calculated by multiplying the counts (dpm) in each trap by the specific activity of the dosing solution. A method detection limit (MDL) of 8.52 dpm above background was determined based on 3 times the standard deviation of 8 matrix spikes following the protocol described in USEPA SW-846. Sporadic counts, just above this MDL, were detected on both the first and second traps from all chambers throughout the study. Figures C-1 and C-2 show the timing and magnitude of these counts on the traps. After TCE comes to an equilibrium with binding sites in the plant tissue, TCE might begin to volatilize in a steady, continuous efflux from open stomates. The erratic, small, trap counts before day 21 do not suggest any steady efflux. However, after day 21, the counts above the detection limit became more frequent. These data suggest that a small amount of TCE began to volatilize from the plant tissue after day 21.

For the thesis discussion, counts above the MDL in the breakthrough trap were subtracted from counts above the MDL in the initial trap for that chamber on that day. (Analysis 1). This was because the trend in the "noise" associated with the charcoal trap data seemed to be similar for both the initial and background traps. By subtracting the background trap from the initial trap, some of this noise was removed. Figures C- 3 and C-6 are a result of Analysis 1.

Ideally, an undosed control chamber would have been run and carried through for the entire 43-d study period. The variability of the control chamber data could have been compared to data from dosed chambers. In this study a poisoned, dosed control chamber (Chamber C) was run, but only for 10 d. The variability in this chamber's volatilized TCE data was quite large and probably doesn't represent the variability across the entire 43-d study period. The variability in dpms measured on the initial traps (7.40 dpm, 0.003 mg TCE equivalents) is almost identical to that measured on the breakthrough traps (7.59 dpm, 0.003 mg TCE equivalents). Comparison of this variability to the observed data is

referred to as Analysis 2. This variability is graphed as a horizontal, dashed line in Figures C-4 and C-7. If data falling below this line are considered "noise" and data above this line are considered "real," the sum of the "real" data from both the initial and breakthrough traps of each chamber would be the total phytovolatilized dpms or mg TCE equivalents. The results of such a calculation are shown in Table C-1.

Because the specific activities of the 1 ppm and 10 ppm dosing solutions were different, it is more appropriate to compare phytovolatilization of radiolabel in each chamber after converting dpms to mg TCE equivalents. Graphs showing the result of this calculation are shown in Figures C-5 to C-7.

It is interesting to calculate the phytovolatilized component of the TSCF on a daily basis, rather than summing up total phytovolatilization at the end of the study. This gives a daily snapshot of how phytovolatilization changes in terms of the amount of water transpired. These values are graphed in Figures C-8 and C-9.

More precise quantification of TSCF values below 0.1 in future studies should include the following modifications to the system: 1) an undosed control chamber, run for the entire study; 2) a higher ratio of hot to cold TCE; and 3) a subsample that is a greater fraction of the total flow.

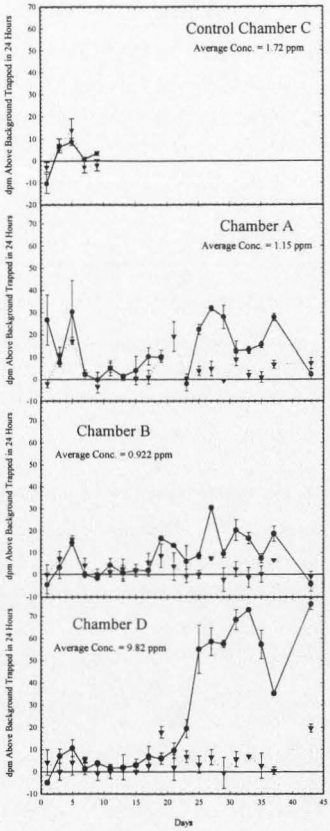


Fig. C-1. Average dpm per trap. Daily raw data for each chamber. Error bars represent the standard deviation of triplicate measurements. Solid lines represent initial traps. Dotted lines represent breakthrough traps. Daily y-axis values are determined by subtracting the average of each day's triplicate trip blank counts (usually around 100 dpm) from the average of that day's triplicate trap counts.

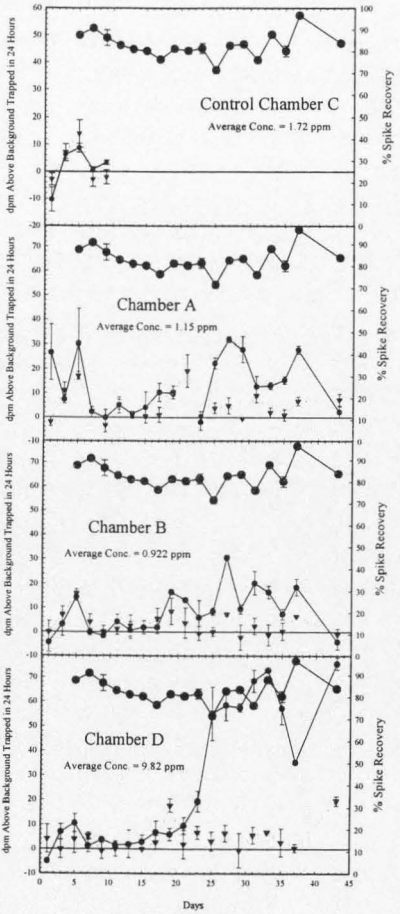


Fig. C-2. Daily spike recoveries. Same as Figure C-1 with one addition. Each time a set of traps was analyzed, a blank trap was spiked with [^{14}C]TCE and eluted.

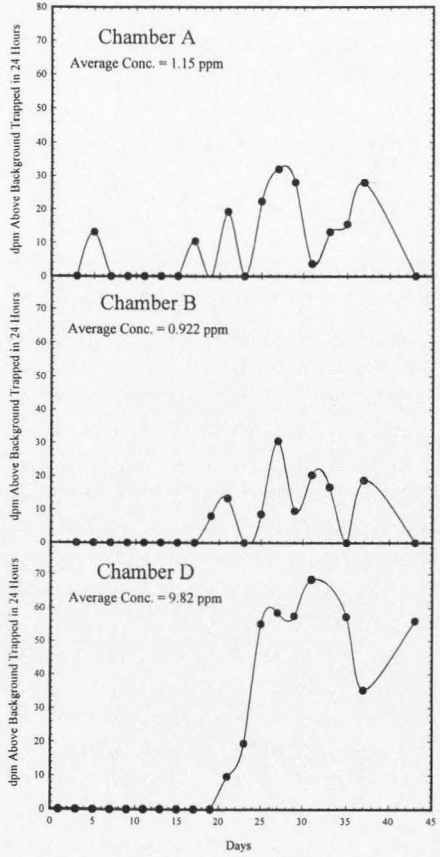


Fig. C-3. Analysis 1. Daily, above MDL, backup trap counts (dpm) are subtracted from the corresponding day's above MDL, initial trap counts. The resulting data are "real." On many days, neither trap had counts above the MDL. Background counts are subtracted from each trap before comparison to the MDL.

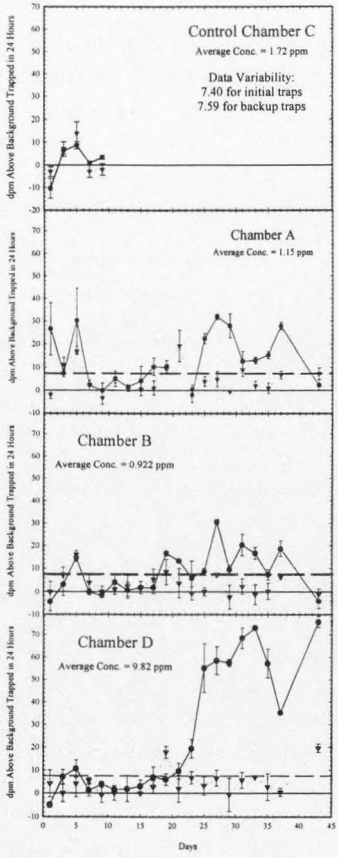


Fig. C-4. Analysis 2. Daily raw data for each chamber (dpm). The horizontal dashed line on each graph represents the standard deviation of the data collected for the control chamber (C). Data falling below this line are "noise." Data above this line are "real."

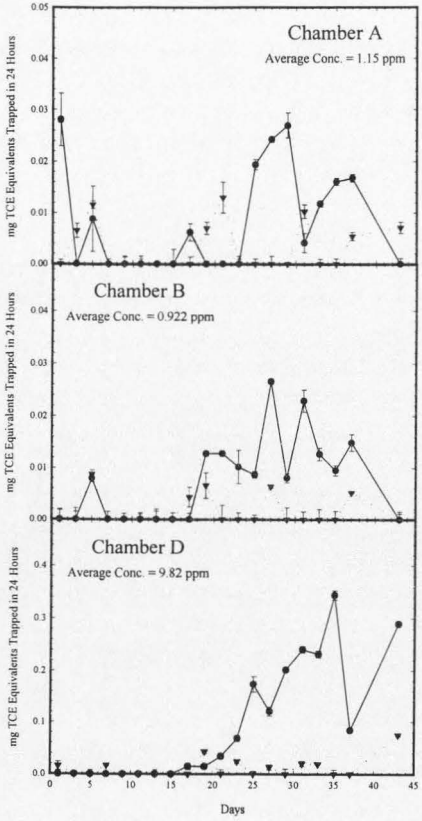


Fig. C-5. Daily phytovolatilized [^{14}C]TCE (mg) for each trap. Solid lines represent initial traps. Dotted lines represent breakthrough traps. Daily y-axis values are determined by subtracting the average of each day's triplicate trip blank counts (dpm) from the average of that day's triplicate trap counts. TCE equivalent mass (mg) is then calculated from the remaining dpms. Note that the scale for Chamber D (10 ppm treatment) is 10 times larger than that for Chambers A and B (1 ppm treatments).

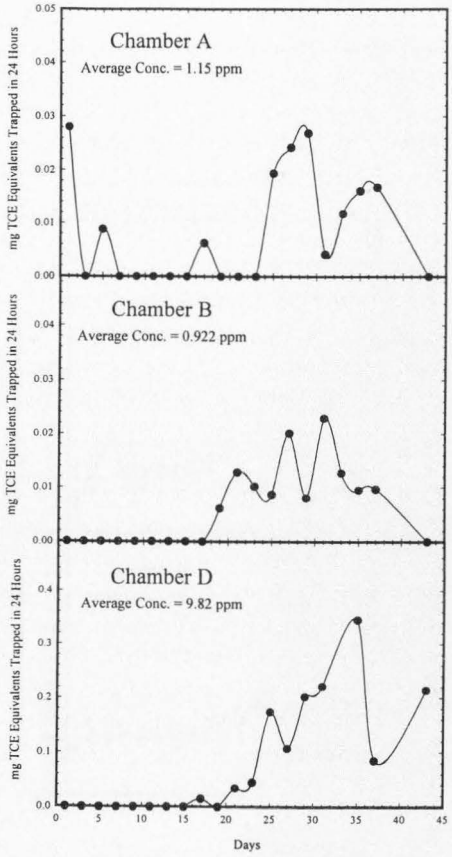


Fig. C-6. Daily phytovolatilized [^{14}C]TCE (mg) by Analysis 1 (similar to Figure C-3). Daily, above MDL, backup trap counts (dpm) are subtracted from the corresponding day's above MDL, initial trap counts. The resulting counts are converted to mg TCE by multiplying the dpm by the specific activity of the [^{14}C]TCE solution.

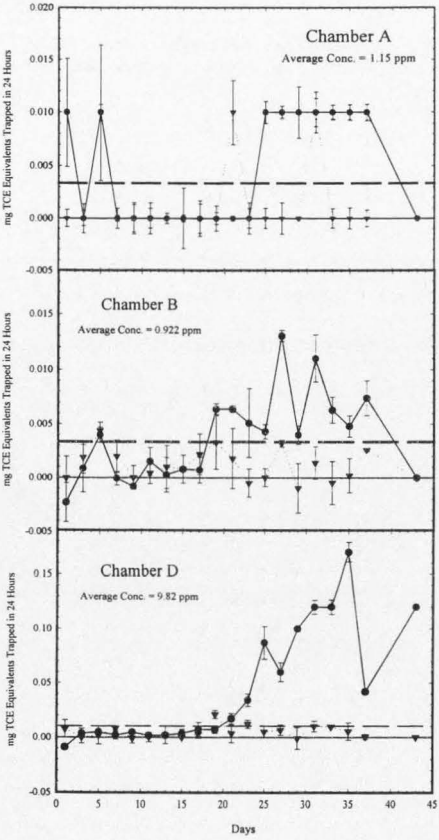


Fig. C-7. Daily phytovolatilized [^{14}C]TCE (mg) by Analysis 2 (similar to Figure C-4). The horizontal dashed line on each graph represents the standard deviation of the data collected for the control chamber (C), converted to mg. Data falling below this line are "noise." Data above this line are "real." Note that the scale for Chamber D (10 ppm treatment) is 10 times larger than that for Chambers A and B (1 ppm treatments).

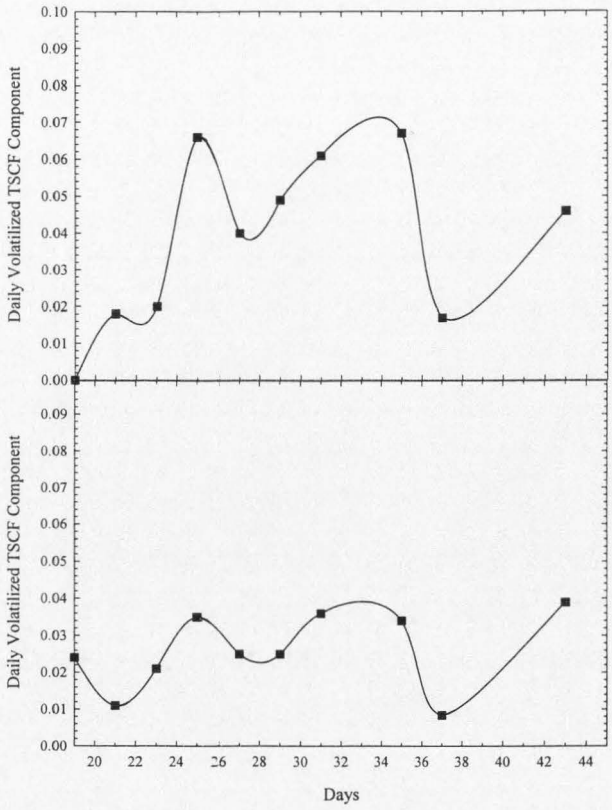


Fig. C-8. Daily phytovolatilization (mg) in the 10-mg L^{-1} chamber (D), according to Analysis 1 (top) and Analysis 2 (bottom), converted to the TSCF component. Each day's data is divided by the amount of water transpired on that day. The quotient is then further divided by the average root-zone concentration. This is only one component of the TSCF and does not take into account any ^{14}C associated with plant tissues.

Table C-1. Comparative results of two analyses. Analysis 1 subtracts the "noise" associated with control chamber data from each chamber's data. Analysis 2 subtracts the backup trap data from the initial trap data.

Chamber	A	B	D
Avg. Root Zone Conc.	1.15 ppm	0.922 ppm	9.82 ppm
mg Phytovolatilized			
Analysis 1	0.12	0.08	1.29
Analysis 2	0.16	0.12	1.65
TSCF Component			
Analysis 1	0.02	0.02	0.03
Analysis 2	0.03	0.03	0.04

Appendix D

Barium Chloride Precipitation/

CO_2 Re-Evolution

STANDARD OPERATING PROCEDURE
CO_2 Trap Sample Precipitation/Re-evolution

Procedures:

1. Add a 10-mL sample (in triplicate) of each CO_2 trap to a 50-mL, disposable, polyethylene centrifuge tube (Fisher Scientific, Pittsburgh, PA).
2. Add 10 mL of a 1.0 M KOH/0.5 M $NaHCO_3$ solution to each of two 50-mL, disposable polyethylene centrifuge tubes: method blank and method spike.
3. Spike an appropriate amount of ^{14}C-labeled $NaHCO_3$ into the "method spike" centrifuge tube.
4. Add 20 mL 1.5 M $BaCl_2$ to each centrifuge tube to form $BaCO_3$ precipitate.
5. Centrifuge all tubes at 7500 rpm for 30 minutes to aid in the settling of the precipitate.
6. For each centrifuge tube:
   a. Vacuum filter the supernatant using a 0.22 micron membrane filter (Corning, Corning, NY).
   b. Place the membrane filter into the centrifuge tube with the precipitate.
   c. Clamp the uncapped centrifuge tube upright atop a stir plate, add a small stir vane, and insert the re-evolution apparatus developed for this procedure (figure next page).
   d. Add 20 mL of a 50% Ready Gel®/40% Methanol/10% MEA solution to trapping apparatus and connect the re-evolution apparatus to the trapping apparatus.
   e. Pull a vacuum of 0.1-0.3 L/min across the combined re-evolution and trapping apparatus.
   f. Re-evolve the $BaCO_3$ precipitate by gradually adding 30 mL 10% (v/v) HCl through the apparatus to the centrifuge tube while stirring.
   g. Keep the re-evolution/trapping system running until one full minute after the last of the precipitate has dissolved.
   h. Remove the re-evolution apparatus and rinse both the tubing and the air sparger with 5% HCl, DDW, and methanol prior to re-evolving the next sample.
   i. Empty the trapping solution into a Maxi scintillation vial.
   j. Rinse the trapping apparatus with 5% HCl, DDW, and methanol prior to adding the trapping cocktail for the next sample.
7. Evaluate each sample using LSC.

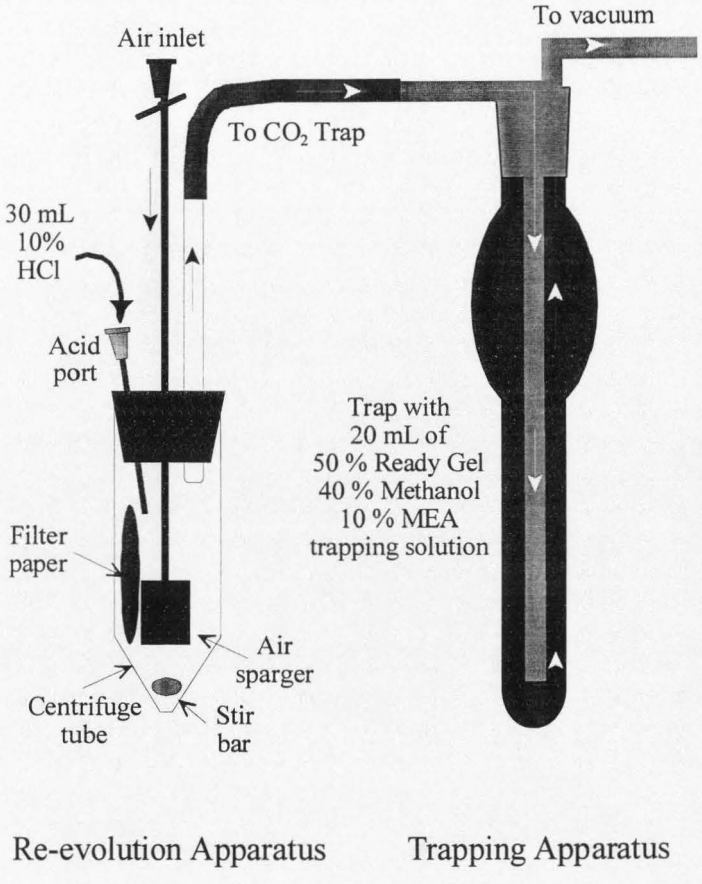


Fig. D-1. Carbon dioxide analysis apparatus.

Appendix E

Carbon Dioxide Trap Tests

Carbon Dioxide Trap Tests

An attempt was made to use dry CO_2 traps in the foliar trapping scheme. Ascarite II (sodium hydroxide-coated silica gel) was selected for use in the dry traps based on its small mesh size. Thomas Scientific, manufacturers of Ascarite, report that its composition is 89-93% NaOH and that its absorptive capacity for CO_2 is about 20%-30% by weight (personal communication).

The first round of tests determined the CO_2 scrubbing capability of an Ascarite trap. Each trap was constructed of a 7.5 cm-long glass tube with an inside diameter of 9.5 mm. A small glass wool plug was inserted into one end of the tube. The tube was filled with 1.5 g Ascarite and tapped along the length of the tube for consistent packing. Another glass plug was inserted in the opposite end to hold the Ascarite in place.

For these initial tests, two Ascarite traps were oriented vertically and placed in series after two water traps of magnesium perchlorate dessicant. Magnesium perchlorate was chosen because it has been shown to have little to no affinity for CO_2 (Trusell and Diehl, 1963). The traps were connected by 3/8" plastic tees so that gas sampling could take place through a septum located in one branch of each tee. An air pump was hooked to the dessicant trap ends of the trapping series and air was blown through the traps at a rate of 100 cc/min. An infrared gas analyzer (IRGA) was used to evaluate the CO_2 concentration in the air stream before the first Ascarite trap, between the two Ascarite traps, and after the second Ascarite trap. The IRGA was calibrated at each sampling event by sampling gas from a tank of known CO_2 concentration. At semi-hourly intervals, triplicate 3-mL samples were pulled from each of the three sampling ports for a total of at least 24 h. Results of these initial tests show that a single, 1.5 g. Ascarite trap efficiently scrubs CO_2 from ambient air for at least 24 h. Tests also confirmed that magnesium perchlorate traps have minimal, if any, affinity for CO_2.

The second round of tests was conducted to determine whether radiolabeled CO_2 could be recovered from Ascarite. For the first set of these tests, radiolabeled bicarbonate was gradually spiked into a strong HCl acid solution. A vacuum pump pulled air through the spiking port, bubbling the labeled bicarbonate into the acid solution. The evolved $^{14}CO_2$ was then pulled through two charcoal traps, two water traps, and finally into two Ascarite CO_2 traps. Mass balances were determined by dissolving the traps completely in deionized water, sampling the dissolved volume, and carrying those samples through a barium chloride precipitation and re-evolution process (Appendix D). Mass balance recoveries for two trials were both below 50%.

For the second set of tests, the charcoal traps were eliminated from the system and only one magnesium perchlorate water trap was used. The water trap is necessary to dry the air before it enters the Ascarite trap. Any moisture in the air turns the Ascarite to a sludge that eventually blocks airflow. Recoveries from these "semi-direct spikes" were also below 50%. Magnesium perchlorate traps were dissolved and counted, but no detectable radiolabel was found. It was suggested that perhaps the HCl solution was too strong and that acid vapors were deactivating the Ascarite. A second round of direct spikes was carried out using a weak acid solution. With a pKa of 6.3, carbonate is 99% transformed to CO_2 at pH 4.3. The acid solution was prepared such that after addition of

the carbonate, the acid pH was between 3 and 4. Mass balances for these semi-direct spikes were also below 50%.

A third set of tests was conducted in biometer flasks. Biometer flasks allow complete reactions to occur without continuous airflow. A known and sufficient mass of Ascarite was placed in one side of the flask while a mixture of labeled and non-labeled (hot and cold) bicarbonate was added to the other side of the flask. Both sides of the flask were stoppered to prevent gaseous efflux and enough dilute HCl was added through a syringe needle with leur-lock tip to acidify the bicarbonate to pH 4. A stir bar ensured complete mixing of the acid/bicarbonate mixture. Mass balances for these tests were also below 50%.

In order to test the validity of using biometer flasks, the biometer flask method was attempted with both NaOH pellets and 2M KOH solution instead of Ascarite. Mass balances for both of these tests were greater than 70% and it was concluded that for our purposes, Ascarite was not the trapping medium of choice.

Liquid CO_2 traps have been implemented in the growth chamber system for all previous trials. At low flow rates, these traps have been shown through IRGA tests to have excellent trapping efficiencies. These traps had not been tested for radiolabel recovery before their use in trials in the growth chamber system. Two identical tests using radiolabel were, therefore, conducted. For each test, $^{14}CO_2$ was generated through the addition of hydrochloric acid into an Erlenmeyer flask containing a mixture of labeled and non-labeled bicarbonate, very similar to the procedure used in the biometer flasks. A vacuum was pulled at a rate of 50 cc/min through the acid addition port, out of the flask, and through two liquid CO_2 traps in series containing 900 mL 2.0 M KOH each. Results showed 81.9% and 89.1% recovery of $^{14}CO_2$ from liquid CO_2 traps. Method spike recoveries for the precipitation and re-evolution procedure were 87.4% and 92.3%.

Calculations of KOH trapping efficiency indicate that 60 d worth of ambient air (400 ppm CO_2), at a flow rate of 100 cc/min could be efficiently scrubbed of CO_2 by only 450 mL 2.0 M KOH. Because this reduced volume would lower detection limits for CO_2, tests identical to those described previously were carried out with liquid traps containing 450 mL 2.0 M KOH each. Results showed 91.9% and 71.8% recovery with method spike recoveries of 87.1% and 82.7%. It remains unclear why the recovery from the second set of reduced volume liquid traps was so low (71.8%). Based on the results of these tests, liquid CO_2 traps containing 450 mL 2.0 M KOH each were used in the growth chamber system.

References

Trusell, F., and H. Diehl (1963). Efficiency of chemical dessicants. *Analytical Chemistry*, **35**:674-675.

Appendix F

Red Color in Hybrid Poplar Leaves

Red Color in Hybrid Poplar Leaves

In previous studies in the plant growth chamber system used for the TCE uptake study, leaves of hybrid poplar turned red. The red color does not appear until plants have been in the chambers for over two weeks and does not appear to have an effect on TCE uptake. One hypothesis formulated was that cold root-zone temperatures caused the red color to appear, as sometimes happens with tomato plants. The root zone area of the system was thus insulated and heat tape was applied to contol root-zone temperature in the most recent study. However, by day 14 of the TCE uptake study, plants began to show some red color in their new leaves.

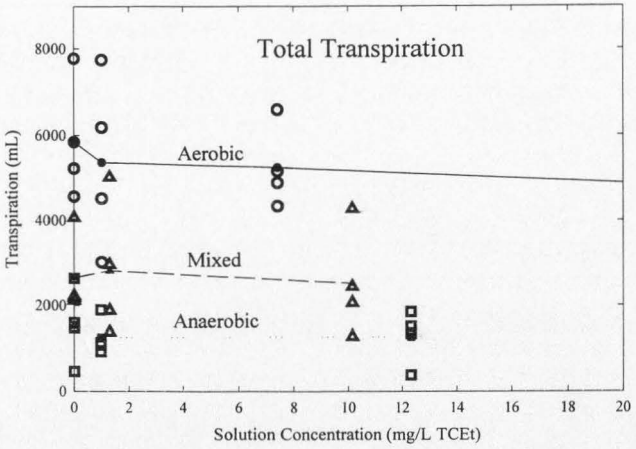
The next hypothesis tested was that the red color was caused by some kind of nutrient deficiency. On day 19, each chamber was "spiked" with $CaNO_3$, KNO_3, KH_2PO_4, $MgSO_4$, and $CuCl_2$ nutrients. The red color persisted and on Day 23, pH control was initiated. Daily addition of 10 to 20 mL of a 0.1 M HNO_3 solution was required to stabilize pH between 5.5 and 6.5. On Day 29, additional nutrients ($ZnCl_2$, H_3BO_3, $CuCl_2$, and Na_2MoO_4) were spiked into each chamber. Within 6 d (by Day 35), the older leaves of all three plants lost most or all of their red color.

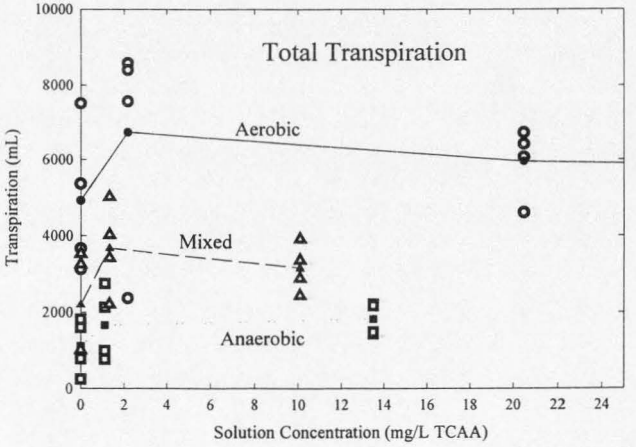
It is difficult to determine whether the red color was "cured" by pH control or by the addition of micronutrients. In future studies in this chamber system, an effort should again be made to insulate plant root zones and stabilize root zone temperatures. pH control should be initiated from the start of the experiment. If the red color does appear again, the addition of micronutrients may help. To further test this, a greenhouse study should be conducted where individual bottles receive different combinations of root-zone temperature, pH, and nutrients.

Appendix G

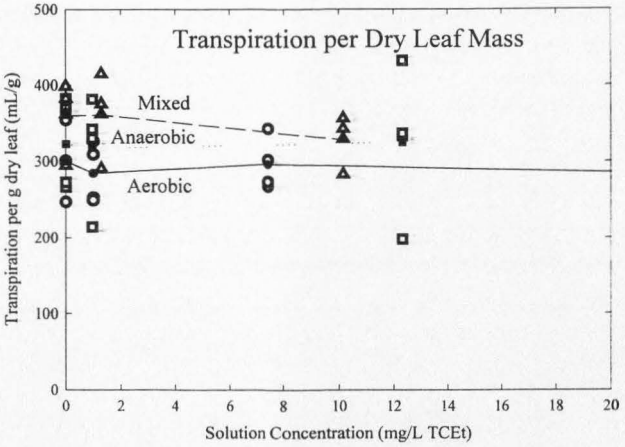
TCAA and TCEt Study Graphs

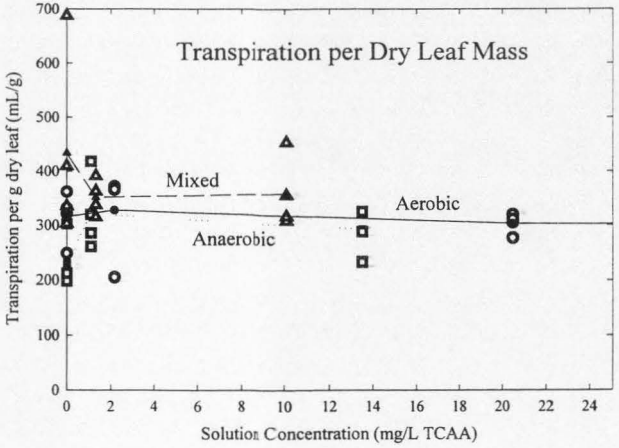
Total water transpired in TCEt (top) and TCAA (bottom) studies.





Total water transpired per leaf dry mass in TCEt (top) and TCAA (bottom) studies.





Appendix H

Methanol Toxicity Study

Toxicity of Methanol

Introduction:

Methanol is often utilized as a co-solvent to deliver and/or dissolve organics in laboratory studies, but its potential toxicity to plants may preclude its use in plant research. The purpose of this study is to determine the effects of varying concentrations of methanol on the relative growth rate (RGR) of sunflower plants.

Materials and Methods:

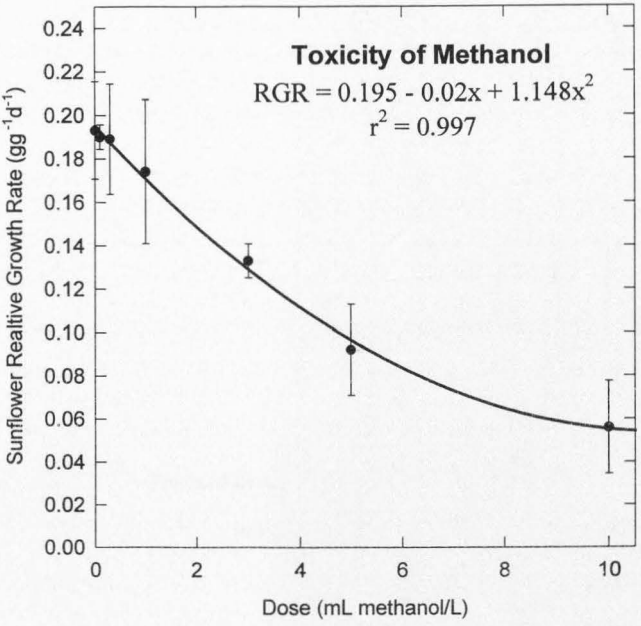
Each sunflower plant was propagated in isolite and transferred a dark, 2 L bottle filled with hydroponic solution. Bottles were individually aerated via an air manifold connected to a diaphragm pump. Methanol was added to bottles in seven different dose levels. Six replicate bottles were dosed at each of the 0, 0.3, 1, 3, and 10 mL/L (0, 7.42, 24.7, 74.2, and 247 mM, respectively) concentrations. Three replicate bottles were dosed at the 0.1 and 5 mL/L (2.5 and 124 mM, respectively) levels. The study ran for two weeks from the day of dosing (day 0), and whole plants were weighed on days 0, 7 and 14 for RGR determination.

Results:

Relative growth rate represents the new mass generated by a plant in terms of the old mass and the amount of time that has passed and is calculated using the following equation:

$$RGR = (\ln M_2 - \ln M_1)/(T_2-T_1)$$

where M_1 = plant mass at time 1, and M_2 = plant mass at time 2.



Conclusions:

As methanol concentration increases, the resulting RGR for sunflowers decreases, indicating that methanol inhibits plant growth. Use of methanol as an organic co-solvent in work with plants is best avoided. However, at concentrations < 1 mL/L it may not pose a significant problem.

Appendix I

Proposed Bioreactor for TCE Fate Studies in a Plant/Soil System

Introduction:

This appendix is part of a proposal to rigorously quantify the fate of TCE in a plant-soil-microbe system using unique bioreactors. TCE may undergo physical, chemical, and biological changes and these present analytical challenges. Plants can bind TCE or mask actual TCE concentrations. Subtle changes in the plant/soil matrix can alter TCE extraction efficiencies. Soil must be packed to a uniform bulk density. Soil water potential, temperature, pH, organic matter, and dissolved oxygen should be measured to facilitate extrapolation to a field environment.

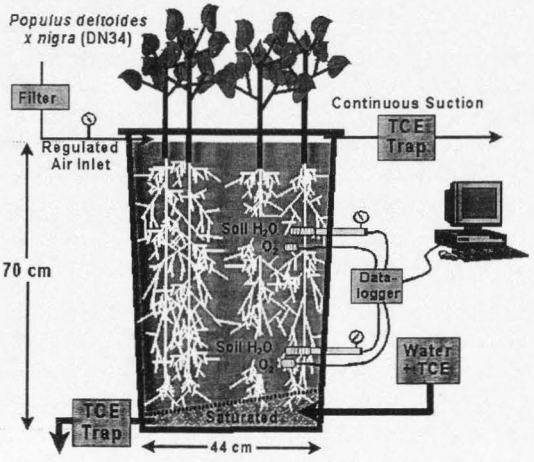
Materials and Methods:

Bioreactor: Each bioreactor will be constructed of a 121-L Rubbermaid® brand refuse container. Lids for each container will be sealed with silicone sealant to prevent leakage of volatilized TCE. Poplar cuttings will be secured in lids with rope caulk for an airtight seal. Further, a small suction of 0.1 L/min (100 cc/min) will be pulled across the top of the container to ensure that any leaks will be directed inward. Air leaving the reactor will pass through a Tenax trap in order to account for TCE volatilized through the bulk soil. Selected cans will have oxygen sensors as well as tensiometers installed at inclined angles various heights along their sides. Holes will be drilled just large enough for each of the sensors to fit through, and will be sealed with a silicone sealant. Datalogging equipment will continuously record sensor readouts.

Soil: Kidman fine sandy loam soil will be used. This soil was selected for its relatively high sand content (63%). Care will be taken to pack each bioreactor in a similar fashion and to obtain in each a bulk density of approximately 1.3 g cm^{-3}. First, the bulk soil will be spread to one large layer, 20-cm thick. Soil will be dried for one day, turned, and dried for a second day before packing. At this point, a gravimetric water content of around 15% is expected. Packing will be done in all containers concurrently, in 10-cm increments. At each increment, soil in the containers will be packed to the desired bulk density.

Poplars: Populus deltiodes x nigra (DN34) will be planted in each reactor. Rooted cuttings measuring 45-60 cm in length and 2-5 cm in diameter will be transferred to containers containing the Kidman soil. Cuttings will be allowed to stabilize in soil media for a minimum of one week. Cuttings in these smaller containers will then be selected for uniformity and transferred to the bioreactors. Three poplar trees will be planted in each bioreactor and lids will then be secured. Trees will be allowed to adapt to the containers for one week before dosing begins.

Treatments: Seven treatments with three replicates each are intended as follows: 1) unplanted reactor with no TCE, 2) planted reactor with no TCE, 3) planted reactor with 1 ppm TCE, 4) planted reactor with 1 ppm TCE and fluctuating depth to soil saturated zone, 5) planted reactor with 10 ppm TCE, 6) planted reactor with TCE and all water added via the surface, and 7) planted reactor with TCE and half of all water added via the surface. With the exception of numbers 6 and 7, water will not drain from the bioreactor. Drainage will occur in reactors to simulate leaching. In numbers 1 through 5, saturated zone levels will be kept constant using a Mariotte bottle system. TCE will be added via syringe in the inlet water line toward the bottom of the reactor.



UPTAKE AND TRANSFORMATION OF TRICHLOROETHYLENE BY HYBRID POPLAR: LABORATORY STUDIES

by

Julie K. Chard

Plant uptake and phytovolatilization of TCE was quantified using a unique laboratory system. [^{14}C]TCE was added to four high-flow, aerated, hydroponic plant growth chamber systems designed to provide high mass recoveries, an optimal plant environment, and complete separation between foliar and root uptake. Hybrid poplar trees were exposed to 1- or 10-mg/L TCE over a 43-d period.

Calculated transpiration stream concentration factors (TSCFs) for TCE were <0.15 for all treatments with roughly 25% attributed to phytovolatilization. The TCE metabolites trichloroethanol (TCEt), trichloroacetic acid (TCAA), and dichloroacetic acid (DCAA) were identified in plant tissues of the 10-mg/L treatment.

Uptake of TCAA and TCEt was quantified using a simpler aerated hydroponic system. TSCFs for TCEt and TCAA were < 0.03. Transformations of TCEt to TCAA and of TCAA to DCAA were evidenced. Transformation of parent compound, coupled with low extractability, may contribute to low TSCFs.