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Fate of Volatile Chlorinated Organic Compounds in a Laboratory Chamber with Alfalfa Plants†

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The fate of two volatile organo chlorinated compounds, 1,1,1-trichloroethane (TCA) and trichloroethylene (TCE), was studied in rhizosphere soil. Laboratory experiments were performed with alfalfa (*Medicago sativa*) growing in sandy silt soil fed continuously with groundwater contaminated with TCA and TCE at 50 and 200 μL/L, respectively. Methane generated in the groundwater provided evidence for anaerobic biodegradation. Groundwater samples indicated that the concentration of TCE decreased with axial position during the steady-state period. The flow rate of the effluent was significantly less than the inlet flow because of active evapotranspiration. Thus, a significant fraction of TCA and TCE disappeared. Headspace analysis of the gas in the enclosed chamber using a FT-IR spectrophotometer showed that small quantities of TCA and TCE migrated into the gas phase above the alfalfa plants; no chlorinated intermediates or methane were found in this gas phase.

Introduction

Chlorinated aliphatic hydrocarbons, such as 1,1,1-trichloroethane (TCA) and trichloroethylene (TCE), are commonly used as metal degreasing agents, as dry cleaning and industrial solvents, as refrigerants, and as fumigants (*1–33*). It is also reported that these chlorinated compounds are the most frequently found contaminants at Superfund sites. Presently, the detection of these pollutants in soils and groundwater of industrial cities is becoming more widespread. This is of concern because TCA and TCE, in particular, are suspected human carcinogens and mutagens (*3*). Therefore, the presence of TCA and TCE in soils and groundwater would constitute a major health risk to the human population relying on groundwater for drinking. The maximum contamination levels (MCL) as enforced under the Safe Drinking Water Act are 100 μg/L for TCA and range from 1 to 5 μg/L for TCE (*3, 4*). Industrial effluents and landfill leachates are also common sources of these compounds in contaminated soils and groundwater (*4*).

Bioremediation of soils and groundwater contaminated with TCA and TCE can be performed *in situ* where contaminants are treated in the place of occurrence (*14–19*). This is one of the most economically attractive remediation methods for cleanup, especially when compared to traditional pump-and-treat technology. Anaerobic conditions that exist in relatively deep subsurface soil environments lead to anoxic biotransformation of TCE prevalent in soils and groundwater. Evidence for microorganisms that could anaerobically transform TCE under methanogenic conditions was provided by Vogel and McCarty (*5*), Belay and Daniels (*6*), and Bouwer and McCarty (*7*). For TCA, biotransformation was observed under both methanogenic (*7–9*) and sulfate-reducing (*9*) conditions. It was shown that anaerobic transformation of chlorinated compounds generally results in the formation of more toxic intermediate products such as dichloroethylenes (DCEs) and vinyl chloride (VC) (*5–9*). As the MCL for VC is only about 1 μg/L (*3, 4*), *in situ* anaerobic transformation of chlorinated compounds is less preferred.

In 1985, Wilson and Wilson (*10*) observed aerobic biodegradation of TCE by enriched methane-utilizing bacteria previously exposed to air containing 0.6% natural gas comprised of 78% methane. Aerobic biotransformation of TCE by bacteria enriched on methane was demonstrated subsequently by other investigators (*11, 12*). Biodegradation of TCE under aerobic conditions was found to occur through a cometabolic mechanism.

Cometabolic Transformation. Cometabolic transformation is a process by which microbes that derive energy and growth by degrading a beneficial primary substrate can concomitantly transform other nonbeneficial substrate analogs (*4–19*). Several investigators have demonstrated *in situ* cometabolic transformation of chlorinated solvents prevalent at contaminated sites. Speitel and Closmann (*13*) showed that biorestoration of unsaturated soils is possible by enriching the microbes with nutrients and a primary growth and energy-yielding substrate. Field studies performed at the Moffett Naval Air Station, California, confirmed aerobic *in situ* biotransformation of TCE (up to 30%) by methane-utilizing bacteria. Groundwater containing dissolved methane and oxygen when injected into the aquifer in alternating pulses, stimulated the growth of the native population of methanotrophic bacteria (*14*).

Hopkins et al. (*15*) reported cometabolic transformation of TCE in the presence of microorganisms growing on phenol as the primary substrate and noted that other researchers had also demonstrated similar cometabolic degradation of TCE by microorganisms growing on other beneficial substrates such as ethylene, propane, cresol, ammonia, and isoprene. Similar cometabolic transformation of TCE in the presence of toluene-utilizing microorganisms was also reported (*16*). Recently, at the St. Joseph, MI, Bendix plant site, investigators have reported *in situ* anaerobic biotransformation of approximately 20% of TCE to ethylene in the presence of acetate as primary substrate (*17–19*).

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Plant-Based Bioremediation. Bioremediation in the presence of vegetation is receiving increasing attention (*20–33*). Vegetation can play a significant role in bioremediating contaminated soils and groundwater (*27–33*). However, research studies to identify the role of vegetation in the bioremediation of chlorinated compounds are limited (*21, 26–28, 33, 34*).

In natural plant ecosystems, indigenous soil microorganisms present in the rhizosphere (root zone of the plants) are found in mutual relationships with plants (*35*). The rhizosphere, which sustains a genetically diverse variety of microbiota is the location for degradation of many organics (*28, 31–33*). The roots of the plants exude a wide spectrum of compounds including sugars, amino acids, carbohydrates, and essential vitamins that may act as growth and energy-yielding substrates for the microbial consortia in the root zone. Exudates may also include compounds such as acetates, esters, and benzene derivatives (*31, 35*).

The process of enriching the *in situ* microbial populations for enhanced degradation of organics by the provision of appropriate beneficial primary substrates supplied by vegetation has been reviewed recently (*28, 33*). Moreover, investigators have also reported enhanced biodegradation of TCE in rhizosphere soils in comparison to non-rhizosphere soils. In 1990, Walton and Anderson (*21*) reported enhanced biodegradation of TCE in the rhizosphere soil as compared to non-vegetated soils without plant roots. They observed from 20–30% of TCE to be mineralized to CO_2 in vegetated soils. Recently, Ferro et al. (*26*) have shown that establishing crested wheatgrass on PCP-contaminated surface soil may accelerate, by as much as 3-fold, the removal of the contaminant. Plant growth was affected when the initial concentration of PCP was 100 mg/kg of dry soil. Stomp et al. (*27*) are studying the uptake and fate of TCE in a poplar hybrid H-11-11 transformed with appropriate genes. They observed up to 85% of TCE removed by these trees grown hydroponically. Wang and Jones (*34*) studied the fate of chlorobenzenes in the presence of carrots and the significance of plant uptake of these compounds from soils. They found that the plant uptake of these compounds was insignificant compared to the amount of compound applied to the soil.

The objectives of this research were to investigate the possible extent of biodegradation of TCE and TCA in the rhizosphere of alfalfa plants growing in a laboratory chamber and to simultaneously monitor the generation of any toxic biodegradation intermediates that might be formed during plant-based bioremediation. Since several others have demonstrated and established earlier that enhanced biodegradation occurs in vegetated soil environments relative to nonvegetated soils (*20, 21, 26–28, 32*), no control experiment was attempted. The movement of water and solutes to root surfaces and the presence of primary substrates to support the microbial population are well-known differences (*23–33*).

Materials and Methods

Chamber Construction. The channel employed earlier for investigating the potential of alfalfa plants to bioremediate soils and groundwater contaminated with phenol was used in this study. The design and construction of the experimental setup was described previously (*24, 32*).

The constructed chamber consisted of two identical U-shaped channels each 10 cm in width, approximately 1.8 m in axial flow length, and 35 cm in depth (Figures 1 and 2). The channels were packed with sandy silt soil (silt <10%) collected near a landfill. Alfalfa plants were established in the soil under laboratory conditions in June 1992. Water fed to the plants was contaminated with pollutants of interest. Four groundwater monitoring wells were placed along the axial length of the channels. Figure 2 shows the overhead view of the laboratory chamber with alfalfa plants, sampling ports, inlets, and outlets. Figure 1 shows the frontal view of the laboratory chamber when it is imagined to be axially extended. The alfalfa plants were provided with illumination from six fluorescent lights placed 40 cm above the ground surface. Plant growth occurred without any photoperiod or seasonal changes. A glass and aluminium enclosure 26 cm high was constructed above the ground level of the chamber.

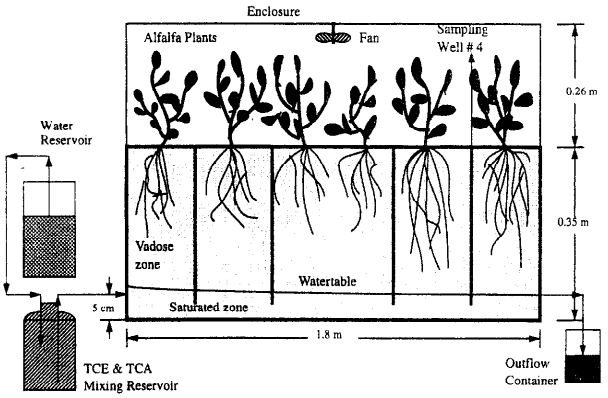

FIGURE 1. Schematic view of the axially extended experimental setup; the actual chamber is 0.9 m long as shown in Figure 2.

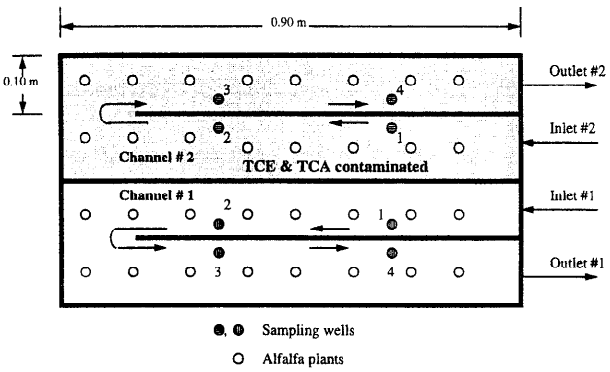

FIGURE 2. Overhead view of the actual experimental unit.

For the present study, TCE (Aldrich Chemical Co., Milwaukee, WI) and TCA (Fisher Scientific Co., Fairlawn, NJ) were both fed to channel 2 (Figure 2), which was formerly fed with phenol at a concentration of 500 mg/L from June 1992 until May 17, 1993. From May 18, 1993 till June 30, 1993, pure water was fed to purge out phenol and study the adsorption–desorption processes. The chlorinated contaminants were then introduced from July 1, 1993, each at 100 μL/L concentration. As TCE and TCA are volatile (Table 1), an effective feeding operation had to be adopted for this system. Calculated amounts of contaminants were daily injected into the 13-L mixing reservoir holding TCE- and TCA-contaminated water while the corresponding amount of pure distilled water to give the desired feed concentrations was added to the pure water reservoir from which water was siphoned into the mixing reservoir (Figure 1).

During chlorinated compound feeding, a step change was made in the inflow concentration of TCA on July 24, 1993 (day 24), from 100 (1.0 mM) to 50 μL/L (0.5 mM).

TABLE 1

Significant Physical and Chemical Properties of TCE and TCA at 26 °C

features	TCE	TCA
structural formula	$Cl_2C{=}CHCl$	$Cl_3C{-}CH_3$
specific gravity (g/mL)[a]	1.47	1.32
solubility (mg/L)[a,b]	1100	1500
vapor pressure (mm of Hg)[a,b]	80	120
log K_{oc}[c]	2.03	2.18
log K_{ow}[d]	2.53	2.47
established condition for biodegradation	methanogenic and aerobic cometabolism	methanogenic

[a] From refs 1 and 43. [b] From refs 1 and 44. [c] K_{oc} is defined as the organic carbon–water partition coefficient and is obtained from refs 1 and 45. [d] K_{ow} is defined as the octanol–water partition coefficient and was obtained from ref 45.

Similarly, on August 7, 1993 (day 38), there was a step change in the inflow TCE concentration from 100 (1.11 mM) to 200 μL/L (2.22 mM). These changes were made to improve the quality of the data that was collected using gas chromatography (GC). This feeding operation was continued until October 14, 1993. The washout of these chlorinated organics was monitored by feeding pure distilled water from October 15, 1993, till December 12, 1993.

Microbial Growth. Stimulation of growth of indigenous microbial consortia occurred in the rhizosphere of alfalfa plants growing in the channel soil when fed with phenol solution in groundwater. The population became well-adapted to the phenol substrate. Observations indicated that 99% of introduced phenol was mineralized in the channel (*24, 32*). It is believed that a specific hydroxylase enzyme, induced in the saturated zone of the channel, facilitated the biodegradation of phenol. The same indigenous microorganisms present in soil were tested for their ability to transform these chlorinated compounds. Except for phenol, no fertilizers or nutrients were added to this soil in the course of the study.

Analysis of Subsurface Chlorinated Compounds. The concentrations of the chlorinated compounds in the groundwater were regularly monitored during the feeding operation. The groundwater samples were collected from inlet, four sampling wells, and outlet. Sampling at the ports was done by drawing 3 mL of groundwater from each well using a 45 cm long by 3 mm o.d. nylon tube fitted to a syringe. The samples were then immediately transferred to nominal 10-mL bottles and capped with a Pierce reactivial screw cap with mininert valves (Pierce Chemical Co., Rockford, IL).

The concentrations in the groundwater samples were analyzed using a gas chromatographic headspace analysis technique (*36*). During the headspace gas analysis, the vials were first shaken to equilibrate the volatile contaminants between the headspace and the aqueous phase and then quickly placed in a water bath at constant temperature (26 °C) for establishing thermodynamic equilibrium. After about 5 min, 1 mL of headspace gas of the vial was collected using a 1000 Series gas-tight Hamilton microliter syringe, equipped with a Teflon-tipped plunger (Hamilton Co., Arlington Heights, IL). The headspace gas was then injected into a GC model Varian aerograph Series 1200 fitted to a flame ionization detector. The injection port temperature was 135 °C, while the column temperature was 200 °C. The stainless steel column, with an o.d. of about 3 mm, was packed with Porapak-R and used nitrogen at 30 mL/min as the carrier gas.

Each sampling bottle was numbered and individually calibrated. The injected headspace gas sample of the bottle responded with a peak that was registered on a recorder attached to the GC. The measured peak heights in conjunction with measurements of standard samples were used for estimating the contaminant concentrations in the groundwater samples obtained from the channel.

Analysis of the Headspace Using FT-IR Instrument. Gas-phase monitoring of TCE and TCA evapotranspired to the headspace of the chamber was reported by Visser (*37*) using the Fourier transform infrared (FT-IR) instrument described previously (*24, 32, 38*). The FT-IR instrument was also used to search the IR spectrum of the headspace gas sample to determine the presence of any volatile intermediates formed during the biodegradation of TCE and TCA. Calibration for TCE, TCA, and methane (CH_4) was also done using FT-IR.

Plant biomass was regularly harvested to 5 cm from the soil surface. Headspace gas samples were monitored immediately after harvesting the above-ground plant biomass for the presence of chlorinated compounds. In one instance, the harvested plants were rapidly transferred to sealed glass jars and equilibrated with 15 mL of aqueous phase. Accumulation of chlorinated organics from the harvested above-ground plant tissues into the headspace of the jars was subsequently monitored using the FT-IR (*37*). Comparisons of the observed FT-IR spectra with corresponding standard spectra were made. FT-IR was also employed to occasionally measure the concentrations of the organics in the groundwater samples. This helped to independently verify the groundwater concentration measurements made using the GC.

Chloride Analysis in Soil and Groundwater. On day 126, soil samples were collected from several locations and at three depths from each location along channels 1 and 2. Assuming all chloride formed to be accumulated in the soil samples as chloride salts, the amount of chloride in each gram of the dry soil sample was estimated. The procedure involved dispersing and equilibrating, for about 45 min, measured amounts of soil in 15 mL of distilled water and then measuring the chloride concentration in the clear suspension (*39*). Groundwater samples were also analyzed for dissolved chloride.

Data Analysis. The experimental data were used to estimate the mean and standard deviation for each contaminant and methane during the steady-state period.

Results and Discussion

Groundwater Concentration Measurements. Figure 3 shows the concentrations of TCA in the groundwater at the inlet and exit at different sampling times. Figure 4a–c shows the concentrations of TCE in the groundwater at various port locations at different sampling times. The first sampling was done on the 48th day from the commencement of the experiment, and the washout study began 106 days after the introduction of the contaminants into the channel.

The system was expected to reach steady state in a few weeks from the time of introducing the contaminants. By steady state, we mean that adsorbed concentrations of TCE and TCA reached constant values, the biodegradation rates of the contaminants and growth rates of the microbes

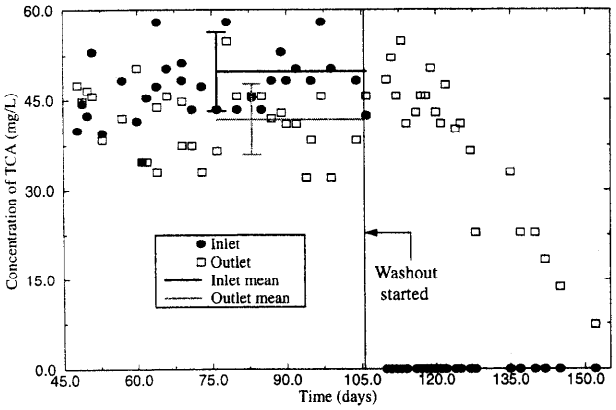

FIGURE 3. Concentration profiles of TCA at inlet and outlet of the channel.

reached a plateau, and all transpiring plants became well-adapted to growth in the presence of the contaminants. As the plants were previously well-adapted to the laboratory chamber conditions during the phenol feeding operation and the indigenous soil microorganisms were also well-acclimated in the channel soil, it should therefore require only a short period of time for the contaminants to saturate adsorption sites in the channel soil and bring the system to a steady state.

Concentration trends for TCE at ports 3, 4, and outlet, shown in Figure 4a and c, indicate that the concentrations were increasing with time. This trend in the concentration of TCE may be attributed to the response to a step change in the inflow TCE concentration made on day 38 from 100 to 200 μL/L. Steady-state operation in the chamber was assumed during the last 15-plus samplings performed before the beginning of the washout study on day 106.

During the gas chromatographic headspace analysis, it was observed that a considerable amount of methane was present in the groundwater samples. The measured groundwater concentrations of methane were in excellent agreement with the groundwater concentrations estimated using the FT-IR instrument. Only traces of methane were detected at port 2. However, methane concentration increased along the length of the channel from port 2 to the outlet. Figure 5 shows the concentration trends for methane in the groundwater obtained from various locations at different periods of time. It was observed that methane concentration tended to remain non-zero at the outlet even after a considerable period from the start of the washout study. However, methane concentration at ports 2, 3, 4, and the outlet declined as the respective TCE concentrations declined.

The means and standard deviations of the steady state measurements recorded for TCA, TCE, and methane are shown in Table 2 and Figures 3–5. The concentration of TCA remained almost uniform along the length of the channel and constant over the steady-state period of operation. However, the TCE concentration decreased by about 27% along the channel from the inlet to the outlet. The concentration at the outlet for either of the contaminants was somewhat higher compared to the concentration measured at port 4 of the channel. This may be attributed to the difference in sampling procedures adopted at the outlet and port locations. Sampling at the outlet was done by drawing 3 mL of groundwater from the exit point whereas sampling at the wells was done by drawing the water from the sampling wells. It is possible that a small portion of the

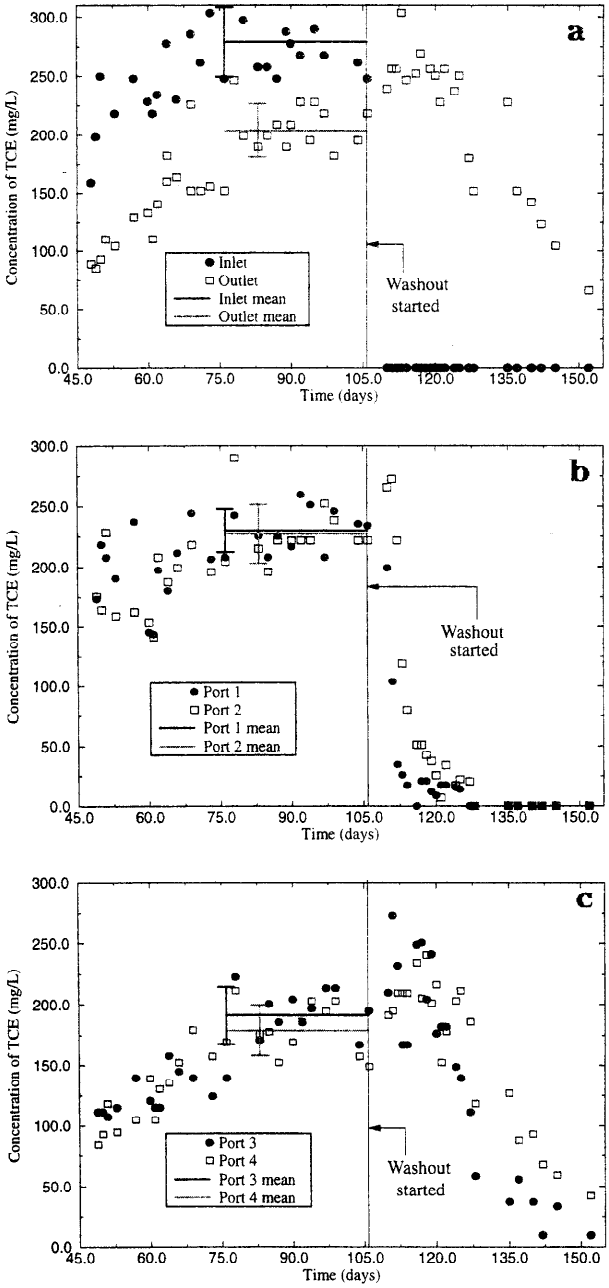

FIGURE 4. (a) Concentration profiles of TCE at inlet and outlet of the channel. (b) Concentration profiles of TCE at port 1 and port 2 of the channel. (c) Concentration profiles of TCE at port 3 and port 4 of the channel.

contaminants may have volatilized into the headspace of the covered but not sealed sampling wells.

The masses of TCA and TCE that were flowing in the groundwater on each day are summarized in Table 3. About 0.33 mmol of TCA and about 1.86 mmol of TCE was fed to the channel on each day during steady state. The corresponding amounts of the contaminants that were flowing out from the channel on each day were 0.07 and 0.37 mmol, respectively. This indicates that about 76% ($\approx$0.26 mmol) of TCA and about 80% ($\approx$1.49 mmol) of TCE were lost each day during the steady-state operation of the system. This loss may be predominantly due to biodegradation and plant uptake. As the system was operating at steady state, losses due to abiotic processes such as adsorption would be negligible. About 0.22 mmol of methane was detected in the groundwater flowing out of the channel on each day during steady-state operation (Table 3).

TABLE 2

Mean Concentrations[a] (in mg/L) of TCA, TCE, and Methane in the Groundwater during Steady-State Conditions

compound	inlet	port 1	port 2	port 3	port 4	outlet
CCl_3–CH_3 (TCA)	49.8 ± 6.3	41.2 ± 8.2	40.0 ± 3.6	38.9 ± 3.0	37.9 ± 7.9	41.6 ± 5.6
CCl_2=CHCl (TCE)	279.1 ± 28.5	224.2 ± 25.6	227.0 ± 23.3	191.2 ± 22.5	179.1 ± 20.0	204.5 ± 21.3
CH_4 (methane)	0.0	0.0	0.6 ± 0.3	10.0 ± 1.6	12.3 ± 2.6	14.4 ± 2.4

[a] All values are mean ± standard deviation (in mg/L); in all cases, $n > 15$.

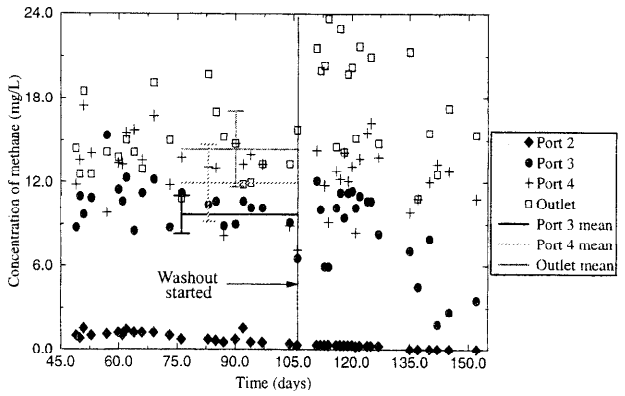

FIGURE 5. Concentration profiles of CH_4 at port 2, port 3, port 4, and outlet of the channel.

TABLE 3

Average Masses of TCA, TCE, and Methane in the Groundwater Flowing through the Chamber during Steady State[a]

compound	inflow concn (mg/L)	inflow amt/day (mmol)	outflow concn (mg/L)	outflow amt/day (mmol)	amt lost/day (mmol)
CCl_3–CH_3 (TCA)	49.8	0.33	41.6	0.07	0.26
CCl_2=CHCl (TCE)	279.1	1.86	204.5	0.37	1.49
CH_4 (methane)	0.0	0.0	14.4	0.22	

[a] Average amount of inflow contaminated water to the system = 875 mL/day; average outflow from the system = 240 mL/day.

During steady state, the amount of contaminated water flowing per day into the channel was about 875 mL with an outflow of only about 240 mL. The difference is the evaporation and net water usage by the transpiring alfalfa plants. On the average, 635 mL/day is attributed to evapotranspirational processes.

The entire channel had six sampling points and uniformly spaced alfalfa plants growing along the axial length. It may therefore be imagined that the complete channel was comprised of five equal sections, with each section representing the channel soil and alfalfa plants between any two sampling points. If the evapotranspiration process is assumed to be uniform over the entire length of the channel, then each section could be assumed to contribute one-fifth of the total evapotranspirational losses occurring in the channel. Therefore, the water usage, i.e., evapotranspirational loss over each section, was calculated to be about 130 mL/day during the steady-state period of operation. This water was drawn up in the vertical direction from the saturated zone into the unsaturated zone. An equal amount was evapotranspired to the atmosphere of the chamber.

The amount of dissolved contaminant associated with the vertical movement of water could also be computed for each section of the channel. Assuming the concentration in each section to be the average concentration between the two adjacent sampling points, the amount of TCE drawn up into the unsaturated region of the channel soil was evaluated, and hence the total amount of the contaminants drawn up into the vadose zone was estimated.

Trichloroethylene balance in the channel during the steady-state operation of the system indicated that approximately 1.05 mmol of TCE was drawn up into the vadose zone on each day. This is about 70% of the total amount of TCE lost each day (1.49 mmol) in the channel. This indicates the significance of the evapotranspiration process, associated with growing alfalfa plants, in vertically drawing up the contaminant to the vadose zone of the channel. It may also be noted that about 0.44 mmol of TCE was lost (biodegraded) in the saturated zone of the channel soil. This implies that during steady state about 30% of the TCE disappearing may be transformed in the saturated region of the channel where anaerobic conditions were observed.

A similar calculation results in an estimated value of 0.24 mmol/day for the dissolved methane that was drawn up into the vadose zone along with groundwater and dissolved contaminants during the evapotranspiration process. It was previously indicated in Table 3 that 0.22 mmol/day of methane was present in the groundwater flowing out from the channel. Therefore, a net amount of about 0.46 mmol of methane was estimated to be generated each day during steady state in the saturated zone of the channel.

If we assume that each mole of TCE is completely biodegraded to yield 1 mol of methane and other products, then 0.46 mmol of methane would be formed from 0.46 mmol of TCE biodegraded anaerobically in the saturated zone. This matched approximately to the amount of TCE that was lost in the saturated zone (0.44 mmol) on each day during steady state. On the basis of methane balances, it was therefore seen that about 30% of TCE may be biodegraded in the saturated zone due to methanogenic activity during steady-state operation of the system.

FT-IR Measurements of Gas Phase in the Chamber. Gas-phase monitoring was accomplished using FT-IR instrumentation by enclosing the chamber and collecting headspace gas spectra, at 15-min intervals, for 2 h. Based on the evapotranspiration rates in the enclosed chamber, at least 0.5 ppm (v/v) of TCA accumulated per hour (≈0.07 mmol/day) in the headspace atmosphere of the enclosed chamber. The amount of TCE accumulated per hour was about 2 ppm (v/v) (≈0.30 mmol/day), about four times the rate of TCA accumulation in the gas phase. The ratio of accumulation of TCA to that of TCE was consistent with the ratio of the amounts of the compounds fed into the channel, suggesting that both were similarly volatilized. It is important to note that when the chamber was enclosed volatilization rates usually were lower than their natural

TABLE 4

Chloride Accumulation Measured along Channel 2, (Expressed as mg of Chloride/kg of Dry Soil)[a]

chamber depth (cm)	distance along length of channel from inlet (cm)			
	20	40	80	150
0–10	79.8	33.7	52.3	56.6
10–20	12.4	11.6	16.3	15.0
20–30	9.6	14.0	20.7	13.5

[a] Control soil, channel 1, gave average values of 20.0 mg/kg at 0–10 cm depth, 14.5 mg/kg at 10–20 cm depth, and 11.0 mg/kg at 20–30 cm depth.

values as water saturates the chamber atmosphere quite rapidly and evapotranspiration rates consequently declined. A refrigeration circulation system enhanced the water flux but was not equal to the evapotranspirational flux of the unenclosed chamber because the outside humidity is generally lower.

The rate of accumulation of these chlorinated compounds was approximately the same subsequent to harvesting the above-ground portion of the plant biomass. This suggests that plant shoots may be contributing less toward transpiration of dissolved TCE and TCA following uptake by roots compared to volatilization through the channel soil. However, removing plants exposes the soil surface to air circulation from the fan in the chamber, which may alter the evaporation rate.

Headspace gas samples showed that methane accumulation in the enclosed chamber atmosphere was below the FT-IR detection limits of about 0.5 μL/L. This indicates that methanotrophic activity was present in the unsaturated zone of the soil. No chlorinated intermediate products were detected in the headspace.

Chloride Analysis in Soil and Groundwater. Chloride analysis of soil and groundwater in channel 2 was undertaken to determine the amount of chloride formation in the channel. Background chloride concentration was estimated by obtaining samples from soil of the same origin in channel 1 that also had transpiring alfalfa plants, which were previously fed with toluene in groundwater (*24, 32*). The chloride accumulation in the soil at different soil sampling points of the channel is given in Table 4. Chloride concentration in the surface soil closest to the inlet showed the highest accumulation of chloride.

The total amount of chloride that accumulated in the channel was estimated based on the chloride concentration measurements. About 32 mmol of chloride accumulated in the channel soil, while about 21 mmol of chloride was found to be associated with all the groundwater flowing out of the channel during the whole study. Therefore, a net total amount of about 53 mmol of chloride was observed to be formed from the chlorinated compounds during the study.

Over the entire experiment, the total amount of TCE input to the system was 165 mmol, the total output was 29 mmol as outflow in the groundwater, and the total amount of vapors entering the gas phase was at least 32 mmol. This indicates that a total loss of 104 mmol of TCE occurred in the channel. This loss may be either due to biodegradation, adsorption, or plant uptake of the compound. Complete degradation of each mole of TCE results in the formation of 3 mol of chloride, provided incomplete biotransformation of TCE to other intermediate chlorinated compounds is assumed to not occur. Based on the chloride accumulation, it can be estimated that 17.5 mmol of the total TCE may be mineralized (≈17% of the total TCE lost) in the channel during the experiment.

TABLE 5

Mass Balances for TCA and TCE (in mmol/day)

	TCA	TCE
inflow amount	0.33	1.86
outflow in groundwater	0.07	0.37
transformed in groundwater	0.06	0.44
outflow in gas phase	0.07	0.30
other losses by difference	0.13	0.75

Headspace Measurements of Cut Plants. The headspace gas analysis of harvested plants was undertaken to detect TCA and TCE in plant tissues or the presence of volatile biotransformation products formed in the plant tissues. Spectra of the headspace gas, however, showed the complete absence of either parent chlorinated compounds or other possible intermediates. Based on the amount of plant water present in the sampling vessel (≈25–50 mL), levels of contaminants above 1% of the groundwater concentration would be detectable in plant biomass. It may therefore be possible that the parent chlorinated compound could be taken up, immobilized, biotransformed, and fixed as waxes and lignins in plant biomass. Alternatively, the compound may be excluded by the plants entirely.

Mass Balances. In Table 5, the results for TCA and TCE are presented in the form of a daily balance. The outflow in the groundwater is based on the average measured flow rate and average measured concentrations for the steady-state period. The estimated amount transformed in the groundwater is based on the average measured concentrations at each port, the average measured flow rates, and the assumption of uniform evapotranspiration along the length of the channel. The outflow in the gas phase is based on the transient fluxes when the chamber is closed in order to measure the concentrations of TCA and TCE in the gas phase. The other losses include transformation in the vadose zone and in the alfalfa plants.

Results without Plants in the Chamber. In later work with TCE fed to one of the channels, the alfalfa plants were removed for a 2-month period. The effluent concentrations of TCE in the groundwater were similar with and without the alfalfa plants. Since the rhizosphere environment was not removed, significant differences may not be expected. Walton and Anderson (*21*) have shown that increased biodegradation of TCE occurs in rhizosphere soil environment compared to soil without plant roots; thus, experiments with sterile soil and without plant roots were not attempted.

Discussion of Results

Chloride measurements indicated that TCE may be aerobically biodegraded near the inlet of the channel because of the increased availability of dissolved oxygen that was flowing into the channel along with the inflow groundwater. Higher accumulations of chloride salts in the surface soil, nearest to the inlet of the channel, suggest that TCE was biotransformed near the entry of this channel and that the subsequently formed chloride was drawn up to the surface soil due to evapotranspiration. After about 60 cm along the length of the channel, methane was detected in the

groundwater. This suggested the presence of methanogenic activity in the saturated zone of the soil once oxygen was depleted. Methane balances indicated that all the methane could be formed due to the biotransformation of TCE to methane, chloride, and other end products in the saturated zone of the soil. It was also seen that small quantities of one or more intermediates with a retention time closer to acetate (no more than 2% of the observed TCE or TCA concentrations) were present in the groundwater. No other intermediates were detected in the groundwater.

Methane generated in the saturated zone of the channel soil was drawn up into the vadose zone due to evapotranspiration associated with transpiring alfalfa plants. The availability of methane in the rhizosphere (vadose zone) may have helped in the enrichment of methanotrophs that feed on methane as a primary substrate. Methanotrophs are usually higher in soils enriched with methane as the primary substrate (*10–14*). Methane concentrations in the gas phase of the enclosed chamber were below the detection limits of the FT-IR instrument, 0.5 μL/L. This indicated that some kind of methanotrophic activity may be responsible for the loss of methane (≈0.24 mmol/day). King (*40*) has reported higher root-associated methanotrophic activity in rhizosphere soils. Holtzapfel-Pschorn et al. (*41*) also observed that methane oxidation (presumably in the rhizosphere) consumed up to 95% of total methane production in vegetated rice paddy soils.

Increased methanotrophic activity in this channel soil may have contributed to the attenuation of TCE by cometabolic transformation in the rhizosphere soil of growing alfalfa plants. Other investigators also found trichloroethylene to be aerobically biodegraded (*10–14*) through a cometabolic transformation mechanism in the presence of methanotrophs.

Root exudates, which contain a wide variety of compounds including phenolic compounds, esters, acetates, carbohydrates, sugars, and amino acids, also enrich rhizosphere microbiota by acting as energy and growth-yielding substrates (*31, 35*). This may have also assisted the diverse microbial community in biotransforming TCE in the channel soil. In fact, TCE was also found to be cometabolically transformed in the presence of substrates such as phenol, toluene, ethylene, propylene, cresol, ammonia, and isoprene (*15, 16*). In general, it may therefore be possible that TCE was cometabolically transformed in the presence of appropriate substrates in the form of either exudates produced from the roots of alfalfa plants or methane generated and drawn up due to evapotranspiration from the saturated zone of the soil. Since vegetation enhances water movement and solute movement toward regions of enriched microbial activity, the potential exists for increased aerobic cometabolic transformation in the rhizosphere compared to soils without plants.

Stomp et al. (*27*) report plant uptake of ^{14}C-labeled TCE and transformation to two nonvolatile polar compounds that were not easily extracted from the plant tissue. Uptake of a portion of the TCE into alfalfa followed by its transformation to nonvolatile compounds could have taken place in the present work; however, the disappearance of TCE is the only evidence presently available.

The feeding operation of the chlorinated compounds, at concentrations extremely toxic to humans, indicated no phytotoxicity. This also indicates the potential for further research into *in situ* bioremediation systems involving vegetation and alfalfa plants in particular.

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Literature Cited

(1) Montgomery, J. H.; Welkom, L. M. *Groundwater Chemicals Desk Reference*; Lewis Publishers: Boca Raton, FL, 1990; pp 515–529.
(2) Gribble, G. W. *Environ. Sci. Technol.* **1994**, *28*, 310A–319A.
(3) *National Primary Drinking Water Regulations; Volatile Synthetic Organic Chemicals*; Environmental Protection Agency: Washington, DC, 1985; 40 CRF Parts 141 and 142, pp 46885–46904.
(4) Vogel, T. M.; Criddle, C. S.; McCarty, P. L. *Environ. Sci. Technol.* **1987**, *21*, 722–736.
(5) Vogel, T. M.; McCarty, P. L. *Appl. Environ. Microbiol.* **1985**, *49*, 1080–1083.
(6) Belay, N.; Daniels, L. *Appl. Environ. Microbiol.* **1987**, *53*, 1604–1610.
(7) Bouwer, E. J.; McCarty, P. L. *Appl. Environ. Microbiol.* **1983**, *45*, 1286–1294.
(8) Vogel, T. M.; McCarty, P. L. *Environ. Sci. Technol.* **1987**, *21*, 1208–1213.
(9) Klecka, G. M.; Gonsior, S. J.; Markham, D. A. *Environ. Toxicol. Chem.* **1990**, *9*, 1437–1451.
(10) Wilson, J. T.; Wilson, B. H. *Appl. Environ. Microbiol.* **1985**, *49*, 242–243.
(11) Henson, J. M.; Yates, M. V.; Cochran, J. W. *J. Ind. Microbiol.* **1989**, *4*, 29–35.
(12) Lanzarone, N. A.; McCarty, P. L. *Ground Water* **1990**, *28*, 910–919.
(13) Speitel, G. E., Jr.; Closmann, F. B. *J. Environ. Eng.* **1991**, *117*, 541–558.
(14) Semprini, L.; Roberts, P. V.; Hopkins, G. D.; McCarty, P. L. *Ground Water* **1990**, *28*, 715–727.
(15) Hopkins, G. D.; Semprini, L.; McCarty, P. L. *Appl. Environ. Microbiol.* **1993**, *59*, 2277–2285.
(16) Fan, S.; Scow, K. M. *Appl. Environ. Microbiol.* **1993**, *59*, 1911–1918.
(17) McCarty, P. L.; Semprini, L.; Dolan, M. E.; Harmon, T. C.; Tiedeman, C.; Gorelick, S. M. In *On site Bioreclamation*; Hinchee, R. E., Olfenbuttel, R. F., Eds.; Butterworth-Heinemann: Boston, MA, 1991; pp 16–40.
(18) McCarty, P. L.; Wilson, J. T. *Bioremediation of Hazardous Wastes: Research, Development, and Field Evaluations*; EPA/600/R-92/126; U.S. EPA: Washington, DC, 1992; pp 47–50.
(19) Semprini, L.; McCarty, P. L.; Dolan, M. E.; Lang, M.; McDonald, T.; Bae, J.; Kitanidis, P. *Bioremediation of Hazardous Wastes: Research, Development, and Field Evaluations*; EPA/600/R-92/126; U.S. EPA: Washington, DC, 1992; pp 43–45.
(20) McFarlane, J. C.; Cross, A.; Frank, C.; Rogers, R. D. *Environ. Monit. Assess.* **1981**, *1*, 75–81.
(21) Walton, B. T.; Anderson, T. A. *Appl. Environ. Microbiol.* **1990**, *56*, 1012–1016.
(22) Paterson, K. G.; Schnoor, J. L. *Water Environ. Res.* **1992**, *64*, 274–283.
(23) Gatliff, E. G. *Remediation* **1994**, *4*, 343–352.
(24) Davis, L. C.; Muralidharan, N.; Visser, V. P.; Chaffin, C.; Fateley, W. G.; Erickson, L. E.; Hammaker, R. M. *Bioremediation through Rhizosphere Technology*; ACS Symposium Series 563; American Chemical Society: Washington, DC, 1994; pp 112–122.
(25) Licht, L. A. *Proceedings of the Air and Waste Management Association*; 86th Annual Meeting; Air and Waste Management Association: Pittsburgh, 1993; Paper No. 93-WA-89.07.
(26) Ferro, A. M.; Sims, R. C.; Bugbee, B. *J. Environ. Qual.* **1994**, *23*, 272–279.

(27) Stomp, A. M.; Han, H. H.; Wilbert, S.; Gordon, M. P.; Cunningham, S. D. *Recombinant DNA Technology II*; Annals New York Academy of Science: New York, 1994; pp 481–491.
(28) Anderson, T. A.; Guthrie, E. A.; Walton, B. T. *Environ. Sci. Technol.* **1993**, *27*, 2630–2636.
(29) Cunningham, S. D.; Berti, W. R. *In Vitro Cell. Dev. Biol.: Plant* **1993**, *29P*, 207–212.
(30) Davis, L. C.; Erickson, L. E.; Lee, E.; Shimp, J. F.; Tracy, J. C. *Environ. Prog.* **1993**, *12*, 67–75.
(31) Shimp, J. F.; Tracy, J. C.; Davis, L. C.; Lee, E.; Huang, W.; Erickson, L. E.; Schnoor, J. L. *Crit. Rev. Environ. Sci. Technol.* **1993**, *23*, 41–77.
(32) Erickson, L. E.; Banks, M. K.; Davis, L. C.; Schwab, A. P.; Muralidharan, N.; Reilley, K.; Tracy, J. C. *Environ. Prog.* **1994**, *13*, 226–231.
(33) Erickson, L. E.; Davis, L. C.; Muralidharan, N. *Thermochim. Acta* **1995**, *250*, 353–358.
(34) Wang, M. J.; Jones, K. C. *Environ. Sci. Technol.* **1994**, *28*, 1260–1267.
(35) Curl, E. A.; Truelove, B. *The Rhizosphere*; Springer-Verlag: Berlin, 1986.
(36) Hachenberg, H.; Schmidt, A. P. *Gas Chromatographic Headspace Analysis*; Heyden: New York, 1977.
(37) Visser, V. P. M.S. Thesis, Kansas State University, Manhattan, KS, 1994.
(38) Marshall, T. L.; Chaffin, C. T.; Hammaker, R. M.; Fateley, W. G. *Environ. Sci. Technol.* **1994**, *28*, 224A–232A.
(39) Aldriano, D. C.; Doner, H. E. *Methods of Soil Analysis, Part 2*, 2nd ed.; Agronomy 9; American Society of Agronomy: Madison, WI, 1982; pp 449–483.
(40) King, G. M. *Appl. Environ. Microbiol.* **1994**, *60*, 3220–3227.
(41) Holtzapfel-Pschorn, A.; Conrad, R.; Seiler, W. *Plant Soil* **1986**, *92*, 223–233.
(42) Trapp, S.; McFarlane, J. C. *Plant Contamination: Modeling and Simulation of Organic Chemical Processes*; Lewis Publishers: Boca Raton, FL, 1995; p 254.
(43) Perry, R. H.; Green, D. W. *Chemical Engineers' Handbook*, 6th ed.; McGraw-Hill: New York, 1984; p 3–43.
(44) Yaws, C. L. *Thermodynamic and Physical Property Data*; Gulf Publishing Company: Houston, TX, 1992; p 217.
(45) Knox, R. C.; Sabatini, D. A.; Canter, L. W. *Subsurface Transport and Fate Processes*; Lewis Publishers: Boca Raton, FL, 1993; p 430.

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