LABORATORY AND FIELD INVESTIGATION OF CHLORINATED SOLVENTS

REMEDIATION IN SOIL AND GROUNDWATER

by

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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

Chlorinated solvents are the second most ubiquitous contaminants, next to petroleum hydrocarbons, and many are carcinogens. Tetrachloroethylene or perchloroethene (PCE) has been employed extensively in the dry cleaning industry and carbon tetrachloride (CT) has been used as a fumigant in grain storage facilities. In this work, remediation feasibility studies were conducted by mesocosm experiments; a chamber was divided into six channels and filled with soil, and plants were grown on top. Each channel was fed with contaminated water near the bottom and collected at the outlet, simulating groundwater flow conditions. The contaminants were introduced starting from March 12, 2004. PCE was introduced at a concentration of about 2 mg/L (~12 μmoles/L) in three channels, two of them with alfalfa plants and the other with grass. CT was introduced at a concentration of about 2 mg/L (~13 μmoles/L) in the other three channels, two of them with alfalfa plants and the other with grass. After the system had attained steady state, the concentrations of PCE and CT at inlet and outlet were monitored and the amount of PCE and CT disappearing in the saturated zone was studied. Since no degradation products were found at the outlet after about 100 days, one channel-each for PCE and CT (with alfalfa) was made anaerobic by adding one liter of 0.2 % glucose solution. The glucose solution was fed once every month starting from July 1, 2004 and continued until February 2005. From October 1, 2004, one liter of 0.1 % emulsified soy oil methyl esters (SOME) was fed to two other channels (with alfalfa), one exposed to PCE and another exposed to CT. The SOME addition dates were the same as that for glucose. The outlet liquid of the channel fed with PCE and SOME started to contain some of the degradation compounds of PCE; however, the extent of degradation was not as great as that of the glucose fed channel. No degradation compounds were observed in the outlet solution of the channel (grass grown on top) in which no carbon and energy supplements were added. Similar trend was observed in the CT fed channels also. KB-1, a commercially available microbial culture (a consortium of *dehalococcoides*) that degrades dichloroethene (DCE), was added through the inlet of the PCE fed channels, but this did not lead to sufficient conversion of DCE. Addition of KB-1 at well 3, located approximately in the middle of the channel, had a greater impact in the degradation of DCE, in both glucose and SOME amended channels, compared to addition at the inlet. KB-1 culture added to the channel

was active even 155 days later, suggesting that there is sustainable growth of KB-1 when provided with suitable conditions and substrates.

A pilot field study was conducted for remediation of a tetrachloroethylene (PCE) contaminated site at Manhattan, KS. The aquifer in the pilot study area has two distinct zones, termed shallow zone and deep zone, with groundwater velocities of about 0.3 m/day and 0.1 m/day. Prior to the pilot study, PCE concentration in groundwater at the pilot study area was about 15 mg/L (ppm) in the deep zone and 1 mg/L in the shallow zone. Nutrient solution comprising soy oil methyl esters (SOME), lactate, yeast extract and glucose was added in the pilot study area for biostimulation, on August 18, 2005. Potassium bromide (KBr) was added to the nutrient solution as a tracer. PCE was converted to DCE under these conditions. To carry out complete degradation of PCE, KB-1, a consortium of *Dehalococcoides*, and a second dose of nutrient solution were added on October 13, 2005. After addition of KB-1, both PCE and DCE concentrations decreased. Nutrients were again injected on March 3, 2006 (with KBr) and on August 1, 2006. The total chlorinated ethenes (CEs) have decreased by about 80 % in the pilot study area due to bioremediation. Biodegradation of CEs continued for a long time (several months) after the addition of nutrients. The insoluble SOME may be retained at the feeding area and provide a long time source of electron donors. Biostimulation and bioaugmentation of PCE contaminated soil and groundwater was evaluated in the laboratory and this technique was implemented successfully in the pilot field study.

Modeling of the tracer study was performed using an advection-dispersion equation (ADE) and traditional residence time distribution (RTD) methods. The dispersion coefficient, groundwater velocity and hydraulic conductivity were estimated from the experimental data. The groundwater velocities vary from 1.5 cm/d to 10 cm/d in the deep zone and 15 cm/d to 40 cm/d in the shallow zone. The velocities estimated from the 2004 tracer study and 2005 tracer study were higher compared to the velocity estimated from the 2006 tracer study, most likely because of microbial growth and product formation that reduced the hydraulic conductivity. Based on data collected from several wells the hydrologic parameter values obtained from tracer studies appear to vary spatially.

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Major Professor
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Dedication

To

My Daughter *Rithika*
My Wife *Sasikala*
My Mother *Santha*

CHAPTER 1 - Chlorinated Solvents Contamination and Remediation: A Review

1.1. Chlorinated Methanes Degradation

Carbon tetrachloride (CT) is a solvent used in the past in the production of refrigeration fluid and propellants for aerosol cans; it was also used as a cleaning fluid, a degreasing agent, and a spot remover. CT was used in fire extinguishers and as a pesticide fumigant to kill insects in grain. Because of its harmful effects, most of these uses were banned in the 1960s; its use as a pesticide was banned in 1986. Today CT is only used in some industrial applications (ATSDR, 2007). Although many uses have been discontinued, the possibility still exists for CT to be released to the environment, primarily through industrial processes or old containers of household cleaning agents containing CT. CT is widely dispersed and persistent in the environment, but is seldom detected in foods (ATSDR, 2007).

The degradation of CT has been shown to occur both in biologically active systems, with direct biological involvement (Hashsham et al., 1995; Criddle et al., 1990b, Egli et al., 1988), and in abiotic systems (Hashsham et al., 1995, Kriegman-King and Reinhard, 1992; Kriegman-King and Reinhard, 1994).

1.1.1. Biodegradation

Degradation of carbon tetrachloride (CT) occurs slowly in the environment, which contributes to the accumulation of the chemical in groundwater (ATSDR, 2007). CT is, therefore, a common groundwater pollutant and a suspected human carcinogen. Although indigenous microorganisms may degrade CT, a common degradation product, chloroform (CF), may be more persistent than CT [Criddle et al., 1990a; Semprini and McCarty, 1992]. CF is readily formed under anaerobic conditions (Hull and Sondrup, 2003). The degradation products of CT, by reductive dechlorination, in addition to CF, are methylene chloride (MC) and chloromethane (CM). For reductive dechlorination to occur, the water has to be anaerobic, i.e., dissolved oxygen must be less than 0.2 mg/L and redox potential should be less than –50 mV. Therefore, to deplete the oxygen present in the groundwater, organic substrates with sufficient or excess biochemical oxygen demand (BOD) have to be supplied. The aerobic microorganisms

while metabolizing, catabolizing or degrading the organic substrates, utilize oxygen as electron acceptor and thus reduce the available oxygen. Further, for reductive dechlorination, electrons and hydrogen ions are necessary to replace the chlorine atoms. All these requirements are taken care of by organic compounds such as glucose, acetate, lactate, molasses, or vegetable oils. Figure 1.1 shows the stepwise reductive dechlorination of carbon tetrachloride to methane, with hydrogen and chlorine forming hydrogen chloride. During reductive dechlorination, therefore, it is not uncommon for the pH of the groundwater to decrease even though the soil matrix can provide some buffering effect. Since the concentration of chlorinated compounds is usually small, there will be no significant change in pH of groundwater.

Subsequent migration of the transformation products to an aerobic environment can lead to oxidation of the products with ultimate complete mineralization of the halogenated aliphatic compounds to chloride ion and carbon dioxide (Hull and Sondrup, 2003). Table 1.1 presents some of the important physical and chemical properties of CT and its degradation products. Chloromethane (CM) is a vapor while the other compounds are liquids at 25ºC. Numerous studies have illustrated that a potential exists for transformation of halogenated aliphatic compounds in groundwater under anaerobic conditions that are conducive to methanogenesis (Hull and Sondrup, 2003). Transformation can occur under unsaturated conditions if the system can be made anaerobic. In highly chlorinated aliphatic compounds (tetrachloroethene, trichloroethene, carbon tetrachloride), transformation occurs through reductive dechlorination. In an anaerobic environment, transformation may stall after partial dechlorination (e.g. dichloroethene, vinyl chloride, chloroform, methylene chloride) (Hull and Sondrup, 2003).

Although CT is easily transformed in many anaerobic environments (Criddle and McCarty, 1991; Bouwer and McCarty, 1983a; Bouwer and McCarty, 1983b; Bouwer and Wright, 1988), attempts at bioremediating this substance have met with limited success. The difficulties seem to result from the toxicity of CT to microorganisms as well as the production of hazardous intermediates, such as CF and MC (Hashsham et al., 1995).

In some studies, nonchlorinated substances such as carbon dioxide (CO_2), carbon monoxide (CO), and formate were produced. Under sulfate reducing conditions, carbon disulfide (CS_2), a compound with known toxicity to humans, has also been identified as a product (Hashsham et al., 1995; Criddle et al., 1990b; Kriegman-King and Reinhard, 1992; Kriegman-King and Reinhard, 1994; Freedman et al., 1995).

Most of the CT biodegradation studies have found substantial quantities of intermediate products resulting from the transformations of CT. Bouwer and McCarty (1983a and 1983b) demonstrated CT biodegradation in methanogenic and denitrifying environments with CF reported as a chief product. Bouwer and Wright (1988) found, in column experiments, that CT biotransformed in a variety of redox environments with generally faster reactions occurring in the more reducing settings. Egli et al. (1987 and 1988) showed that CT, CF and MC adversely affected the ethanogens and sulfate reducers in their systems. In a controlled field experiment, Semprini et al (1992) established denitrifying conditions in a section of an aquifer where CT was injected later. Approximately 30-60 % of the CT was converted to CF, but the most rapid transformation rates were observed in the absence of nitrate, leading the researchers to suggest that the most active CT transforming microorganisms were not denitrifiers.

Criddle et al (1990a) and others (Tatara et al., 1993; Dybas et al., 1998; Mayotte et al., 1996) identified a denitrifying organism, *Pseudomonas* sp. strain KC, that is capable of transforming CT without accumulation of significant quantities of CF. However, this organism is apparently sensitive to the concentrations of various metals (e.g., Fe, Cu, Co, Mo) (Tatara et al., 1993) and may not be widely distributed in aquifers (Criddle et al., 1990a). The data from Devlin and Muller (1999) indicate that the $CF:CS_2$ ratio of 2:1 is indicative of a reaction with amorphous FeS. Another, more practical, implication that follows from the consistency of the 2:1 $CF:CS_2$ ratio is that it may be useful as an indicator of abiotic CT transformation in sulfate reducing environments, when background levels of CF and CS_2 are low. Since CT is subject to both biodegradation and abiotic transformations, this distinction could be important in the interpretation and design of remediation programs.

Biodegradation of CT with vitamin B_{12} was investigated in some of the studies. Cysteine was used as a reductant in CT transformation mediated by vitamin B_{12} at room temperature in the pH range of 4 -14 (Chiu and Reinhard, 1996). The transformation of CT, CF, and MC was examined in batch systems containing vitamin B_{12}, *Shewanella alga* strain BrY, and an electron donor. Transformation of both CT and CF was observed, while no significant change in the MC concentration was detected. Carbon monoxide was a major product of CT transformation. No significant transformation of CT or CF was detected when vitamin B_{12} was not present in the system. This and another work (Workman et al., 1997) demonstrated that a metal-reducing

bacterium, with no apparent ability to transform CT or CF directly, mediates the reduction of vitamin B_{12}, which in turn catalyzes the transformation of CT (Workman et al., 1997).

There generally are not many bacteria available to degrade fumigants, including CT, upon initial exposure (Witt et al., 1999). Since high concentrations kill bacteria, biodegradation depends on several conditions, including the concentration of fumigants, availability of necessary energy sources, other nutrients, and presence of suitable bacteria (Davis and Erickson, 2002).

Denitrifying strain *Pseudomonas stutzeri* KC was able to remove 50-80 % of CT in field conditions (Dybas et al., 1998). McQuillan et al. (1998) described intrinsic remediation of CT driven by spilled gasoline, whereby the CT levels dropped 100-fold within two years. Witt et al. (1999) demonstrated in laboratory model columns that supplementation with acetate could enhance the removal of CT by the indigenous bacterial population, but better results were obtained when specific inoculant was added. Gregory et al (2000) successfully used Fe(0) and methanogenic organisms to dehalogenate CT in laboratory experiments.

Zou et al (2000) investigated microbial degradation kinetics of CT under reducing conditions for different cultures, fed with 1,2-propanediol, dextrose, propionaldehyde, or acetate and nitrate, in the anaerobic step of an anaerobic/aerobic operation sequence. Methanogenesis was inhibited under aerobic conditions. Koons et al (2001) found that the cell exudates from the methanogen *Methanosarcina thermophila* are active in the degradation of CT and CF. The rates of degradation under these various stimulated conditions are much more rapid than the generally estimated half-life, suggesting that with appropriate conditions, degradation can occur relatively fast.

The Interstate Technology Regulatory Council (ITRC) – *In Situ* Bioremediation (ISB) Team published a guidance document that describes a systematic approach to ISB for CT in groundwater (ITRC, 2002). Removal of CT through ISB typically occurs through a reductive process whereby an electron donor is introduced into the subsurface. Reductive pathways for CT have been documented to occur primarily through direct reductive dechlorination, cometabolic reductive dechlorination, and cometabolic denitrification. CT found at silos may be in conjunction with nitrate contamination that serves as an electron acceptor during ISB of CT. ITRC's ISB team guidance document describes regulatory concerns, provides a description of

treatability tests, and feasibility; and defines the contaminant's pervasiveness, risks, sources, and site parameter criteria important to the evaluation of ISB for CT (Faris and ITRC, 2002).

Cervantes et al (2004) found that addition of both humic acids and humic analogue 2,6-anthraquinone disulphonate (AQDS) at sub-stoichiometric levels increased the first-order rate of conversion of CT up to 6-fold, leading to an increased production of inorganic chloride, which accounted for 40-50 % of the CT initially added. Considerably less dechlorination occurred in sludge incubations lacking humic substances. The accumulation of a chlorinated ethene, perchloroethylene (up to 9 % of added CT), is also reported for the first time as an end-product of CT degradation. A humus-respiring enrichment culture (composed primarily of a *Geobacter sp.*) derived from the granular sludge also dechlorinated CT, yielding products similar to the AQDS-supplemented granular sludge consortium. The results indicate that the formation of reduced humic substances by quinone-respiring microorganisms can contribute to the reductive dechlorination of CT (Cervantes et al., 2004).

Field-Scale Biodegradation

Devlin and Muller (1999) conducted a field experiment in which carbon tetrachloride (CT) was found to transform to chloroform (CF) and carbon disulfide (CS_2) in a ratio of about 2:1. The field experiment was conducted to assess the efficacy of a bioremediation scheme for treating CT in groundwater. The authors suggest that the 2:1 ratio may be a useful tool for distinguishing abiotic transformations from biodegradation in sulfate reducing environments where FeS is actively precipitated (Devlin and Muller, 1999).

A field demonstration project (Dybas et al., 2002) was undertaken in which strain KC was introduced to an aquifer containing CT and nitrate, at Schoolcraft, MI. Intermittently, the following materials were added to maintain strain KC: inoculation, acetate, alkali and phosphorus. With subsurface pH adjustments, 60-80 % CT removal was achieved and strain KC was reportedly assimilated into the aquifer community (Dybas et al., 1998).

Phanikumar and Hyndman (2003) examined the interplay between sorption and bioavailability with pulsed injection of nutrients based on a mechanistic model of microbially mediated reactive transport. They considered two case studies involving the biodegradation of CT, as well as a chemically induced degradation system, to evaluate the effects of bioavailability. The contaminant mass degraded per unit pumping was shown to be significantly higher for pulsed injection of substrates than with continuous injection.

Kirtland et al (2003) combined isotopic measurements in conjunction with traditional chemical techniques to assess in-situ biodegradation of trichloroethylene (TCE) and CT at the Savannah River Site, SC. Vadose zone chlorinated hydrocarbons, ethene, ethane, methane, O_2, and CO_2 concentrations were analyzed.

1.1.2. Phytoremediation

The fate of carbon tetrachloride (CT) during phytoremediation with poplar trees was assessed by examining the transpiration of CT from leaves, diffusion from soil, tree trunks, and surface roots, and accumulation of chloride ion in soil and plant tissues (Wang et al., 2004). Feedwater containing 12-15 mg/L CT was added to the field test beds planted with poplar, and over 99 % of the CT was removed. Microbial mineralization of CT was not enhanced in soils from the root zones as compared to unvegetated soils. Hence, the authors concluded that uptake and dechlorination of CT by plant tissues is likely the primary mechanism for phytoremediation by poplar (Wang et al., 2004). Davis and Erickson (2002) have conducted a comprehensive review of the potential for phytovolatilization of fumigants including CT.

1.1.3. Iron

Chlorinated solvents in groundwater are known to undergo reductive dechlorination reactions with Fe (II)-containing minerals and with corroding metals in permeable-barrier treatment systems (Zhang et al., 2004). Balko and Tratnyek (1998) and Zwank et al (2005) have also reported abiotic degradation of CT with iron.

1.2. Health Hazard of CT

Chronic exposure to carbon tetrachloride (CT) at concentrations above the maximum contaminant level (MCL) could result in an estimated low to moderate increased lifetime risk for developing cancer (Prosperie et al., 2000). EPA's MCLs are chemical specific maximum concentrations allowed in drinking water delivered to the users of a public water system; they are considered protective of public health over a lifetime (70 years) of exposure at an ingestion rate of two liters per day. The setting of MCLs may also be influenced by available technology and economic feasibility. Although MCLs only apply to public water supply systems, they are used to help assess the public health implications of contaminants found in water from other sources (Prosperie et al., 2000).

CT tends to volatilize (move into the air) from tap water used for showering, bathing, cooking, and other household uses inside a home (McKone, 1987). Thus, people whose tap water is contaminated with CT can be exposed to it through ingestion, inhalation, or dermal contact (absorption through the skin) (Prosperie et al., 2000).

Exposure to high concentrations of CT can cause liver, kidney, and central nervous system damage. If exposure is low and then stops, the liver and kidneys can repair the damaged cells and function normally again. The liver is especially sensitive to CT (Prosperie et al., 2000).

Chloroform (CF) is found at approximately 50 % of all Superfund sites identified by the EPA. CF can cause cancer, and may damage the liver, kidneys, and endocrine and respiratory systems. CF may also cause birth defects and miscarriages (Frohman et al., 2004).

1.3. Chlorinated Ethenes Contamination

Chlorinated solvents have been widely used as degreasers in various industries and as fumigants in grain storage facilities. Past disposal methods and handling practices for chlorinated solvents have contributed to wide spread contamination in soil and groundwater. The largest use for tetrachloroethylene or perchloroethene (PCE) is in dry cleaning (Kovacs et al., 2001) and textile operations, accounting for an estimated 60 percent of all PCE use in the US in 1991 (Sutfin, J.A. 1996). Drycleaning/textile processing accounted for 36% of PCE usage in the U.S. during 1998 (SCRD, 2007) and 12 % during 2004 (HSIA, 2005). Dry cleaning chlorinated solvents are the second most ubiquitous contaminants, next to petroleum hydrocarbons. In the United States, soil and groundwater at approximately 400,000 sites are contaminated with chlorinated solvents (Sutfin, J.A. 1996). PCE and trichloroethylene (TCE) were the fourth and second most frequently detected organic pollutants at U.S. National Priorities List (NPL) or Superfund sites, with PCE identified at 771 and TCE identified at 852 of the 1430 NPL sites as of September 1997 (US EPA, 1998a). EPA has planned to phase out the use of PCE as a dry cleaning solvent and all existing PCE dry cleaning machines in co-residential facilities will be prohibited in the U.S. after 2020 (US EPA, 2006).

Morrison et al (2006) present the chemistry of the most commonly used chlorinated solvents, degradation pathways for these compounds, and forensic techniques available for source identification and age dating.

1.4. Chlorinated Ethenes Degradation

When chlorinated solvents are released to soil, they will be subject to evaporation into the atmosphere, leaching into the groundwater, and sorption to soil. Biodegradation is nature's way of recycling wastes, or breaking down organic matter into nutrients that can be used by other organisms. This process can occur under aerobic and anaerobic conditions (Cupples et al., 2004). Table 1.2 to Table 1.4 present some of the important physical and chemical properties of PCE and its degradation compounds. The hydrogeologic environment significantly affects the fate and transport of chlorinated solvents in the subsurface. There are several physical, chemical, and biological mechanisms that affect the behavior of contaminants in the subsurface and they can be either destructive or non-destructive. Non-destructive fate and transport mechanisms are advection, sorption, dispersion, dilution by recharge, and volatilization. Destructive mechanisms are biological (primary growth substrate utilization, use as electron acceptor, and cometabolism) and abiotic (reductive dechlorination with metals).

1.4.1. Biodegradation

Bioremediation, both natural and enhanced has proven to be a powerful approach for remediating chlorinated solvents, including PCE. In recent years, it has become apparent that biologically mediated degradation mechanisms may be important for chlorinated solvents; for example, iron sulfide may reduce PCE to acetylene (Haas and Wiedemeier, 2004). An anaerobic bacterium, *coccoid* Strain 195, reduces the toxic pollutants tetrachloroethene and trichloroethene (TCE) to nontoxic ethene gas (Maymo-Gatell *et al.,* 1997).

PCE is known to be degraded under anaerobic conditions (Cupples et al., 2004; Lee et al., 1998). Since PCE is the electron acceptor in reductive dechlorination, this process is also called halorespiration. The dominant electron acceptors are O_2, NO_3, Fe^{3+}, SO_4^{2+} and CO_2 in that order. For reductive dechlorination to occur the above electron acceptors need to be depleted sufficiently to allow PCE to be utilized as an electron acceptor. It has been shown that chlorinated solvents are biodegraded via three different mechanisms:

1. Reduction reactions (Electron Acceptor) where halorespiration is the only known mechanism that degrades PCE (Haas and Wiedemeier, 2004).
2. Oxidation reactions (Electron Donor) by either aerobic respiration (DCE and VC) or Fe (III) reduction (DCE and VC) (Haas and Wiedemeier, 2004).

3. Cometabolism (TCE) (Haas and Wiedemeier, 2004).

For many years anaerobic biological processes were reputed to be more sensitive than aerobic processes to toxic substances such as chlorinated aliphatic hydrocarbons (CAH) and thus a poor choice for treating water containing these compounds (Parkin, 1999). This was especially true for water containing PCE or TCE because vinyl chloride, a human carcinogen, is produced when these two compounds are degraded anaerobically. Aerobic treatment with organisms containing oxygenase enzyme systems, which could fortuitously degrade a wide variety of chlorinated aliphatics (but not PCE), was favored. Recently, however, several enrichments and organisms (Maymo-Gatell et al., 1997) have been isolated that will convert PCE and TCE into ethene and ethane, as shown by field data (Major et al., 2002). Because of this evidence, anaerobic processes are now considered a significant alternative treatment for CAH contamination.

Mixtures of CEs

Parkin (1999) studied the effect of mixtures of CAHs on biotransformation of individual organic compounds and the potential for a combined methanogen-iron (Fe(D)) system to improve CAH bioremediation. At the concentration ranges tested, the presence of a mixture of CAHs seems to decrease the rate of transformation of individual organics. However, there are important exceptions; in some cases a mixture of CAHs seems to facilitate transformation of an individual organic compound. Combination of an active methanogenic population with Fe(D) increases the rate and extent of transformation of carbon tetrachloride and chloroform. Results with PCE and 1, 1, 1-trichloroethane are less clear.

1.4.1.1. Tetrachloroethene

Chemical formula   Chemical Structure

C_2Cl_4
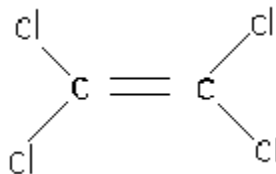
Ellis and Anderson (2003) reported that the anaerobic pathways of PCE degradation are less well understood (Figure 1.2). Only the first enzyme (tetrachloroethene reductive

) in this pathway has been isolated in *Dehalospirillum multivorans.* It also catalyzes the reductive dehalogenation of trichloroethene to cis-1, 2-dichloroethene (Neumann et al., 1996). Other organisms implicated in anaerobic PCE biodegradation include *Sporomusa Ovata* (Terzenbach and Bluat, 1994), and *Dehalobacter restrictus* TEA (Wild et al., 1996). Most organisms studied convert trichloroethene to cis-1, 2-dichloroethene (DCE); *Dehalococcoides ethenogenes* 195 is reported to also produce the trans isomer of DCE. TCE can be reductively dehalogenated (through both cis and trans DCE) by the CO dehydrogenase from *Methanosarchina thermophila* (Jablonski and Ferry, 1992).

The degradation rates of PCE and its intermediate compounds, vary depending on the specific microorganisms and the nutrients present in the site. Usually, the rate limiting step is the degradation of cis 1,2-DCE to vinyl chloride (Nakashima et al., 2002). Typical values of the first-order degradation constant and half-lives of PCE and its degradation compounds calculated for a site in Japan are given in Table 1.5. The HRC (Hydrogen Releasing Compounds such as lactates, provided by Regenesis Co) had a positive impact on the degradation rates.

1.4.1.2. Trichloroethene

Chemical formula   Chemical Structure

C_2HCl_3
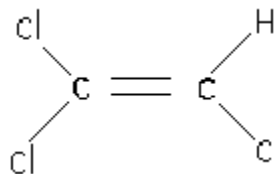
Trichloroethylene (TCE) is found in approximately 60 % of all Superfund sites identified by the EPA. Zhang et al (2006) found that a microbial culture reduced TCE to ethane through 1,1-DCE as a dominant intermediate rather than cis-DCE, in the presence of ampicillin. They concluded that the culture contained at least two TCE-dechlorinating populations.

1.4.1.3. Dichloroethenes

1,1-Dichloroethene:

Chemical formula   Chemical Structure
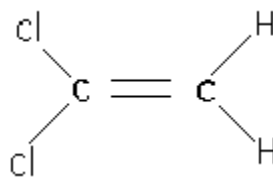
$C_2H_2Cl_2$

Trans 1,2-Dichloroethene:

Chemical formula   Chemical Structure

$C_2H_2Cl_2$

cis 1,2-Dichloroethene:

Chemical formula   Chemical Structure

$C_2H_2Cl_2$
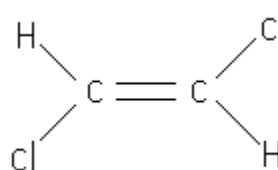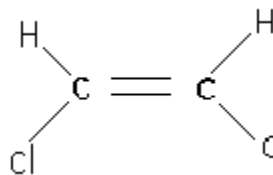
1.4.1.4. Vinyl Chloride

Chemical formula C_2H_3Cl
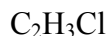
Vinyl Chloride is found in approximately 60% of all Superfund sites identified by the EPA.

Dehalococcoides/KB-1

In-situ bioremediation is used as an alternative to such traditional methods as groundwater pump-and-treat for treating groundwater contaminant plumes. This involves

stimulating indigenous bacteria by adding electron donors and/or nutrients to the subsurface to increase bacterial growth yielding faster degradation rates. A variety of electron donors such as acetate, lactate (Cox et al., 2002; Ellis et al., 2000; McMaster et al., 2001; RTDF Update, 1997), methanol, ethanol (Cox et al., 2002), molasses and vegetable oils (Newman and Pelle, 2006) have been used for biostimulation of microbes that degrade chlorinated solvents (Grindstaff, 1998, Harkness et al., 1999).

Bioaugmentation with cultures containing *Dehalococcoides* is an effective means of remediating chlorinated ethenes contaminated sites where halorespiring organisms are not naturally present or abundant (Ellis et al., 2000; Major et al., 2002). Many studies have characterized reductively dechlorinating communities (Harkness et al., 1999; Flynn et al., 2000; Hohnstock-Ashe et al., 2001; Richardson et al., 2002; Dennis et al., 2003; Rossetti et al., 2003; Aulenta et al., 2004; Gu et al., 2004; Macbeth et al., 2004; Freeborn et al., 2005; Yang et al., 2005; Duhamel and Edwards, 2006). Fermenters transform electron-donating substrates to hydrogen and acetate, which are then used by *Dehalococcoides*, often regarded as the primary halorespiring population. Sulfate-reducers, acetogens, and methanogens are competitors for available hydrogen. Yet perhaps these other populations assist in the dechlorination process, either directly or by providing essential micronutrients to halorespiring species. Duhamel and Edwards (2007) suggest that understanding the functions of various populations in mixed communities may explain why *Dehalococcoides* spp.are active at some sites and not others, and may also assist in optimizing the growth of bioaugmentation cultures, both in the laboratory and in the field.

Duhamel and Edwards (2007) studied the population dynamics of a mixed microbial culture dechlorinating trichloroethene (TCE), cis-1,2-dichloroethene (cDCE), 1,2-dichloroethane (1,2-DCA), and vinyl chloride (VC) to ethene. Both *Geobacter* and *Dehalococcoides* populations grew during TCE dechlorination to cDCE, but only *Dehalococcoides* populations grew during further dechlorination to ethene. The cell yields for *Dehalococcoides* determined in this study were similar on an electron equivalent basis regardless of the chlorinated compound transformed. *Geobacter* population was likely responsible for approximately 80 % of the TCE dechlorinated to cDCE in this experiment.

Daprato et al (2007) enriched three anaerobic, dechlorinating consortia capable of degrading PCE to ethene. The 16S rRNA gene-based analyses demonstrated that enrichment

with PCE resulted in dechlorinating communities dominated by *Dehalococcoides* and *Dehalobacter*, and that up to four different PCE dechlorinating organisms coexisted in one consortium. Several halorespiring organisms dechlorinate PCE-to-*cis*-DCE (Daprato et al., 2007), but for bioremediation to be successful, complete dechlorination to ethene must be achieved. Prior to this work (Daprato et al., 2007), only two *Dehalococcoides* strains had been isolated that are capable of halorespiration of DCEs to ethene, while using hydrogen as their electron donor (He et al., 2003; Sung et al., 2006).

KB-1, a consortium of *Dehalococcoides spp*, has been proven to be effective both in the laboratory (Ibbini et al., 2006) and field (Major et al., 2002). These microbes are strictly anaerobic and require conditions such as dissolved oxygen (DO) less than 0.2 mg/L and redox potential less than -50 mV. KB-1 was purchased from SiREM, Ontario, Canada, for the work reported here and the work by Ibbini et al (2006) and Ibbini et al (2007).

1.4.2. Phytoremediation

The Remediation Technologies Development Forum (RTDF) was established in 1992 as a forum for government, industry and academia to collaborate on the development of cost-effective hazardous waste characterization and treatment technologies. The Phytoremediation of Organics Action Team was established in 1997, as one of a number of RTDF Action Teams to further the RTDF's goals. This team had published a report on the evaluation of phytoremediation for management of chlorinated solvents in soil and groundwater (RTDF, 2005). Specifically this document is designed to briefly introduce phytotechnologies; identify potential applications of phytoremediation to control, transform, or manage chlorinated volatile organic compounds (CVOCs) in soil and groundwater; show how to conduct a preliminary assessment to determine if a particular site is a good candidate for phytoremediation; and describe monitoring options and show how to assess the effectiveness of phytoremediation at full-scale field implementation.

A relationship, called the "transpiration stream concentration factor" (TSCF), which represents the translocation of groundwater contaminants to the plants' transpiration stream, ranges from 0.02 to 0.75 for TCE (Burken and Schnoor 1998; Davis et al. 1999; Orchard et al., 2000; and Ma and Burken, 2002). Hu et al (1998) found that it was necessary to consider a 25-fold sorption to dry matter in poplar stems to account for the retardation in migration of TCE.

Separate experiments showed that TCE does not bind to cellulose, so that the retardation effect may be primarily from sorption to lignin, or dissolution in lipids (Davis et al., 1998). When alfalfa plants in soil systems were fed with TCE and trichloroethane (TCA), there was both transformation, in some cases, and transfer to the atmosphere.

1.4.2.1. Rhizosphere Biodegradation

Rhizosphere degradation is the breakdown of organic contaminants within the rhizosphere – a zone of increased microbial activity and biomass at the root-soil interface. Plant roots secrete and slough substances such as carbohydrates, enzymes, and amino acids that microbes can utilize as a substrate. Contaminant degradation in the rhizosphere may also result from the additional oxygen transferred from the root system into the soil causing enhanced aerobic mineralization of organics and stimulation of co-metabolic transformation of chemicals (Anderson et al., 1993).

The fate of TCE was investigated in laboratory settings (Walton and Anderson, 1990) by comparing degradation of TCE in both rhizosphere soil and non-vegetated soil collected from a TCE-contaminated site. The results showed that TCE degrades faster in rhizosphere soils. Anderson and Walton (1995) also reported that TCE mineralization was greater in soil rooted with the Chinese lespedeza, loblolly pine, and soybeans than in non-vegetated soil.

Additional research on CVOC fate in the rhizosphere has shown varying results. Chlorinated pesticides were shown to have enhanced degradation in the rhizosphere (Shann, 1995), and a loss of TCE and 1,1,1-trichloroethane (TCA) was observed in the rhizosphere of alfalfa (Narayanan et al., 1995). Higher numbers of methanotrophic bacteria, which have been shown to degrade TCE, were detected in rhizosphere soils and on roots of *Lespedeza cuneata* and *Pinus taeda* than in unvegetated soils (Brigmon et al., 1999). Orchard et al. (2000) detected TCE metabolites in the roots of hybrid poplar saplings suggesting rhizosphere degradation and concluded that the greatest degradation of TCE occurred in the rhizosphere. However, Newman et al., (1999) observed no degradation of TCE in the rhizosphere of hybrid poplars. Similarly, Schnabel et al. (1997) observed no degradation of TCE in the rhizosphere of edible garden plants. Studies have indicated that wetland vegetation and rhizosphere microbial communities can effectively treat chlorinated compounds (Dhanker et al., 1999; Bankston et al., 2002; Nzengung et al., 1999; and Kassenga, 2003).

Eberts et al (2005) demonstrated in a field scale project, at a site in Fort Worth, Texas, that eastern cottonwood trees (*Populus deltoides*) delivered enough dissolved organic carbon to the underlying aquifer to lower dissolved oxygen concentrations and subsequently to initiate in situ reductive dechlorination of TCE. The depth to water in the aquifer was less than 3 m. The biodegradation rate constants for TCE increased up to 100-fold. Li et al (2005) determined the uptake of PCE and TCE by roots and shoots of ryegrass seedlings and found that it increases with time of exposure to the aqueous solution.

1.4.2.2. Hydraulic Control

A great deal of research has focused on the use of trees—poplar trees, in particular—to intercept shallow groundwater plumes (Wang et al., 1999; Jones et al., 1999; Thomas and Krueger, 1999; Tossell et al., 1998; Gordon, 1998; Newman et al., 1999; Compton et al., 1998; and Quinn et al., 2001). Most of these studies have shown that trees can extract large enough quantities of groundwater to depress the water table, locally inducing flow toward the trees. This depression can be sufficient to create a hydraulic barrier or hydraulic control. Hydraulic control mitigates potential risks by controlling offsite transport of CVOCs and providing more opportunity for the four mechanisms of phytoremediation (volatilization, rhizosphere degradation, plant degradation and phytoextraction) to remediate the CVOCs. Proper hydraulic control involves the selection and planting of vegetation to intercept and transpire large quantities of groundwater or surface water.

1.4.3. Iron

Chlorinated solvents in groundwater are known to undergo reductive dechlorination reactions with Fe (II)-containing minerals and with corroding metals in permeable-barrier treatment systems (Zhang et al., 2004). Several laboratory (Butler and Hayes, 1999; Butler and Hayes, 2001; Dries et al., 2004; Ebert et al 2006; Lee and Batchelor, 2002) and field studies (Wilkin et al., 2002) were conducted for using Zero-Valent Iron as permeable reactive barrier in the remediation of chlorinated ethenes.

1.5. Health Hazard of CEs

PCE inhalation has acute effects like irritation of the upper respiratory tract and eyes, kidney dysfunction, dizziness, headache, sleepiness and unconsciousness. The major effects from

chronic inhalation exposure to PCE are neurological effects, including sensory symptoms such as headaches, cognitive and motor neurobehavioral functioning and color vision decrements. It also causes reproductive effects such as spontaneous abortions and reduced fertility. It has increased risk of cancer and liver problems. Some of the health hazards of skin contact with PCE are skin irritation and eye irritation (Irwin, 1997).

Exposure to TCE affects the central nervous system. At very high levels of exposure such as might occur in an enclosed space or during a spill, TCE can injure the liver and kidneys. TCE vapor in the air can irritate eyes, nose, and throat. TCE can cause cancer and may damage the nervous system, liver, and lungs. It may also cause adverse reproductive and developmental impacts, and damage to the cardiovascular and immune system.

Exposure to high concentrations of 1,2-dichloroethylene vapor can cause humans to become dizzy and lightheaded and to pass out. Long-term exposure may damage the liver. Contact can irritate the skin and eyes. The vapor may irritate the nose, throat and lungs. 1,2-Dichloroethylene is a flammable and reactive chemical and is a fire and explosion hazard.

Acute exposure of humans to high levels of vinyl chloride via inhalation has resulted in effects on the central nervous system (CNS), such as dizziness, drowsiness, headaches, and giddiness. Vinyl chloride is reported to be slightly irritating to the eyes and respiratory tract in humans. Liver damage may result in humans from chronic exposure to vinyl chloride, through both inhalation and oral exposure. Inhaled vinyl chloride has been shown to increase the risk of a rare form of liver cancer (angiosarcoma of the liver) in humans. Vinyl chloride may damage the liver and immune and nervous systems (Frohman et al., 2004).

1.6. Field Scale Remediation of PCE

Harnessing the metabolic activity of halorespiring bacteria in contaminated aquifers is achieved through the introduction of electron donors that stimulate anaerobic organisms, which are capable of producing hydrogen. Hydrogen production is necessary because it is the only electron donor that *Dehalococcoides* can utilize. While direct hydrogen addition for the stimulation of *Dehalococcoides* and other halorespiring bacteria has been attempted (Newell et al., 2000), the injection of fermentable substrates (lactate, molasses, hydrogen releasing compound (HRC), emulsified vegetable oil, chitin, etc.) is a more common approach (Ellis et al., 2000; Major et al., 2001; Adamson et al., 2003). The anaerobic microbial community enriched

by these fermentable substrates may include fermentative organisms that produce hydrogen and organic acids, dechlorinators that use the hydrogen (and in some cases organic acids), and other bacteria capable of utilizing hydrogen and organic acids (i.e., methanogens (Yang and McCarty, 1998), sulfate reducers (Mazur and Jones, 2001), and iron reducers (Lu et al., 2001)).

In-situ bioremediation is used as an alternative to such traditional methods as groundwater pump-and-treat for treating groundwater contaminant plumes. This involves stimulating indigenous bacteria by adding electron donors and/or nutrients to the subsurface to increase bacterial growth and degradation rates. A variety of electron donors such as acetate, lactate (Cox et al., 2002; Ellis et al., 2000; McMaster et al., 2001; RTDF Update, 1997), methanol, ethanol (Cox et al., 2002), molasses and vegetable oils have been used for biostimulation of microbes that degrade chlorinated solvents (Grindstaff, 1998, Harkness et al., 1999). A number of organic substrates such as benzoate and butyrate also support this process. Ethanol, lactate, and butyrate, which are fermented directly to hydrogen without the production of methane, may also promote dechlorination (Lee et al., 1997).

The use of inexpensive substrates reduces anaerobic bioremediation costs. Inexpensive, complex substrates such as molasses, a wastewater containing formate, acetate, propionate, cheese whey permeate (a waste product from the manufacture of cheese), corn steep liquor (an inexpensive product used for fermentation and that is produced by steeping corn in water), and the dissolved organic fraction of chicken manure were shown to support reductive dechlorination of PCE to VC in microcosm studies (Lee et al., 1997). Several bioremediation pilot scale studies (Abriola et al., 2005; Ellis et al., 2000; Major et al., 2002) and a physical-chemical remediation pilot study (Cox et al., 2002) have been conducted in the past in the United States.

It appears that if a dechlorinating population is present at a site, almost any fermentable substrate can be effective in stimulating its activity. However, because of the variable responses to substrates by organisms from different sites, laboratory or small-scale field studies are necessary to confirm that a particular substrate will support dechlorination at the site. No substrate that reliably supports complete dechlorination at all sites has been identified to date.

Monitored natural attenuation (MNA) has recently emerged as a viable groundwater remediation technology in the United States (Witt et al., 2002). Witt et al (2002) examined the potential for MNA of PCE and TCE in groundwater and aquifer sediments at Dover Air Force Base (Dover, DE) test site. Reductive dechlorination likely dominated in the anaerobic portion

of the aquifer where PCE and TCE levels were observed to decrease with a simultaneous increase in cis-1,2-dichloroethene (cis-DCE), vinyl chloride (VC), ethene, and dissolved chloride. Near the anaerobic/aerobic interface, concentrations of cis-DCE and VC decreased to below detection limits, presumably due to aerobic biotransformation processes. Therefore, the contaminant and daughter product plumes present at the site appear to have been naturally attenuated by a combination of active anaerobic and aerobic biotransformation processes (Witt et al., 2002). Rectanus et al (2007) conducted experiments to determine if the indigenous organic carbon in aquifer sediments could support reductive dechlorination of chlorinated ethenes.

In this work, a biostimulation/bioaugmentation pilot scale design was developed and implemented for a PCE contaminated site in Manhattan, KS.

1.7. Biostimulation/Bioaugmentation Design

1.7.1. Biostimulation

Biostimulation involves addition of nutrients such as oxygen (aerobic remediation) or hydrogen (anaerobic remediation) to the subsurface to degrade the contaminants (Regenesis, 2003b). From its introduction in 1994, Oxygen Releasing Compounds (ORC™) have been used at 9,000 sites in the United States and in 20 countries. Similarly, Hydrogen Releasing Compounds (HRC™) have been used, since 1999, at 475 sites worldwide (Regenesis, 2003b). A fewer number of sites have used HRC, not only because of its shorter time on the market, but because there are less sites requiring "anaerobic management" relative to "aerobic management." Nevertheless, when one looks at all the sites where electron donors have been applied, the 475 sites treated with HRC™ represents a majority of the total number treated anaerobically (Regenesis, 2003b).

Anaerobic bioremediation has been recognized in recent years as one of the primary attenuation mechanisms by which a number of contaminants can be contained and/or remediated. Contaminants amenable to anaerobic bioremediation include chlorinated solvents such as PCE and TCE, metals such as hexavalent chromium, and pesticides such as chlordane. Hydrogen-releasing compound (HRC™) consists of soy-oil methyl esters and lactic acid (North et al., 2001; Murray et al., 2001). The use of a variety of electron donors to accelerate natural attenuation is becoming a standard procedure. As the frequency of use of these protocols increases, certain issues are surfacing and becoming the subject of more intense examination.

One of the major issues is the phenomenon of incomplete dechlorination, such as the case of cis-DCE appearing in concentrations higher than the parent materials and that it can persist. One or more of the following may happen: 1) unknown sources are providing a constant feed of parent material, 2) rates of degradation of parent compounds are faster than those of daughter compounds ("kinetic disparity") leading to accumulation, and 3) differences in solubility from parent to daughter products make the latter more prevalent in the dissolved phase. Once these issues are examined, only then it is appropriate to search for valid inhibition phenomena; to that end we can cite both the biological explanation (absence of necessary microorganisms) and the lesser recognized geochemical explanation (elevated reduction of iron, which blocks electron flow to DCE) (Regenesis, 2003b).

In terms of solutions, with biological limitations one can bioaugment or, for both biological and/or geochemical limitations, if the pool of DCE is present without significant parent material, then a switch to aerobic conditions can be made. All of these solutions are "second tier," because the very important conclusion is that the vast majority of sites displaying a sluggish pattern of DCE attenuation may simply need "more time and more electrons" (Regenesis, 2003b).

North et al (2001) report that injection of a polylactate ester (HRC) into a plume of PCE-contaminated ground water beneath a dry cleaning facility effectively changed the chemistry of the aquifer to an anaerobic and nutrient-rich environment, thus accelerating reductive dechlorination of chlorinated solvent compounds. In the core of the plume, PCE concentrations decreased by up to 99 % in the first year after injection of the HRC. Continued ground water monitoring indicated that more than one year after injection, the majority of the treated area remains anaerobic and PCE concentrations continue to decrease (North et al., 2001). HRC has been used as a slow release electron donor to enhance natural biological destruction of chlorinated solvents. Completed pilot tests show that HRC can effectively enhance the natural attenuation of chlorinated solvents with very efficient degradation rates, an obvious requirement for economic site cleanup (Murray et al., 2001). Enhanced bioremediation, using hydrogen release compound (HRC™), was applied to a site in Japan contaminated with chlorinated solvents (Nakashima, 2002). Thirty-six days after HRC injection, the concentrations of PCE and TCE in groundwater at the downstream well decreased to some extent; however the concentrations of *cis*-1,2-DCE, *trans*-1,2-dichloroethylene (*trans*-1,2-DCE) and vinyl chloride (VC) increased.

After 78 to 107 days, concentrations of DCE and VC showed a decreasing trend, as well as PCE and TCE concentrations. After that, concentrations of all VOCs kept decreasing until the HRC in the subsurface was exhausted. The attenuation ratio of the PCE concentration at the downstream well reached 97 % to 99 % and that of TCE reached 80 to 93 % (Nakashima, 2002).

Where practicable, complete coverage of the source and nearby areas with HRC will likely be more effective at mitigating impacts, than a barrier wall only design. In addition, the potential for the influx of untreated aerobic water near the plume margins should be evaluated to ensure adequate amounts of the treatment agent are injected in areas where fresh water influx may be significant. Given the apparent impact of a fresh water flux on the effectiveness of the HRC at creating favorable conditions, the economics of treating an area larger than the plume itself compared to the cost of potential multiple injections should be considered in designing an injection approach (Regenesis, 2003b).

There are a number of advantages to using time-release strategies. Implementation of the appropriate time-release system can eliminate major capital and operational costs associated with mechanical systems, because it is delivered into the aquifer only once or twice a year. The use of simple, ubiquitous push-point injection technology makes application fast, directed, and minimally disruptive to site operations. Project design is simplified since there is no need for the design of aboveground treatment process and equipment. Since chlorinated hydrocarbon sources are difficult to locate, a continuous, highly diffusible series of organic substrates can increase the effectiveness of contact and biodegradation. The primary design issues are (1) amount of substrate required to support biodegradation of a given amount of contaminant and (2) number of delivery locations needed to effectively distribute electron donor within the contaminant plume (Regenesis, 2003b).

Laboratory and field studies conducted by Borden (2003) have shown that injection of Edible Oil Substrate (EOS®) into the subsurface can provide an effective, low-cost alternative for the enhanced anaerobic bioremediation of chlorinated solvents, nitrate, perchlorate, acid mine drainage, and heavy metals. Many edible oils are insoluble in water and are only slowly biodegradable under anaerobic conditions. As a consequence, these oils can provide an inexpensive, slow-release source of organic carbon for aquifer bioremediation.

Borden (2003) reported that at Altus Air Force Base (AFB), Oklahoma, a pilot-study was conducted to evaluate the suitability of EOS® injection for stimulating reductive dechlorination

of TCE. Historical releases of degreasing agents resulted in a 5000-ft long chlorinated solvent plume with TCE concentrations reaching 78 mg/L in the source area. Over the 13-month interval since EOS® injection, TCE declined from 1300 µg/L to below the detection limit (BDL) in the center injection well. In a well 20 ft down-gradient of the barrier, TCE had declined from 1,600 µg/L to BDL, cDCE from 900 to 73 µg/L, with increases in VC from 440 to 1,770 µg/L and ethene from 6.9 to 510 µg/L. An analysis of total costs over a 30 year life cycle suggested that edible oil barriers may be a very cost effective alternative for controlling plume migration (Borden, 2003).

1.7.2. Bioaugmentation

Lookman et al (2007) successfully conducted a field test where ground water transfer from one site (showing complete natural reductive dechlorination of chlorinated ethenes to ethene) induced full reductive dechlorination at another site polluted with tetrachloroethene and its partial dechlorination products trichloroethene and *cis*-dichloroethene (cDCE).

Weiss and Cozzarelli (2008) reviewed the experimental approaches and microbial/molecular methods for investigating the controls on microbially mediated degradation processes in contaminated aquifers. This paper discusses emerging technologies and recent state-of-the-art studies that serve as models for integrating microbiological approaches with more traditional geochemical and hydrogeologic approaches to better understand the controls on contaminant fate. Weiss and Cozzarelli (2008) emphasize the need for characterizing the structure and function of the microbial populations at contaminated sites. Understanding the mechanisms responsible for biodegradation allows for the enhancement of these processes through an engineering approach.

KB-1 (Consortium of Dehalococcoides)

KB-1™ is a commercially-available dehalorespiring microbial culture that has been used at more than 20 sites in 10 states to improve the performance of PCE and TCE bioremediation for both source area and plume remediation in porous media and fractured rock environments. Major et al. (2002) and Cox et al. (2002) have reported that *Dehalococcoides* strains in KB-1™ can migrate in aquifers. The rate of migration is relatively slower than groundwater flow velocities. The extent of migration and activity of migrating cells is likely dependent on the distribution of the solubilized electron donor in comparison with chloroethenes. KB-1™ has

been demonstrated to work at high concentrations of PCE/TCE/cDCE and VC. KB-1™ is being used to enhance the disappearance of PCE and TCE in source areas. High sulfate concentrations may impact the activity of KB-1™ due to competition of sulfate reducing bacteria (SRB) for available electron donor and hydrogen. However, research has shown that bacteria in the KB-1™ consortia are able to out-compete SRB when PCE/TCE predominates. No inhibition was observed in moderately brackish water. Moderate concentrations of chloroform and 1,1,1-trichloroethane can inhibit KB-1™ activity (Sirem, 2004b).

Phylogenetic analysis of KB-1™ suggests that it consists predominantly of microorganisms that commonly inhabit subsurface environments, including many drinking water aquifers. The KB-1™ culture has not been genetically modified in any manner; it is simply an enrichment derived from naturally occurring bacteria found in soil and groundwater. Microbial screening has confirmed that the KB-1™ culture does not contain a number of known human pathogens. Introduction of KB-1™ to subsurface environments would not be expected to significantly alter subsurface microbial conditions, but would supplement the natural microbiota with bacteria, particularly *Dehalococcoides* that can promote the rapid and complete dechlorination of PCE and TCE to ethene. Following degradation of the chlorinated solvents, these bacteria (which respire chlorinated solvents) would become inactive. Furthermore, the sensitivity of this microbial culture to oxygen would prevent its spread into aerobic environments within aquifer systems. For field demonstrations/applications, the fate of the KB-1™ culture can be tracked using 16S RNA molecular profiling and using denaturing gradient gel electrophoresis (DGGE) analysis to confirm and monitor its distribution, transport, survival and attenuation at the subject site (Sirem, 2004a, b).

Cl   H   H   H   H

$$Cl-\underset{\underset{Cl}{|}}{\overset{\overset{Cl}{|}}{C}}-Cl \ \overset{H_2 \quad HCl}{\searrow} \ Cl-\underset{\underset{Cl}{|}}{\overset{\overset{H}{|}}{C}}-Cl \ \overset{H_2 \quad HCl}{\searrow} \ Cl-\underset{\underset{Cl}{|}}{\overset{\overset{H}{|}}{C}}-H \ \overset{H_2 \quad HCl}{\searrow} \ Cl-\underset{\underset{H}{|}}{\overset{\overset{H}{|}}{C}}-H \ \overset{H_2 \quad HCl}{\searrow} \ H-\underset{\underset{H}{|}}{\overset{\overset{H}{|}}{C}}-H$$

CT   CF   MC   CM   Methane
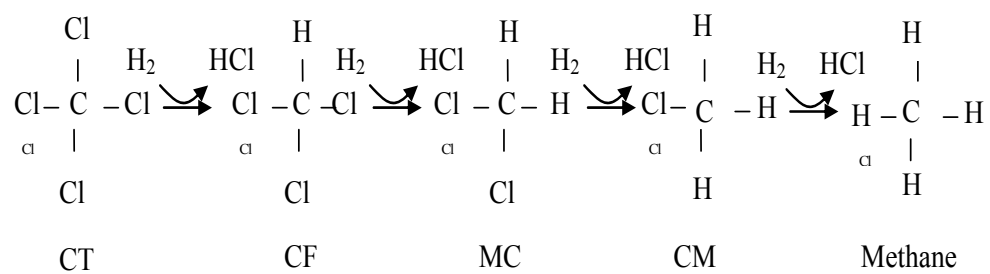
Figure 1.1. Stepwise reductive dechlorination of carbon tetrachloride to methane.

```
                    Tetrachloroethene (PCE)
               Dehalococcoides ethenogenes 195
                 Dehalospirillum multivorans
                      Sporomusa ovata
                   Dehalobacter restrictus TEA
                 Desulfitobacterium sp. PCE-S
                              |
                              |  tetrachloroethene
                              |  reductive dehalogenase
                              |
                              v
   +----------------- TCE --------------------------+
   |              only D.ethanogenes 195            |
   |                 |                              |
   |  trichloroethene |  trichloroethene            |
   |  reductive       |  reductive                  |
   |  dehalogenase    |  dehalogenase               |
   |                  |                             |
   |                  |                             |
   v                  v                             |
 cis-1,2-Di-      [trans-1,2-Di-                    |
 chloroethene]     chloroethene]                    |
   |                  |                             |
   |                  |                             |
   |  dichloroethene  |  dichloroethene   trichloroethene
   |  reductive       |  reductive        reductive
   |  dehalogenase    |  dehalogenase     dehalogenase
   |                  |                             |
   |                  |                             |
   |                  v                             |
   +----> Vinyl chloride                            |
              |                                     |
              |  vinyl chloride                     |
              |  reductive dehalogenase             |
              |                                     |
              v                                     |
          Ethylene <------------------------------+
              |
              |
              V
          To the
      Acetylene Pathway
```

Figure 1.2. PCE degradation products by various microorganisms and enzymes[#].

[#](Ellis and Anderson, 2003).

Table 1.1. Physical and chemical properties[#] of CT and its degradation products.

Property	CCl_4	$CHCl_3$	CH_2Cl_2	CH_3Cl
Molecular weight	153.8	119.4	84.9	50.5
Boiling point (°C)	76.5	62	40	-23.8
Vapor pressure (mm Hg) at 25°C	115	195	447	4373
Dimensionless Henry's constant at 25°C (gas/liquid)	1.24	0.15	0.09	0.36
Density (gm/cc) at 25°C	1.6	1.48	1.32	0.92
Solubility in water (g/L) at 25°C	0.8	8.1	20.0 (20°C)	6.3
Log K_{oc}	2.62	1.64	1.3	1.4
Log K_{ow}	2.78	1.9	1.3	0.91
Maximum Contaminant Level (MCL) in water (μg/L)	5	100	5	NA
(μM)	0.03	0.84	0.06	

[#] Faris and ITRC, 2002; Knox et al., 1993; Schwarzenbach et al., 1993; Spectrum Laboratories, 2006; Verschueren, 1996.

Table 1.2. Physical and chemical properties[#] of PCE and its degradation products, TCE and VC.

Property	PCE	TCE	VC
Molecular weight	165.83	131.40	62.5
Physical state (at room temperature)	Liquid	Liquid	Gas
Color	Colorless	Colorless	Colorless
Melting point (°C)	-22.4	-87.1	-153.8^0C
Boiling point (°C)	121	86.7	-13.37^0C
Vapor pressure at 25°C (mm Hg)	18.47	74	2580
Density (gm/cc)	1.6227	1.465	0.916
Henry's law constant at 25°C (atm-m^3/mol)	0.018	0.01172	1.2
Solubility in water at 25°C (mg/L)	150	136.6	1110
$\log K_{ow}$ (L/kg) 25°C	3.4	2.42	1.36
$\log K_{oc}$ (L/kg) 25°C	[2.2-2.7]	[2.03-2.66]	[0.9-1.99]
Maximum Contaminant Level (MCLin water (µg/L)	5	5	2
(µM)	0.03	0.04	0.03

ATSDR, 1997; ATSDR, 2004; Canada health online, 1992; ITRC, 2005; Knox et al., 1993; U.S. EPA, 1994.

Table 1.3. Values of the physical-chemical properties[#] of the dichloroethenes.

Parameter	1,1-DCE	trans 1,2-DCE	cis 1,2 -DCE
Molecular weight	96.95	96.95	96.95
Physical state (at room temperature)	Liquid	Liquid	Liquid
Color	colorless	colorless	colorless
Melting point (°C)	-122.6	-49.4	-81.5
Boiling point (°C)	31.6	47.7	60.3
Vapor pressure (mm Hg) at 20°C	500	265	180
Density (gm/cc)	1.214	1.257	1.2837
Henry's law constant at 25°C (atm-m^3/mol)	0.026	0.0094	0.0041
Solubility in water (mg/L)	2250	6300	3500
logK_{ow} (L/kg) 25°C	1.84	2.09	1.86
K_{oc} (L/kg) 25°C	65	59	35
Maximum Contaminant Level (MCL) in water (µg/L)	7	100	70
(µM)	0.07	1.03	0.72

[#] Gossett, 1987; ITRC, 2005; Knox et al., 1993; USACE, 2002.

Table 1.4. Values of the physical-chemical properties[#] of Methane, Ethene and Ethane

Parameter	Methane	Ethene	Ethane
Molecular weight	16	28	30
Physical state (at room temperature)	Gas	Gas	Gas
Color	colorless	colorless	colorless
Melting point (°C)	-182.5	-169.4	-182.8
Boiling point (°C)	-164	-103.8	-88.2
Vapor pressure (atm)	275 (at 15°C)	80 (at 15°C)	38 (at 21°C)
Specific Gravity (Air = 1)	0.97	0.97	1.047
Henry's law constant at 25°C (atm-m^3/mol)	0.72	0.21	0.54
Water Solubility (mg/L)	22 (at 25°C)	131 (at 25°C)	26 (at 20 °C)
Log K_{ow}	1.09	1.13	1.32

[#] Chemfinder, 2004; ChemIDPlus, 2004; Chemfinder, 2004; OSHA, 2003; SRC, 2005;

Chemistry Daily, 2008; Concoa, 2008; Hudson, 2004; U.S. EPA, 2007.

Table 1.5. Calculated first-order degradation coefficient and half-life of PCE and its degradation compounds at a chlorinated solvents contaminated site in Japan[#].

Compound	Natural Condition	After HRC Injection	
	Day 0	Day 107	Day 379
First Order Degradation Constant			
PCE	0.15 1/year	55 1/year	55 1/year
TCE	0.8 1/year	95 1/year	75 1/year
DCE	0.2 1/year	0.9 1/year	20 1/year
VC	-	0.12 1/year	200 1/year
Half-life			
PCE	4.6 years	4.6 days	4.6 days
TCE	0.87 years	2.7 day	3.4 day
DCE	3.5 years	0.77 years	12.6 days
VC	-	5.8 years	1.3 days

[#]Nakashima et al., 2002**.**

CHAPTER 2 - An Experimental Laboratory Study on Biostimulation of Carbon Tetrachloride Contaminated Groundwater

2.1. Introduction

In this work, the degradation of carbon tetrachloride (CT) was studied in a six-channel soil column system. The degradation of CT is mainly limited by the availability of electron donors. Hence, two different substrates were used in this study, to create the necessary reducing conditions favorable for CT transformation. Soil samples were collected at several depths in the channels to determine the vertical distribution of CT and degradation products. Headspace samples were analysed to check if any compounds diffused through the vadose zone into the gas phase above the soil surface. A tracer study was also conducted in the channels, at the beginning of the biodegradation study, to understand the flow pattern and the residence time distribution of the tracer. This information was used in the analysis of the fate of the CT that enters through the inlet.

2.2. Materials and Methods

2.2.1. Experimental System or Mesocosm

A chamber was divided into six channels; each channel was 110 cm long, 65 cm high and 10 cm wide. Channels 4, 5 and 6 were used for the CT study. The channels were filled with alluvial silty sand soil (< 10% silt) up to ~ 60 cm. The soil was collected from a site near a landfill in Riley County, Kansas in 1993 (Zhang, 1999). Alfalfa was grown in channels 5 and 6, while fescue grass was grown in channel 4. A pair of fluorescent tube lights (40 W) for each channel, placed at a height of 50 cm above the soil surface, provides the light source for the plants. The photosynthetically active radiation (PAR) at 40 cm from the soil surface (the average plant height) was about 160 $\mu E/m^2/s$ (measured by using a L1-188B Integrating Quantum Radiometer/Photometer) (Zhang, 1999). For further details on soil type and organic carbon content, see Secion 3.2.1.

The inlet solution was fed at 5 cm above the bottom of the channels (see Figure 2.1). Watering of plants started on November 5, 2003. During the initial period the plants were observed, without contaminants in the soil. Until March 11, 2004, only distilled water was fed into the channels and the plants were growing well.

The contaminants were introduced starting from March 12, 2004. CT was introduced at a concentration of about 2 mg/L (~13 μmoles/L) in three channels, two of them with alfalfa plants and the other with fescue grass. The height of saturated zone in each channel was controlled by position of the end of each outlet tube (25 cm in this system). The volume of water in each channel corresponding to 25 cm of saturated zone is estimated to be 8.25 L, assuming a porosity of 0.3. Plants were harvested at the beginning of each month by cutting the top portion; after harvest, the plant height was approximately 10 cm. At the end of the month, the fescue grass and alfalfa grew to a height of approximately 50 cm and 70 cm, respectively. Initially, nicotine hemisulfate solution was sprayed, weekly, on channel 4 to destroy the pest aphids on the grass. Later, this pesticide solution was sprayed on all the channels. The pesticide solution was prepared by adding 10 mL of nicotine hemisulfate salt (40 % aqueous solution (w/v), Sigma, St. Louis, MO) to 500 mL of distilled water. From February 2007, 1% solution of potassium salt of a fatty acid was used as insecticide. After the initial preparation of inlet contaminant solution, 100 mL of CT stock solution (~40 mg/L of CT) and 900 mL of distilled water were added every day.

The amount of nitrogen, phosphorus and potassium (NPK) consumed by the plants and removed by harvesting since the beginning of the experiment, was computed. A fertilizer solution was prepared with required NPK and one liter of the solution was applied to the top soil of each channel.

Even though plants were grown in the system, the study primarily focused on the saturated zone of the channels and not on rhizosphere effects. The plants served as a natural pump, evapotranspiring some of the contaminated water and thus, influencing the residence time and movement of the compounds in the channels.

The flow rate of the contaminant solution inside the channel varied in a 24-hour period. Immediately after watering, the flow rate was maximum and at the end of a 24-hour period, the flow rate was minimum. Based on the bromide peak arrival time during the month of June 2004,

the flow rate was estimated to be approximately 98 cm/d (3.3 ft/d) for channel 4, 77.5 cm/d (2.6 ft/d) for channel 5, and 88 cm/d (2.9 ft/d) for channel 6.

There were five monitoring wells containing sintered alumina with polyethylene tubing for channel 6 through which groundwater samples could be collected from near the bottom of the channel (see Figure 2.1). The wells were placed at a distance of 17, 35, 60, 73 and 100 cm from the inlet. The depth of the wells was 60 cm and the bottom of each well was about 0.2 cm from the bottom of the channel. Wells were not installed in Channels 4 and 5.

2.2.2. Inlet/Outlet Analysis

After introducing the contaminant solution on March 12, 2004, the concentrations of the contaminants were analysed at the inlet and the outlet of the channels. A 10 mL sample was collected from the inlet tube at the entrance to each channel. A 10 mL sample was collected from the outlet of each channel. Samples were collected using a 10 mL syringe and transferred to a 25 mL vial and closed with a mininert cap immediately. After shaking, the compounds in the sample were allowed to partition into the headspace and attain equilibrium. The headspace samples were analysed with a gas chromatograph (GC).

2.2.3. Tracer Study

A tracer study was conducted, using potassium bromide (KBr), to determine the residence time distribution of the contaminants which enter at the inlet. The corresponding volume of outlet liquid, was also measured because it varies with the plant size, and therefore, time of the month. The outlet liquid volume is a better parameter for estimating the tracer response because the time for peak of bromide concentration depends on the growth stage of the plants. In the tracer study, 150 mL of KBr solution, at a concentration of 100 mg/L (10.05 mg as bromide), was injected at the inlet of each channel through separating funnels, on June 4, 2004. Since the plants were harvested on June 1, 2004, the evapotranspiration was relatively small and consequently the volume of outlet liquid was relatively large. Samples were collected from the outlet at intervals of 3 hrs, 6 hrs, 12 hrs and 24 hrs on the first day and second day. On third and fourth day, two samples, at 5 hrs and 24 hrs, were collected. From then on, each day a sample was collected, for 14 days, and analysed for bromide concentration. The mean residence time for the bromide in a channel was estimated from the expression (Levenspiel, 1999)

$$\tau = \frac{\sum t_i C_i \Delta t_i}{\sum C_i \Delta t_i}$$

<div align="right">….(2.1)</div>

where,

τ = mean residence time, days

C_i = concentration of bromide in the ith sample, mg/L

t_i = time at which the ith sample was collected, day

Δt_i = Difference between $(i+1)^{th}$ time and i^{th} time, days

Some of the bromide will be transported upward through the soil column due to evapotranspiration by plants. Hence, some bromide gets trapped in the soil column above the primary flow path. The mass of bromide trapped in the soil is estimated based on the daily water uptake of plants and the concentration of the bromide in the outlet for that day. The composite concentration of the bromide entering the vadose zone in the upflow water is assumed to be the same as the composite concentration of bromide in the outlet liquid. When two or more samples were collected during the initial period of study, the average concentration of bromide was used to calculate the mass of bromide transported upward.

2.2.4. Biostimulation by Glucose and Soy Oil Methyl Esters

Since no degradation was observed after 100 days, supplements were added to stimulate growth of indigenous microbes, to create anaerobic conditions, and also for supplying hydrogen. Introduction of one liter of 0.2 % glucose solution (Dextrose, anhydrous, Fisher Chemicals, Fairlawn, NJ) as an electron donor into channel 5 (alfalfa grown on top) resulted in anaerobic conditions in the channel. The glucose solution was fed once every month starting from June 30, 2004 and continued until February 2005 and once on June 27, 2006. From September 1, 2006 until March 2007, 18 doses of cheese whey (1 mL in 1 L distilled water) were added to channel 5 through the inlet. From October 1, 2004, one liter of 0.1 % emulsified soy oil methyl esters (SOME) was fed to channel 6 (alfalfa grown on top). SOME was sometimes injected in the wells instead of the inlet, since it tends to stay near the inlet. The glucose and cheese whey addition dates and SOME addition dates are listed in Table 2.1. The supplement solution was fed at the inlet, unless mentioned otherwise. Corn starch was fed, inadvertently, instead of glucose in December, January, and February as shown in Table 2.1.

2.2.5. Soil Sample Analysis

After 96 days of exposure to CT solution, soil samples were collected, at a distance of 30 cm from the inlet, at four different depths from the soil surface, viz. 0-8 cm, 12-20 cm, 22-30 cm and 34-42 cm, from all three channels fed with CT. The samples were collected with a soil core and iron rods of various lengths. Soil collected at each depth was transferred to a 25 mL vial and immediately closed with mininert cap and analysed with GC. The sample headspace was analysed for CT and degradation compounds and the concentration in the aqueous phase of the soil was estimated using a calibration curve and a mass balance (See section 2.2.5.1). The gas phase volume, available for partitioning, is obtained by subtracting the sum of the volumes occupied by water and soil from the total volume of the vial.

The soil sample with the vial is dried in a vacuum oven (Thelco vacuum oven, Precision Scientific Co., Chicago, IL) at 80ºC for 24 hours and the dry weight of soil is determined (see Tables 2.6 and 2.8). The difference between the mass of vial with moist soil and dry soil yields the mass of water present in the soil sample. The difference between the mass of vial with dry soil and the tare mass of vial gives the mass of dry soil.

Calculation of CT Concentration in Soil Samples

The total mass of CT present in the soil sample initially is

$$TM_{CT} = m_{ds} C_{s1} + V_w C_{w1} + V_{g1} C_{g1} \qquad \qquad \dots (2.2)$$

where

TM_{CT} = Total mass of CT

m_{ds} = mass of dry soil

C_{s1} = concentration of CT adsorbed to soil before partitioning into headspace

V_w = volume of aqueous phase

C_{w1} = concentration of CT in the aqueous phase

V_{g1} = volume of gas phase in the soil sample

$$= n \left[\frac{m_{ws}}{\rho_b} \right] - V_w \qquad \qquad \dots (2.3)$$

n = porosity of the soil = 0.3 (assumed)

m_{ws} = mass of wet soil

ρ_b = bulk density of the soil = 1.6 gm/cc (assumed)

C_{g1} = concentration of CT in the gas phase of the soil sample

Assuming equilibrium concentrations in all three phases of the soil sample,

$$C_{s1} = K_{d,CT}\, C_{w1} \qquad\qquad \ldots (2.4)$$

$$C_{g1} = C_{w1}\, H_{CT} \qquad\qquad \ldots (2.5)$$

where

$K_{d,CT}$ = partition coefficient of CT between soil organic matter and aqueous phase

$\qquad = K_{oc} * f_{oc}$

K_{oc} = partition coefficient of CT between organic matter and aqueous phase

$\qquad\quad = 417$ L/kg

f_{oc} = fraction of soil organic matter (see Table 2.9 for values for each channel)

H_{CT} = dimensionless Henry's constant of CT = 1.24 at 25˚C

Rewriting Eqn (2.2) in terms of aqueous phase concentrations, using the relationships Eqns. (2.4) and (2.5)

$$TM_{CT} = m_{ds}\, K_{d,CT}\, C_{w1} + V_w\, C_{w1} + V_{g1}\, H_{CT}\, C_{w1}$$

or

$$TM_{CT} = C_{w1}\, (m_{ds}\, K_{d,CT} + V_w + V_{g1}\, H_{CT}) \qquad\qquad \ldots (2.6)$$

After transferring the soil sample to the vial, due to the available headspace, partitioning of compounds takes place. After partitioning, the total mass of CT is,

$$TM_{CT} = m_{ds}\, C_{s2} + V_w\, C_{w2} + V_{g2}\, C_{g2} \qquad\qquad \ldots (2.7)$$

$$V_{g2} = 25 - \left[\frac{m_{ws}}{\rho_b}\right] + V_{g1} \qquad\qquad \ldots (2.8)$$

Rewriting Eqn (2.7) in terms of gas phase concentrations, using relationships Eqns (2.4) and (2.5),

$$TM_{CT} = m_{ds}\, K_{d,CT}\, C_{g2}/\, H_{CT} + V_w\, C_{g2}/\, H_{CT} + V_{g2}\, C_{g2} \qquad\qquad \ldots (2.9)$$

or

$$TM_{CT} = (C_{g2}\, /\, H_{CT}\,)\, (m_{ds}\, K_{d,CT} + V_w + V_{g2}\, H_{CT}) \qquad\qquad \ldots (2.10)$$

Since the total mass of CT is same before and after partitioning, equating Eqn (2.6) and (2.10) yields C_{w1}

$$C_{w1} = \frac{C_{g2}}{H_{CT}}\left[\frac{m_{ds}\, K_{d,CT} + V_w + V_{g2}\, H_{CT}}{m_{ds}\, K_{d,CT} + V_w + V_{g1}\, H_{CT}}\right] \qquad\qquad \ldots (2.11)$$

Since C_{g2} is the concentration of CT analysed in GC, the aqueous phase concentration of CT in the soil samples can be obtained from Eqn (2.11). Using the same procedure as above, the aqueous phase concentrations of CF, MC and methane can be obtained.

A sorption study was also conducted to estimate the fraction of organic carbon content (f_{oc}) in the soil samples collected on day 151. After analyzing for the chlorinated methanes' concentrations, the soil samples were spiked with 1 mL of PCE-CT standard mixture. The standard was prepared by injecting 10 µL of PCE liquid and 10 µL of CT liquid in a clean amber glass bottle of volume 4.2 liters. The concentrations of PCE and CT in the standard bottle are, therefore, 3.83 mg/L and 3.76 mg/L. One mL of this gas mixture is then spiked onto the soil samples and a control. The control was prepared with 5 gms of glass beads (3 mm diameter, Arthur H. Thomas Co., Philadelphia, PA) and 1 mL of distilled water. The organic carbon fraction (f_{oc}) is then estimated by mass balance. The above equations are used for estimating the organic carbon fraction in the soil, with organic carbon fraction (f_{oc}) as the unknown parameter.

2.2.6. Analytical Method

Chlorinated compounds and methane were analysed using a gas chromatograph (HP 5890 Series II, Wilmington, DE) equipped with a Flame Ionization Detector (FID) and a HP-1 column (Dimethyl Polysiloxane matrix, 30 m x 0.53 mm, Agilent Technologies, Wilmington, DE). Hydrogen was the carrier gas. The injector temperature was set at 200˚C and detector temperature was set at 300˚C. Sample volume of 100 µL was injected in the column at 100˚C and run for 5 minutes. The run temperature was chosen based on the arrival time and good separation of compounds. For higher temperatures, the compounds elute faster but the peaks may be closer or even overlap. For lower temperatures, the separation of compounds is good but the elution will be slow. For the above conditions and gas flow rate of 1.5 mL/min, the elution times of CT, CF, MC and methane are approximately 1.4 min, 1.1 min, 0.85 min and 0.6 min. The detection limits for CT, CF, MC and methane, in the gas phase, with above conditions and instrument, are 4.3 µg/L (0.028 µM), 2.7 µg/L (0.023 µM), 1.5 µg/L (0.018 µM) and 0.12 µg/L (0.0075 µM), respectively. Trial samples of CT and reaction products were run with different temperatures 80˚C, 100˚C, 110˚C, and a temperature program with 35˚C for 5 min and ramp to 245˚C at the rate of 10˚C/min.

For the tracer analysis, the outlet liquid collected was transferred to a 1.5 mL centrifuge tube and centrifuged at 10,000 rpm for 2 minutes. The supernatant was then transferred to a 2 mL clear vial with white septum and threaded black cap (National Scientific Company, Rockwood, TN) for bromide analysis using an ion chromatograph (Dionex DX500 Series, Sunnyvale, CA) equipped with a conductivity detector and analytical column (Ionpac, AS9-HC, 4 x 250 mm). The eluent solvent was 9 mM sodium carbonate at a flow rate of 1 mL/min. The elution times of chloride, bromide, nitrate and sulfate were approximately 6.3 min, 9.5 min, 11 min and 18 min, respectively. The sample volume injected was 25 μL and each sample was run for 20 minutes.

2.3. Results and Discussion

2.3.1. Tracer Studies

The time and the outlet liquid volume at which peak concentration of bromide occurred and the residence time distribution for each channel, are shown in Figure 2.2 and Table 2.2. The mean residence time (MRT) was estimated from the residence time distribution of tracer in the channel (Levenspiel, 1999). The peak of bromide concentration in the outlet occurred between 1.08 to 1.46 days corresponding to an outlet liquid volume of 1.1 L to 1.4 L and mean residence time of 2.8 to 3.6 days.

Table 2.3 presents the cumulative outlet liquid volume, mass of bromide eluted in the outlet, estimated mass of bromide transported to vadose zone by upflow due to evapotranspiration and the total bromide accounted for by these processes and the recovery percentage. The amount of water present in the saturated zone can be calculated as follows:

Volume of the saturated zone $= 110 \times 10 \times 25 = 27,500$ mL $= 27.5$ L

Assuming a porosity of 0.3, the volume of water $= 27.5 \times 0.3 = 8.25$ L

However, the bromide elutes corresponding to an active liquid volume of ~ 1.3 L.

The height of flow zone corresponding to the volume of 1.3 L of water is $1300/(0.3*110*10) = 3.93$ cm. There is some channeling, therefore, in the saturated zone associated with flow from the inlet to the outlet. The entire amount of bromide (10.05 mg) introduced at the inlet was not recovered at the outlet because a portion of the solution is transported upward due to evapotranspiration and another portion is deposited in the saturated zone. The recovery ranges from 62 % to 74 %. The experiment was carried out for only 14 days.

The bromide was still eluting at low concentrations at the end of the experiment. In Table 2.4, the solution recovery is compared to the bromide recovery. A lower recovery is expected for the tracer when added as a pulse.

The evapotranspiration in each channel varies over the monthly period due to harvesting at the beginning of each month. Because of this, the outlet liquid volume also varies and, consequently, the mean residence time of the compounds in the channel. Table 2.5 presents the variation for the daily outlet liquid volume, which was recorded every day throughout the duration of the experiment, for channels 4, 5 and 6. It varied from 0.9 L – 0.6 L for channel 4, 0.9 L – 0.6 L for channel 5, and 0.9 L – 0.4 L for channel 6, from the beginning to the end of one month.

2.3.2. Inlet/Outlet

Figures 2.3 through 2.5 show the inlet CT, outlet CT and degradation compound concentrations for channels 4, 5 and 6. Where no hydrogen donor was added (channel 4, Figure 2.3), the outlet concentrations of the degradation compounds were small, during most of the sampling events, and the concentrations of CT in the inlet and outlet were almost similar. There is no clear evidence that biodegradation was occurring in this channel.

Figure 2.4 shows the CT degradation in the glucose treated channel. One liter of 0.2 % (w/v) glucose solution was added every month starting from day 110 (June 30, 2004) until day 236 (November 3, 2004). On days 266, 299 and 328, cornstarch suspension (1 L of 0.2 % w/v) was added. Glucose solution was later added on day 837. Forty days after the first addition of glucose, the outlet CT started to decrease gradually and reached a low concentration (less than 2 μM) by day 230. Chloroform (CF) appeared, but never exceeded a concentration of 3 μM. Methylene Chloride (MC) was also detected but mostly remained less than 1 μM. Even after stopping the feeding of glucose on day 328, CT degradation continued. Glucose could be stored as polysaccharides and cell materials and released slowly to supply electron donor for dechlorination, while the starch provided a slow release source of glucose. To completely reduce one mole of CT to methane, 8 moles of electrons are needed. One mole of glucose ($C_6H_{12}O_6$) can, theoretically, supply 24 moles of electrons (Hutchinson, 2006; Shrout and Parkin, 2006) that can reduce 6 moles of CT. Total mass of CT fed in a month is 13 μM $*$ 30 L = 390 μmoles. The mass of glucose necessary for degrading 390 μmoles of CT is 390/3 = 130 μmoles = 23.4 mg. In

each feeding, 2 gm or 2000 mg of glucose was added. All of the glucose fed may not be available for CT reduction since some of it may be washed out in the outlet, some may be used by other microorganisms, and some of it may be taken upward by evapotranspiration. Assuming that 10 % of glucose fed was used for reductive dechlorination of CT that would still provide 200 mg glucose or reduction power for 8 months of CT fed.

As shown in Figure 2.4, the total chlorinated methanes (CMes) in the outlet dropped to about 2 μM by day 246 and remained at a lower concentration through day 500, when the values started to increase most likely due to lack of hydrogen donor and carbon source. After about day 600, the outlet CT concentration was between 5 and 10 μM and the concentration of CF was mostly about 1 μM. Although the outlet CT concentration started to increase, it did not reach the inlet level. Glucose solution added on day 837 resulted in a decrease of outlet CT concentration until day 885. However, after the depletion of glucose, the outlet CT concentration increased again to the value of inlet CT. Continuous detection of CF and outlet CT concentration less than inlet CT concentration indicated on-going biodegradation in the channel.

After the role of glucose was studied and understood well, it was decided to change the supplement in this channel. Cheese whey, a byproduct of cheese manufacturing, was chosen. One liter of cheese whey solution, 0.1 % (v/v), was fed to this channel starting September 1, 2006, day 903, and continued about every 10 days. Until March 18, 2007, 18 doses of cheese whey were injected. The outlet CT concentration started to decrease from day 965, almost 60 days from the beginning of cheese whey addition. From day 994 the outlet CT concentration remained between 2 to 5 μM. The outlet CT concentration did not decrease to low levels (below 1 μM) as is the case with glucose, however, CF concentration was well below 2 μM. Cheese whey sugars consists primarily of lactose at a concentration of ~5 % (Ghaly et al., 2003). One mole of lactose ($C_{12}H_{22}O_{11}$) can, theoretically, supply 48 moles of electron that can reduce 6 moles of CT. Total mass of CT fed in a month is 13 μM * 30 L = 390 μmoles. The mass of lactose necessary for degrading 390 μmoles of CT is 390/6 = 65 μmoles = 22.2 mg. In each feeding, 1 mL of cheese whey was added that contains 5 % of lactose or 50 mg, i.e., about 150 mg in a month. This is approximately seven times the theoretical requirement for degradation of CT. All of the lactose fed may not be available for CT reduction since some of it may be washed out in the outlet and some of it may be taken upward by evapotranspiration. Assuming that 10 % of lactose fed was used for reductive dechlorination of CT that would provide 15 mg lactose or

reduction power for only 68 % of a month supply of CT. Glucose appeared to be a better supplement than cheese whey for CT degradation under these conditions. However additional tests need to be conducted with different concentrations and time intervals of injection to better understand the differences between the two supplements.

Figure 2.5 shows the CT degradation pattern in the SOME fed channel. One liter of 0.1 % SOME (v/v) was added every month starting from day 203 (October 1, 2004) until day 445 (May 31, 2005). Outlet CT decreased to low levels within 40 days after the first dose of SOME addition, unlike the slow response in the glucose amended channel. CF and MC were formed but CF was not detected above a concentration of 2.7 μM. Similar results were observed by Witt et al (2001) in a laboratory column study to evaluate the potential for intrinsic bioremediation of CT and related chlorinated methanes. Transient metabolites (CF and MC) were occasionally observed over the course of the study. However, in other work, complete dechlorination of CT was reported in most microcosms at the end of a one-year study (Witt et al., 2001).

In channel 6, MC increased and decreased regularly (see Figure 2.5). This may be due to the variation of the mean residence time of the liquid in the channel. During the start of a month, the plants were harvested, and therefore, the evapotranspiration rate was less. In these days, most of the water flowed out and, therefore, the mean residence time was less. However, at the end of the month, when the plants were larger, the evapotranspiration rate was higher and the daily effluent volume was lower. This led to higher mean residence times and consequently, higher degradation of MC. In this channel, the inlet CT was not completely degraded as in the glucose fed channel. The outlet concentration was below 1 μM until day 621 even after feeding was stopped on day 445. To completely reduce one mole of CT to methane, 2 moles of hydrogen are needed. SOME consists of linoleic, oleic, palmitic, linolenic and stearic fatty acids in that order of predominance. One mole of SOME with mean molecular weight 292.2 (Marion, 2007) can, theoretically, supply 16 moles of hydrogen that can reduce 8 moles of CT. Total mass of CT fed in a month is 13 μM * 30 L = 390 μmoles. The mass of SOME necessary for degrading 390 μmoles of CT is 390/8 = 48.8 μmoles = 14.2 mg. In each feeding, 0.87 gm or 870 mg of SOME was added. All of the SOME fed may not be available for CT reduction since some of it may be washed out in the outlet and some of it may be taken upward by evapotranspiration and a part of it may be adsorbed to soil matrix and not available for reduction of CT. Assuming that 10 % of

SOME fed was used for reductive dechlorination of CT that would still provide 87 mg of SOME or reduction power for 6 months of CT fed.

It was assumed that SOME, being hydrophobic and not soluble in water, may experience flow restrictions or adsorb to the soil organic matter at the initial portion of the channel and not be distributed very well along the length of the channel. Since, according to this assumption, electron donors were not as available beyond well 1 (most of the SOME appeared to stay between the inlet and the first well), a dose of SOME (100 mL of 1 % SOME (v/v)) was added to well 3 (60 cm from inlet) on day 445. After this addition, the total CMes in the outlet decreased and remained lower from day 550 to day 740, except for a couple of sampling dates. After day 750, the substrates were most likely depleted and the concentration of CT started to increase at the outlet. However, it took up to day 825 for the concentration of CT in the outlet to rise above 10 μM, and the modest concentrations of CF and MC provided evidence for some ongoing biodegradation. SOME emulsion was again added on days 837, 957 (injected through well 2), 990 (well 1) and 1020 (well 3). The concentration of CT decreased due to these supplement additions.

2.3.3. Well Samples

The schematic of the channel and the monitoring wells are shown in Figure 2.1. On day 438 (May 24, 2005), the total chlorinated methanes (Total CMes) in channel 6 are shown as a function of position to decrease from ~12 μM to ~ 7 μM (Figure 2.6). Most of the CT decreased in the initial portion of the channel. Chloroform was produced, but the concentration was less than 1.5 μM and remained at that value throughout the length of the channel. Methylene chloride persisted in the channel and the outlet solution comprised mostly MC. Analysis of well samples on day 495 (July 21, 2005) revealed that the addition of SOME, to well 3 on day 445, led to considerable decrease of MC in the outlet (Figure 2.7). Figures 2.8 and 2.9 show that the concentrations remained low on days 590 and 614, respectively.

By day 741 (March 22, 2006), the SOME stored/sorbed in the channel may be nearly depleted and, therefore, the outlet CT increased (Figure 2.10). In the well samples, the concentration of total CMes in well 1 and well 2 were less, compared to well 3, in Figures 2.7 through Figure 2.10. The well samples may not be representative of the solution in the channel that leads to the outflow. The channel was 10 cm wide but the samples were collected from 0.5

cm diameter wells, which are about 0.2 cm from the bottom of the chamber. The channeling may result in depletion of hydrogen donor along the flow path to the outlet.

The wells were analysed on additional days but the results are not shown. For results on days 382, 395, 409, 460, 555, 644, 686, 712, 775, 804, 831, 864, 928 and 984 please see the supplement.

2.3.4. Soil Samples

The mass of aqueous phase and the moisture content fraction, dry weight of soil, and the concentration of CT in soil samples collected on day 96, June 25, 2004, are presented in Tables 2.6 and 2.7. The mass of aqueous phase increases with depth (Table 2.6) as expected, since the water diffuses through the vadose zone and evaporates through the soil surface, thus creating a gradient in moisture content. However, the mass of aqueous phase (channel 4 and 6, Table 2.6) and dry soil (Table 2.6) at depth 4 (34-42 cm) may be less than depth 3 because the soil sample below the saturated zone is usually wet and some of the collected soil drips while transferring to vial.

The field capacity of sandy soil is in the range 0.04-0.08 cm^3/cm^3 (Morgan et al., 2001). Assuming the bulk density of soil in the channel is 1.6 g/cm^3, the gravimetric field capacity of sandy soil is in the range 0.025-0.05 g/g. The moisture content of the soil samples from channels 4, 5, and 6 were in the range 0.02 to 0.17 on day 96 and 0.01 to 0.18 on day 151. The observed moisture content is greater than the minimum field capacity for sandy soil, except for the top soil in channel 5; the values were 0.02 on day 96 and 0.01 on day 151. The moisture content in channel 5 in the soil samples were consistently lower than the values observed in channels 4 and 6 during all sampling events.

CT was not detected in channel 5 at the depths that were investigated. In channels 4 and 6, at 37 cm depth, the concentrations of CT are 1.37 and 0.47 μM (Figure 2.11). No reaction intermediates were detected since, prior to day 96, nutrients were not added.

The moisture content and its fraction, and dry weight of soil for samples collected on day 151, August 10, 2004, are presented in Table 2.8. A sorption study was also conducted to estimate the fraction of organic matter content (f_{oc}) in the soil samples. After analyzing for the chlorinated methanes' concentrations, the soil samples were spiked with 1 mL of the PCE-CT standard mixture. The standard was prepared by injecting 10 μL of PCE liquid

and 10 μL of CT liquid in a clean amber glass bottle of volume 4.2 liters. The concentrations of PCE and CT in the standard bottle are, therefore, 3.83 mg/L and 3.76 mg/L. One mL of this gas mixture is then spiked onto the soil samples and a control. The control was prepared with 5 gms of glass beads (3 mm diameter, Arthur H. Thomas Co., Philadelphia, PA) and 1 mL of distilled water. The f_{oc} is then calculated by a mass balance and is listed in

Table 2.9. Typically, the organic matter content is high at the surface of a vegetated soil due to foliage, and decreases with depth. This trend is observed in channels 5 and 6, but not the case with channel 4. For modeling the same soil system, Zhang (1999) has used a value of 1 % for f_{oc}.

CT was not detected in the soil samples from channels 4 and 5. In channel 6, the concentrations were 0, 0.065, 0.089 and 0.166 μM with increasing depths (Figure 2.12). The concentration of CT increases with depth and the inlet/flow regime concentration is ~13 μM.

Figure 2.13 shows the concentration of CT in channels 4 and 6, on day 230, October 28, 2004. CT was not detected in channel 5. The concentration of CT increases from 0 to 0.35 μM and 0.69 μM in channels 4 and 6. On day 354, no CT or intermediate products were detected in channels 5 and 6 (Figure 2.14), since CT is degraded (see Figures 2.4 and 2.5).

Figures 2.15 through 2.19 show the concentrations of CT and reaction intermediates analyzed on day 812, June 12, 2006 and on day 996, December 3, 2006. In general, the concentrations increased with depth; however, the samples collected even in the deepest soil (saturated zone) did not have the same concentration as the flow regime at the bottom 5 cm of the channel (which is ~ 20 cm below the deepest soil sample collected).

2.4. Conclusions

Channeling occurs in the bottom zone of the channels leading to lower mean residence times for the tracer solution in the channels. Peak bromide concentration in the outlet occurred between 1.08 to 1.46 days corresponding to an outlet liquid volume of 1.1 L to 1.4 L, for the tracer study conducted immediately after harvesting plants. Since the outlet liquid flow rate will be less at the end of a month cycle (when plants are fully grown), the expected peak time would be longer, at the end of a month, than the peak time reported for the beginning of June 2004.

The maximum contaminant levels in drinking water are 0.03 μM for CT, 0.84 μM for CF and 0.06μM for MC. In channels 5 and 6, CT was below MCL during six and one sampling events respectively, out of 83 sampling events. After the addition of supplements, CF was below MCL during 16 out of 60 sampling events from day 146 to day 1106 in channel 5 and MC was below MCL during 46 out of 60 sampling events. After the addition of SOME, MC was below MCL during 5 out of 58 sampling events from day 226 to day 1106 in channel 6. The end point of MCL is usually a strict standard and, therefore, based on risk assessment, higher concentration of end point termed alternate cleanup level (ACL) may be permitted and used in field remediation (Anderson et al., 2004). The desired levels can be achieved by increasing the concentration of supplements and frequency of feeding.

Supplements such as glucose, corn starch, cheese whey and SOME stimulated the indigenous microbes and helped in the degradation of carbon tetrachloride (CT). However the pattern and rate of degradation of CT were different for different supplements. As a result, the degradation compound ratios were not the same in the glucose/corn starch/cheese whey and SOME amended channels. In both glucose and SOME fed channels, the degradation continued several days after stopping the feeding of supplements. The soil matrix and the microorganisms were able to store the supplements/degradation products of supplements and provide a long-term source of carbon and hydrogen. This fact is very important in the design of remediation systems in field sites and it determines the frequency of supplement addition. Glucose was found to be a better supplement than cheese whey for CT degradation; however, further study is necessary to determine if the concentration and frequency of cheese whey addition can improve CT degradation.

The outlet MC in SOME and CT fed channel depended on the mean residence time, unlike the glucose fed channel, where the concentration of MC was similar irrespective of the time of month. Most of the degradation process took place in the initial portion of the SOME fed channel where SOME was present, most likely due to flow restriction or sorption of SOME to soil organic matter. The concentration of MC was generally higher in the channel with SOME compared to the channel with glucose. In the soil sample analysis, CT was not detected in the vadose zone but found in the saturated zone in appreciable concentrations, in channels 4 and 6. This study demonstrated that the supplements glucose and SOME are effective substrates that can be added to CT contaminated groundwater to promote degradation of CT.
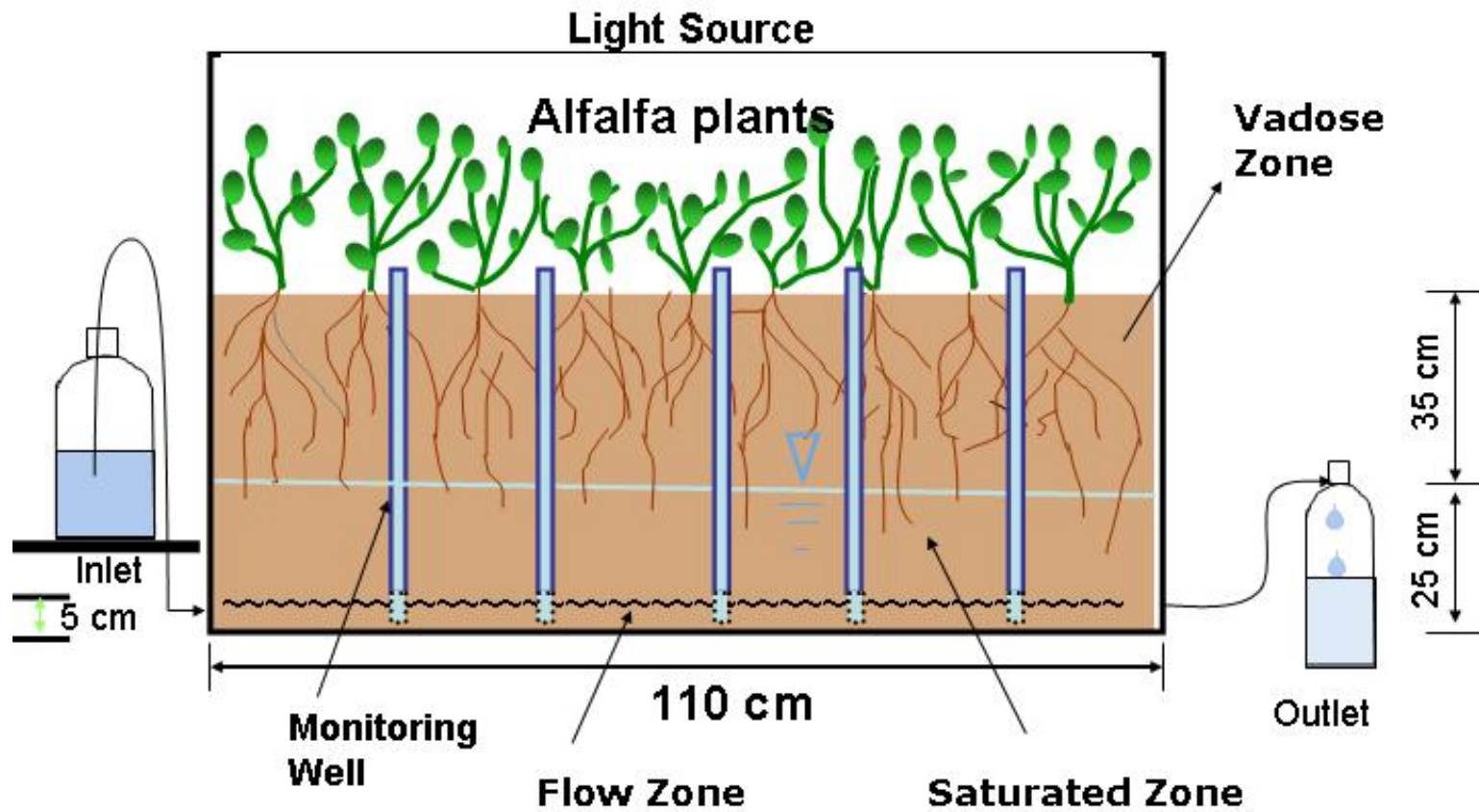
Figure 2.1. Schematic and cross section of a channel in the six-channel system.
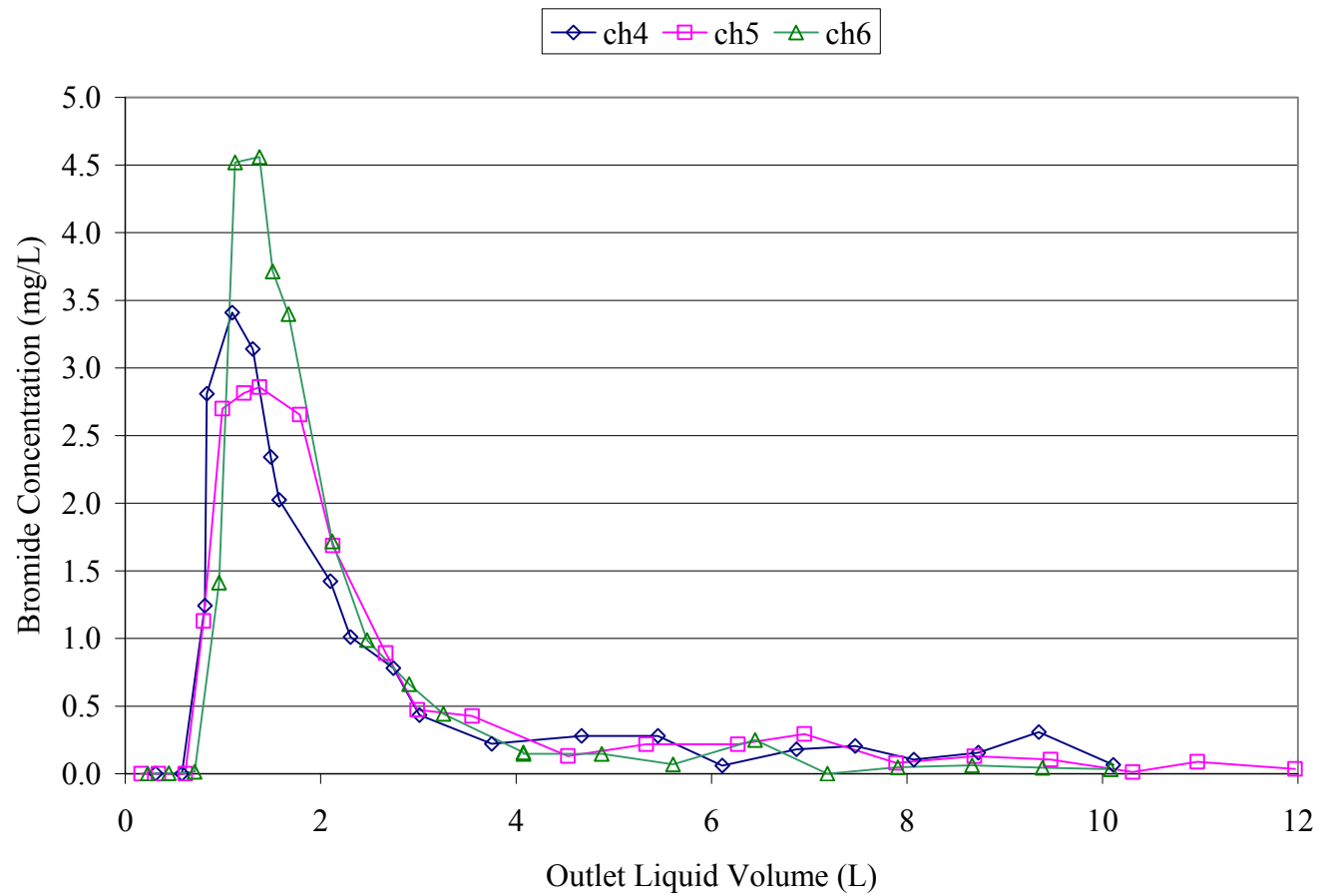
Figure 2.2. Concentration of bromide in the outlet liquid vs cumulative outlet liquid volume for channels 4, 5 and 6. Inlet bromide concentration = 100 mg/L.
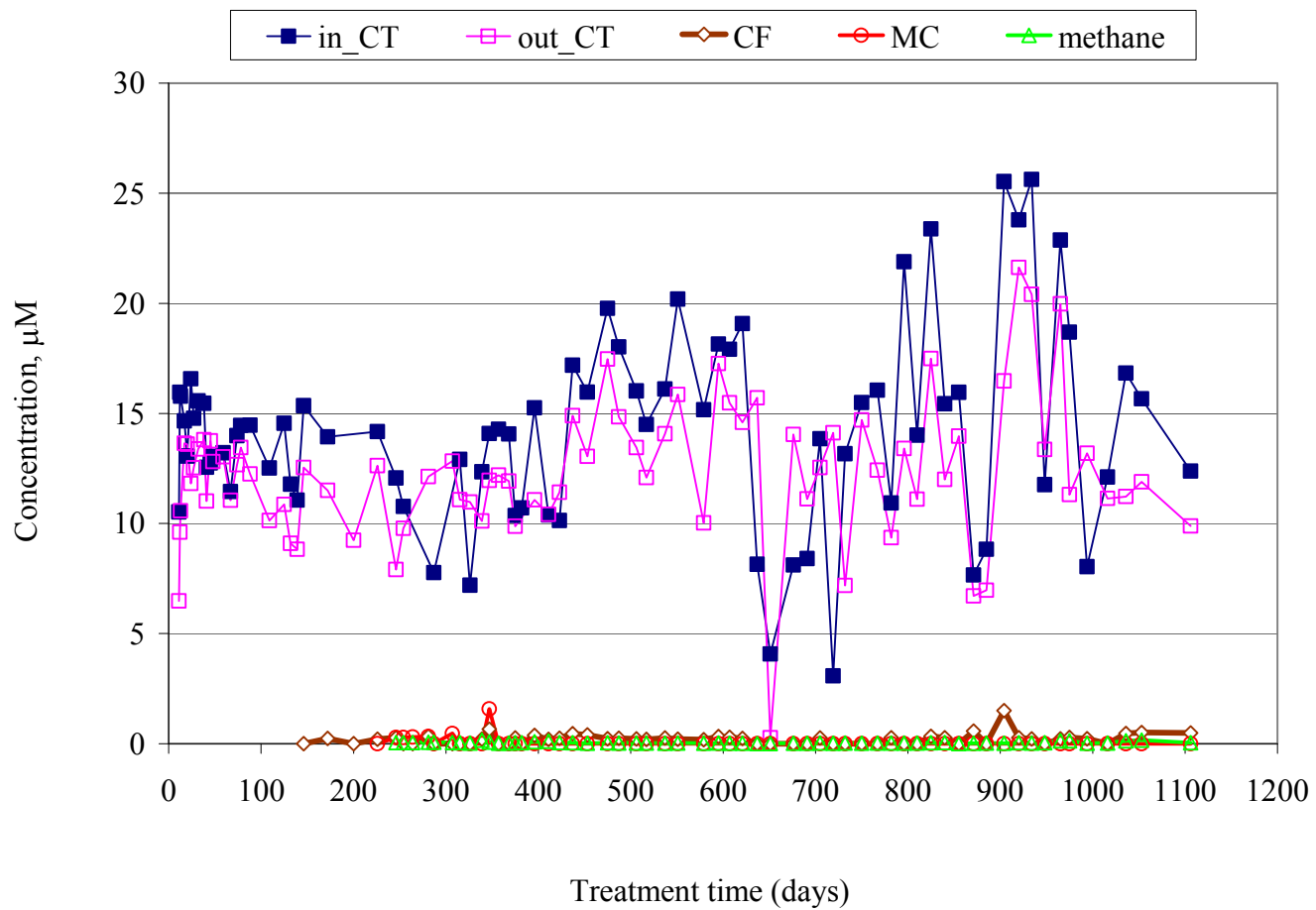
Figure 2.3. Inlet CT and outlet CT, CF, MC and methane concentrations for channel 4 (control). Water samples taken on indicated days after beginning (March 12, 2004) exposure.
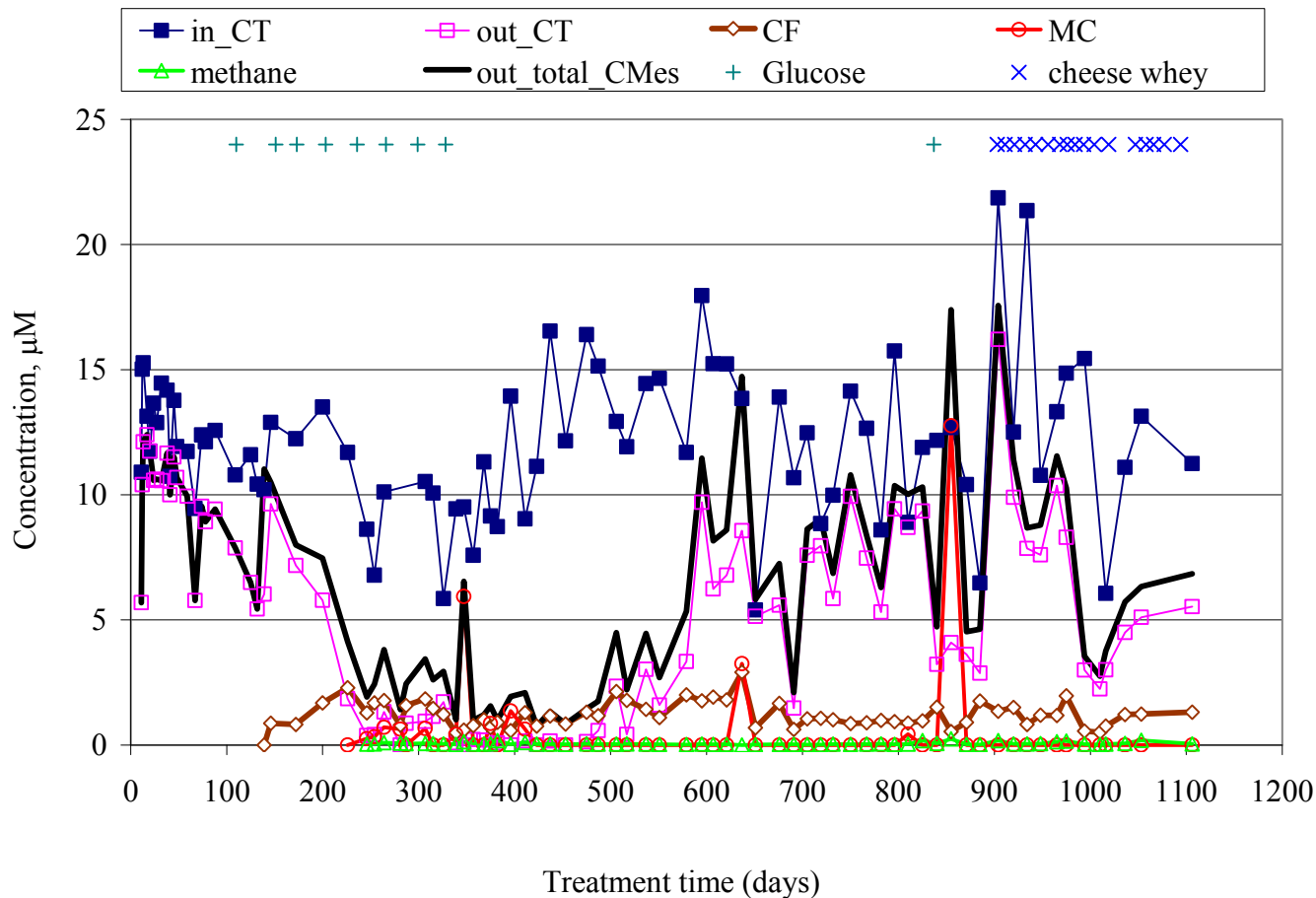
Figure 2.4. Inlet CT and outlet CT, CF, MC and methane concentrations for channel 5. Water samples taken on indicated days after beginning (March 12, 2004) exposure. Glucose solution was added on days 110, 151, 173, 203, 236 and 837; corn starch on days 266, 299 and 328. Cheese whey was added on days 903, 911, 921, 932, 943, 956, 968, 976, 984, 993, 1004, 1019, 1047, 1058, 1077, 1094 and 1111.

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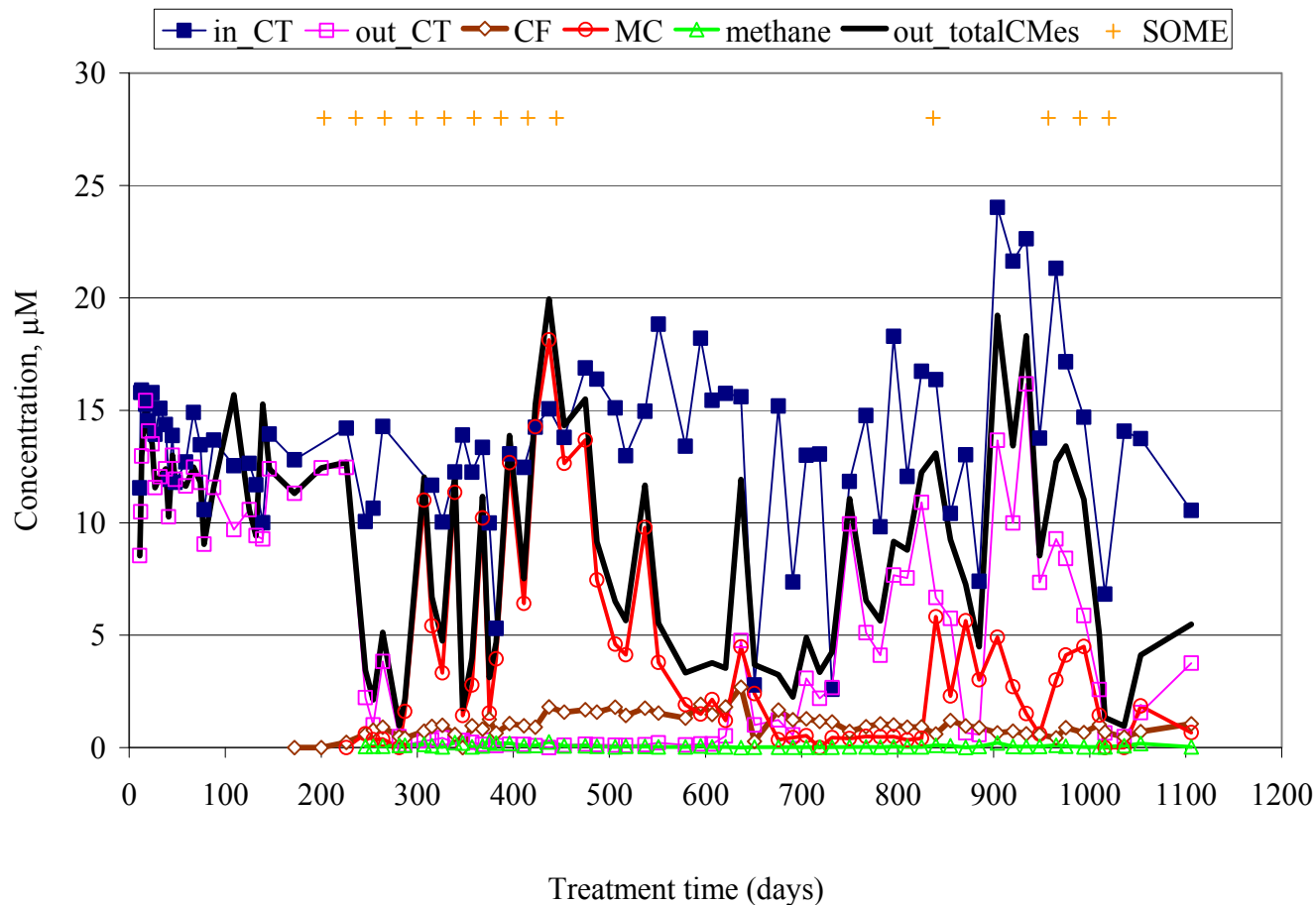
Figure 2.5. Inlet CT and outlet CT, CF, MC and methane concentrations for channel 6. Water samples taken on indicated days after beginning (3/12/2004) exposure. Soy Oil Methyl Esters (SOME) added on days 203, 236, 266, 299, 328, 359, 387, 415, 445 (well 3), 837, 957 (well 2), 990 (well 1) and 1020 (well 3).
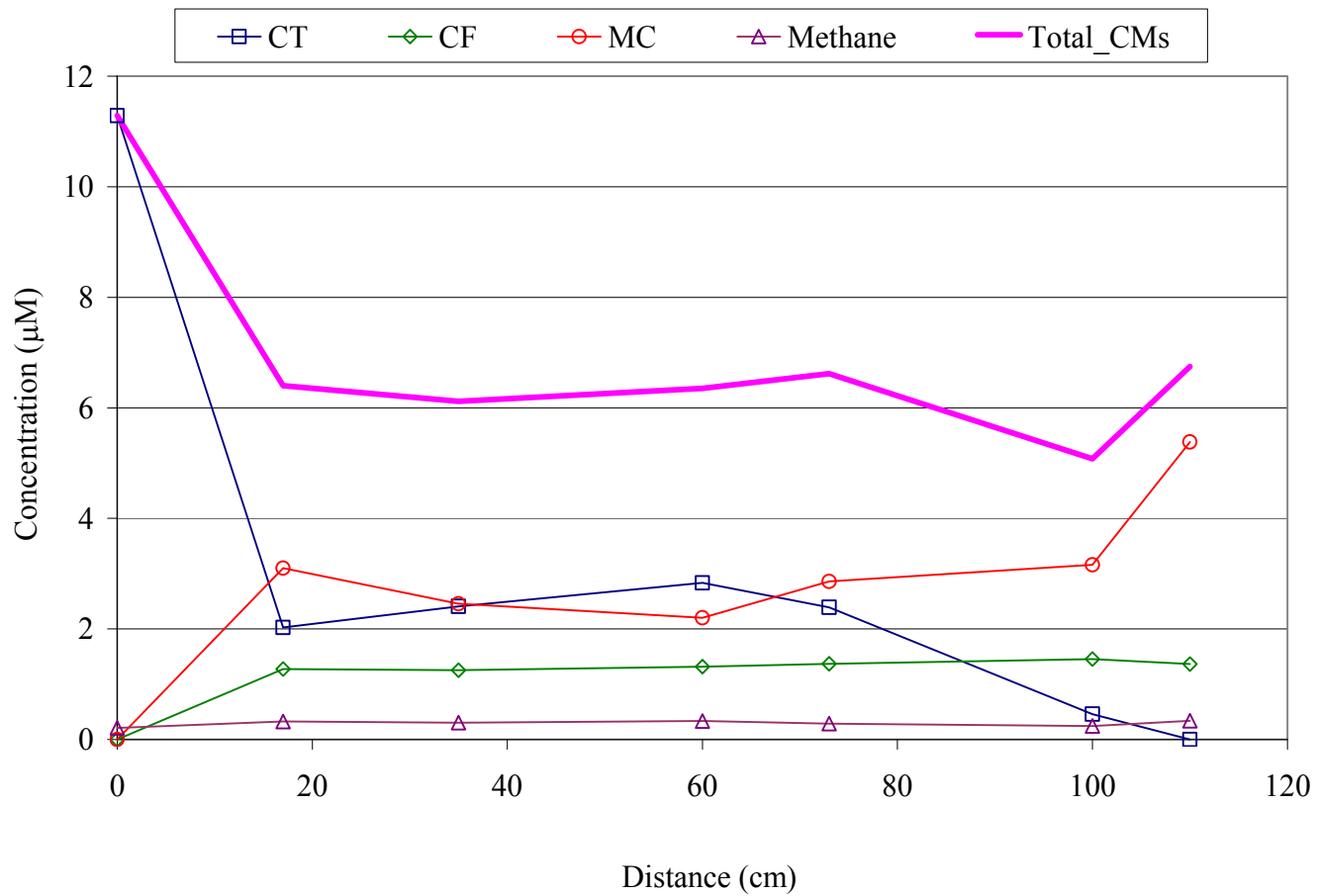
Figure 2.6. Variation of CT and degradation compounds with distance along channel 6 on day 438, 5/24/05. Soy Oil Methyl Esters (SOME) added on days 203, 236, 266, 299, 328, 359, 387 and 415.
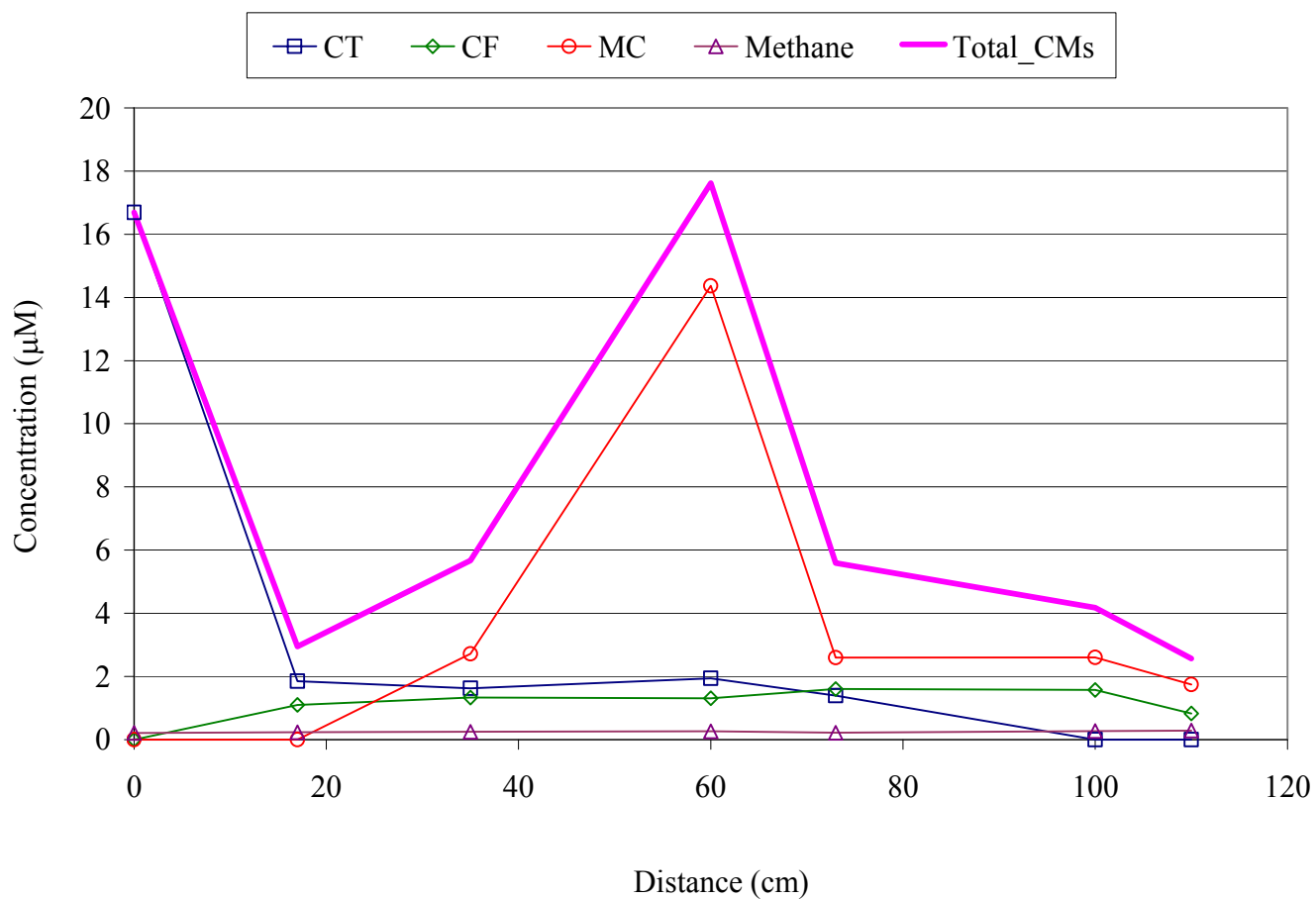
Figure 2.7. Variation of CT and degradation compounds with distance along channel 6 on day 495, 7/21/05. Soy Oil Methyl Esters (SOME) added on days 203, 236, 266, 299, 328, 359, 387, 415 and 445 (well 3).
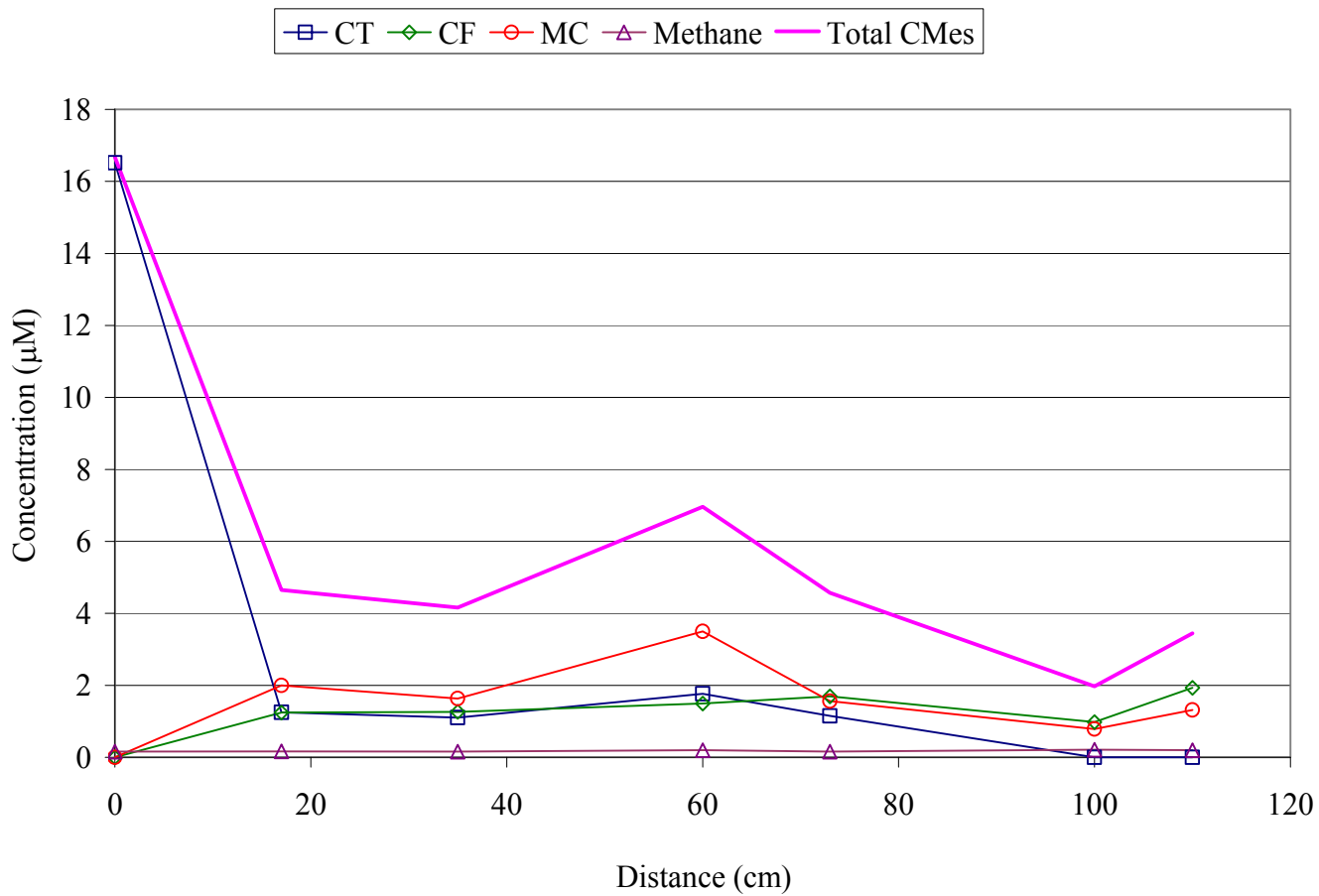
Figure 2.8. Variation of CT and degradation compounds with distance along channel 6 on day 590, 10/23/05. Soy Oil Methyl Esters (SOME) added on days 203, 236, 266, 299, 328, 359, 387, 415 and 445 (well 3).
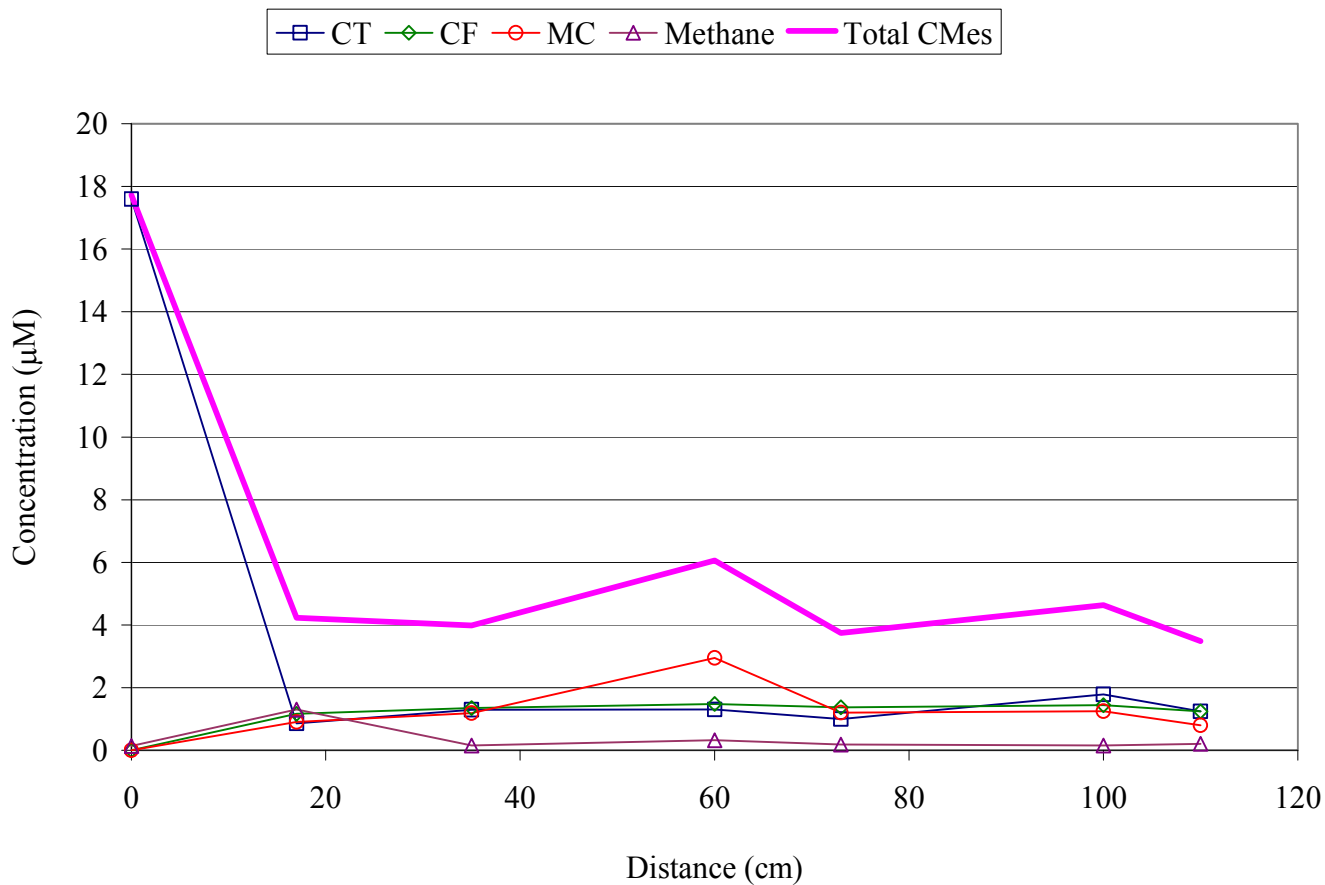
Figure 2.9. Variation of CT and degradation compounds with distance along channel 6 on day 614, 11/16/05. Soy Oil Methyl Esters (SOME) added on days 203, 236, 266, 299, 328, 359, 387, 415 and 445 (well 3).
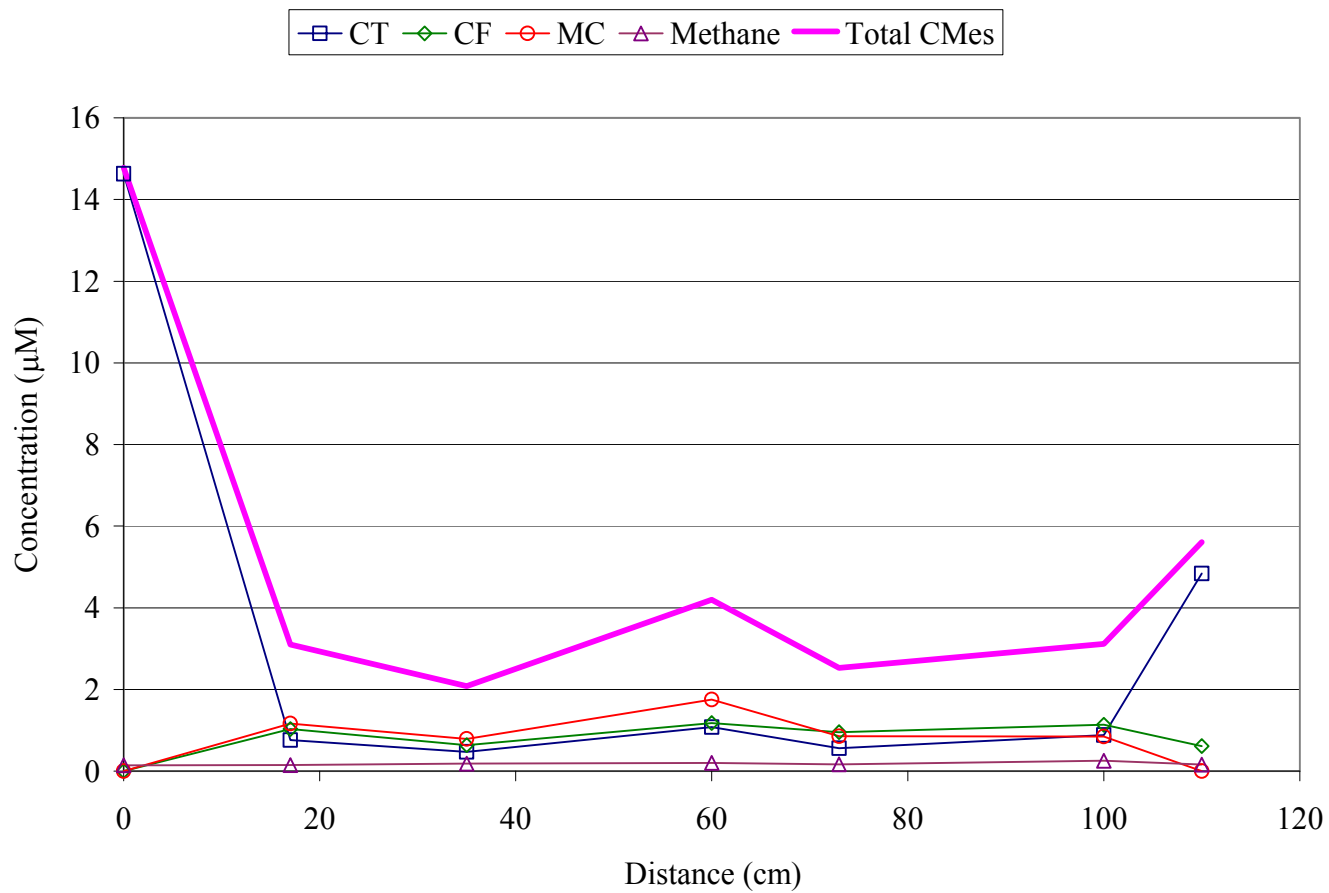
Figure 2.10. Variation of CT and degradation compounds with distance along channel 6 on day 741, 3/22/06. Soy Oil Methyl Esters (SOME) added on days 203, 236, 266, 299, 328, 359, 387, 415, 445 (well 3).
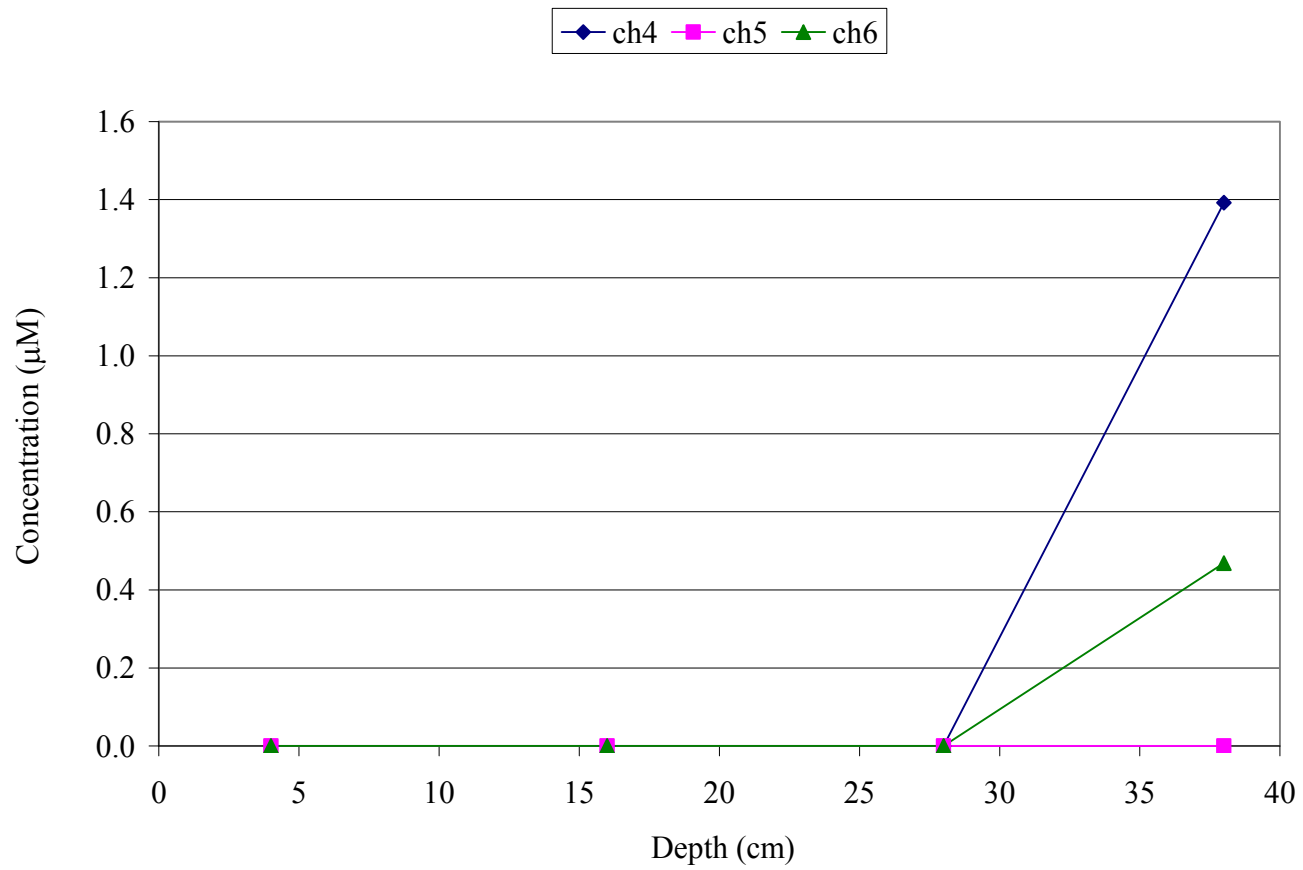
Figure 2.11. CT concentration in the soil samples of channels 4, 5 and 6, on June 16, 2004, day 96. Inlet concentration is ~ 13 µM. Soil samples were collected at a distance of 30 cm from inlet.
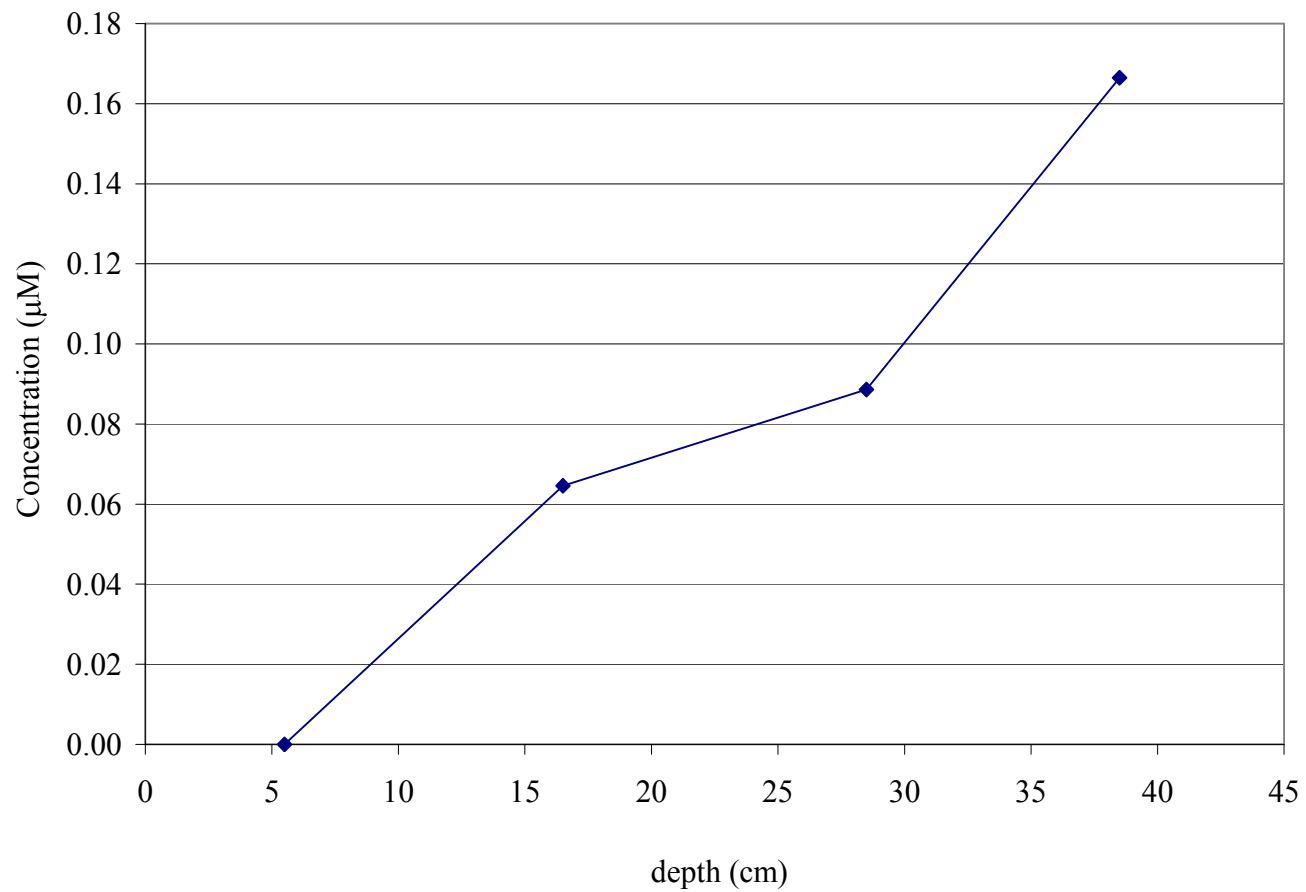
Figure 2.12. CT in channel 6 soil, as a function of depth from the surface of the soil, on day 151, August 10, 2004. CT was not detected in channels 4 and 5. Soil samples were collected at a distance of 57 cm from inlet.
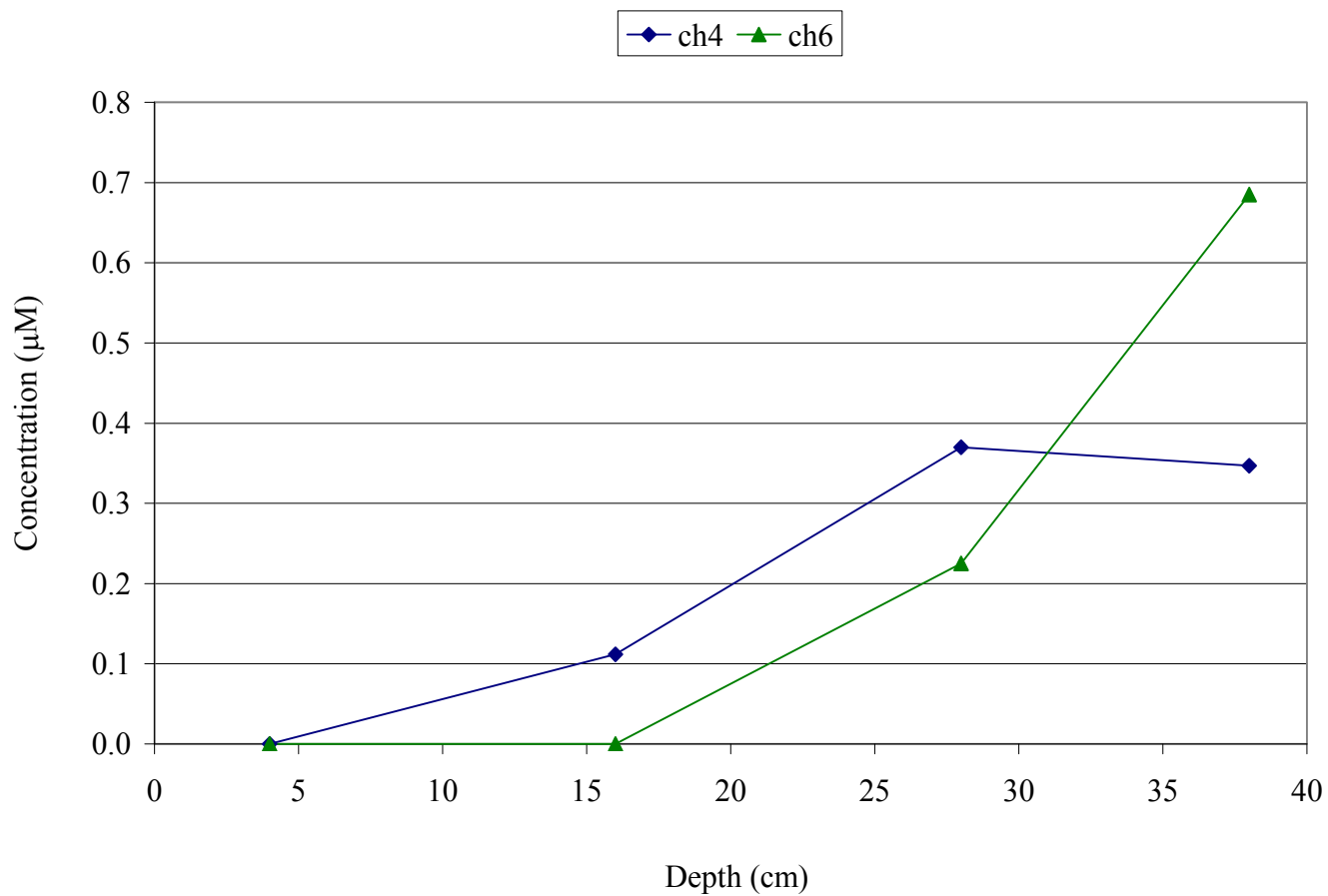
Figure 2.13. CT concentration, as a function of depth from the soil surface, in the soil samples of channels 4 and 6, on day 230, October 28, 2004. Soil samples were collected at a distance of 65 cm from inlet. Inlet concentration is ~ 13 μM. CT was not detected in channel 5.
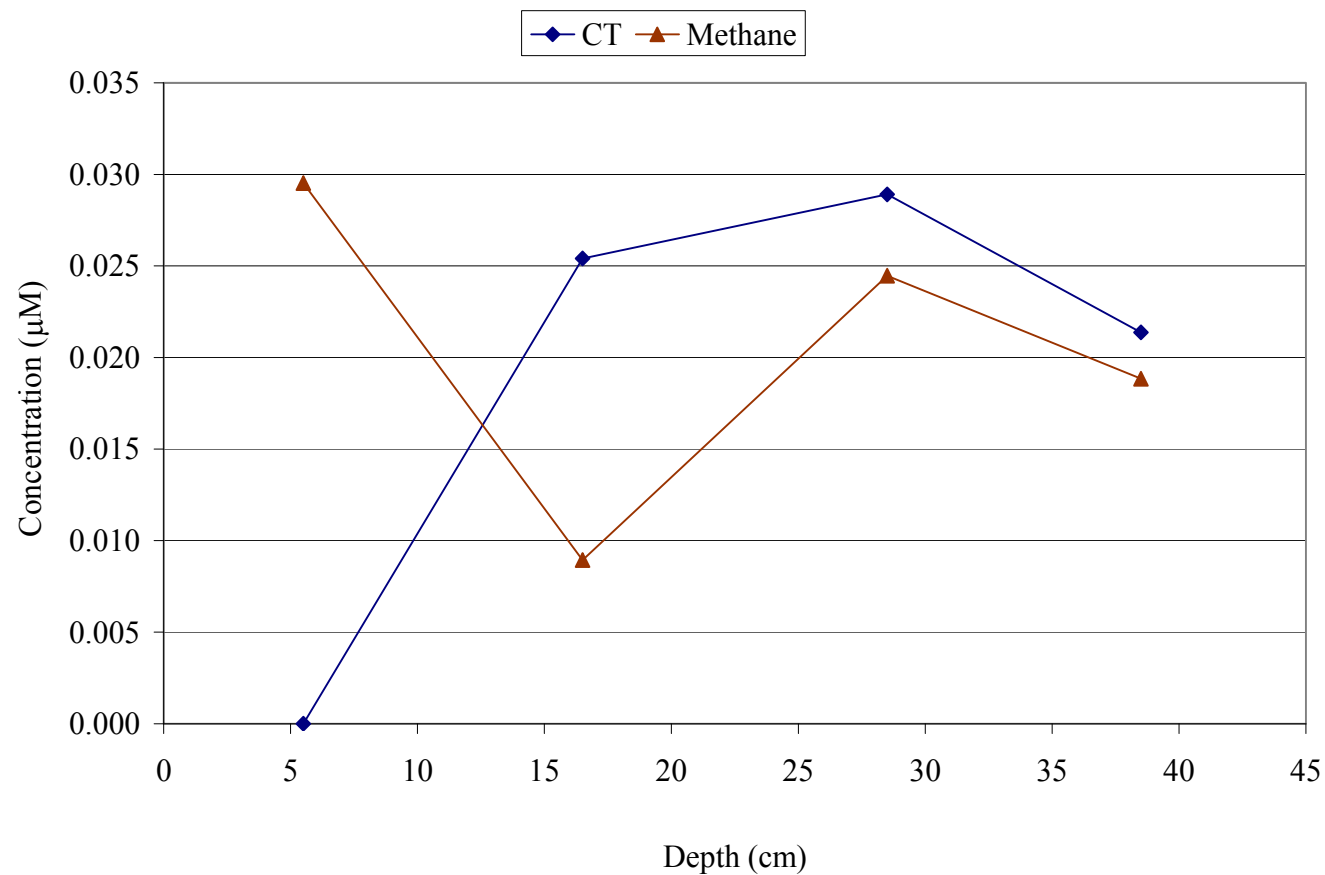
Figure 2.14. CT and methane concentration in channel 4 soil, as a function of depth from the surface of the soil, on day 354, March 1, 2005. Soil samples were collected at a distance of 90 cm from inlet. CT and reaction intermediates were not detected in channel 5 and channel 6.
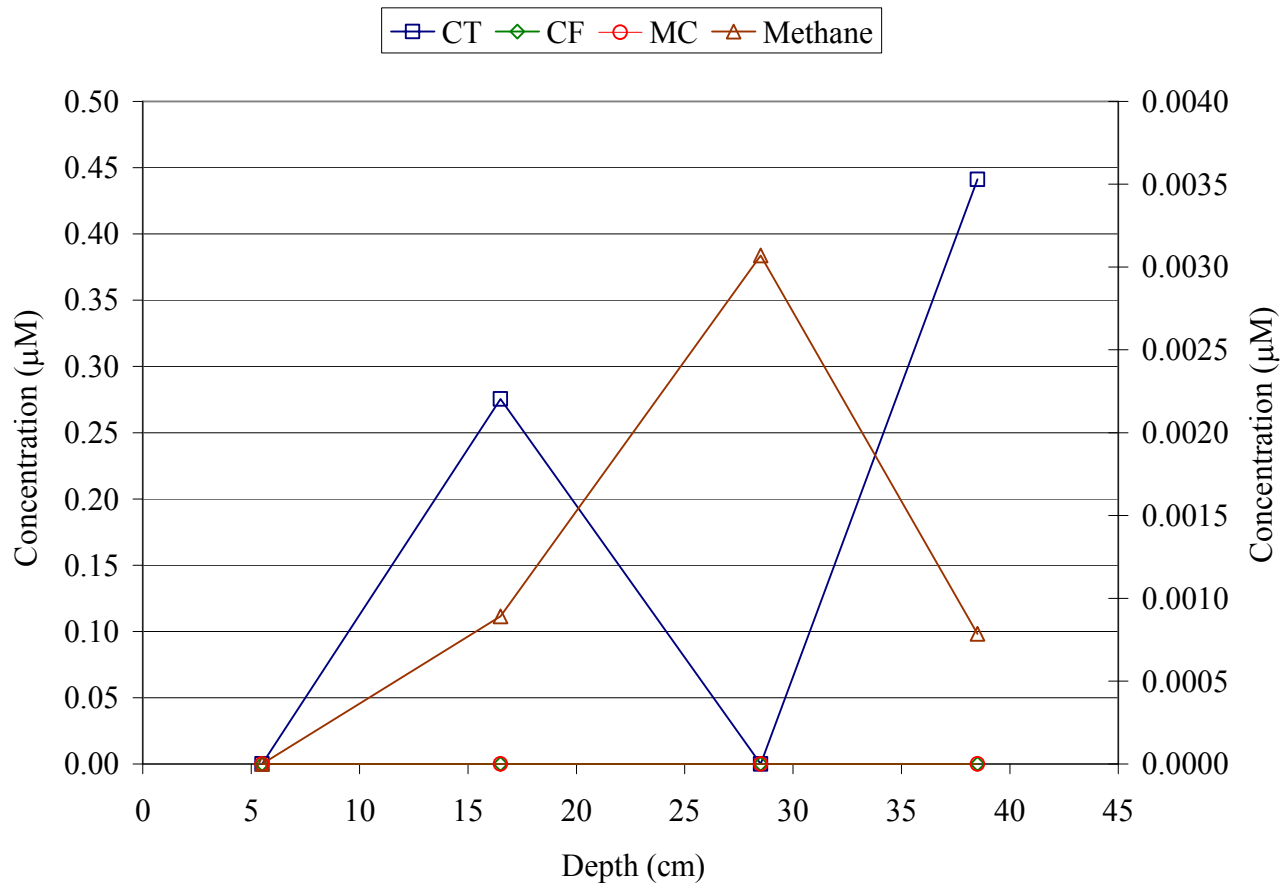
Figure 2.15. Chlorinated methanes (CMes) and methane profile, in channel 4 soil, as a function of depth from the surface of the soil on day 812, June 12, 2006; Methane concentrations are on right hand (secondary) y-axis. Soil samples were collected at a distance of 28 cm (depths 4.5 cm and 16.5 cm) and 37 cm (depths 28.5 cm and 38.5 cm) from inlet.
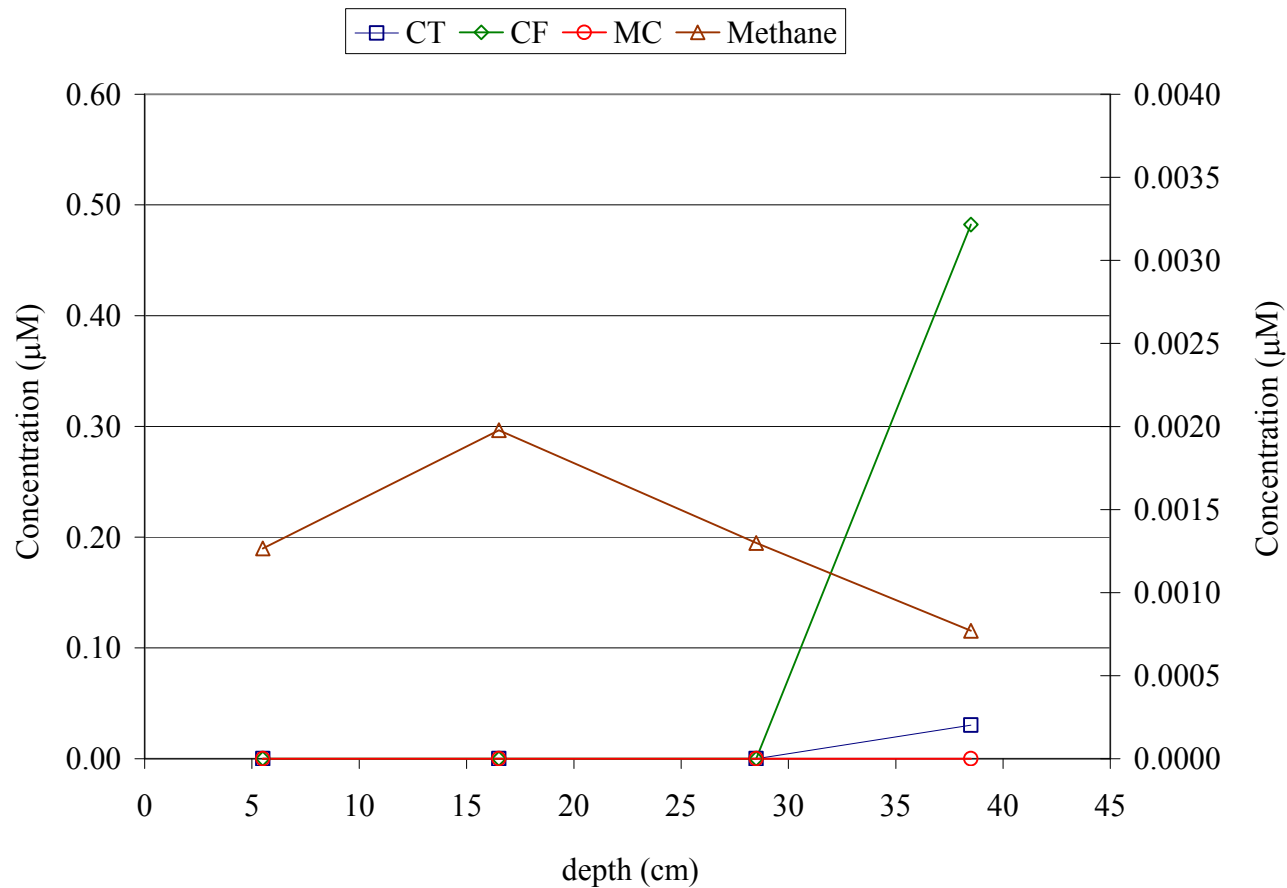
Figure 2.16. Chlorinated methanes (CMes) and methane profile, in channel 5 soil, as a function of depth from the surface of the soil on day 812, June 12, 2006; Methane concentrations are on right hand (secondary) y-axis. Soil samples were collected at a distance of 12 cm from inlet.
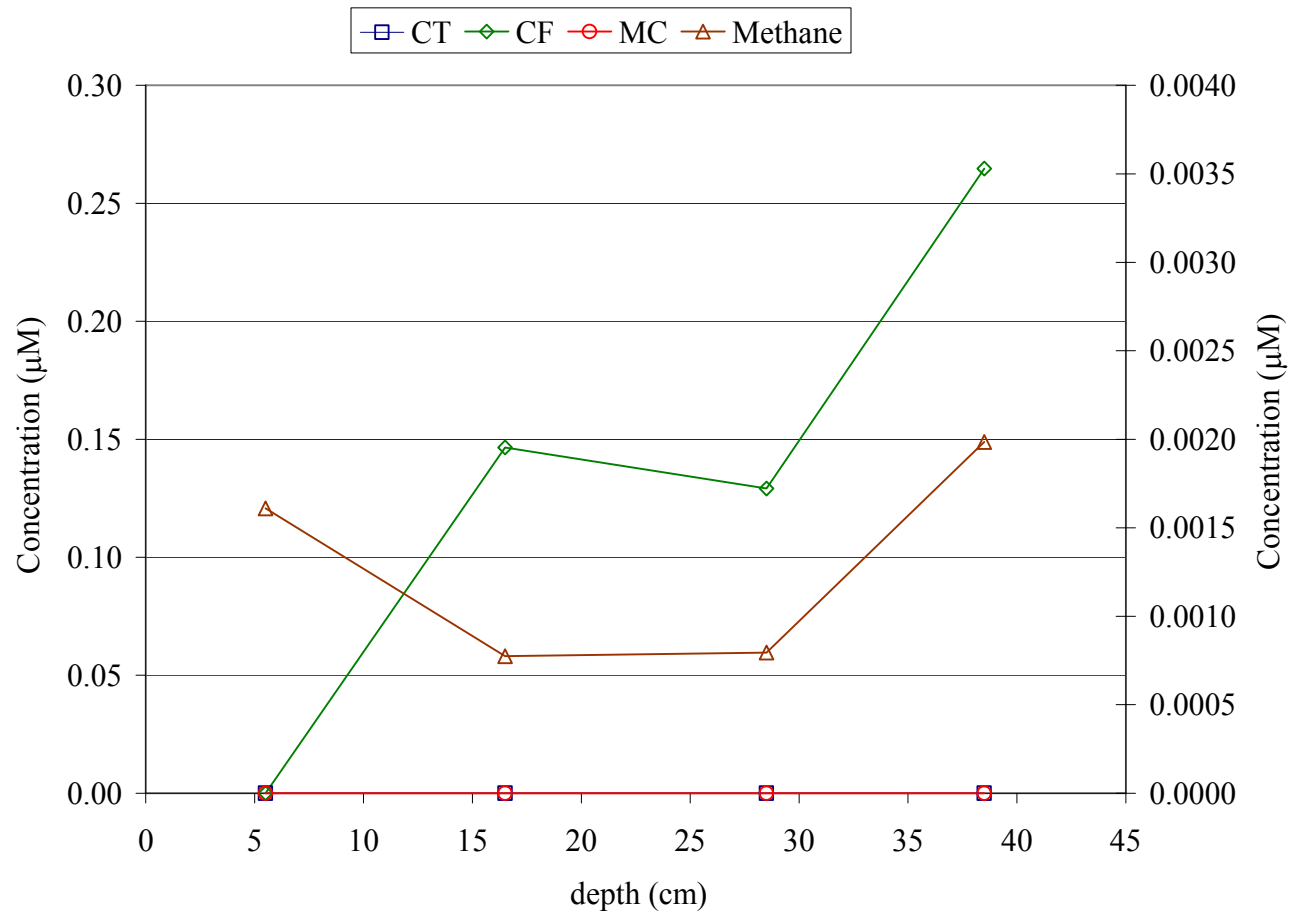
Figure 2.17. Chlorinated methanes (CMes) and methane profile, in channel 6 soil, as a function of depth from the surface of the soil on day 812, June 12, 2006; Methane concentrations are on right hand (secondary) y-axis. Soil samples were collected at a distance of 12 cm from inlet.
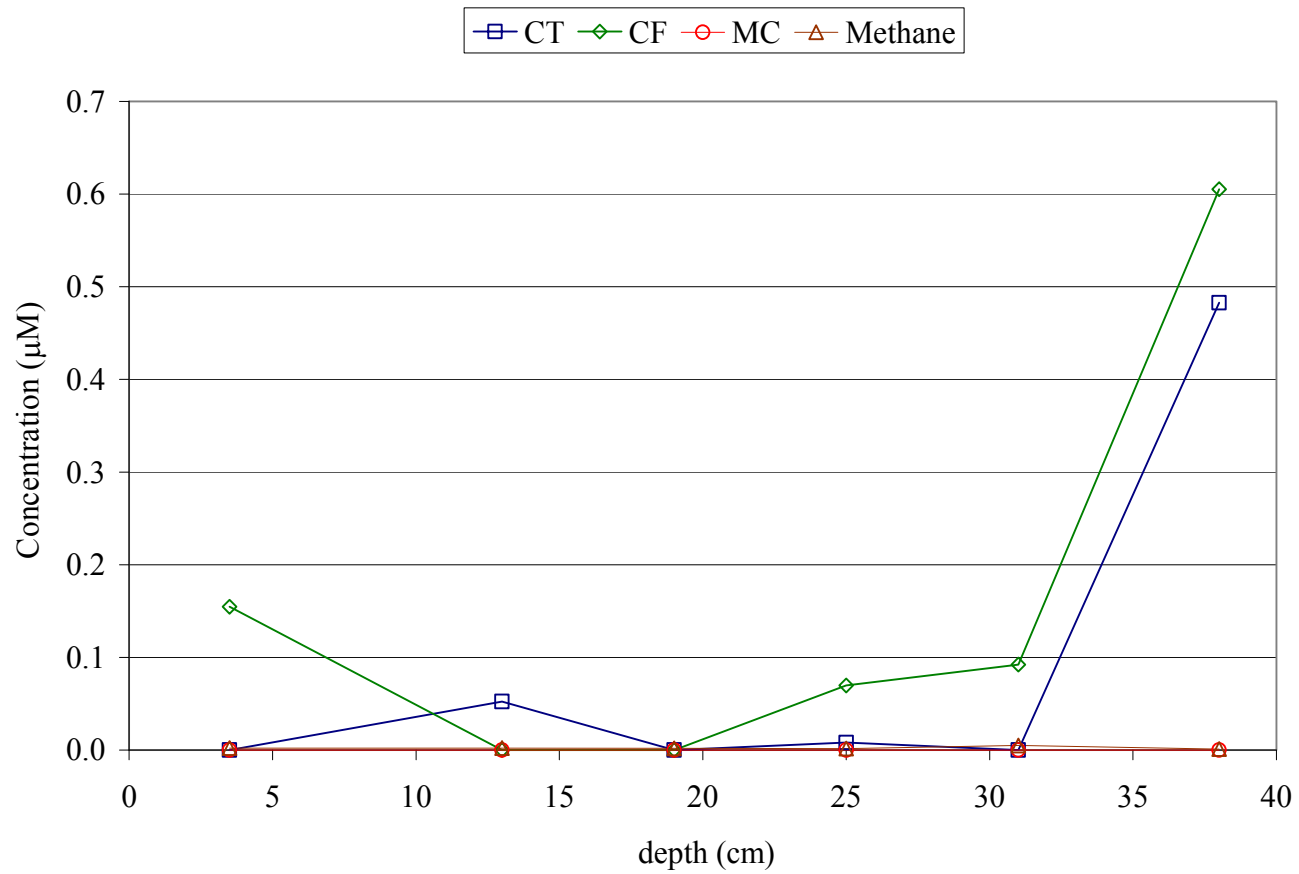
Figure 2.18. CMes and methane profile, in channel 5 soil, as a function of depth from the surface of the soil on day 995, December 3, 2006. Soil samples were collected at a distance of 26 cm from inlet.
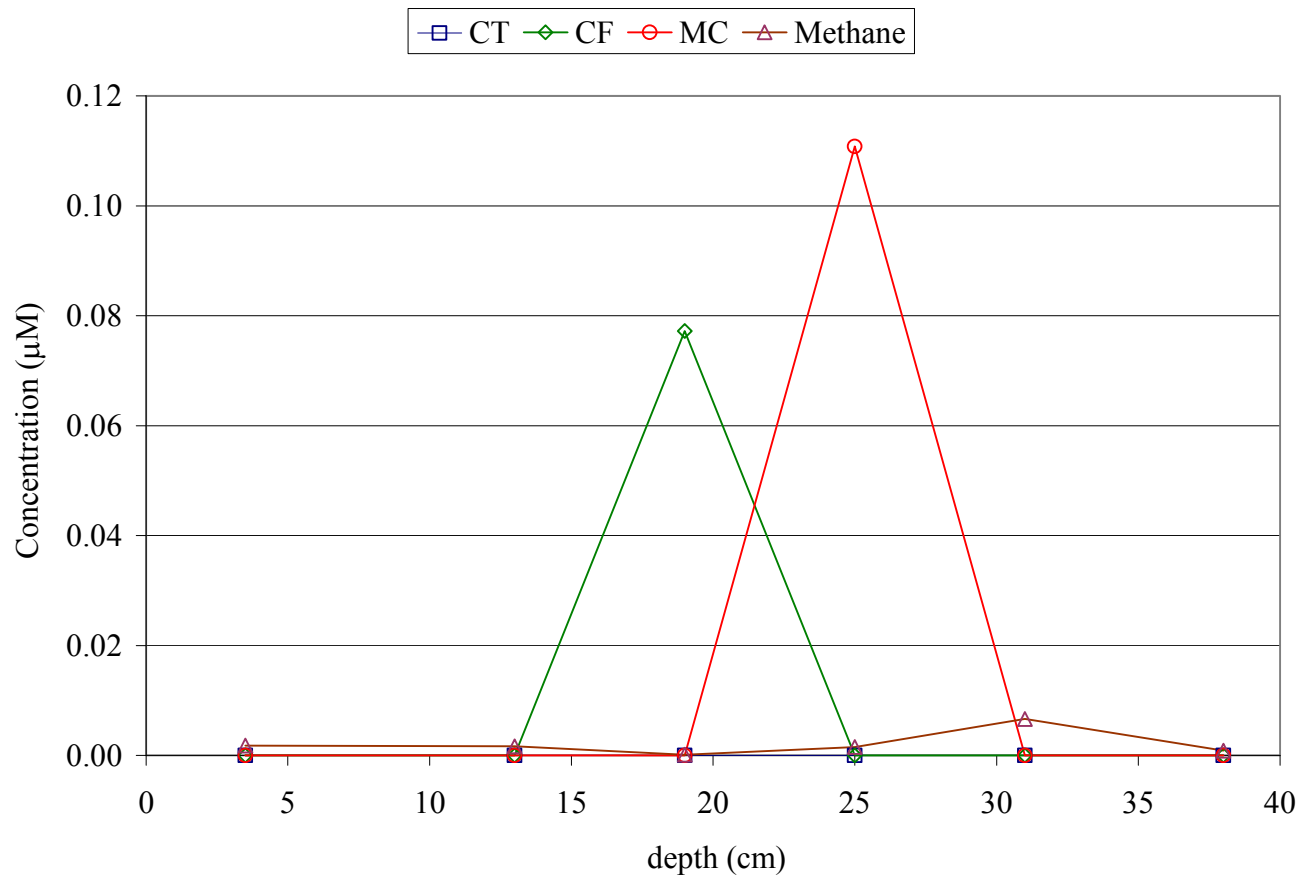
Figure 2.19. CMes and methane profile, in channel 6 soil, as a function of depth from the surface of the soil on day 995, December 3, 2006. Soil samples were collected at a distance of 12 cm from inlet.

Table 2.1 Supplements feeding history for channels 5 and 6.

Date	Day	Channel 5	Channel 6
2004			
June 30	110	G	
August 10	151	G	
September 1	173	G	
October 1	203	G	S
November 3	236	G	S
December 3	266	CS*	S
2005			
January 5	299	CS*	S
February 3	328	CS*	S
March 6	359		S
April 3	387		S
May 1	415		S
May 31	445		S (well 3)#
2006			
June 27	837	G	S
September 1	903	CW	
September 9	911	CW	
September 19	921	CW	
September 30	932	CW	
October 11	943	CW	
October 24	956	CW	
October 25	957		S (well 2)#
November 5	968	CW	
November 13	976	CW	
November 21	984	CW	

Date	Day	Channel 5	Channel 6
November 27	990		S (well 1)
November 30	993	CW	
December 17	1004	CW	
December 26	1019	CW	
December 27	1020		S (well 3)
2007			
January 23	1047	CW	
February 3	1058	CW	
February 11	1066	CW	
February 22	1077	CW	
March 11	1094	CW	
March 28	1111	CW	
April 12	1126	CW	
April 22	1136	CW	

Key: G - Glucose; CS – Corn Starch; CW – Cheese Whey; S – SOME or Soy Oil Methyl Esters.

* From December 3, 2004 to February 3, 2005, Corn Starch was added instead of glucose, inadvertently.

On day 445, May 31, 2005, 100 mL of 1 % SOME was injected into well 3 of channel 6, through nylon tubing; the solution was fed near the bottom of the channel.

Table 2.2 Tracer peak concentration in outlet and mean residence time for channels 4, 5 and 6.

Channel	Volume of outlet liquid (L)*	Peak bromide concentration time (days)*	Bromide peak concentration in outlet liquid (mg/L)	Mean residence time (days)[#]
4	1.095	1.125	3.41	3.6
5	1.375	1.42	2.86	3.4
6	1.375	1.25	4.56	2.8

*Values are the liquid volume and residence time associated with the peak concentration.

[#] Mean residence time was estimated from the residence time distribution (RTD) model.

Table 2.3 Mass balance for tracer study in channels 4, 5 and 6, for the 14 day period.

Channel	Cumulative volume of outlet water (L)	Cumulative water uptake by plants (L)	Bromide in outlet liquid (mg)	Bromide trapped in soil by upflow* (mg)	Total bromide[#] accounted (mg)	Percent recovery (%)
4	10.11	4.744	5.26	2.20	7.46	74
5	11.97	2.285	5.42	0.82	6.24	62
6	10.09	3.271	5.50	1.85	7.35	73

*Estimated based on the assumption that the bromide concentration in the upflow due to evapotranspiration is the same as that in the outlet liquid for a particular day.

[#]The mass of bromide added at the inlet was 10.05 mg.

Table 2.4. Bromide recovery vs solution recovery in channels 4, 5 and 6, for the 14 day period, from June 4, 2004 to June 18, 2004.

Channel	Volume of outlet liquid over total liquid fed (L/L)	Solution recovered (%)	Bromide in outlet liquid (mg) out of total 10.05 mg	Bromide recovered (%)
4	10.11/14.84	68	5.26	52
5	11.97/14.25	84	5.42	54
6	10.09/13.36	76	5.50	55

Table 2.5. Effect of evapotranspiration on the residence time of compounds in channels 4, 5 and 6; Estimated from data in June 2004.

Channel	Variation in the daily exit liquid volume (L/day) for 1 month period	Volume of exit liquid for peak concentration of tracer (L)	Peak time (days)	Estimated variation in the time for the volume of liquid (corresponding to peak bromide concentration) to exit at the start and the end of a month (days)*
4	0.8-0.6	1.095	1.125	1.4-1.8
5	0.9-0.6	1.375	1.42	1.5-2.3
6	0.8-0.4	1.375	1.25	1.7-3.4

*This value is obtained by dividing the volume of exit liquid corresponding to peak concentration by the volume of liquid collected at the outlet each day, at the beginning and the end of June 2004. For example, for channel 4, the time taken for collecting 1.095 L of liquid at the outlet in the beginning of June 2004 is 1.095/0.8 = 1.4 days and similarly, the time taken for collecting 1.095 L of liquid at the outlet at the end of June 2004 is 1.095/0.6 = 1.8 days.

Table 2.6. Mass of aqueous phase, moisture content fractions, and dry weight of soil in the soil samples collected on day 96 from channels 4, 5 and 6. Soil samples were collected at a distance of 30 cm from inlet.

	Depth (cm)	4	5	6
Mass of aqueous phase (gm)	0-8	0.646	0.0847	0.556
	12-20	1.301	0.2248	1.1177
	24-32	1.2986	0.2	1.2941
	34-42	1.0876	0.6138	1.1905
	Depth (cm)	4	5	6
Moisture content fraction (w/w)	0-8	0.11	0.02	0.17
	12-20	0.15	0.05	0.15
	24-32	0.16	0.04	0.16
	34-42	0.17	0.11	0.17
	Depth (cm)	4	5	6
Mass of dry soil (gm)	0-8	5.0123	4.8131	2.7889
	12-20	7.2423	4.6755	6.2645
	24-32	6.9205	5.3338	6.6048
	34-42	5.4111	5.1084	6.0009

Table 2.7. Concentration of CT in the soil samples of channels 4, 5 and 6, collected on June 16, 2004, day 96. Inlet concentration ~ 13 μM. Soil samples were collected at a distance of 30 cm from inlet.

Sample	Depth (cm)	Concentration of CT (μM)		
		Channel 4	Channel 5	Channel 6
1	0-8	0.00	0.00	0.00
2	12-20	0.00	0.00	0.00
3	24-32	0.00	0.00	0.00
4	34-42	1.4	0.00	0.47

Table 2.8. Mass of aqueous phase, moisture content fractions, and dry weight of soil in the soil samples (day 151) collected from channels 4, 5 and 6. Soil samples were collected at a distance of 52, 57 and 57 cm from inlet for channels 4, 5 and 6 respectively.

	Depth (cm)	4	5	6
Mass of aqueous phase (gm)	0-8	0.7775	0.0196	0.7136
	12-20	0.8195	0.197	0.9969
	24-32	0.9443	0.561	1.0036
	34-42	0.7958	0.6056	1.0857
	Depth (cm)	4	5	6
Moisture content fraction (w/w)	0-8	0.18	0.01	0.14
	12-20	0.13	0.03	0.14
	24-32	0.14	0.08	0.15
	34-42	0.13	0.09	0.15
	Depth (cm)	4	5	6
Mass of dry soil (gm)	0-8	3.5984	2.7366	4.406
	12-20	5.3886	5.4419	6.3735
	24-32	5.732	6.2972	5.9163
	34-42	5.1985	6.3515	6.3307

Table 2.9. Fraction of organic matter content (as %) in CT channel soil samples (day 151).

Sample	Depth (cm)	4	5	6
1	0-8	0.55	0.44	0.34
2	12-20	0.25	0.63	0.48
3	24-32	0.45	0.66	0.37
4	34-42	0.47	0.75	0.27

CHAPTER 3 - An Experimental Laboratory Study on Bioremediation of Tetrachloroethene Contaminated Groundwater

3.1. Introduction

In this work, the degradation of tetrachloroethene (PCE) was studied in a six-channel soil chamber system. The degradation of PCE is mainly limited by the availability of electron donors. Two different substrates were used in this study, to create the necessary reducing conditions favorable for PCE transformation. Soil samples were collected at several depths in the channels to determine the vertical distribution of PCE and degradation products. Headspace samples were analysed to check if any compounds diffused through the vadose zone to the soil surface. A tracer study was also conducted in the channels, at the beginning of the biodegradation study, to understand the flow pattern and the residence time distribution of the tracer. This information was used in the analysis of the fate of the PCE that enters at the inlet.

3.2. Materials and Methods

3.2.1. Experimental System or Mesocosm

The experimental system consisted of six independent channels that were constructed with steel bottoms, side panels, and end panels. Each channel was 110 cm long, 10 cm wide and 65 cm high with soil depth of 60 cm. The channels were packed with alluvial silty sand soil (less than 10 % silt) collected near the Riley County landfill in Riley County, Kansas. This soil system was used for contaminant fate studies previously (Zhang, 1999). Channels 1, 2 and 3 were used for the PCE study. Alfalfa was grown in channels 1 and 2, while fescue grass was grown in channel 3 (Figure 3.1). The channels were filled with alluvial silty sand soil (< 10% silt) up to ~ 60 cm. The soil was collected from a site near a landfill in Riley County, Kansas in 1993 (Zhang, 1999). Soils were collected from three different soil zones and filled in the channels. The organic carbon contents of the soil collected from the field are 1.8% in the top depth (0-15 cm), 1.0% in the middle depth (15-40 cm), and 0.3% in the bottom depth (below 40 cm). The types of soil collected from the field are 90% sand and 10% silt in the top depth, 91.5% sand and 8.5% silt in

the middle depth, and 96% sand and 4% silt in the bottom depth (below 40 cm) (Narayanan, 1994).

Alfalfa was grown in channels 5 and 6, while fescue grass was grown in channel 4. A pair of fluorescent tube lights (40 W) for each channel, placed at a height of 50 cm above the soil surface, provides the light source for the plants (Figure 3.1). The photosynthetically active radiation (PAR) at 40 cm from the soil surface (the average plant height) was about 160 $\mu E/m^2/s$ (measured by using a L1-188B Integrating Quantum Radiometer/Photometer) (Zhang, 1999). The inlet water was fed at 5 cm above the bottom of the channels (see Figure 2.1). Watering of plants started from November 5, 2003. During the initial period the plants were observed, without contaminants in the soil. Until March 12, 2004, only distilled water was fed into the channels and the plants were growing well.

The contaminant (PCE) was introduced starting from March 12, 2004 at a concentration of about 2 mg/L (~12 μmoles/L) in three channels, two of them with alfalfa plants and the other with fescue grass. The height of saturated zone in each channel was controlled by the vertical position of the end of each outlet tube (25 cm in this system). The volume of water per channel corresponding to 25 cm of saturated zone is estimated to be 8.25 L, assuming a porosity of 0.3. Plants were harvested at the beginning of each month by cutting the top portion; after harvest, the plant height was approximately 10 cm. At the end of the month, the fescue grass and alfalfa grew to a height of approximately 50 cm and 70 cm, respectively.

Initially, nicotine hemisulfate solution was sprayed, weekly, on channel 3 to destroy the pest euphids on the grass. Later, this pesticide solution was sprayed on all the channels. The pesticide solution was prepared by adding 10 mL of nicotine hemisulfate salt (40 % aqueous solution (w/v), Sigma, St. Louis, MO) to 500 mL of distilled water. From February 2007, 1 % solution of potassium salt of a fatty acid was used as insecticide.

The amount of nitrogen, phosphorus and potassium (NPK) consumed by the plants and removed by harvesting since the beginning of the experiment, was computed. A fertilizer solution was prepared with required NPK and one liter of the solution was applied to the top soil of each channel.

After the initial preparation of inlet contaminant solution at 2 mg/L it was maintained by adding 100 mL of ~40 mg/L PCE stock solution (prepared from PCE 99 % purity, Sigma-Aldrich, St. Louis, MO)) and 900 mL of distilled water, everyday. This stock solution was

necessary to maintain approximately 2 mg/L in the inlet solution to each channel. As the inlet solution flows out of the bottle, the volume of the headspace in the inlet bottle increases, and air is drawn into the inlet bottle through a needle provided at the top of the inlet bottle. Approximately one L of air enters the inlet bottle in the course of a day and the PCE concentration in the headspace is maintained by mass transfer of PCE from the liquid phase. Because of the loss of PCE to the gas phase, the inlet concentration of PCE into the channels is not constant in a 24-hour period. However, for each day, the variation in the inlet PCE concentration is almost the same. The dimensionless Henry's constant of PCE at room temperature (25°C) is 0.72. There is a loss of PCE while adding 1 L of water and contaminant solution the next day because one liter of gas phase leaves the inlet bottle.

Even though plants were grown in each channel, the study primarily focused on the saturated zone of the channels and not on rhizosphere effects. The plants served as a natural pump, evapotranspiring some of the contaminated water and thus, influencing the residence time of the compounds in the channels.

The flow rate of the contaminant solution inside the channel varied in a 24-hour period. Immediately after watering, the flow rate was maximum and at the end of a 24-hour period, the flow rate was minimum. Based on the bromide peak arrival time during the month of June 2004, the flow rate was estimated to be approximately 37 cm/d (1.2 ft/d) for channel 1, 100 cm/d (3.3 ft/d) for channel 2, and 79 cm/d (2.6 ft/d) for channel 3.

There were five monitoring wells containing sintered alumina with polyethylene tubing for channel 1 and glass tubes with fritted glass at the bottom along the length of channel 2 through which groundwater samples could be collected from near the bottom of the channel (see Figure 2.1). In channel 1, the wells were placed at a distance of 13, 35, 60, 75 and 100 cm from the inlet. In channel 2, the wells were placed at a distance of 12, 32, 59, 77 and 100 cm from the inlet.

3.2.2. Inlet/Outlet Analysis

After introducing the contaminant solution on March 12, 2004, the concentrations of the contaminants were analysed at the inlet and the outlet of the channels. Ten mL samples were collected from the inlet tube at the entrance to each channel and from the outlet of each channel. Samples were collected using a 10 mL syringe and transferred to a 25 mL glass vial (22 mL

nominal volume, Supelco, Bellefonte, PA) and closed with a mininert cap immediately. The vials were shaken manually and the compounds in the sample were allowed to partition into the headspace and attain equilibrium concentrations. The headspace samples were analysed with a gas chromatograph (GC).

3.2.3. Tracer Study

A tracer study was conducted, using potassium bromide (KBr), to determine the residence time distribution, the time of the bromide peak at the outlet, and the corresponding volume of outlet liquid. The outlet liquid volume, on a particular day, depends on the plant size, and varies with the time of the month. The outlet liquid volume is a better parameter for estimating the tracer response because the time for peak of bromide concentration depends on the growth stage of the plants. In the tracer study, 150 mL of KBr solution, at a concentration of 100 mg/L (67 mg/L as bromide), was injected at the inlet of each channel through separating funnels. Samples were collected from the outlet after 3 hrs, 6 hrs, 12 hrs and 24 hrs on the first day and at the same times on the second day. Since the inlet solution level was higher after daily watering, the flow rate is also higher at the beginning of a day. As the head decreases, the flow rate decreases and becomes smaller near the end of the 24-hour period. On the third and fourth days, two samples, at 5 hrs and 24 hrs, were collected. From then on, each day a sample was collected, for 14 days, and analysed for bromide concentration. The mean residence time for the bromide in a channel is estimated from the expression (Levenspiel, 1999)

$$\tau = \frac{\sum t_i C_i \Delta t_i}{\sum C_i \Delta t_i} \qquad \qquad ....(3.1)$$

where,

τ = mean residence time, days

C_i = concentration of bromide in the ith sample, mg/L

t_i = time at which the ith sample was collected, day

Δt_i = Difference between (i+1)[th] time and i[th] time, days

Some of the bromide will be transported upward through the soil column due to evapotranspiration by plants. Hence, some bromide gets trapped in the unsaturated zone. The mass of bromide trapped in the soil is estimated based on the daily water uptake of plants and the concentration of the bromide in the outlet for that day. The daily composite concentration of the

bromide moving up into the vadose zone in the evapotranspired water is assumed to be the same as the daily composite concentration of bromide in the outlet liquid. When two or more samples were collected, on a day, during the initial period of study, the average concentration of bromide was used to estimate the mass of bromide transported upward.

3.2.4. Biostimulation by Glucose, Corn Starch and Soy Oil Methyl Esters

Since no degradation was observed after 100 days of introduction of the contaminant, supplements were added to stimulate growth of indigenous microbes, to create anaerobic conditions, and also for supplying hydrogen by fermentation. Introduction of 100 mL of 2 % (w/w) glucose solution (Dextrose, anhydrous, Fisher Chemicals, Fairlawn, NJ) as an electron donor into channel 2 (alfalfa grown on top) resulted in anaerobic conditions in the channel. The glucose solution was fed once every month starting from June 30, 2004 and continued until February 2005 and intermittently on several occasions until January 1, 2007. From October 2004, the same mass of glucose (2 g in one liter) was fed over a longer period of time to distribute it more efficiently. Totally, 12 doses of glucose solution were added. Corn starch was fed, inadvertently, instead of glucose on days 266, 299, 328 and 522. From October 1, 2004, one liter of 0.1 % emulsified soy oil methyl esters (SOME) was fed to channel 1 (alfalfa grown on top). SOME was sometimes injected in the wells instead of the inlet, since it tends to stay at the inlet due to its negligible solubility in water (SoyGold MSDS, 1998). The glucose and corn starch addition dates for channel 2 and SOME addition dates for channel 1 are listed in Table 3.1. The supplement solution was fed at the inlet, unless mentioned otherwise. Information on SOME is presented in Tables 3.2a and 3.2b.

3.2.5. Bioaugmentation with KB-1

Since the degradation of PCE stopped at the stage of DCE in the six-channel system, it was concluded that the native microbes were not able to degrade DCE. Hence, KB-1, a consortium of *Dehalococcoides spp.*, was added to channel 1 and 2. Since KB-1 is strictly anaerobic, it has to be injected in an oxygen free manner and the channels were operated to maintain that condition. For this purpose, it was planned to inject some amount of anaerobic water before injecting KB-1 and also to chase the KB-1 with the anaerobic water. The water for this purpose was obtained from the inlet of the channels. When the inlet tube of the channel was

pulled out, the solution from the channel drips out. Another tube, with same diameter as inlet tube, is connected to the inlet of the channel and 300 mL of solution from near the inlet of the channel was collected in a 300 mL amber bottle while flushing with nitrogen gas, to maintain it oxygen free. One gm of glucose was then added to this solution and used as anaerobic water. Before adding KB-1, 100 mL of this solution is injected at the inlet. Fifty mL of this solution was taken in a 50 mL syringe and 2 mL of KB-1 culture was then injected into the syringe, thus mixing the culture with 50 mL of solution. This solution was then injected into the inlet of the channel and then chased with 150 mL of the glucose solution. The inlet tube from the inlet contaminant reservoir is then reconnected to the inlet of the channel.

3.2.6. Soil Sample Analysis

After 95 days of exposure to PCE solution, soil samples were collected, at a distance of 32 cm from the inlet, at four different depths from the soil surface, viz. 0-8 cm, 12-20 cm, 22-30 cm and 34-42 cm, from all three channels fed with PCE. The samples were collected with a soil core and iron rods of various lengths to reach the desired depth. Soil collected at each depth was transferred to a 25 mL vial and immediately closed with mininert cap, shaken well manually, and headspace was analysed with a gas chromatograph (GC). The sample headspace was analysed for PCE and degradation compounds and the concentration in the aqueous phase of the soil was estimated using a calibration curve and a mass balance as shown below. In the calculation, the gas phase volume, available for partitioning, is obtained by subtracting the sum of the volumes occupied by water and soil from the total volume of the vial.

The soil sample with the vial is dried in a vacuum oven (Thelco vacuum oven, Precision Scientific Co., Chicago, IL) at 80°C for 24 hours and the dry weight of soil was determined. The difference between the mass of vial with moist soil and dry soil yields the mass of water present in the soil sample. The difference between the mass of vial with dry soil and the tare mass of vial gives the mass of dry soil.

In order to estimate the aqueous phase PCE concentration in the soil samples, the following equations are used.

The total mass of PCE present in the soil sample initially is

$$TM_{PCE} = m_{ds} \, C_{s1} + V_w \, C_{w1} + V_{g1} \, C_{g1} \qquad \qquad \text{... (3.2)}$$

where,

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TM_{PCE} = Total mass of PCE

m_{ds} = mass of dry soil

C_{s1} = concentration of PCE adsorbed to soil before partitioning into headspace

V_w = volume of aqueous phase

C_{w1} = concentration of PCE in the aqueous phase

V_{g1} = volume of gas phase in the soil sample

$$= n\left[\frac{m_{ws}}{\rho_b}\right] - V_w \qquad \ldots (3.3)$$

n = porosity of the soil = 0.3 (assumed)

m_{ws} = mass of wet soil

ρ_b = bulk density of the soil = 1.6 gm/cm^3 (assumed)

C_{g1} = concentration of PCE in the gas phase of the soil sample

Assuming equilibrium concentrations in all three phases of the soil sample,

C_{s1} = $K_{d,PCE}\, C_{w1}$ $\qquad \ldots (3.4)$

C_{g1} = $C_{w1}\, H_{PCE}$ $\qquad \ldots (3.5)$

$K_{d,PCE}$ = Partition coefficient of PCE between soil and aqueous phase

= $K_{oc}*\, f_{oc}$ $\qquad \ldots (3.6)$

K_{oc} = Partition coefficient of PCE between organic carbon and aqueous phase

= 282 L/kg at 25°C

f_{oc} = Organic carbon fraction in soil

H_{PCE} = Dimensionless Henry's constant of PCE = 0.72 at 25°C

Rewriting Eqn (3.2) in terms of aqueous phase concentrations, using the relationships Eqns. (3.4) and (3.5)

$$TM_{PCE} = m_{ds}\, K_{d,PCE}\, C_{w1} + V_w\, C_{w1} + V_{g1}\, H_{PCE}\, C_{w1}$$

or

$$TM_{PCE} = C_{w1}\, (m_{ds}\, K_{d,PCE} + V_w + V_{g1}\, H_{PCE}) \qquad \ldots (3.7)$$

After transferring the soil sample to the vial, due to the available headspace, partitioning of compounds takes place. After partitioning, the total mass of PCE is,

$$TM_{PCE} = C_{w2}\, m_{ds}\, C_{s2} + V_w\, C_{w2} + V_{g2}\, C_{g2} \qquad \ldots (3.8)$$

$$V_{g2} = 25 - \left[\frac{m_{ws}}{\rho_b}\right] + V_{g1} \qquad \qquad \ldots (3.9)$$

Rewriting Eqn (3.8) in terms of gas phase concentrations, using relationships Eqns (3.4) and (3.5),

$$TM_{PCE} = m_{ds}\ K_{d,PCE}\ C_{g2}/\ H_{PCE} + V_w\ C_{g2}/\ H_{PCE} + V_{g2}\ C_{g2} \qquad \ldots (3.10)$$

or

$$TM_{PCE} = (C_{g2}\ /\ H_{PCE}\ )\ (m_{ds}\ K_{d,PCE} + V_w + V_{g2}\ H_{PCE}) \qquad \ldots (3.11)$$

Since the total mass of PCE is same before and after partitioning, equating Eqn (3.7) and (3.11) yields C_{w1}

$$C_{w1} = \frac{C_{g2}}{H_{PCE}}\left[\frac{m_{ds}\ K_{d,PCE} + V_w + V_{g2}\ H_{PCE}}{[m_{ds}\ K_{d,PCE} + V_w + V_{g1}\ H_{PCE}]}\right] \qquad \ldots (3.12)$$

Since C_{g2} is the concentration of PCE analysed in GC, the aqueous phase concentration of PCE in the soil samples can be obtained from Eqn (3.12). Using the same procedure as above, the aqueous phase concentrations of TCE, DCE, VC and methane can be obtained.

A sorption study was also conducted to estimate the fraction of organic matter content (f_{oc}) in the soil samples collected on day 150. After analyzing for the chlorinated ethene concentrations, the soil samples were spiked with 1 mL of PCE-CT standard mixture. The standard was prepared by injecting 10 μL of PCE liquid and 10 μL of CT liquid (Certified A.C.S., Fisher Scientific Co., Fairlawn, NJ) in a clean amber glass bottle of volume 4.2 liters and allowed to completely vaporize. The concentrations of PCE and CT in the standard bottle are, therefore, 3.83 mg/L and 3.76 mg/L. One mL of this gas mixture is then spiked onto the soil samples and a control. The control was prepared with 5 gms of glass beads (3 mm diameter, Arthur H. Thomas Co., Philadelphia, PA) and 1 mL of distilled water in a 25 mL vial. After allowing at least one hour to reach equilibrium, the concentration in the gas phase was measured. The organic carbon fraction (f_{oc}) is then estimated by mass balance using the literature value for K_{oc}. The above equations are used for estimating the organic carbon fraction in the soil, with organic carbon fraction (f_{oc}) as the unknown parameter in Eqn (3.6).

3.2.7. Channel Soil Flux Analysis

Headspace samples from the channels were obtained by placing 400 mL containers on the surface of the soil on the channels. After 4 hours, 0.5 mL of the gas phase in the container is

drawn and analysed in the GC. This technique was successfully used by Zhang (1999) for analyzing the concentration of methyl tert-butyl ether (MTBE) in the same system.

3.2.8. Microcosm Study

In the six-channel system, PCE was converted to DCE but the native microbes were not able to degrade DCE during the time taken for inlet solution to reach the outlet. The native microbes may degrade DCE, but the rate of degradation may be too slow to be significant. To determine whether the native microbes can degrade DCE given sufficient time, we conducted microcosm studies using the outlet solution from channels 1 and 2. It was assumed that outlet water would contain the chlorinated ethene-degrading microorganisms which could be used in the microcosms, instead of KB-1. Since these microorganisms are presumably anaerobic, the collection of the outlet water had to be carried out in an oxygen free manner. For this purpose two 80 mL flow through samplers) were connected at the outlet (see Figure 3.2) and 20 mL of the solution from these gadgets were transferred to 25 mL vials with argon flushing at the surface of the water in the vial (microcosm). The microcosms contained 20 mL of solution and 5 mL of headspace. On October 29, 2004, the first batch of three microcosms was prepared, one being control, the second supplemented with glucose 0.005 % and the third with lactate 0.425 %. All concentrations are expressed as volume/volume.

On November 1, 2004, a second batch of six microcosms was prepared: control, glucose 0.01 %, lactate 0.85 %, yeast extract 0.01 %, glucose 0.01 %+ yeast extract 0.01 %, and lactate 0.85 % + yeast extract 0.01 %. On December 1, 2004, a third batch of six microcosms was prepared by the same procedure described above: control, soy oil methyl esters (SOME) 0.1 %, SOME 0.1 % + yeast extract 0.01 %, yeast extract 0.01 %, glucose 0.01 %+ yeast extract 0.01 %, and lactate 0.085 % + yeast extract 0.01 %. Yeast extract is a source of microbial nutrients. The microcosms were kept in the laboratory under the room tubelights and maintained at room temperature of 25°C.

The batch 1 microcosms were spiked with 0.5 mL of ~40 mg/L PCE (stock solution used for daily watering of plants in the six channel system) on day 14; batch 2 microcosms were spiked on day 11 and batch 3 microcosms on the same day of preparation.

New Microcosms were prepared, on March 5, 2005 with channel 1 (PCE and SOME fed) outlet liquid (batch 4). On day 23, 10 µL of KB-1, a consortium of *Dehalococcoides*, (SiREM

Lab, Ontario, Canada) was added to batch 4 microcosms. This sample of KB-1 was obtained from SiREM Lab in May 2004 and was kept under refrigeration.

3.2.9. Analytical Methods

Concentrations of chlorinated compounds and methane were measured using a gas chromatograph (HP 5890 Series II, Wilmington, DE) equipped with a Flame Ionization Detector (FID) and a HP-1 column (Dimethyl Polysiloxane matrix, 30 m x 0.53 mm, Agilent Technologies, Wilmington, DE). Hydrogen was the carrier gas. The injector temperature was set at 200°C and detector temperature was set at 300°C. Sample volume of 100 μL was injected in the column at 100°C and run for 5 minutes. The run temperature was chosen based on the arrival time and good separation of compounds. For higher temperatures, the compounds elute faster but the peaks may be closer or even overlap. For lower temperatures, the separation of compounds is good but the elution will be slow. Trial samples of PCE and reaction intermediates were run with different temperatures 80°C, 100°C, 110°C, and a temperature program with 35°C for 5 min and ramp to 245°C at the rate of 10°C/min. The elution times of PCE for the above temperature programs are approximately 4.1 min, 2.7 min, 2.2 min and 2.6 min. Isothermal temperature of 100°C was chosen and with gas flow rate of 1.5 mL/min, the elution times of PCE, TCE (99+ % purity, Aldrich Chemical Co. Inc., Milwaukee, WI), DCE (ChemService, West Chester, PA) and methane (Matheson Gas Products, East Rutherford, NJ) are approximately 2.7 min, 1.6 min, 1.1 min and 0.6 min. The detection limits, in the gas phase, with above conditions and instrument (assuming the minimum reliable area count is 500), are 6.6 μg/L (0.04 μM) for PCE, 4.9 μg/L(0.04 μM) for TCE, 3.8 μg/L (0.04 μM) for cis-DCE and 0.6 μg/L (0.04 μM) for methane. PCE and its degradation compounds, TCE, 1,1,1-TCA, 1,1,2-TCA, 1,1-DCA, 1,2-DCA, 1,1-DCE, cis 1,2-DCE, and methane were calibrated in this gas chromatograph; TCA is trichloroethane and DCA is dichloroethane. There was some difficulty in developing a calibration graph for vinyl chloride due to its availability in methanol. Various techniques were tried such as adding water to dissolve the methanol, adding sodium hydroxide (NaOH) to convert methanol to methoxylate, and analyzing after keeping the samples in a freezer. However, the masking of vinyl chloride peak by methanol peak could not be overcome. We observed that the chlorinated ethenes PCE, TCE and DCE resulted in the same response in the gas chromatograph for equal molar concentrations, approximately 13,000 area counts for 1 μM. We utilized this

finding to obtain the calibration curve for vinyl chloride. The concentrations of vinyl chloride shown in the graphs in this work is based upon the above calibration.

For the bromide tracer analysis, the outlet liquid collected was transferred to a 1.5 mL centrifuge tube and centrifuged at 10,000 rpm for 2 minutes. The supernatant was then transferred to a 2 mL clear vial with white septum and threaded black cap (National Scientific Company, Rockwood, TN) for bromide analysis using an ion chromatograph (Dionex DX500 Series, Sunnyvale, CA) equipped with a conductivity detector and analytical column (Ionpac, AS9-HC, 4 x 250 mm). The eluent solvent was 9 mM sodium carbonate at a flow rate of 1 mL/min. The elution times of chloride, bromide, nitrate and sulfate were approximately 6.3 min, 9.5 min, 11 min and 18 min, respectively. The sample volume injected was 25 μL and each sample was run at room temperature for 20 minutes.

3.3. Results and Discussion

3.3.1. Tracer Studies

The time and the outlet liquid volume at which the peak concentration of bromide occurred and the mean residence times (MRTs) for each channel, are shown in Figure 3.3 and Table 3.3. The MRT was estimated from the residence time distribution of tracer in the channel (Levenspiel, 1999). The peak of bromide concentration in the outlet occurred between 1 to 3 days corresponding to an outlet liquid volume of 1 L to 2.8 L.

Table 3.4 presents the cumulative outlet liquid volume, mass of bromide eluted in the outlet, estimated mass of bromide transported to vadose zone by evapotranspiration and the total bromide accounted for by these two losses and the recovery percentage. The amount of water present in the saturated zone can be calculated as follows:

Volume of the saturated zone $= 110 \times 10 \times 25 = 27,500 \text{ cm}^3 = 27.5 \text{ L}$

Assuming a porosity of 0.3, the volume of water $= 27.5 \times 0.3 = 8.25 \text{ L}$

However, the bromide elutes corresponding to exit volume of ~ 1.3 L. Therefore the estimated height of flow zone is only $(1.3 \times 25/8.25) = 3.9 \text{ cm} \sim 4 \text{ cm}$. Therefore, the flow may take place only within a height of 4 cm, assuming the width is completely available for flow. Suppose there is channeling and the actual flow height is, say 10 cm, the corresponding effective porosity can be calculated as 0.12, instead of the actual value of about 0.3.

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The entire amount of bromide (10.05 mg) introduced at the inlet was not recovered at the outlet because a portion of the solution, with bromide, is transported upward due to evapotranspiration and some of the bromide remained in the saturated zone due to channeling. As shown in Table 3.4, the bromide recovery ranges from 42 % to 70 %. Table 3.5 shows that the fraction of solution that leaves in the outlet is larger than the fraction of bromide recovered in the outlet. The experiment was carried out for 14 days. However, the bromide was still eluting at low concentrations when the experiment was terminated. The bromide that remained in the saturated zone after 14 days was not analysed and therefore, not used in estimating the recovery. The effluent bromide concentrations, after the end of 14 days, were 0.28 mg/L for channel 1, 0.07 mg/L for channel 2, and 0.19 mg/L for channel 3. In all three channels, the concentration of bromide was 0 mg/L, before the start of the experiment. There are two contributions for pore volume: volume external to soil particles and volume associated with internal void space. The bromide that passes through rapidly is associated with the external pore volume and the bromide within internal void space or held by clay particles by ionic or Van der Waal forces (Reddi and Inyang, 2000) may take more time to elute.

The rate of evapotranspiration in each channel varies over the monthly period due to harvesting at the beginning of each month. Because of this, the outlet liquid volumetric flow rate also varies and, consequently, the mean residence time of the compounds in the channel varies with plant size. Table 3.6 presents the variation of mean residence time at the start and end of each month. The outlet liquid volume which was recorded every day throughout the duration of the experiment, for channels 1, 2 and 3, varied from 0.9 L – 0.6 L, 0.9 L – 0.4 L, 0.8 L – 0.6 L respectively, from the beginning to the end of one month, during June 2004.

3.3.2. Inlet/Outlet

Figures 3.4, 3.5 and 3.6 show the inlet PCE, outlet PCE and degradation product concentrations for glucose/corn starch fed channel, SOME fed channel and control channel respectively. In Figures 3.4a and 3.5a, a solid line is used to show the concentration of the sum of the chlorinated ethenes (CEs).

3.3.2.1. Glucose/Corn Starch Fed Channel

Forty days after first feeding glucose solution, the outlet PCE concentration started to decrease and eventually reached a low concentration (less than 1 μM) in 3 months (Figure 3.4a).

Correspondingly, the concentration of cis 1,2-DCE increased in the outlet liquid. For an inlet concentration of about 12 μmoles PCE/L, the outlet water had concentrations of about 10 μmoles/L of cis 1,2-DCE and methane concentrations ranging upto 135 μmoles/L. A mass balance of the inlet and outlet liquid revealed that the methane (Figure 3.4b) in the outlet water could have been generated from the glucose added. From the bromide tracer data, the peak arrival time in the glucose fed channel varied from about 1.2 days at the beginning of the month to an estimate value of 2.7 days at the end of the month.

Glucose addition led to conversion of PCE mainly to DCE and not other degradation products (Figure 3.4a). Breakdown of DCE is usually the rate limiting step in the degradation of PCE (Daprato et al., 2007). The mean residence time in this channel may not be sufficient for the DCE to degrade further. With glucose, almost the entire inlet PCE is converted to DCE; however with SOME, more of the entering PCE exits in the outlet (Figure 3.5a). Since SOME is sparingly soluble in water, the microbial degradation may be limited by availability of SOME in the portion of the channel downgradient to well 1. Different microbial populations are likely supported by the supplements, glucose and SOME. The glucose feeding was stopped after day 328, February 3, 2005. After day 400, concentrations of outlet PCE and TCE started to increase and DCE decreased. Outlet PCE in channel 2 increased and by day 437, it had reached 30 % of inlet value. However, even by day 475 (~ 150 days after stopping glucose feeding) PCE degradation was observed. Mass balance calculations and stoichimetric requirements (see end of this section) indicate that one dose of glucose solution is sufficient for about 8 months of PCE entering the channel. More than 190 days after the last dose of glucose, PCE degradation was taking place even though the extent of conversion was dropping gradually with appearance of TCE (Figure 3.4b) also at the outlet between days 437 to 551. The mean residence time for this channel from bromide studies was found to be 3.6 days from the experiment conducted during the first half of June 2004. If the same ratio of peak arrival time of tracer at the beginning and end of a month is assumed for the MRT, then the MRT for the flow pattern at the end of a month will be (2.7/1.2)*3.6 = 8.1 days. If the average value of 5.85 days is chosen, then in terms of MRT, the glucose added was sufficient for 190/5.85 ~ 32 MRTs, i.e., it takes 32 MRTs for the glucose and glucose derived stored material to be washed away or to be depleted. From the tracer studies, we know that it takes 4 MRTs for the bromide concentration to decrease to a small value. If we assume that it takes 10 MRTs for the most readily available soluble glucose to wash

out of the channel, then the remaining 22 MRTs are associated with the glucose deposits away from the channeling pathway, sorbed glucose, glucose derived stored materials and endogenous decay. Some of the food added was converted to biomass and when the channel runs out of food, endogenous decay may also come into play and provide the carbon and energy required for the biomass. Glucose may be converted into other compounds and stored; this may slowly be released and supply electron donor for PCE degradation. PCE concentration in the outlet increased but did not reach the inlet value. The inlet PCE is 12 μM which is converted to about 3 μM TCE, 8 μM DCE and remaining 1 μM PCE flows out.

Glucose solution was again added on several days as shown in Figure 3.4a and Table 3.1. After the addition of glucose on day 522 and day 564, the outlet PCE and TCE concentrations started to decrease. By day 551 DCE concentration increased and reached the value of inlet PCE concentration by day 579. KB-1 was injected at the inlet on day 591 but it did not have any immediate significant effect. This culture of KB-1 was stored in the refrigerator for more than a year and therefore, may not have been active.

There was an increase in PCE and decrease in DCE around days 650 and 750, due to depletion of carbon and energy source. Glucose solution was added whenever the above happened to maintain the outlet PCE concentration at low levels.

KB-1 was again added at the inlet on day 810 resulting in a decrease of DCE by more than 50 %. The concentration of DCE was maintained between 3.3 μM and 8.8 μM between days 810 to 948. KB-1 may not be dispersed throughout the length of the channel. KB-1 migrates very slowly in soils (Major et al., 2002) and spreads in the soil by growth of KB-1 biomass. After reduction of DCE to ~50 %, KB-1 and/or energy source may not be available in the rest of the flow path. Hence, KB-1 culture was introduced into well 3, halfway along the channel, on day 958. Glucose solution was also added on days 994 and 1025 into well 1 and well 2, respectively, instead of at the inlet. After day 994, DCE concentration reached low levels and then was maintained below 1.5 μM until the end of the experiment (day 1113).

Mass balance for a monthly basis: Glucose fed channel

The assumptions made in the following mass balance are:

1. Solubility of oxygen in water is 8 mg/L, though it can vary based on temperature and altitude.

2. Complete consumption of oxygen is required for the onset of PCE degradation, though it is not necessary.

The amount of PCE added in a month is 12 μM * 30 L = 360 μmoles.

Amount of oxygen entering the channel in a month = 8 mg/L * 30 L = 240 mg or 240/32 = 7.5 mmoles.

Glucose in one dose = 2 gm = 11.1 mmol

One mole of glucose required for complete consumption of six moles of oxygen

Glucose required for complete consumption of oxygen = 7.5/6 = 1.25 mmol

Glucose available for supplying hydrogen for dehalogenation = 11.11 - 1.25 = 9.86 mmoles

One mole of glucose can theoretically supply 24 moles of electrons. Eight moles of electrons are required to dehalogenate one mole of PCE. Therefore, one mole of glucose can supply hydrogen for 3 moles of PCE. However, Dawson et al (2007) suggest that the theoretical supply of hydrogen (electron equivalent) by glucose is 4 moles when the conversion is to acetic acid, CO_2 and hydrogen.

In general, redox reactions involve the transfer of electrons between two chemical species (ITRC, 2005) and the oxidized compound provides electrons (i.e., the electron donor). The oxidation of a simple carbohydrate (CH_2O) electron donor is represented by

$$CH_2O + H_2O \rightarrow CO_2 + 4H+ + 4e-$$
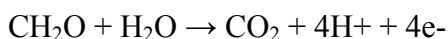
The electrons are transferred to the species undergoing reduction (i.e., the electron acceptor). Multiple electron acceptors are present in most groundwater environments including oxygen, ferric iron, and sulfate. However, the electron acceptors of particular interest are the contaminants undergoing reductive dechlorination. For example, the reduction of PCE to ethene is given by

$$C_2Cl_4 + 4H^+ + 8e^- \rightarrow C_2H_4 + 4Cl^-$$
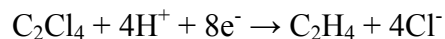
The net stoichiometry of these redox reactions indicates that two moles of the simple carbohydrate electron donor are required to dechlorinate one mole of PCE to ethene. The stoichiometry of these redox processes may be used to calculate the quantity of electron donor required to meet the total electron donor demand exerted by all electron acceptors (AFCEE, 2004).

PCE that can be dehalogenated by 9.86 mmol of glucose = 9.86*3 = 29.6 mmol; however, only 0.36 mmol of PCE was added each month. 29.6/0.36 = 82 months of PCE supply. However, the added glucose is also utilized for oxygen consumption. If we assume that 0.12 mmoles of glucose is needed for the PCE degradation and 1.25 mmoles is needed for oxygen consumption each month, then the glucose supplied is sufficient for 11.11/1.37 = 8.1 or approximately 8 months of inlet PCE. However, some of the glucose will wash out of the system, some will support growth of other microorganisms, and some will be converted to methane.

3.3.2.2. SOME Fed Channel

From October 1, 2004, channel 1 was fed with one liter of 0.1 % emulsified soy oil methyl esters (SOME). The SOME addition dates are listed in Table 3.1. The outlet liquid of the channel fed with PCE and SOME started to show some of the degradation compounds of PCE; however, the lag time for starting PCE degradation was longer compared to the glucose fed channel. This could be due to availability of SOME to the microbes along the length of the channel; SOME, being hydrophobic and only slightly soluble in water, may stay at the inlet, the point of injection, in contrast to the glucose solution which is distributed better throughout the channel. The peak time predicted for input to the PCE and SOME fed channel varied from about 3 days at the beginning of the month to about 6 days at the end of the month (Table 3.6).

In channel 1, the outlet PCE concentration decreased after the addition of SOME (Figure 3.5a) with conversion of PCE to TCE, DCE, VC and methane. The DCE and TCE in the outlet increased with treatment time. Maximum concentrations reached were 2.8 μM TCE, 17 μM DCE, 1.2 μM VC and 2 μM methane. SOME solution was added monthly in channel 1, and the low outlet PCE concentration with high DCE concentration in the outlet was observed to continue.

SOME solution was added in channel 1 inlet on May 1, 2005 (day 415). Since the entire inlet PCE was not transformed in channel 1, it is possible that the SOME fed at the inlet could be trapped in the initial portion of the channel. On May 3, 2005 (day 417), in addition to the SOME fed at the inlet, 100 mL of 1 % SOME solution was directly injected into well 3, at a distance of 60 cm from inlet. This addition had a significant effect on the PCE and other product concentrations at the outlet as shown in Figures 3.5a and 3.5b. After SOME solution was added

on May 3, 2005 (day 417), feeding of SOME was stopped to study the capability of the channel to carry on PCE degradation with the electron donor stored over the past eight months. In the period from day 411 to day 475, the outlet concentration of PCE decreased from about 5 μM to 0.2 μM, TCE decreased from about 2.5 μM to 0.1 μM, DCE increased from about 5 μM to 17 μM and methane increased from about 0.5 μM to 9 μM (Figure 3.5). SOME solution was added on nine occasions prior to day 417 and the tenth dose was on day 594. With this SOME, the microorganisms were capable of degrading PCE until day 954, i.e., for 364 days. The mean residence time for this channel from bromide studies was found to be 6.2 days based on the experiment conducted during the first half of June 2004. If the same ratio of peak arrival time of tracer at the beginning and end of a month is assumed for the MRT, then the MRT for the flow pattern at the end of a month will be (4.6/3.1)*6.2 = 9.3 days. If the average value of 7.75 days is chosen, then in terms of MRT, the glucose added was sufficient for 364/7.75 ~ 47 MRTs, i.e., it takes 47 MRTs for the SOME and SOME derived stored material to be washed away or to be depleted. From the tracer studies, we know that it takes about 3 MRTs for most of the bromide to wash out of the channel. Since SOME does not dissolve appreciably in water, it appears to be retained in the channel longer than a soluble substrate. A portion of the food added was converted to biomass and when the channel runs out of food, endogenous decay may also come into play and provide the carbon and energy required for the biomass. SOME solution was added again on days 991 and day 1025 to maintain low redox condition in the channel, which was conducive to KB-1. The outlet solution had nearly equimolar amounts of DCE as inlet PCE, and the native microbes were not capable of degrading DCE further. To degrade DCE, KB-1 was added at the inlet on day 605 and day 811. But this addition did not have significant effect on the outlet DCE. SOME and KB-1 may stay at the inlet and not be available in the rest of the channel length to degrade DCE. Based on this hypothesis, KB-1 was added into well 3 (60 cm from inlet of channel) on day 958. This addition had a significant effect: By day 994 DCE decreased to 7.9 μM from about 16 μM on day 975, and it continued to decrease and reached a value of 5.8 μM by day 1113. KB-1 was able to degrade DCE with both SOME and glucose supplements, however, with glucose DCE concentration reached lower values (less than 1 μM).

Mass balance for a monthly basis: SOME fed channel

SOME in one dose = 1 mL

Density of SOME at 25°C = 0.87 gm/cc (SoyGold 2000 Solvent, 1998)

Mass of SOME in one dose = 0.87/292 = 2.98 mmol

The molecular weight of SOME is obtained from the relative weight percent of each fatty acid in SOME (See Table 3.2b). The value is approximately 292.

One mole of SOME required for complete reduction of 25 moles of oxygen

SOME required for complete consumption of 7.5 mmoles of oxygen = 7.5/25 = 0.3 mmol

SOME available for supplying electrons for dehalogenation = 2.98 - 0.3 = 2.68 mmoles

One mole of SOME can approximately supply 102 moles of electrons. Eight moles of electrons are required to dehalogenate one mole of PCE. Therefore, one mole of SOME can supply electrons for approximately 12 moles of PCE.

PCE that can be dehalogenated by 2.68 mmol of SOME = 2.68*12 = 32.16 mmol; however, only 0.36 mmoles of PCE was fed each month and it requires 0.03 mmoles of SOME. Total SOME required each month is then 0.03+0.3 = 0.33 mmoles. Thus, the SOME supplied is sufficient for 2.98/0.33 = 9.03 months or approximately 9 months of inlet PCE.

3.3.2.3. Control Channel

The outlet solution of the channel (grass grown on top) in which no energy supplements were added, showed no degradation compounds of PCE (Figure 3.6). The outlet PCE concentration was almost the same as inlet PCE concentration. Based on the study of these three channels, it can be concluded that the PCE degradation is certainly an electron-donor-limited process, which benefits from adding supplements such as glucose and SOME.

3.3.3. Well Samples

Wells were already present in channel 1, while new wells were installed in channel 2. Five wells, approximately 15 cm apart, were located between inlet and outlet. Using a nylon tube and a 3 mL syringe, groundwater samples (2 mL) were collected from each well, near the bottom of the channel, i.e., at a depth of 60 cm. Five wells were located between inlet and outlet in channels 1 and 2.

3.3.3.1. Channel 1 (SOME)

As shown in Figure 3.7, for channel 1, on day 382, relative to the inlet, the concentration of PCE was much smaller in wells 1 and 2 and correspondingly, the concentration of DCE increased with distance until well 2. However, after well 3, DCE concentration remained almost the same (Figure 3.7). The outlet concentrations of PCE and DCE differ from the concentrations in well 5. This indicates that there is channeling and the samples from the wells differ from those in the main channel of flow. The outlet liquid is more of a composite sample combining the effects of fast flowing and slow flowing zones of the channel.

The SOME injected into well 3 of the channel, on May 3, 2005 (day 417) had a significant impact on CEs transformation (Figure 3.8 and Figure 3.9). In Figure 3.8 and Figure 3.9, the measured concentration of DCE ($\sim$ 20 μM) is higher than the molar amount of inlet PCE (12 μM). Inlet PCE concentration was higher than 15 μM during the period day 326 to 368. CEs may have accumulated by being distributed into the SOME phase that was added earlier and on day 417. Pfeiffer et al (2005) measured the oil:water partition coefficients of dissolved CEs into Soy Oil in batch tests and found the values ranged from 22 to 1200 with increasing chlorination. Soutter and McBean (2007) point out that the chlorinated ethenes concentration in groundwater may be significantly greater due to partitioning into the organic phase where both contaminants are present. In this work, chlorinated compounds that are present in the oil phase of a sample will release into the gas phase during the analysis. This will cause the measured value to appear to be larger than the actual concentration in the aqueous phase. In Figure 3.5a also, the DCE concentration (greater than15 μM) was higher than the inlet PCE concentration during the period from day 437 to day 537. In Figure 3.8, there is a sharp decrease in concentration of DCE from well 3 (20 μM) to well 4 (13.5 μM). PCE concentration decreased drastically (12.9 μM to 2.9 μM) from inlet to well 1 and remained at that level until well 3. In Figure 3.8, there is also a decrease in PCE concentration from well 3 (2.4 μM) to well 4 (1.1 μM), likely due to the SOME fed into well 3. The outlet concentrations of PCE, TCE and VC reached very low values. Methane concentrations were also higher after feeding SOME at well 3 on day 417, increasing from about 3 μM on day 382 to 19 μM on day 456. As shown in Figure 3.9, on day 456, methane concentration was highest at well 4 indicating that it is from the fermentation of SOME added at well 3. By day 496, the PCE concentration, shown in Figure 3.10, was reduced to a low value by well 3, and DCE concentration remained steady from well 3 to well 5 likely due to lack of PCE.

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Addition of SOME on day 594 and KB-1 on day 605 at the inlet may have impacted the degradation of PCE and DCE, as shown for day 614 in Figure 3.11. The low concentrations of PCE and TCE at wells 4, 5 and at the outlet are similar to results in Figure 3.10; however, there is a decrease in DCE at well 5 and the outlet relative to the results in Figure 3.10. Methane concentration at well 4 remained between 13 μM and 18 μM during the period day 456 to day 614. On days 644 and 712, DCE concentration remained relatively steady along the length of the channel as shown in Figures 3.12 and 3.13. In Figure 3.13, PCE concentration at the inlet is only 5.1 μM, which could be due to analytical problem or a slow leakage from the vial.

Even though SOME feeding was stopped by day 417 and added once on day 594, degradation of PCE continued in channel 1 until day 897. On day 864, most of the inlet PCE (15 μM to less than 1 μM) was converted to DCE as shown in Figure 3.14. However on day 897, only about 50 % of inlet PCE was converted to DCE, based on the outlet, as shown in Figure 3.15. This may be due to the lack of SOME in channel 1.

A culture of KB-1 was again introduced at the inlet on day 811 for channel 1, but resulted in no apparent change in product distribution except for a decrease in DCE at well 5 and the outlet on day 864 as shown in Figure 3.14. However, introduction of KB-1 into well 3 on day 958 had a significant impact. On day 984, the DCE concentration increased to 10.5 μM at well 3 but decreased to 2.6 μM at the outlet as shown in Figure 3.16. Total concentration of chlorinated ethenes (CEs) decreased by 80 %. Prior to day 958, KB-1 injected at the inlet may not have been available or only in limited locations from well 2 onwards, to degrade DCE. KB-1 migrates in soil slowly and also grows along the flow path (Major et al., 2002).

3.3.3.2. Channel 2 (Glucose)

In channel 2, the degradation of PCE was gradual: the concentration of PCE decreased gradually and that of DCE increased gradually from the inlet to the outlet. The samples collected on day 409 (Figure 3.17) comprised mostly PCE and DCE. However, samples collected on day 438 (Figure 3.18) contained greater concentrations of TCE and PCE and smaller concentrations of DCE since the glucose and corn starch solution was nearly depleted. Corn starch added on day 522 had a significant effect and by day 555, the outlet contained predominantly DCE and very little PCE and other CEs as shown in Figure 3.19. One more dose of glucose solution on day 564

resulted in rapid formation of DCE from PCE and most of the inlet PCE was converted to DCE by well 3. KB-1, added on day 591 at the inlet did not have much impact in converting DCE as seen in Figure 3.20 and Figure 3.21. Glucose solution was fed to the inlet of the channel on days 676 and 800. KB-1 was added on day 810, at the inlet. This KB-1 had some effect and the DCE in the outlet on day 831 was less than 50 % of the molar amount of inlet PCE as shown in Figure 3.22. The total CEs also decreased to less than 50 % by day 831 (Figure 3.22). However, by day 897, PCE and DCE were present in the outlet and total CEs had decreased only by 30 % (Figure 3.23) because glucose was not supplied after day 800. To completely convert DCE to end products and decrease total CEs to low levels, glucose solution was added on days 901 and 955 and KB-1 was added on day 958 into well 3 instead of at the inlet. Since DCE is produced from PCE uniformly along the length of the channel, KB-1, if injected at the inlet, may not be available to degrade DCE in the later portion of the channel. This addition had a significant impact and the total CEs decreased by 85 % on day 984 (Figure 3.24). On day 1123 (4/10/07), the well samples were collected in triplicates from the wells and the results are shown in Figure 3.25. The coefficients of variation of the concentrations for the triplicates in five wells, the inlet and the outlet ranged from 0.7 % to 49 % for PCE, 0 % to 87 % for TCE, 1 % to 34 % for DCE and 8 % to 43 % for methane. For example, PCE concentration at the inlet was 10.5±0.08 µM, TCE concentration at well 3 was 0.3±0.04 µM, DCE concentration at well 3 was 2.04±0.25 µM, and methane concentration at well 4 was 7.71±0.81 µM.

In Figure 3.25, most of the decrease in PCE concentration occurred between the entrance and well 3 and after that PCE concentration remained almost constant. DCE concentration was low and reached a peak of 2 µM at well 3 and then decreased and reached a final value of 1.7 µM at the outlet. The KB-1 added at the inlet and glucose solution added into wells 2 and 3 on days 994 and 1025, respectively, helped carry out the degradation of PCE and DCE until well 3 and KB-1 that was injected into well 3 on day 958 helped carry out the degradation of remaining PCE and DCE to bring the total CEs to low levels at the outlet. The total CEs decreased from 10.6 µM to 2.8 µM (74 %) along the length of the channel. TCE was formed but at very low levels and the maximum concentration reached was 0.3 µM at well 3 and well 5. Concentration of methane ranged from 1 to 8 µM.

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3.3.3.3. *Spatial Variations*

In the well samples, the concentration of PCE was often less compared to the outlet, and some of the downgradient wells had higher concentration of PCE than upgradient wells. For example, in Figure 3.7, the reported PCE concentration in well 2 is higher than well 1 and the outlet PCE concentration is higher than that in the wells. The samples may not be representative of the entire channel water; the channel was 10 cm wide and there is vertical variation also. The sample was collected from a 0.5 cm diameter well, with an uncertain region of influence. The observed values indicate that channeling occurs and spatial variations of concentrations are present. Some of the variations may also be due to experimental error.

Results from channel 1 wells showed that most of the activity happens within the first 15 cm of the channels suggesting that the SOME fed, at the inlet, remained mostly in the initial part of the channel causing greater population of microbes in that location. However, in channel 2, PCE concentration decreases and DCE concentration increases gradually along the length of the channel.

Channel 1 wells were analysed on additional days but the results are not shown for all sampling events. For results on days 395, 409, 555, 590, 686, 741, 775, 804, 831, 897 and 928 please see the supplement. Channel 2 wells were analysed on additional days but the results are not shown for all sampling events. For results on days 395, 456, 496, 590, 644, 686, 712, 775, 804, 864 and 928 please see the supplement.

3.3.4. Soil Surface Concentrations

In all three channels the above described outlet concentrations represent the concentrations in the ground water. A significant fraction of input water migrates vertically through the soil. The headspace was collected in 400 mL containers placed at the surface of the soil at several locations along the length of the channel. Duplicate samples were collected on the following dates: July 30, 2004, August 1, 2004, August 2, 2004, October 30, 2004, November 3, 2004, November 29, 2004, December 27, 2004, February 2, 2005, March 2, 2005 and April 3, 2005. The concentrations of PCE were the same as the background levels in the room where the six channels are kept. No other chlorinated compounds were detected at the soil surface.

3.3.5. Soil Samples

The mass of aqueous phase and the moisture content as a fraction, dry weight of soil, and the concentration of PCE for soil samples collected on day 95, June 15, 2004, and day 150, August 10, 2004, are presented in Tables 3.7 and 3.8, and Figures 3.26 and 3.27. Results are presented for only two sampling events, day 95 and day 150, for illustration purpose. For other days, please see the supplement.

In channels 1 and 2, the moisture content increases with depth as expected, since the water diffuses through the vadose zone and evaporates through the soil surface, thus creating a gradient in moisture content. In channel 3, however, the moisture content remains almost steady with increase in the depth of the channel due to application of Hoaglands solution on topsoil. From 12/3/2003 until 3/1/2004, once in two days, 0.5 liter of 1x Hoaglands solution was added to channel 3. From 3/8/2004 until 4/4/2004, once a week, 0.5 liter of 1x Hoaglands solution was added and from 4/13/2004 to 12/10/2004, 0.5 liter of 2x Hoaglands solution was added once a week.

The field capacity of sandy soil is in the range 0.04-0.08 cm^3/cm^3 (Morgan et al., 2001). Assuming the bulk density of soil in the channel is 1.6 g/cm^3, the gravimetric field capacity of sandy soil is in the range 0.025-0.05 g/g. The moisture content of the soil samples from channels 1, 2, and 3 were in the range 0.03 to 0.22 on day 95 and 0.02 to 0.19 on day 150. The observed moisture content is greater than the minimum field capacity for sandy soil.

Figure 3.26 shows that PCE concentration increases with depth, in all three channels, until 28 cm and then increases drastically from depth 28 cm to 38 cm, due to transition from vadose zone to saturated zone. The water table starts at 35 cm below the soil surface. In channels 1, 2 and 3, the concentrations of PCE at depth 38 cm were 2.5 μM, 3.4 μM and 1.8 μM respectively (Figure 3.26). No reaction intermediates were detected since prior to day 95, nutrients were not added.

The moisture content and its fraction, and dry weight of soil for samples collected on day 150, August 9, 2004, are presented in Table 3.8. A sorption study was also conducted to estimate the fraction of organic matter content (f_{oc}) in the soil samples. The value of f_{oc}, which was estimated by a mass balance, based on the literature value of K_{oc}, is presented in Table 3.9. Typically, the organic matter content is higher at the surface of a vegetated soil due to foliage and root mass and exudates, and decreases with depth. However, there is no definite trend

observed in channels 1 and 3. For modeling the same soil system, Zhang (1999) has used a value of 1 % for f_{oc}.

On day 150 (8/9/04), PCE concentrations in the lower soil samples, at 38 cm depth, were 0.18 µM in channel 1, 0.49 µM in channel 2, and 2.03 µM in channel 3 (Figure 3.27). Samples were collected at a distance of 55 cm from inlet. The concentration of PCE in channels 1 and 2 remained almost constant with depth; however, for channel 3 it increases with depth.

Figure 3.28 shows the concentration of PCE in channels 1, 2 and 3, on day 229, October 27, 2004. In channel 1, PCE concentration is higher at depth 2 (13-20 cm) relative to depths 3 and 4. However, PCE increases uniformly along the depth in channels 2 and 3, and there was the usual drastic increase from depth 3 to depth 4, the transition from vadose to saturated zone. Since it was 119 days since the first dose of glucose, biodegradation was taking place and DCE was found in the lower depth at a concentration of 0.25 µM. DCE was not found in the vadose zone where it can be easily degraded under aerobic conditions. Moreover, the concentration was not high in the lower depth.

On day 353, the concentrations of PCE in channels 1 and 2 are small in all of the soil samples (Figure 3.29). In channel 2, PCE concentration increased from 0 at depth 1 to 0.08 µM by depth 2 and then remained almost constant until depth 4. DCE concentrations in channel 2 were larger than PCE concentrations except near the soil surface. It is interesting to note that DCE was present in depths 2 and 3 in the vadose zone. The concentration of PCE in channel 3 exhibited the same trend as in the previous figures.

Figure 3.30 shows the concentrations of CEs in the aqueous phase of channel 2 soil samples collected at the same depth and several locations along the length of the channel on day 385, 4/1/05. Soil samples were collected at 37 cm depth at all locations except at 101 cm from inlet which was collected at 42 cm depth. The samples were collected while installing wells in channel 2. These results can be compared to concentrations in the well samples in Figure 3.17, the difference being that the well samples were collected at 60 cm from the soil surface. In both figures, PCE concentration decreases and correspondingly DCE concentration increases along the length of the channel. TCE concentration was in the range 0.16 to 1.5 µM in the soil samples whereas in the well samples it was in the range 0 to 0.9 µM.

Figures 3.31 through 3.36 show the concentrations of PCE, reaction intermediates and methane analyzed on day 812, 6/12/2006 and on day 955, 10/23/2006 (channel 1) and day 987,

11/26/06 (channels 2 and 3). In general, the concentrations increased with depth; however, the samples collected even in the deepest soil (saturated zone) did not have the same concentrations as the samples from the flow regime at the bottom 5 cm of the channel (which is ~ 20 cm below the deepest soil sample collected), except for channel 3 on day 987 (Figure 3.36).

3.3.6. Microcosm Studies

Microcosm studies of the outlet water from both channels were carried out to estimate the half-life of PCE with different supplements and to determine the feasibility of DCE degradation when provided longer time. This is based on the assumption that some representative fraction of relevant microorganisms are in the free suspension, not as a biofilm. The concentrations of glucose and yeast extract (YE) are expressed as percent w/v and the concentrations of lactic acid and SOME are expressed as percent v/v. Table 3.10 and Table 3.11 provide a summary of the results for batches 1, 2, 3 and 4. The percentage decrease in PCE and DCE in the microcosms is listed and variation in the concentration of methane is given. The first order rate constants and half-life of PCE in microcosms for Batch 1 and 2 are given in Table 3.12 and for Batch 3 and 4 in Table 3.13. The regression coefficients for first order reaction model for all microcosms are listed in Table 3.14. Most of the regression coefficient values were above 0.80 suggesting that first order kinetics can be used with a reasonable degree of confidence.

In batch 1, there was not much difference in PCE degradation rate between the control (Figure 3.37) and the glucose (0.005 % w/v) amended microcosm. However, PCE disappearance was ~ 1.2 times faster in lactic acid (0.425 % w/v) amended microcosm (Figure 3.38) compared to the control and glucose amended microcosm. In batch 2 also, there was not much difference in the rates of degradation of PCE in control and 0.01 % w/v glucose amended (Figure 3.39) and 0.01 % w/v yeast extract (YE) amended microcosm. However, the microcosms amended with glucose+yeast extract and lactic acid+yeast extract (Figure 3.40) exhibited up to twice as great a rate of degradation compared to the control. In general, after 180 days there was not appreciable decrease in PCE concentration in batches 1 and 2; DCE concentration, however, remained steady. Since there is not much activity taking place, analysis of batch 1 and 2 microcosms was discontinued after day 234 (batch 1) and day 231 (batch 2).

In batches 1 and 2 microcosms, those amended with lactate performed well, whereas in batch 3, microcosm amended with SOME performed well. Variations in methane concentrations suggest that there may be methanotrophs present in the microcosms. For the glucose microcosms, comparison of batch 2 (glucose 0.01 %) and batch 3 (glucose 0.01 %+ yeast extract 0.01 %) suggests that the half-life of PCE degradation is higher in the presence of yeast extract. It is not clearly understood whether there were sufficient microbes in batch 3 microcosm or yeast extract acted in any inhibitory manner. In the batch 3 microcosm amended with (lactate 0.085 % + yeast extract 0.01 %), all compounds were disappearing in few days which may be due to leak in the vial, rather than degradation; moreover, if we compare to batch 2 microcosm (lactate 0.85 % + yeast extract 0.01 %), where lactic acid is at 10 times higher concentration, we expect a longer half-life.

The control in batch 3 exhibited faster degradation (~ 75 % PCE disappeared in 100 days) but it could possibly be a gradual leak. In the microcosm amended with 0.1 % SOME more than 90 % PCE disappeared in 30 days when PCE was respiked on day 86. It is not clear whether it is due to a leak in the microcosm. The microcosm amended with 0.1 % SOME and 0.01 % YE produced large amounts of VC and appreciable amounts of degradation compounds such as TCE and DCE (Figure 3.41). TCE and VC reached a peak at about 50 days and then started to decrease and reached a low value after 200 days. However, only 25 % of PCE had disappeared after 200 days and DCE remained almost steady. In the microcosms amended with SOME, PCE usually increases first and then decreases. This is because some of the PCE (being a non-aqueous phase liquid (NAPL)) was incorporated into the hydrophobic SOME initially. After SOME starts to be consumed, the PCE is released to the aqueous phase. In the microcosm amended with 0.01 % YE, PCE and DCE decreased slowly up to day 85, but afterwards, decreased sharply and reached almost zero by day 140 (Figure 3.42). In the microcosm amended with 0.01 % glucose and 0.01 % YE, ~ 55 % PCE disappeared in 200 days. Microcosm amended with 0.085 % lactic acid+0.01 % YE was a leaky one. No inhibition was observed in microcosms with higher concentrations of substrates glucose and lactate. Table 3.10 summarizes the results for batches 1, 2 and 3.

The results from the microcosm studies differ from the earlier results based on data from channels 1 and 2. The rate of degradation of PCE is larger in the channels because of the soil and

the biofilms associated with the soil. The microenvironment needed for degradation may be much better in the biofilms.

In batch 4, even after the addition of KB-1, there was not much difference in the rate of degradation of PCE. KB-1 may not be alive or its activity could have decreased substantially due to long storage. Table 3.11 summarizes the results from microcosm studies (batch 4). Microcosm amended with 0.01 % yeast extract was a leaky one and, therefore, the results are not presented. The results for microcosm amended with lactic acid and lactic acid+YE are shown in Figures 3.43 and 3.44 respectively.

Analysis of batch 3 and batch 4 microcosms was discontinued after 268 days and 174 days respectively. DCE remains steady in the microcosms and it is concluded that the microorganisms from the six-channel system were not capable of reducing DCE further even if provided sufficiently long time.

3.4. Conclusions

Supplements such as glucose, corn starch, and SOME stimulated the indigenous microbes and helped in the degradation of tetrachloroethene (PCE). The pattern and rate of degradation of PCE varied with different supplements. The relative concentrations of degradation products were different in the glucose/corn starch and SOME amended channels. Glucose was found to be a better supplement than SOME with respect to the onset time for PCE degradation. Because glucose is soluble in water, it is distributed more rapidly. PCE concentration decreased to below the MCL value of 0.03 μM in the glucose fed channel on some dates and the minimum value in the SOME fed channel was 0.074 μM on day 825, almost 2.5 times the value of MCL.

Most of the degradation process took place in the initial portion of the SOME fed channel, since SOME likely stayed near the inlet of the channel, due to sorption and retarded flow. SOME is a NAPL which does not flow freely like an aqueous solution. It may also sorb to soil organic matter in the initial portion of the channel. In the soil sample analysis, PCE concentration was low in the vadose zone, but it was present in the saturated zone in appreciable concentrations. This study demonstrated that the supplements glucose and SOME are effective substrates that can be added to PCE contaminated groundwater to promote degradation.

The mesocosm studies have shown that there is a significant residual effect of introduced carbon supplements. Mass balance and stoichiometric requirements of substrates for degradation

of PCE suggest that about 10 % of the nutrient supplements glucose and SOME are used in the process and the remaining substrate is used to consume oxygen and to support the growth of the microbes. In the soil system, the microbes which are present as a biofilm absorb glucose and SOME, store polysaccharides, and have a reservoir of carbohydrates to use to reduce PCE to DCE. The process of endogenous decay can also provide a source of food. There are also effects of distributed flow and channeling. These phenomena influence the length of time the system operates effectively and degrades PCE after food is added. Because SOME has very low solubility in water, it is retained near the point of injection, and it is able to provide needed substrate for a relatively long time compared to a soluble substrate such as glucose, which washes out with the effluent.

Addition of KB-1 at well 3 had significant impact in the degradation of DCE, in both glucose and SOME amended channels. KB-1 added on day 958 at well 3 was active even on day 1113 (155 days later) suggesting that there is sustainable growth of KB-1 when provided with suitable conditions and substrates.

The well sample results impart an idea of how SOME should be applied in the field: it should be injected at several points down-gradient rather than at a single point.

The native microorganisms, in free suspension in the exit solution, from the six-channel system were not capable of reducing the DCE further even when provided sufficiently long time in microcosms. DCE remained steady in the microcosms and it is concluded that microorganisms capable of reducing DCE to ethene must be added to degrade DCE. Thus, the microcosm results and the results from the channel studies show that addition of nutrients which provide hydrogen and microorganisms that can degrade DCE are both necessary for dechlorination of PCE to ethene.

Figure 3.1. Photograph of six-channel system taken from outlet. The outlet solution is collected using 2L pop bottles.

Figure 3.2. Two flow through samplers connected to the outlet of channel 1. The outlet solution is collected, anaerobically, for preparing microcosms.
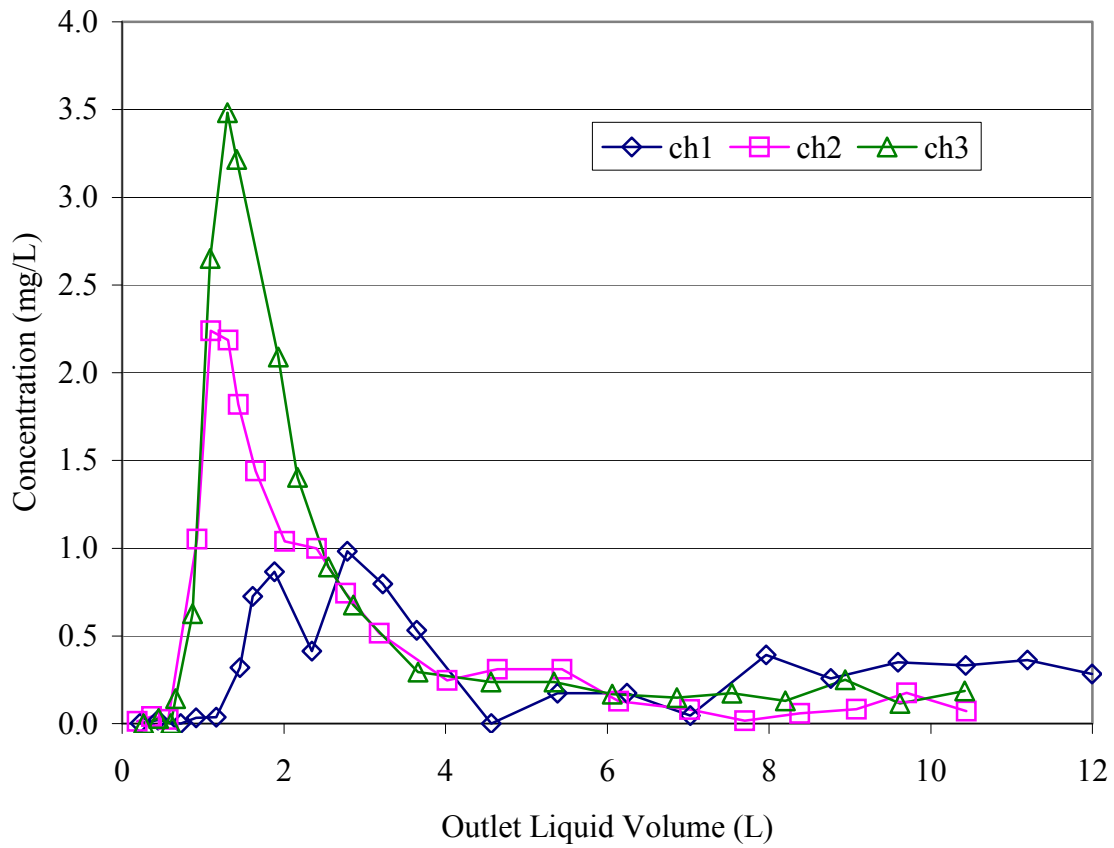
Figure 3.3. Concentration of bromide in the outlet liquid vs cumulative outlet liquid volume for channels 1, 2 and 3. Inlet bromide concentration = 67 mg/L.
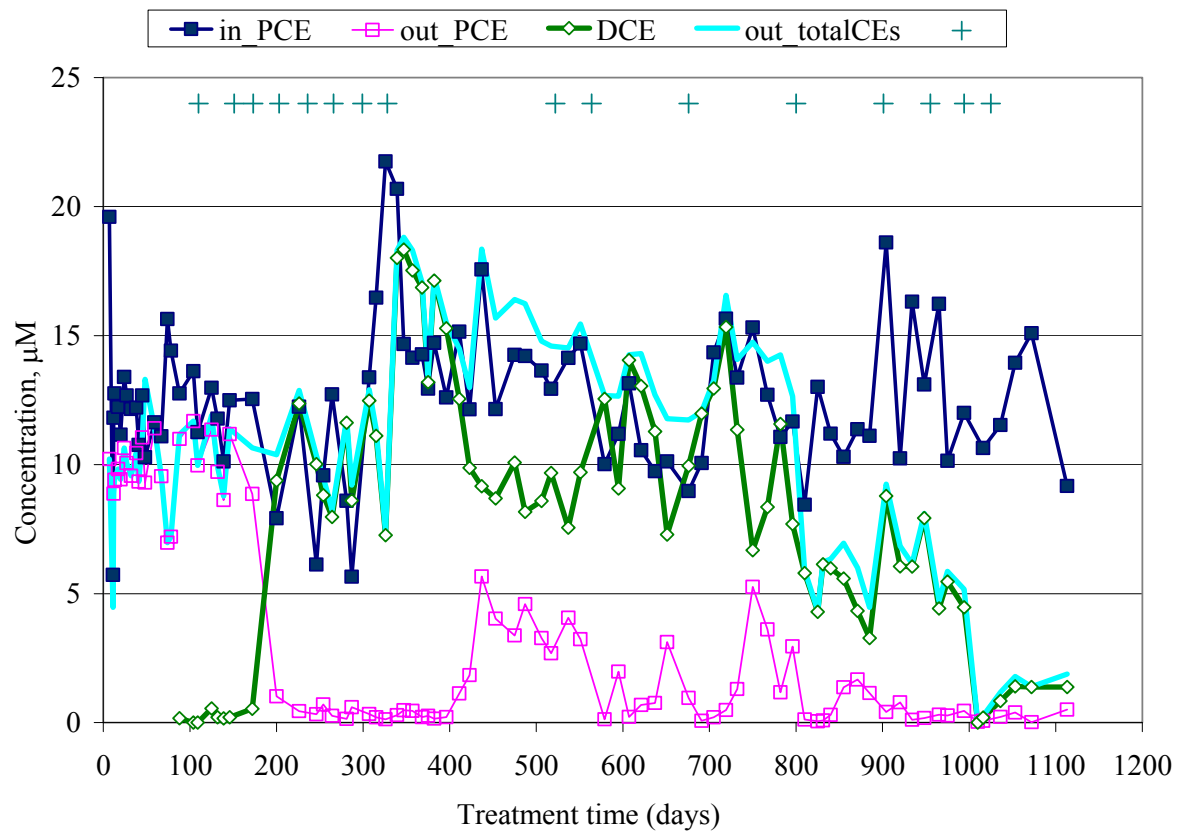
Figure 3.4a. Inlet PCE and outlet PCE and DCE concentrations for channel 2. Glucose solution was added (indicated by + symbols) on day 110, 151, 173, 203, 236, 564, 676,799, 901, 955, 994 (well 1) and 1025 (well 2). Corn starch was added on days 266, 299 328 and 522. KB-1 was added on days 591, 810 and 958 (well 3).
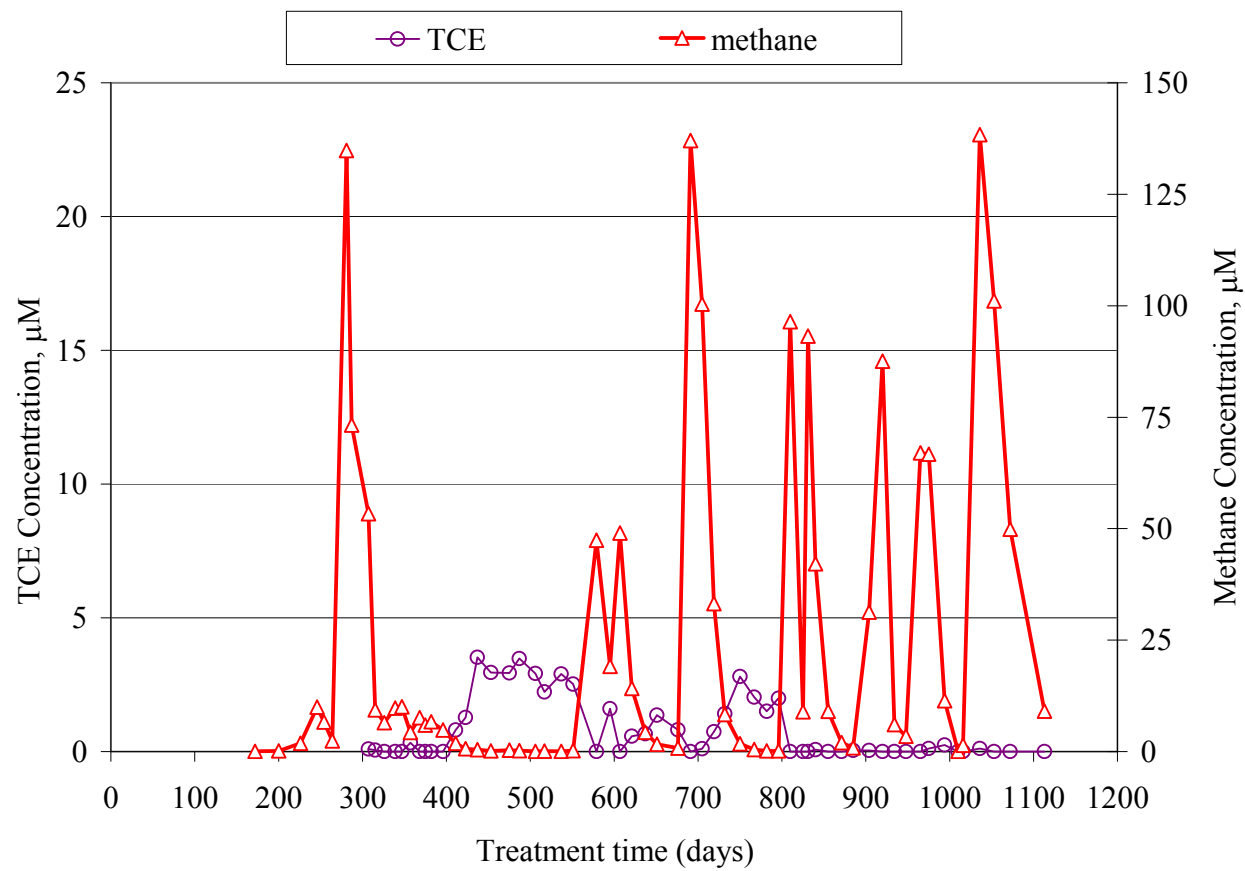
Figure 3.4b. Outlet TCE and methane concentrations for channel 2. See Figure 3.4a for glucose and KB-1 additions.
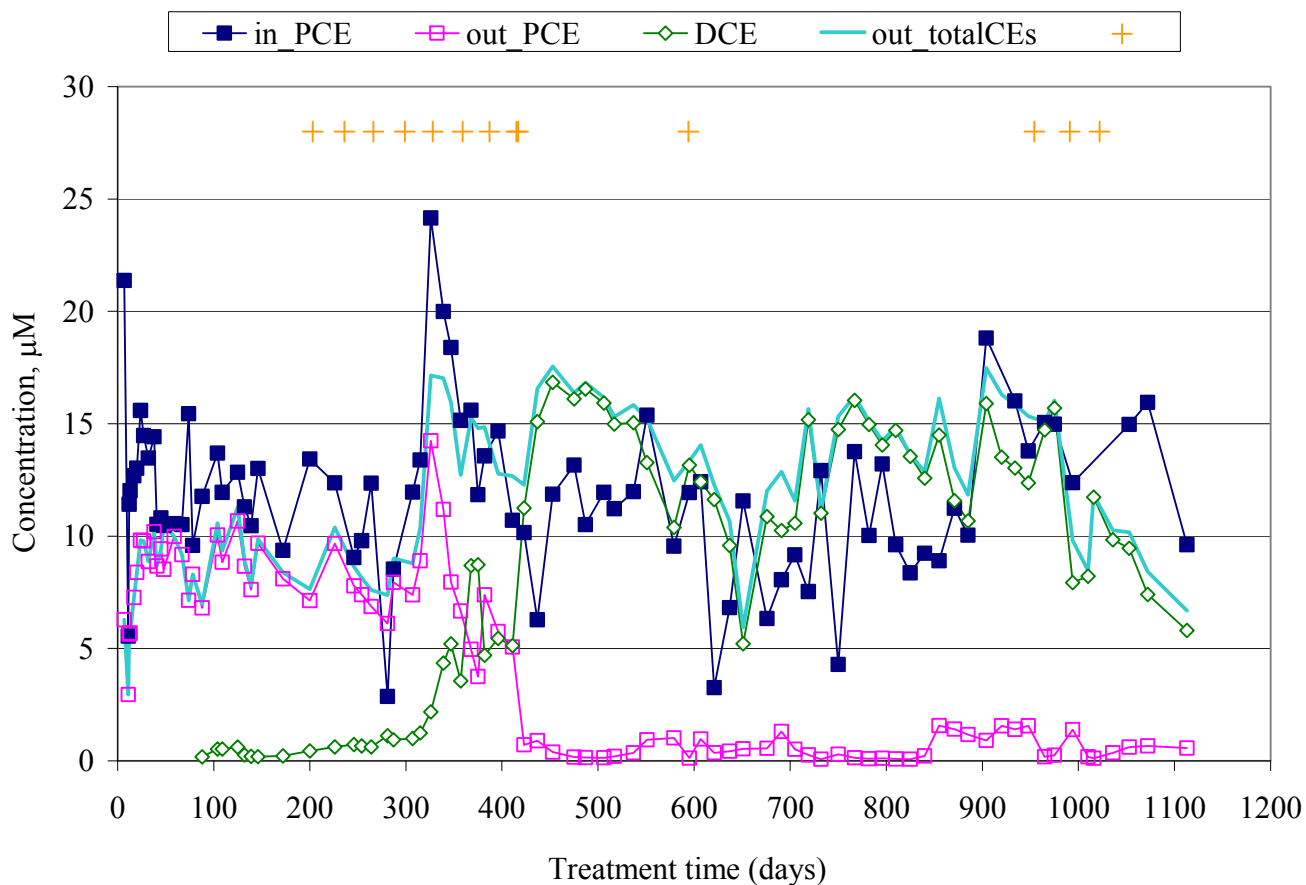
Figure 3.5a. Inlet PCE and outlet PCE, DCE, and total CEs concentrations for channel 1. Water samples taken on indicated days after beginning (March 12, 2004) exposure. Soy Oil Esters were added (indicated by + symbols) on days 203, 236, 266, 299, 328, 359, 387, 415, 417 (well 3), 594, 954 (well 3), 991 (well 3) and 1022 (well 1). KB-1 was added on day 605, 811 and 958 (well 3).
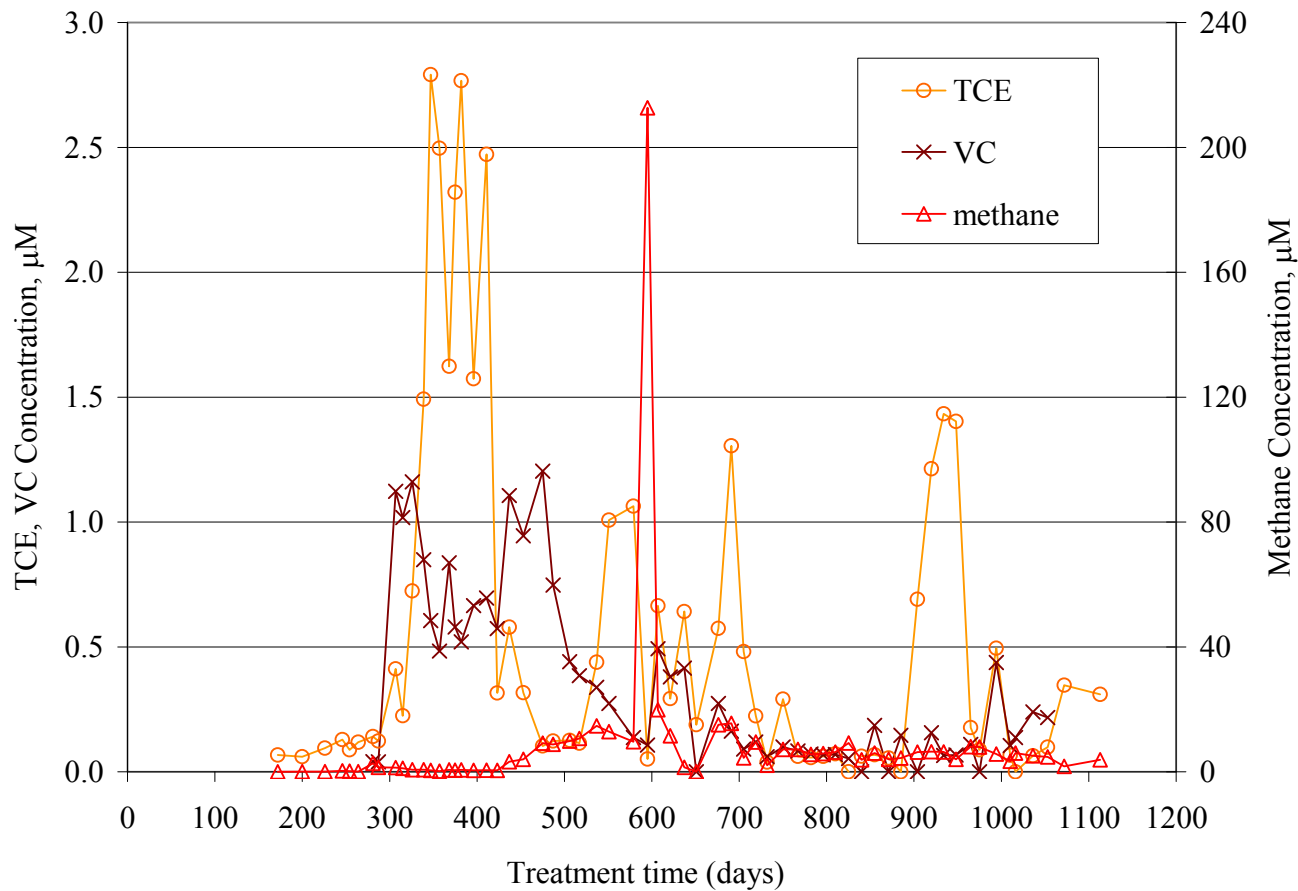
Figure 3.5b. Outlet TCE, VC and methane concentrations (right hand y-axis) for channel 1. Water samples taken on indicated days after beginning (March 12, 2004) exposure. See Figure 3.5a for times of SOME and KB-1 additions.
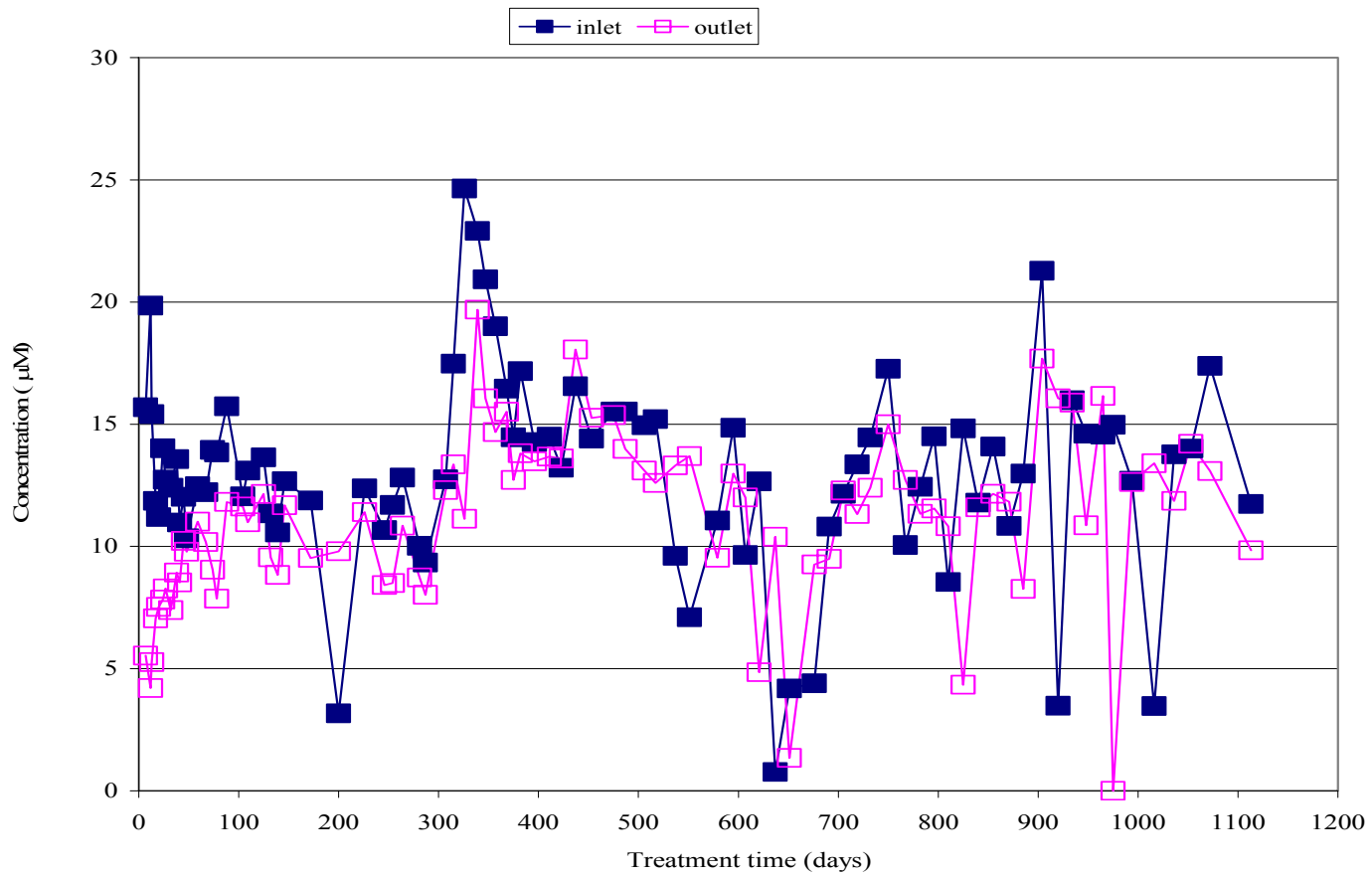
Figure 3.6. PCE concentrations in inlet and outlet of channel 3. Water samples taken on indicated days after beginning exposure (March 12, 2004).
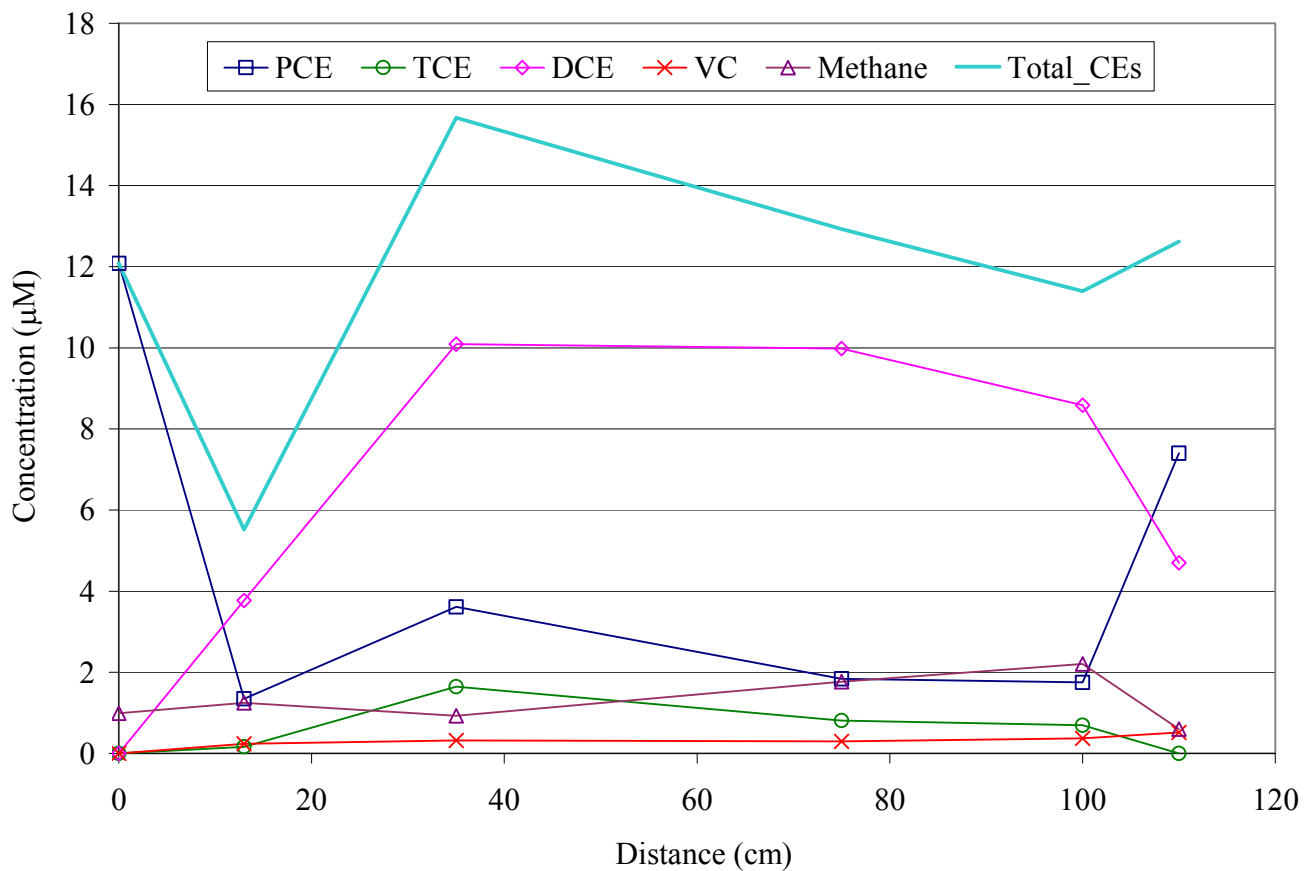
Figure 3.7. Variation of PCE and degradation compounds with distance along channel 1 on day 382, 3/29/05. Data for well 3 is not available. Soy Oil Methyl Esters (SOME) were added on days 203, 236, 266, 299, 328, and 359.
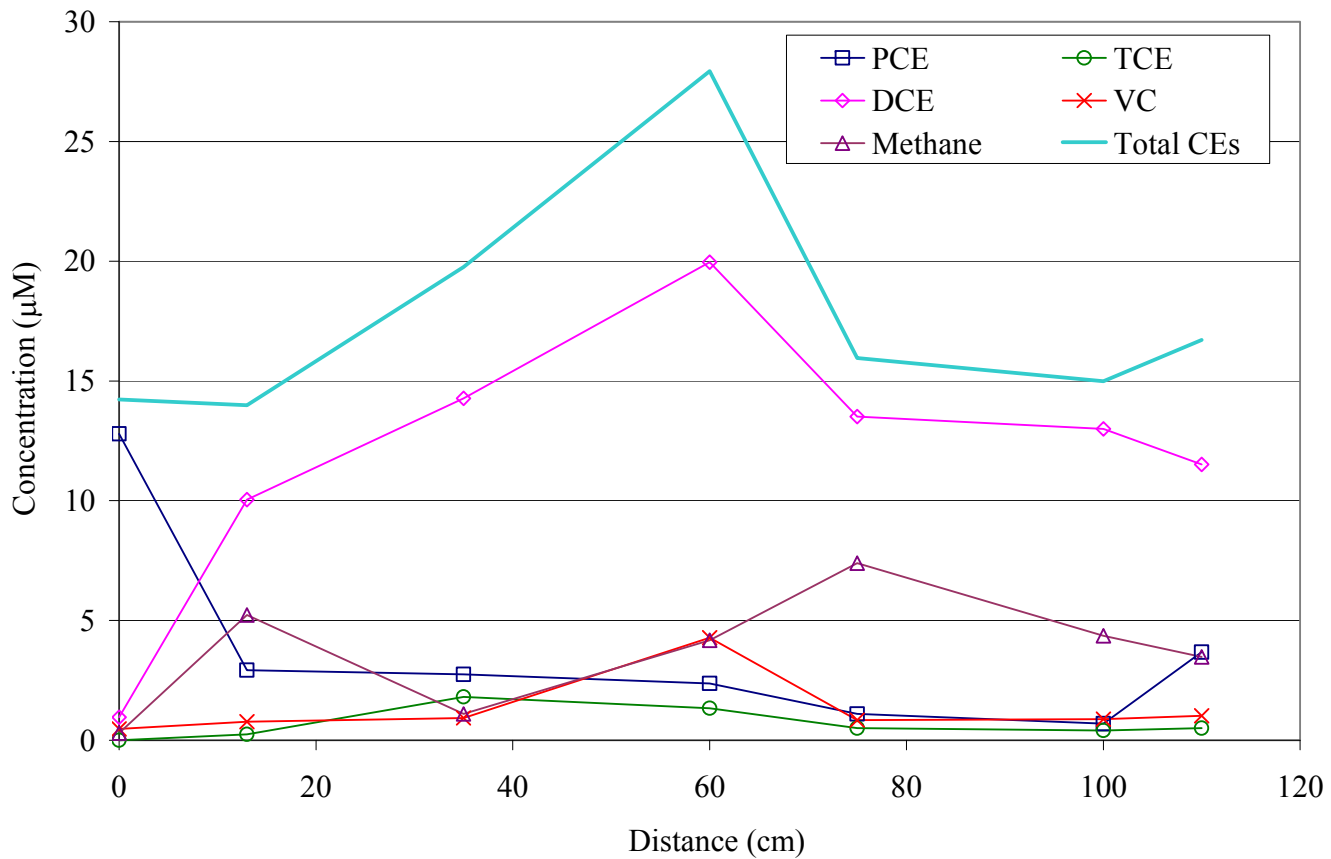
Figure 3.8. Variation of PCE and degradation compounds with distance along channel 1 on day 438, 5/24/05. Soy Oil Methyl Esters (SOME) were added on days 203, 236, 266, 299, 328, 359, 387, 415, and 417 (well 3).
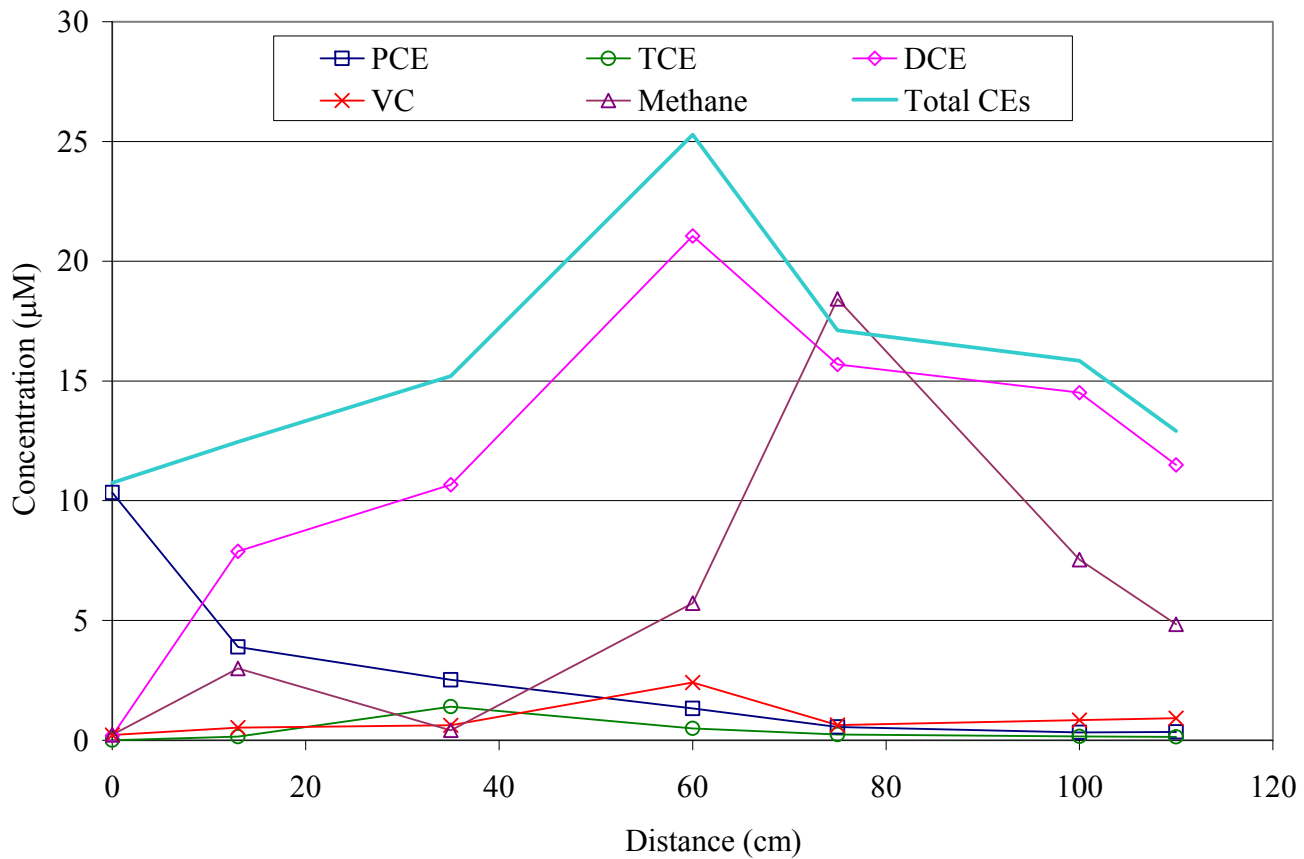
Figure 3.9. Variation of PCE and degradation compounds with distance along channel 1 on day 456, 6/11/05. Soy Oil Methyl Esters (SOME) were added on days 203, 236, 266, 299, 328, and 359, 387, 415, and 417 (well 3).
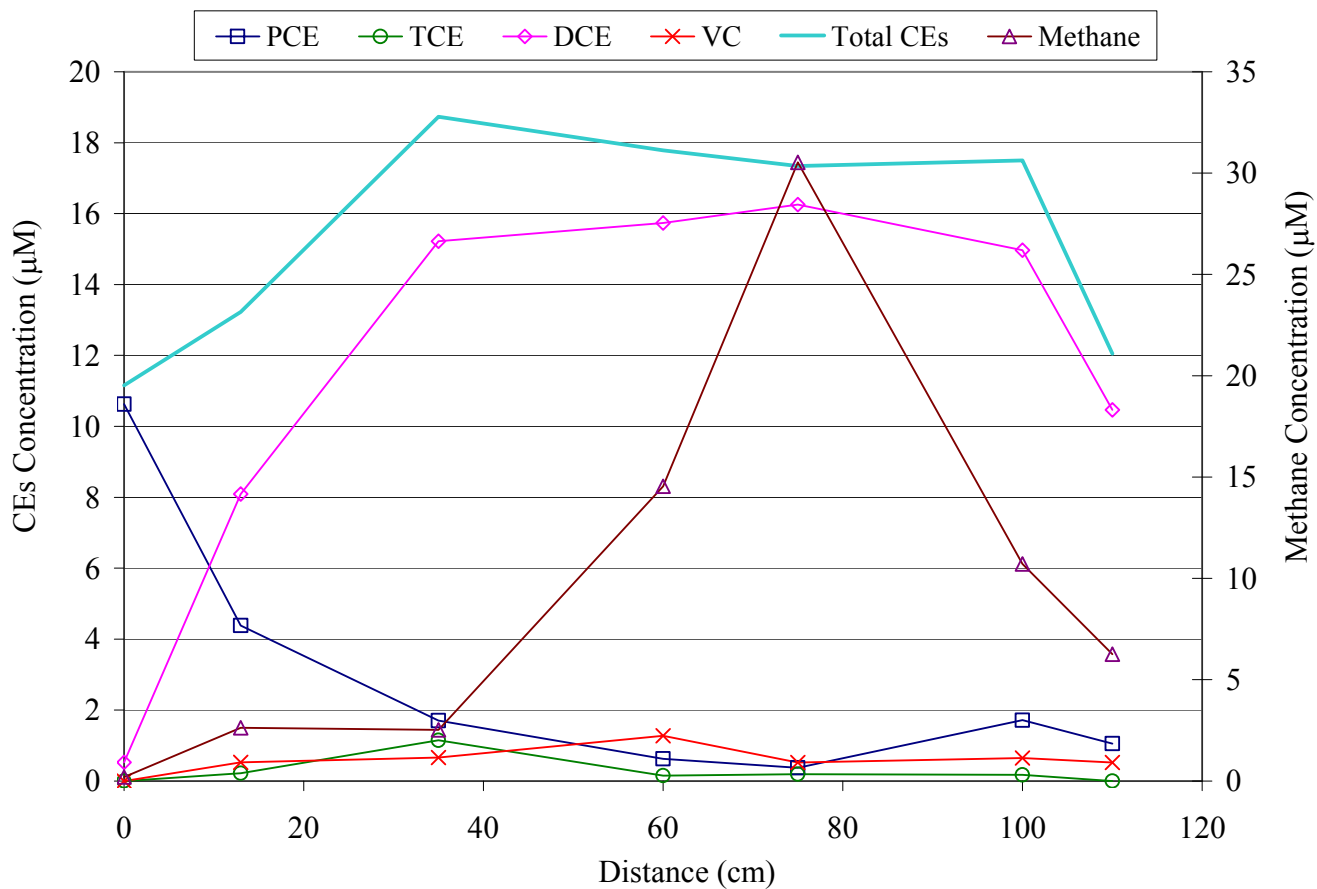
Figure 3.10. Variation of PCE and degradation compounds with distance along channel 1 on day 496, 7/21/05; Methane concentration on right-hand (secondary) y-axis. Soy Oil Methyl Esters (SOME) were added on days 203, 236, 266, 299, 328, 359, 387, 415, and 417 (well 3).
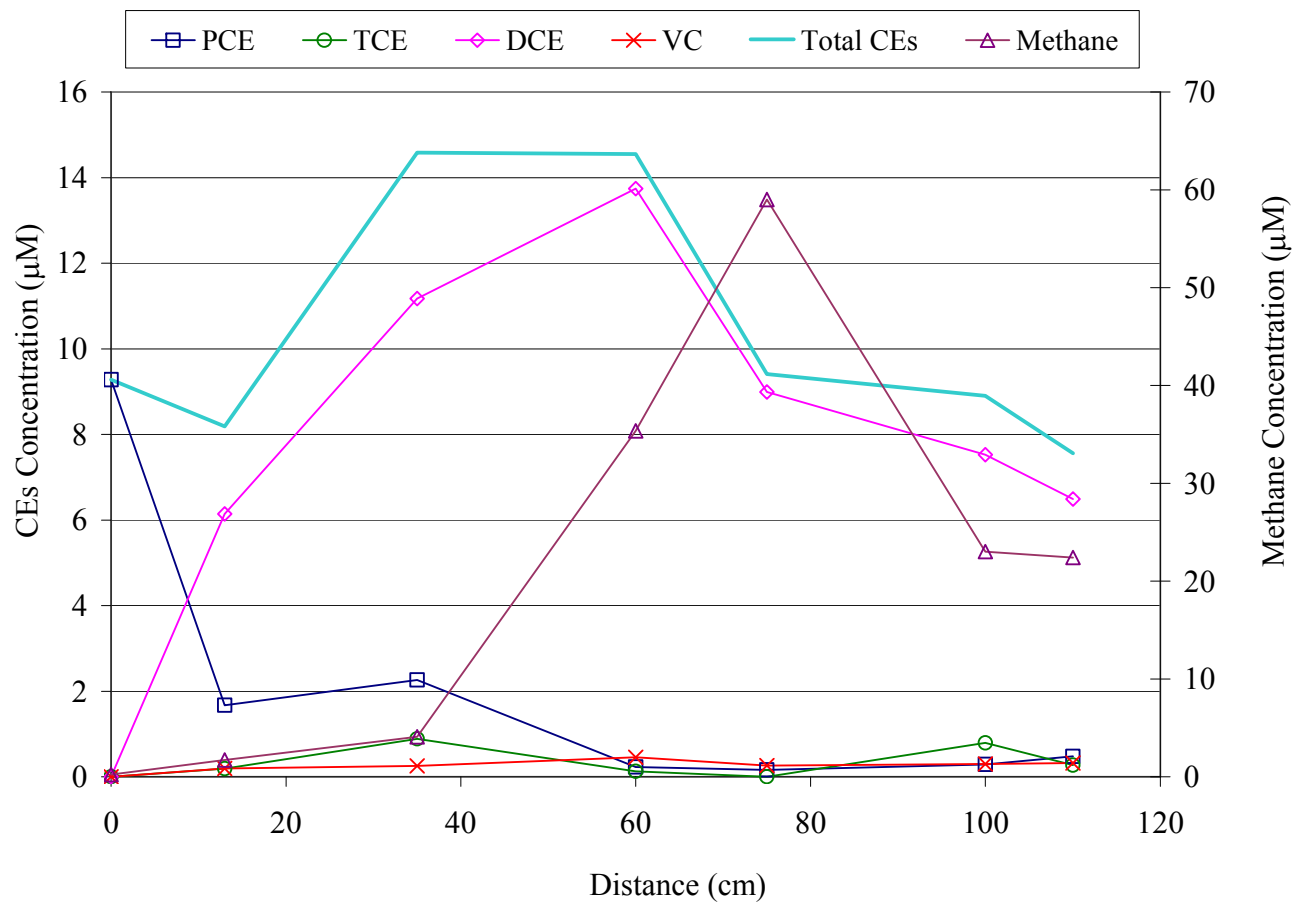
Figure 3.11. Variation of PCE and degradation compounds with distance along channel 1 on day 614, 11/16/05; Methane concentration on right-hand (secondary) y-axis. KB-1 was injected on day 605, 11/7/05. Soy Oil Methyl Esters (SOME) were added on days 203, 236, 266, 299, 328, 359, 387, 415, 417 (well 3) and 594.
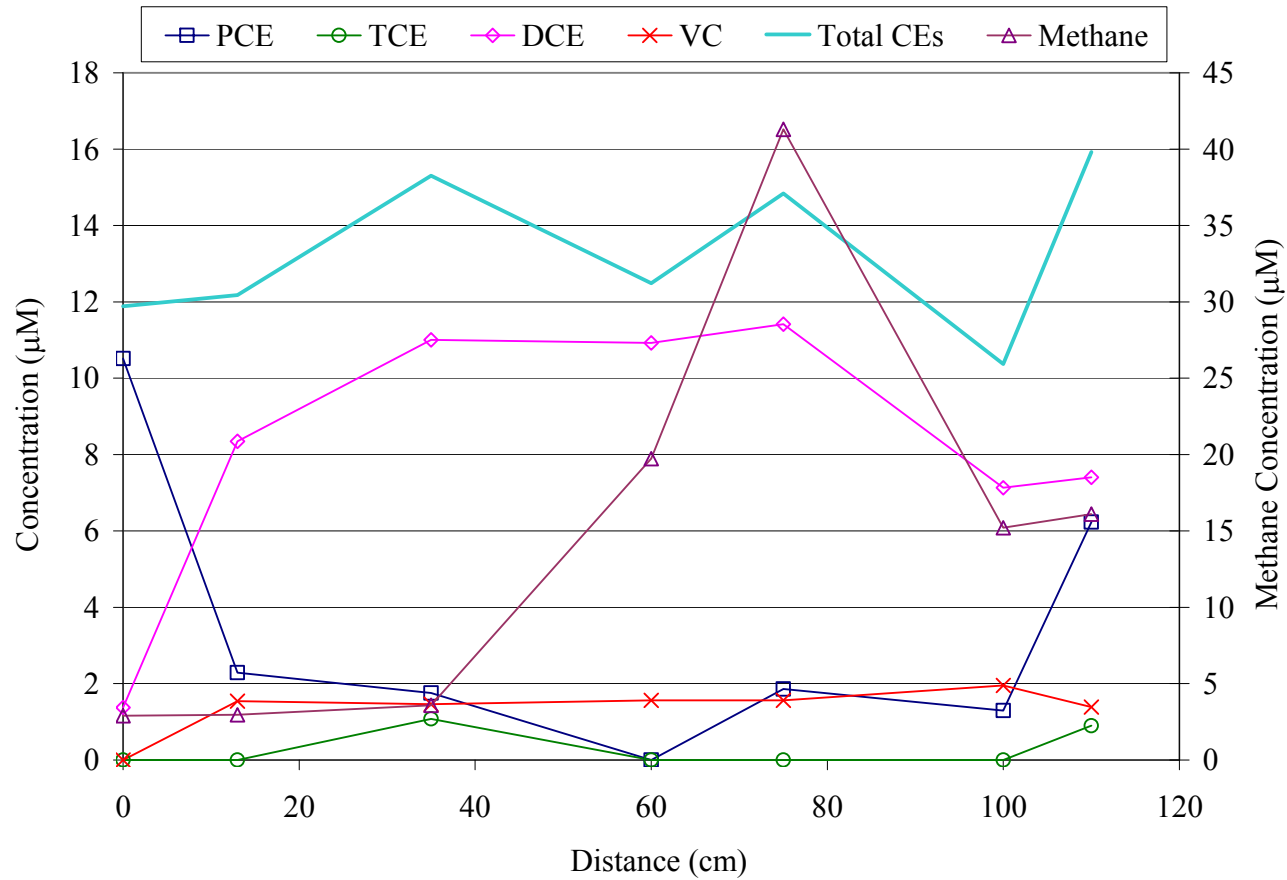
Figure 3.12. Variation of PCE and degradation compounds with distance along channel 1 on day 644, 12/15/05; Methane concentration on right-hand (secondary) y-axis. KB-1 was injected on day 605, 11/7/05. Soy Oil Methyl Esters (SOME) were added on days 203, 236, 266, 299, 328, 359, 387, 415, 417 (well 3) and 594.
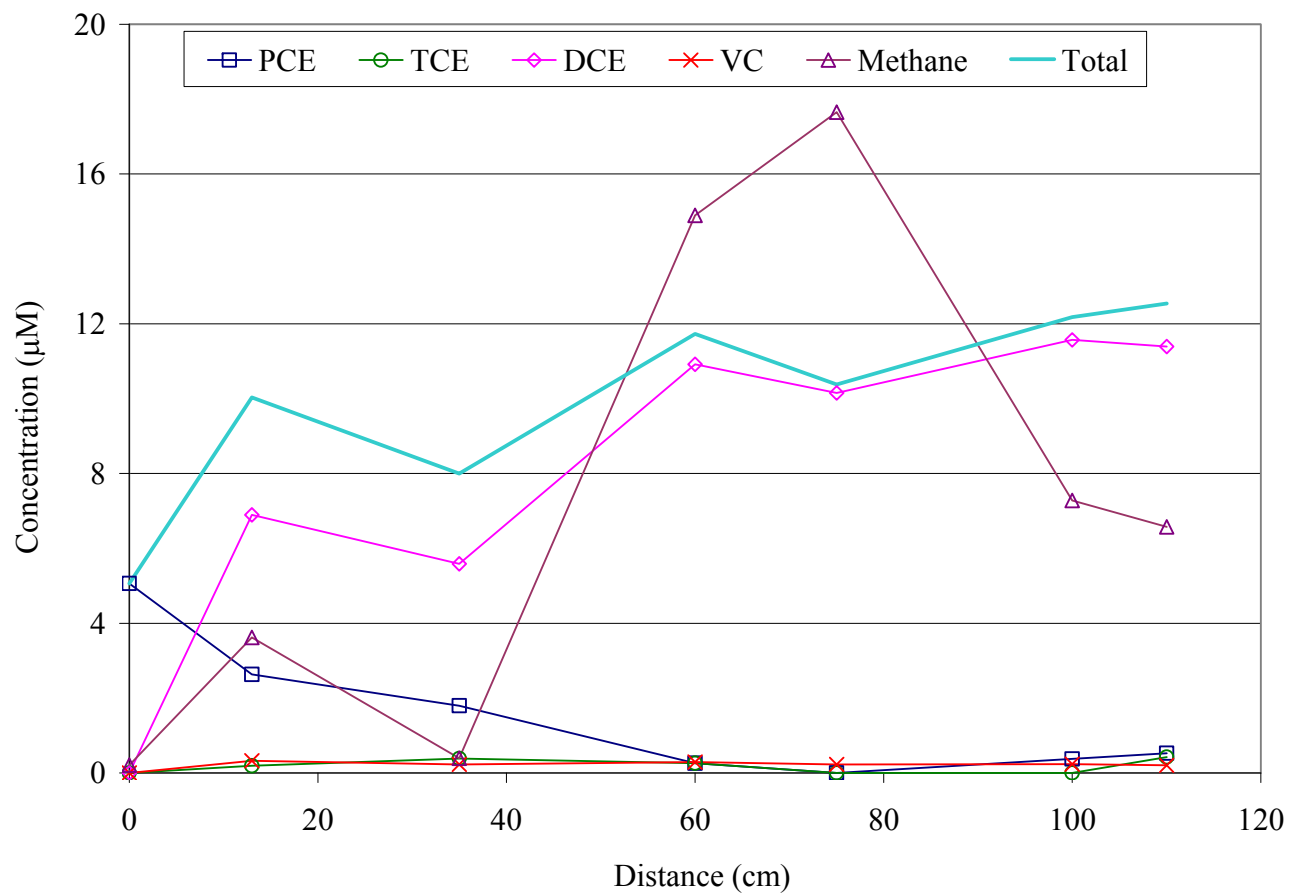
Figure 3.13. Variation of PCE and degradation compounds with distance along channel 1 on day 712, 2/21/06. KB-1 was injected on day 605, 11/7/05. Soy Oil Methyl Esters (SOME) were added on days 203, 236, 266, 299, 328, 359, 387, 415, 417 (well 3), and 594.
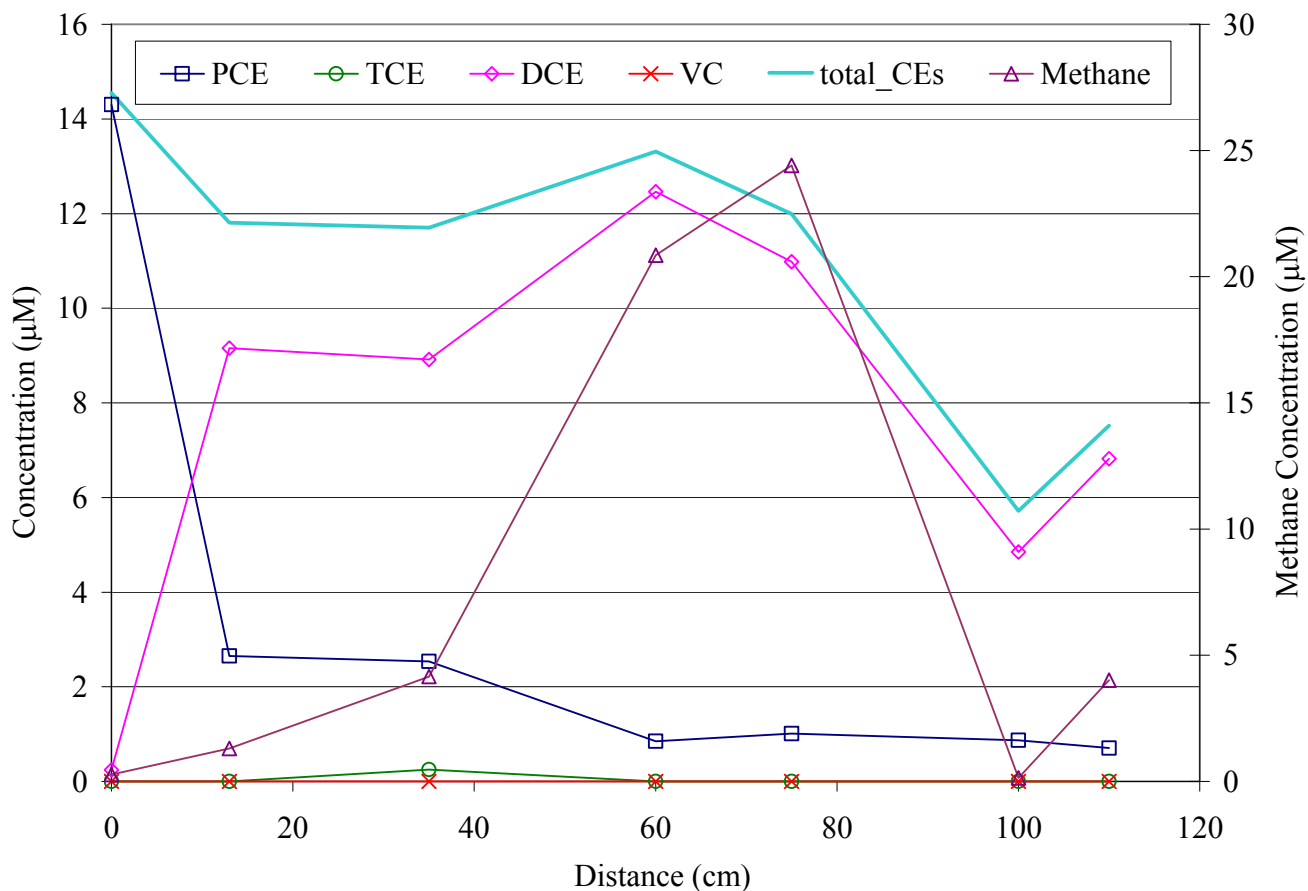
Figure 3.14. Variation of PCE and degradation compounds with distance along channel 1 on day 864, 7/24/06; Methane concentration on right-hand (secondary) y-axis. KB-1 was injected on day 605, 11/7/05 and day 811, 6/1/06. Soy Oil Methyl Esters (SOME) were added on days 203, 236, 266, 299, 328, 359, 387, 415, 417 (well 3) and 594.
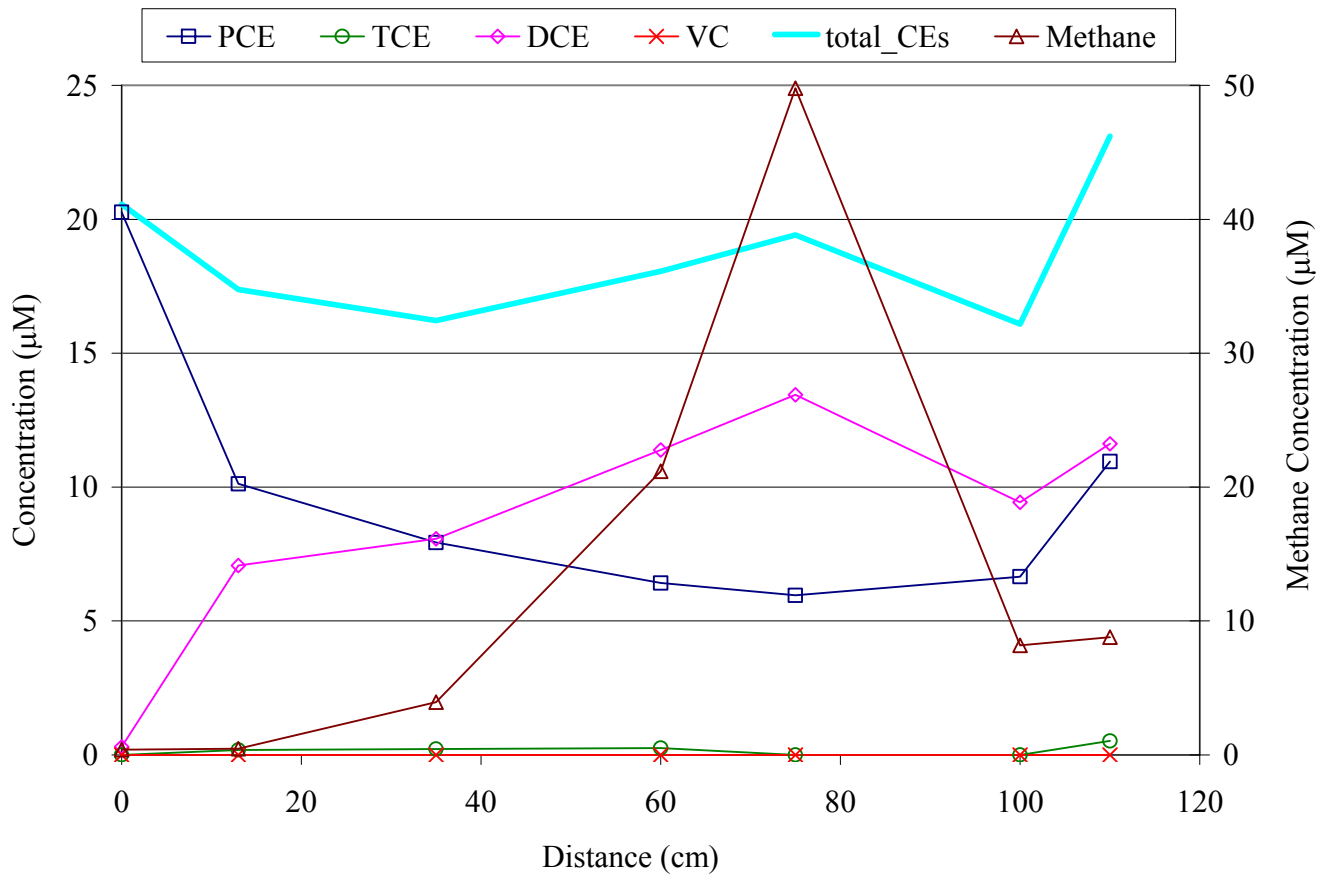
Figure 3.15. Variation of PCE and degradation compounds with distance along channel 1 on day 897, 8/26/06; Methane concentration on right-hand (secondary) y-axis. KB-1 was injected on day 605, 11/7/05 and day 811, 6/1/06. Soy Oil Methyl Esters (SOME) were added on days 203, 236, 266, 299, 328, 359, 387, 415, 417 (well 3) and 594.
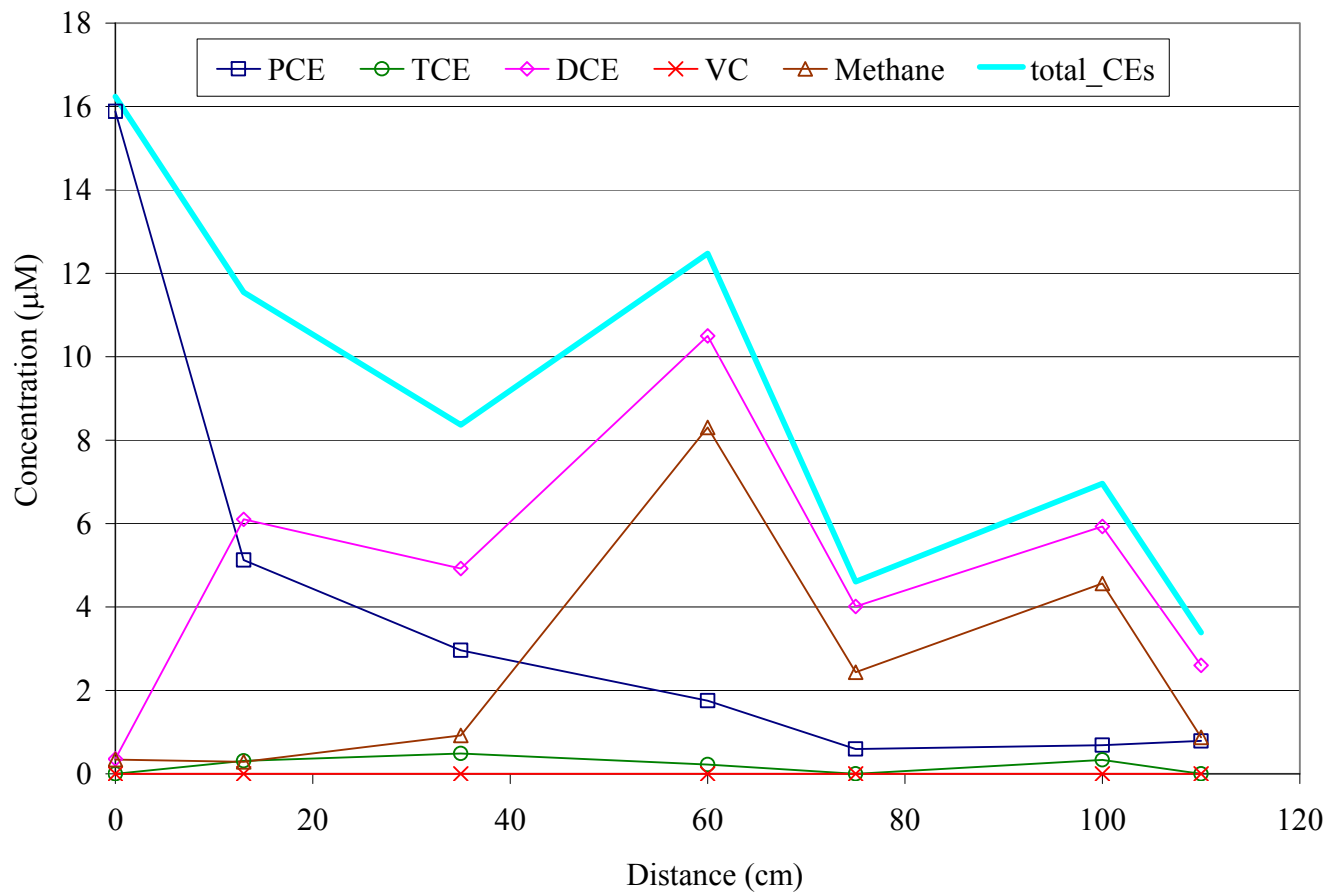
Figure 3.16. Variation of PCE and degradation compounds with distance along channel 1 on day 984, 11/23/06. KB-1 was injected in the inlet on day 605, 11/7/05 and day 811, 6/1/06, and into well 3 on day 958, 10/26/06. Soy Oil Methyl Esters (SOME) were added on days 203, 236, 266, 299, 328, 359, 387, 415, 417 (well 3), 594 and 954.
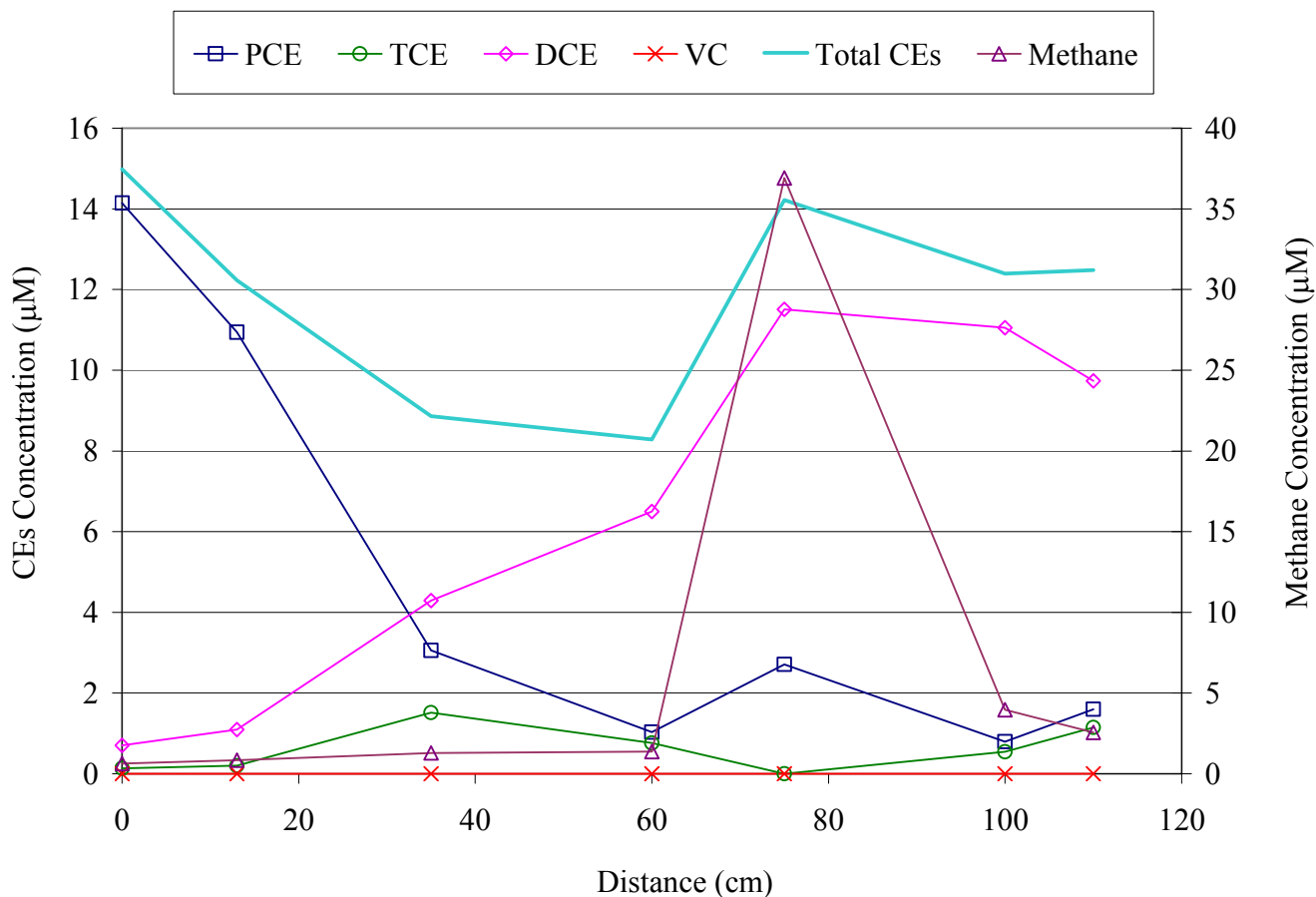
Figure 3.17. Variation of PCE and degradation compounds with distance along channel 2 on day 409, 4/25/05; methane concentration on right-hand (secondary) y-axis. Glucose solution was added on days 110, 151, 173, 203, and 236. Corn starch was added on days 266, 299 and 328.
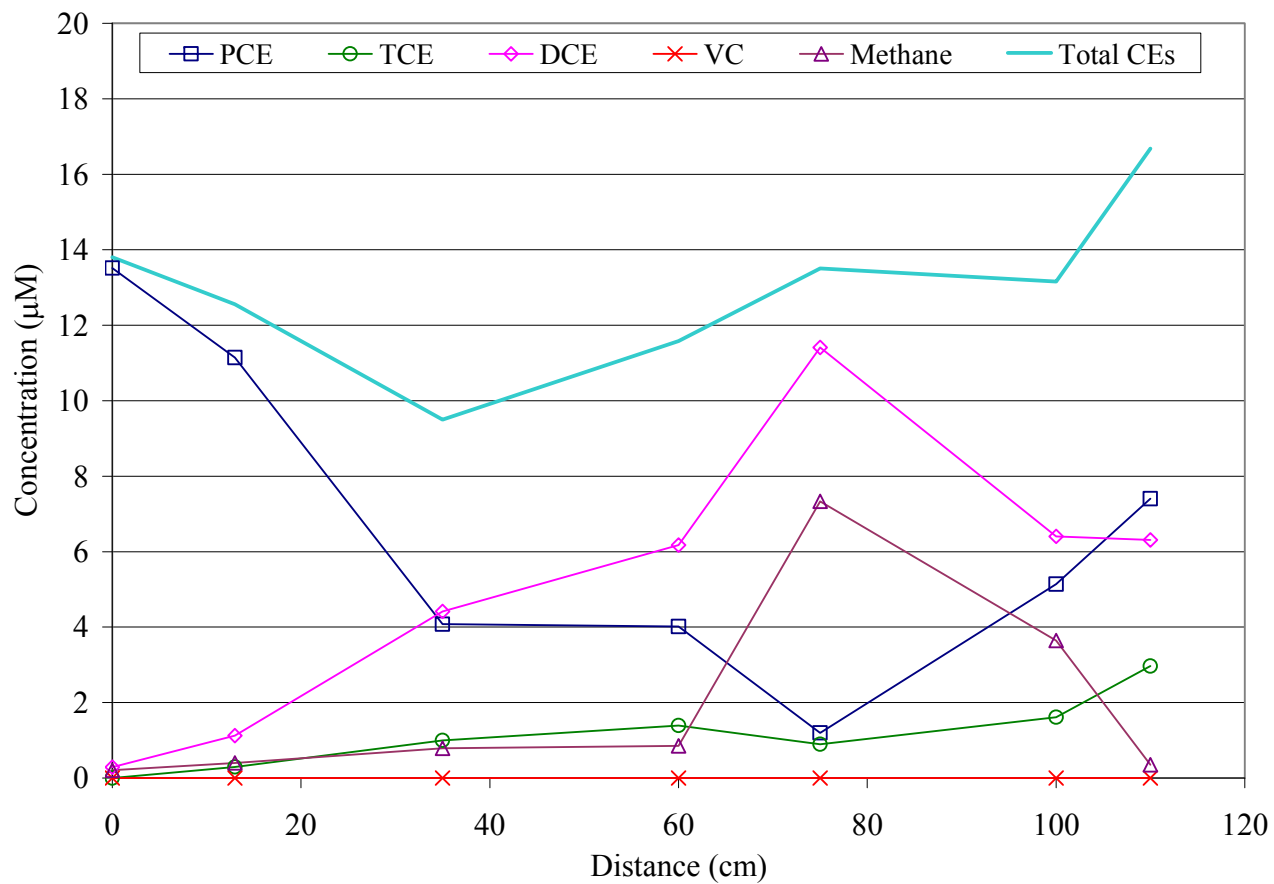
Figure 3.18. Variation of PCE and degradation compounds with distance along channel 2 on day 438, 5/24/05; Glucose solution was added on days 110, 151, 173, 203 and 236. Corn starch was added on days 266, 299 and 328.
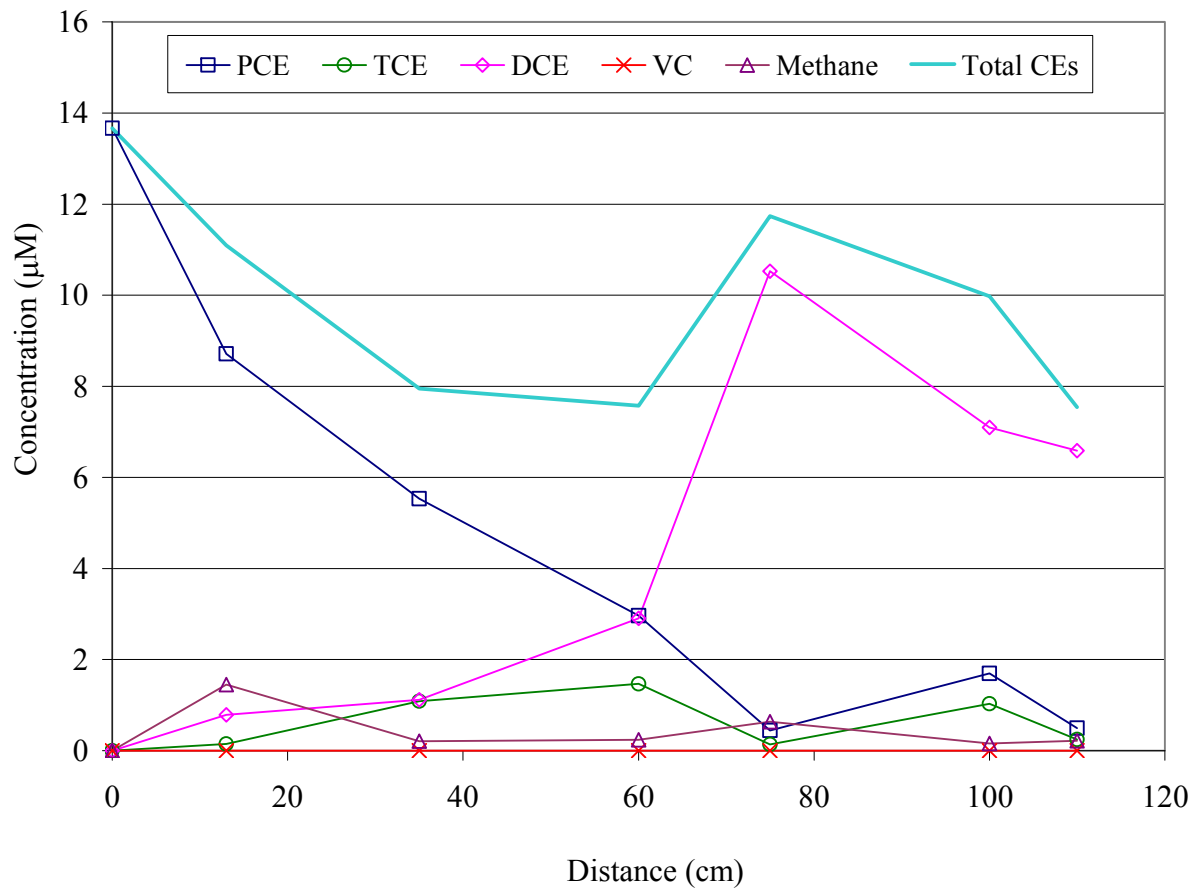
Figure 3.19. Variation of PCE and degradation compounds with distance along channel 2 on day 555, 9/18/05; Glucose solution was added on days 110, 151, 173, 203 and 236. Corn starch was on days 266, 299, 328 and 522.
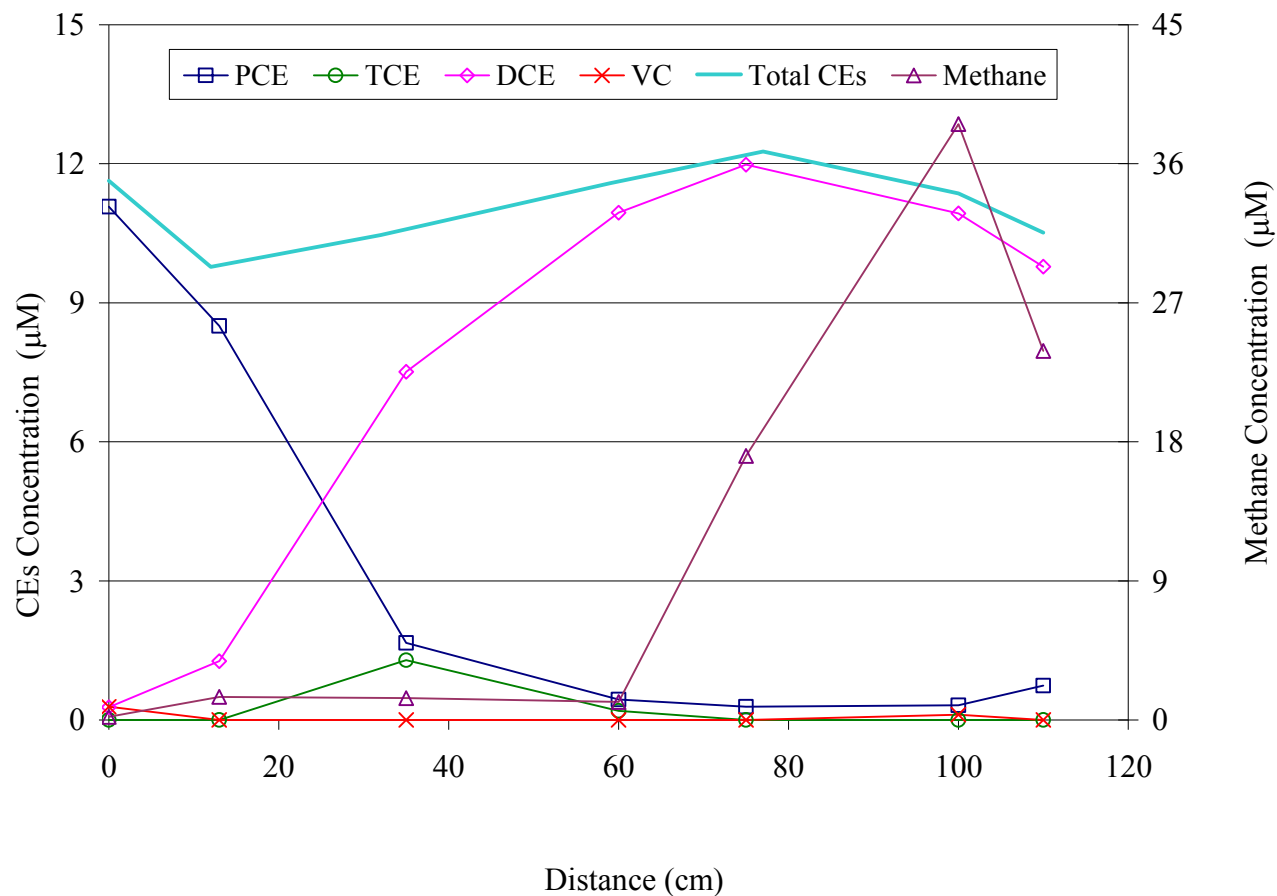
Figure 3.20. Variation of PCE and degradation compounds with distance along channel 2 on day 614, 11/16/05. Methane on right-hand (secondary)y-axis. KB-1 was injected on day 591, 10/24/05; Glucose solution was added on days 110, 151, 173, 203, 236 and 564. Corn starch was added on days 266, 299, 328 and 522.
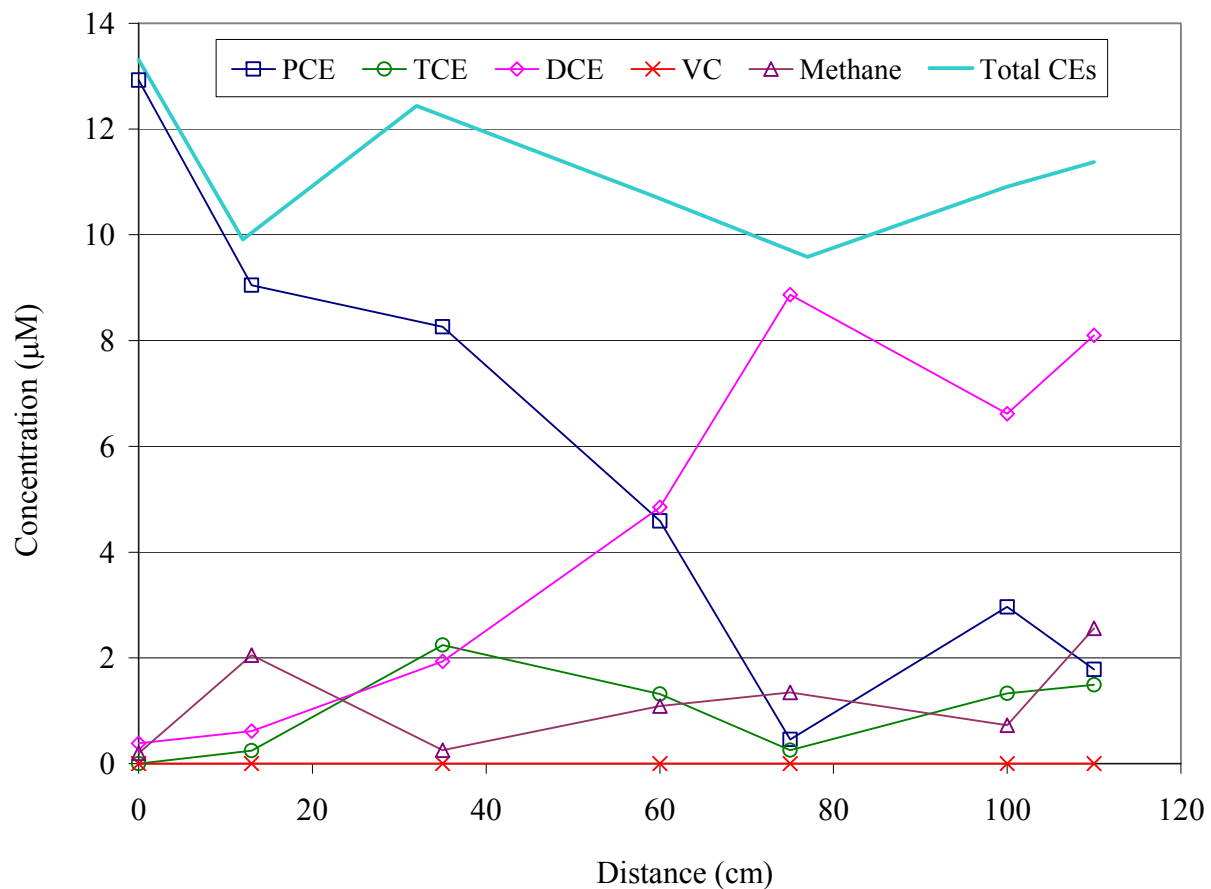
Figure 3.21. Variation of PCE and degradation compounds with distance along channel 2 on day 741, 3/22/06. KB-1 was injected on day 591, 10/24/05; Glucose solution was added on days 110, 151, 173, 203, 236, 564 and 676. Corn starch was added on days 266, 299, 328 and 522.
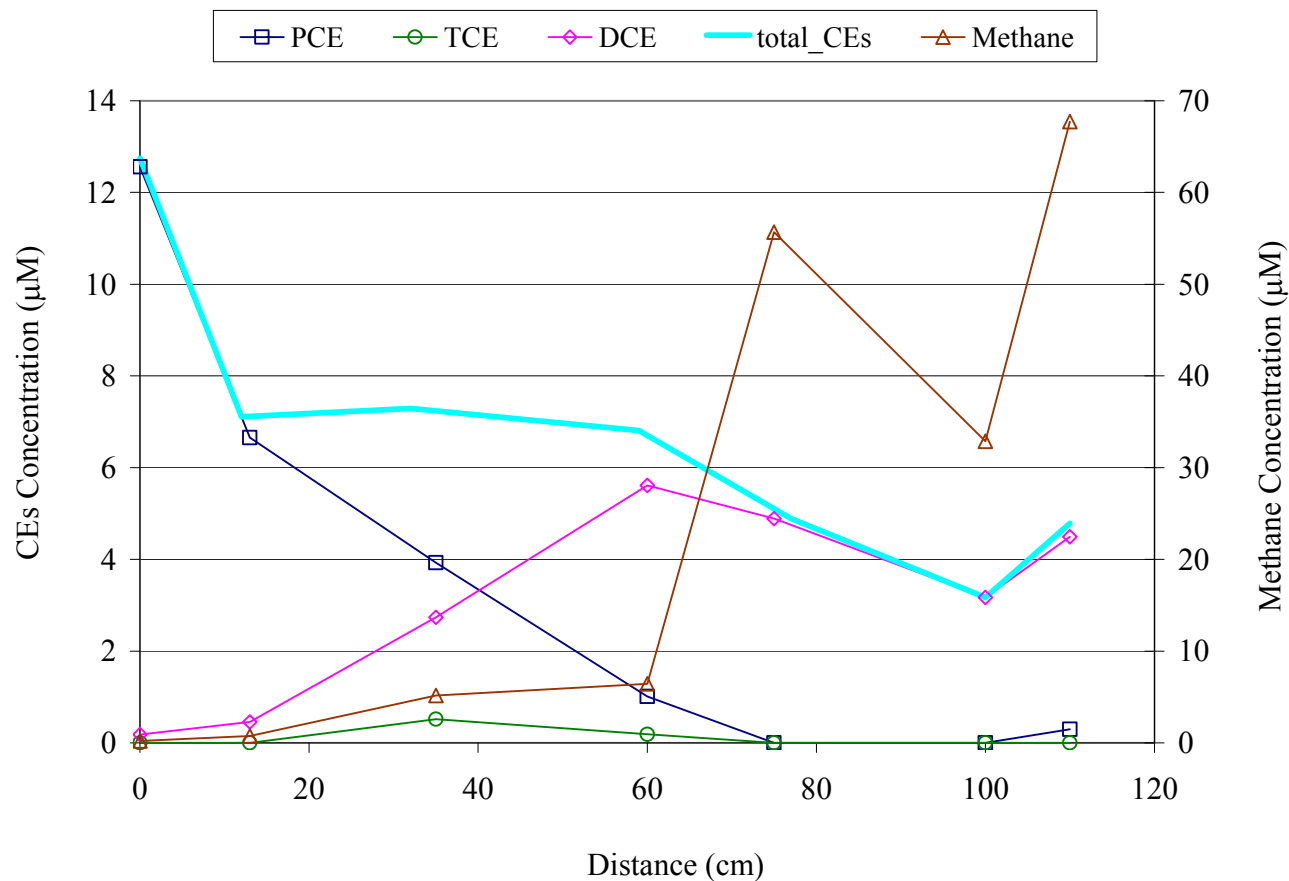
Figure 3.22. Variation of PCE and degradation compounds with distance along channel 2 on day 831, 6/21/06. Methane on right-hand (secondary)y-axis. KB-1 was injected on 10/24/05 (day 591) and 5/31/06 (day 810); Glucose solution was added on days 110, 151, 173, 203, 236, 564, 676 and 800. Corn starch was added on days 266, 299, 328 and 522.
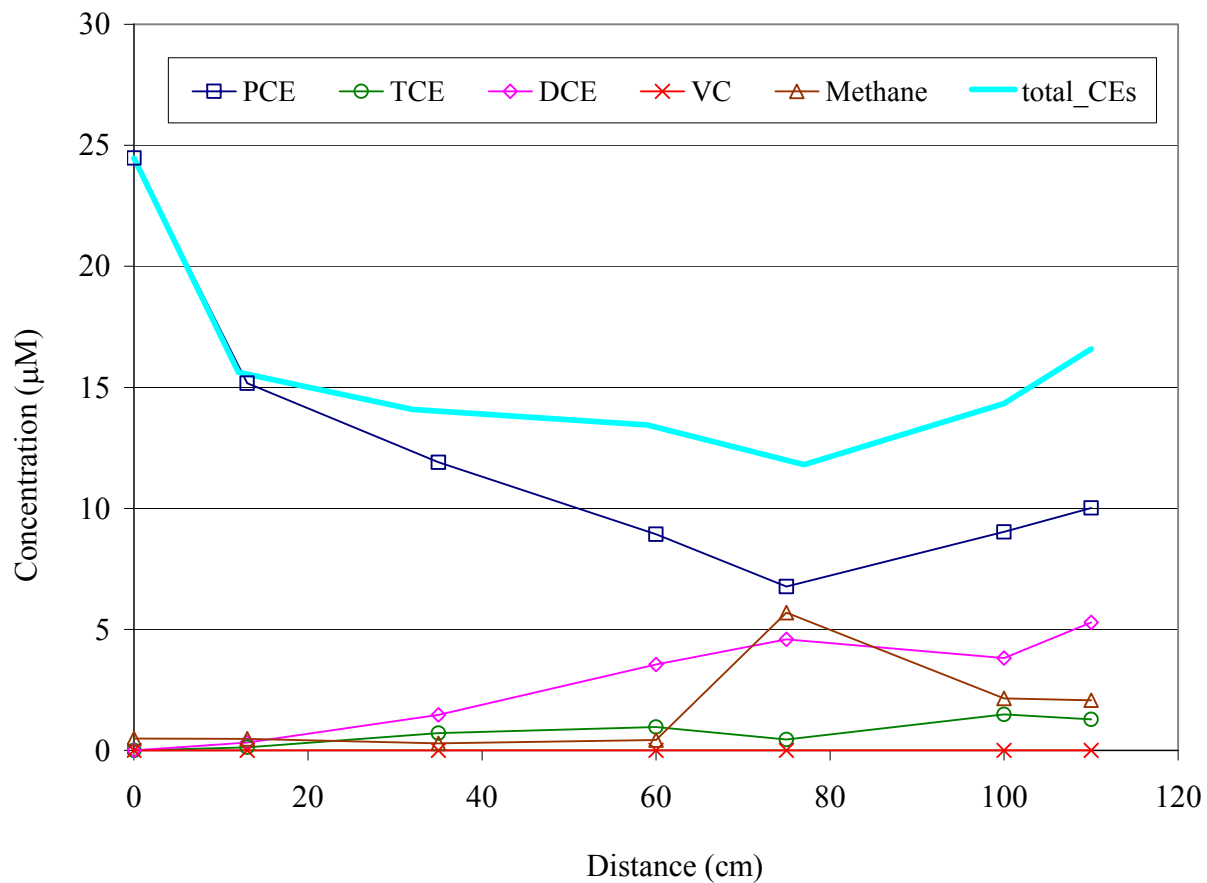
Figure 3.23. Variation of PCE and degradation compounds with distance along channel 2 on day 897, 8/26/06. KB-1 was injected on 10/24/05 (day 591) and 5/31/06 (day 810). Glucose solution was added on days 110, 151, 173, 203, 236, 564, 676 and 800. Corn starch was added on days 266, 299, 328 and 522.
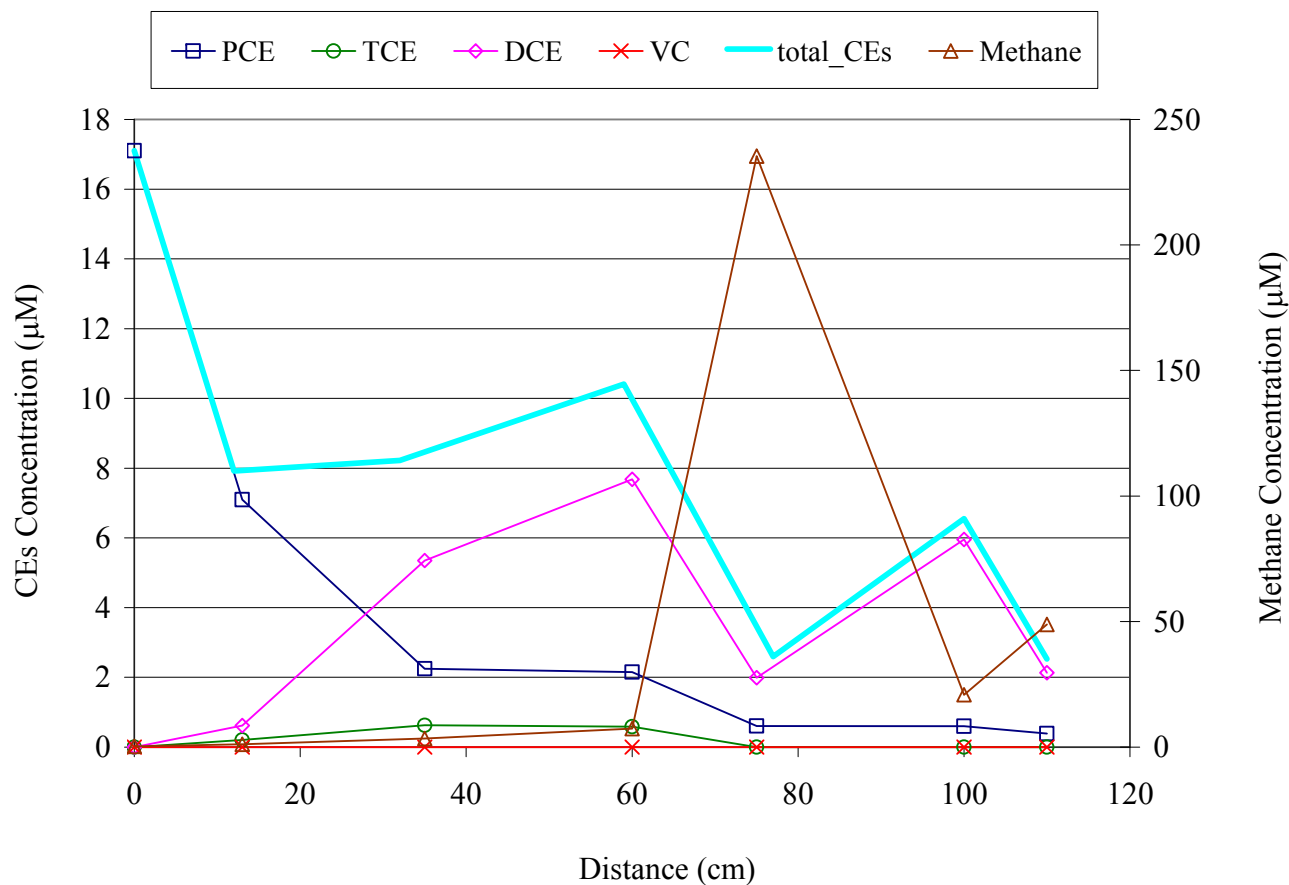
Figure 3.24. Variation of PCE and degradation compounds with distance along channel 2 on day 984, 11/23/06. Methane on right-hand (secondary)y-axis. KB-1 injected on 10/24/05 (day 591) and 5/31/06 (day 810) and 10/26/06 (day 958, into well 3). Glucose solution was added on days 110, 151, 173, 203, 236, 564, 676, 800, 901 and 955. Corn starch was added on days 266, 299, 328 and 522.
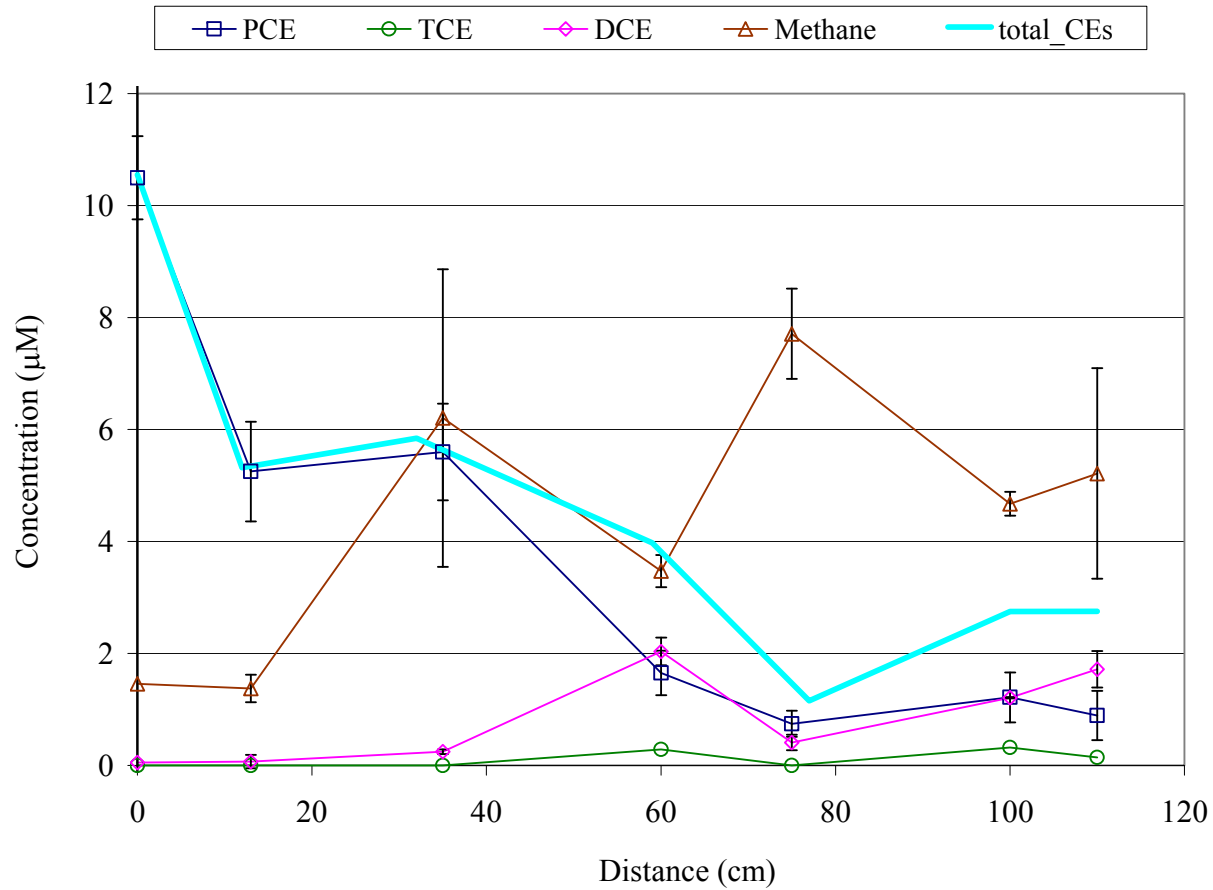
Figure 3.25. Variation of PCE and degradation compounds with distance along channel 2 on day 1123, 4/10/07. KB-1 was injected on 10/24/05 (day 591) and 5/31/06 (day 810) and 10/26/06 (day 958, into well 3). Glucose solution was added on days 110, 151, 173, 203, 236 564, 676, 800, 901, 955, 994 (well 1) and 1025 (well 2). Corn starch was added on days 266, 299, 328 and 522.
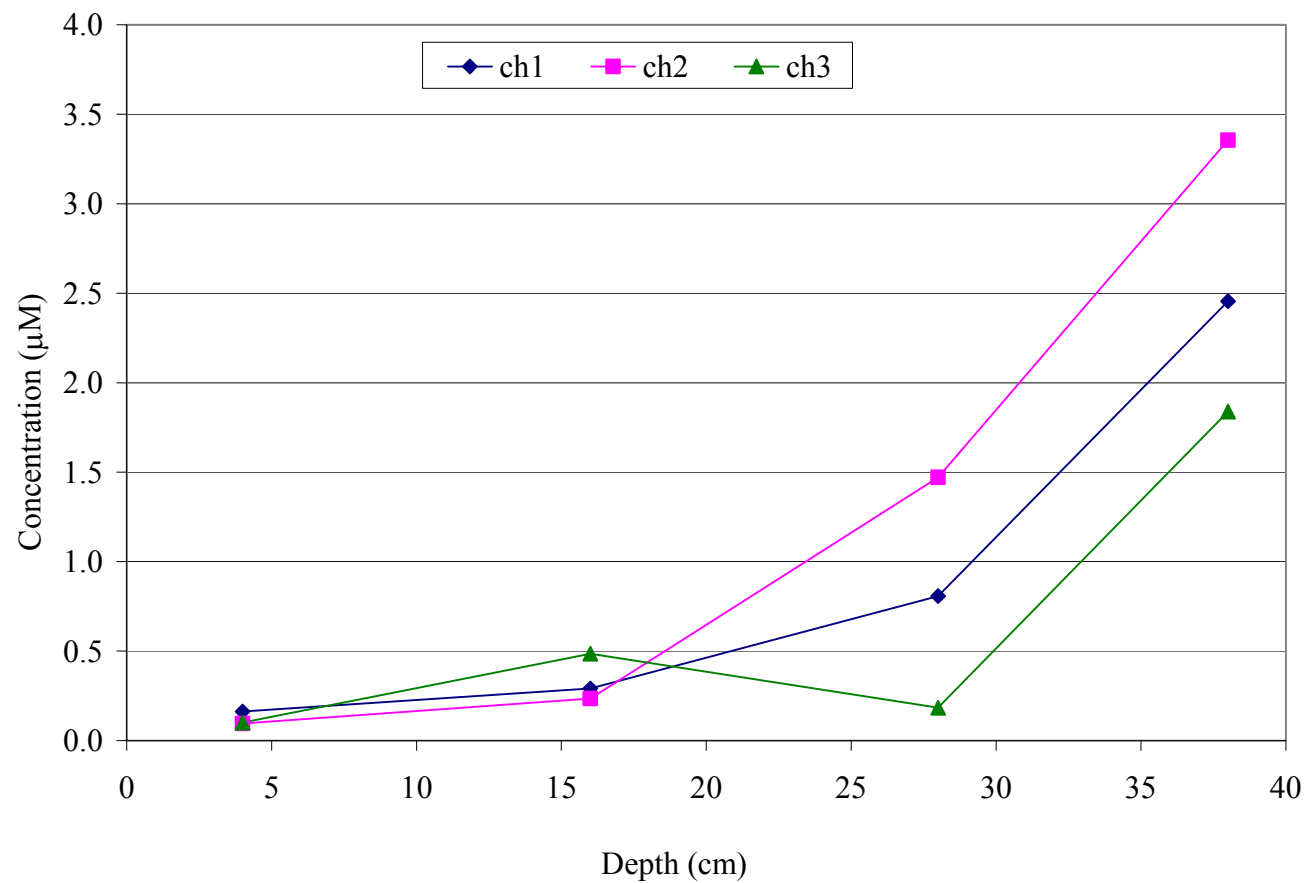
Figure 3.26. PCE concentration in the the aqueous phase of soil samples of channels 1, 2 and 3; June 15, 2004, day 95. Inlet concentration is ~ 12 μM. Samples collected at a distance of 32 cm from inlet.
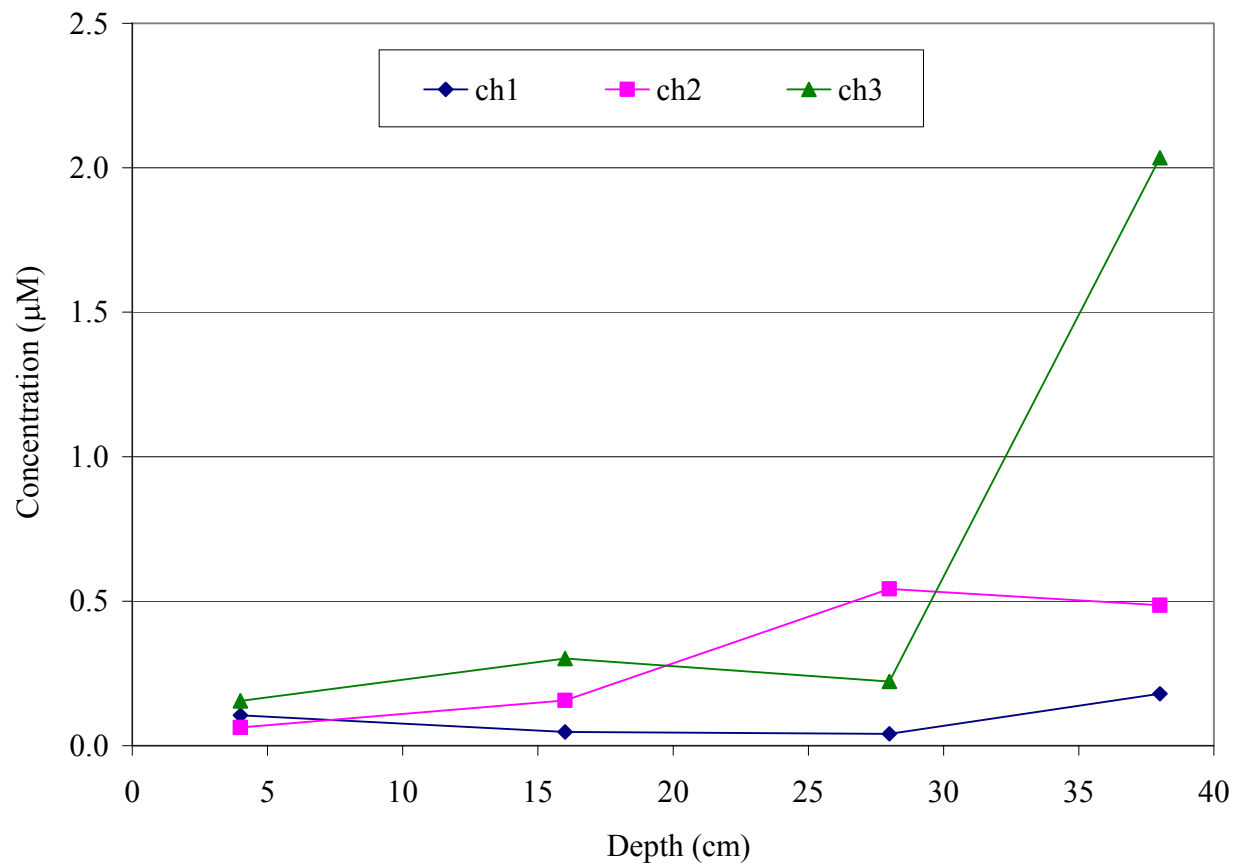
Figure 3.27. PCE concentration in the the aqueous phase of soil samples of channels 1, 2 and 3; August 9, 2004, day 150. Inlet concentration is ~ 12 μM. Samples were collected at a distance of 55 cm from inlet.
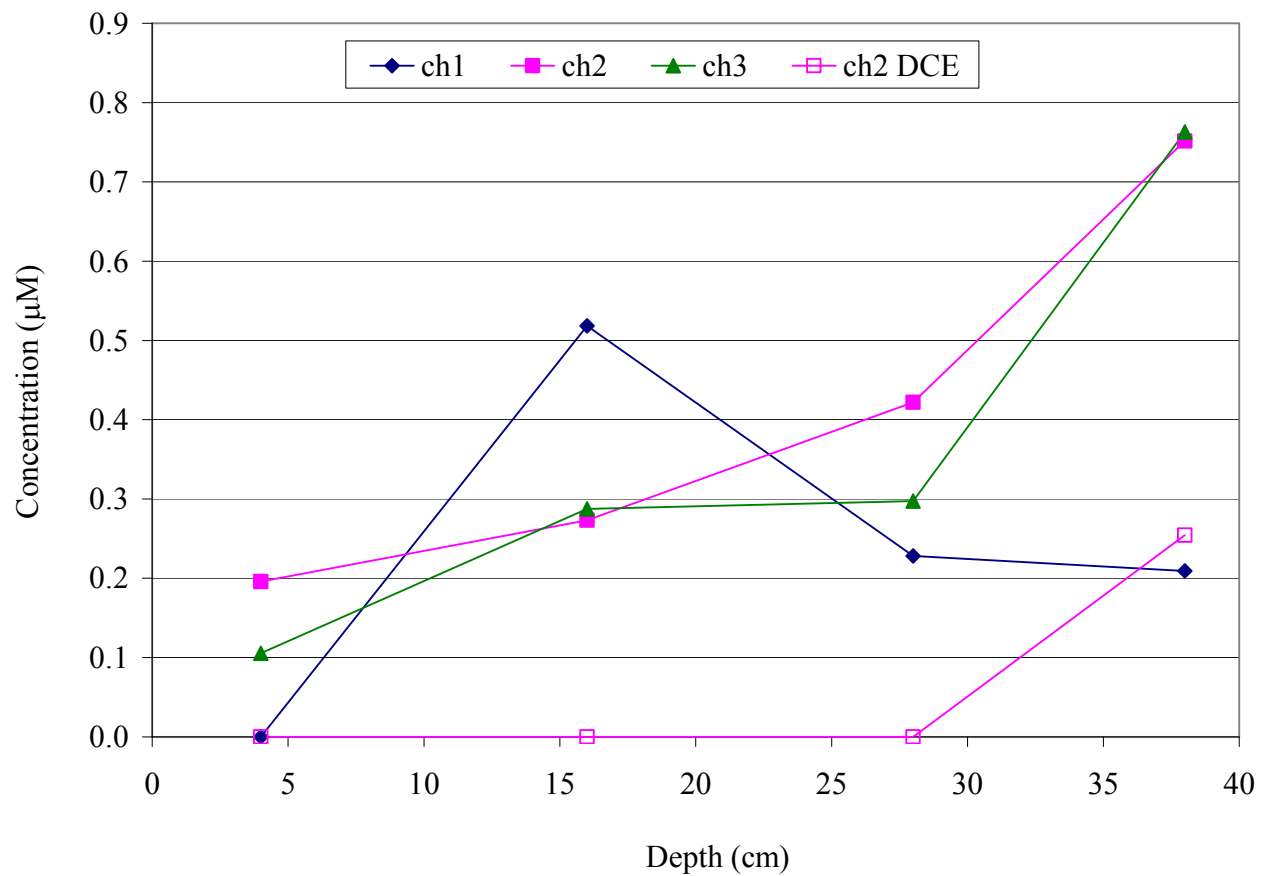
Figure 3.28. PCE and DCE concentrations in the aqueous phase of soil samples on day 229, 10/27/04. Inlet concentration is ~ 12 μM. Samples were collected at a distance of 65 cm from inlet.
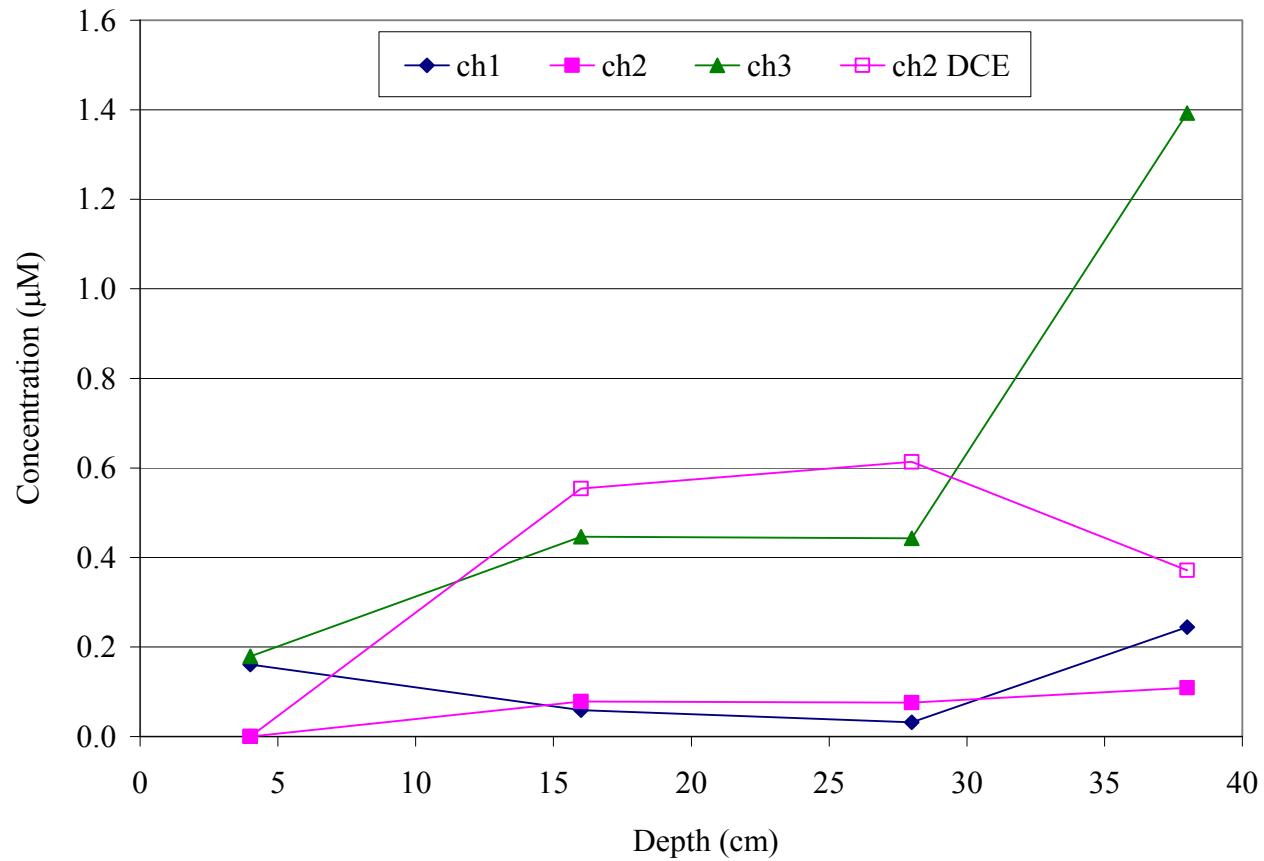
Figure 3.29. PCE and DCE concentrations in the aqueous phase of soil samples on day 353, 2/28/05. Inlet concentration is ~ 12 μM. Samples were collected at a distance of 90 cm (channel 1 and 2) and 100 cm (channel 3) from inlet.
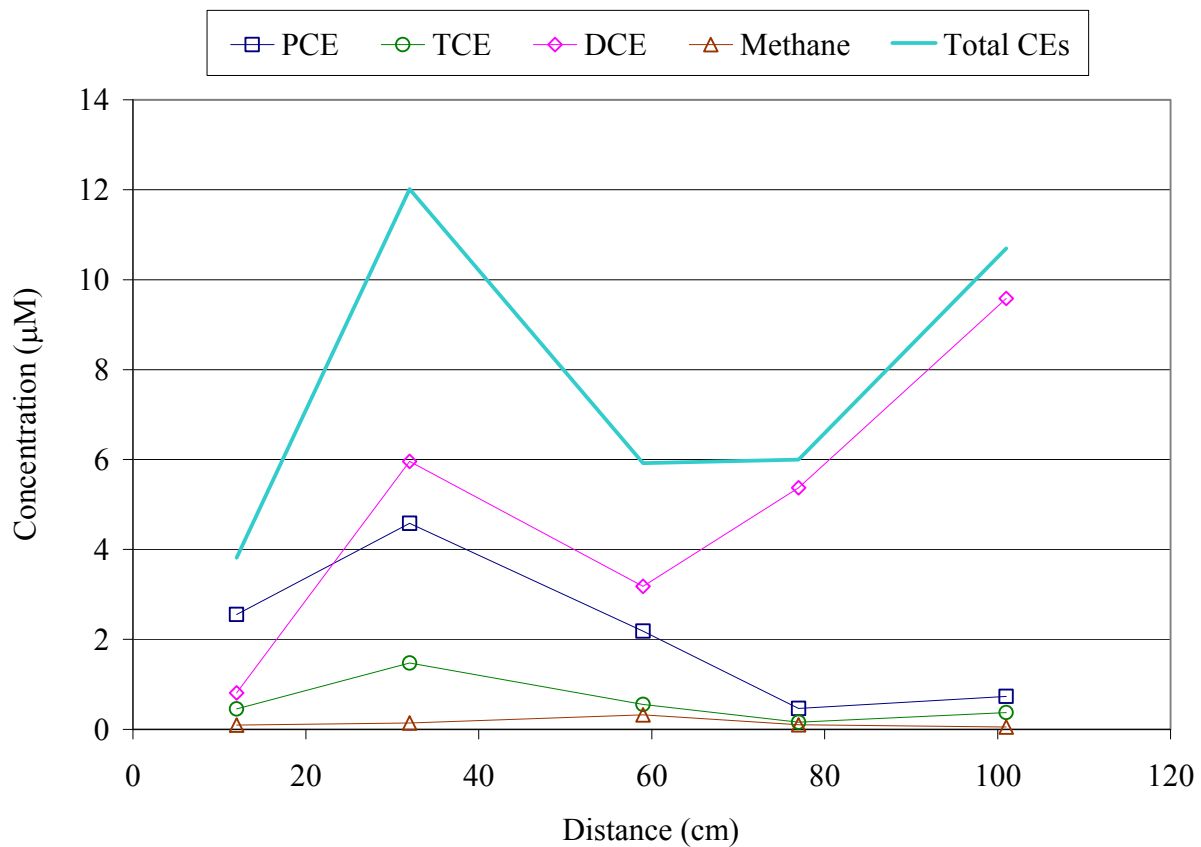
Figure 3.30. CEs and Methane profile, in the aqueous phase of channel 2 soil, vs length of the channel on day 385, 4/1/05. Soil samples were collected at 37 cm depth at all locations except 101 cm which was collected at 42 cm depth. The samples were collected while installing wells in channel 2.
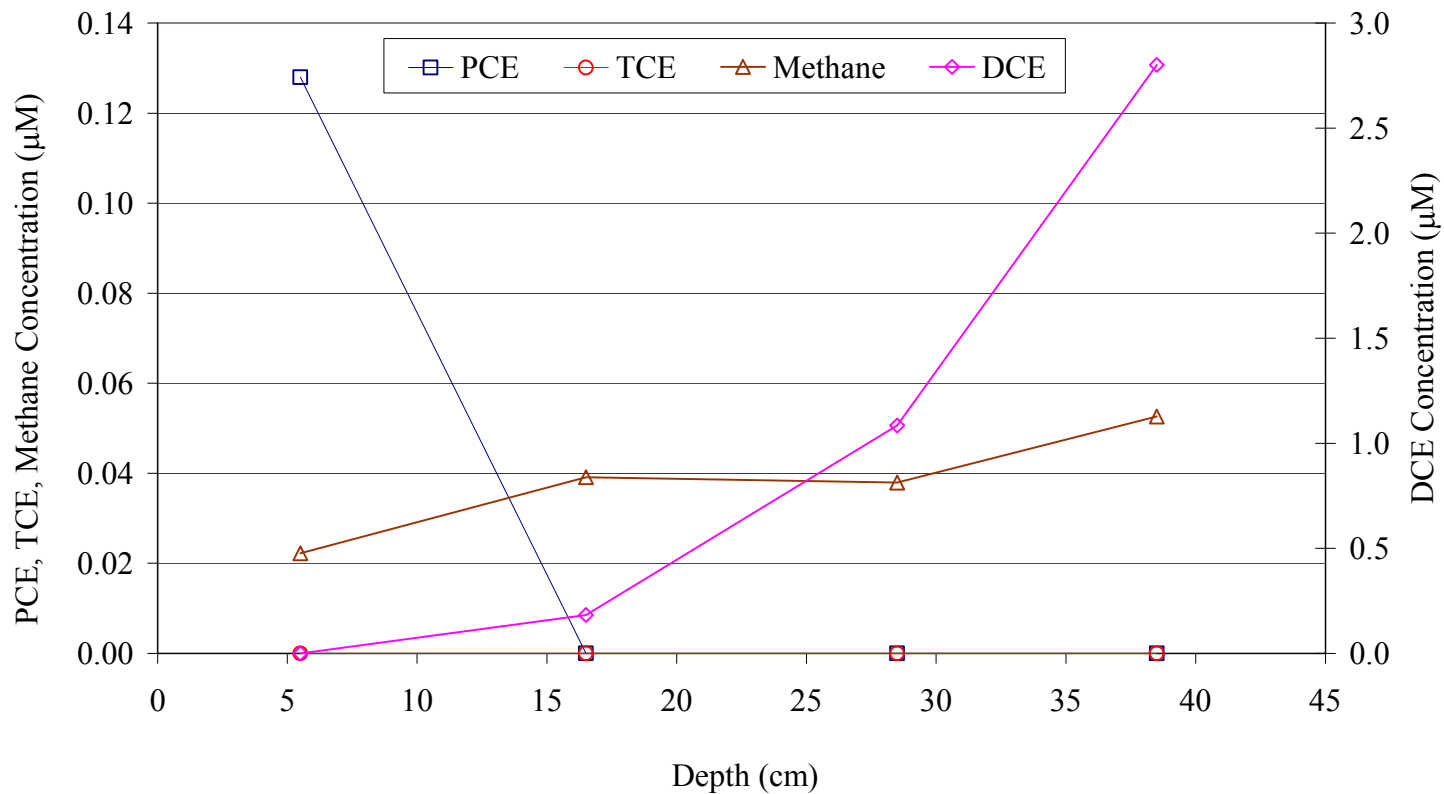
Figure 3.31. CEs and Methane profile, in the aqueous phase of channel 1 soil, vs depth from the surface of the soil on day 812, 6/12/06; DCE right-hand (secondary) y-axis. Soil samples were collected at a distance of 10 cm from inlet.
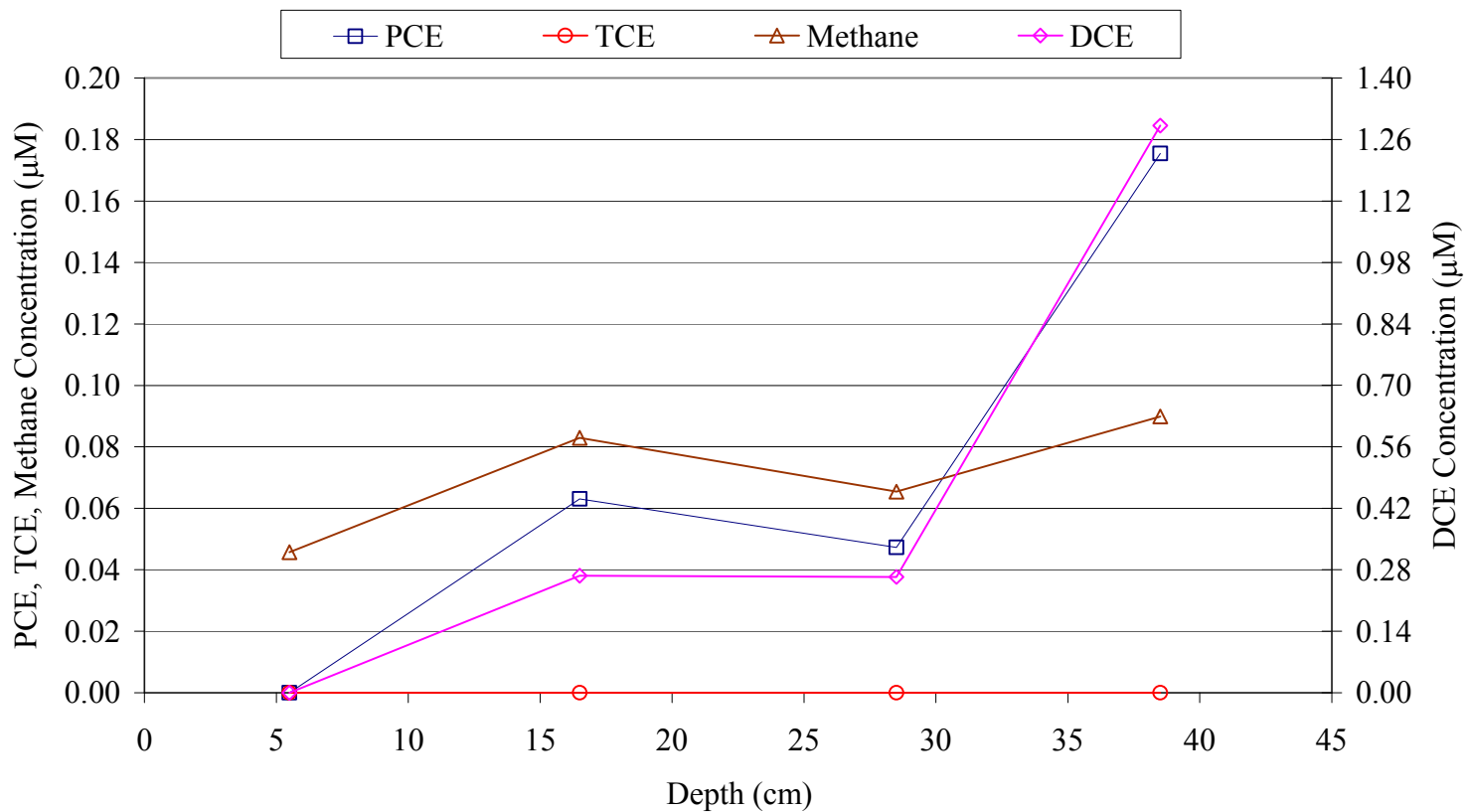
Figure 3.32. CEs and Methane profile, in the aqueous phase of channel 2 soil, vs depth from the surface of the soil on day 812, 6/12/06; DCE right-hand (secondary) y-axis. Soil samples were collected at a distance of 14 cm from inlet.
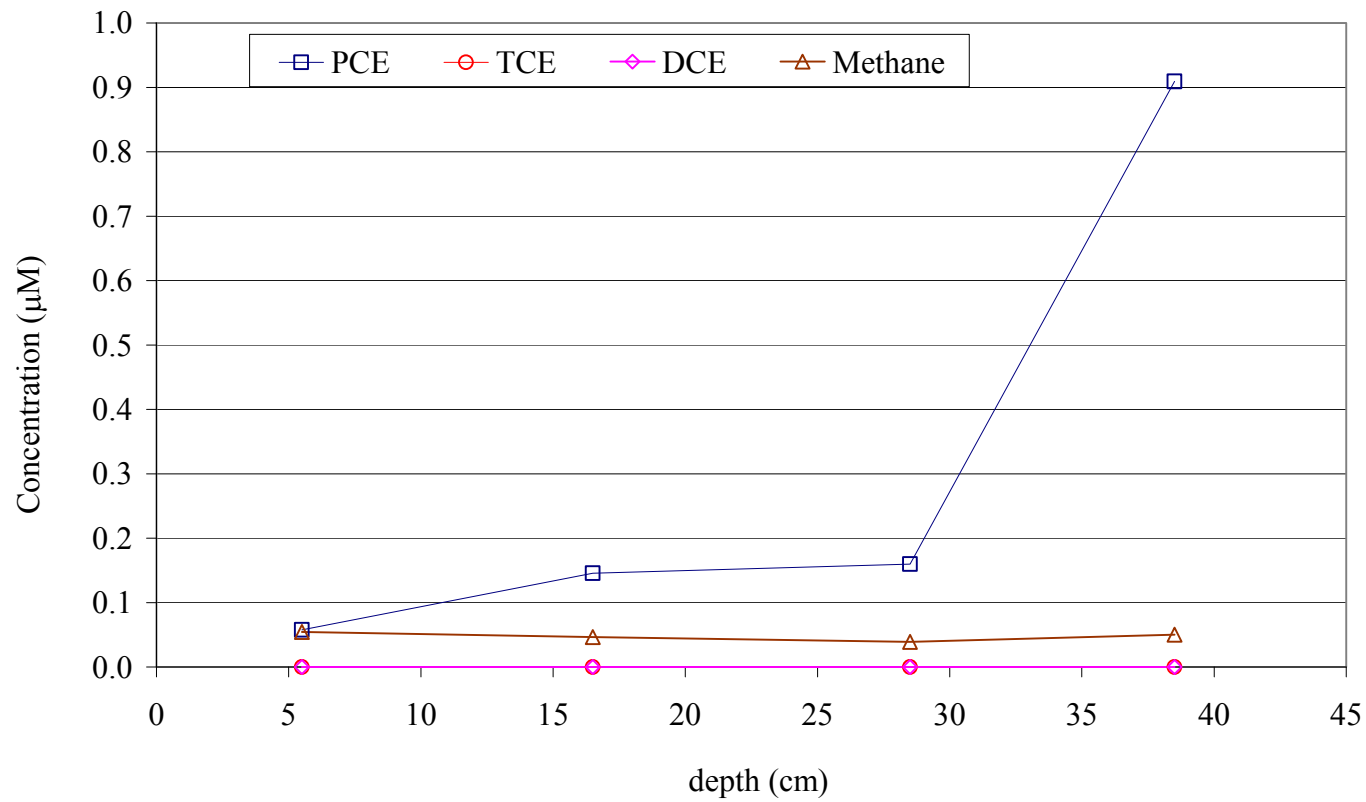
Figure 3.33. CEs and Methane profile, in the aqueous phase of channel 3 soil, vs depth from the surface of the soil on day 812, 6/12/06. Soil samples were collected at a distance of 30 cm from inlet.
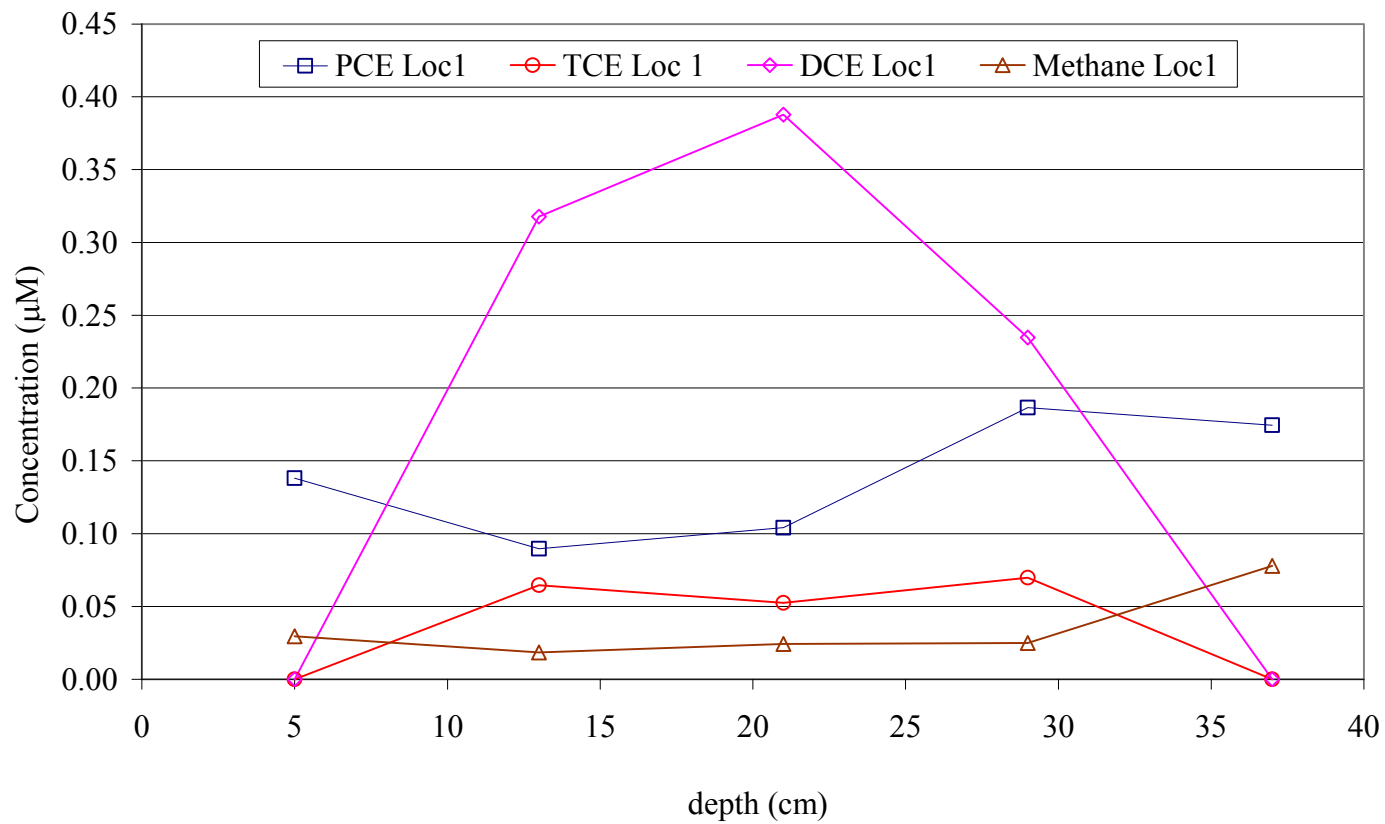
Figure 3.34a. CEs and methane profile, in the aqueous phase of channel 1 soil, vs depth from the surface of the soil on day 955, 10/23/06. Soil samples were collected at a distance of 18 cm from inlet.
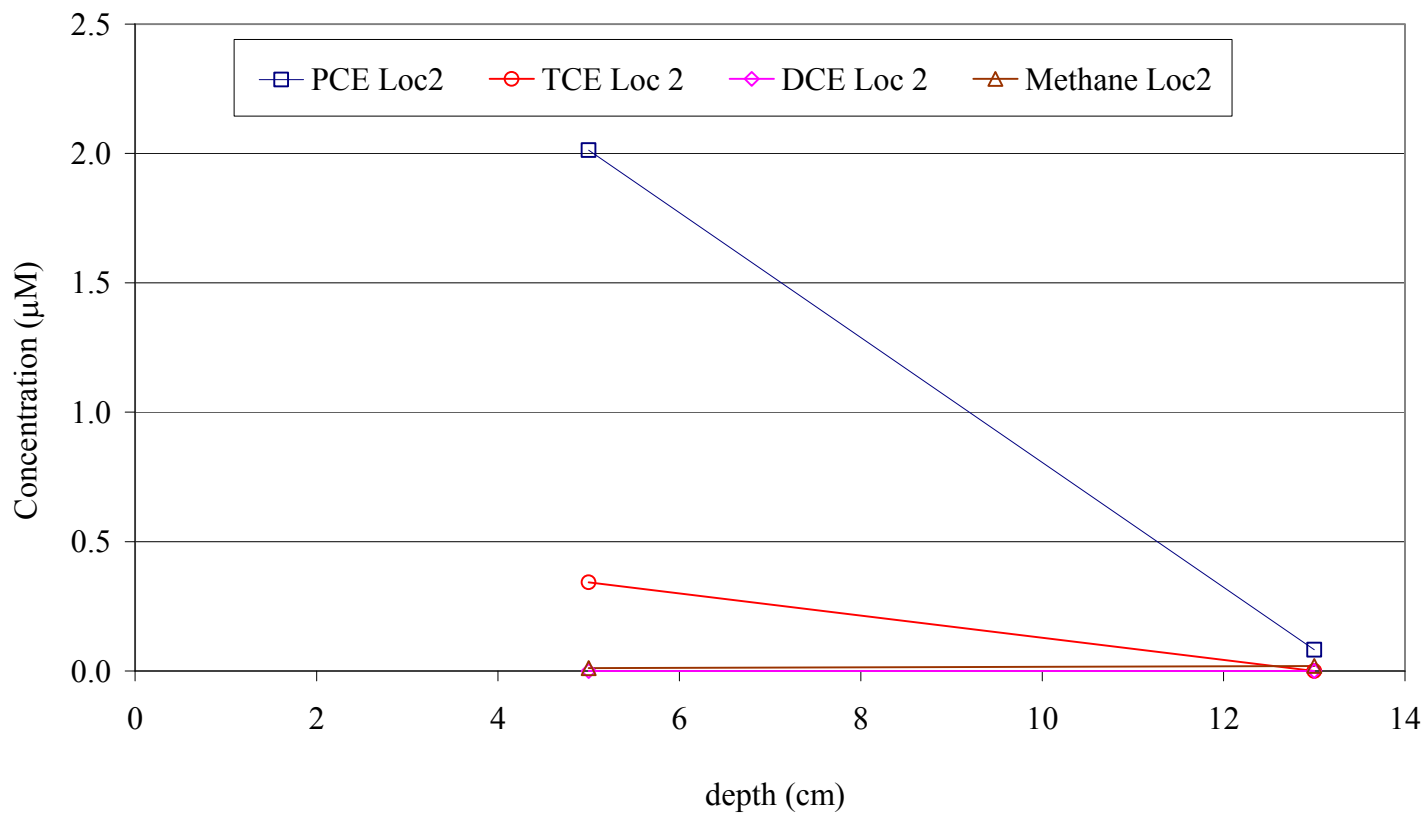
Figure 3.34b. CEs and methane profile, in the aqueous phase of channel 1 soil, vs depth from the surface of the soil on day 955, 10/23/06. Soil samples were collected at a distance of 20 cm from inlet.
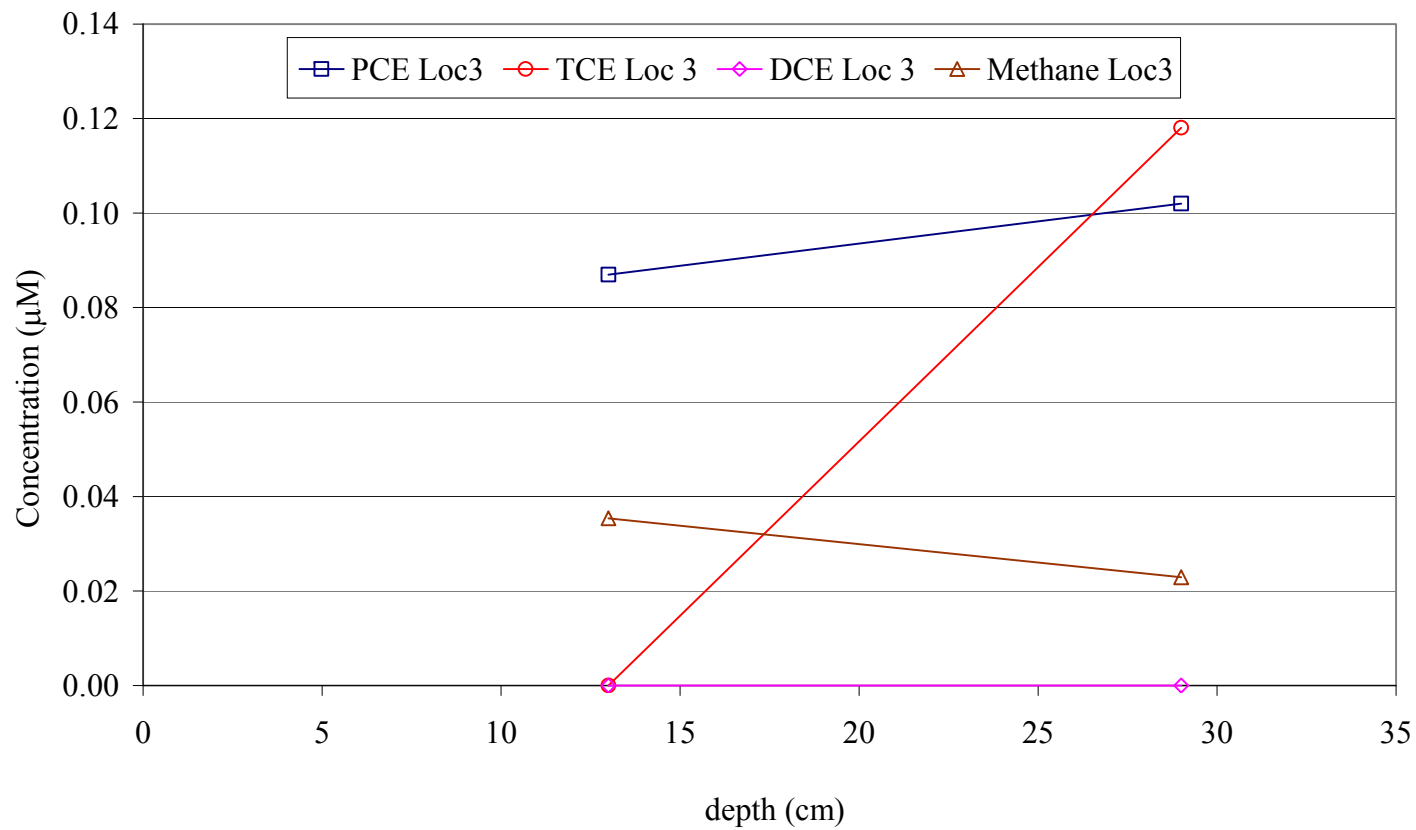
Figure 3.34c. CEs and methane profile, in the aqueous phase of channel 1 soil, vs depth from the surface of the soil on day 955, 10/23/06. Soil samples were collected at a distance of 75 cm from inlet.
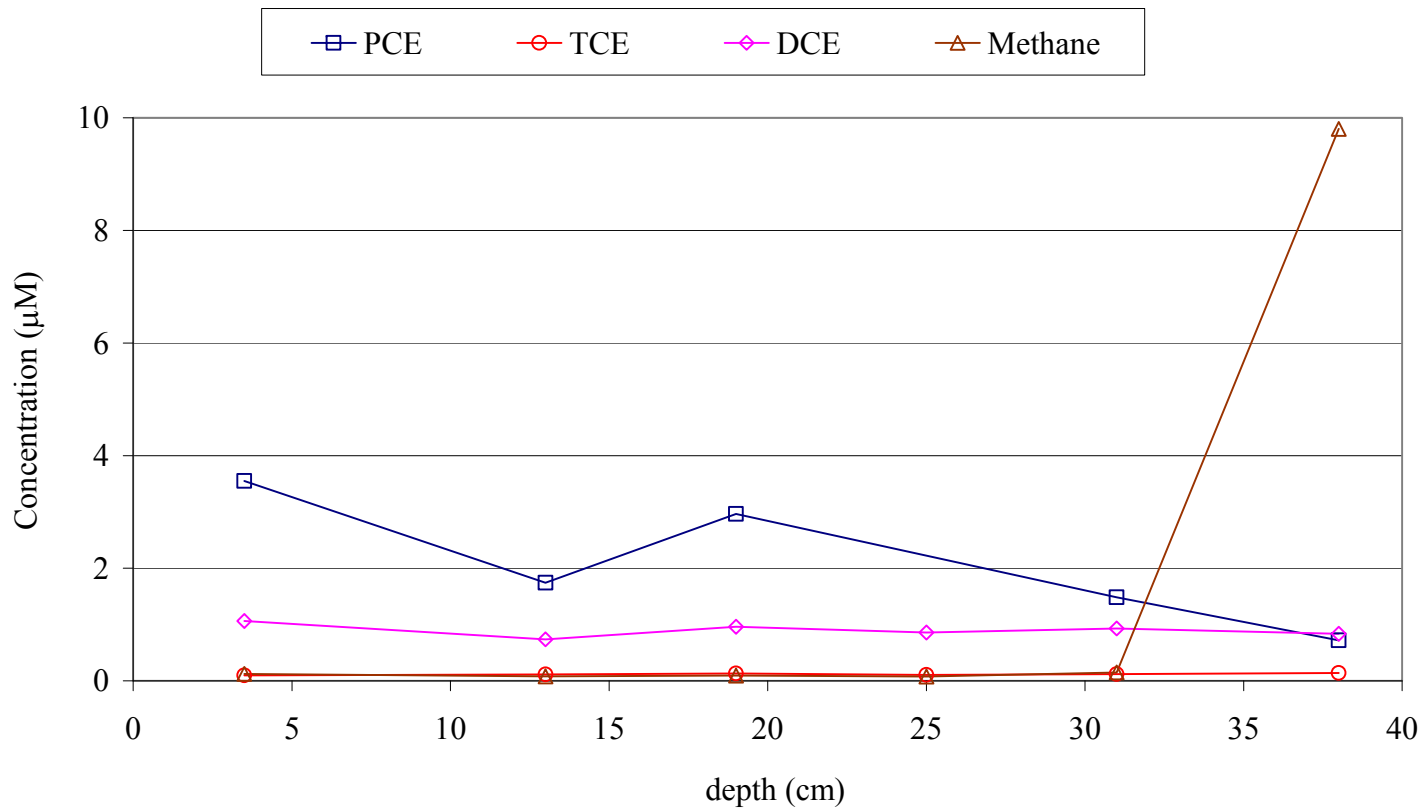
Figure 3.35. CEs and methane profile, in the aqueous phase of channel 2 soil, vs depth from the surface of the soil on day 987, 11/26/06. Soil samples were collected at a distance of 32 cm from inlet.
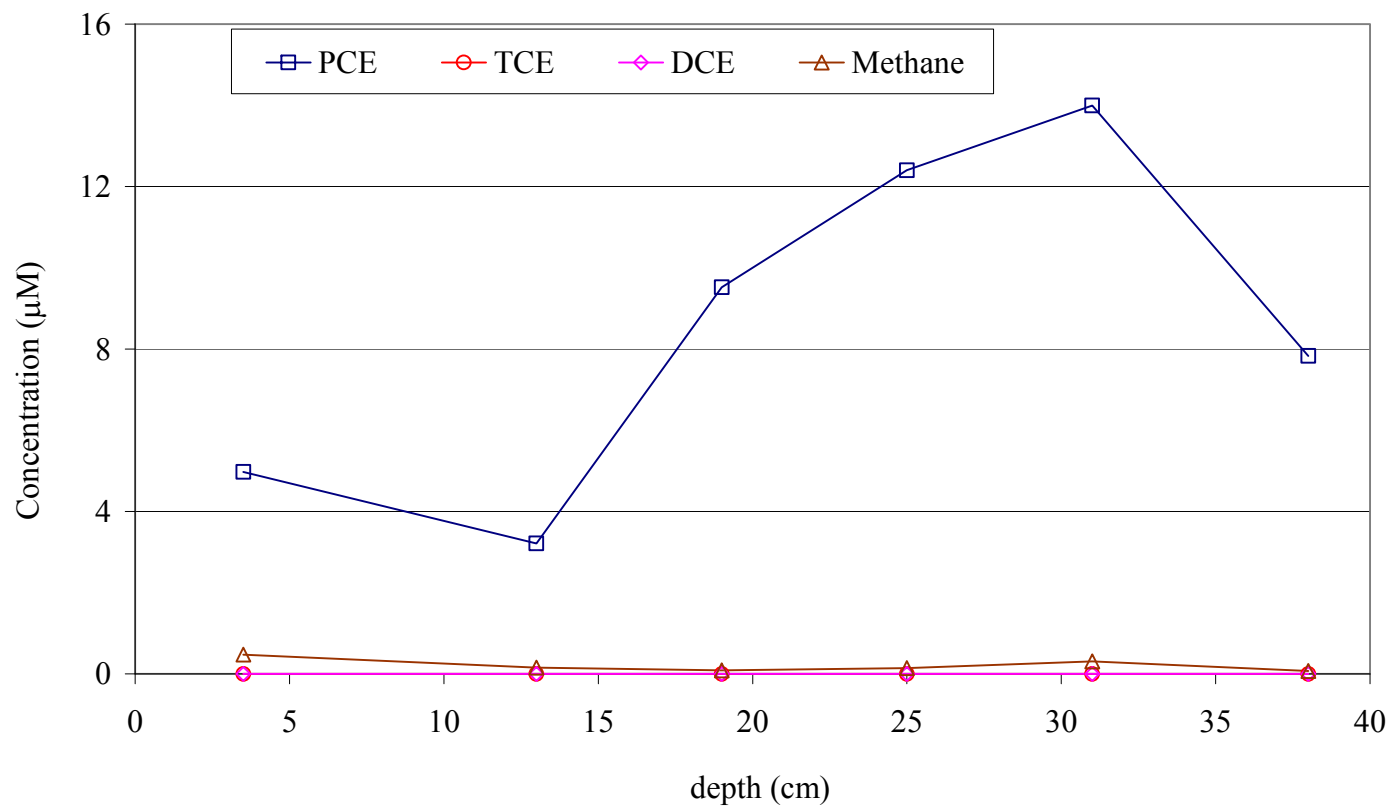
Figure 3.36. CEs and Methane profile, in the aqueous phase of channel 3 soil, vs depth from the surface of the soil on day 987, 11/26/06. Soil samples were collected at a distance of 27 cm from inlet.
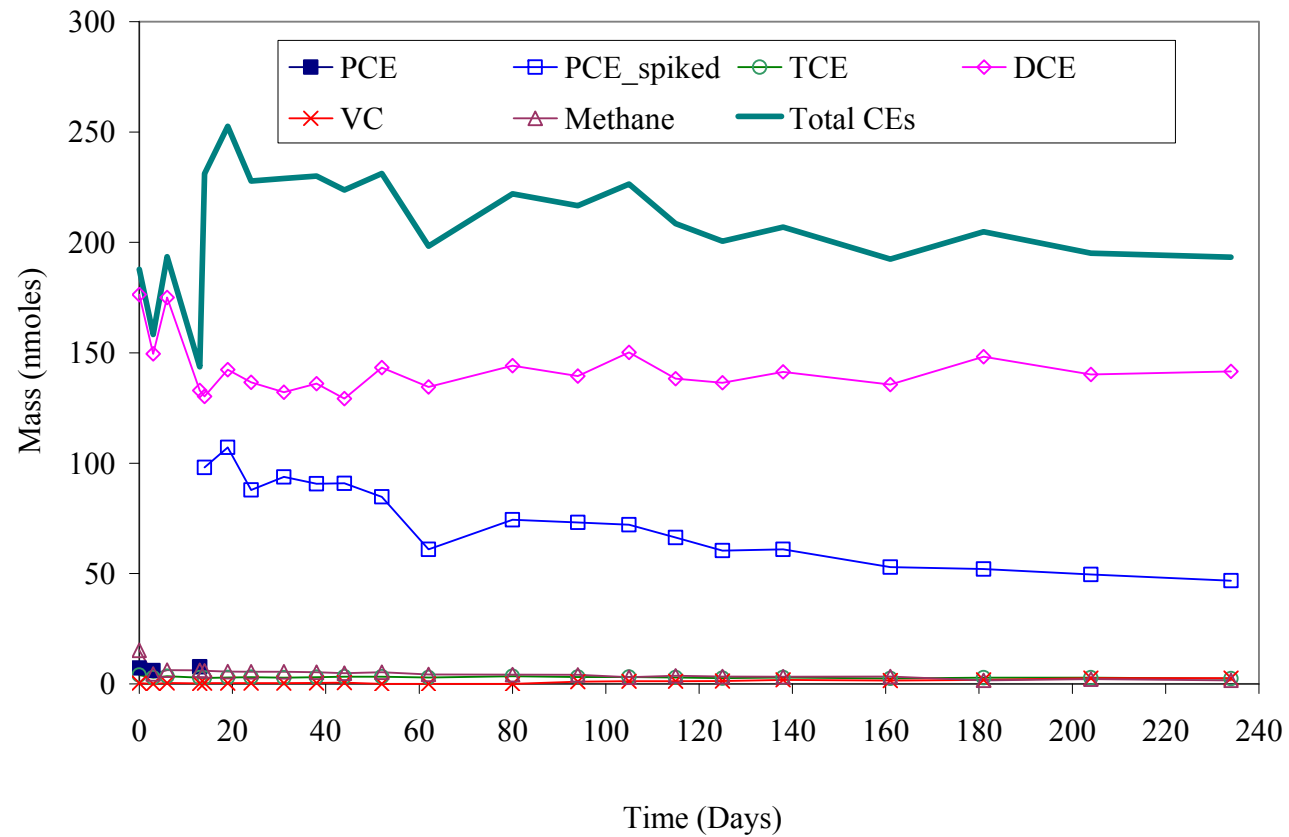
Figure 3.37. Variation of PCE, DCE and methane in microcosm control, Batch 1; PCE spiked on day 14.
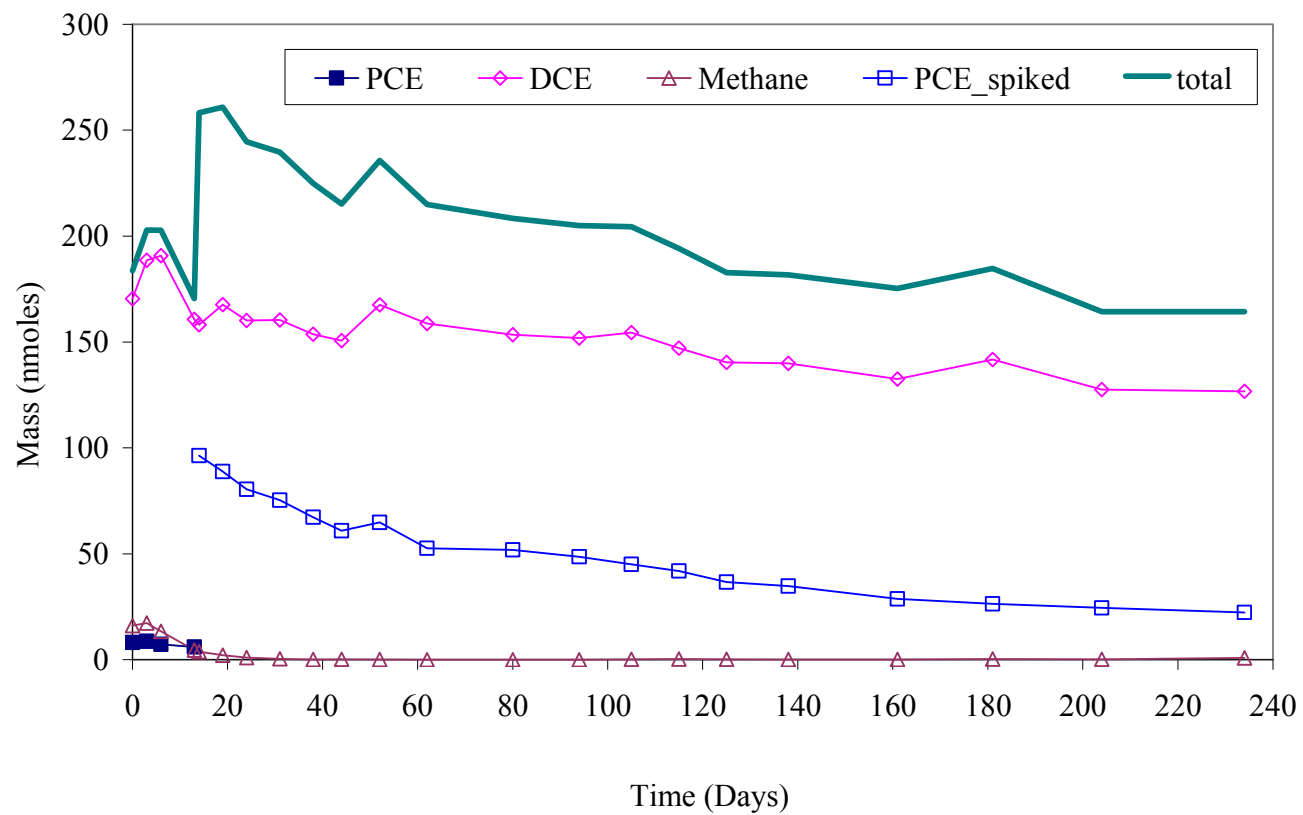
Figure 3.38. Variation of PCE, DCE and methane in microcosm with lactic acid 0.425 %, Batch 1; PCE spiked on day 14.
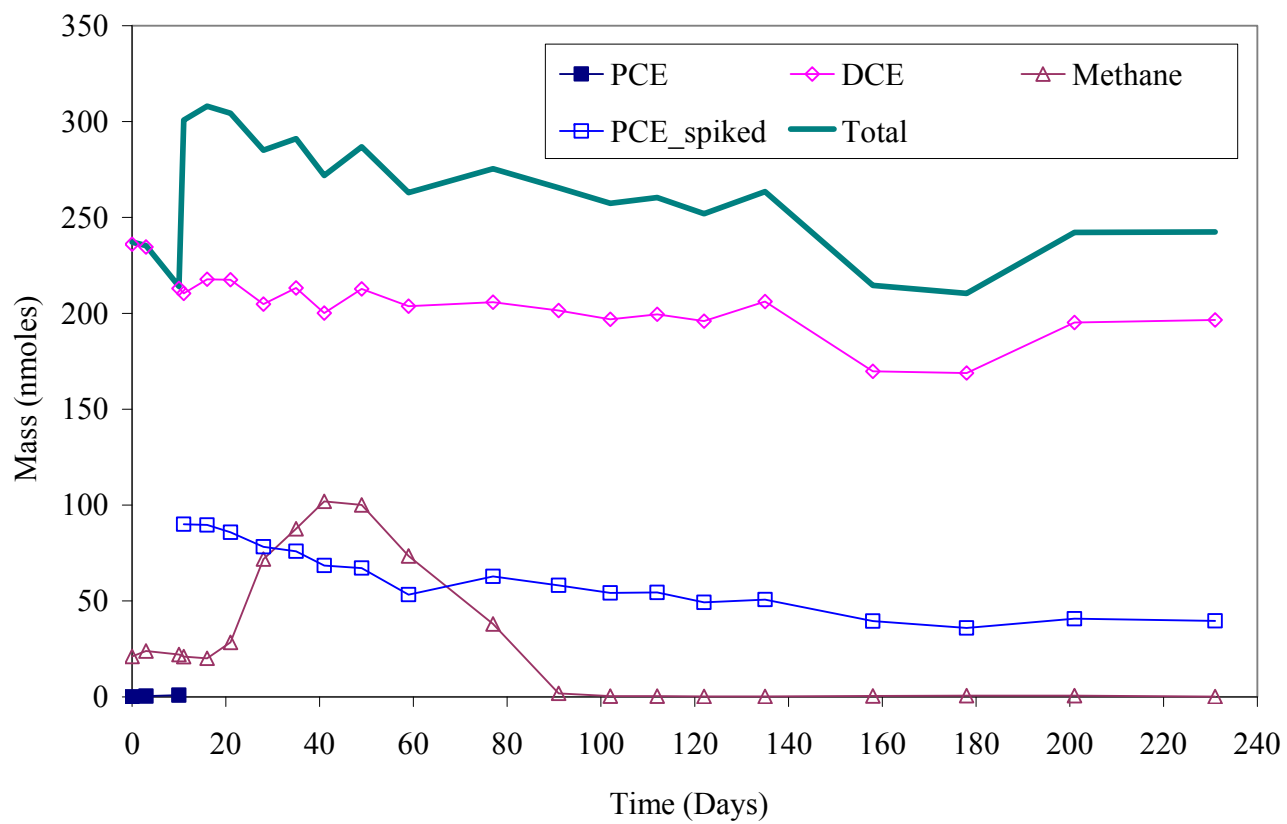
Figure 3.39. Variation of PCE, DCE and methane in microcosm with glucose 0.01 %, Batch 2; PCE spiked on day 11.
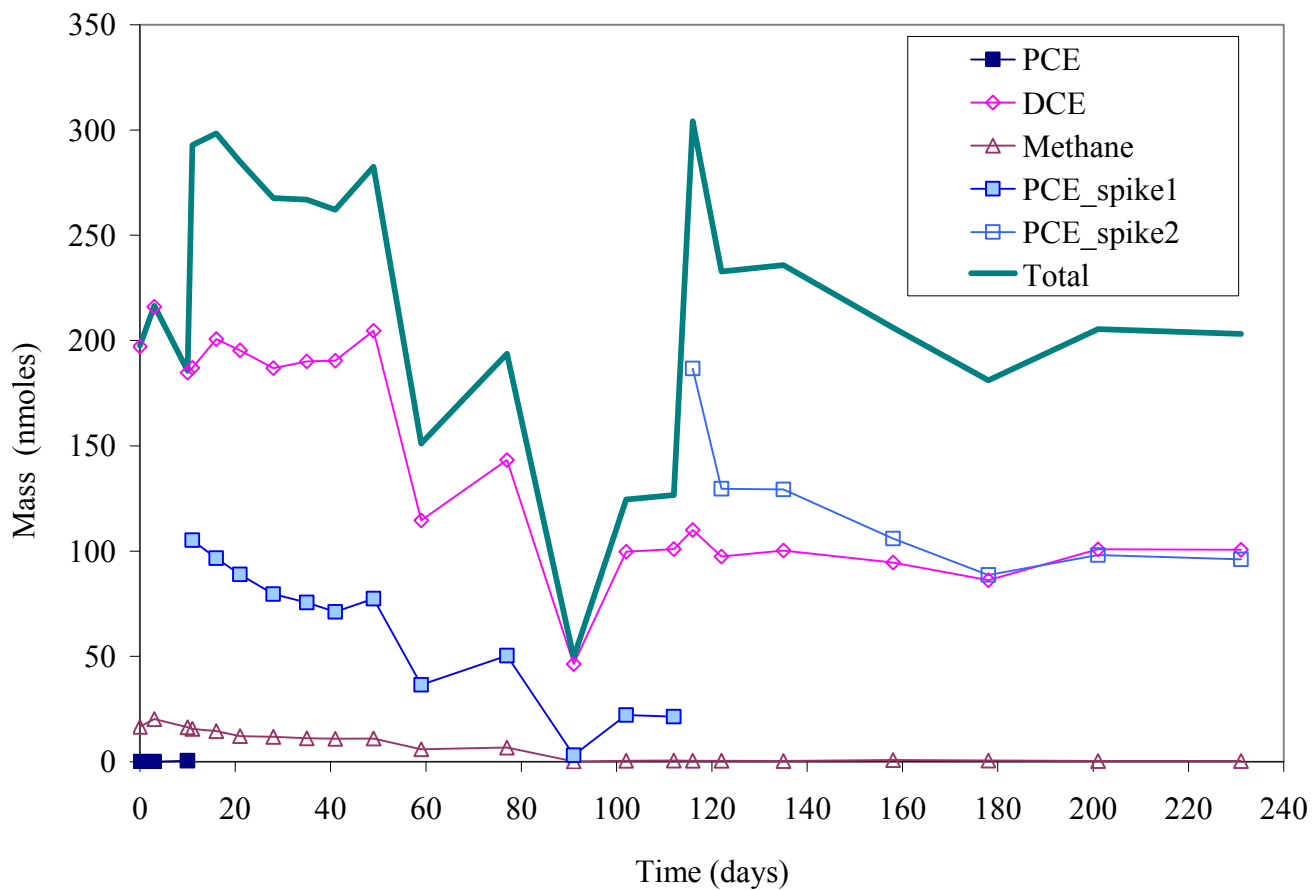
Figure 3.40. Variation of PCE, DCE and methane in microcosm with lactic acid 0.85 %+yeast extract 0.01 %, Batch 2; PCE spiked on day 11 and day 116.
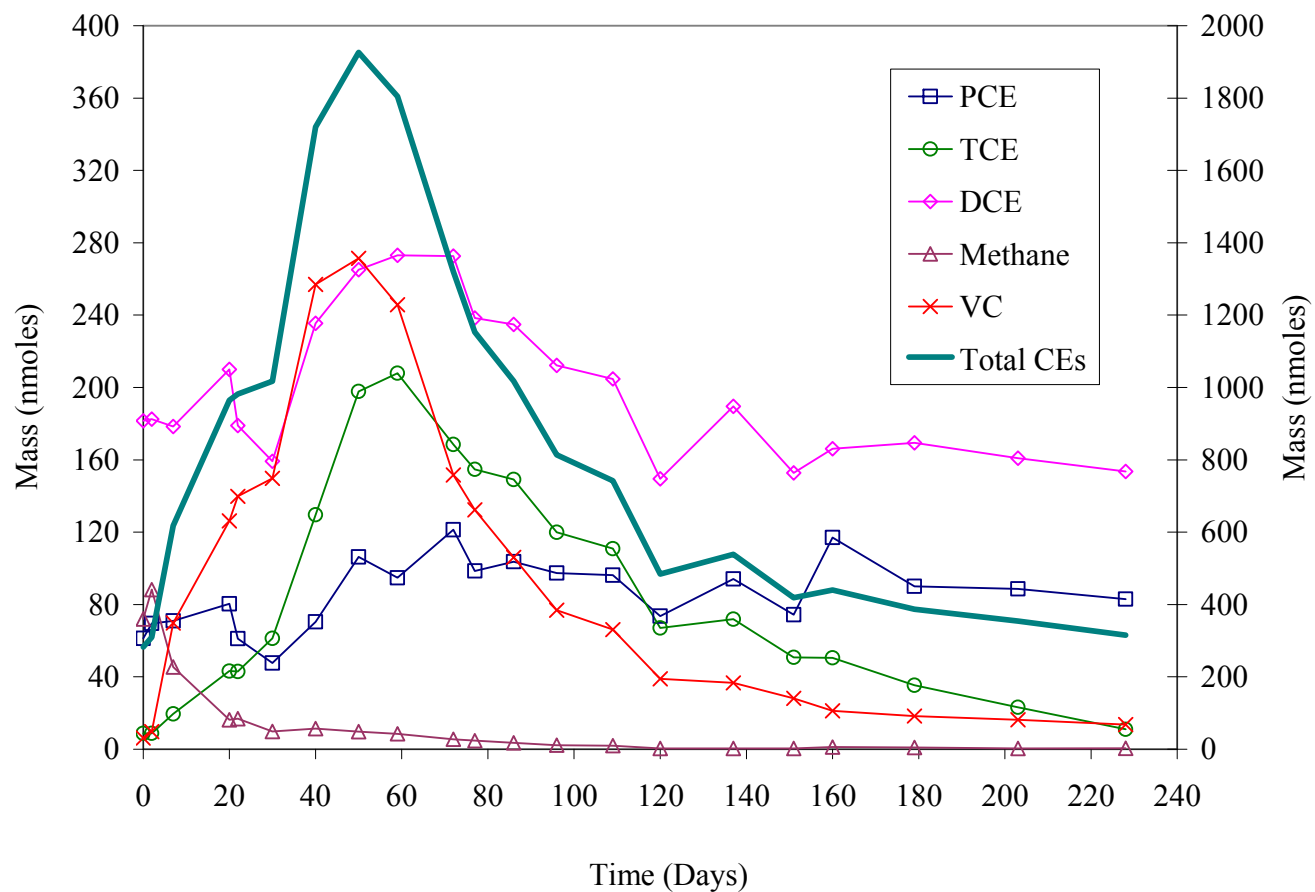
Figure 3.41. Variation of PCE, DCE and methane in microcosm with soy oil methyl ester 0.1 % and yeast extract 0.01 %, Batch 3; PCE spiked on day 0; VC and Total CEs right-hand (secondary)y-axis.
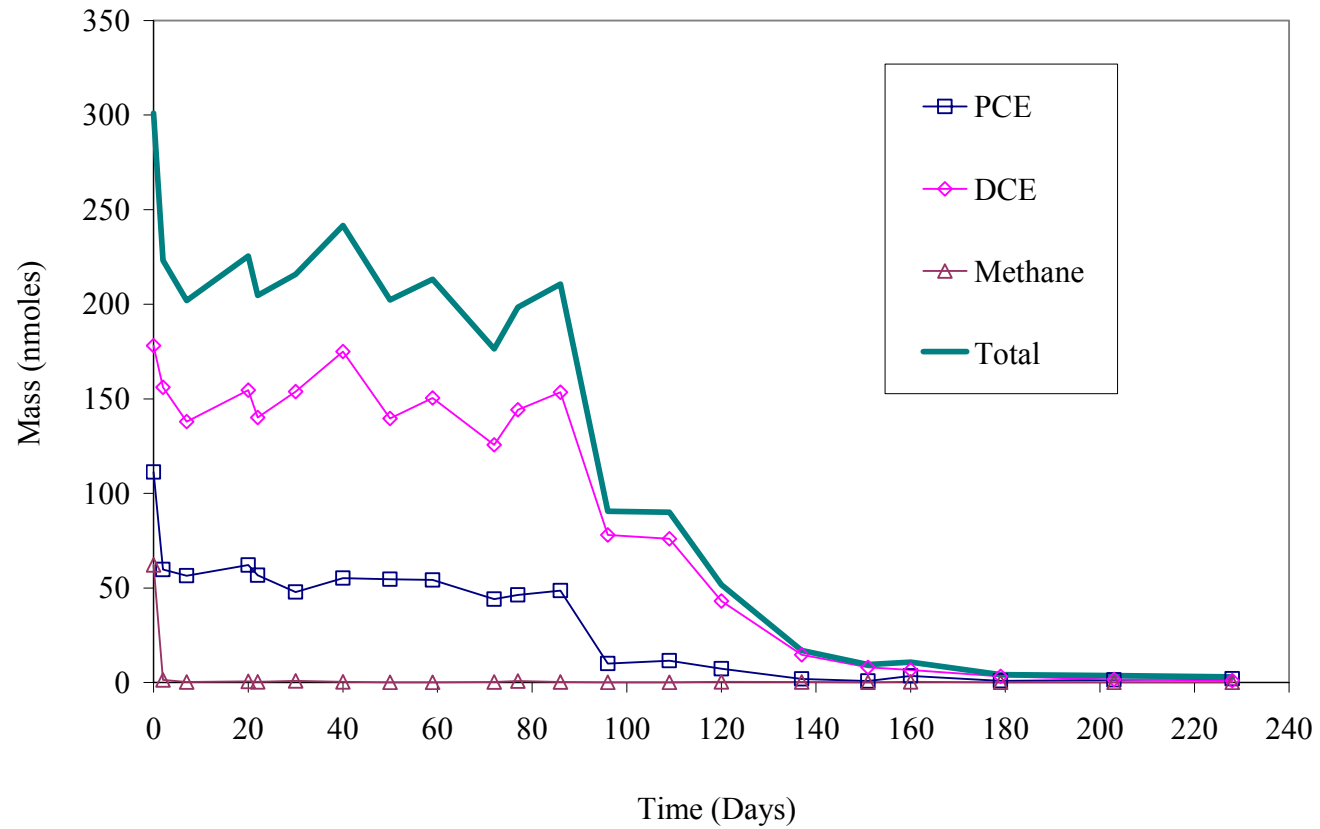
Figure 3.42. Variation of PCE, DCE and methane in microcosm with yeast extract 0.01 %, Batch 3; PCE spiked on day 0.
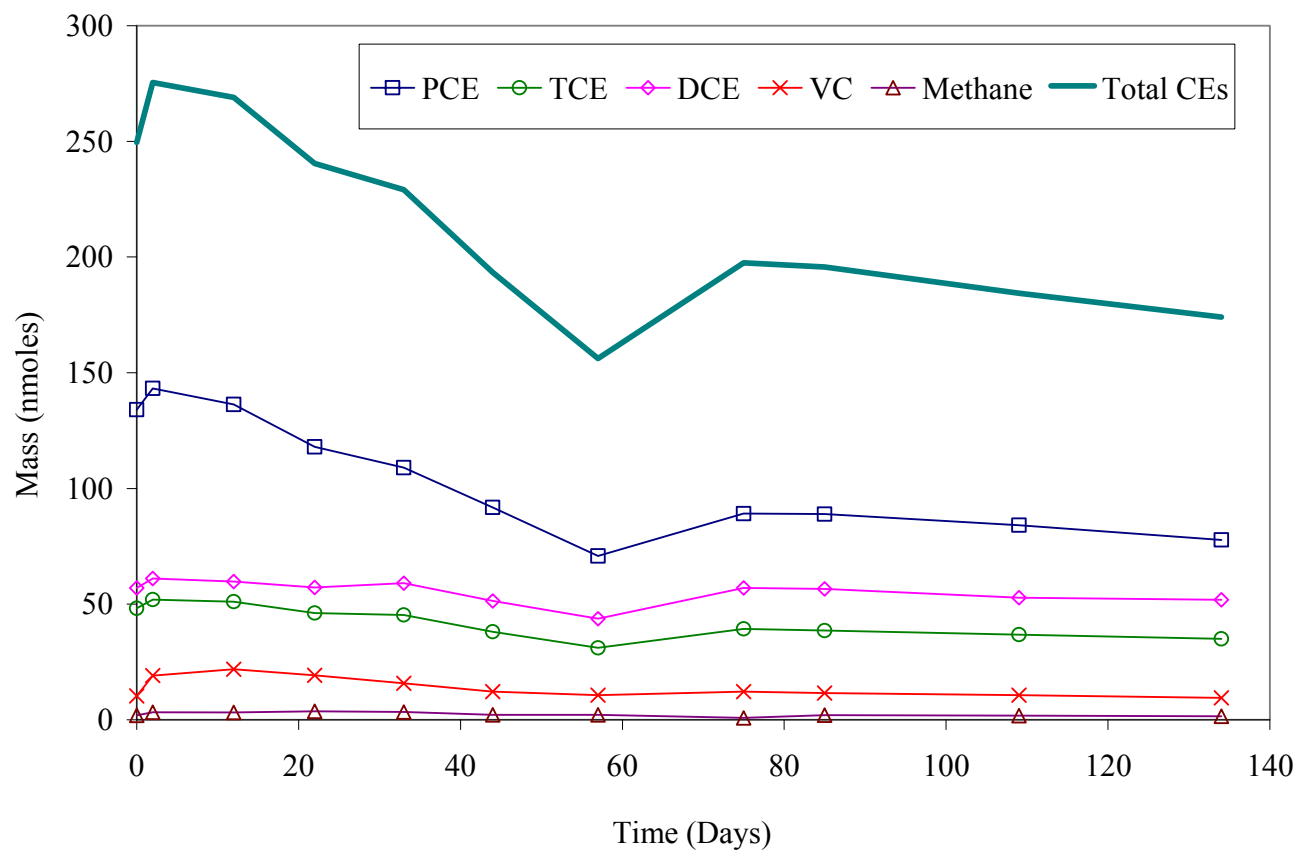
Figure 3.43. Variation of PCE,TCE, DCE, VC and methane in microcosm with lactic acid 0.085 %, Batch 4 (channel 1 outlet liquid); KB-1 was added on day 23.
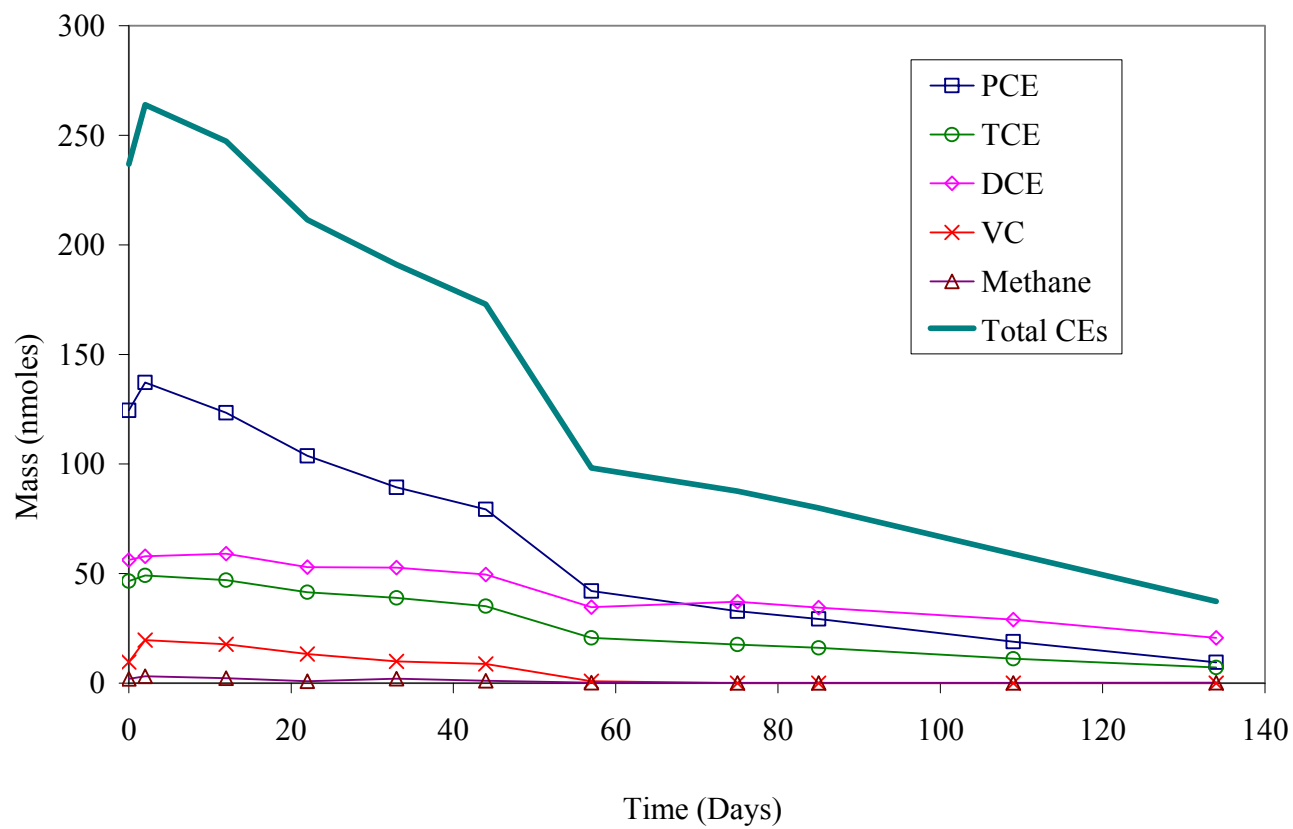
Figure 3.44. Variation of PCE,TCE, DCE, VC and methane in microcosm with lactic acid 0.085 % and yeast extract 0.01 %, Batch 4 (channel 1 outlet liquid); KB-1 was added on day 23.

Table 3.1. Supplements feeding history for channels 1 and 2. 100 mL containing 2 % glucose was fed to channel 2 on days 110, 151 and 173. From October 1, 2004, one liter of 0.2 % glucose was fed to channel 2 and one liter of 0.1 % SOME was fed to channel 1 as indicated in this table.

Date	Day	Channel 1	Channel 2
2004			
June 30	110		G
August 10	151		G
September 1	173		G
October 1	203	S	G
November 3	236	S	G
December 3	266	S	CS
2005			
January 5	299	S	CS
February 3	328	S	CS
March 6	359	S	
April 3	387	S	
May 1	415	S	
May 3	417	S (well 3)	
August 16	522		CS
September 27	564		G
October 24	591		KB-1
October 27	594	S	
November 7	605	KB-1	
2006			
January 16	676		G
May 20	800		G
May 31	810		KB-1

Date	Day	Channel 1	Channel 2
June 1	811	KB-1	
August 30	901		G
October 22	954	S (well 3)	
October 23	955		G
October 26	958	KB-1 (well 3)	KB-1 (well 3)
November 28	991	S (well 3)	
November 30	993		
December 1	994		G (well 1)
December 29	1022	S (well 1)	
January 1	1025		G (well 2)

Key: G - Glucose; CS – Corn Starch; CW – Cheese Whey; S – SOME or Soy Oil Methyl Esters;

KB-1 – *Dehalococcoides spp*.

* From December 3, 2004 to February 3, 2005, Corn Starch was added instead of glucose, inadvertently.

On day 445, May 31, 2005, 100 mL of 1 % SOME was injected into well 3 of channel 6, through a nylon tubing; the solution was fed near the bottom of the channel.

Table 3.2a. Physical and chemical properties of soy oil methyl ester (SOME).

Property	Value
Molecular weight (avg)	292.2
C:H:O (molar ratio)	19:34:2
Odor	Light Vegetable Oil Odor
Melting point (°C)	-1
Boiling point (°C)	315
Flash Point (°C)	218
Density (g/cc)	0.89
Solubility in water at 25°C (mg/L)	Negligible
Vapor Pressure (mm Hg) at 20°C	1.8

(Purcell et al., 1995; Marion, 2007).

Table 3.2b. Distribution of fatty acids and their chemical formula in SOME.

S.No.	Fatty Acid	Wt %	Mol. Wt.	Formula
1	Palmitic	12	270.46	$C_{15}H_{31}CO_2CH_3$
2	Stearic	5	298.52	$C_{17}H_{35}CO_2CH_3$
3	Oleic	25	296.50	$C_{17}H_{33}CO_2CH_3$
4	Linoleic	52	294.48	$C_{17}H_{31}CO_2CH_3$
5	Linolenic	6	292.46	$C_{17}H_{29}CO_2CH_3$

(Purcell et al., 2007; Marion, 2007).

Table 3.3. Tracer peak volume, time, and concentrations in outlet and mean residence time for channels 1, 2 and 3.

Channel	Volume of outlet liquid (L)*	Arrival time for peak bromide concentration (days)*	Bromide peak concentration in outlet liquid (mg/L)	Mean residence time (days)[#]
1	2.8	3.0	0.98	6.2
2	1.1	1.1	2.24	3.6
3	1.3	1.4	3.48	3.5

*Values are the liquid volume and residence time associated with the peak bromide concentration.

[#] Mean residence time was estimated from the residence time distribution (RTD) model.

Table 3.4. Mass balance for tracer study in channels 1, 2 and 3, for the 14 day period, from June 4, 2004 to June 17, 2004.

Channel	Cumulative volume of outlet liquid (L)	Cumulative water uptake by plants (L)	Bromide in outlet liquid (mg)	Bromide trapped in soil by evapotranspiration* (mg)	Total bromide accounted (mg)	Percent recovery (%)
1	12.00	2.26	3.63	0.65	4.18	42
2	10.44	3.31	4.10	1.09	5.05	50
3	10.42	4.43	5.28	1.94	7.06	70

*Estimated based on the assumption that the bromide concentration in the upflow due to evapotranspiration is the same as that in the outlet liquid for a particular day.

Table 3.5. Bromide recovery vs solution recovery in channels 1, 2 and 3, for the 14 day period, from June 4, 2004 to June 18, 2004.

Channel	Volume of outlet liquid over total water fed (L/L)	Solution recovered (%)	Bromide in outlet liquid (mg) out of total 10.05 mg	Bromide recovery (%)
1	12/14.25	84	3.63	36
2	10.44/13.75	76	4.10	41
3	10.42/14.85	70	5.28	53

Table 3.6. Effect of evapotranspiration on the residence time of compounds in channels 1, 2 and 3; estimated based on data from June 2004.

Channel	Variation in the daily exit liquid volume (L) in June 2004	Volume of exit liquid for peak concentration of tracer (L)	Arrival time for peak bromide concentration (days)	Estimated variation of peak time in June 2004 (days)*
1	0.9-0.6	2.8	3.0	3.1-4.6
2	0.9-0.4	1.1	1.1	1.2-2.7
3	0.8-0.6	1.3	1.4	1.6-2.2

*This value is obtained by dividing the volume of exit liquid corresponding to peak concentration by the volume of liquid collected at the outlet each day, at the beginning and the end of June 2004. For example, for channel 1, the time taken for collecting 2.8 L of liquid at the outlet in the beginning of June 2004 is 2.8/0.9 = 3.1 days and similarly, the time taken for collecting 2.8 L of liquid at the outlet at the end of June 2004 is 2.8/0.6 = 4.6 days.

Table 3.7. Mass of aqueous phase, moisture content fraction, and dry weight of soil in the soil samples collected (day 95, 6/15/04) from channels 1, 2 and 3. Soil samples were collected at a distance of 32 cm from inlet.

	Depth (cm)	Channel 1	Channel 2	Channel 3
Mass of aqueous phase (gm)	0-8	0.107	0.2648	0.813
	12-20	0.5679	0.6173	1.1723
	24-32	1.1074	1.0571	1.0098
	34-42	1.4171	1.048	1.14
	Depth (cm)	Channel 1	Channel 2	Channel 3
Moisture content fraction (w/w)	0-8	0.03	0.09	0.22
	12-20	0.11	0.13	0.16
	24-32	0.14	0.15	0.15
	34-42	0.17	0.16	0.16
	Depth (cm)	Channel 1	Channel 2	Channel 3
Mass of dry soil (gm)	0-8	3.8336	2.8461	2.911
	12-20	4.5872	3.9656	6.3053
	24-32	6.6012	6.1796	5.511
	34-42	7.1149	5.5757	5.9594

Table 3.8. Mass of aqueous phase, moisture content fraction, and dry weight of soil in the soil samples (day 150, 8/9/04) collected from channels 1, 2 and 3. Soil samples were collected at a distance of 55 cm from inlet.

	Depth (cm)	Channel 1	Channel 2	Channel 3
Mass of aqueous phase (gm)	0-8	0.0762	0.3053	0.842
	12-20	0.3304	1.0073	0.6614
	24-32	0.1544	1.4701	0.1917
	34-42	1.2475	0.8139	1.5187
	Depth (cm)	Channel 1	Channel 2	Channel 3
Moisture content fraction (w/w)	0-8	0.02	0.11	0.18
	12-20	0.06	0.14	0.19
	24-32	0.03	0.16	0.16
	34-42	0.15	0.14	0.15
	Depth (cm)	Channel 1	Channel 2	Channel 3
Mass of dry soil (gm)	0-8	3.7087	2.5069	3.7402
	12-20	5.3799	6.322	2.894
	24-32	5.5651	7.68	1.0295
	34-42	6.9037	5.0599	8.7644

Table 3.9. Organic carbon content (as %) in channel 1 and 3 soil samples (day 150, August 9, 2004).

Sample	Depth (cm)	Channel 1	Channel 3
1	0-8	0.37	0.76
2	12-20	0.36	0.38
3	24-32	0.53	0.66
4	34-42	0.15	0.06

Table 3.10. Summary of results from microcosms, Batch 1, 2 and 3.

No.	Microcosm	Percentage decrease		Methane
		PCE	**DCE**	
Batch I (166 days)				
1	control	59	33	Slow decrease
2	0.005 % glucose	60	40	Decreased 80 %
3	0.425 % lactate	77	40	decreased to zero in 40 days
Batch II (166 days)				
1	control	58	22	Decreased to zero in 100 days
2	glucose 0.01 %	60	30	Increased first and decreased to zero in 90 days
3	lactate 0.85 %	Slow leak in microcosm		
4	yeast extract 0.01 %	63	27	First order decrease
5	glucose 0.01 %+ yeast extract 0.01 %	21 % in first 80 days 46 % in 78 days after respiking	decreased to zero in 10 days	decreased to zero in 3 days
6	lactate 0.85 % + yeast extract 0.01 %	80 % in first 100 days 54 % in 62 days after	60	Decreased to zero in 90 days

No.	Microcosm	Percentage decrease		Methane
		respiking		
		PCE	**DCE**	
Batch III (158 days)				
1	control	76 % decrease in 77 days, then remained steady	35	decreased to zero in 50 days
2	SOME 0.1 %	Decreased quickly; could be leak		
3	SOME 0.1 % + yeast extract 0.01 %	All compounds increased and then decreased		
4	yeast extract 0.01 %	PCE and DCE remained steady for about 86 days and then decreased to zero after 137 days		-
5	glucose 0.01 %+ yeast extract 0.01 %	47	20	Decreased to zero in 70 days
6	lactate 0.085 % + yeast extract 0.01 %	Leak in microcosm		

Table 3.11. PCE and DCE attenuation in batch 4 microcosms after day 134.

Microcosm	Decrease in PCE (%)	Decrease in DCE (%)
control	67	27
SOME 0.1 %	99	40
SOME 0.1 % + yeast extract 0.01 %	71	-8
yeast extract 0.01 %	leak	Leak
glucose 0.01 %+ yeast extract 0.01 %	59	37
lactate 0.085 %	42	9
lactate 0.085 % + yeast extract 0.01 %	92	63

Table 3.12. First order rate constants and half-life of PCE in microcosms, Batch 1 and 2.

	Rate constant ($* 10^{-3}$ per day)		Half-Life (days)	
	Batch 1	Batch 2	Batch 1	Batch 2
Control	3.6	4.1	193	169
Glucose	3.4	4.1	204	169
Lactic acid	6.6	Leaky vial	105	Leaky vial
Yeast extract		4		173
Glu + YE		3.8 and 6.3		182 and 110
LA + YE		19.4 and 4.8		35 and 144

Table 3.13. First order rate constants and half-life of PCE in microcosms, Batch 3 and 4.

	Rate constant (* 10^{-3} per day)		Half-Life (days)	
	Batch 3	Batch 4	Batch 3	Batch 4
Control	9.7	7.8	71	89
SOME	Leaky vial		Leaky vial	
SOME + YE	-		-	
YE	22.4	Leaky vial	31	Leaky vial
Glu + YE	3.7	6.8	187	102
LA + YE	Leaky vial	4.6	Leaky vial	151
LA		19.8		35

NA – Not Applicable

Table 3.14. Regression coefficients for first order reaction model.

	Batch 1	Batch 2		Batch 3	Batch 4
Control	0.90	0.78	Control	0.84	0.92
Glu	0.85	0.89	SOME	Leaky vial	-
LA	0.97	Leaky vial	SOME + YE	-	-
YE		0.86	YE	0.83	Leaky vial
Glu + YE		0.46, 0.92	Glu + YE	0.84	0.87
LA + YE		0.90, 0.64	LA + YE	Leaky vial	0.70
			LA		0.98

CHAPTER 4 - Hydrogeologic Characterization and Groundwater Modeling for a Tetrachloroethene Contaminated Site

4.1. Introduction

It is important to have a sound knowledge of the hydrogeology of the site before planning a remediation strategy. Typically, a tracer study is conducted to determine the groundwater flow direction and velocity. The geochemistry (ion concentrations) of groundwater can also play a vital role in developing the remediation plan and therefore, the geochemical parameters are also analysed. In this work, several tracer studies using potassium bromide, were conducted in a tetrachloroethene contaminated site at Manhattan, KS. The hydraulic conductivity was estimated, based on the tracer studies and transient well pumping data. The site geology was characterized by others (Terracon, 2004), and we studied the anion concentrations in the groundwater.

4.2. Materials and Methods

4.2.1. Location

The subject property is located at 1227 Bluemont Ave., which is situated in the NW Quarter of Section 18, Township 10S, Range 8E in Manhattan, Kansas (Terracon, 2004). This is part of the Aggieville district of Manhattan, a predominantly commercial and entertainment area. Dry cleaning contaminants from another source area, a dry cleaning establishment on 12th Street, may be commingled with the main plume at some locations down-gradient. This facility is approximately 400 ft down gradient of the 1227 Bluemont Ave. site. Due to the proximity of the facility to the source area, the two sources of contamination have been considered to create a common plume. The facility formerly located at 1227 Bluemont Ave. is the targeted location for this study and it is up-gradient of the facility on 12th Street. A GIS map of the study area is shown in Figure 4.1 and additional details on the wells are shown in Figures 4.2 and 4.3.

Contamination Release Characteristics/Historical Concentrations

The saturated zone at the site consists of silty-clay that is underlain by permeable sands in the lower portion of the aquifer. Chlorinated solvents, both dissolved and NAPL, may be present based on historical presumptive evidence; they are likely adsorbed and present with silty-clay

soil particles and provide a continuous source of contamination. PCE has degraded to trichloroethylene (TCE) within 400 ft of the source area indicating reductive dechlorination is occurring at this site. However, complete reductive dechlorination to ethene/ethane did not appear to be occurring under natural conditions.

An Environmental Site Assessment (ESA) during 2004 indicated that concentrations of PCE in groundwater are well above the Maximum Contaminant Level (MCL) of 5 μg/L. The source area PCE contamination at the Bluemont Ave. facility was detected at 50,000 μg/L using an on-site field gas chromatograph and at 35,000 μg/L from a fixed lab analysis (Terracon, 2004). Analytical results of collected groundwater samples indicated that most of the PCE is primarily within one block of the source. However, concentrations of cis-DCE in the groundwater in excess of the MCL of 70 μg/L were detected at a distance approximately one-mile down gradient of the source areas. Chlorinated solvents have been detected (above the MCLs) in two of the City of Manhattan's public water supply (PWS) wells, located approximately 1.2 miles down gradient of the source (Terracon, 2004) .

In 2004, the highest PCE concentration in water at the source area was about 35,000 μg/L; however, PCE was not found beyond three blocks from the source. As the plume moves eastward the PCE concentration decreases and both TCE and DCE are present. After about 5 blocks, DCE usually exceeds TCE at several depths. Along the plume, concentrations up to a maximum of 4000 ppb of TCE, 1780 ppb of DCE and 10 ppb of VC in groundwater were detected (Terracon, 2004). Soil samples collected at the 1227 Bluemont Ave. site at four different locations had concentrations in the range 244 to 2837 mg/kg of PCE and about 2 mg/kg of TCE and DCE (Terracon, 2004).

4.2.2. History

Cinderella Dry Cleaners operated at 1227 Bluemont Ave., Manhattan, KS, from 1967-1997. PCE was utilized as a dry-cleaning solvent at this facility. The drycleaner is no longer in operation. Following a property transfer in 1997, the site was converted to Coco Bolos Restaurant. The history of this facility as well as other dry cleaning facilities in the vicinity is discussed by Davis (2007).

4.2.3. Lithology

Lithologic studies were conducted by Terracon, Wichita, Kansas (Terracon, 2004) to determine the soil characteristics at the site. The subsurface cross sections with soil types along the depth, were constructed from four probe logs and boring logs from monitoring wells MW-7D, MW-8D, MW-11D and MW-12D. Information developed from the site investigation indicates subsurface materials near the site primarily consists of clay (hydraulic conductivity 10^{-7} – 10^{-9} cm/s), with interbedded silty clay and clayey silt from about ground level to about 40 feet below ground surface (bgs). This material is underlain by sand to a depth of approximately 55 feet bgs. The cone penetrometer (CPT) probes were to be advanced to bedrock, anticipated at approximately 55 to 60 feet bgs, or probe refusal, whichever occurred first. However, at CPT-1 (near MW-5D) the rig was apparently not adequately centered over the probe rods and probe refusal was encountered at a depth of 35 feet. The other three CPT probes encountered probe refusal at depths that ranged from 44.5 to 46 feet bgs, and bedrock was not encountered in the four CPT probes performed (Terracon, 2004).

The soil used in the laboratory study was primarily sand with less than 10% silt (Zhang, 1999). Soil samples collected during the boring for MW-9D was analysed at the Soil Testing Laboratory at Throckmorton Hall, Kansas State University. Soil over a 5-ft depth was homogenized and the results are presented in Table 4.1 with each 5-ft depth of soil identified by the middle depth for that section. In Table 4.1, from a depth of 2.5 ft to 47.5 ft, the sand content increased from 12% to 36%, silt content varied between 34% to 54% with higher silt content in the deep zone, and clay content decreasing from 48% at the top to 12% at the depth of 42.5 ft and increasing to 22% at 47.5 ft. The soil in the bottom samples in the pilot study area can be compared to the soil used in the laboratory study, eventhough the sand content is less and clay content high in the field soil. The average velocity of contaminant solution in the six-channel system, based on the arrival of bromide peak, was 1.2 ft/d in channel 1, 3.3 ft/d in channel 2 and 2.6 ft/d in channel 3. The velocities in the six-channel system was about an order of magnitude higher than the values observed in the field.

The aquifer is found to have two distinct zones, termed shallow zone and deep zone. Groundwater flows toward the east-northeast direction. The depth to water for the shallow zone is in the range 11 - 15 feet bgs. The groundwater thickness (or height) of the shallow zone is about 20 ft and the thickness of the deep zone is about 20 ft. The deep zone is from about 35 feet

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bgs to about 55 feet bgs. Due to pressure at the top of the deep zone, the water table in the wells rises more than 15 ft. The monitoring wells in the shallow zone were 30 ft deep and screened from 10 ft to 30 ft bgs. The monitoring wells in the deep zone were 55 ft bgs and screened from 45 ft to 55 ft bgs.

4.2.4. Fall 2004 Tracer Study

The location of the monitoring wells is shown in Figure 4.1 as a GIS map and in Figures 4.2 and 4.3 as a schematic. A tracer study was initiated in the deep zone, on August 2, 2004. Approximately 200 L of water was pumped from MW-8D into a large rectangular plastic tank and mixed with 150 g potassium bromide (KBr) to obtain a bromide concentration of approximately 500 mg/L. This solution was injected into MW-8D, at three different depths of 40 ft bgs, 45 ft bgs and 50 ft bgs in approximately equal volumes.

Frequency of sampling was adjusted, based on monitoring results, with daily sampling of the first well (MW-9D) approximately 10 ft down-gradient, until the peak of bromide appeared there, and sampling at longer intervals thereafter. The sampling interval was greater (2-3 days) for MW-10D, approximately 20 ft down-gradient from the injection well MW-8D. Samples were collected at multiple depths, viz., 40 ft bgs (which is in the unscreened part of the well), 45 ft bgs and 50 ft bgs through the screened portion of the well, to check for preferential flow.

Once the peak of bromide had passed the wells MW-9D and MW-10D, another tracer study was initiated in the shallow zone. Injection occurred on September 16, 2004, in MW-8S, using the same approach as previously described. The injection depths in the shallow zone were 17 ft bgs, 22 ft bgs and 27 ft bgs. Samples were collected from the monitoring wells at these three depths.

4.2.5. Fall 2005 Tracer Study

A bioremediation pilot study was carried out beginning Fall 2005 at a location down-gradient from the source, as shown in Figure 4.3. In the nutrient solution injected for bio-stimulation of native microbes, a tracer, potassium bromide, was also added. Drilling for installation of injection wells at four locations, each 4 ft apart in the north-south direction and between MW-8 and MW-9 was started on Monday, August 15, 2005 and completed by Wednesday, August 17, 2005; see Figure 4.3. Two wells were installed at each injection location, one each for deep and shallow zones of the aquifer. The screening depths in the

injection wells were 50-55 ft bgs and 42-47 ft bgs for deep zone and 30-35 ft bgs and 23-28 ft bgs for the shallow zone, as depicted in the schematic Figure 4.3. Figure 4.4 is a photograph of the injection points taken just after installation of the injection wells. Groundwater from MW-10S and MW-10D was pumped into four barrels (55 gal or ~ 200 L each) for preparing nutrient solution and anaerobic chase water. Two hundred grams of potassium bromide was added to the nutrient solution (concentration = 670 mg bromide/L) as a tracer to monitor the groundwater flow and also to understand the possible flow of the nutrients.

Injection of nutrient solution and chase water was carried out on Thursday, August 18, 2005 (Day 0). The solutions were injected first in the shallow zone and then in the deep zone. In the shallow zone, each injection well received 50 L of nutrient solution (containing 670 mg/L of bromide) followed by 50 L of chase water. In all the shallow zone injection wells, only siphoning (gravity flow) was used to inject the solutions. In the deep zone, a rotor pump was used to inject the solution in Loc D, but in Loc C there was overflow when solution was pumped. Hence siphoning was used at the deep in injection well at Loc C. Since the injection rate was too slow in the deep zone, only 30 L of nutrient solution was injected in Loc C while Loc A and Loc B received 60 L each of nutrient solution.

Groundwater samples were collected from monitoring wells at depths (all values are bgs) 18 ft (top), 23 ft (middle) and 28 ft (bottom) for shallow zone and 42 ft (top), 47 ft (middle) and 51 ft (bottom) for deep zone. Samples were collected daily for the first week and from then onwards once in two days for the next two weeks and from then onwards twice a week. The samples were analyzed for both chlorinated ethenes (CEs) and ions as described below in Section 4.2.9. Analytical Method.

The samples at three depths across the screened interval served as replicate samples, at locations where there was sufficient dispersion and mixing to result in uniform concentration, and helped in determining variation in flow rate in the aquifer across the screened interval. Because the nutrients were injected at different and non-overlapping depths, there may be different effects at different depths within the aquifer depending on the extent of vertical mixing. At locations where there not sufficient dispersion and mixing, the concentrations of CEs and anions varied with depth.

4.2.6. Spring 2006 Tracer Study

In light of the results of the Fall 2005 tracer study and the observation that reducing power was depleted in the down-gradient wells, it was decided to introduce a new tracer along with additional nutrients. We anticipated that the hydraulic conductivity in the vicinity of the nutrient injection wells may have been altered due to formation of biofilms and microbial products. To understand whether the aquifer had been altered and the ground water flow pattern had changed, a second dose of potassium bromide tracer and third dose of nutrients were injected on March 3, 2006 (day 197) using the same procedure described above.

4.2.7. Pumping Tests

Pumping tests were carried out in MW-8D and MW-10S to determine the hydraulic conductivity of the soil at the pilot study area. Groundwater was pumped out of MW-10S and while pumping out, the decrease in groundwater level vs time was recorded. The groundwater that was pumped out was injected back into MW-10S and the rise in the groundwater level vs time was recorded. This experiment was conducted on two different dates, 8/16/2005 and 12/21/2006. The rated pumping capacity of the pump used is 12 L/min, however, the actual pumping rate was less, due to the depth from which water was pumped out, as estimated from the time taken to pump out 200 L of water. The depth at which groundwater was pumped out was 20 ft bgs for MW-10S and 30 ft bgs for MW-8D. The actual rates at which water were pumped out of MW-10S, were 5.6 L/min and 4.3 L/min during Fall 2005 and Fall 2006 experiments. The flow rate at which water was pumped into MW-10S was 7.4 L/min.

Pumping test was carried out in MW-8D on 12/21/2006. While pumping out water from MW-8D, there was no appreciable change in depth to water. The pump capacity (4.3 L/min at a depth of 30 ft bgs) may be too low to impact the groundwater level.

4.2.8. Models Used in the Study

4.2.8.1. Dilution Model

In this model, the tracer, when injected, was assumed to spread in a cylindrical fashion concentric to the injection well. The tracer is then diluted or washed away by the flow of groundwater. The injection solution volume is considered as the system volume and treated as a

continuous stirred tank, where the concentration of the tracer inside the system is assumed the same (Levenspiel, 1999). A mass balance for the system yields,

$$\begin{bmatrix} \text{Accumulation of tracer} \\ \text{in the system volume} \end{bmatrix} = \begin{bmatrix} \text{Tracer entering} \\ \text{the system} \end{bmatrix} - \begin{bmatrix} \text{Tracer exiting} \\ \text{the system} \end{bmatrix}$$

Expressed in terms of the system parameters,

$$V \frac{dC}{dt} = 0 - Q * C \tag{4.1}$$

where,

V = volume of the fluid in the system (cylinder with radius equal to the radius of influence of injection)

C = concentration of the tracer at time t

Q = flow rate of the fluid through the system, i.e., Volumetric flow rate of groundwater through the cross section of the system = v * A

where,

v = actual velocity of groundwater = Darcy velocity/Porosity of the soil

A = cross sectional area available for the fluid to flow

Since the tracer solution is assumed to spread in a cylindrical fashion, the cross section is a rectangle, the height of which is the screening depth and the width is the diameter of the cylinder. The area available for flow is only 30 % of this rectangle due to porosity of the soil.

The analytical solution for Eqn (4.1) is

$$C = C_0 e^{\left(-\frac{Q}{V}t\right)} \tag{4.2}$$

with the initial condition,

C_0 = concentration of the tracer at time t = 0, or the injection concentration

V/Q = time constant of the system

4.2.8.2. Advection-Dispersion Model

The one-dimensional advection dispersion equation (ADE) that governs the concentration of a conservative tracer is (Reddi and Inyang, 2000),

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} \tag{4.3}$$

where,

C = concentration of the substance at time t and distance x, from the source

D = dispersivity of the groundwater

v = actual velocity of the groundwater = Darcy velocity/Porosity of the soil

The initial and boundary conditions are

C(x, t) = 0   t<0

C(∞, t) = 0   t ≥ 0

The solution for Eqn (4.3), with the above conditions, for a slug of tracer input, is given by (Reddi and Inyang, 2000)

$$C = \frac{M}{\sqrt{4\pi Dt}} \exp\left[-\frac{(x-vt)^2}{4Dt}\right]$$ (4.4)

where,

M = mass of the substance spilled/injected per unit area

In developing the above model for the injection well 8S, the distance x, at which the tracer concentration is observed is assumed to be 1 cm. For the purpose of estimating the area of the spill/injection, the tracer when injected, was assumed to spread in a cylindrical fashion concentric to the well.

4.2.8.3. Residence-Time Distribution Model

Another model, based on the residence time distribution (RTD) of the tracer, was used to fit the tracer data for MW-9S and MW-10S (Levenspiel, 1999). In this model, the time spent by the individual molecules of tracer or the time taken for a fraction of the tracer injected to reach a particular point or travel a particular distance, is taken into account. The distribution of these times for the stream of fluid at a monitoring well location is called the residence time distribution (RTD) of the fluid. For a detailed treatment of the model development, see Levenspiel (1999).

The mean residence time for the bromide, at a particular monitoring well, is estimated from the expression (Levenspiel, 1999)

$$\tau = \frac{\sum t_i C_i \Delta t_i}{\sum C_i \Delta t_i}$$ ....(4.5)

where,

τ = mean residence time, days

C_i = concentration of bromide in the ith sample, mg/L

t_i = time at which the ith sample was collected, day

Δt_i = difference between $(i+1)^{th}$ sampling time and i^{th} sampling time, days

The dimensionless form of the advection dispersion equation, is

$$\frac{\partial C}{\partial \theta} = \left(\frac{D}{vL} \right) \frac{\partial^2 C}{\partial z^2} - \frac{\partial C}{\partial z} \tag{4.6}$$

where,

C = concentration of the tracer at time t and distance x from the source

z = dimensionless distance = x/L

D = dispersion coefficient of the fluid; sum of molecular and mechanical dispersivites

v = velocity of the fluid

L = distance, from the source, at which the tracer concentration is measured

D/vL = vessel dispersion number = 1/Peclet number; the Peclet number (Pe) is a measure of the relative importance of advection to dispersion.

$\theta = t/\tau$ = dimensionless time

For small extents of dispersion, the solution for the dimensionless concentration, C_θ, is given by (Levenspiel, 1999)

$$C_\theta = \frac{1}{2\sqrt{\pi(D/vL)}} \exp\left[-\frac{(1-\theta)^2}{4(D/vL)} \right] \tag{4.7}$$

where,

$$C_\theta = \text{Dimensionless concentration} = \frac{\tau \, C_i}{\sum C_i \Delta t_i} \tag{4.8}$$

The accuracy of the model is better for smaller values of D/vL (usually less than 0.01), i.e., when dispersion effect is smaller compared to the advection effect.

4.2.8.4. Theis' Transient Well Model

The Girinski potential, Φ_w, in groundwater for two-dimensional radial flow to a point source in an infinite, homogeneous aquifer is given by (Haitjema, 1995)

$$\Phi_w = -\frac{Q}{4\pi} E_1(u) + \Phi_0 \tag{4.9}$$

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where,

Q = Discharge of pumping rate of groundwater from well

Φ_0 = Initial Girinski potential, estimated from the groundwater elevation or potential at infinity or before the start of the pumping process

$E_1(u)$ = The exponential integral referred to as well function W(u)

$$E_1(u) = W(u) = \int_u^\infty \frac{e^{-\xi}}{\xi} d\xi \qquad (4.10)$$

$$u = \frac{S_s r_w^2}{4kt} = \text{lower limit of integration} \qquad (4.11)$$

where,

r_w = Distance from the well where the potential is measured = 0.03 m = 0.104 ft

k = Hydraulic conductivity of the aquifer

t = Time of pumping

S_s = Specific storage of the aquifer = 0.001/m = 0.0003/ft

The value of specific storage was estimated from the equation (Strack, 1999)

$$S_s = \rho \, g \, (m_v + n \, \beta) \qquad (4.12)$$

where,

ρ = Density of groundwater = 1000 kg/m^3

g = Gravitational force = 9.81 m/s^2

m_v = Coefficient of volume compressibility of the soil = 10^{-7} m^2/N

For clay, m_v is in the range 10^{-6} to 10^{-8} m^2/N, for sandy soil, m_v is in the range 10^{-7} to 10^{-9} m^2/N.

n = porosity of the soil

β = coefficient of volume compressibility of water = 10^{-10} m^2/N

For small values of u ($\ll$ 1), the exponential integral (well function) simplifies to

$$E_1(u) = W(u) = - \ln(u) - 0.5772 \qquad (4.13)$$

The Girinski potential is estimated from the following relationships (Haitjema, 1995):

$$\Phi = \frac{1}{2} k\varphi 2 \qquad \text{for unconfined aquifer}$$

$$\Phi = kH\varphi - \frac{1}{2} k\varphi 2 \qquad \text{for confined aquifer} \qquad (4.14)$$

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where,

φ = Potential or groundwater elevation

H = Height or thickness of the confined aquifer

The initial Girinski potential is estimated from the potential (groundwater elevation) at infinity or before starting the pumping process. The model was used to fit the field data by varying the hydraulic conductivity. The shallow zone was considered as an unconfined aquifer and the deep zone was considered as a confined aquifer.

4.2.9. Analytical Method

The depth to water in monitoring wells was measured by ??? water meter (co, place) with graduation of 0.05 ft. The groundwater samples were collected at three different depths (all values are bgs) – for shallow wells, 18 ft (5.5 m), 23 ft (7 m) and 28 ft (8.5 m) and for deep wells, 42 ft (12.8 m), 47 ft (14.3 m) and 51 ft (15.5 m). A three stage rotor pump (WSP-12V-3, Length 14.5", Diameter 1.6", Maximum Flow Capacity 12.1 L/min, Waterra, Bellingham, WA), powered by a car battery, attached to 55 ft (16.8 m) of a general purpose clear PVC tubing [3/8 inch ID x 1/16 inch wall thickness (9.5 mm x 1.6 mm), Fisherbrand, Pittsburgh, PA] was used to collect the groundwater samples. Approximately 3 L of groundwater were pumped out of a well before collecting the groundwater sample. The groundwater is allowed to fill a plastic cup (approximately 0.5 L) and it overflows to a bucket on which the cup is hooked. Samples were collected by immersing a 15 mL (nominal volume, actual volume is 16.5 mL) glass vial (Supelco, Bellefonte, PA) into the plastic cup while the groundwater was flowing into it. After the vial was filled with groundwater it was closed immediately with a mininert cap. The cap of the vial was loosened and 5 mL of the liquid was taken out of the vial through the septum of the mininert cap using a 10 mL syringe, while air entered the headspace of the vial. The 5 mL liquid was transferred to a wide-mouthed glass vial and closed with a screw-cap and stored in a cold room which was used for anion analysis.

For the tracer analysis, 1.5 mL of the 5 mL liquid was transferred to a 1.5 mL centrifuge tube and centrifuged at 10,000 rpm for 2 minutes in a ??? centrifuge (co, place). The supernatant was then transferred to a 2 mL clear vial with white septum and threaded black cap (National Scientific Company, Rockwood, TN) for analysis of bromide and other anions using an ion chromatograph (Dionex DX500 Series, Sunnyvale, CA) equipped with a conductivity detector

and an analytical column (Ionpac, AS9-HC, 4 mm x 250 mm). The eluent solvent was 9 mM sodium carbonate at a flow rate of 1 mL/min. The ion elution times were approximately 6.3 min for chloride, 9.5 min for bromide, 11 min for nitrate and 18 min for sulfate, respectively. The sample volume injected was 25 µL and analysis was run at room temperature for 20 minutes.

4.3. Results and Discussion

The location of the monitoring wells is shown in Figures 4.1 to 4.3. Based on bromide arrival times or from modeling, the estimated groundwater velocities (Darcy velocity/porosity of the soil) are presented in Table 4.2. The mean values of velocity are calculated from data for MW-9D and MW-10D. For the deep zone of the aquifer, the means from 2004 (0.65 ft/d or 0.2 m/d) and 2005 (0.125 ft/d or 0.038 m/d) study are compared using statistical tests (lsd, Tukey, Bonferroni and Scheffe) and found to be significantly different (Ott, 2001). The p-value is 0.0076 (the value should be greater than 0.05 for the means not to be significantly different). The software that was used for running the test was Statistical Analysis Software (SAS) 9.1.3, Servio Pack 4, XP-PRO platform, SAS Institute, Inc., Cary, NC.

4.3.1. Groundwater Elevation

One of the major factors affecting the groundwater table level is the precipitation. Table 4.3 lists the amount of rainfall (inches) recorded at Manhattan, KS, during the period May 2003 to April 2007 (KSU Research and Extension, http://av.vet.ksu.edu/webwx/, 2008). Tables 4.4 and 4.5 report the measured groundwater elevation levels in the pilot study area for the deep zone and the shallow zone respectively. Figure 4.5 shows the groundwater level (+1000 ft (304.8 m)) for selected shallow and deep wells as a function of time. The site is just a few feet over 1000 ft (304.8 m) above mean sea level (MSL). In general, the groundwater table in the shallow zone is approximately 5 ft (1.52 m) above that in the deep zone. The groundwater level increased from August 2003 to August 2004 and then had a decreasing trend until March 2006, except for one event in June 2005. The groundwater levels increased from March 2006 until June 2006 and then decreased until August 2006. The variations are mainly impacted by rainfall events. In the shallow zone, the groundwater levels were in the range 1011 ft (308.2 m) to 1016 ft (309.7) above MSL and in the deep zone the groundwater levels were in the range 1007 ft (306.9 m) to 1010 ft (307.9 m) above MSL. Groundwater elevation in MW-14S is consistently less than the

values for MW-14D, contrary to the observations at monitoring wells 7 to 12. The groundwater elevations at MW-14S are significantly lower than those at MW-12S. There is not sufficient data to explain these observations. There is no evidence that groundwater at MW-12S and MW-14S are connected or that there is significant flow from MW-12S to MW-14S.

The groundwater elevation gradients (ft/1000 ft or m/1000 m) between MW-8 and MW-12 and between MW-12 and MW-14 are shown in Figures 4.6 and 4.7, respectively, during the period August 2003 to February 2007. The gradient in the shallow zone is about one order of magnitude higher than that in the deep zone. Based on several measurements of groundwater levels during this period, the mean values of gradients between MW-8 and MW-12 are 0.0094 in the shallow zone and 0.0006 in the deep zone. The mean values of gradients between MW-12 and MW-14 are 0.012 in the shallow zone and 0.0012 in the deep zone. The general flow direction is to the east in both zones.

4.3.2. Fall 2004 Tracer Study

4.3.2.1. Deep Wells

At the injection well MW-8D, bromide was diluted by the flowing groundwater and the concentration decreased rapidly in an exponential manner. The bromide concentration decreased from about 500 mg/L to 50 mg/L in approximately 8 days, as shown in Figure 4.8. After 28 days, the injected bromide had been almost completely washed out from MW-8D. The time constant for the first order decrease of concentration is obtained based on the time taken for 63.2 % response or when the concentration is 37.8 % of the initial concentration. Since the injected concentration is 500 mg/L, 500 * 0.378 = 184 mg/L and from Figure 4.8, this value occurs at approximately 4 days, based on the data at mid-depth. The time constant is the time taken for one flushing of the system volume, at the given flow rate.

Breakthrough of bromide occurred at day 9 for MW-9D, approximately 10 ft (3.05 m) down-gradient, with a peak at day 15 and return to baseline by day 47; the tracer had a longer tail and the concentration curve is not symmetrical with respect to the peak time as shown in Figure 4.9. For MW-10D, approximately 20 ft (6.1 m) down-gradient from the injection well, only a very weak peak was detected at about 28 days, as shown in Figure 4.10. It is not statistically significant compared to the observed background concentrations, which are mostly in the range of 0.3 - 0.6 mg/L.

The dilution model was used to fit the bromide concentration in MW-8D, the injection well. The actual velocity that best fits the model is 0.12 m/day (0.4 ft/day) as shown in Figure 4.11. The tracer solution of 200 L is assumed to spread in a cylindrical fashion, with height 3.05 m (10 ft; the height of the screening in the well) and radius 0.26 m. The volume occupied by these dimensions is 0.647 m^3 or 647 L assuming a soil porosity of 0.3. The area of the rectangle perpendicular to the flow is then 3.05 m * 0.52 m = 1.586 m^2. Due to the porosity of the soil, the actual area available for flow is 0.3 * 1.586 = 0.48 m^2. The model results for actual velocities of 0.06 m/d (0.2 ft/d), 0.18 m/d (0.6 ft/d) and 0.24 m/d (0.8 ft/d) are shown in the supplement. The velocity can also be obtained from the time constant estimated above. Since time constant = V/Q = 4 days, Q = V/4 = 200/4 = 50 L/day = 0.05 m^3/day. The velocity is, therefore, Q/A = 0.05/0.48 = 0.1 m/day (0.34 ft/day), which is in good agreement with the value estimated from the dilution model.

Based on the arrival times at MW-9D (15 days) and MW-10D (28 days, though the peak may not be decisive), the actual velocities are estimated as 0.186 m/d (0.62 ft /d) and 0.204 m/d (0.68 ft/d). Table 4.2 lists the velocities estimated based on the results at each deep well. Similar methods were used to obtain the values in Table 4.2 for the shallow wells.

The bromide concentrations in MW-7D, MW-11D and MW-12D, shown in Figures 4.12 to 4.14, are similar to the background level, between 0.2 – 0.6 mg/L in MW-7D, 0.1 – 0.5 mg/L in MW-11D, and 0.2 – 0.8 mg/L in MW-12D. Sampling was discontinued after 63 days in MW-12D. A peak may have been detected at this well if monitoring had been continued, as was found later in tracer study during Fall 2005, where a peak was detected at this well 240 days after bromide injection at the injection wells. As shown in Figure 4.3, the injection wells which were used for injection in the Fall 2005 study are located about 5 ft (1.52 m) down-gradient of MW-8D.

4.3.2.2. Shallow Wells

At the injection well MW-8S, the bromide was diluted by the flowing groundwater and the concentration decreased rapidly in an exponential manner from the initial 500 mg/L to 50 mg/L in approximately 3.5 days, as shown in Figure 4.15, which is almost twice as fast as MW-8D. After 14 days, the injected bromide had been almost completely washed out from MW-8S. This suggests a more rapid flow in the shallow wells than in the deep wells. Such a result was not

expected, given the very low reported permeability of the soil in the shallow zone, in spite of the apparent groundwater elevation gradient being 10 fold greater than that in the deep zone (~1/100 in the shallow zone compared to ~1/1000 in the deep zone). The time constant for the first order decrease of concentration is obtained from the time taken for 63.2 % response or when the concentration is 37.8 % of the initial concentration. Since the injected concentration is 500 mg/L, 500 * 0.378 = 184 mg/L and from Figure 4.15, this value occurs at approximately 1.95 days, based on the data at mid-depth (22 ft bgs).

The peak of bromide concentration occurred at day 9 for MW-9S, approximately 10 ft down-gradient, as shown in Figure 4.16. Assuming a tortuosity factor of 1, the groundwater velocity is, therefore, 9.25 ft/9 days = 1.03 ft/d (0.31 m/d). Since the shallow zone comprises more clay than deep zone, it was presumed that it would take more time than the deep zone, for the bromide to appear. Hence, the sampling interval was greater. However, the bromide appeared sooner than in the deep zone and that is the reason there are only a few data points before the peak. The groundwater elevation gradient in the pilot study area is approximately 10 times higher in the shallow zone compared to that in the deep zone (see Figures 4.5 to 4.7). There may be lenses of sand or silt in the shallow zone causing preferential flow paths resulting in higher velocity of groundwater.

The peak bromide concentration for MW-9S was 32.3 mg/L at the bottom depth compared to only 2.7 mg/L in MW-9D. The groundwater flow direction could be more towards east in the shallow zone and we may have detected the bromide that was near the center of the tracer plume traveling eastward. Another reason for higher concentration may be that the lateral dispersivity is higher in the deep zone compared to shallow zone. However, the bromide concentrations were almost the same in all three depths in MW-9D, whereas there is a distinct difference in the bromide concentrations in MW-9S, with the values increasing with depth. From Figures 4.9 and 4.16, the vertical dispersion of groundwater between MW-8D and MW-9D was higher compared to that between MW-8S and MW-9S. For MW-10S, approximately 20 ft down-gradient, the peak of bromide occurred at about 22 days, as shown in Figure 4.17, with return to baseline by day 65. Assuming a tortuosity factor of 1, the groundwater velocity is, therefore, 19 ft/22 days = 0.87 ft/d (0.27 m/d)The peak concentration was 14 mg/L, suggesting a dilution factor of only two between MW-9S and MW-10S, assuming the groundwater flow is eastward. The dilution factor for bromide for approximately the same distance of travel from MW-8S to

MW-9S is 500/32 ~ 16, suggesting that the bromide detected at MW-10S may not have necessarily traveled through MW-9S.

The bromide concentration curve for MW-8S was modeled based on the advection-dispersion equation (Reddi and Inyang, 2000), as shown in Figure 4.15. The screening interval of the well where the tracer was injected is 305 cm (27 ft to 17 ft bgs). The circular area over which the bromide is assumed to spread due to injection, was estimated to be 2123 cm^2 or the radius of influence (ROI) is 26 cm. The value of dispersivity used in the model is 2.9 * 10^{-3} cm^2/s. Velocity of groundwater is estimated based on the time for peak tracer concentration in MW-10S which is located 20 ft from injection well. The ADE model was also fitted for the data for MW-10S, as shown in Figure 4.18. The value of dispersivity used in the model is 4.6 * 10^{-3} cm^2/s. Perfect et al (2002) have reported that the typical value of dispersivities (in cm^2/s) are in the range 1 to 0.1 for clay, 0.1 to 0.001 for silt and < 0.001 for sand. The dispersivities estimated based on data from MW-8S and MW-10S suggest that the soil in this area may be sandy silt.

The velocity can also be obtained from the time constant estimated above. Since time constant = V/Q = 2 days, Q = V/2 = 200/2 = 100 L/day = 0.1 m^3/day. The velocity is, therefore, Q/A = 0.1/0.48 = 0.21 m/day or 0.68 ft/day. This value is in close agreement with the value of 0.27 m/day (0.87 ft/d) based on the data from MW-10S.

The experimental data for three depths and the RTD model curve is shown in Figures 4.19 and 4.20 for MW-9S and MW-10S, respectively. The mean residence time (MRT) was estimated to be 9 days for MW-9S and 27.4 days for MW-10S, based on data at mid-depth. For MW-9S, the tracer breakthrough curve is almost symmetrical; therefore, the MRT is very close to the peak arrival time. The symmetrical nature of the curve suggests that the groundwater travels almost in a plug flow manner, from MW-8S to MW-9S. For MW-10S, the MRT is not the same as the bromide peak time since the tracer breakthrough curve is not symmetrical. The velocity and dispersivity were estimated from the model as 31.3 cm/day and 10.1 * 10^{-3} cm^2/s for MW-9S and 26.6 cm/day and 4 * 10^{-3} cm^2/s for MW-10S suggesting a silty soil with equal amounts of sand and clay (Perfect et al., 2002). The Peclet numbers (vL/D) are 10.1 and 45.4 based on data from MW-9S and MW-10S. Peclet numbers near zero occur for very small velocities and larger values indicate larger velocities and less axial mixing. The numbers are high suggesting that advection is significant compared to dispersion. Since the value of 'L' varies for each monitoring well, caution must be exercised in comparing Peclet numbers between

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monitoring wells. However, a comparison of Peclet numbers over time at the same monitoring well reveals a change in the pattern of groundwater flow. There was no significant peak of bromide at MW-12S, though on day 34, the bromide concentrations at three depths were approximately twice the background concentration, as shown in Figure 4.21.

Each type of model can be used to fit the data from the other monitoring wells also. However, for illustration purposes, for each model, results are presented for selected monitoring wells only. Since the models only consider flow in the axial direction, they are only useful to model the response in the direction of flow.

4.3.2.3. Other Inorganic Compounds/Ions

In addition to bromide, the concentrations of other anions chloride, nitrate and sulfate were analyzed in the ion chromatograph. The results presented below are based on samples collected during August 2004 to December 2004.

The concentrations of nitrates in deep wells were below the detection limit (0.1 mg/L). In the shallow wells the concentrations were approximately 21 mg/L for MW-8S and MW-9S and ranged from 11 – 19 mg/L in MW-10S, 5 – 20 mg/L in MW-7S, 18 – 20 mg/L in MW-11S and 5 – 22 mg/L in MW-12S. The nitrate concentrations were higher in the top and decreased with depth from ground surface. The graphs showing nitrate concentration vs time are given in the supplement (Figures S.4.4 to S.4.9). The units are mg nitrate/L.

In the deep wells the sulfate concentrations at the middle depth ranged from 220 - 260 mg/L for MW-8D, 250 - 280 mg/L for MW-9D, 230 – 260 mg/L in MW-10D, 200 – 250 mg/L in MW-7D and MW-11D, and 220 – 230 mg/L in MW-12D. In the shallow wells the sulfate concentrations at the middle depth were in the range 220 - 230 mg/L for MW-8S, MW-9S, and MW-10S, 150 – 200 mg/L in MW-7S, and 190 – 210 mg/L in MW-12S. Overall, the sulfate concentration in the deep zone were about 40 mg/L higher than that in the shallow zone. The graphs for sulfate concentrations, at the middle depth for selected days, are given in the supplement (Figures S.4.10 and S.4.11).

In the deep wells the chloride concentrations at the middle depth ranged from 70 - 120 mg/L for MW-8D, 90 - 100 mg/L for MW-9D, 70 – 100 mg/L in MW-7D, and consistently about 90 mg/L in MW-10D and MW-12D. In the shallow wells the chloride concentrations at the middle depth were in the range 140 - 150 mg/L for MW-8S, 130 – 150 mg/L for MW-9S, 100 –

150 mg/L in MW-10S, 90 – 130 mg/L in MW-7S, and 115 – 135 mg/L in MW-12S. Overall, the chloride concentrations in the shallow wells were about 40 mg/L more than those in the deep zone. The graphs for chloride concentrations, at the middle depth for selected days, are given in the supplement (Figures S.4.12 and S.4.13).

4.3.3. Fall 2005 and Spring 2006 Tracer Study

4.3.3.1. Shallow Wells

The bromide that was injected into the injection wells reached MW-8S as a result of the radius of influence of injection and associated dispersion, as shown in Figures 4.22, 4.22a and 4.22b. After injection, there was a dilution effect due to dispersion and groundwater flow. For clarity, the bromide concentration in MW-8S is shown in Figures 4.22a and 4.22b for these bromide injections. During the first injection on 8/18/05, bromide concentrations measured after two days indicate that concentration increases with increasing depth bgs and returned to background concentration within a week. Based on the data from the bottom depth, the time constant is approximately 1.7 days (response time for initial concentration to decrease to 7.8 * 0.378 = 2.95 mg/L). In Figure 4.22b, the bromide concentration after two days of injection was much higher compared to the first injection in Figure 4.22a, considering even that the injection concentration was almost double in the second injection on 3/3/06, day 197. The time taken for return to baseline was also larger in the second injection, 22 days vs 7 days. Based on the data from the bottom depth, the time constant is approximately 1.8 days (response time for initial concentration to decrease to 28 * 0.378 = 10.6 mg/L). The time constants for MW-8S estimated from the tracer studies conducted at three different time periods (1.95, 1.7 and 1.8 days) were comparable.

In the shallow zone, the groundwater flow is predominantly eastward. In Figure 4.23, the data for MW-9S has a small bromide peak, with concentration between 1 and 1.5 mg/L (background concentration of 0.3 mg/L) at about 5 days and reached background concentration in 15 days. The second injection on day 197 did not lead to a higher peak in MW-9S as shown in Figure 4.23. The peak of bromide was about 1 mg/L, however, it took 11 days to reach the peak and 28 days to return to baseline. The biofilms formed during the previous nutrient injections, on 8/18/05 and 10/13/05, appear to have decreased the hydraulic conductivity of the soil between the injection points and MW-9S and the bromide may have taken a different route due to that

(see Table 4.6). In Table 4.6, in general, the hydraulic conductivity decreased with time for any given well. This is due to the addition of nutrients causing biofilms. As shown in Table 4.2, the estimated groundwater velocity is lower in Spring 2006.

The injection wells were sampled on day 46 and ions were analyzed. The bromide concentrations in the shallow zone were 0.6 mg/L at Loc A (25 ft bgs), 86 mg/L at Loc B (25 ft bgs), and 16 mg/L at Loc D (32 ft bgs). Some of this bromide may have been pushed to the surrounding monitoring wells during subsequent injections.

In Table 4.7, the estimated values of Peclet number decreased with time for 9S, 10S and 12D. Since L is constant for a given well, v/D decreased with time. This is due to decrease in velocity caused by biofilms. The approximate flow rates of solution into the injection wells decreased from the early studies to the later experiments as shown in Table 4.8, corroborating the phenomenon of biofilm formation. The observed arrival times for bromide tracer are shown in Table 4.9. Since the value of 'L' varies for each monitoring well, caution must be exercised in comparing Peclet numbers between monitoring wells. However, a comparison of Peclet numbers over time at the same monitoring well reveals a change in the pattern of groundwater flow.

There is not much vertical variation in concentration of bromide in MW-9S compared to MW-10S (Figure 4.24) corroborating the assumption that the bromide reaching MW-10S may not have passed through MW-9S. The bromide concentrations remained almost at the background level in MW-9S, especially after the injection of nutrient solution on day 347, except for a few small peaks. The bromide peak is much greater during the Fall 2004 tracer study as shown in Figure 4.16. These results suggest that the groundwater flow in the shallow zone is almost eastward and the low concentration of bromide detected in MW-9S, the nearest well down-gradient of injection wells, may be due to detecting only the edge of the bromide plume.

MW-10S had a bromide peak at about 18 days, with concentration up to 13 mg/L, as shown in Figure 4.24. The peak concentration in MW-10S is much higher than in MW-9S, which is closer to the injection points. Some of the bromide in the groundwater, appears to have bypassed MW-9S. However, the velocity of groundwater flow based on MW-9S (1 ft/day or 0.3 m/day) and MW-10S (0.83 ft/day or 0.25 m/day) data is approximately the same, considering that the injection was done at four different points. It is not possible to determine the source of the bromide at monitoring wells, since it could be from any of the four injections points though it is likely from the closest two injection points. In estimating the velocities, the distance is

measured from the line joining the four injection points (north-south), which is perpendicular to the line in which the monitoring wells are located (east-west). In MW-10S, the concentrations of bromide were higher with increasing depth. For example, at the peak, day 18, the concentration at top (18 ft), middle (23 ft) and bottom (28 ft) samples were 2 mg/L, 7 mg/L and 13 mg/L respectively. This indicates that there was not much vertical dispersion of bromide injected at depths 23-28 ft and 30-35 ft. Although the concentrations at different depths were different, the time at which the peak of bromide occurred was consistent, likely due to lack of significant variation of hydraulic conductivity in the vertical direction in the depth range of 15 to 30 ft (4.5 to 9 m).

On day 194, there was a spike of bromide concentration at MW-10S (Figure 4.24). For injecting nutrient solution and anaerobic chase water on day 197, 400 liters of groundwater was pumped out from MW-10S. This event could have drawn groundwater from all directions including some of the bromide from a relatively stagnant region up-gradient to MW-10S. Unlike the first bromide injection results, two peaks were observed during the second injection, first on day 229 and the second on day 265. The concentrations were also not as high as during the first injection, in spite of twice the injected bromide concentration. The bromide concentration reached a peak of 2.5 mg/L and 2.9 mg/L during the first and second peaks following the injection on day 197. The variation in concentration with depth was not significant. The Spring 2006 tracer study indicated that the site hydrogeology was significantly modified due to injection of nutrients and growth of biofilms. It took a long time for the bromide concentration to decrease and even before it reached the background level, there was again a small peak (1.7 mg/L) due to injection of nutrient solution on day 348. The residual bromide trapped in or around the injection wells was likely released due to the hydraulic force of injection. The bromide concentration reached the background level by day 450, i.e., the bromide had been above background level at MW-10S for more than 230 days after the breakthrough occurred on day 219. This suggests that the bromide plume must be wide and also likely from more than one injection well.

The bromide level in MW-12S remained consistently around the background level, 0.2 mg/L to 0.4 mg/L, as shown in Figure 4.25.

4.3.3.1. Deep Wells

In the deep zone, the bromide injected through the injection wells, on 8/18/2005, reached MW-8D due to the injection process and then it was washed away, as shown in Figure 4.26. As observed during the Fall 2004 tracer study, the time taken for dilution was greater in MW-8D compared to MW-8S. Moreover, the bromide reached background level in MW-8S in 10 days, but this was not the case in MW-8D. The bromide level in MW-8D has remained fairly steady since the second nutrient injection in October 2005. It never returned to baseline, as shown in Figure 4.26, after its initial spike, decline and rebound from the first feeding in August 2005, suggesting that there is in effect a biobarrier formed causing little or no flow through that well. The injection wells were sampled on day 46 and ions were analyzed. The bromide concentrations in the deep zone were 12 mg/L at Loc A (42 ft bgs), 2.4 mg/L at Loc B (48 ft bgs), 61 mg/L at Loc C (50 ft bgs), and 6.6 mg/L at Loc D (51 ft bgs). The sampling depth is given in the parenthesis after the location. These results suggest that the bromide was still present at the injection wells 46 days after the injection.

As described in ITRC (2002), bio-fouling is attributed to the increase in microbial populations and perhaps more importantly, to the creation of extra cellular polysaccharides by cells. These slimy polysaccharides are important for the accumulation of microorganisms on surfaces or within porous media and can contribute significantly to bio-fouling of an injection well. A portion of a nutrient amendment contributes to the creation of new bacteria (biomass). Eventually, continued unchecked bacterial growth is likely to reduce circulation and injection of the amendment, and may lead to a plugged formation or injection well (i.e., bio-fouling). In bioremediation, various operating strategies have been devised to minimize this potentially undesirable outcome. These methods are not formalized, but rather various engineering approaches have been used over the years. No one approach is a clear solution. However, it is an issue that must be considered in system design and operation (ITRC, 2002).

At MW-9D, the bromide peak was delayed (80 days vs 50 days) relative to the first injection during Fall 2005, and the peak response was much smaller despite introducing twice as much bromide in this tracer study (Figure 4.27). After the fourth dose of nutrients on day 348, the bromide concentration increased at MW-9D. Until day 500, the bromide concentration at middle depth (47 ft bgs) was higher than top depth (42 ft bgs) which in turn was higher than the bottom depth (51 ft bgs). It is likely that the bromide injected at the injection well screening

depth of 42 – 47 ft bgs (Loc A and Loc C) was reaching MW-9D in larger amounts than the bromide injected at depth of 50 – 55 ft bgs (Loc B and Loc D). It is interesting to note that the bromide concentration in the middle and bottom samples started to increase after day 550. Bromide may be trapped in the soil and biofilms at the injection locations and released slowly which was observed on day 602, 405 days after the last injection of bromide on day 197. As mentioned above, 46 days after the first injection of bromide, there was a significant concentration of bromide still present at the injection locations. The bromide concentration in the bottom samples are higher than the middle samples, indicating that the bromide may be from the injection depths 50-55 ft bgs.

In MW-10D, the breakthrough occurred at about day 60 and the bromide peak occurred at day 95, at a smaller concentration of 1.3 mg/L, with a broad plateau, indicating a dispersed continued source, and returned to baseline by day 175, as shown in Figure 4.28. The results suggest that the groundwater flow in the deep zone is rather slower than in the shallow zone, probably due to lack of a sufficient gradient and pockets of higher conductivity in the shallow zone. During Spring 2006, the bromide peak was much larger (18 ppm vs 1.3 ppm) than in the previous tracer study (Figure 4.28). However, the apparent time of peak arrival was comparable in both injections.

The combined results for the wells 9 and 10 suggest that alternative flow paths must be present at different times, such that MW-10D is being fed a more undispersed plug of tracer in this study than in the previous study while for well 9D the tracer is more dispersed this time, or the MW-9D may be at the edge of the tracer plume. Given our limited understanding of this aquifer, our best interpretation is that perhaps growth of biofilms has altered the resistance to flow of some paths in the deeper zone. For the shallow zone similar effects may be present but the variation of water table as a function of rainfall may influence both rates and paths of flow.

In Figure 4.29, there was a distinct peak at MW-12D, at a concentration of 2.3 mg/L at the bottom depth, even higher than that in MW-10D, in spite of being located approximately 60 ft down-gradient of MW-10D. This is presumed to have come from the first tracer injection since it arrived within 20 days of the third nutrient injection (second dose of tracer) and moreover, a flowrate of 120 cm/d is not plausible for this site. The dispersion in the travel path from the injection wells to MW-12D was not large enough to dilute the bromide concentration to the background level. Since there were four injection wells the bromide source may have been from

different injection wells for MW-10D and MW-12D. Also the paths of travel from injection wells to MWs are different and in the case of MW-10D, the concentration may not reflect the value at the center of the plume. The bromide plumes from each injection well could possibly be mixed with each other down-gradient, depending upon the extent of lateral dispersion. The peak of bromide tracer at MW-12D was between day 220-230 (depending on depth of measurement in the well). The distance is 75 ft from injection point. Thus the flow rate of 1/3 ft/d is estimated.

In Table 4.7, the estimated values of Peclet number decreased with time for 12D. Since L is constant for a given well, v/D decreased with time. This is due to decrease in velocity caused by biofilms. The approximate flow rates of solution into the injection wells decreased from the early studies to the later experiments as shown in Table 4.8, corroborating the phenomenon of biofilm formation. The observed arrival times for bromide tracer are shown in Table 4.9. Since the value of 'L' varies for each monitoring well, caution must be exercised in comparing Peclet numbers between monitoring wells. However, a comparison of Peclet numbers over time at the same monitoring well reveals a change in the pattern of groundwater flow.

The bromide concentration in MW-5D, MW-7S, MW-7D, MW-11S, and MW-11D remained only at the background levels and the results are shown in the supplement.

The field tracer studies and the laboratory results suggest that channeling and non-uniform flow occurs in the field as well as in the laboratory.

4.3.3.1.1. Nitrate Concentrations

The concentrations of nitrates in deep wells were below the detection limit (0.1 mg/L). In the shallow wells the background concentrations prior to the pilot study were approximately 15 mg/L for MW-8S, MW-9S and MW-10S, approximately 13 mg/L in MW-12S as shown in Figures 4.30 to 4.33. The nitrate concentrations in MW-8S are shown in Figure 4.30. In the nutrient solution, only yeast extract contains nitrogen at 8.8 % (w/w), mostly in the organic form. The amount of yeast extract added on day 0 is 200 g in nutrient solution and 10 g in chase water, i.e., 1 g YE/L in nutrient solution and 0.05 g YE/L in chase water or 88 mg N/L in nutrient solution and 4.4 mg N/L in chase water. However, most of the nitrogen is in the form of amino acids in yeast extract. The nutrients injected on day 0 had a significant impact on the concentration of nitrate. The value dropped rapidly to almost zero at the middle and bottom depths though there was not much decrease in the concentration at the top depth. Nitrate is an

electron acceptor in the fermentation of the nutrients by the native microorganisms. As the added nutrients were diluted, the concentration of nitrate increased and reached 80 % of the background level by day 16. The trend in nitrate concentration was exactly inverse as that for bromide (and the nutrients) concentration and understandably so. When the bromide concentration was higher, that implies the nutrient concentration was also higher and therefore, due to microbial activity, the nitrate concentration becomes less. As the groundwater flows and dilutes the bromide and nutrient concentration, the nitrate concentration increases. This trend was observed during each injection on days 56, 197 and 348. However, unlike the first injection, the nitrate concentration at the top depth also decreased to low levels on days 197 and 348.

The peak of bromide in MW-9S, during the first and second injections, occurred after 4 days and 11 days. There was a corresponding trough in nitrate concentrations around those days since the nutrients also travel with the tracer though not as fast. The dip in nitrate concentrations occurred after 8 days and 14 days, as shown in Figure 4.31. There was also a dip in nitrate concentrations from day 95 to 146 and day 249 to 296 probably due to nutrients flowing from a farther injection well which had taken longer time to reach MW-9S. Even though no tracer was added on day 348, nutrients were added and this effect was shown on day 355, by a decrease in nitrate concentrations. However, between day 50 and day 208, the nitrate concentrations in MW-9S were between 20 – 23 mg/L with lower concentrations from day 90 to day 140(see Figure 4.31). After day 208, the nitrate concentration started decreasing gradually until day 450, with dip in concentration on days 211, 250, 280, 355 and 410. The low concentration may be due to nutrients reaching MW-9S from different injection wells or reduced groundwater reaching MW-9S.

The bromide-nitrate concentration correlation is more classically depicted in Figures 4.24 and 4.32 for MW-10S. During the Fall 2005 study, the nitrate concentration was not only inversely related to bromide concentration but also followed the same vertical trend. The concentration of bromide gets higher with increasing depth, whereas, the nitrate concentration was lower with depth, suggesting that the nutrients injected did not disperse much in the vertical direction. For MW-10S it appears that after the feeding on day 197, nitrate levels became very low. That would be consistent with the relatively rapid appearance of a high bromide tracer concentration if nutrients were able to allow microbes to reduce nitrate in that zone. In Figure 4.33, for MW-12S a decrease in nitrate occurred at a time consistent with the travel from first

nutrient injection, although no detectable bromide peak was observed. Nitrate concentrations at MW-7S was not steady, starting at 13 mg/L and increasing to 30 mg/L by day 77 and later decreased to 13 mg/L by day 180 and remained in the range 6 – 16 mg/L. Nitrate concentrations remained steady between 9 – 13 mg/L in MW-11S until day 450. The nitrate concentrations for MW-7S and MW-11S are given in the supplement. The variation in the background concentration of nitrate in the shallow wells may be due to several reasons including precipitation, foliage (Fenn et al., 2005), possible mixing with water from mid-campus creek which infiltrates into the subsurface. Run off directly from the Kansas State University campus and surrounding town from application of fertilizers to lawns and plants may also contribute to variation of nitrate concentrations. The figures showing nitrate concentration vs time for other wells are given in the supplement. The units are mg nitrate/L.

The nitrate concentration profiles also support the finding that the groundwater reaching a down-gradient monitoring well may not have traveled through an up-gradient monitoring well. Nitrate concentrations at MW-10S was very low (< 0.1 mg/L) from day 220 to day 290, which should appear in MW-12S from day 280 to day 350. However, the nitrate concentrations at MW-12S were not below 6 mg/L. Similarly, the nitrate concentrations at MW-9S were not below 0.1 mg/L from day 210 to day 280.

4.3.3.1.1. Sulfate and Chloride Concentrations

The figures showing sulfate and chloride concentrations vs time for the monitoring wells are given in the supplement. The units are mg/L.

4.3.4. Hydraulic Conductivity Estimation from Pumping Tests

Figures 4.34 to 4.36 show the results of the pumping tests and Theis' transient well model (Haitjema, 1995) used to fit the field data. On 8/17/2005, due to pumping water out of MW-10S, the groundwater elevation (all values are based on the assumption that the shallow zone bottom is at 35 ft bgs and considered as reference elevation zero) in that well decreased from 21.3 ft to 18.75 ft in 24 minutes and then decreased gradually to 18.18 in 50 minutes (see Figure 4.34). After 50 minutes, there was an increasing trend. Some of the resistances in the sub-surface may have been overcome and preferential flowpaths may have been created by pumping. The initial Girinski potential, corresponding to the initial potential of 21.29 ft (6.5 m), is 1133.2

ft^3/d (32.1 m^3/d). The value of groundwater elevation or potential is obtained based on a shallow zone bottom at 35 ft bgs (10.67 m bgs) and using Eqn. (4.14) for unconfined aquifer.

For the pumping tests on 12/21/2006, the decrease in groundwater elevation in MW-10S was rapid until 4.11 minutes. However, the groundwater elevation increased from 4.18 minutes to 6.58 minutes, and then the phenomenon of exponential decrease in elevation is repeated. The initial Girinski potential, corresponding to the initial potential of 20.14 ft (6.14 m), is 709.83 ft^3/d (20.1 m^3/d). The hydraulic conductivities estimated using the Theis' transient well model and based on MW-10S data for the pumping out cases, are $1.8 * 10^{-3}$ cm/s and $1.2 * 10^{-3}$ cm/s on 8/17/05 and 12/21/06.

The estimated hydraulic conductivity, for the pumping in case for MW-10S, based on data on 12/21/06, was $8.83 * 10^{-4}$ cm/s. The initial Girinski potential, corresponding to the initial potential of 20.14 ft (6.14 m) and a hydraulic conductivity of 2.5 ft/d ($8.83 * 10^{-4}$ cm /s) is 507 ft^3/d (14.4 m^3/d).

Using Darcy's law, the velocities can be estimated from the measured groundwater elevation gradient and the hydraulic conductivities obtained above. Darcy's law states that

$$v_d = k \left(\frac{d\varphi}{dx}\right) = v * n \tag{4.15}$$

where,

v_d = Darcy or superficial velocity

k = hydraulic conductivity of the soil

φ = groundwater elevation

x = horizontal distance between two points where groundwater elevation is measured

v = actual velocity

n = porosity of the soil

The groundwater elevation gradient measured on or close to the date of pumping test was used for estimating the velocity. The groundwater elevation gradient in the shallow zone between MW-8S and MW-10S are 0.0095 on 8/18/05 and 0.0105 on 11/28/06. The velocities based on these data are 0.048 and 0.037 ft/d for pumping out cases in MW-10S and 0.026 ft/d for pumping in case in MW-10S. These velocities are about 15 times smaller than those estimated from the tracer results. In the Theis' solution, assumptions such as homogeneous aquifer were made in the model. Also for estimating specific storage, literature values were used which are only an

approximation. These factors might have led to the difference in the velocities estimated from the above two methods.

4.5. Conclusions

The tracer study shows that the groundwater flow in the aquifer is predominantly towards the east in both the shallow zone and the deep zone. The estimated groundwater velocity in the shallow zone is approximately 3-fold greater than that in the deep zone. The static head gradient is about one order of magnitude larger in the shallow zone. The hydraulic conductivity is, therefore, 3-fold greater in the deep zone than that in the shallow zone. Based on the observed static head gradient and the estimated hydraulic conductivities from pumping tests, the velocity is estimated to be larger in the shallow zone of the aquifer as observed. Lenses of higher permeability regions may be present in the shallow zone as there is evidence of non-uniform flow.

The times taken for the bromide to reach MW-9D, MW-10D and MW-12D are consistent. The estimated velocity decreased from the 2004 study to the more recent studies due to biofilms associated with the biodegradation process. The nitrate concentration variations associated with nitrate being used as an electron acceptor are consistent with the times of nutrient addition and the bromide data. The organic substrates flowing with the bromide provide food for the organisms to consume with nitrate as electron acceptor. The nitrate concentrations indicate there is seasonal variation in the ion concentrations which depend on factors such as rainfall and surface runoff.

The groundwater reaching down-gradient wells may not have traveled through the up-gradient wells.

Figure 4.1. GIS map of site and monitoring well locations.
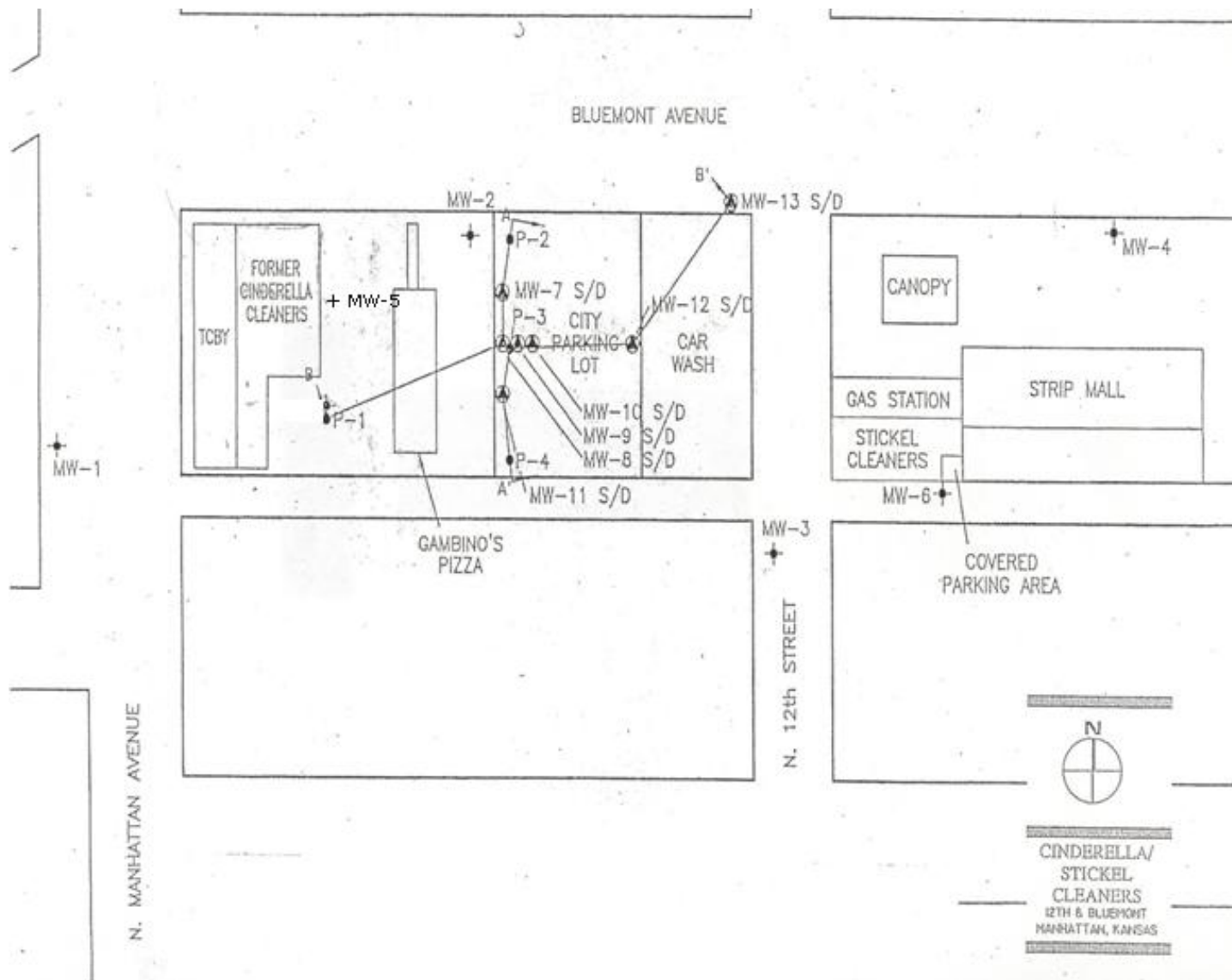
Figure 4.2. Location of monitoring wells (Terracon, 2004).
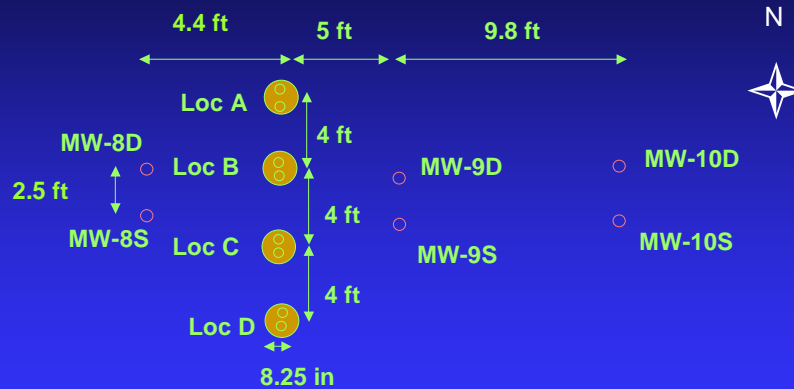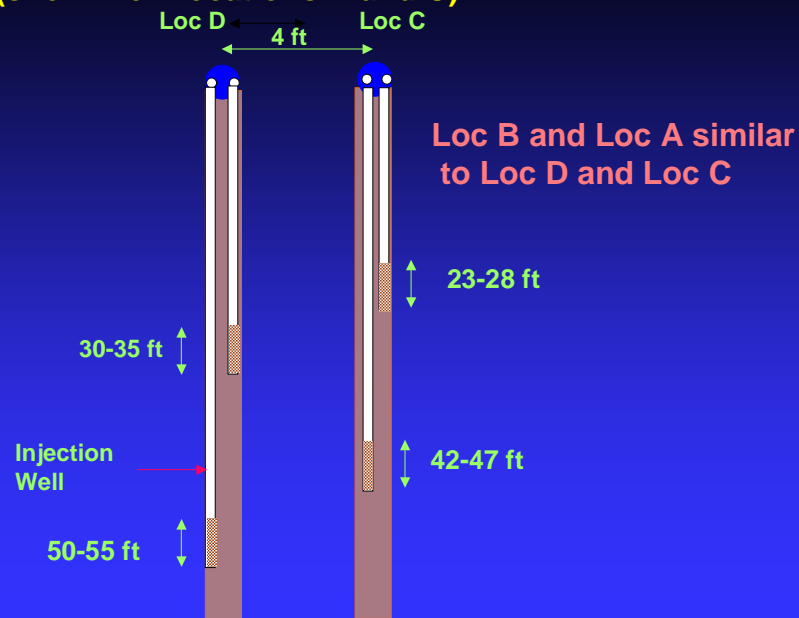
Figure 4.3. Plan and elevation of the injection points at the pilot study area. (Above) Schematic description of injection wells A, B, C, and D showing their locations with respect to monitoring wells 8, 9 and 10 deep (D) and shallow (S). (Below) The screening intervals for A are similar to C, and those for B are similar to D.

Figure 4.4. Photograph of the injection locations; each location has two injection wells, one for shallow zone and one for deep zone.
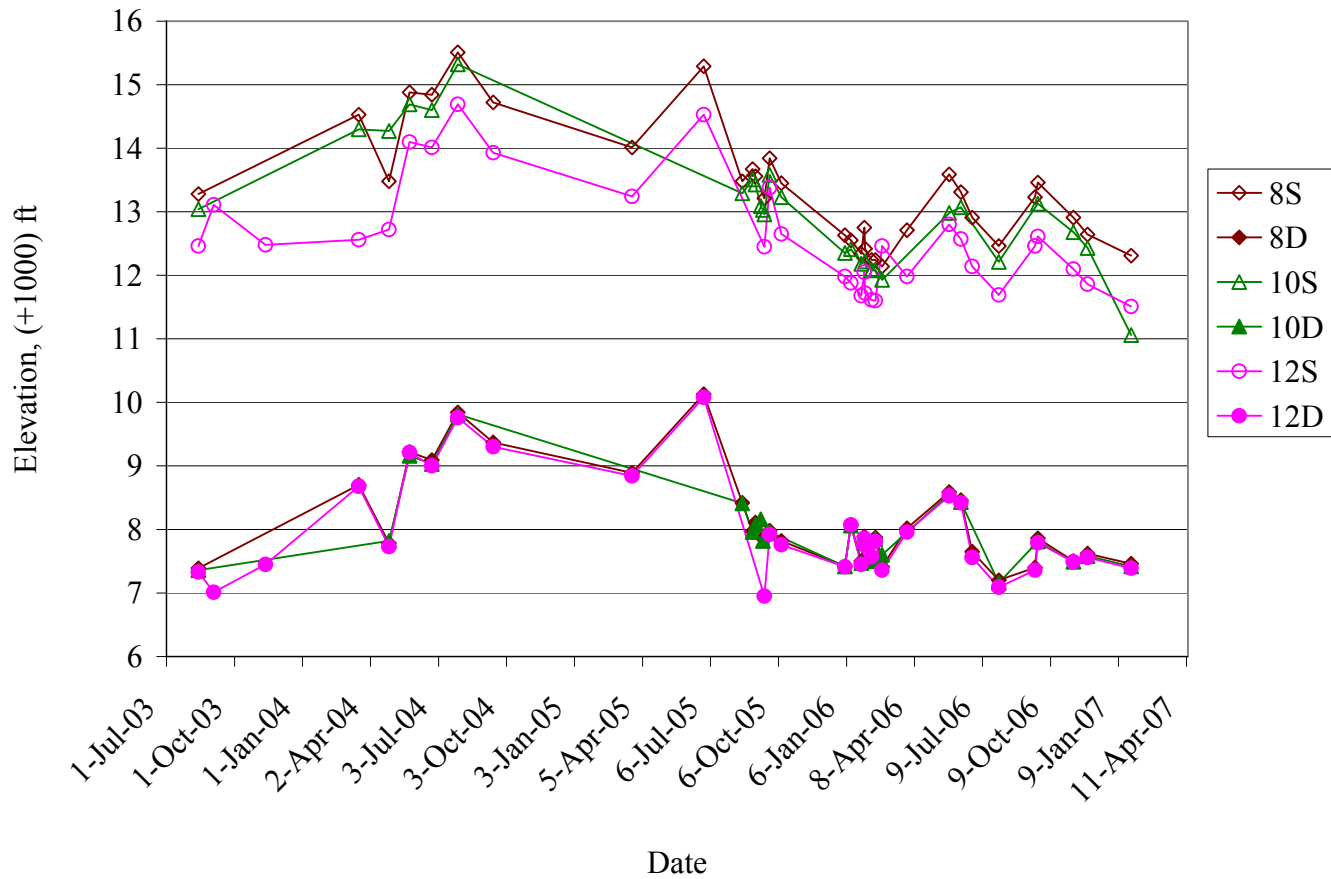
Figure 4.5. Groundwater elevation data for the monitoring wells at the pilot study area. The elevation at the site surface is approximately 312 m (1025 ft).
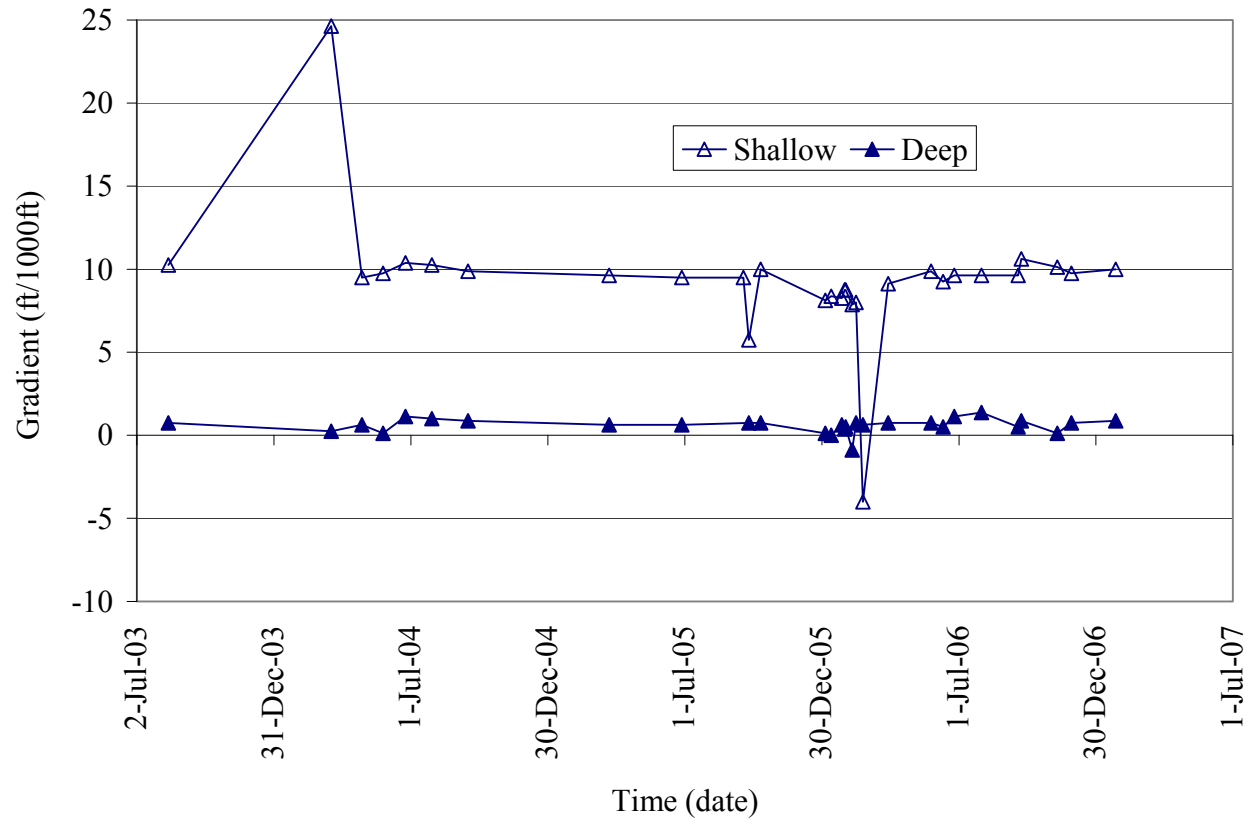
Figure 4.6. Elevation gradient (MW8-MW12) for shallow and deep zones. Distance between MW-8 and MW-12 is ~ 24.4 m (80 ft).
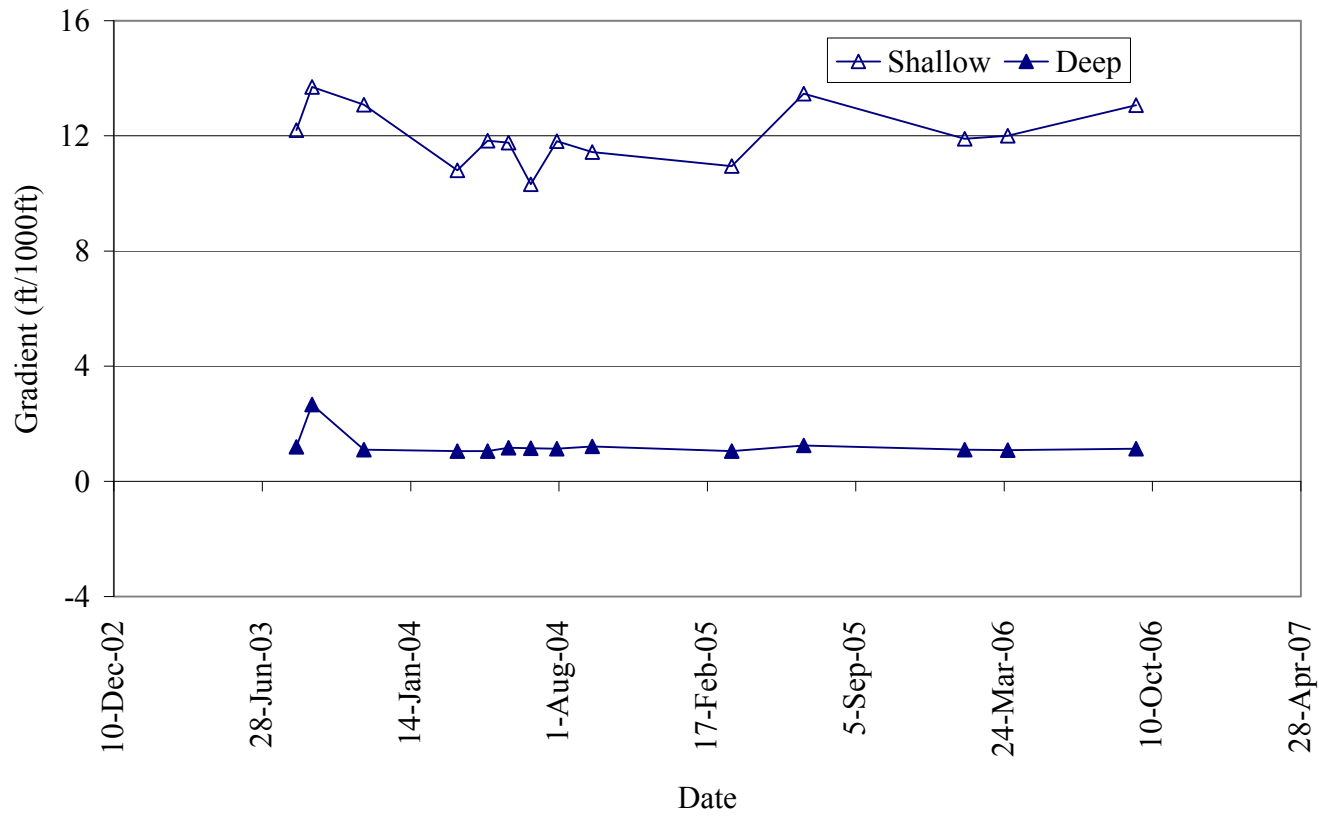
Figure 4.7. Elevation gradient (MW12-MW14) for shallow and deep zones. Distance between MW12 and MW14 is ~ 183 m (600 ft).
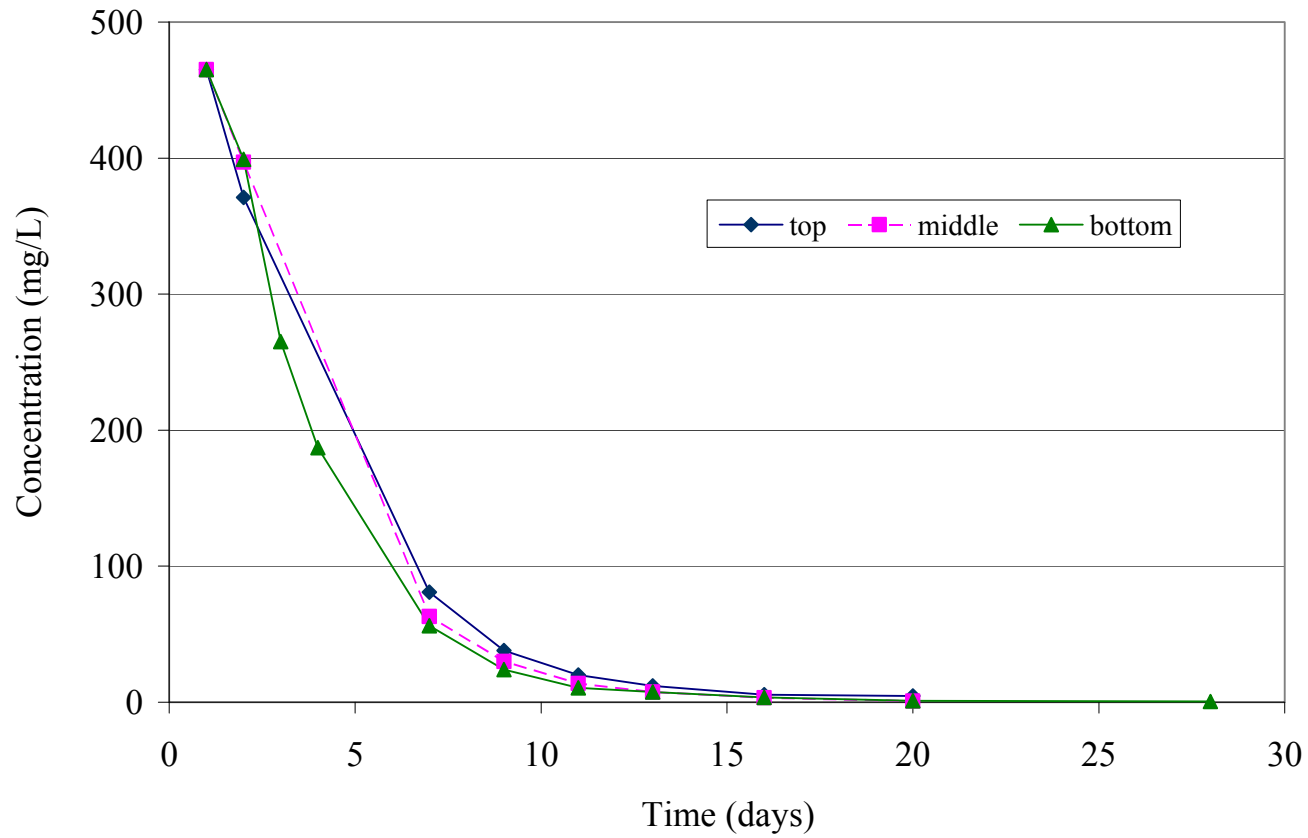
Figure 4.8. Bromide concentrations in MW-8D (injection well); injection concentration ~ 500 mg/L, 8/2/2004, day 0.
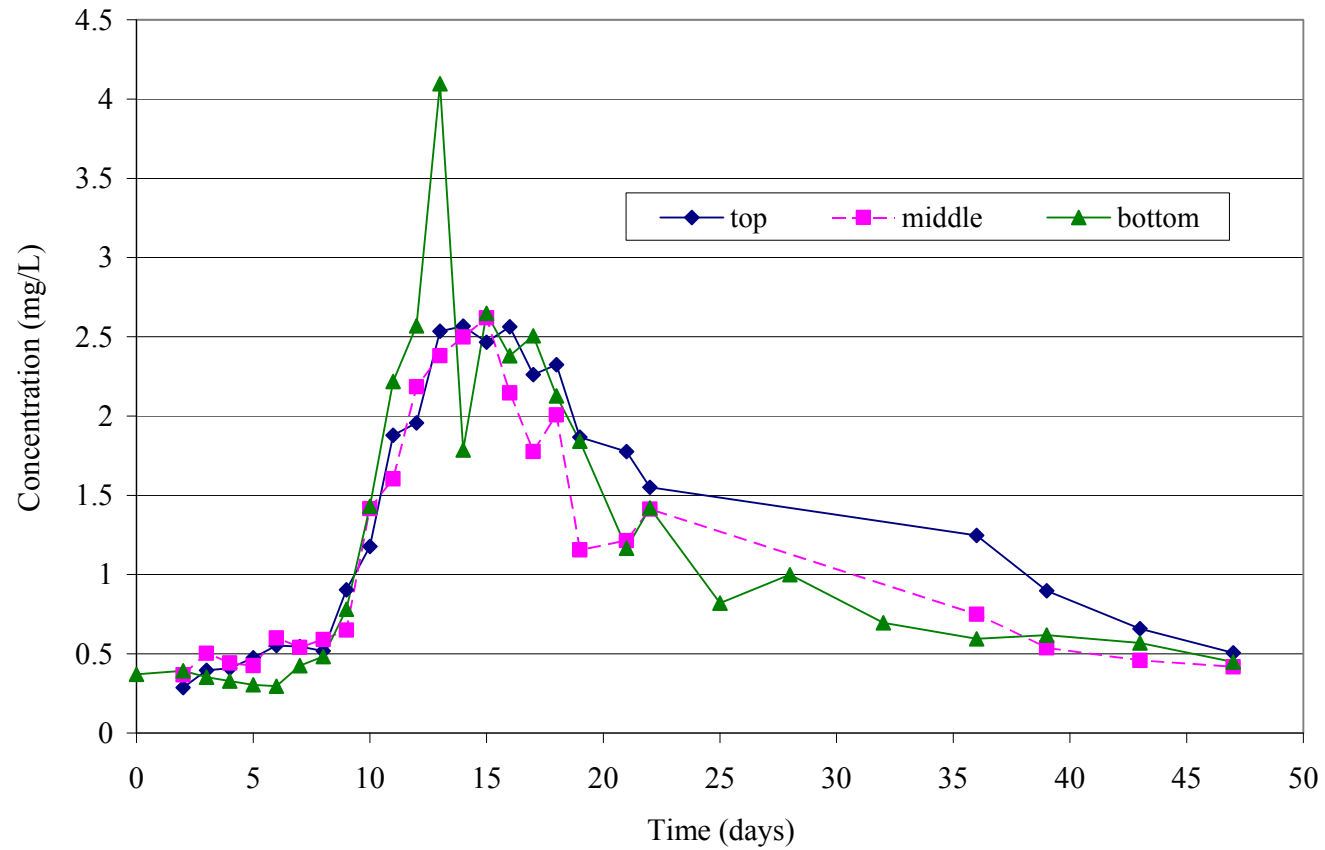
Figure 4.9. Bromide concentrations in MW-9D, 10 feet from injection well MW-8D; injection concentration ~ 500 mg/L, 8/2/2004, day 0.
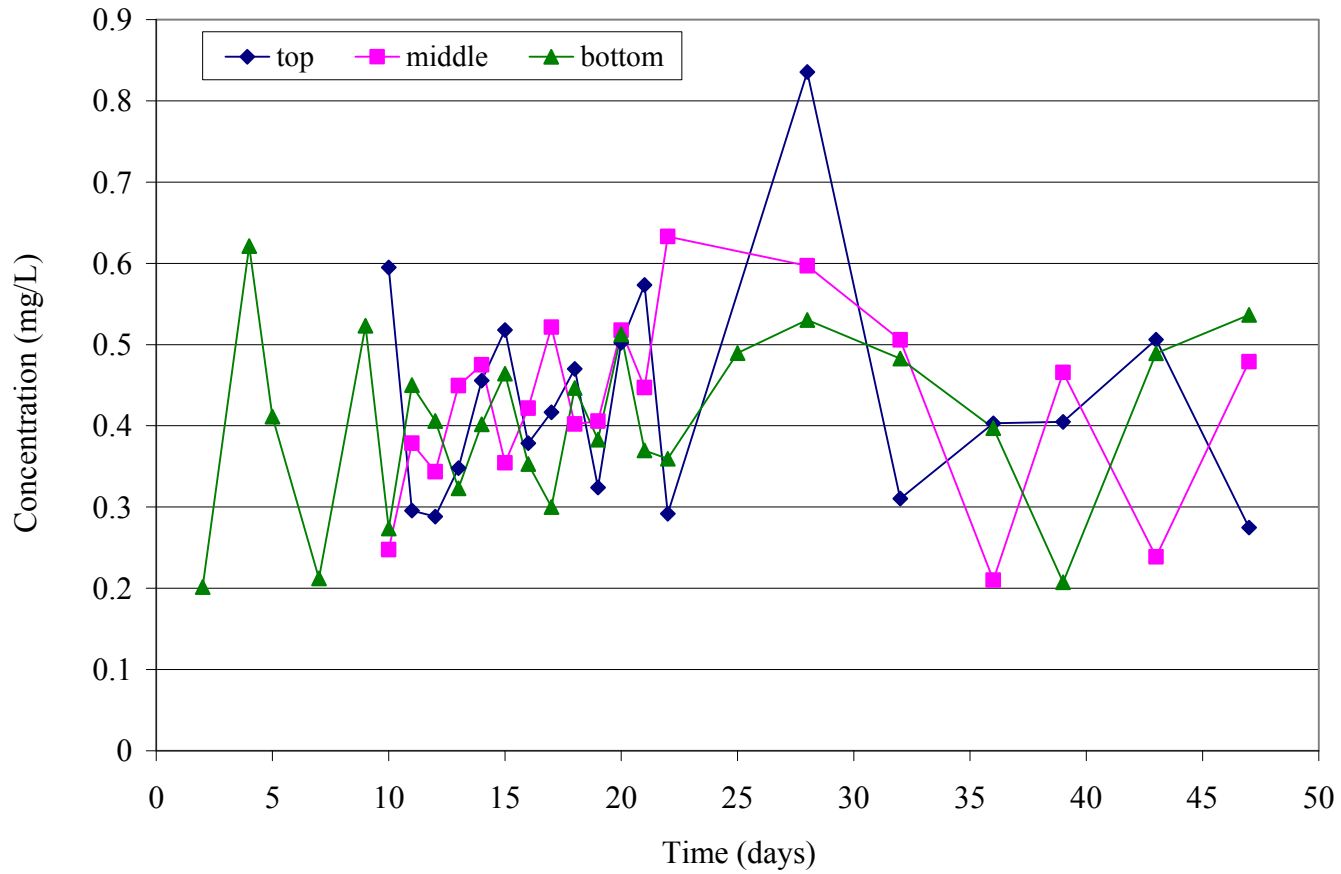
Figure 4.10. Bromide concentrations in MW-10D, 6.1 m (20 ft) from injection well MW-8D; injection concentration ~ 500 mg/L, 8/2/2004, day 0.
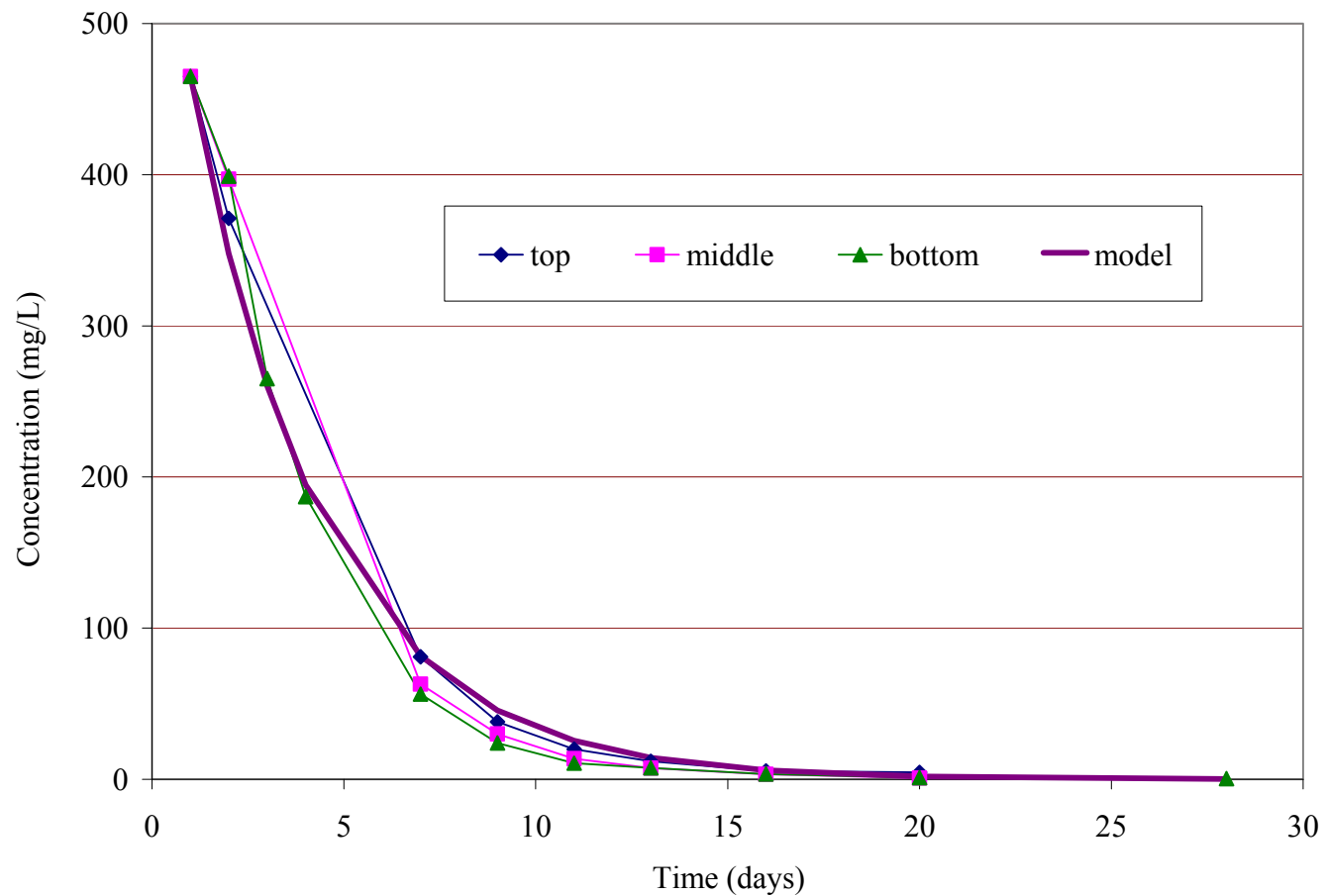
Figure 4.11. Bromide concentrations in MW-8D (injection well); Injected concentration ~ 500 mg/L, 8/2/2004, day 0; groundwater velocity for dilution model is 0.12 m/day (0.4 ft/day); estimated time constant from model = 3.4 days; time constant from experimental data ~ 4 days.
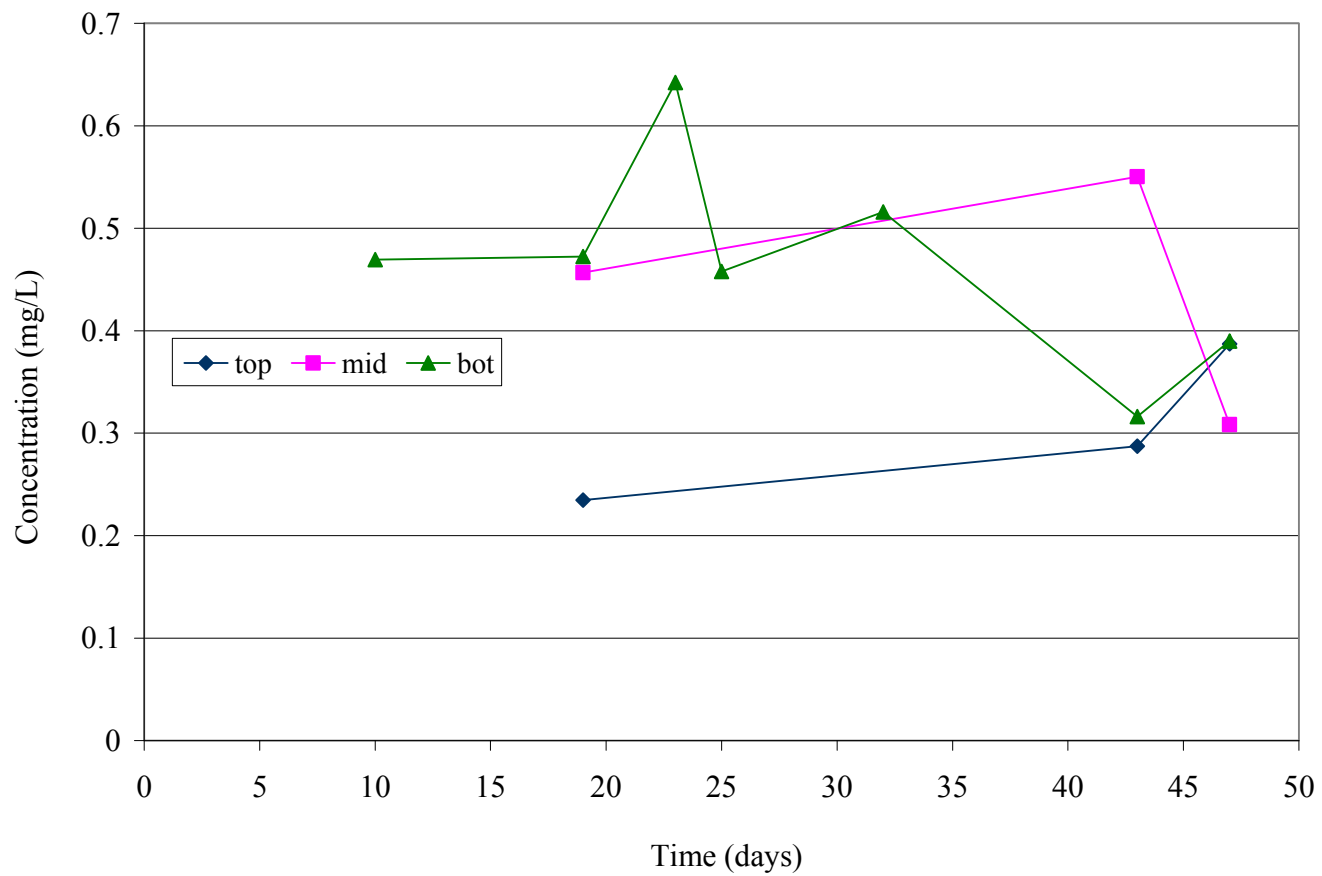
Figure 4.12. Bromide concentrations in MW-7D, located 6.1 m (20 ft) north of injection well 8D; 8/2/2004, day 0.
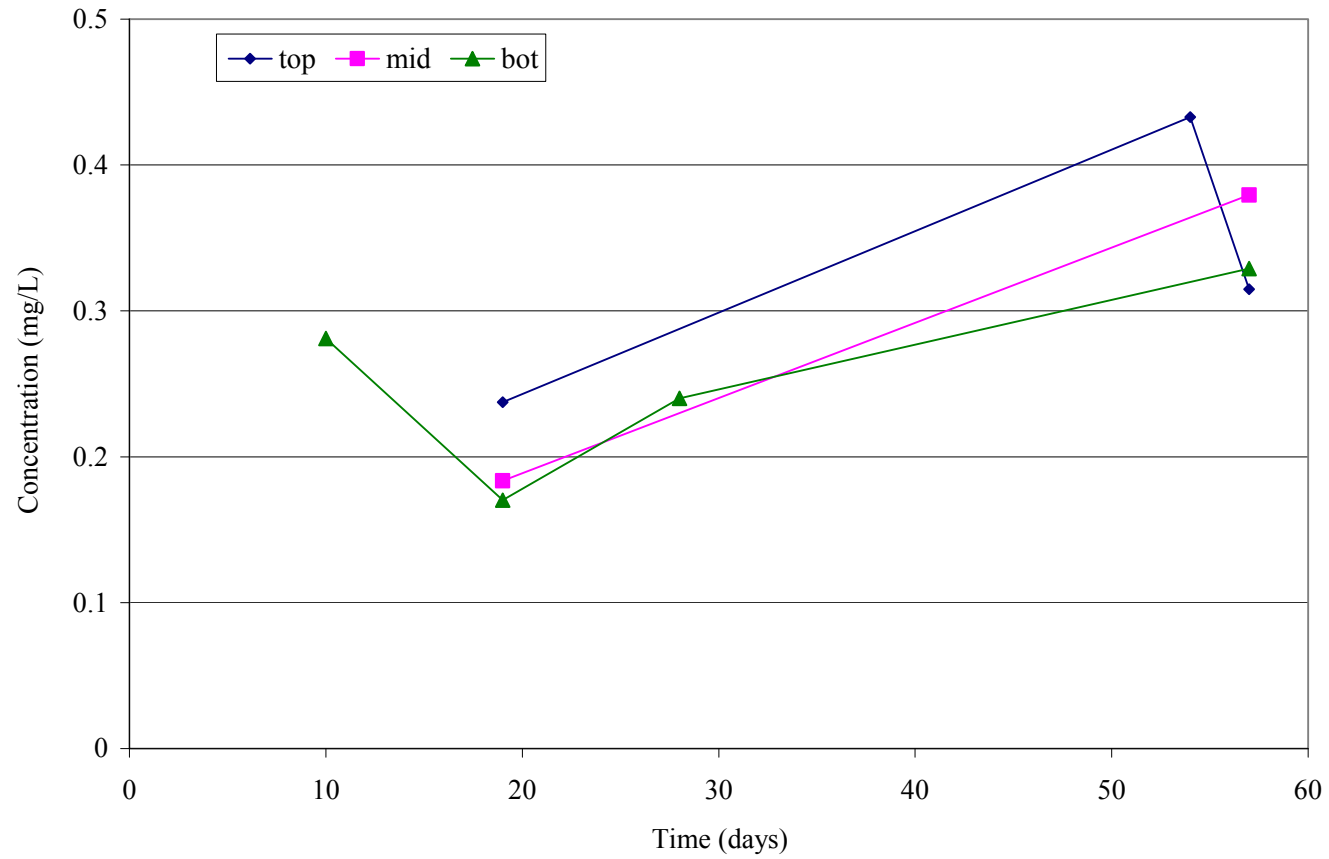
Figure 4.13. Bromide concentrations in MW-11D, which is located 7 m (23 ft) south of well 9D or 3 m (10 ft) east and 7 m (23 ft) south of injection well 8D.

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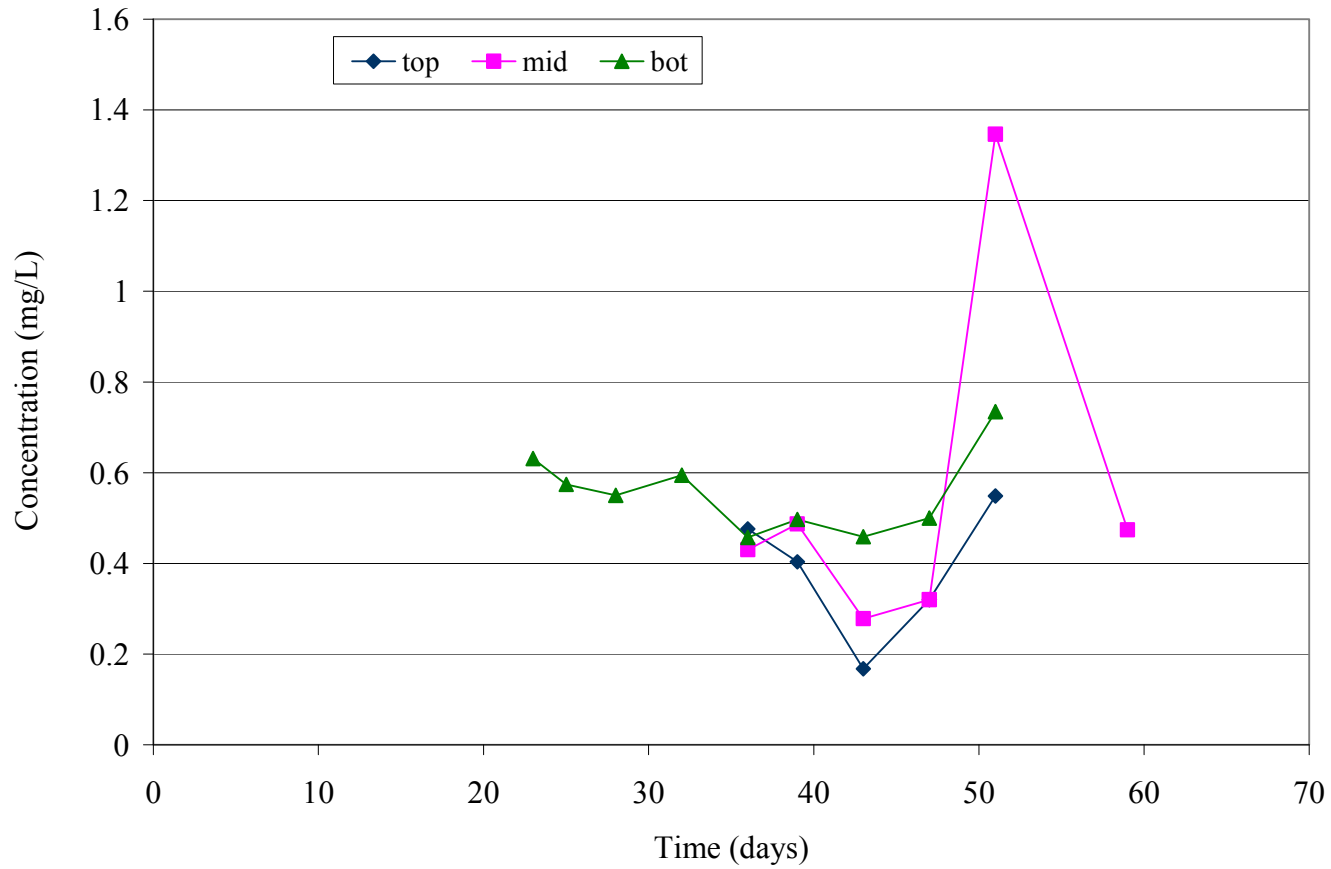
Figure 4.14. Bromide concentrations in MW-12D, located 24.4 m (80 ft) east from injection well 8D; injection concentration ~ 500 mg/L, 8/2/2004, day 0.
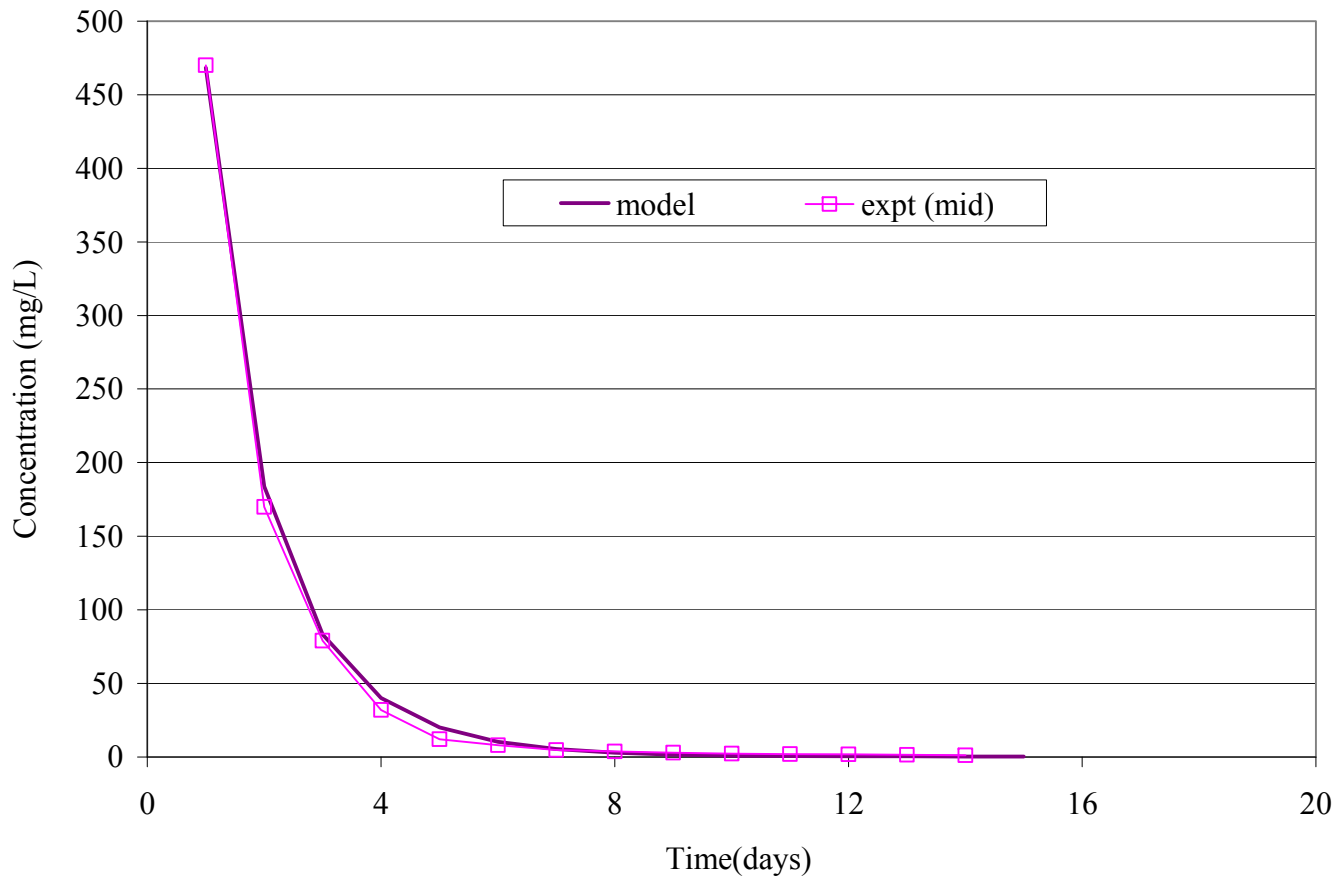
Figure 4.15. Bromide concentrations at mid-depth in MW-8S (injection well); injected concentration is ~ 500 mg/L, 9/16/2004, day 0; Model (Advection Dispersion Equation) parameters: D = 250 cm^2/d; v = 24.3 cm/d; ROI = 26 cm, A = 2122 cm^2.
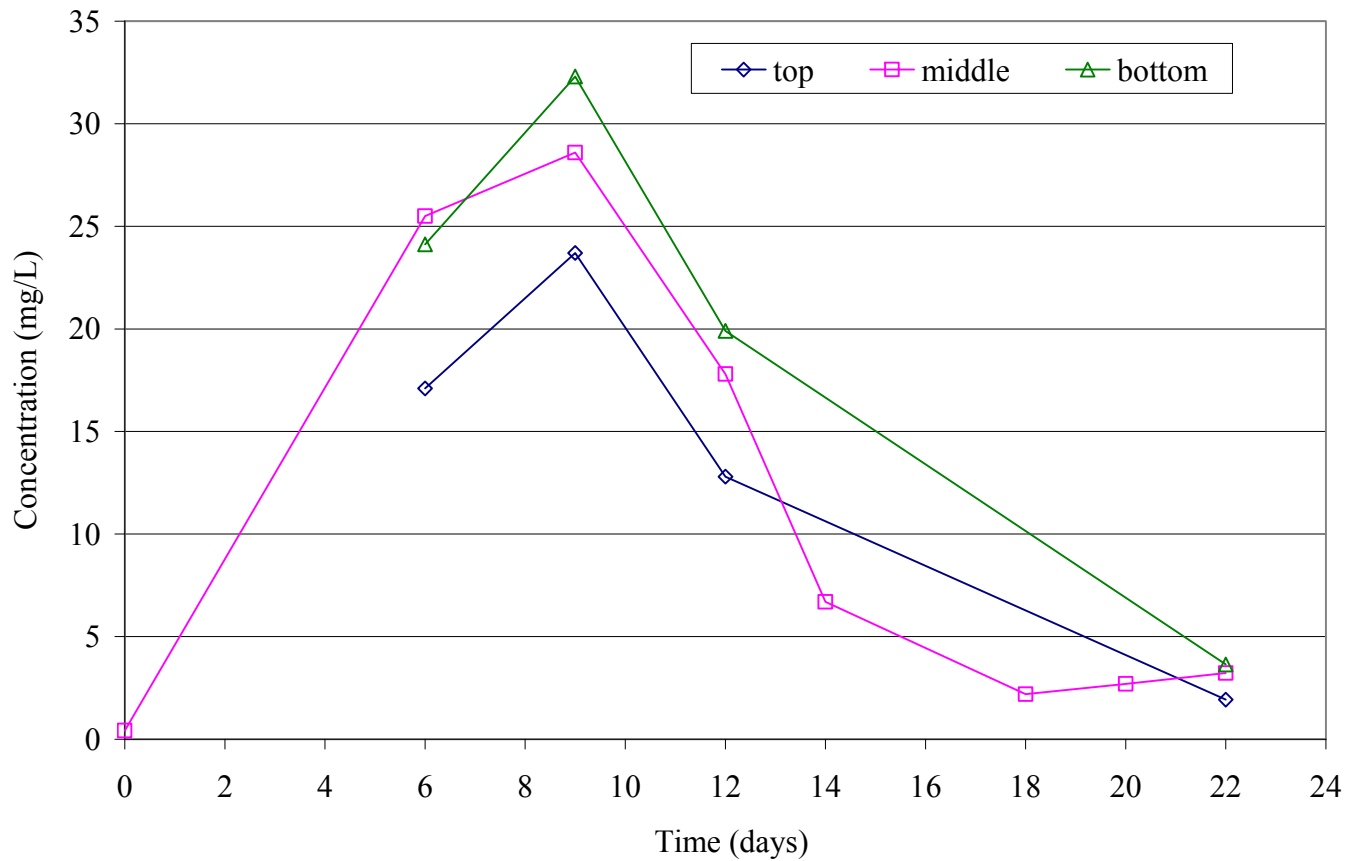
Figure 4.16. Bromide concentrations in MW-9S, ~ 10 feet east from injection well 8S; injection concentration is ~ 500 mg/L, 9/16/2004, day 0. Background concentration was 0.4 mg/L.
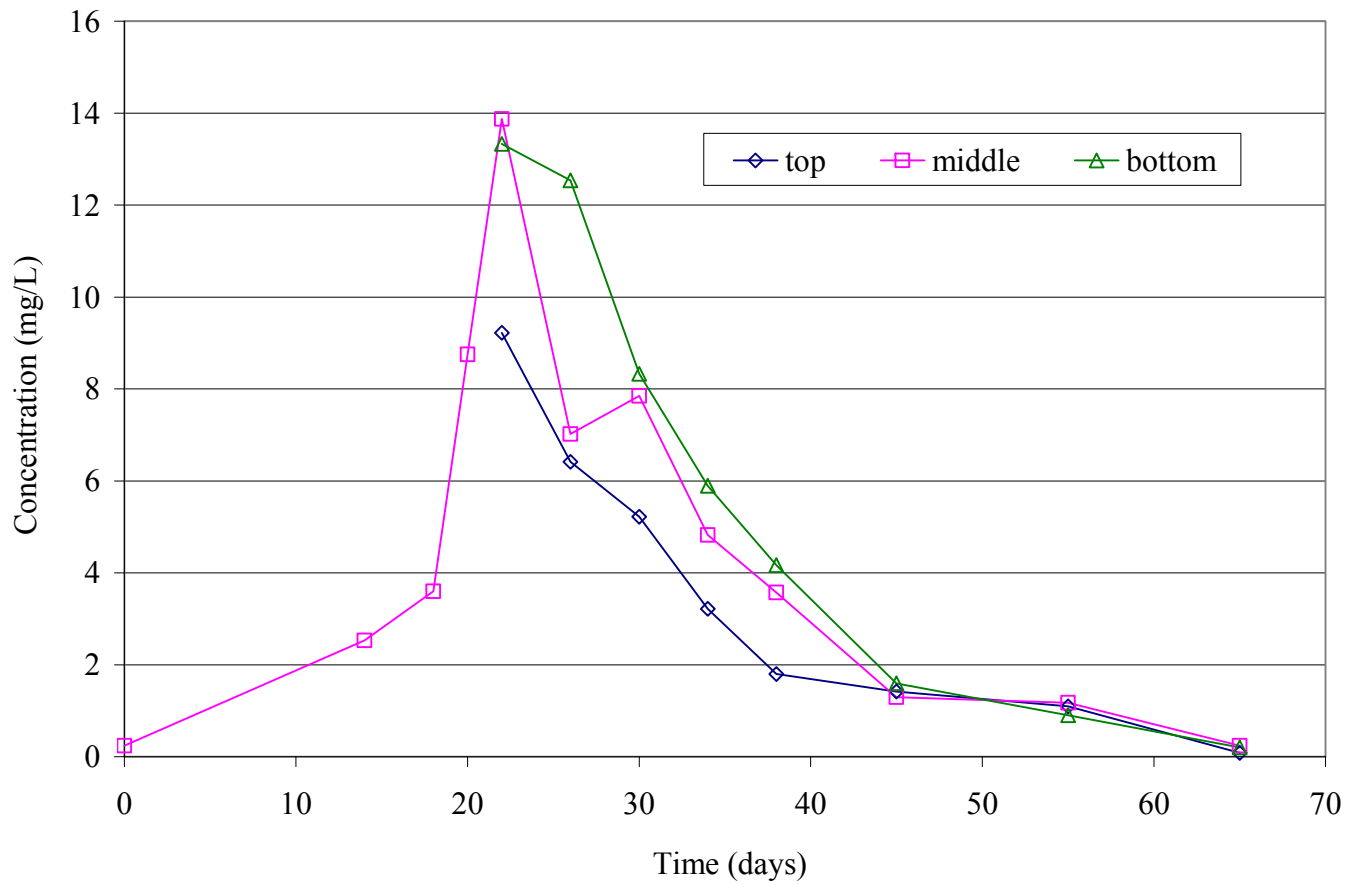
Figure 4.17. Bromide concentrations in MW-10S, 6.1 m (20 ft) east from injection well MW-8S; injection concentration is ~ 500 mg/L, 9/15/2004, day 0.
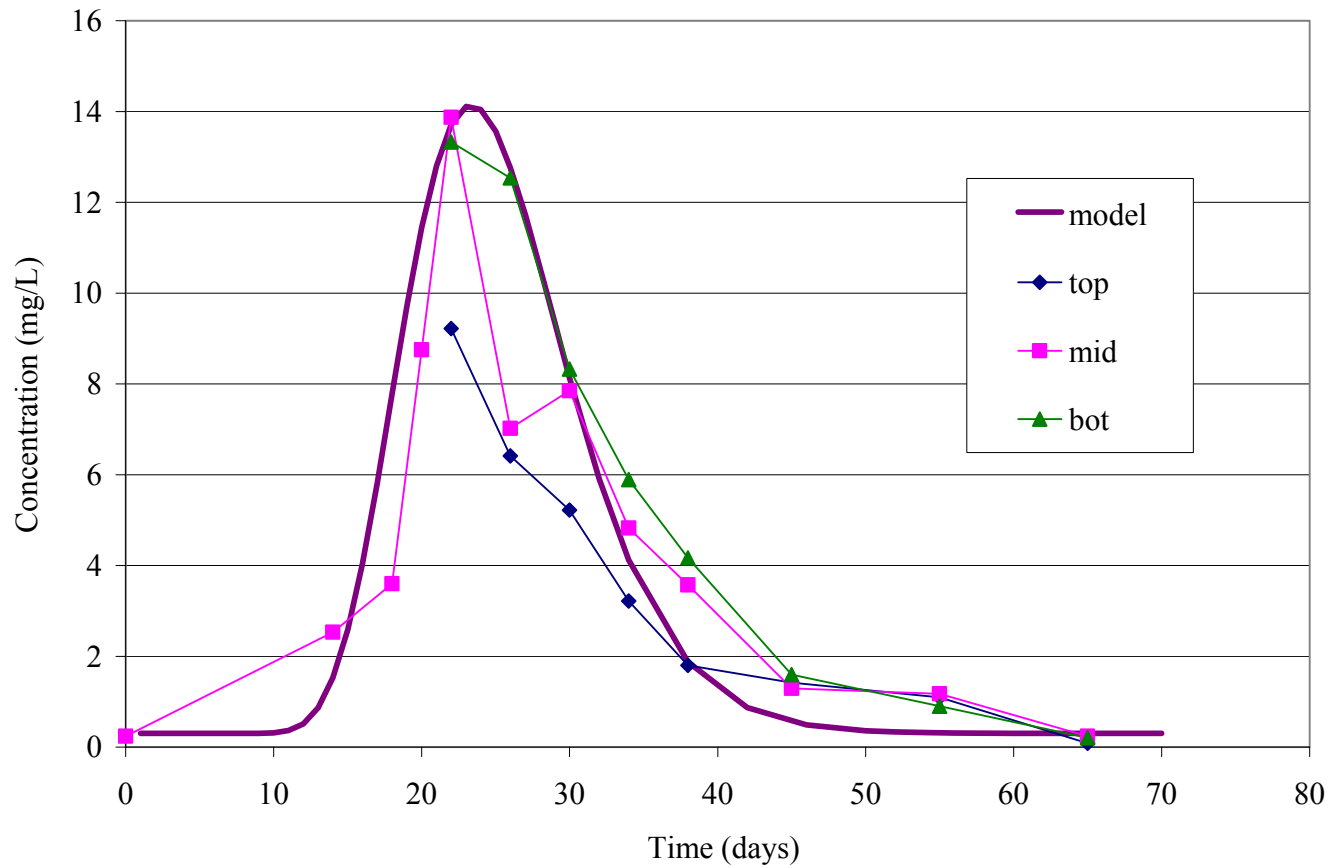
Figure 4.18. Breakthrough curve for well 10S fitted with ADE model, Fall 2004 study; x = 584 cm; D = 394 cm^2/d; v = 24.3 cm/d; Fitted ROI = 82 cm (initial estimated ROI = 26 cm), A = 21226 cm^2 (10 * initial estimated area).
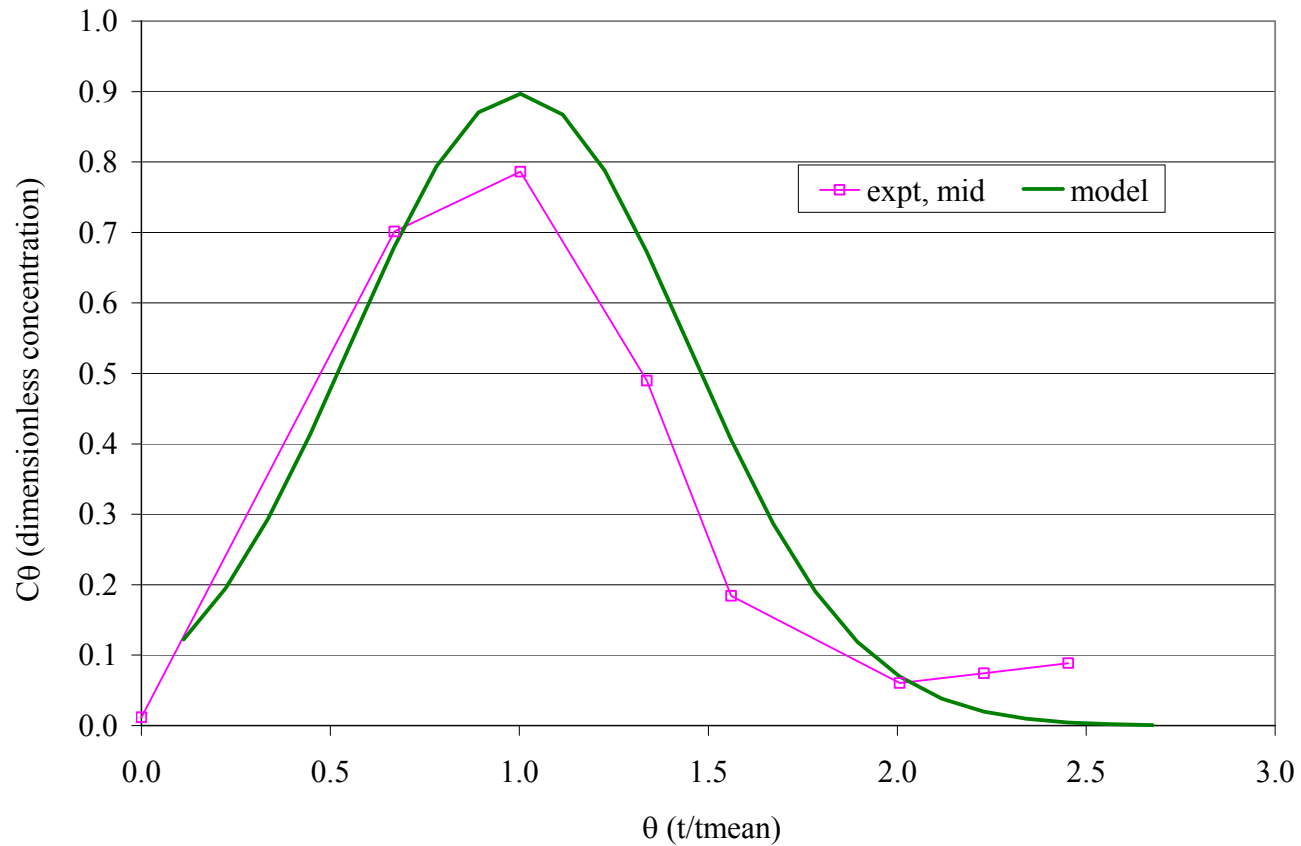
Figure 4.19. Comparison of residence time distribution (RTD) model and experimental results (mid-depth data), for well 9S, tracer study, Fall 2004. $t_{mean} = \tau = 9$ **days; Velocity = 31.3 cm/d; Dispersivity = 875 cm^2/d; Distance from injection well = 282 cm; D/uL = 0.1.**
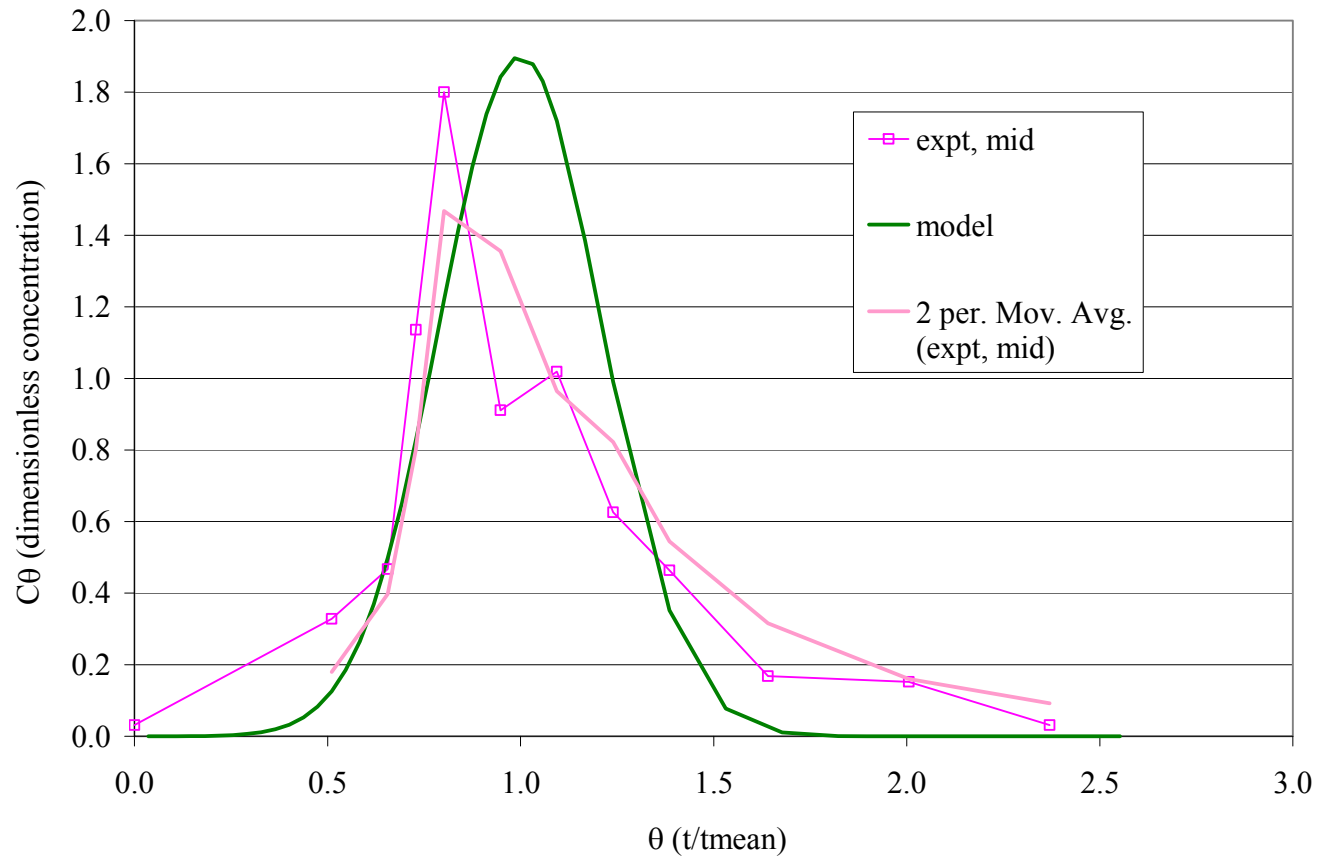
Figure 4.20. Comparison of residence time distribution (RTD) model and experimental results (mid-depth data with two-point moving average curve), for well 10S, tracer study, Fall 2004. $t_{mean} = \tau = 27.4$ days; Velocity = 26.6 cm/d; Dispersivity = 342 cm^2/d; Distance = 584 cm; D/uL = 0.02.
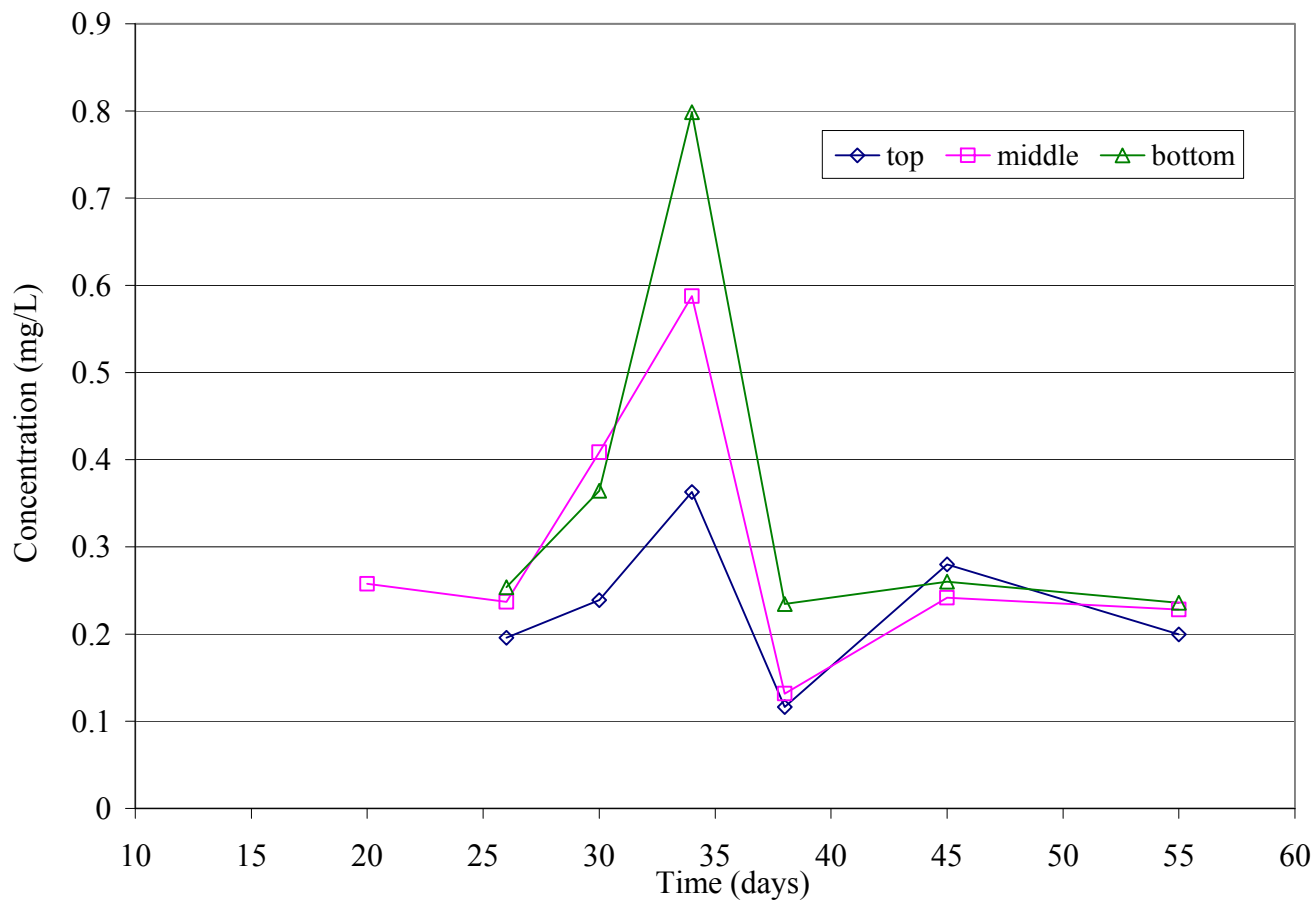
Figure 4.21. Bromide concentration in MW-12S; ~ 24.4 m (80 ft) east from injection well 8S; injection concentration is ~ 500 mg/L, 9/16/2004, day 0.
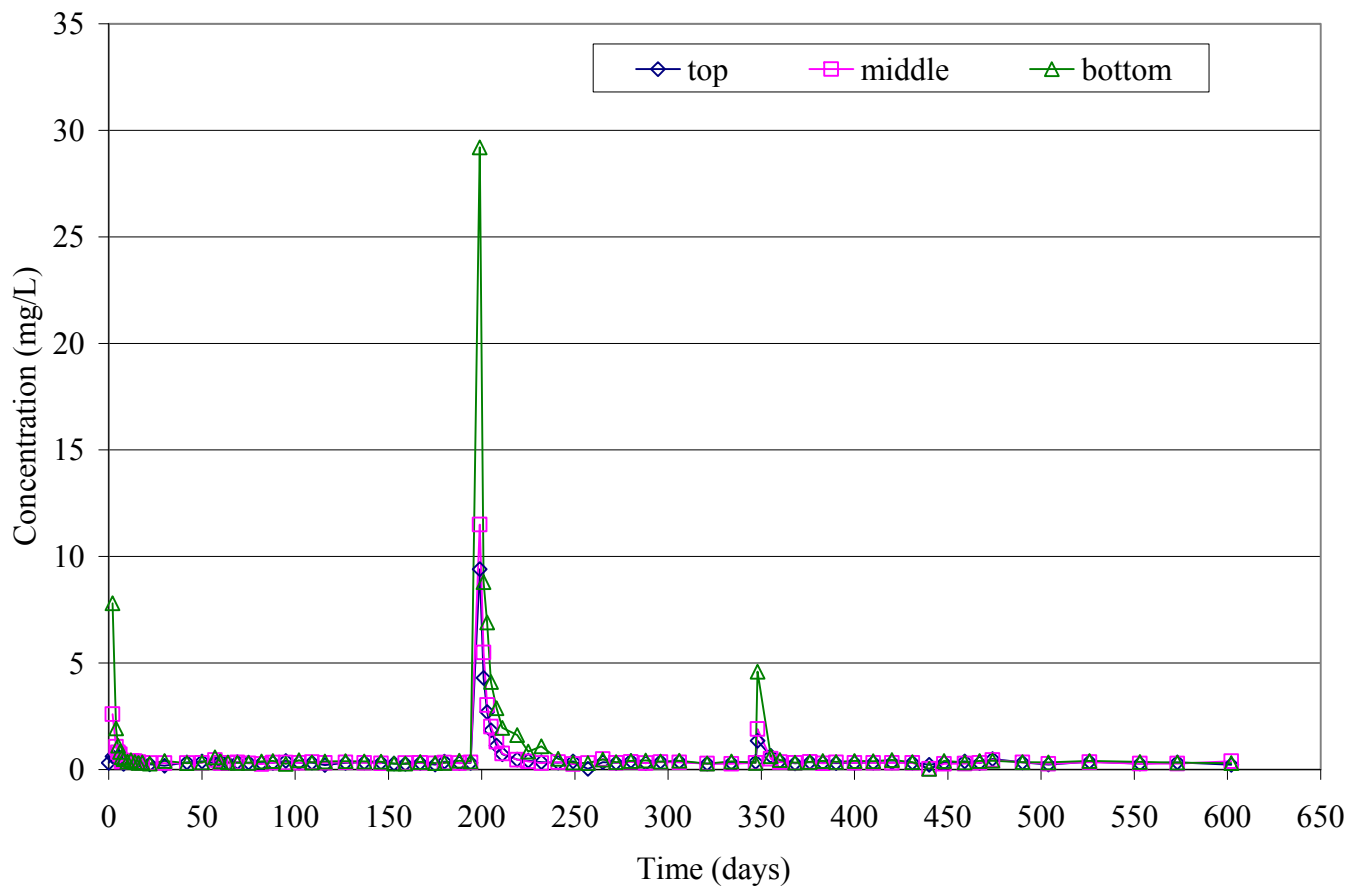
Figure 4.22. Bromide concentrations in MW-8S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005) (Injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006) (Injected bromide concentration = 1340 mg/L); and injection of nutrients on day 348 (Aug 1, 2006).
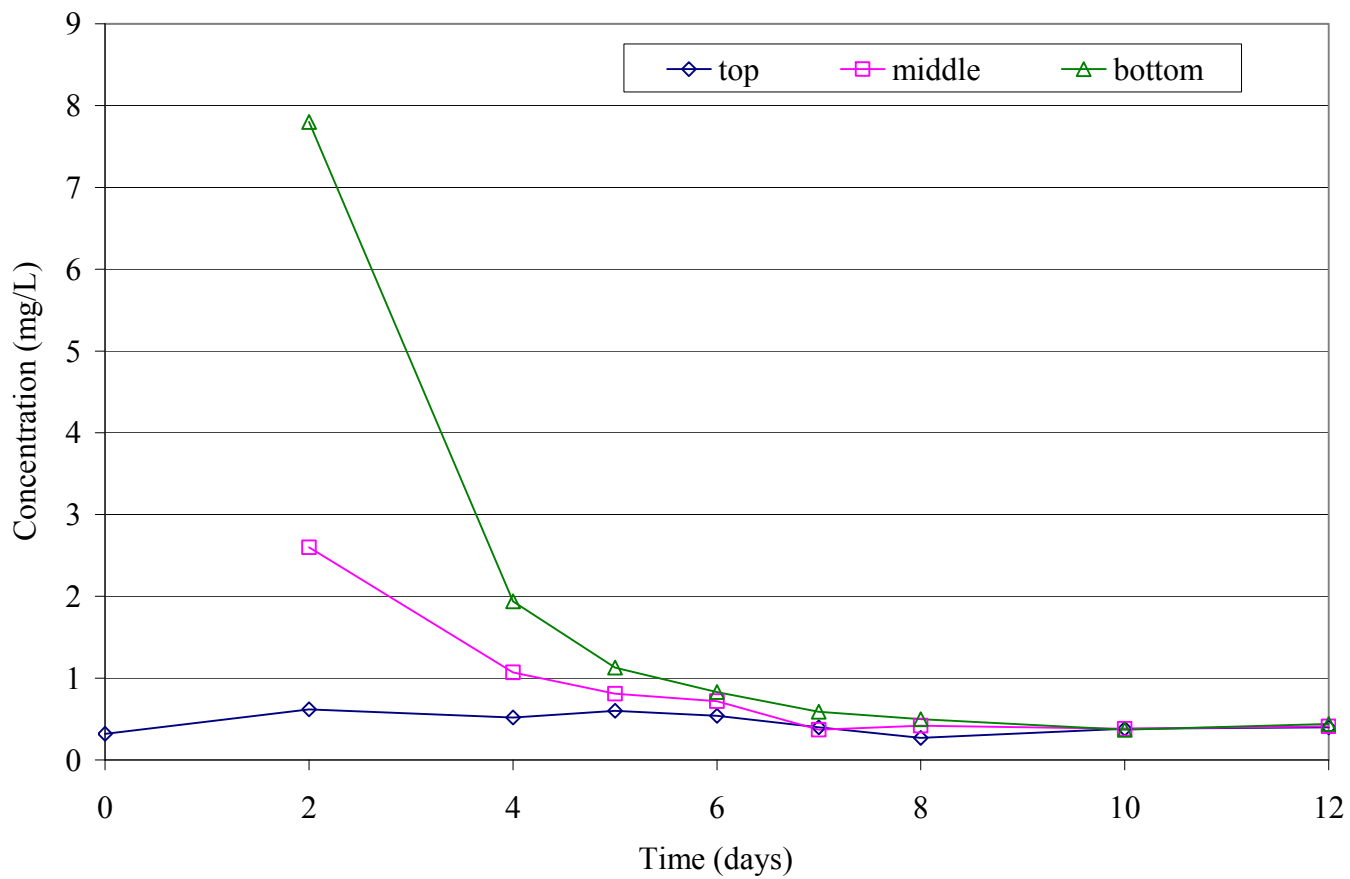
Figure 4.22a. Bromide concentrations in MW-8S; Injection of nutrients between MW-8 and MW-9 on day 0 (August 18, 2005) (Injected bromide concentration = 670 mg/L).
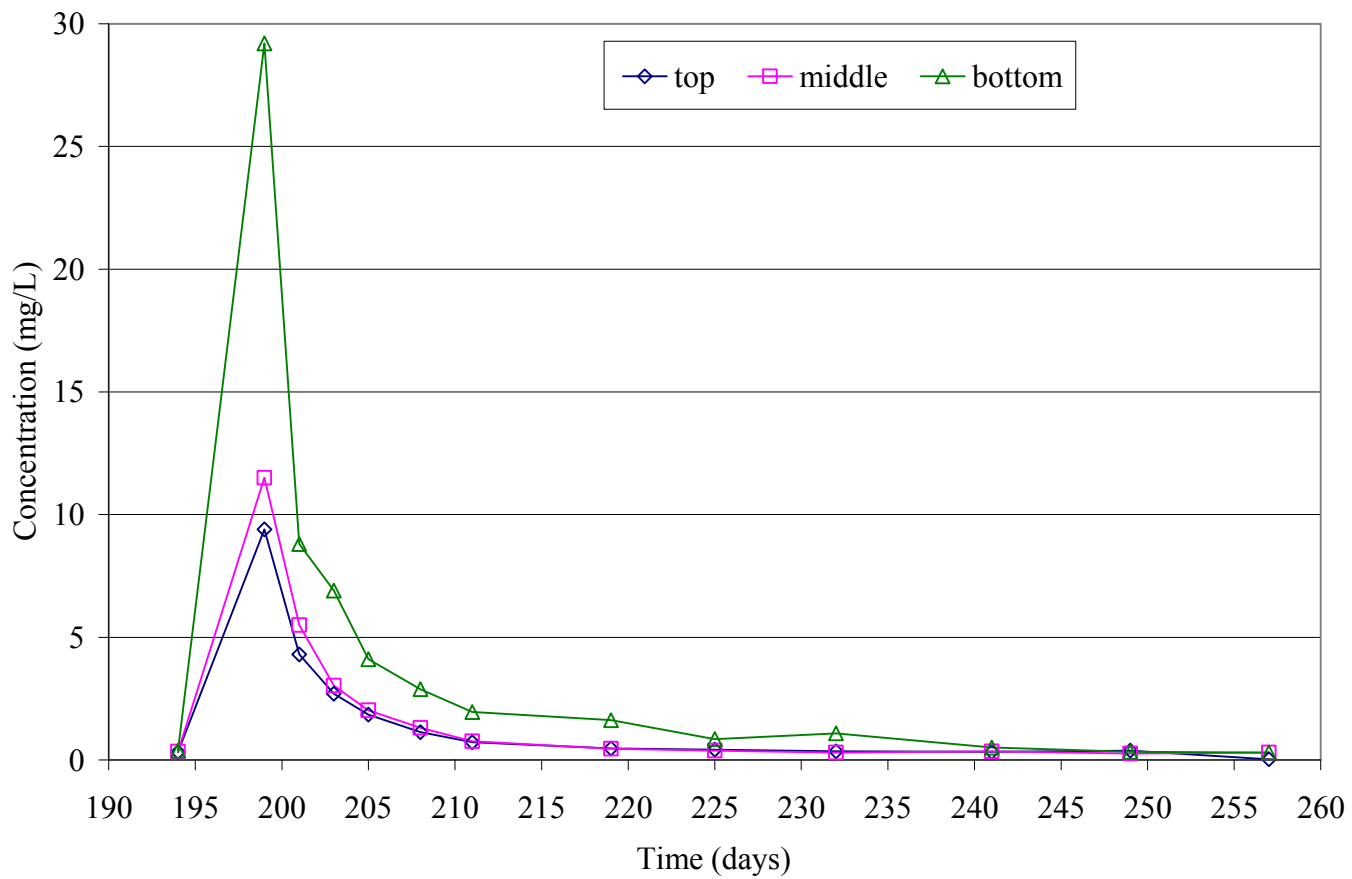
Figure 4.22b. Bromide concentrations in MW-8S; Injection of nutrients between MW-8 and MW-9 on day 197 (Mar 3, 2006) (Injected bromide concentration = 1340 mg/L).
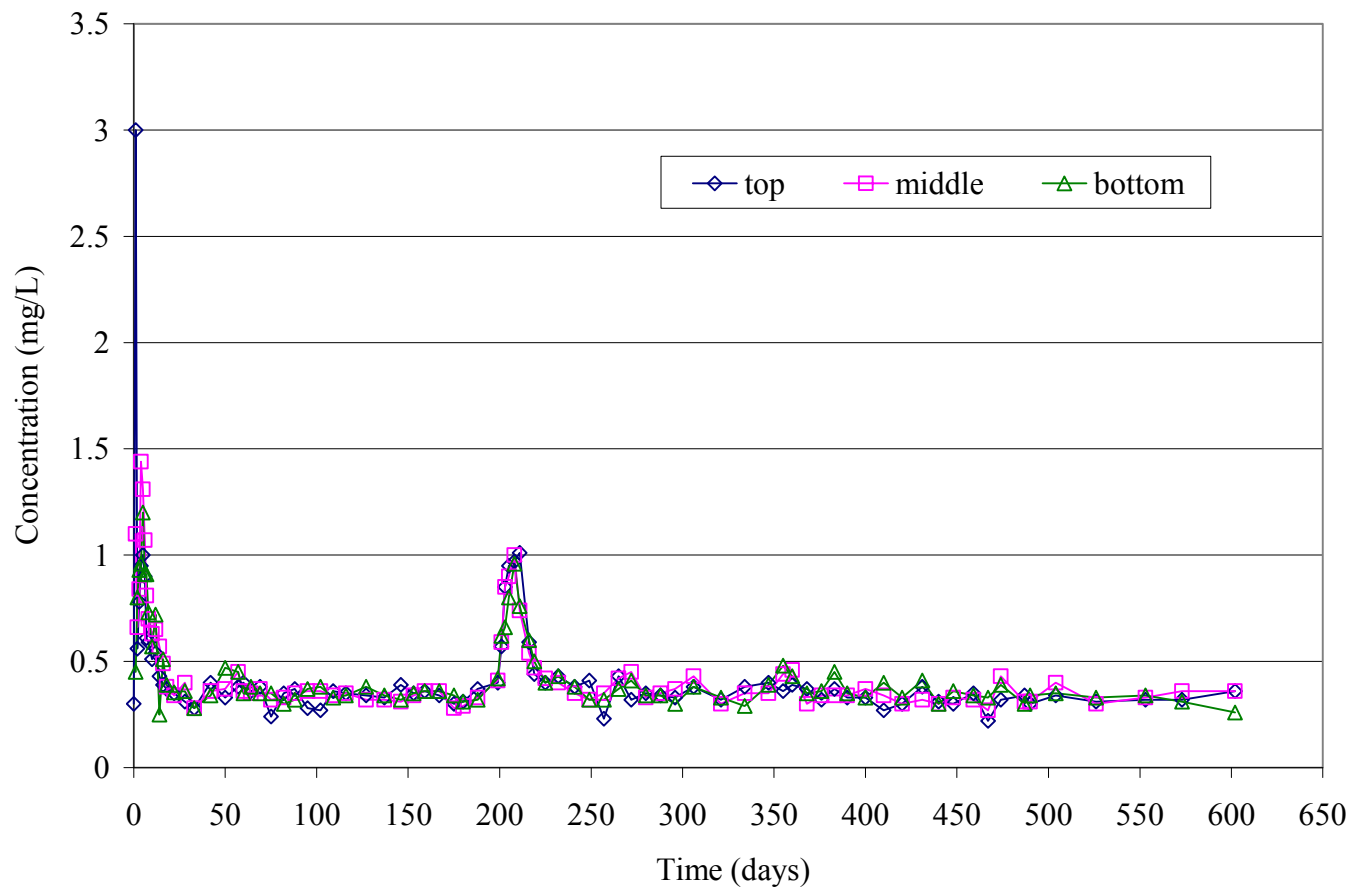
Figure 4.23. Bromide concentrations in MW-9S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005) (Injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006) (Injected bromide concentration = 1340 mg/L); and injection of nutrients on day 348 (Aug 1, 2006).
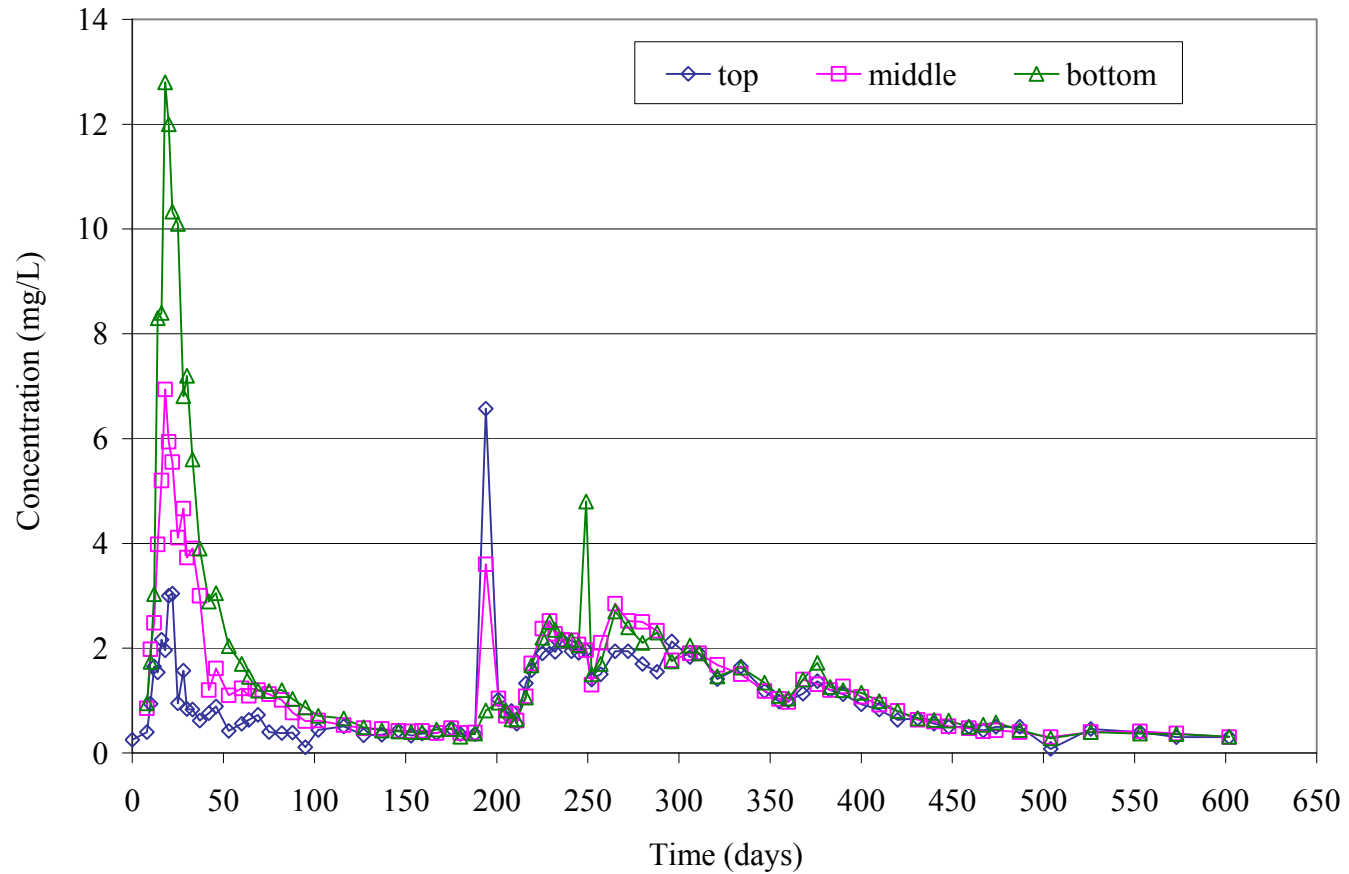
Figure 4.24. Bromide concentrations in MW-10S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005) (Injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006) (Injected bromide concentration = 1340 mg/L); and injection of nutrients on day 348 (Aug 1, 2006).
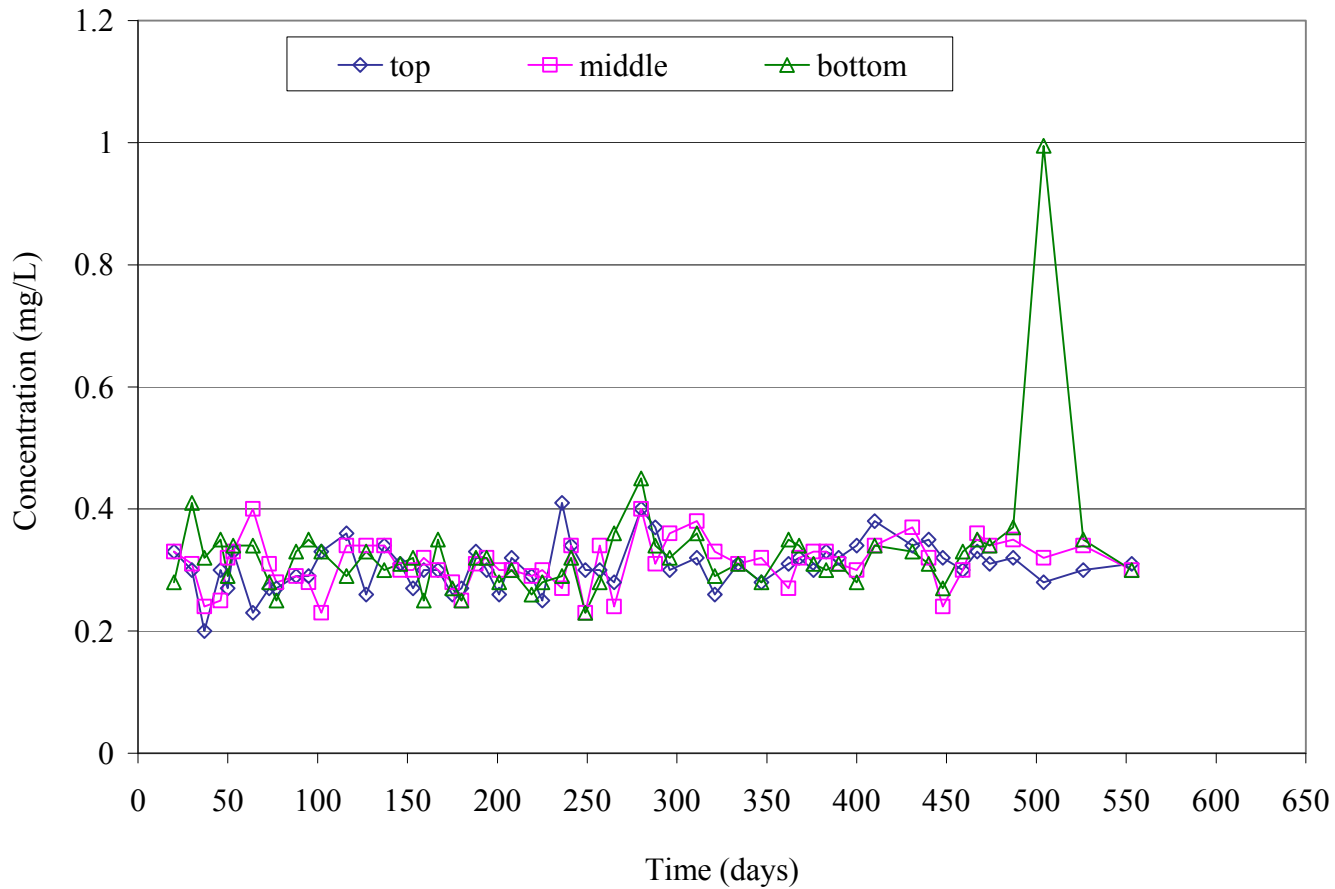
Figure 4.25. Bromide concentrations in MW-12S (75 ft from injection location); Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005) (Injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006) (Injected bromide concentration = 1340 mg/L); and injection of nutrients on day 348 (Aug 1, 2006).
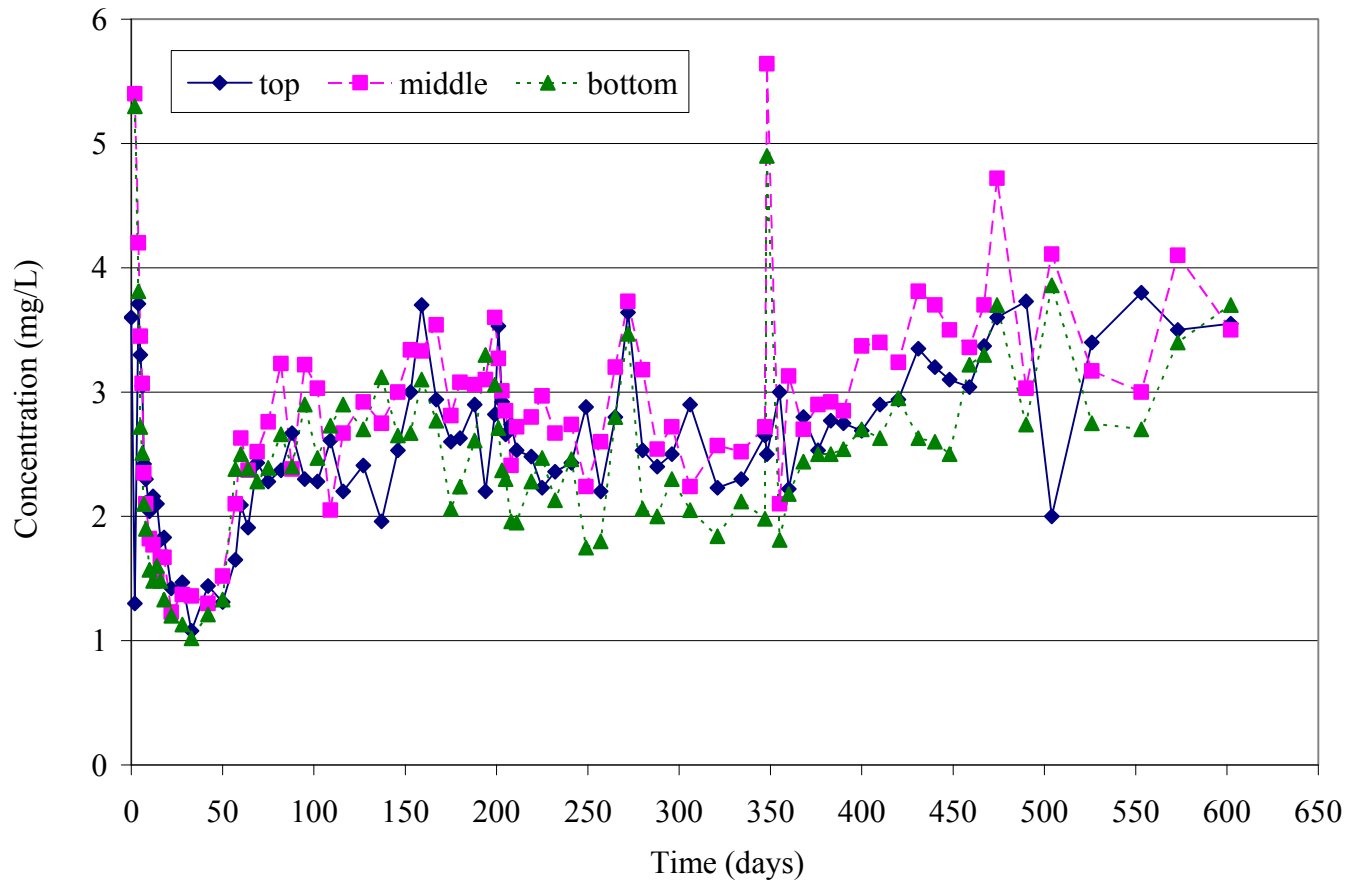
Figure 4.26. Bromide concentrations in MW-8D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005) (Injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006) (Injected bromide concentration = 1340 mg/L); and injection of nutrients on day 348 (Aug 1, 2006).
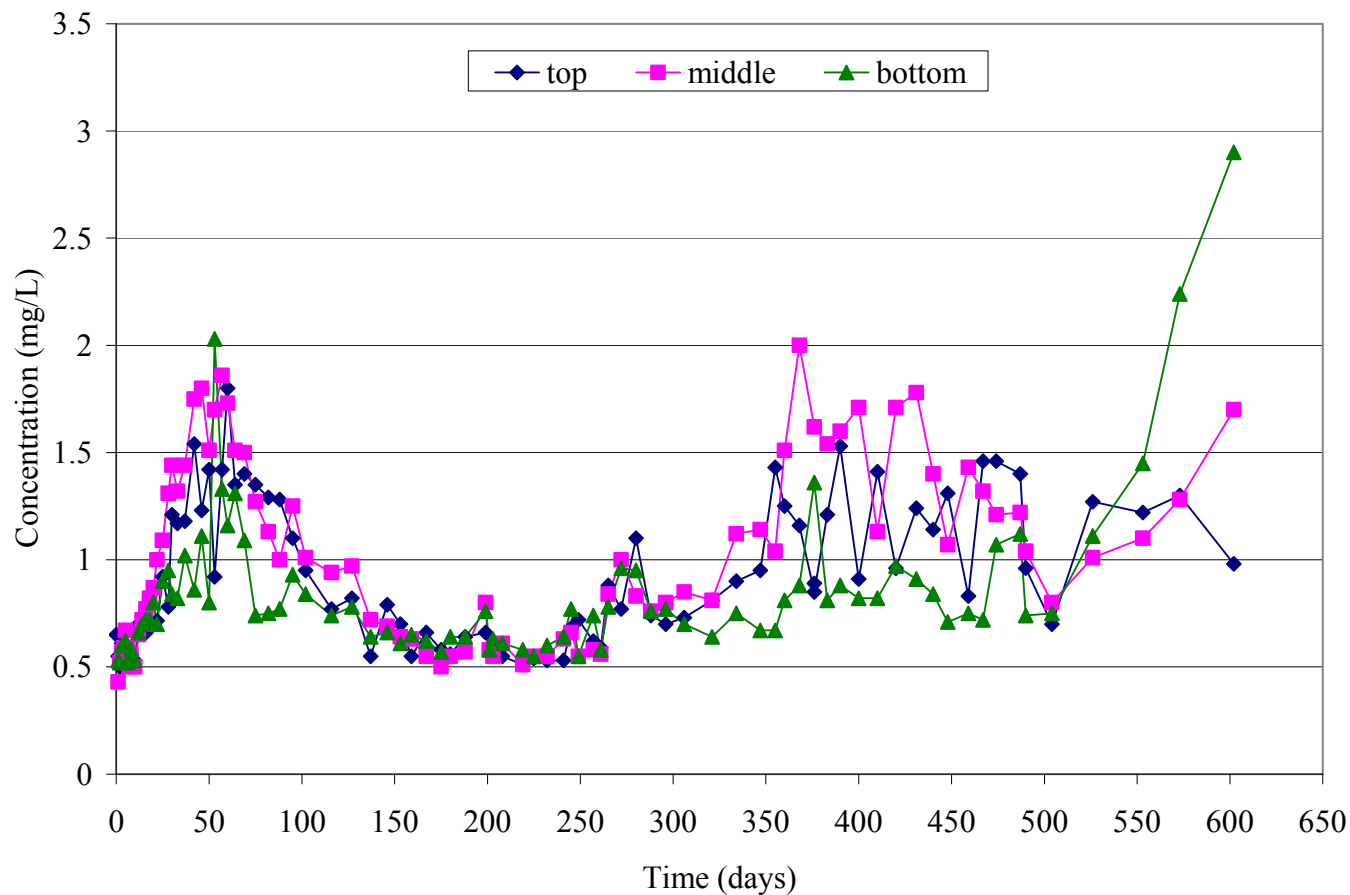
Figure 4.27. Bromide concentrations in MW-9D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005) (Injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006) (Injected bromide concentration = 1340 mg/L); and injection of nutrients on day 348 (Aug 1, 2006).
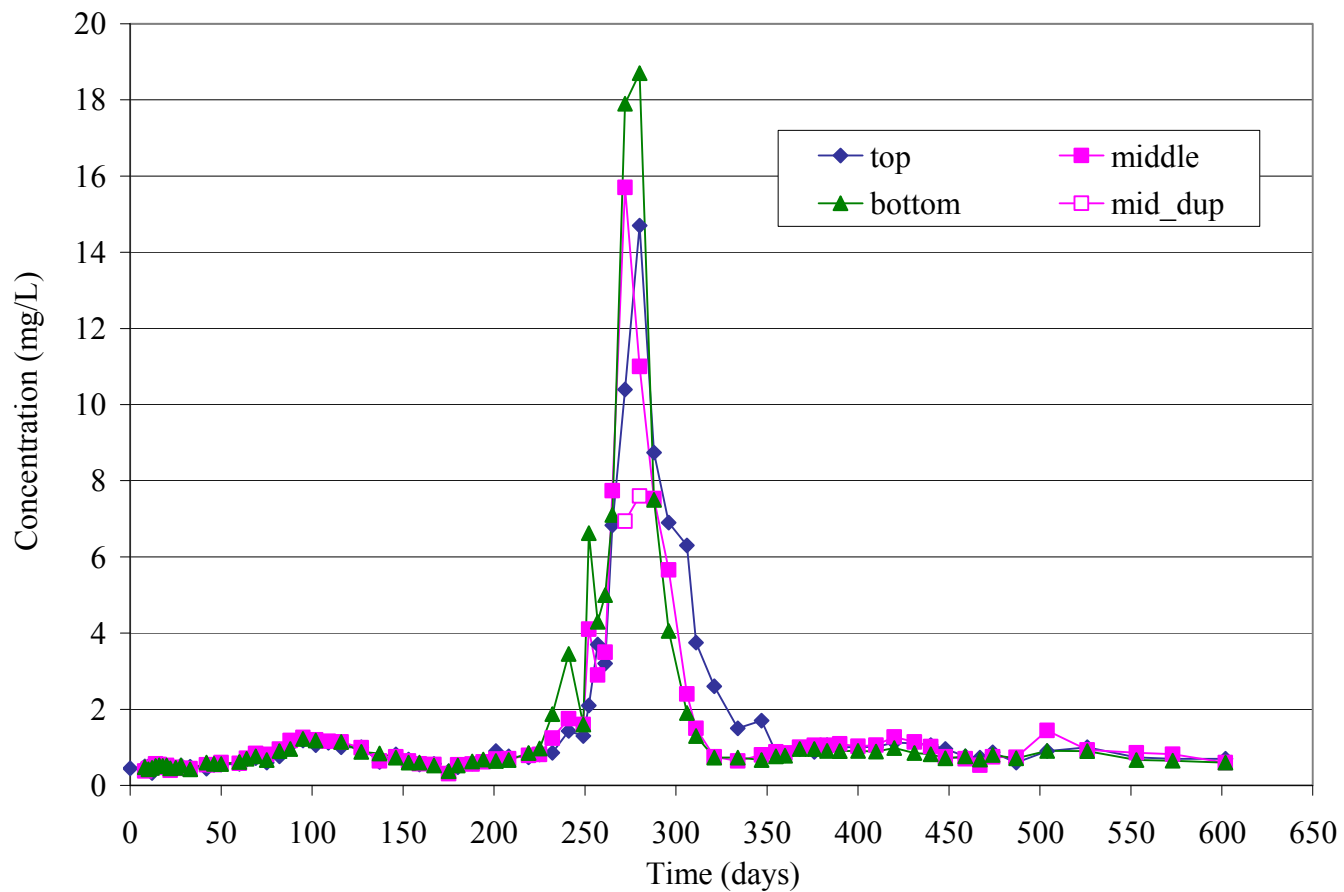
Figure 4.28. Bromide concentrations in MW-10D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005) (Injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006) (Injected bromide concentration = 1340 mg/L); and injection of nutrients on day 348 (Aug 1, 2006).
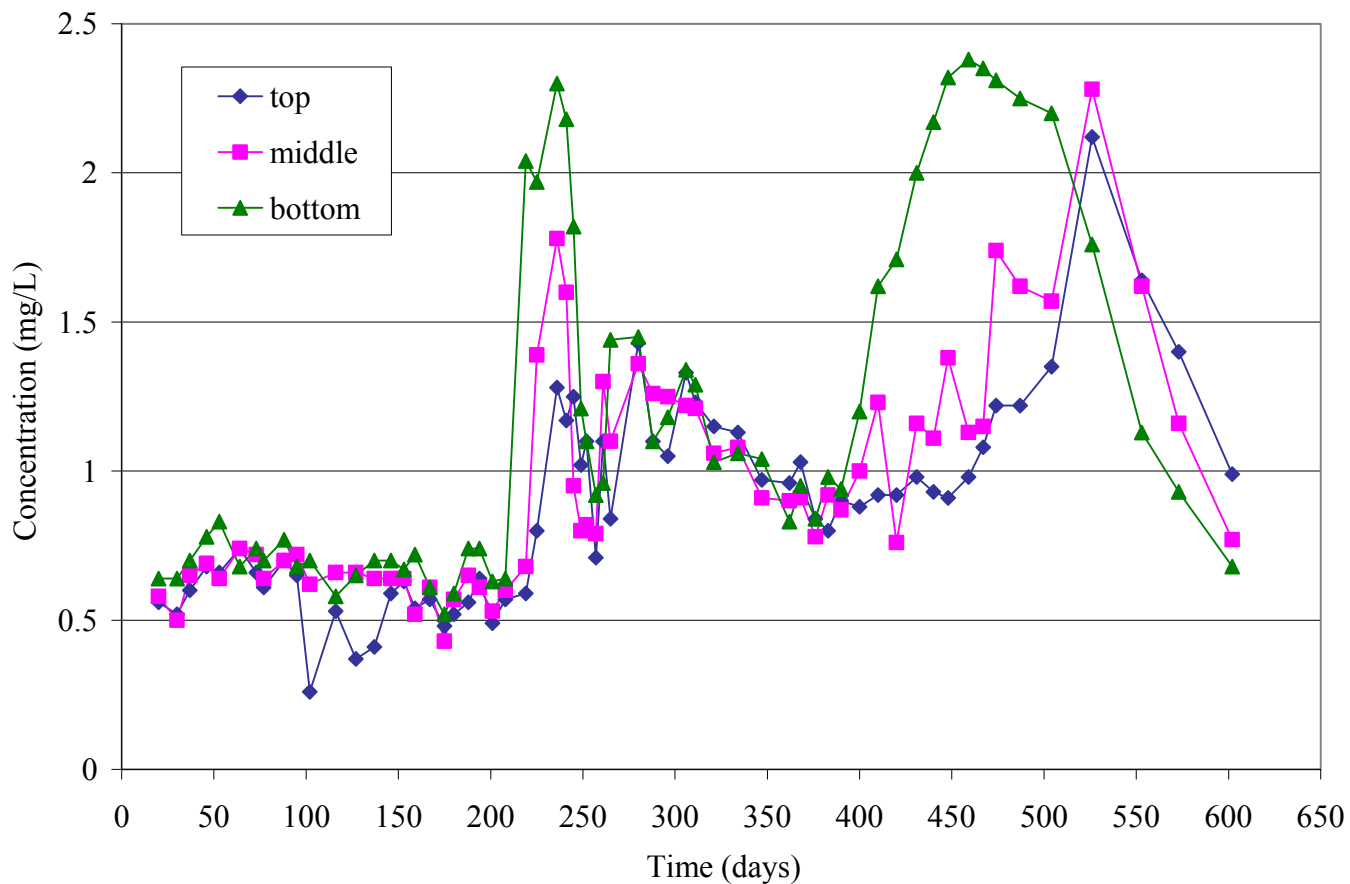
Figure 4.29. Bromide concentrations in MW-12D (75 ft from inj pt). Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005) (Injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006) (Injected bromide concentration = 1340 mg/L); and injection of nutrients on day 348 (Aug 1, 2006).
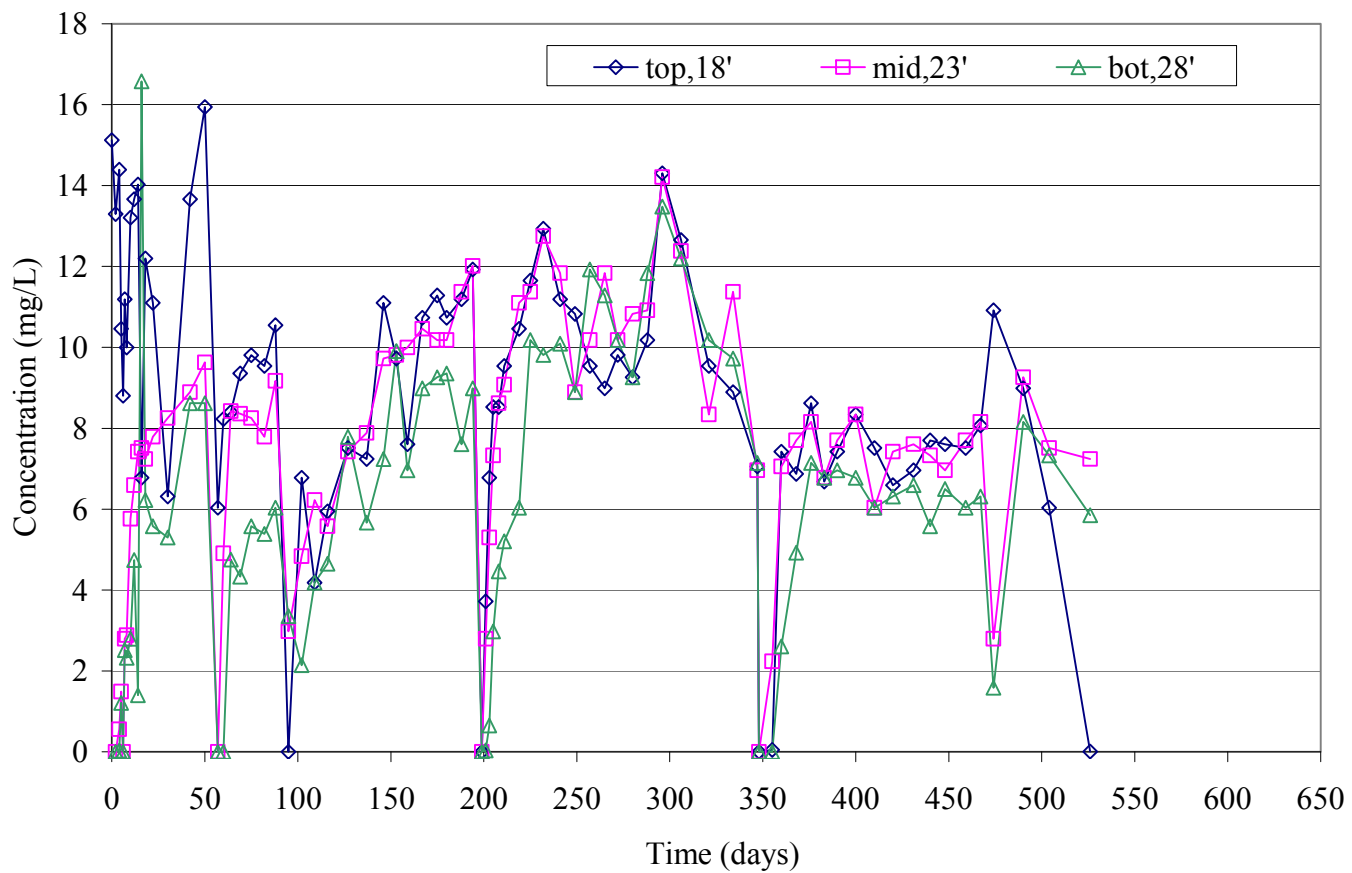
Figure 4.30. Nitrate concentrations in well MW-8S; Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005) , day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).
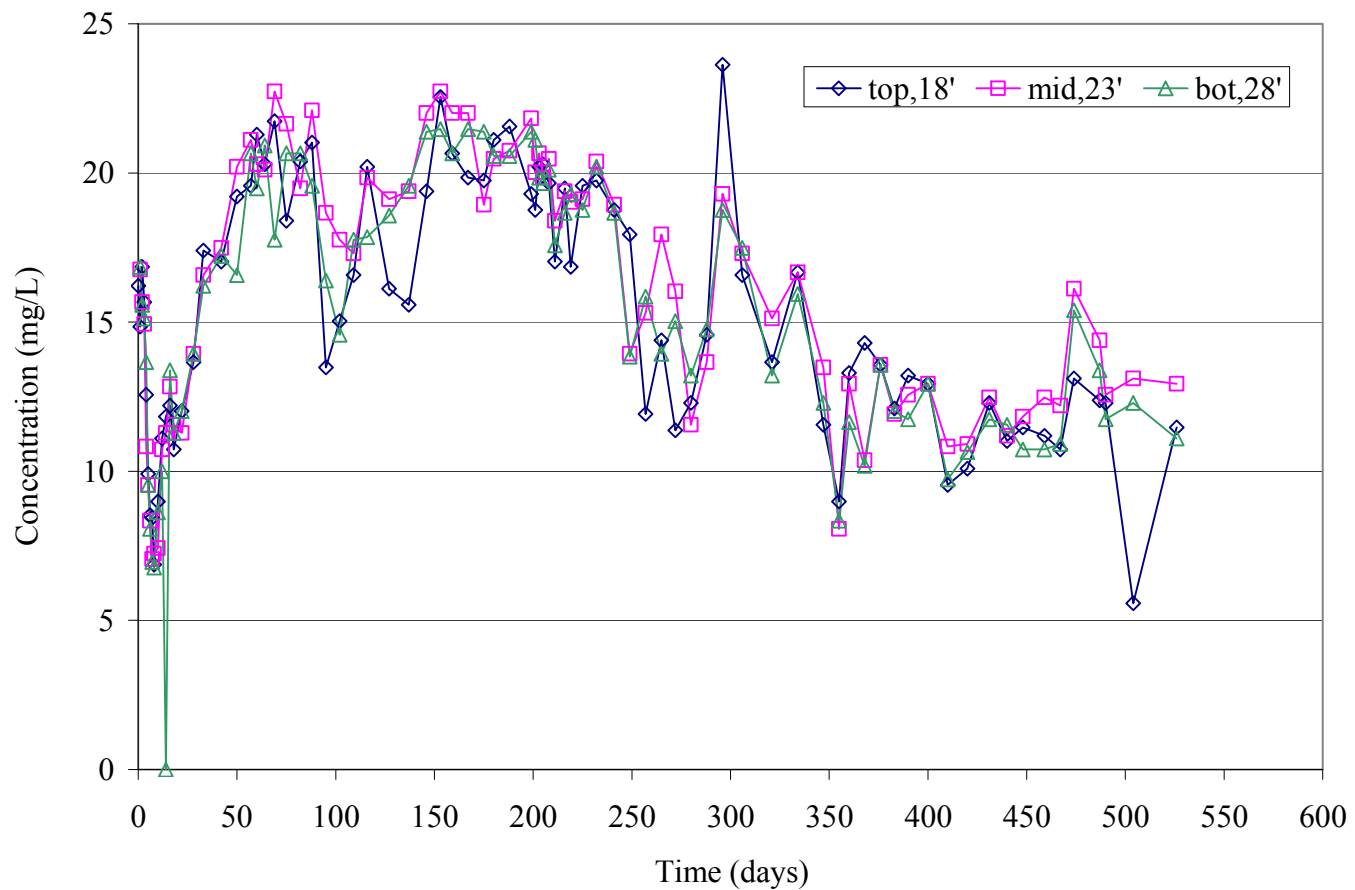
Figure 4.31. Nitrate concentrations in well MW-9S; Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005) , day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).
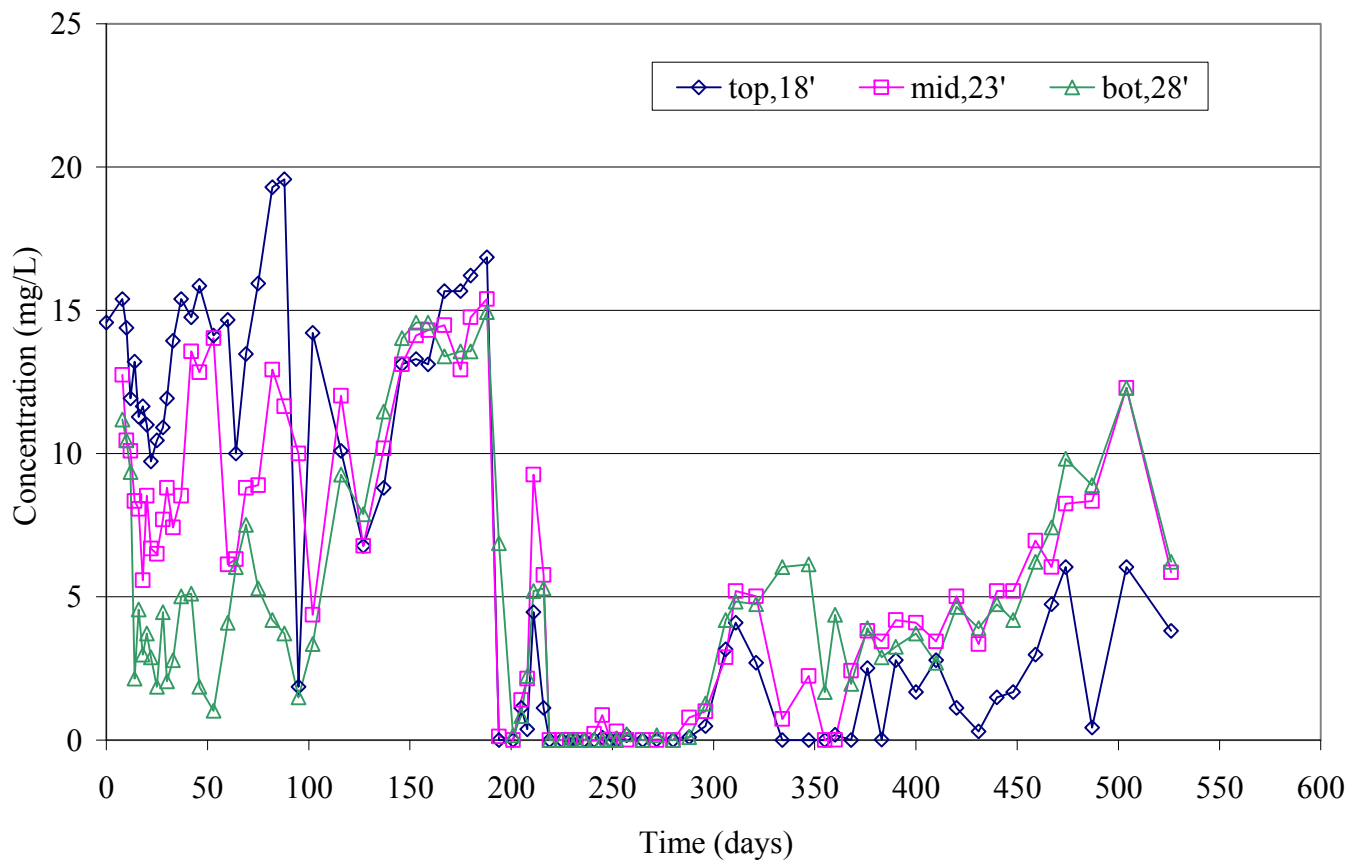
Figure 4.32. Nitrate concentrations in well MW-10S; Injection of nutrients between MW-8 and MW-9 on day 0 (August 18, 2005), day 56 (Oct 13, 2005) , day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).
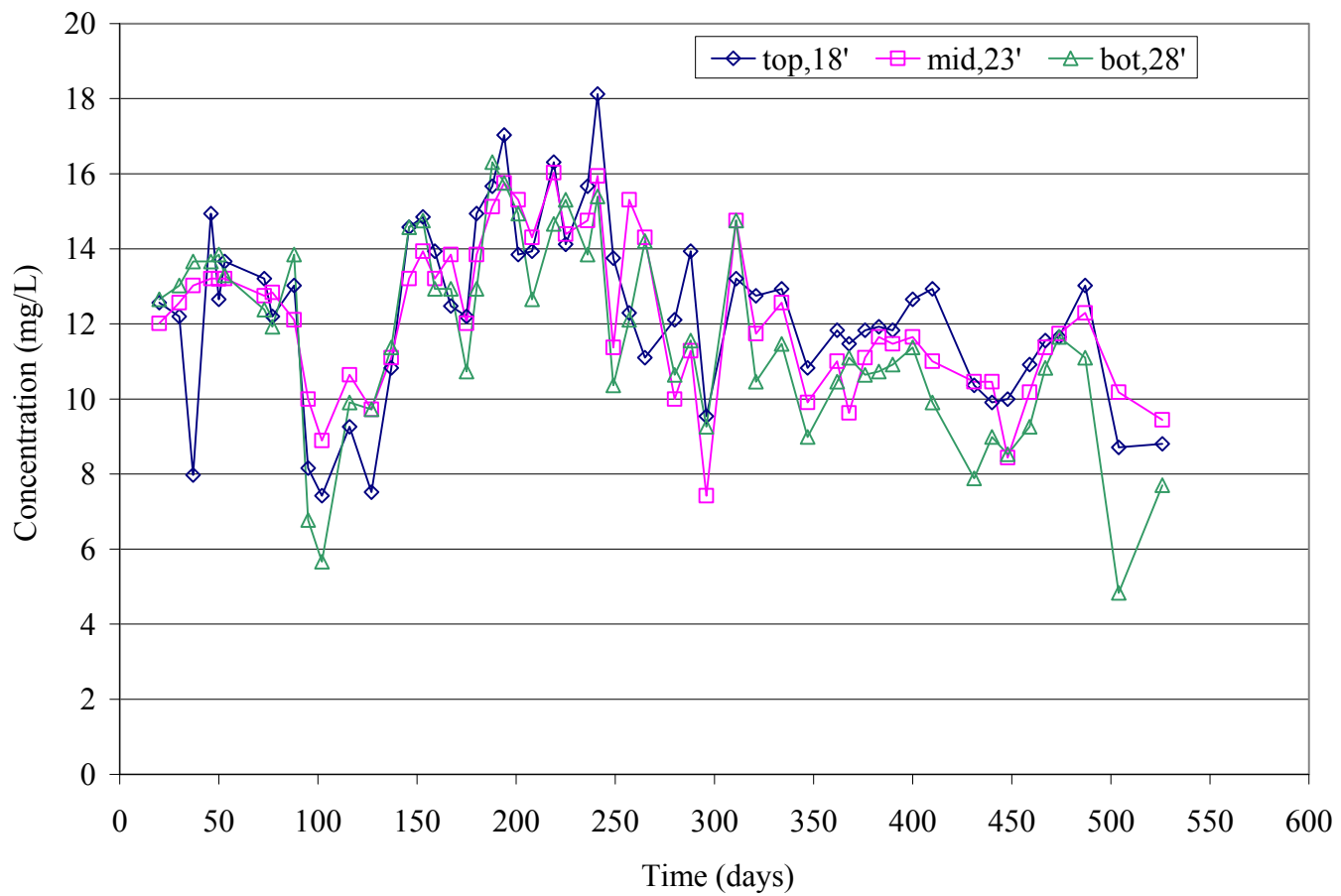
Figure 4.33. Nitrate concentrations in well MW-12S; Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005) , day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).
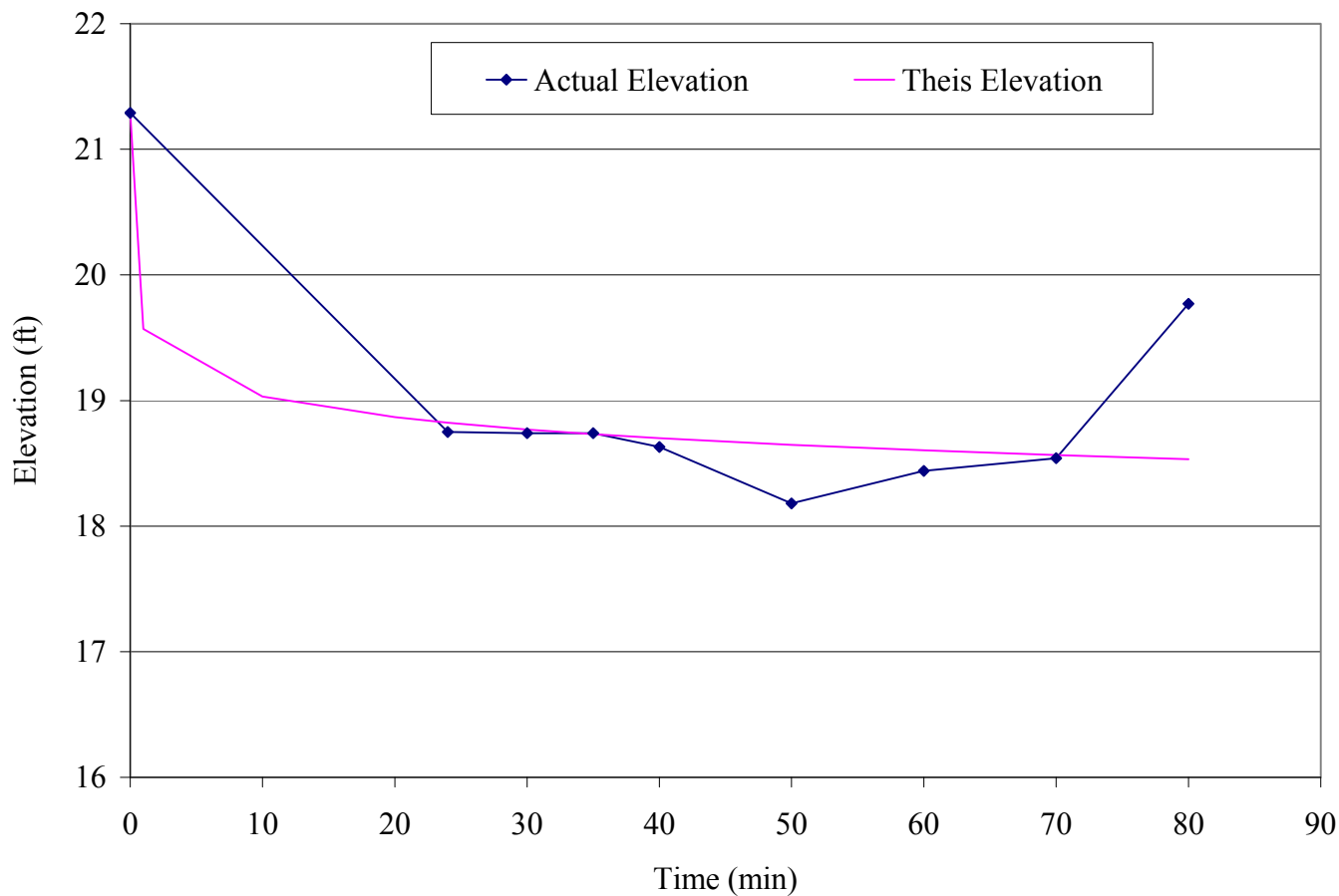
Figure 4.34. Theis' solution for well 10S, water was pumped out (at average flow rate of 5.6 L/min) continuously throughout the experiment (8/17/2005). Groundwater elevation started to increase after 50 minutes, likely due to overcoming resistances in the soil for groundwater flow caused by the suction force of pump, leading to higher permeability.

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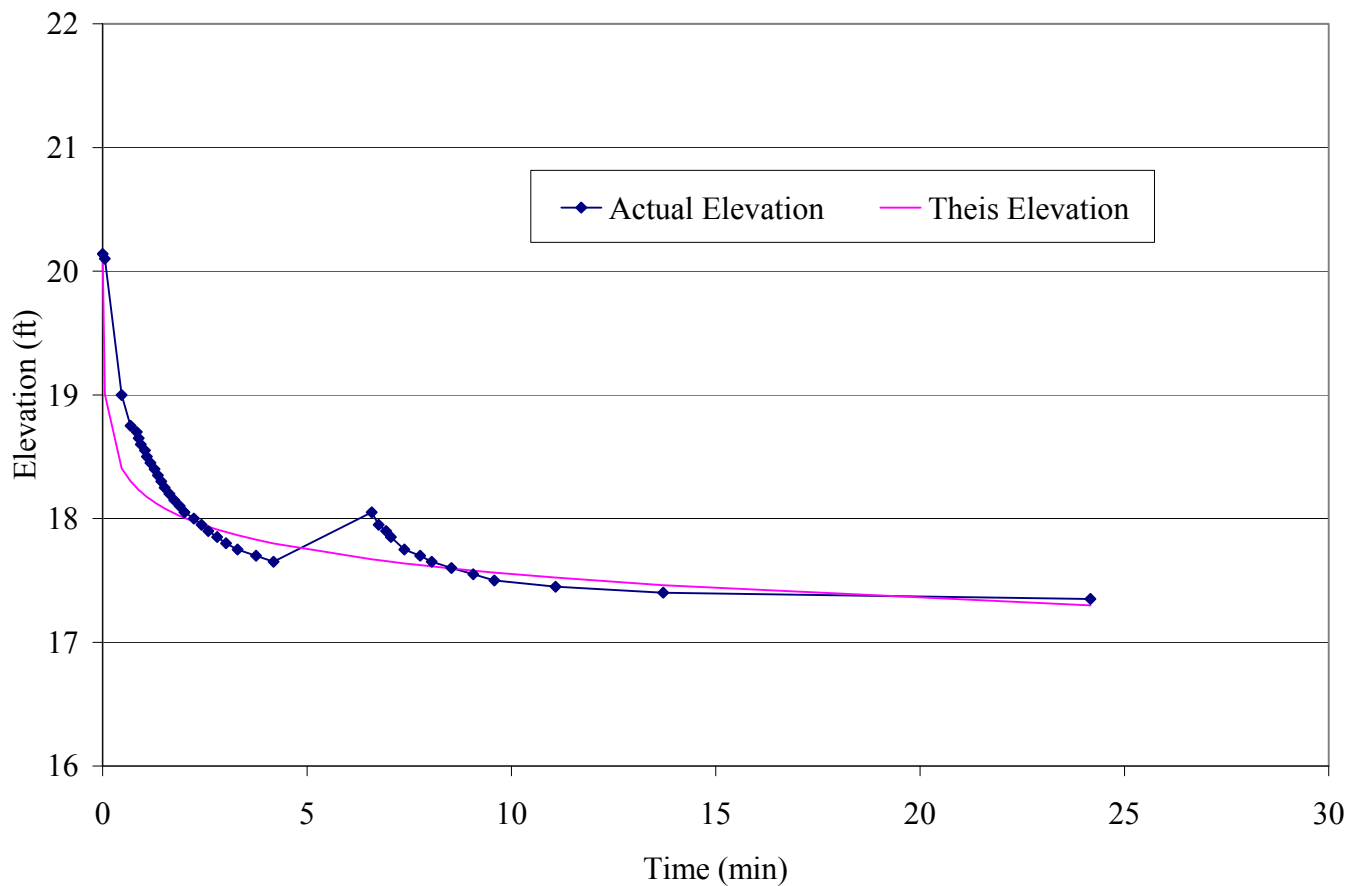
Figure 4.35. Theis' solution for well 10S; water was pumped out (at average flow rate of 4.3 L/min) continuously throughout the experiment (12/21/06). Groundwater elevation increased from 4.18 minutes to 6.58 minutes during which time elevation data was not collected. The increase may likely be due to overcoming resistances in the soil for groundwater flow caused by the suction force of pump, leading to higher permeability.
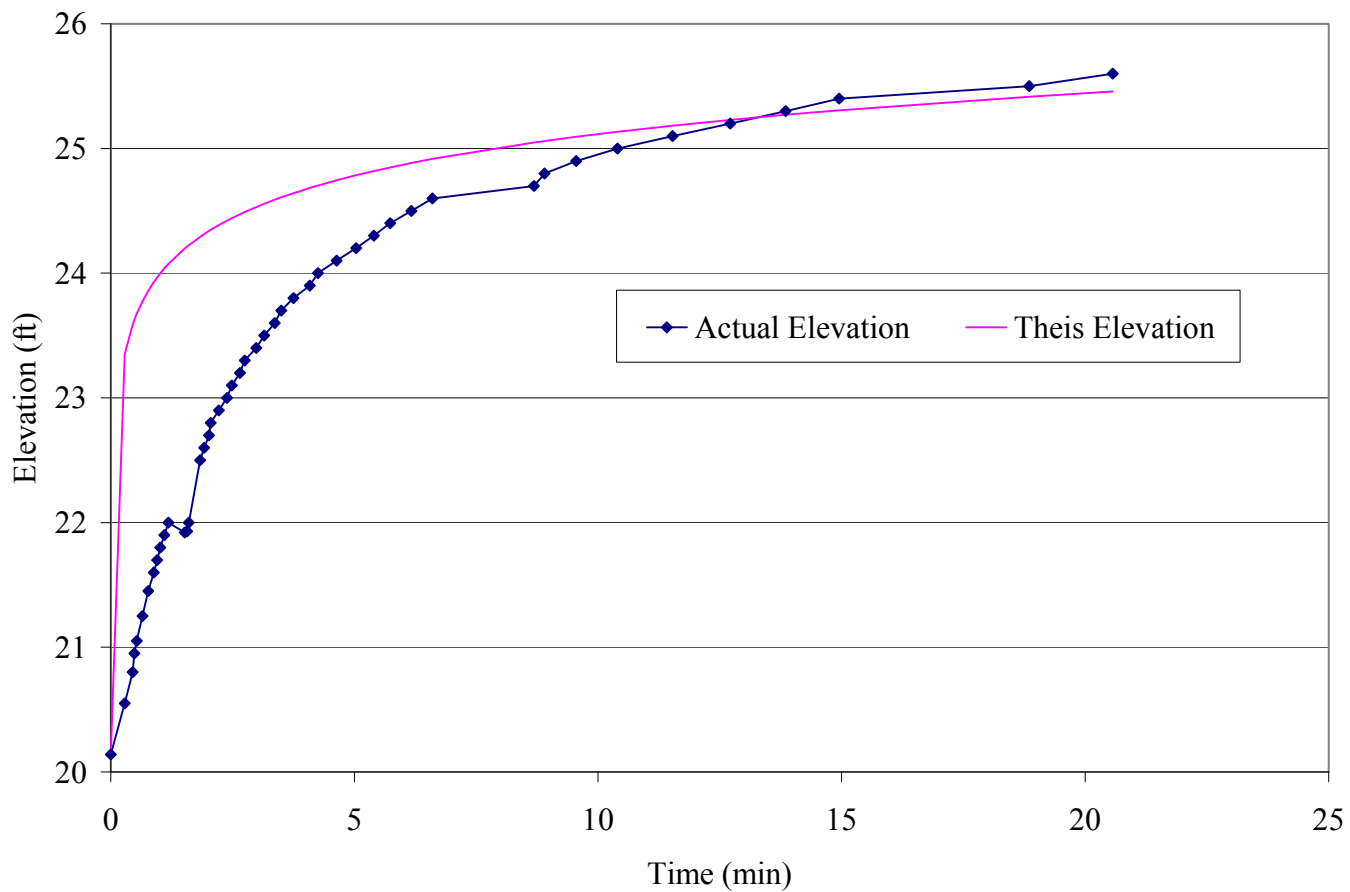
Figure 4.36. Theis' solution for well 10S; water was pumped in (at average flow rate of 7.4 L/min) continuously throughout the experiment (12/21/06).

Table 4.1. *Characteristics of soil samples collected on August 5, 2003 during installation of MW-9D.

Sample ID	Texture			#Total C %	#Total N %	NO$_3$-N ppm	pH	Bray-1 P ppm	K ppm	Na ppm
	% Sand	% Silt	% Clay							
2.5	12	40	48	0.66	0.052	2.9	7.0	21	520	8
7.5	14	40	46	0.72	0.036	2.2	7.5	28	476	16
12.5	12	34	54	0.40	0.036	3.0	7.6	41	550	31
17.5	10	50	40	0.36	0.045	1.3	7.7	51	422	15
22.5	14	46	40	0.17	0.029	2.0	7.6	62	389	18
27.5	16	54	30	5.29	0.355	1.2	7.1	20	196	17
32.5	30	48	22	0.49	0.051	1.1	8.2	39	145	11
37.5	30	54	16	0.08	0.022	1.1	8.1	45	186	9
42.5	38	50	12	0.06	0.013	1.2	8.2	41	137	7
47.5	36	42	22	0.03	0.001	1.1	8.2	24	73	6

*Analysed by Soil Testing Laboratory, Throckmorton Hall, Kansas State University; Samples collected by Terracon, Wichita.

#Total N and C are expressed as % (g/100 g dry weight soil)

Table 4.2. Estimated* actual (Darcy velocity/porosity) groundwater velocities (ft/d) at the pilot study area.

Monitoring Well	Study Period		
	Fall 2004	Fall 2005	Spring 2006
8S	0.8	1.0	0.56
9S	1.03	1.25	0.45
10S	0.87	0.83	0.47
12S	-	0.74[#]	0.76[#]
8D	0.4	0.0033	~ 0
9D	0.62	0.09	0.06
10D	0.68	0.16	0.18
12D	-	0.32	0.23

*Estimated by dilution model for MW-8S and MW-8D, Fall 2004; estimated by ADE model for MW-8S and MW-8D, 2005-06; estimated by peak arrival time of tracer for other monitoring wells.

[#]Since bromide was not detected at MW-12S, the velocity was estimated from nitrate reduction data and assuming nutrients traveled at the same velocity as bromide.

Table 4.3. *Monthly rainfall (inches) in Manhattan for the period May 2003 to April 2007. The data was recorded at the Kansas State University Campus, Manhattan, KS.

Year Month	2003	2004	2005	2006	2007
Jan		0.86	0.85	0.5	0.68
Feb		1.54	2.96	0.01	0.95
Mar		4.86	0.84	2.93	4.01
Apr		2.43	0.67	3.46	3.08
May	2.4	2.45	1.45	2.85	
Jun	7.1	6.42	11.81	1.44	
Jul	2.36	6.89	2.26	4.1	
Aug	4.88	5.54	5.61	10.21	
Sep	2.97	1.02	4.36	1.99	
Oct	1.96	1	3.27	1.95	
Nov	0.81	1.37	0.68	0.07	
Dec	0.97	0.42	0.78	0.43	

*KSU Research and Extension, http://av.vet.ksu.edu/webwx/, 2008.

Table 4.4. Groundwater elevation (listed value + 1000 ft above MSL) in the deep zone.

Date	MW-5	MW-7D	MW-8D	MW-9D	MW-10D	MW-11D	MW-12D	MW-14D
3-Apr-02	8.44							
13-Aug-03		7.42	7.39	7.48	7.36	7.39	7.33	6.61
3-Sep-03				7.09			7.01	5.41
12-Nov-03	7.63						7.45	6.79
17-Mar-04			8.7				8.68	8.05
27-Apr-04	8.72	7.8	7.78		7.82	7.8	7.73	7.1
25-May-04	8.35	9.22	9.22	9.24	9.16	9.18	9.21	8.51
24-Jun-04	9.1	9.08	9.09	9.05	9.03		9	8.31
29-Jul-04	9.85	9.94	9.84	9.86	9.81		9.76	9.08
15-Sep-04	9.29		9.37				9.3	8.57
22-Mar-05	8.91		8.89				8.84	8.21
27-Jun-05	10.22		10.13				10.08	9.33
18-Aug-05			8.42	8.42	8.41			
1-Sep-05			7.97	7.98	7.96			
5-Sep-05	8.12	8.21	8.11	8.12	8.07	8.1		
12-Sep-05	8.12			8.19	8.16	8.17		
15-Sep-05		7.85	7.86	7.85	7.82			
17-Sep-05				8.03			6.95	
24-Sep-05		7.97	7.98	7.97	7.96		7.92	
10-Oct-05			7.82	7.81			7.76	
4-Jan-06			7.42	7.45	7.42		7.41	
12-Jan-06			8.07	8.11	8.06		8.07	
26-Jan-06			7.5	7.49	7.47		7.45	
30-Jan-06	7.93		7.89				7.86	7.2
31-Jan-06			7.79	7.8	7.78		7.75	
9-Feb-06			7.5	7.51	7.5		7.57	
14-Feb-06			7.87	7.89	7.85		7.81	
23-Feb-06			7.41	7.43	7.6		7.36	
29-Mar-06	8.06		8.02				7.96	7.31
25-May-06	8.62	8.6	8.59	8.61	8.56		8.53	
10-Jun-06			8.46	8.49	8.43	8.45	8.42	
25-Jun-06	7.67	7.65	7.65				7.56	
31-Jul-06	7.22	7.19	7.2	7.16	7.16	7.17	7.09	
18-Sep-06	7.44		7.4				7.36	6.68
22-Sep-06	7.87	7.86	7.86	7.87	7.81		7.79	
9-Nov-06		7.52	7.5	7.51	7.49		7.49	
28-Nov-06	7.67	7.61	7.62	7.62	7.58		7.56	
26-Jan-07			7.46	7.45	7.42		7.39	
21-Feb-07			7.23	7.25	7.22		7.18	6.61
22-Feb-07	7	5.97						5.41

Table 4.5. Groundwater elevation (listed value + 1000 ft above MSL) in the shallow zone.

Date	MW-7S	MW-8S	MW-9S	MW-10S	MW-11S	MW-12S	MW-14S
3-Apr-02							
13-Aug-03	13.35	13.28	13.12	13.04	13.2	12.46	5.14
3-Sep-03			13.98			13.11	4.89
12-Nov-03						12.48	4.63
17-Mar-04	14.59	14.53		14.3		12.56	6.08
27-Apr-04	13.6	13.48	13.44	14.27	13.46	12.72	5.62
25-May-04	14.93	14.88	14.8	14.69	14.77	14.1	7.04
24-Jun-04	14.89	14.84	14.7	14.6		14.01	7.82
29-Jul-04	15.32	15.51	15.53	15.32		14.69	7.6
15-Sep-04		14.72				13.93	7.07
22-Mar-05		14.01				13.24	6.67
27-Jun-05		15.29				14.53	6.45
18-Aug-05		13.48	13.38	13.29			
1-Sep-05		13.67	13.57	13.51			
5-Sep-05	13.77	13.56	13.42	13.43	13.46		
12-Sep-05			13.21	13.09	13.22		
15-Sep-05	13.27		13.13	13.03			
17-Sep-05		13.21		12.96		12.45	
24-Sep-05	13.87	13.84	13.72	13.58		13.38	
10-Oct-05		13.45	13.34	13.23		12.65	
4-Jan-06		12.63	12.54	12.35		11.98	
12-Jan-06		12.55	12.48	12.41		11.88	
26-Jan-06		12.34	12.28	12.18		11.68	
30-Jan-06		12.75				12.05	4.91
31-Jan-06		12.42	12.32	12.22		11.72	
9-Feb-06		12.24	12.18	12.08		11.61	
14-Feb-06		12.24	12.2	12.08		11.6	
23-Feb-06		12.14	12.03	11.93		12.46	
29-Mar-06		12.71				11.98	4.78
25-May-06	13.58	13.59	13.43	12.98		12.8	
10-Jun-06		13.31	13.19	13.07	13.2	12.57	
25-Jun-06	12.88	12.91				12.14	
31-Jul-06	12.48	12.46	12.32	12.21	12.32	11.69	
18-Sep-06		13.23	26.93			12.46	4.62
22-Sep-06	13.48	13.46	13.32	13.13		12.61	
9-Nov-06	12.93	12.91	12.78	12.68		12.1	
28-Nov-06	12.71	12.64	12.53	12.43		11.86	
26-Jan-07		12.31	12.17	11.06		11.51	
21-Feb-07		12.28	12.18	12.06		11.55	5.14
22-Feb-07	12.33						4.89

Table 4.6. Estimated hydraulic conductivities (listed value * 10^{-5} m/s) at the pilot study area. The hydraulic conductivity is estimated from groundwater elevation gradient and velocity using Darcy's law, unless indicated otherwise.

Monitoring Well	Study Period		
	Fall 2004	Fall 2005	Spring 2006
8S	7.4	10.7	10.1
9S	73.2	16.1	4.8
10S	7.0	9.3, 180[#]	5.2, 120[#], 88[#]
12S	ND	[&]9.3	[&]8.5
8D	28.2	0.38	~0
9D	43.8	10.4	6.4
10D	36	11.1	8.2
12D	ND	43.3	27.5

[#] From Theis' solution
ND – Tracer was not detected
[&]Bromide was not detected; velocity was estimated from nitrate data

Table 4.7. Estimated Peclet Numbers (vL/D, dimensionless) at the pilot study area.

Monitoring Well	Study Period		
	Fall 2004	Fall 2005	Spring 2006
9S	10.1	8.7	5.5
10S	45.4	24.3	7.6
12S	ND	ND	ND
9D	37	8.2	24
10D	19.4	16	163
12D	-	943	100

ND – No Data; tracer was not detected at MW-12S

Table 4.8. Approximate flow rates (L/hr) of injection solution.

		Loc A	Loc B	Loc C	Loc D
8/18/05					
Nutrient	**Shallow**	**200**	**200**	150	**200**
	Deep	100	**25**	67	**429**
Chase	**Shallow**	150	120	176	136
	Deep	100	**25**	50	**429**
10/13/05					
Nutrient	**Shallow**	100	**68**	**115**	88
	Deep	100	**40**	60	**150**
Chase	**Shallow**	67	**60**	67	**75**
	Deep	100	**43**	50	**120**
3/3/06					
Nutrient	**Shallow**	91	79	**200**	86
	Deep	86	**33**	75	**120**
Chase	**Shallow**	**97**	55	73	**52**
	Deep	**100**	**33**	30	75
8/1/06					
Nutrient	**Shallow**	55	75	100	**120**
	Deep	26	**5**	27	25
Chase	**Shallow**	**100**	75	67	60
	Deep	11	**5**	15	23

Table 4.9. Comparison of bromide arrival times (days) at monitoring wells. The distance is approximate and measured along the east-west direction, starting from the line joining the injection wells in the north-south direction.

Well	Distance (ft)	Arrival Time (days)	
		Fall 2005	Spring 2006
9S	5	5	11
9D	5	53	48
10S	15	18	32
10D	15	95	83
12S	75	-	-
12D	75	236	262

CHAPTER 5 - A Field Study of Bioremediation of Tetrachloroethene Contaminated Groundwater

5.1. Introduction

In this work, the biodegradation of tetrachloroethene (PCE) was demonstrated in the field by a pilot study. Biostimulation and bioaugmentation were used to enhance the rate and extent of biodegradation. Many dry cleaning facilities have used PCE in the past (SCRD, 2007). Due to improper disposal and accidental spill, many former dry cleaner sites are contaminated by PCE.

Field scale pilot studies of PCE remediation have been carried out mostly in sites where the groundwater velocities are lower than 0.1 ft/day, using a recirculation system (Major et al., 2002). In this work, a pilot scale bioremediation study was done in a flowing aquifer with velocity up to 1 ft/day. Vegetable oils and other organic compounds have been used as electron donors in PCE bioremediation by other researchers, however, soy oil methyl esters (SOME) was not used extensively. In this work, SOME and lactic acid were used as electron donors.

5.2. Materials and Methods

5.2.1. Site Description and Problem Statement

The site location, history, lithology and contaminant release characteristics are described in Chapter 4 and explained in more detail elsewhere (Davis, 2007; Terracon, 2004).

Dry cleaning operations during the period from 1967 to 1997 at the Cinderella Cleaners site in Manhattan, Kansas have contaminated the soil and groundwater with tetrachloroethene (PCE). Chlorinated solvents, that may be adsorbed (to the silty-clay soil particles) and present as a non-aqueous phase liquid (NAPL) provide a constant source of contamination of groundwater. The contaminated groundwater plume extends approximately one mile down gradient of the site and impacts public water supply wells near the leading edge of the plume. PCE has degraded to trichloroethene (TCE) within 400 ft of the source area indicating reductive dechlorination is occurring at this site. However, complete reductive dechlorination to ethene/ethane/methane does not appear to be occurring.

5.2.2. Bioremediation Design for the Cinderella Dry Cleaners Site

The total amount of PCE present at the source and in the plume, from source to a down-gradient distance of 780 ft is estimated as approximately 1000 kg; the calculations are presented in Appendix 5.A.

Although the theoretical electron demand is modest, many times more substrate is actually required because of competition for the electron donors by the organisms carrying out the reductive dechlorination and other organisms in the consortium (McCarty, 1996). Lee et al (1997) reported that a minimum of about 60 mg/L of total organic carbon (TOC; mg C/L) of any of the inexpensive substrates or yeast extract was necessary to support dechlorination beyond DCE in microcosm studies with the Victoria, Texas, soils (Lee et al., 1997). The concentrations of electron donors necessary for dechlorination depend on the microbial consortia, substrate, chlorinated solvent concentrations, other electron acceptor concentrations and relative efficiency of conversion of the substrate to drive reductive dechlorination.

In this pilot study, for the purpose of estimating the amount of electron donor to be added in the field, the following simplifying assumptions were made: the microbes degrade only dissolved PCE and not the NAPL phase or sorbed phase contaminants; the aqueous phase PCE is completely degraded to its final daughter product ethene. The basis for estimating the appropriate amount of nutrients, viz., lactic acid and soy oil methyl esters (SOME), is presented in Appendix 5.B.

5.2.3. Bioremediation

5.2.3.1. Fall 2005

Nutrient injection

Tables 5.1 and 5.2 list the amount of each supplement added to the nutrient and chase water during the injection on August 18, 2005. Soy oil methyl ester (SOME) acts as a slow electron donor while glucose and lactate act as fast electron donors to reduce the redox potential of groundwater. Yeast extract (YE) was added as a source of vitamins and minerals. The screening depths of the deep injection wells were 50-55 ft and 42-47 ft; for shallow injection wells, the screening depths were 30-35 ft and 23-28 ft (see Figure 4.3). Groundwater from MW-10S and MW-10D was pumped into four 55 gal barrels (approximately 200 L each) for preparing nutrient solution and anaerobic chase water. Injection of nutrient solution and chase water into

the injection wells was carried out on Thursday, August 18, 2005 (Day 0). The injection process is explained in detail in Chapter 4, Section 2.5.

KB-1 injection

The KB-1 culture was purchased from SiREM, Ontario, Canada. After determining that the area around the injection wells had become reduced [based on the measured oxidation reduction potential (ORP) and dissolved oxygen (DO) values], and after migration of the tracer to down-gradient wells, a culture of KB-1 was injected through the injection wells, on October 13, 2005. The injection of KB-1 was preceded, and followed by, injection of low concentration of nutrient water. Each injection well first received 50 L of nutrient solution. The nutrient solution was prepared using groundwater from MW-9 that had been incubated, from 10/10/2005, with 1 L of SOME, 40 g of glucose, 0.5 L of lactate and 200 g of yeast extract in a 55 gal (208 L) barrel. Then each injection well received 5 L of KB-1 culture injected by pressurizing the KB-1 vessel using a nitrogen cylinder. A photograph of the pilot study area taken during the injection of KB-1 is shown in Figure 5.1. Injection of KB-1 was followed by injection of 50 L of the anaerobic chase water. The anaerobic chase water was prepared similar to the nutrient solution, described above, but without the SOME. No tracer was added during this injection (October 13, 2005) because the study of tracer injection on August 18, 2005, was in progress.

5.2.3.2. Spring 2006

By mid-February 2006, sampling by KDHE personnel indicated that the reducing power in MW-9 and MW-10 was becoming depleted (DO increased and ORP became positive), requiring a third injection of nutrients in March 2006. Groundwater from MW-10S and MW-10D was pumped into four barrels for preparing nutrient solution and anaerobic chase water. Table 5.3 and Table 5.4 list the amount of each supplement added to the nutrient and chase water.

5.2.3.3. Fall 2006

After demonstrating that lactic acid and SOME were successful in reducing PCE levels in samples collected from the pilot study area, we planned to use cheese whey as nutrient source in the field. Groundwater from MW-10 was used to dilute cheese whey for injection. Laboratory microcosm studies conducted by Ibbini et al (2006) suggested that high concentrations of cheese whey are inhibitory of reductive activity, while low concentrations are very stimulatory.

Inhibition at higher concentrations presents a problem for field work because higher concentrations must be introduced to account for dispersion that occurs within the aquifer. In the microcosm studies, the optimal concentration of cheese whey for PCE degradation (with and without KB-1) was 0.025 %, with inhibition occurring at concentrations greater than 0.1 % (Ibbini et al., 2006).

Taking into account the above fact and also considering the groundwater flow and associated dispersion, it is difficult to come up with a recommended injection concentration for cheese whey. Based on bromide tracer patterns during 2004 to 2006, injected solutions may dilute 4-100 fold for every foot of groundwater flow in deep zone and 2-200 fold for every foot of groundwater flow in the shallow zone. If we consider a 10-50 fold dilution, injection of about 0.5 % cheese whey would disperse to the effective concentration range of 0.05 to 0.01 %. In microcosms, 0.1 % cheese whey gave a long lag before reduction of PCE (Ibbini et al., 2006). While 0.05 % whey supplies very little carbon, it is sufficient to fully reduce the levels of PCE typically observed in the field. Cheese whey contains about 50 g/L of sugars (mostly lactose). Dilution of cheese whey to 0.5 % results in a concentration of about 250 mg/L or approximately 1.4 mM glucose equivalent. Depending on detailed reaction paths, this may be enough to reduce more than 1.4 mM of PCE because each glucose molecule provides a dozen active hydrogen molecules (H_2) as NADH, and each PCE needs four H_2. Therefore, when diluted to 0.05 % whey there is in principle enough reducing power for 130 μM of PCE. The microcosm studies were conducted by Ibbini et al (2006) with about 10 μM PCE and even 0.01 % cheese whey proved to be effective. Inhibition and competition by organics and ions present in the aquifer were tested by microcosm studies with water from the site; it was found that there is no significant inhibition or competition by sulfate reducing bacteria.

On July 31, 2006, one day before the fourth nutrient injection, one liter of cheese whey, 40 g of glucose and 10 g of yeast extract were added to 200 L of groundwater pumped into a 55 gal barrel. The nutrient solution was prepared in four such barrels. On August 1, 2006 each injection well received 100 L of nutrient solution.

5.2.4. Analytical Method

Chlorinated compounds and methane were analysed using a gas chromatograph (HP 5890 Series II, Wilmington, DE) equipped with a Flame Ionization Detector (FID) and a HP-1 column

(Dimethyl Polysiloxane matrix, 30 m x 0.53 mm, Agilent Technologies, Wilmington, DE). Hydrogen was the carrier gas. The injector temperature was set at 200˚C and detector temperature was set at 300˚C. Sample volume of 100 µL was injected into the column at 100˚C and run for 5 minutes. The run temperature was chosen based on the arrival time and good separation of compounds. For higher temperatures, the compounds elute faster but the peaks may be closer or even overlap. For lower temperatures, the separation of compounds is good but the elution will be slow.

Resazurin, which is a sodium derivative of a three-ringed nitro aromatic compound, is a reducing-oxidizing indicator that turns colorless under reducing conditions (redox potential less than -110 mV) and will turn blue in the presence of oxygen (redox potential greater than 0 mV) and varying hues of pink for redox potential in the range -110 mV to 0 mV (Karakashev et al., 2003). In this work, 25 µL of 1 mg/L resazurin was added to each groundwater sample collected in a 15 mL vial. The variation of color of the sample is observed immediately, after one hour and after 24 hours. To create headspace for gas analysis, the cap of the vial is loosened and 5 mL of the liquid is taken out of the vial using a 10 mL syringe, while air enters the headspace of the vial. The 5 mL liquid is transferred to a wide-mouthed glass vial and closed with a screw-cap and stored in a cold room. From this 5 mL liquid, 1.5 mL was used for ion analysis (see Chapter 4, Section 4.2.9).

5.3. Results and Discussion

5.3.1. Fall 2005

In the resazurin test, MW-8D showed the most significant changes in color indicating that the water is reduced, with oxidation-reduction potential (ORP) less than -50 mV. The DCE concentration was higher in these samples. The dissolved oxygen (DO) and ORP data collected by KDHE from 8/19/2005 to 9/14/2005 suggested that adding the nutrients had a significant effect, with ORP decreasing by about 100 mV and percent DO decreasing to very low levels. The ORP in MW-8D decreased from -136 mV on 8/23/2005 to -257 mV on 9/14/2005 and in MW-9D the ORP decreased from -103 mV to -248 mV during the same period.

As mentioned in chapter 4, section 3, the groundwater level variations were 5 ft in the shallow zone and 3 ft in the deep zone, during the period July 2003 to January 2007. These

variations can be quite important in the leaching of the NAPL phase contaminant from the unsaturated zone. Infiltration of rainfall and surface water can also result in dissolution of vadose zone contaminants and transporting the compounds to the groundwater table below.

5.3.1.1. Chlorinated Ethenes (CEs) Degradation: Deep Wells

In Figure 5.2, the molar concentrations of PCE and DCE at MW-8D are plotted for all three depths. There was a significant increase in the DCE levels (18 μM to 64 μM by day 28), especially in the top samples (42 ft) which is not in the screened zone of the well MW-8D. After day 28, the DCE concentration started to decrease. The PCE concentrations decreased from day 10 until day 28 and then started to increase. The nutrients that reached MW-8D, by the hydraulic force of injection, may be washing out resulting in a rebound of PCE and a drop in DCE levels. However, it is clear that the nutrients added had a significant reducing effect. Figure 5.2a presents the average concentrations of PCE, TCE, DCE, and total CEs in MW-8D. The reported concentrations represent the average of the concentrations at three depths. Figure 5.2b shows the results after averaging three consecutive data points to obtain lines with less variation.

After the injection of KB-1 and nutrients on day 56 (October 13, 2005), there was a rapid decrease in the (depth averaged) PCE concentrations in MW-8D (nominally up-gradient), from about 44 μM on day 50 to about 9 μM by day 64 (see Figure 5.2a). The DCE concentrations show a corresponding increase from about 9 μM to 47 μM during the same period, consistent with the decrease in PCE. From day 64 until day 188, the average PCE concentrations remained at low levels between 4 μM to 12 μM, whereas DCE concentrations decreased gradually, during the same time period, as shown in Figure 5.2a. The continued presence of bromide (see Figure 4.26), as mentioned in the tracer study at this well, indicates that there was a nearly stagnant condition at this location, possibly due to build up of biomass, by repeated nutrient injections. The ORP measured by KDHE in January 2006 was consistent with the continued presence of highly reducing conditions at the well. Part of the initial decrease in the CE concentrations may also be due to partitioning into the SOME phase (Pfeiffer et al., 2005; Lookman et al., 2007). The effect of adding KB-1 can be understood by observing the concentration trend in PCE and DCE together. From day 28 to day 56, before addition of KB-1, the concentration of total chlorinated ethenes (CEs) remained above 50 μM. After the injection of KB-1 on day 56 until day 180, the concentrations of both PCE and DCE decreased leading to a decrease in total CEs.

The total CEs decreased from 75 μM on day 18 to 22 μM (70 % reduction) on day 180. The lag time for the onset of PCE degradation after the second injection is also less (18 days for the first injection to almost immediate response in the second injection). The microbial population in the vicinity of MW-8D was likely low before the first injection of nutrients on day 0, and it took two weeks for the microbial population to grow significantly to start degrading PCE. However, this large microbial population was present during the second injection and therefore, once the nutrients were injected on day 56, the PCE degradation rate was enhanced. TCE concentration did not vary significantly; it remained steady in the range 2 μM to 11 μM. In the batch studies conducted by Ibbini et al (2006) and Ibbini et al (2007), the lag time for biodegradation of PCE were observed to be approximately 10 days for the microcosms with KB-1 and 20 days for the microcosms with native microorganisms.The rate of conversion of TCE to DCE is rapid compared to the rate of dechlorination of DCE.

A comparison of the laboratory and field results reveals that the lag time for PCE degradation was higher in the laboratory. In the laboratory study, the electron donor was added once a month, about 8 times the required stoichiometric quantity. However, in the field, the amount of electron donors added was theoretically sufficient for degrading PCE for about 170 months assuming the oxygen content of the groundwater in the field is zero. In the laboratory, a major portion of electron donors added was utilized for the consumption of oxygen in inlet water. Due to lower velocities, the electron donors added in the field are not transported rapidly and not washed away. In the laboratory, a portion of the glucose may have been washed out of the channels. The above factors may be responsible for the longer lag time in the laboratory study.

In Figure 5.3, the molar concentrations of PCE and DCE at MW-9D are plotted for all three depths. Figure 5.3a presents the average concentrations of PCE, TCE, DCE, and total CEs in MW-9D. The concentrations were obtained by taking the mean of the concentrations at three depths. In Figure 5.3b the lines were obtained by averaging three consecutive points in order to smooth the data. The mean concentration of PCE started to decrease from day 25 and reached lower levels by day 57, as shown in Figure 5.3a. There was some increase in DCE concentration but not equal to the loss of PCE. The total CEs decreased from 98 μM on day 25 to 47 μM by day 37. The mean DCE concentration reached a peak of about 49 μM by day 57 while the PCE concentration decreased to about 7 μM. After day 57, the mean DCE concentration decreased

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and mean PCE concentration started to increase, likely due to depletion of carbon source. It took 20 days for the effect of second injection of nutrients to be observed at MW-9D compared to immediate response at MW-8D. The nutrients may have reached MW-8D by hydraulic force of injection whereas at MW-9D the nutrients reached due to a combination of both the hydraulic force of injection and the groundwater flow. TCE concentration increased from the background level of 2.7 μM to 8 μM but remained less than 8 μM throughout the duration of this study. Conversion of TCE to DCE is rapid enough such that TCE concentrations remained below 8 μM. Following injection of KB-1, there were somewhat different responses according to sampling depth across the screened interval, but overall there was a slow further decline of both PCE and DCE so that the total CEs decreased to less than 20 μM by day 188. PCE concentrations at the top depth (42 ft bgs) remained low and DCE levels at that depth were higher. With increase in depth, PCE concentrations were higher and DCE concentrations were lower. The nutrients injected were likely more available at lesser depth and also since the screening of the well is from 45 ft to 55 ft bgs, the groundwater in the top samples (42 feet bgs) may experience less dispersion compared to the greater depths. After about day 75 to day 200, both PCE and DCE concentrations decreased, likely due to the injection of KB-1 on day 56. The samples collected at MW-9D, MW-10D and MW-12D reflect both the transformation near the well and transformations that occurred upstream prior to the flow to the well.

In MW-10D, there were no significant changes in the CE levels until day 60, as shown in Figures 5.4, 5.4a, and 5.4b. The initial PCE concentration was about 60 μM and a less than detectable concentration of DCE. Sixty days after the first injection of nutrients, PCE concentration started to decrease and reached a value of about 4 μM by day 100. DCE concentration also correspondingly increased. The timeframe correlates well with MW-9D, where the beginning of decrease in PCE concentration occurred from day 25. There is a lag of about 35 days, corresponding to a velocity of approximately 10 ft/35 days = 0.3 ft/day, as observed typically in the deep zone (see Table 4.1). The peak of DCE concentration occurred at day 95 after the PCE concentration declined to a low value. This peak appeared about 38 days after the corresponding peak at MW-9D; this DCE peak may be due to both formation of DCE near MW-10D and DCE produced upstream. The estimated velocity based on the DCE peaks at MW-9D and MW-10D is 10 ft/38 days = 0.26 ft/day. The groundwater at MW-10D may not be exactly the same as the groundwater that passed through MW-9D; however, it may be the

groundwater that flowed in the vicinity of MW-9D. There was a slight increase in TCE levels, from a starting concentration of 3 µM on day 10 to about 10 µM on day 82, as shown in Figure 5.4a (average for three depths); TCE is converted rapidly to DCE and hence not observed at higher concentrations. The decrease in PCE concentration and subsequent increase was consistent with the tracer arrival times. The concentrations of the CEs at three depths were not significantly different at MW-10D compared to MW-8D and MW-9D. The standard deviation of PCE concentrations, considering the samples collected at three depths as triplicates, for all sampling events until day 200 was computed for MW-8D, MW-9D, MW-10D and MW-12D. The number of sampling events until day 200 is 38 for MW-8D, 44 for MW-9D, 33 for MW-10D, and 21 for MW-12D. The mean values of the standard deviations are 11.8±7.4 µM for MW-8D, 8.6±5.4 µM for MW-9D, 5.1±3.6 µM for MW-10D and 10.9±7.2 µM for MW-12D. The average standard deviation of PCE concentrations at three depths, for data from day 0 to day 200, at MW-8D is more than twice the value at MW-10D and at MW-9D the value is 1.7 times the value at MW-10D. These data suggest that the chlorinated ethenes mix well vertically as they travel with the groundwater from MW-8D to MW-10D. The variation of PCE concentrations at three depths at MW-12D, from day 20 to day 188, was however higher than those for MW-10D. This may likely be due to varying degree of dispersion and mixing of untreated groundwater at different depths, during the flow for about 60 ft from MW-10D.

It is interesting to note that the bromide peak and the DCE concentration peak (and the nadir in PCE concentration) are synchronous at MW-9D and MW-10D. The peak of bromide occurs on days 57, 95 and 236 for wells MW-9D, MW-10D and MW-12D, respectively (see Figure 4.27 to 4.30). The peak of average DCE concentration occurs on exactly the same days as those for bromide for each well. This suggests that bromide, soluble nutrients and DCE travel at the same velocity and there is not much retardation (due to sorption to soil carbon) of nutrients and DCE. The soil organic carbon fraction is usually very low below the organic topsoil (usually the top few feet of soil) and decreases with increasing depth. Since the chlorinated solvent plume was flowing for several years through the pilot study area, the adsorption sites in the soil organic carbon may have been saturated with the contaminants.

In MW-12D, as shown in Figures 5.5, 5.5a, and 5.5b, an increase of DCE was evident at 200-220 days with a corresponding decline in PCE. This matches the timing of appearance of

bromide, presumably from the first injection on day 0. There is a decrease in DCE and total CEs after 400 days, and the concentration of CEs falls below 20 μM at 600 days.

Comparison of the concentrations at MW-12D with those at MW-9D and MW-10D suggests that the flow to MW-12D includes inflow from outside the core plume where PCE and DCE are being degraded. Figure 5.5a exhibits a decline in PCE concentration after 200 days and a further decline after 300 days. The DCE concentration increases after 200 days and then increases further after 300 days. If the decline in DCE concentration after 450 days is due to KB-1 in the vicinity of MW-12D, it took about 400 days for KB-1 to travel from the point of injection to MW-12D. This rate of movement of KB-1 is about half the velocity of the groundwater.

In Figure 5.6, for MW-7D, PCE concentrations were similar to the values before the implementation of the pilot study; the PCE concentrations in most of the samples remained in the range 20 to 40 μM. The concentration in the bottom depth was lower than the values in the top and middle depths. DCE concentration remained steady between 2 μM to 8 μM. There was significant variation in the PCE concentration at MW-7D, due to the variation near the source, showed in Figure 5.8 for MW-5D. This variation at the source may likely be a major reason for the variation at MW-7D since MW-7D is down-gradient of MW-5D. These results indicate that there is no flow or dispersion that causes the injected nutrients to reach MW-7D. These results show that the decline in PCE concentration in the pilot study area (MW-8D, MW-9D and MW-10D) is due to biochemical transformation rather than disappearance of the source of PCE.

PCE concentrations at MW-11D were low, compared to the pilot study area, as shown in Figure 5.7. This location, which is 23 feet south of MW-9D, more or less defines the south end of the CE plume from the former Cinderella dry cleaners. The concentrations of PCE and DCE fluctuated in the range from 0.3 to 5 μM. The fluctuation may likely be due to both the fresh groundwater flowing at that point and the effect of introduction of nutrients at the pilot study area. DCE concentrations were low in MW-11D, and in many of the samples the value was less than the MCL of DCE, 0.72 μM. The higher values of DCE near 100 days may be due to nutrients that were added in the study area, possibly reaching MW-11D. The sampling for MW-11D was not as frequent as other wells because it was in the center of a parking space in the City parking lot and had to be skipped many times when a vehicle was parked there.

An upgradient well to the pilot study are, MW-5D, exhibited variable behavior, as depicted in Figure 5.8. The PCE concentration in the deepest samples was consistently lower than the value in the top samples; values were between 60 to 120 μM until day 120 in the deepest samples. In the middle sampling depth, levels fluctuated from 30 to 380 μM, averaging about 180 μM. In the top, the concentrations declined from 500 μM in the beginning to about 70 μM by day 250 and then increased before they decreased to less than 20 μM. MW-5D displayed a very wide range of concentrations at different sampling depths over time. Levels were up to 400 μM at the start of the field study; by day 300, the values were close to 150 μM. Monthly rainfall values and the water table level (+1000 ft above MSL) are shown as well because of the potential impact of rainfall and water table level variation on contaminant release from the unsaturated zone. There is no obvious relationship between rainfall and PCE concentration, however, PCE concentration appears to increase with increase in rainfall, with a lag period of about 60 days. The lag period may be due to the time taken for dissolution of PCE in the vadose zone and travel downwards to the aquifer. The horizontal travel time from the source to the well may also contribute to the lag time. The values are larger at the start of the field study and generally decrease with time. The higher concentrations in the top samples shown in Figure 5.8 suggest that there is PCE in the unsaturated zone that is made available by rainfall and/or variations in the water table.

The results for DCE in Figure 5.8a suggest that there may be flow from the injection wells to MW-5D. The background DCE concentration is very low as shown in Figure 5.8a, from day 0 to 150. Corresponding to a decrease in PCE concentration, the concentration of DCE increased after day 150 and reached a maximum value of 76 μM (bottom sample) on day 245, which is about 50 % of the PCE concentration at this depth; however, most of the values of DCE concentration lie in the range 0 to 50 μM, which is about 20 % of the PCE concentration at this location. The trend in PCE and DCE concentration between days 350 and 450 is opposite indicating evidence of biodegradation. The average of the hydraulic gradient between MW-5D and MW-8D is estimated as $2 * 10^{-4}$. The value for the same parameter between MW-5D and MW-9D is also very small (estimated as $-8 * 10^{-4}$) indicating the groundwater flow is very slow or may even be towards MW-5D on some occasions. The average hydraulic gradient is estimated from the groundwater elevation at the above locations measured during the period Spring 2004 to

Fall 2006. When groundwater flow is slow, there may be a variety of pathways and pressure forces that impact fluid movement. Comparison of Figures 5.8 and 5.8a shows that the two largest values of DCE concentration are associated with rainfall events. It may be possible for rainfall to carry nutrients from the shallow aquifer to MW-5D. The stormwater with organic compounds, could be entering into MW-5D and provide the carbon source for microbes to degrade PCE and DCE. Before our extensive sampling of MW-5D during pilot study, the well was not opened often and hence may be sealed sufficiently to prevent infiltration of stormwater. During frequent sampling, that required frequent opening and closing of the well cover, the cover and the sealing were subject to wear and tear creating pathways for stormwater to enter the well.

At 248 days, in May 2006, the recorded value for groundwater elevation is1008.62 ft at MW-5D and 1008.61 at MW-9D as displayed in Table 4.2. Both values are larger than the values in earlier months because of the rainfall. Similarly, at 400 days, during September 2006, there is a rainfall event and groundwater elevations are larger compared to July 2006. In September 2006, the values are 1007.87 ft at MW-5D and 1007.87 ft at MW-9D. We do not know of any pumping from wells or other forces that would cause flow from the injection wells to MW-5D. The pathways associated with any preferential flow of rainfall to the deep aquifer are not known.

The data in Table 4.2 show that the groundwater elevations are generally slightly higher in MW-5D compared to MW-8D and MW-9D; however, there are dates when the values are similar as well. There are also measured values where the elevation at MW-5D is lower.

5.3.1.2. Chlorinated Ethenes (CEs) Degradation: Shallow Wells

In the shallow wells MW-8S to MW-10S, there were no significant changes in the chlorinated ethene levels in the first 30 days as shown in Figures 5.9 to 5.11, 5.9a to 5.11a, and 5.9b to 5.11b. In MW-8S (See Figures 5.9, 5.9a, and 5.9b), there were small increases in DCE concentration after the August 2005 and October 2005 nutrient injections, with total CEs only about 9 μM for the average of the three depths across the screened interval. Following the third nutrient injection (day 197), levels of DCE rose at MW-8S while PCE concentration declined. This was a transient response corresponding to the spike of bromide, and therefore the nutrients, in the same well. Later the DCE concentration gradually declined and the PCE concentration rebounded because the groundwater flowed through MW-8S and diluted the nutrients. The concentration of DCE increased for a short period following the injection at 348 days.

In Figures 5.10, 5.10a and 5.10b, for MW-9S, there was only a small change in mean PCE concentration from about 9 μM to values mostly in the range 4 μM to 6 μM between day 28 to day 208 and a moderate increase of DCE with peaks on days 18, 64 and 102. The DCE concentration peaks on days 18 and 64 correspond to the nutrient injections on day 0 and 56, respectively. The peak on day 102 may be due to nutrients from a farther injection well or the reduced groundwater from MW-8S reaching MW-9S. The average PCE concentration varied from 3 μM to 9 μM but DCE concentration remained almost steady and in the range 0.5 to 2.5 μM. There was no significant response at MW-9S to the feeding on day 197. However, the feeding on day 348 resulted in a significant decrease in PCE concentration.

In Figures 5.11, 5.11a, and 5.11b, for MW-10S, the concentration of PCE decreased from an initial average value of more than 12 μM to values of about 8 μM. The average concentration of DCE increased from about 1.5 μM to 5 μM, by day 95. The average PCE concentration increased from day 102 and eventually reached the value of 13 μM and DCE concentration decreased to 2 μM by day 188. The total CEs remained almost steady in MW-10S between 12 μM to 18 μM, except for a short duration from day 460 to day 570. There was a striking response to the third feeding, on day 197, with PCE levels reaching zero and DCE levels rising to those previously observed for PCE. Later, there was a rebound to the previous state with more PCE than DCE after 300 days. It took only 14 days for the PCE concentration to decrease in MW-10S after the third injection on day 197, however, it took more than 100 days for the PCE concentration to decrease drastically, after the fourth injection of nutrients, on day 348.

A comparison of the total CEs in MW-8S, MW-9S and MW-10S reveals that the groundwater observed at MW-10S may not be the same groundwater that passed through MW-8S and MW-9S. The approximate total CE concentrations in MW-8S, MW-9S and MW-10S are 9 μM, 8 μM, and 14 μM, respectively. A decrease of CE concentration in a down-gradient well can be explained by biodegradation, adsorption and dispersion, however, a higher value leads to the conclusion that the groundwater may have traveled through a different path other than the up-gradient well. The concentrations of PCE that enter the pilot study area are smaller in the shallow zone compared to the deep zone, but well above the MCLs of 0.03 μM (PCE), 0.04 μM (TCE), and 0.72 μM (DCE).

For MW-12S, the response was gradual with a decline of average PCE concentration from about 7 µM to about 3 µM by day 600 with DCE remaining fairly steady at about 4 µM (see Figures 5.12, 5.12a, and 5.12b). The variation in PCE concentration may be due to other factors such as variation of PCE concentration at the source, changes in water table levels, and infiltration of surface water. The DCE concentrations during the initial days indicate that conversion of PCE to DCE was taking place before nutrients were added. The degradation may have been happening near MW-12S or upgradient.

In MW-7S, PCE and DCE concentrations fluctuated over time and there was no obvious trend (see Figure 5.13). The CE concentrations, however, were only about one-tenth of the values in MW-7D. PCE concentrations were about 3 µM and DCE concentrations were about 1 µM, with higher and lower concentrations observed on some dates. It is unlikely that this location is affected by nutrient injection due to the distance to the North from the injection wells of 23 ft and also the direction of groundwater flow being to the East which is perpendicular to the direction from the injection wells to MW-7S.

In Figure 5.14, the PCE concentrations at MW-11S were slightly higher than MW-11D, which is opposite to the trend in the immediate pilot study area, about 20 ft to the North of MW-11S. The concentration varies between 3 µM and 7 µM and remains steadier compared to that in MW-11D. DCE concentrations also remained steady and in the range 0.4 µM to 2 µM. PCE concentrations were below 4 µM during the periods day 93 to day 306, and day 390 to day 602, except for a few samples. Compared to the background concentrations of about 5 µM, the decrease in PCE is not significant and it is difficult to conclude whether the small changes in the concentrations are seasonal or due to the effect of feeding at the pilot study area.

Interpreting the patterns of contaminant concentration is complicated by the dynamics of flow. The plume is migrating with reaction occurring simultaneously, so that an observed concentration at any down-gradient well represents all of the dispersion and reaction processes between the point of plume origination and the well. An examination of historical data from KDHE contractors' sampling and our study indicates that the ratio of DCE/PCE has changed dramatically following nutrient addition. As shown in Figure 5.15, the ratio increased drastically for monitoring wells located in the pilot study area (PSA) and MW-12D influenced by nutrient addition, however, MW-7D located north of the PSA was not affected by nutrient feeding. The

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ratio of DCE to PCE remained low and fairly steady at 0.15, indicating no evidence of biodegradation of PCE. The DCE to PCE ratio was highest for MW-10D reaching a value of 22 on day 95, compared to a value of the order of 0.01 before the start of the pilot study. Lower values of the ratio in the later part of the study were due to decrease in both DCE and PCE concentrations rather than increase of PCE concentration only. There was also a significant loss of total chlorinated ethenes at all wells in the pilot study area.

As shown in Figure 5.16, the DCE/PCE ratio did not vary significantly in the shallow zone of the PSA except at MW-10S. The ratio increased drastically for MW-10S after the third nutrient injection on day 197. The DCE/PCE ratio reached a peak value of 135 compared to the background value of about 0.2. The ratio was high at MW-10S from day 220 to day 250. The values of the ratios at MW-9S and MW-10S confirm the inference that the groundwater at MW-10S may not have traveled through MW-9S. A comparison of the values of the ratio at several monitoring wells indicates the nutrient addition had a significant effect on the shallow zone PCE and DCE concentration. At MW-9S, the background DCE/PCE ratio was about 0.1 and increased up to 0.3 after the first nutrient addition. There were several peaks after the second nutrient addition, and value reached 0.63 on day 102. After day 210 until day 400, the ratio remained fairly steady about 0.2. After day 400 there was a significant decline in PCE concentration resulting in increase of the DCE/PCE ratio. There were several peaks after day 400 and the value reached 0.8 on day 573.

Each nutrient injection had a significant impact in the DCE/PCE ratio at MW-8S. The response at MW-8S was drastic compared to the down-gradient well MW-9S. From a background ratio of 0.2, the value reached 0.3 after first injection, 0.95 after second injection, 1.3 after third injection and 1.2 after fourth injection. Besides the peaks, the ratio was in the range 0.3 to 0.5 throughout the duration of the study which is higher than the background.

The DCE/PCE ratio remained fairly steady at MW-12S except for a few values. The ratio was in the range 0.2 to 1.0 indicating that there is sufficient mixing of untreated or partially treated water during the travel from the PSA to MW-12S. The background ratio at MW-7S was higher compared to the pilot study area and the values were about 0.4. There was also a significant loss of total chlorinated ethenes at all wells in the pilot study area.

Observed PCE concentrations in all wells varied with time, even in locations where no nutrients were injected. Variations may be due to several factors including changes in groundwater level, rainfall, nutrient addition, and KB-1 injection.

There must be different mixing processes of contaminated water and untreated water flowing into each zone of the aquifer because the entering PCE concentrations observed in the deep wells at MW-8D and MW-9D as represented by early samples are about 60 µM; however, the PCE concentrations in the shallow wells are much lower, in the range of 6-15 µM. The data for PCE concentration at various depths also provides an idea of the PCE distribution at the source. PCE may be present as NAPL phase in the vadose zone and in the deep zone, acting as a source. The shallow zone source of PCE is only the dissolution occurring when NAPL PCE travels downward from vadose zone to deep zone.

5.3.1.3. Comparison with KDHE Contractor's Data

The concentrations reported by KSU personnel were equal to or higher than those reported by KDHE's Contractors in most of the comparable sampling dates. At MW-5D the concentrations analyzed at KSU laboratory were 386.7 µM and 47.4 µM for samples collected, at 44 ft bgs, on 2/1/2006 and 4/4/2006, respectively. At the same monitoring well, the concentrations reported by the contractors were 108.4 µM and 28.9 µM for samples collected (likely at 44.5 ft bgs) on 1/30/2006 and 3/30/2006, respectively. The Contractors typically collect samples at 5 ft from the bottom of a monitoring well. We do not have the information on the exact depth at which the contractors collect their samples. Moreover there were several different contractors employed by the KDHE during the period 2003 to 2006. The variation may be due to differences in concentrations in the samples, sampling depth, sampling method, and analytical methods. KSU laboratory analyses results may be higher than the Contractor's results due to proximity of the laboratory to the site, which was conducive to immediate analysis, whereas the samples collected by KDHE's and their Contractors were shipped to a distant laboratory that may have caused a loss in the volatile organic compounds due to storage and transportation time. An exact comparison is also not possible due to difference in sample collection dates, although a significant variation is not expected in the groundwater within a few days. A detailed comparison of the concentrations obtained in the KSU laboratory and KDHE's Contractors' laboratory for several wells and dates was presented by Davis (2007).

5.3.2. Spring 2006

The concentrations of chlorinated ethenes (CEs) in deep wells MW-8, 9, 10 and 12 are shown in Figures 5.2 through 5.5 and 5.2a through 5.5a. The data is shown for PCE and DCE in Figures 5.2 through 5.5 at all three depths and the average concentration is shown in Figures 5.2a through 5.5a. The arrival time of the tracer precisely coincided with the arrival time of DCE and the front of PCE disappearance, indicating that there is no appreciable retardation within the aquifer for the CEs and the nutrients. If either DCE or nutrients were appreciably sorbed to aquifer materials, there would be a measurable retardation of their arrival times relative to the KBr tracer. Comparison of arrival times mentioned above indicates that sorption may not be a major factor in the aquifer or the sorption sites may already be saturated since the contaminated water has been flowing for several years. There is loss of a significant fraction of the total chlorinated ethenes (CEs) (Figures 5.2a to 5.5a) though high concentrations of ethene or methane were not observed. For MW-8D (Figure 5.2) there was an increase in DCE corresponding to a decline of PCE after each feeding, followed by a rebound. However, the total chlorinated ethenes decreased substantially. For MW-9D (Figure 5.3) there was also little response, but it is important to note that the level of PCE observed after the third nutrient feeding (day 197) was less than 10 % of what it was a year earlier.

For MW-10D (Figure 5.4), there was a surprisingly fast response to the third feeding. Previously PCE concentration had declined and DCE concentration had risen upon feeding, and then rebounded. After the third nutrient injection, PCE concentration declined and DCE concentration increased again until day 220 and again from days 230 to 270. PCE concentration rebounded from day 280 to day 330 but did not reach the previous levels. is beginning again as PCE levels are rising and DCE levels were declining slowly. After the fourth nutrient injection on day 348, until day 602, both PCE and DCE concentrations were below 10 μM in most of the samples.

In MW-12D, an increase in DCE concentration was evident at 200-220 days with a corresponding decline in PCE. This matches the timing of appearance of bromide (see Figure 4.29), presumably from the first injection on day 0. If the water in MW-12D is coming from up-gradient well areas, the levels of PCE ought to reflect the corresponding results in MW-10D six months earlier. The decline in PCE concentrations observed at MW-12D at 200-220 days was observed at MW-10D at 60-90 days. From day 200-300, the DCE concentrations at MW-10D

were in the range 20-30 μM. At MW-12D, from day 350-450, the DCE concentrations were in the range 25-35 μM, indicating that the groundwater reaching MW-12D flowed through or in the vicinity of MW-10D. The total CEs at MW-12D remained fairly steady until day 350 and then there was a gradual decrease from about 60 μM to 20 μM.

5.4. Conclusions

Biodegradation of PCE is an electron donor limited process in the study site. Addition of electron donors such as SOME, glucose and lactic acid stimulated the native microbes to convert PCE to DCE. Injection of KB-1 enhanced conversion of DCE to end products such as methane or ethene. The results indicate that the KB-1 cultures may have been distributed by fluid motion to MW-9D, MW-10D and MW-12D, for example. Steady concentrations of sulfate indicate that the dehalogenating microorganisms out compete sulfate reducing bacteria (SRBs) for electron donor. SRBs may not be present in sufficient numbers.

Since CEs were found in MW-10D even though there was not much in 9D, a significant portion of the groundwater at 10D may not have passed through the area near MW-9D. There is clear evidence that nutrients and KB-1 have impacted groundwater samples collected at MW-10D and MW-12D. It is possible that nutrients may have traveled with the tracer to the downgradient wells and that biodegradation of PCE to DCE took place at or near these wells, or the groundwater from the upgradient wells and injection wells may be reaching the downgradient wells.

The arrival time of the tracer precisely coincided with the arrival time of DCE and the front of PCE disappearance, indicating that there is no appreciable retardation within the aquifer. If either DCE or PCE were appreciably sorbed to aquifer materials, there would be a measurable retardation of their arrival times relative to the KBr tracer.

The PCE present at the site of contamination provides a long term source for the plume. The data for MW-5D indicate that the upper portion of the deep zone of the aquifer is affected by the source of contamination more than the lower portion of the deep zone. This suggests that the source of contamination may be in the unsaturated zone above the deep aquifer. A different approach such as chemical oxidation using potassium permanganate or soil vapor extraction should be considered to remediate the vadose zone contamination.

The measured concentrations of PCE and DCE in the deep zone of the aquifer provide supporting evidence that flow in the aquifer near the points of nutrient addition was altered because of microbial growth and microbial product formation which reduced the hydraulic conductivity.

The general results of the pilot study demonstrate that both nutrient addition and KB-1 addition were beneficial. The resulting concentrations of PCE, TCE, and DCE were much lower because of the added nutrients and cultures. The resulting concentrations were well above the desired end point (MCLs) because of the mixing of treated water with other water that is untreated or partially treated.

Figure 5.1. Photograph taken during the injection of KB-1. Nitrogen cylinder was used to pressurize the KB-1 vessel to inject KB-1 into the wells.

Figure 5.2. Concentrations of PCE and DCE in MW-8D. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (August 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1,2006).

Figure 5.2a. Mean Concentrations (avg for three depths) of CEs in MW-8D. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).

Figure 5.2b. Data points are mean concentrations (avg for three depths) of CEs in MW-8D. Lines were obtained by averaging three consecutive points from both X and Y axes data. Injection of nutrients was carried out on days 0, 56, 197, and 348.

Figure 5.3. Concentrations of PCE and DCE in MW-9D. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (August 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1,2006).

Figure 5.3a. Mean Concentrations (avg for three depths) of CEs in MW-9D. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).

Figure 5.3b. Data points are mean concentrations (avg for three depths) of CEs in MW-9D. Lines were obtained by averaging three consecutive points from both X and Y axes data. Injection of nutrients was carried out on days 0, 56, 197, and 348.

Figure 5.4. Concentrations of PCE and DCE in MW-10D. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (August 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348, 8/1/06.

Figure 5.4a. Mean Concentrations (avg for three depths) of CEs in MW-10D. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
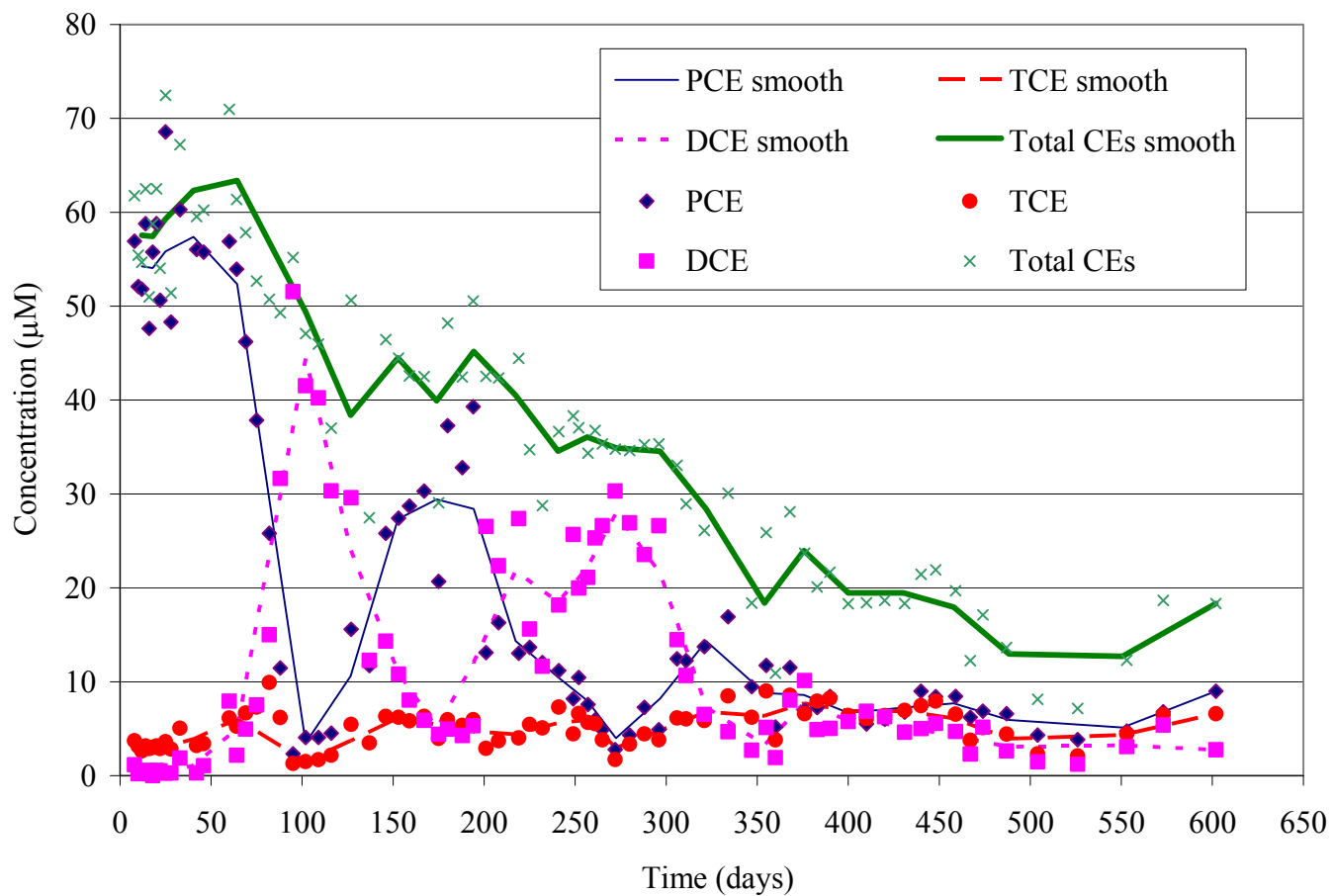
Figure 5.4b. Data points are mean concentrations (avg for three depths) of CEs in MW-10D. Lines were obtained by averaging three consecutive points from both X and Y axes data. Injection of nutrients was carried out on days 0, 56, 197, and 348.
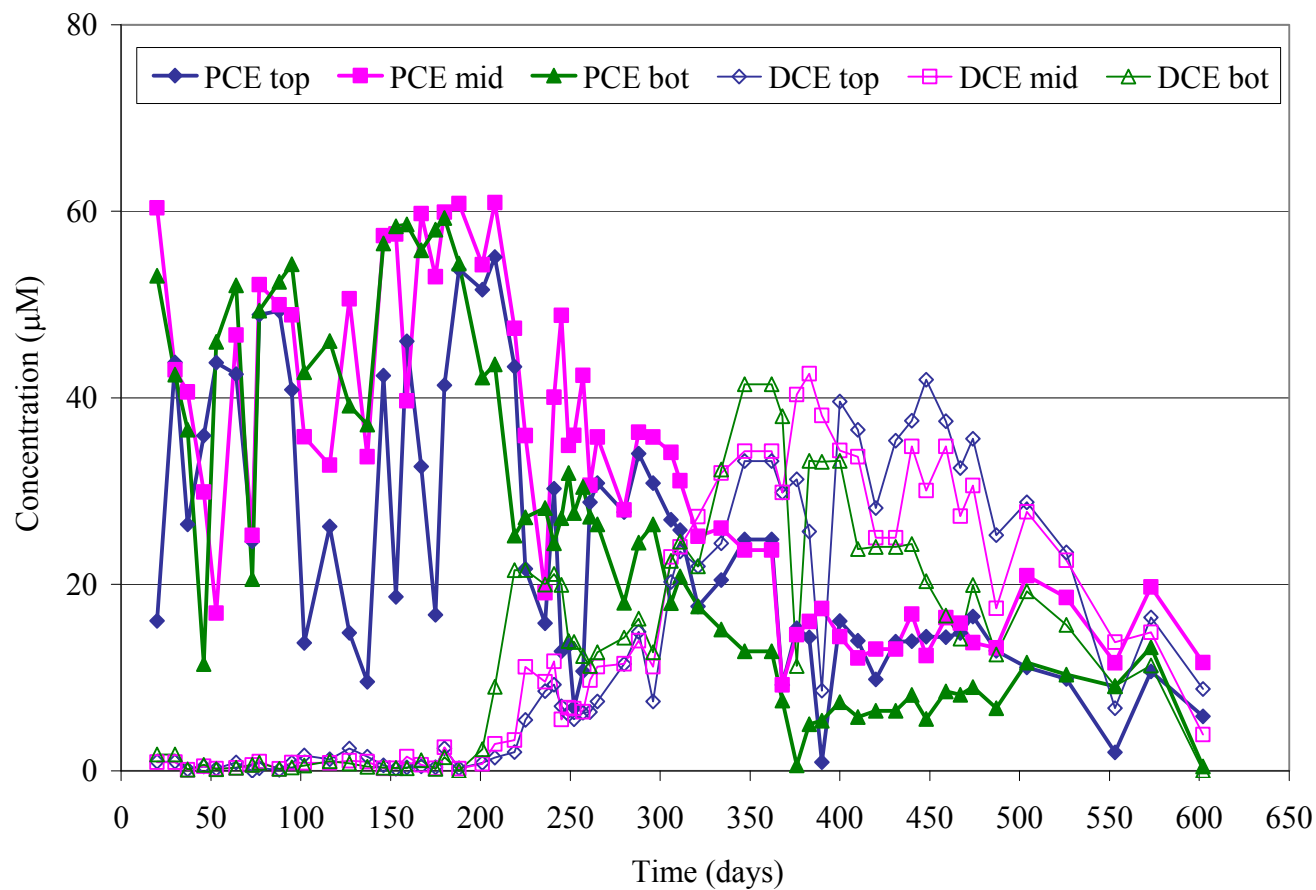
Figure 5.5. Concentrations of PCE and DCE in MW-12D. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (August 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
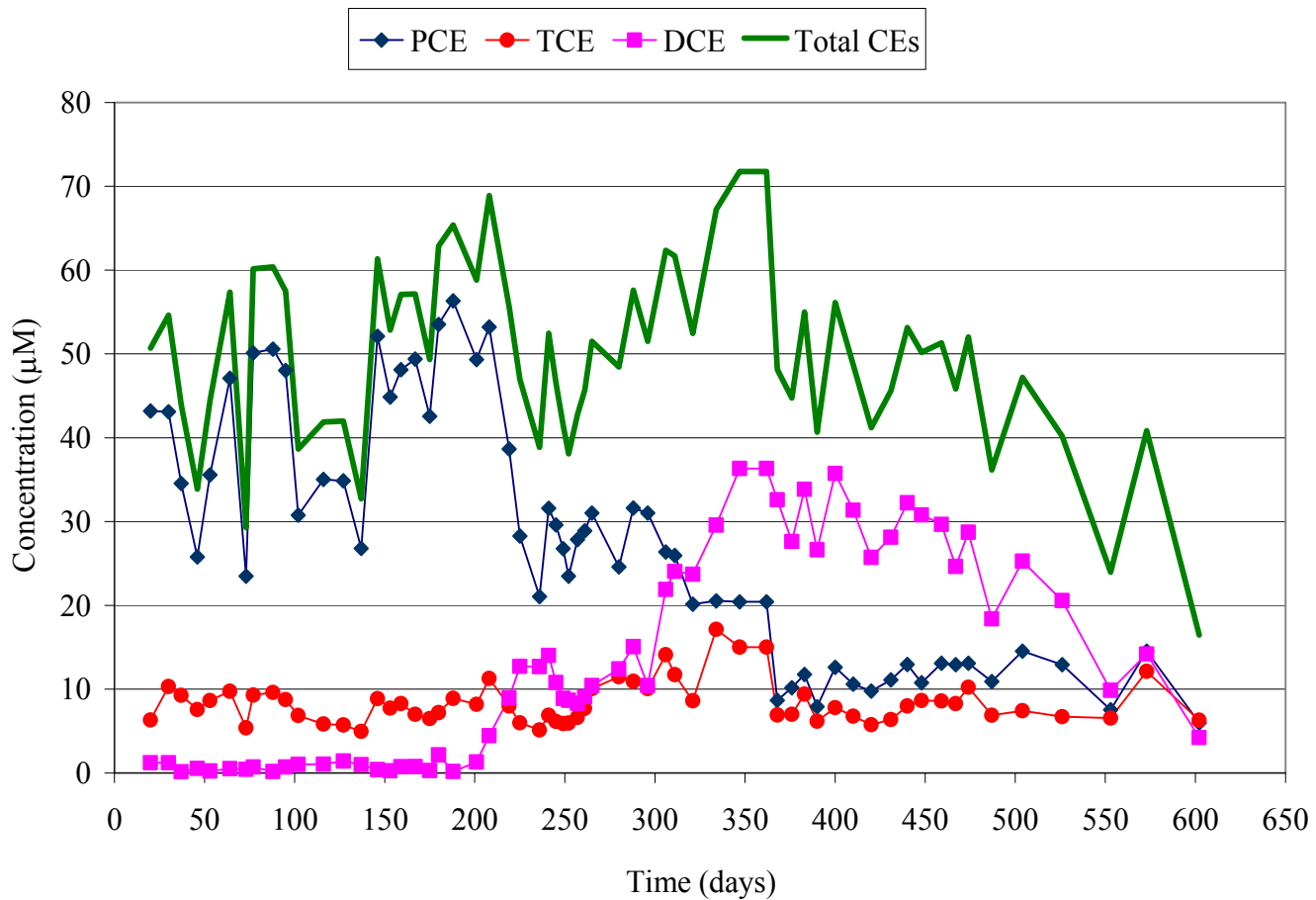
Figure 5.5a. Mean Concentrations (avg for three depths) of CEs in MW-12D. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
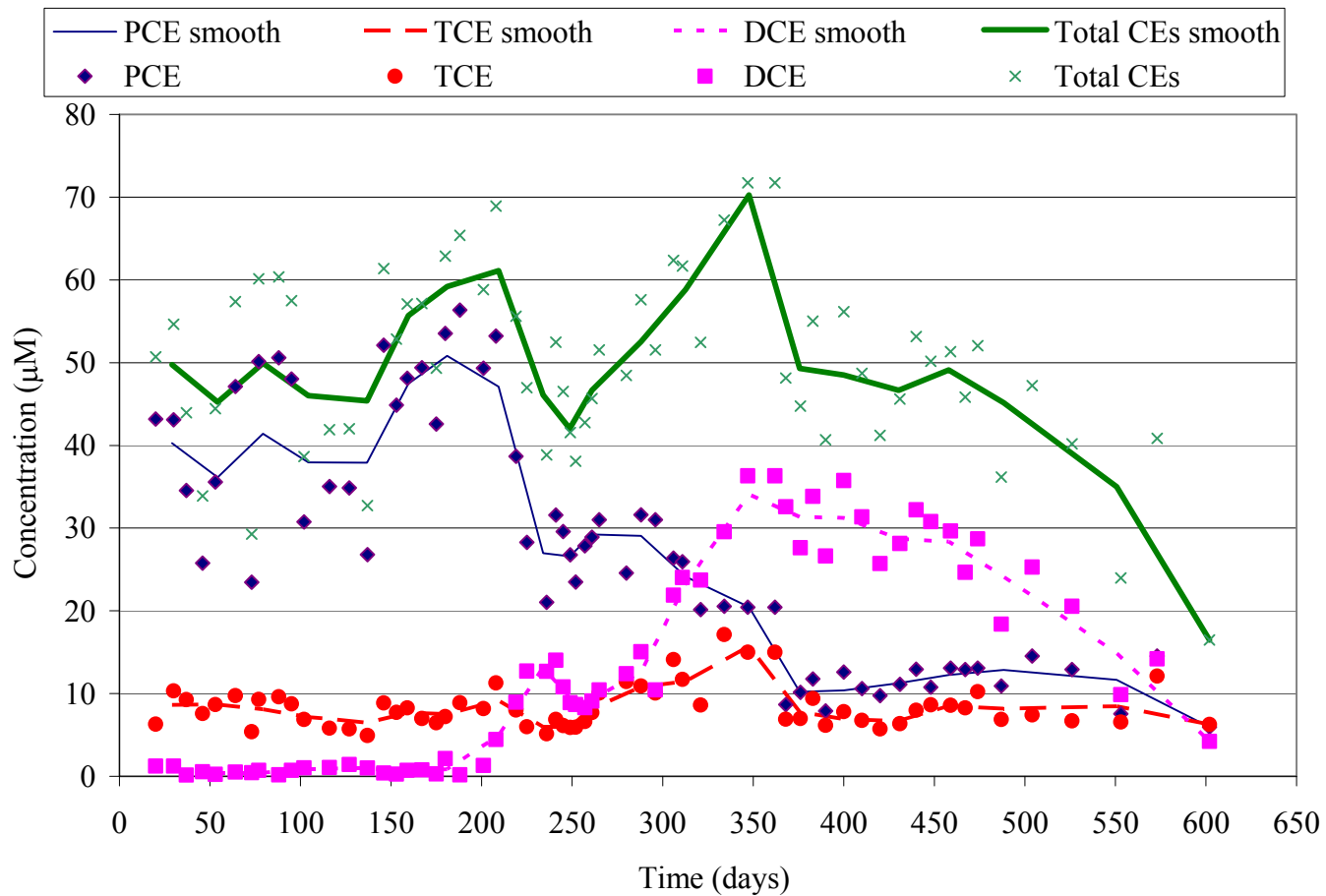
Figure 5.5b. Data points are mean concentrations (avg for three depths) of CEs in MW-12D. Lines were obtained by averaging three consecutive points from both X and Y axes data. Injection of nutrients was carried out on days 0, 56, 197, and 348.
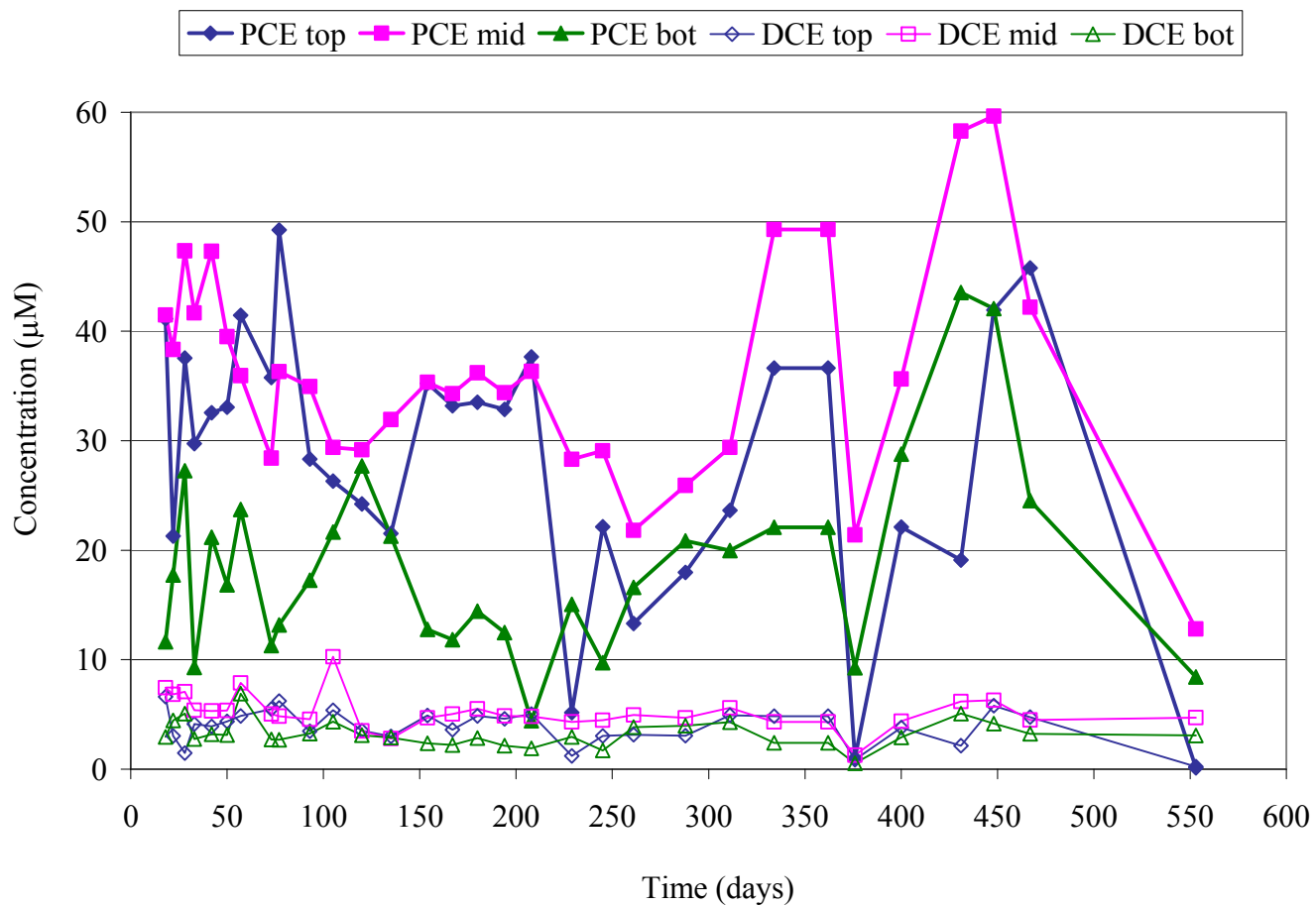
Figure 5.6.Concentrations of PCE and DCE in MW-7D. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
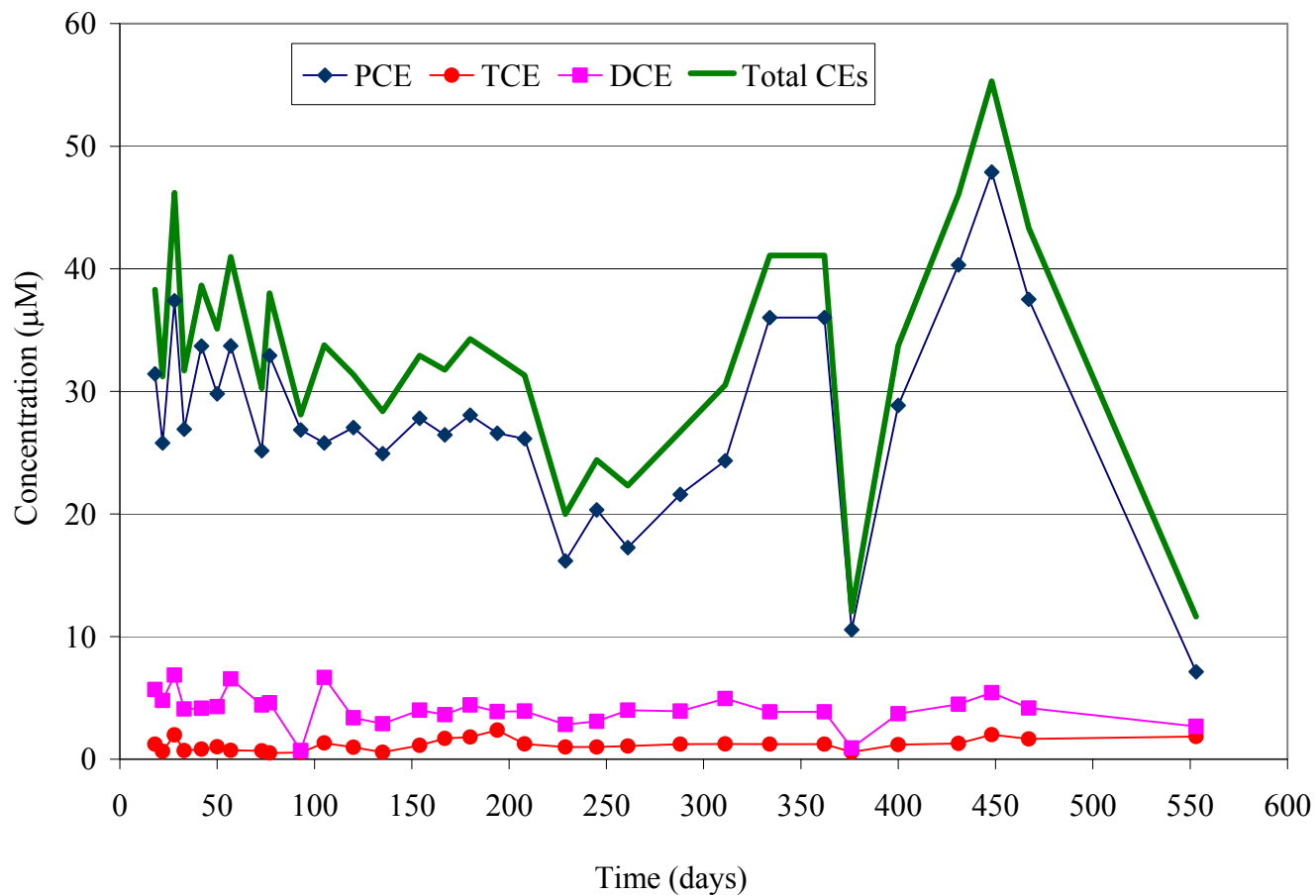
Figure 5.6a. Mean Concentrations (avg for three depths) of CEs in MW 7D. Injection of nutrients and bromide between MW 8 and MW 9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and Bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
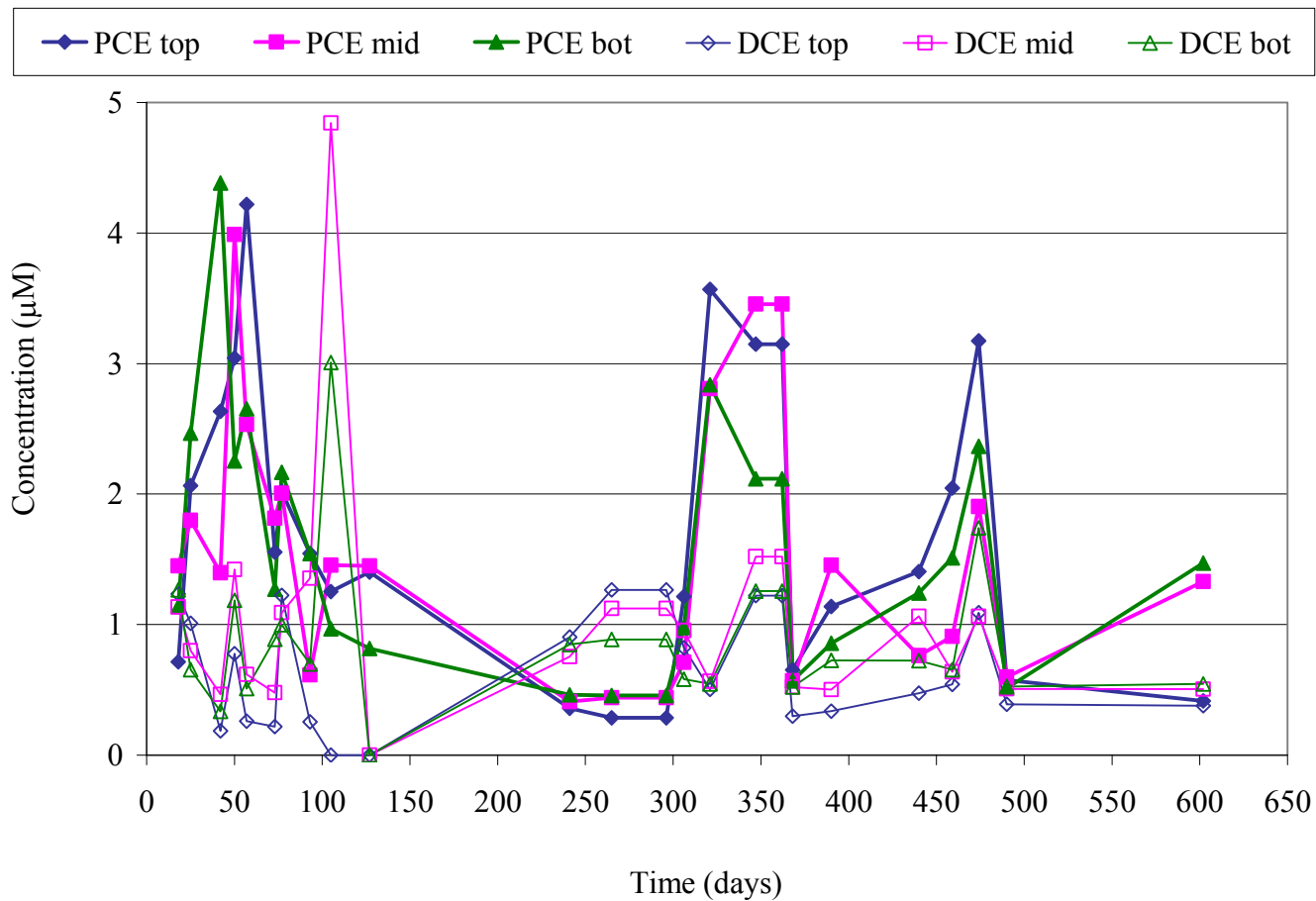
Figure 5.7. Concentrations of PCE and DCE in MW-11D. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1,2006).
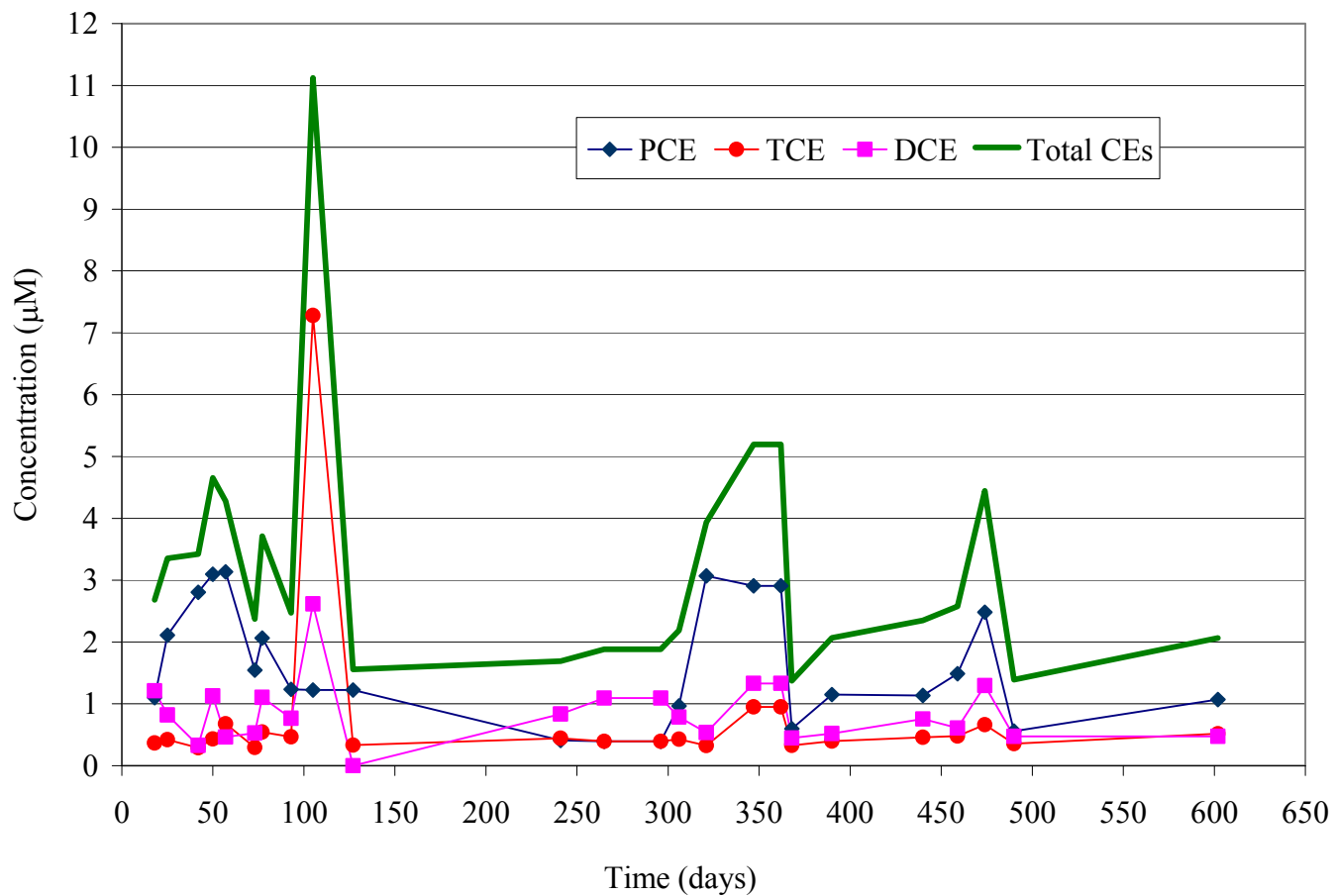
Figure 5.7a. Mean Concentrations (avg for three depths) of CEs in MW-11D. Injection of nutrients and bromide between MW 8 and MW 9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and Bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1,2006).
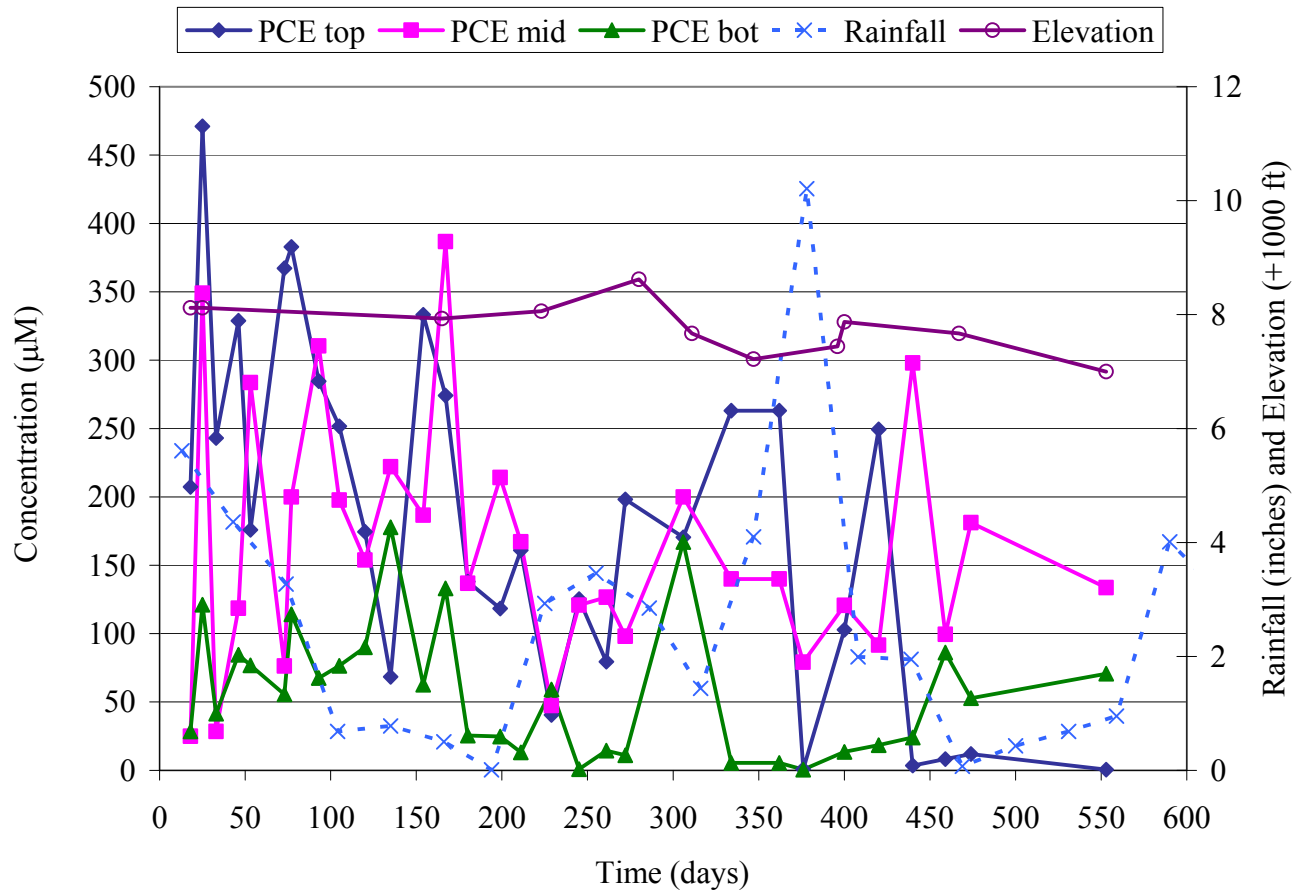
Figure 5.8. Concentrations of PCE in MW 5D. Monthly rainfall values are shown in inches.
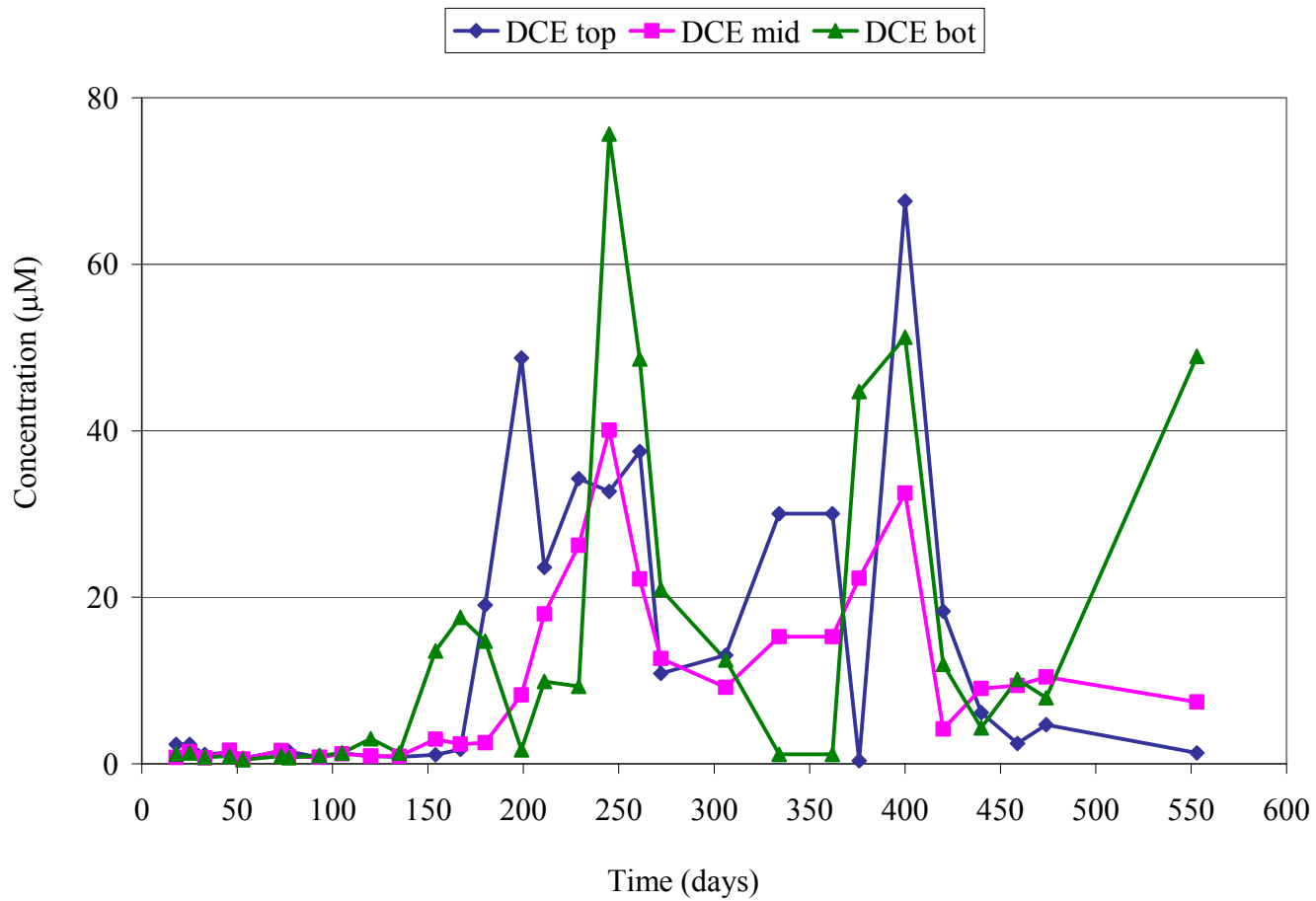
Figure 5.8a. Concentrations of DCE in MW 5D. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (August 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
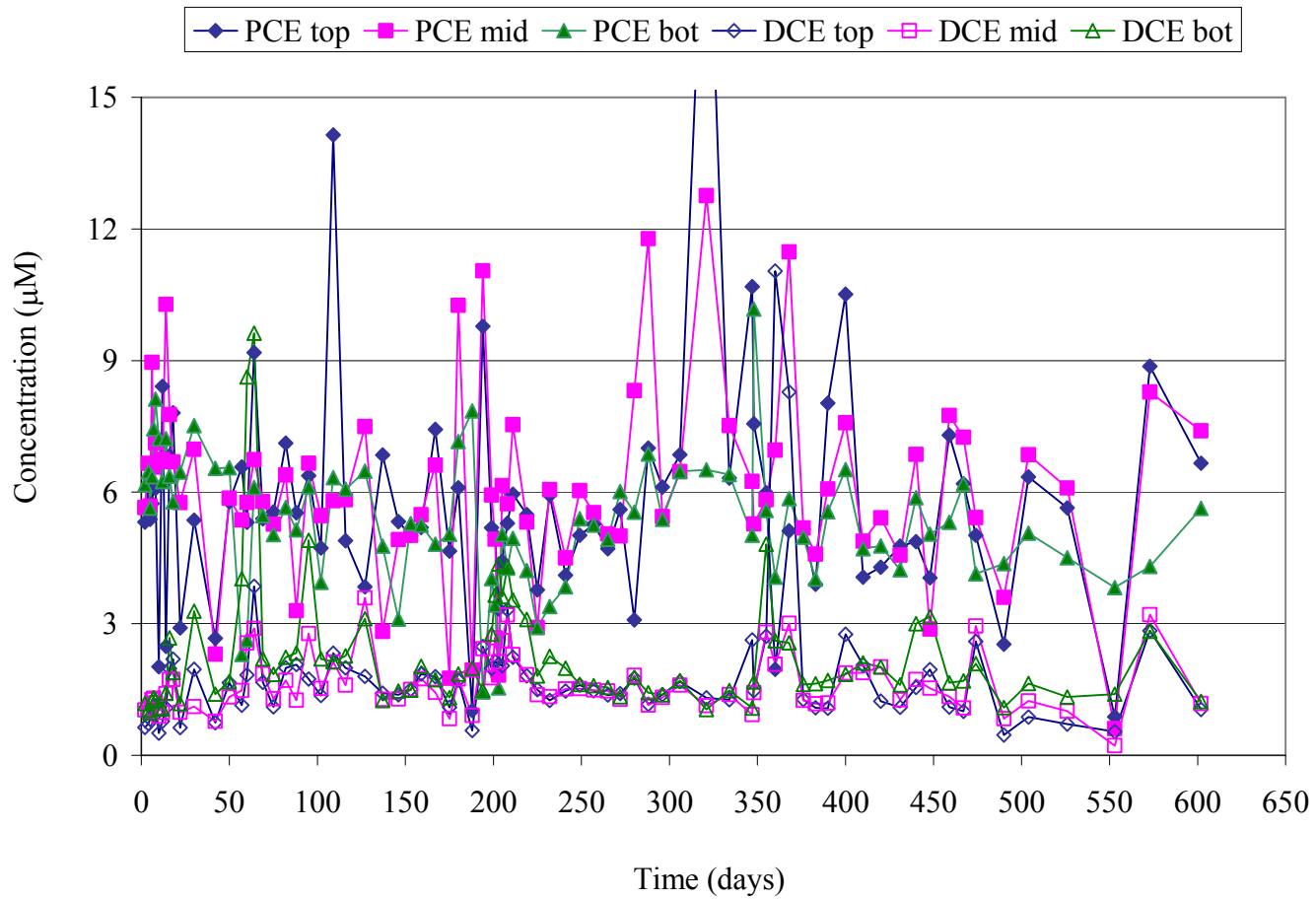
Figure 5.9. Concentrations of PCE and DCE in MW-8S. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
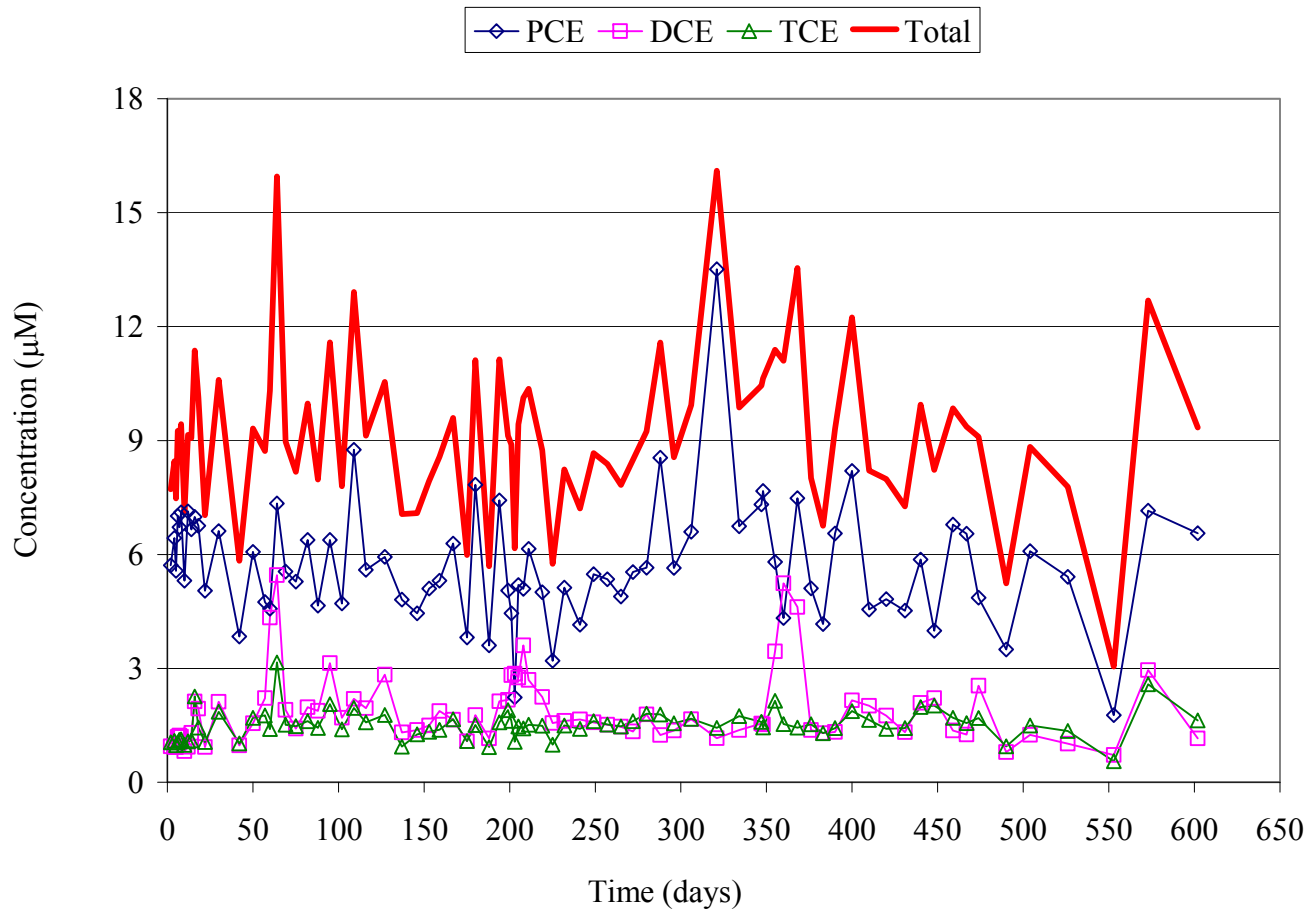
Figure 5.9a. Mean Concentrations (avg for three depths) of CEs in MW-8S. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
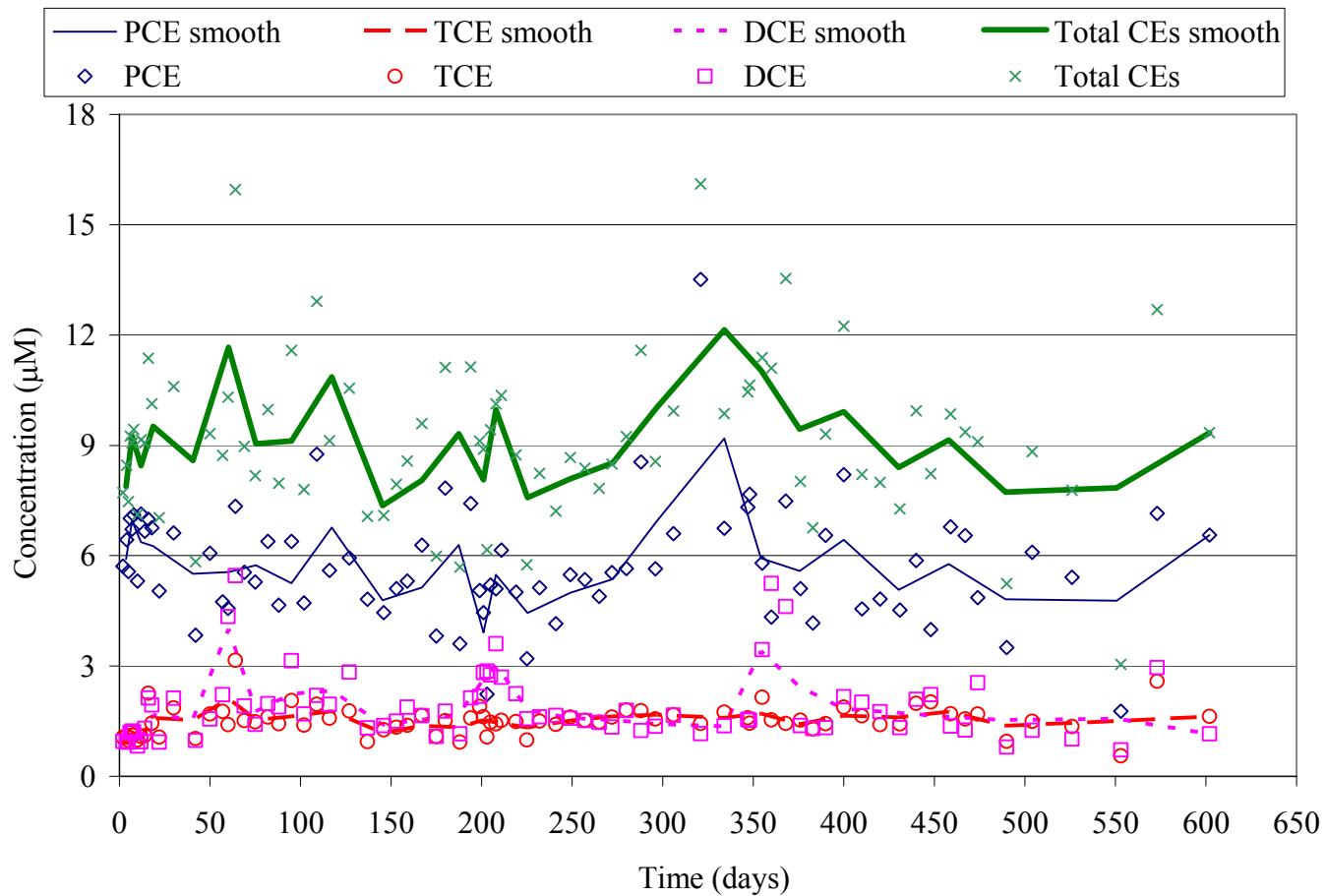
Figure 5.9b. Data points are mean concentrations (avg for three depths) of CEs in MW-8S. Lines were obtained by averaging three consecutive points from both X and Y axes data. Injection of nutrients was carried out on days 0, 56, 197, and 348.
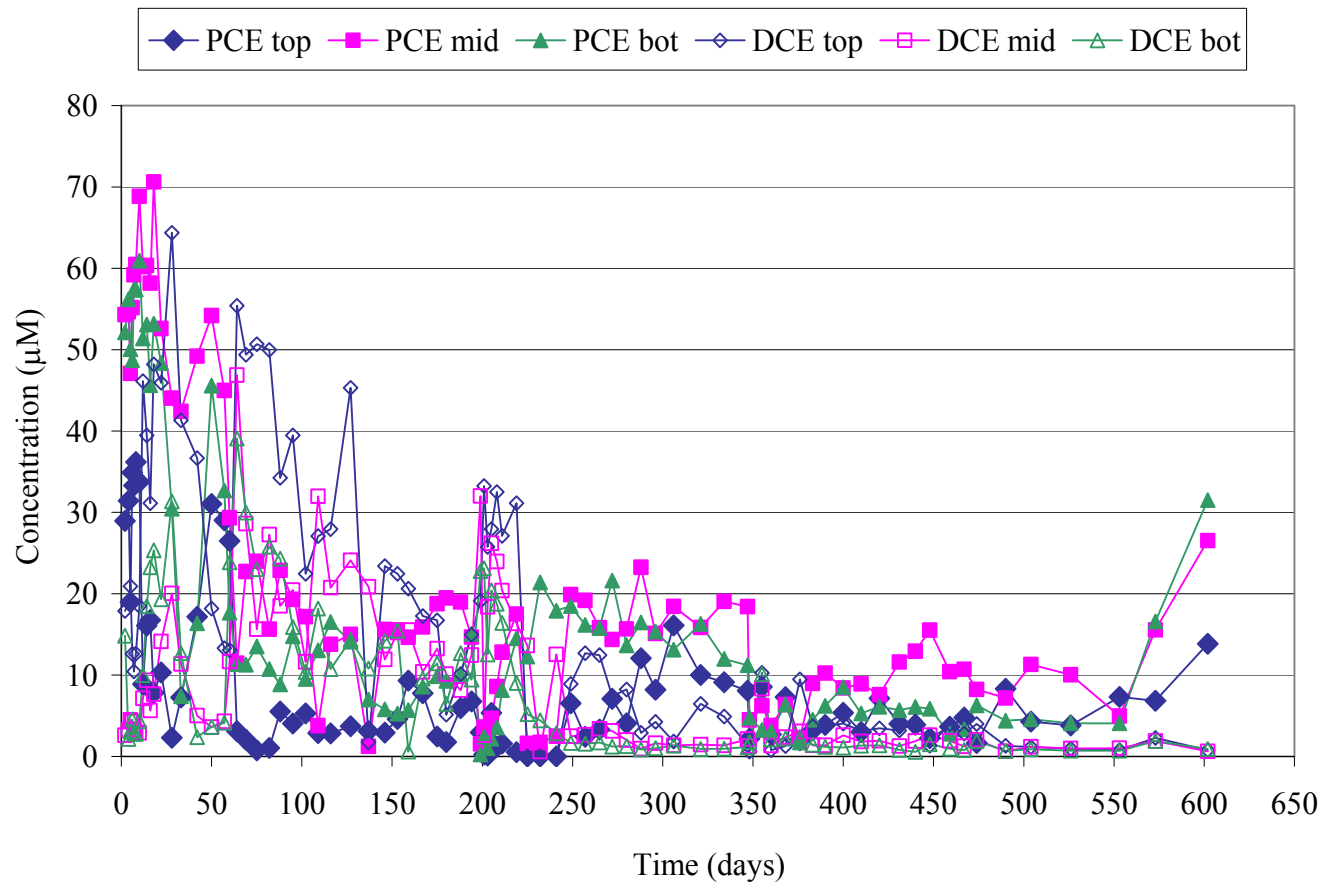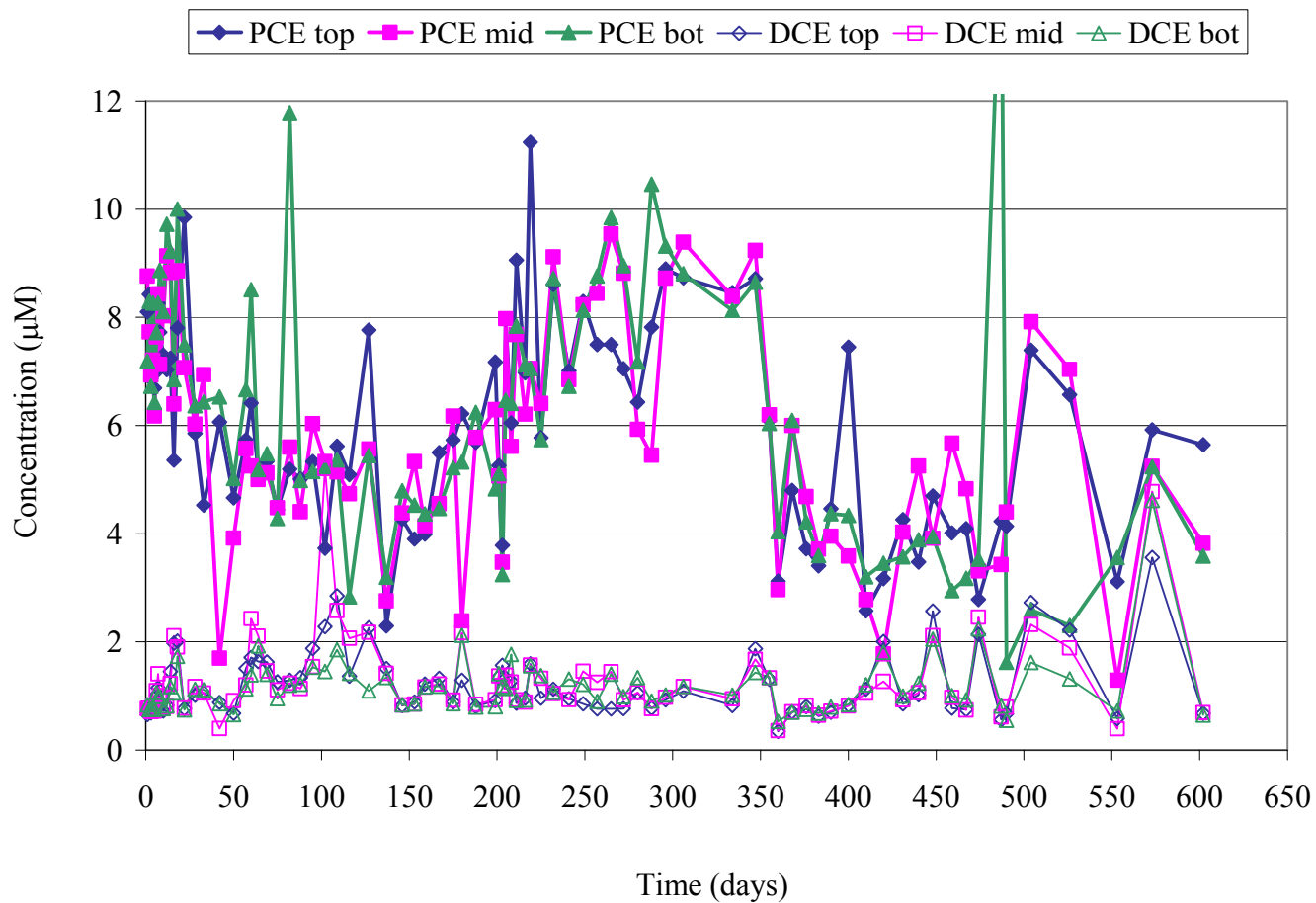
Figure 5.10. Concentrations of PCE and DCE in MW-9S. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
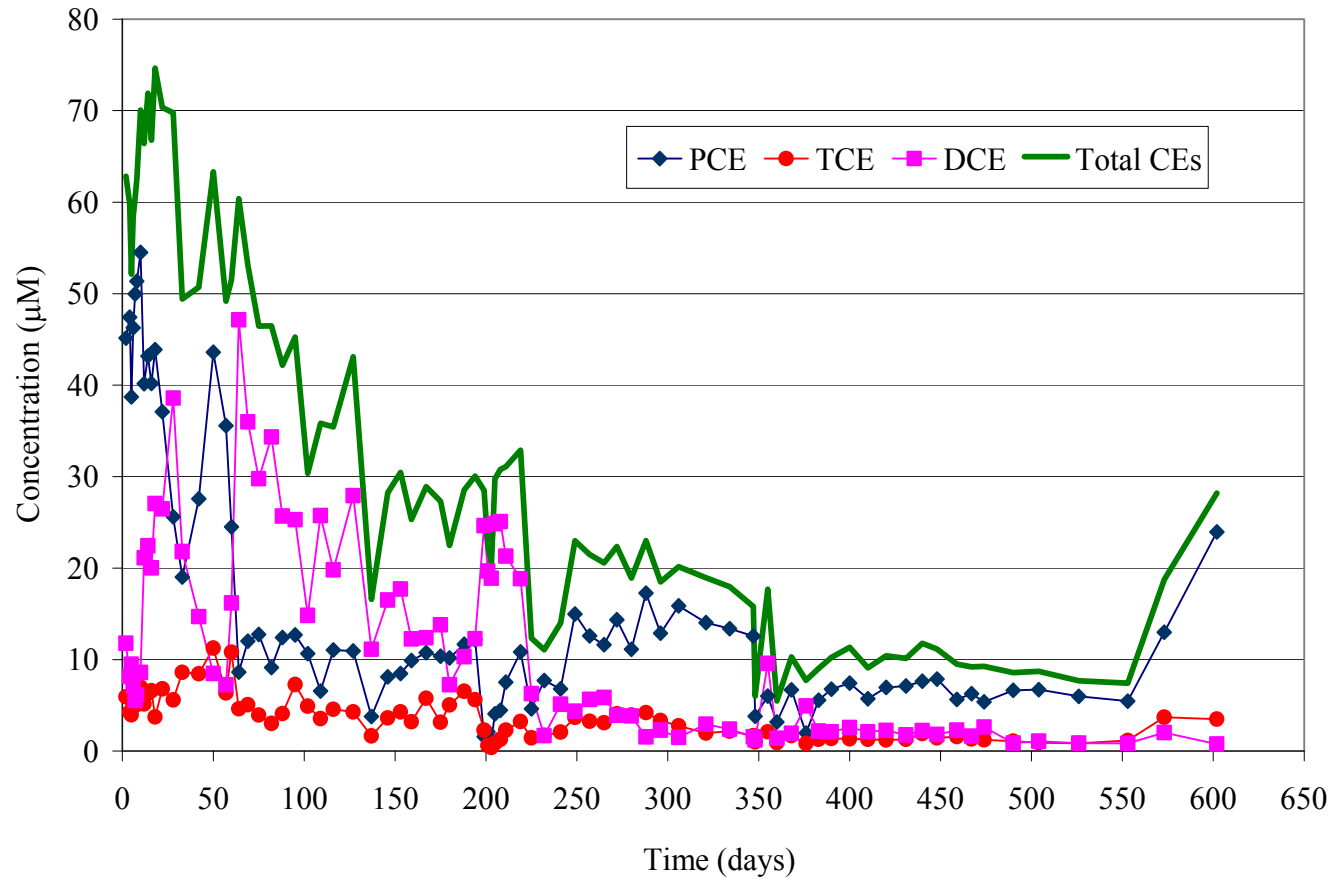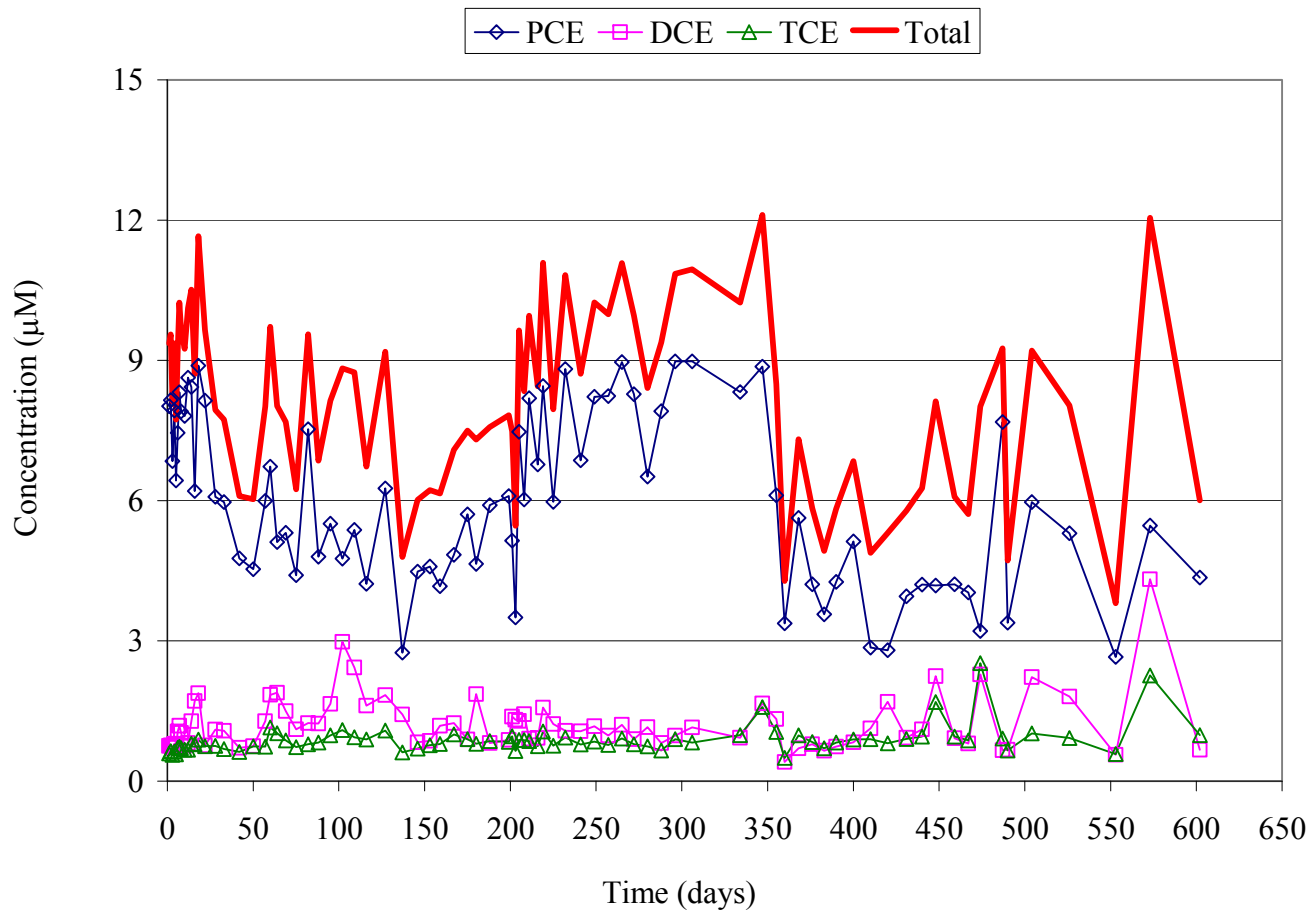
Figure 5.10a. Mean Concentrations (avg for three depths) of CEs in MW-9S. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
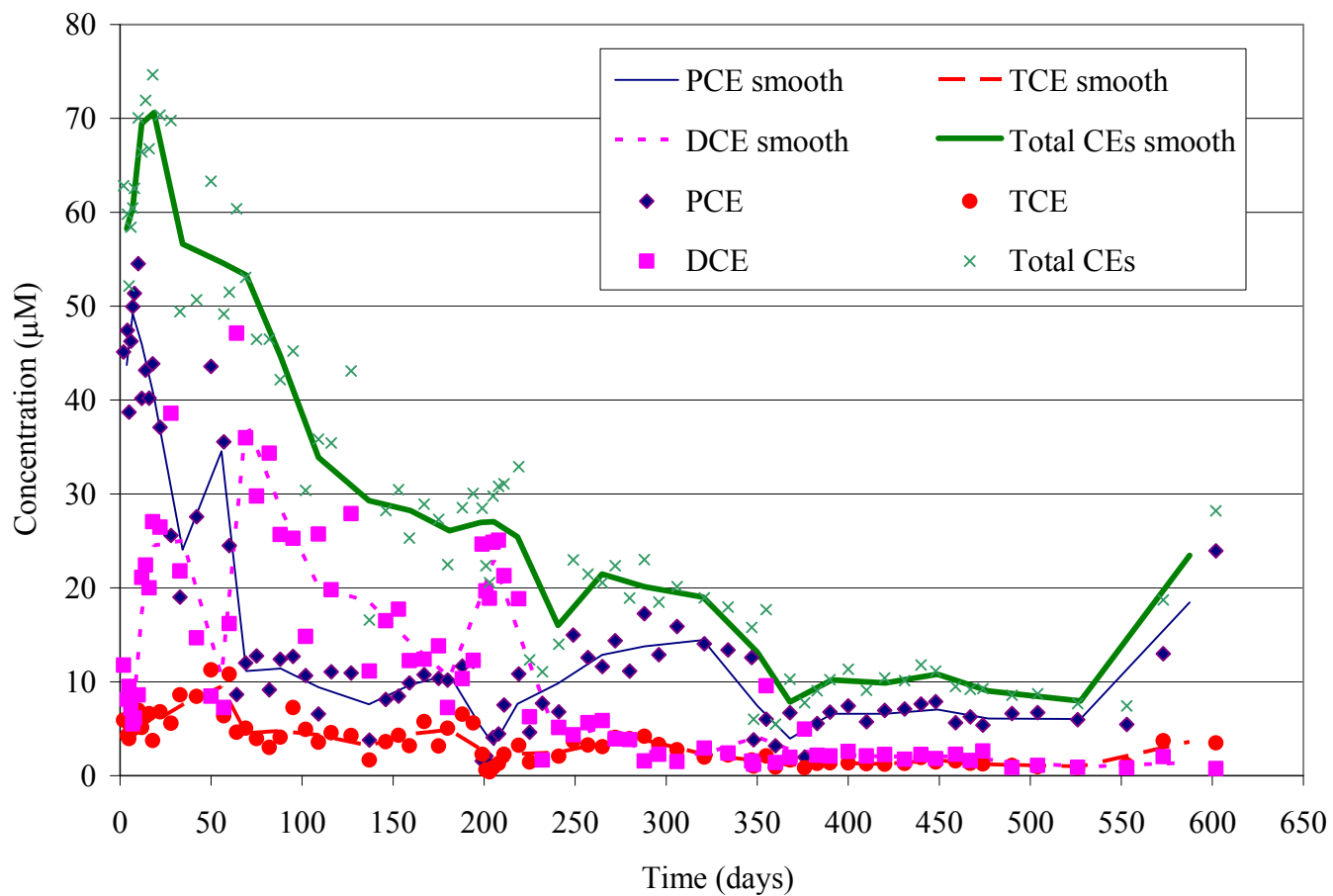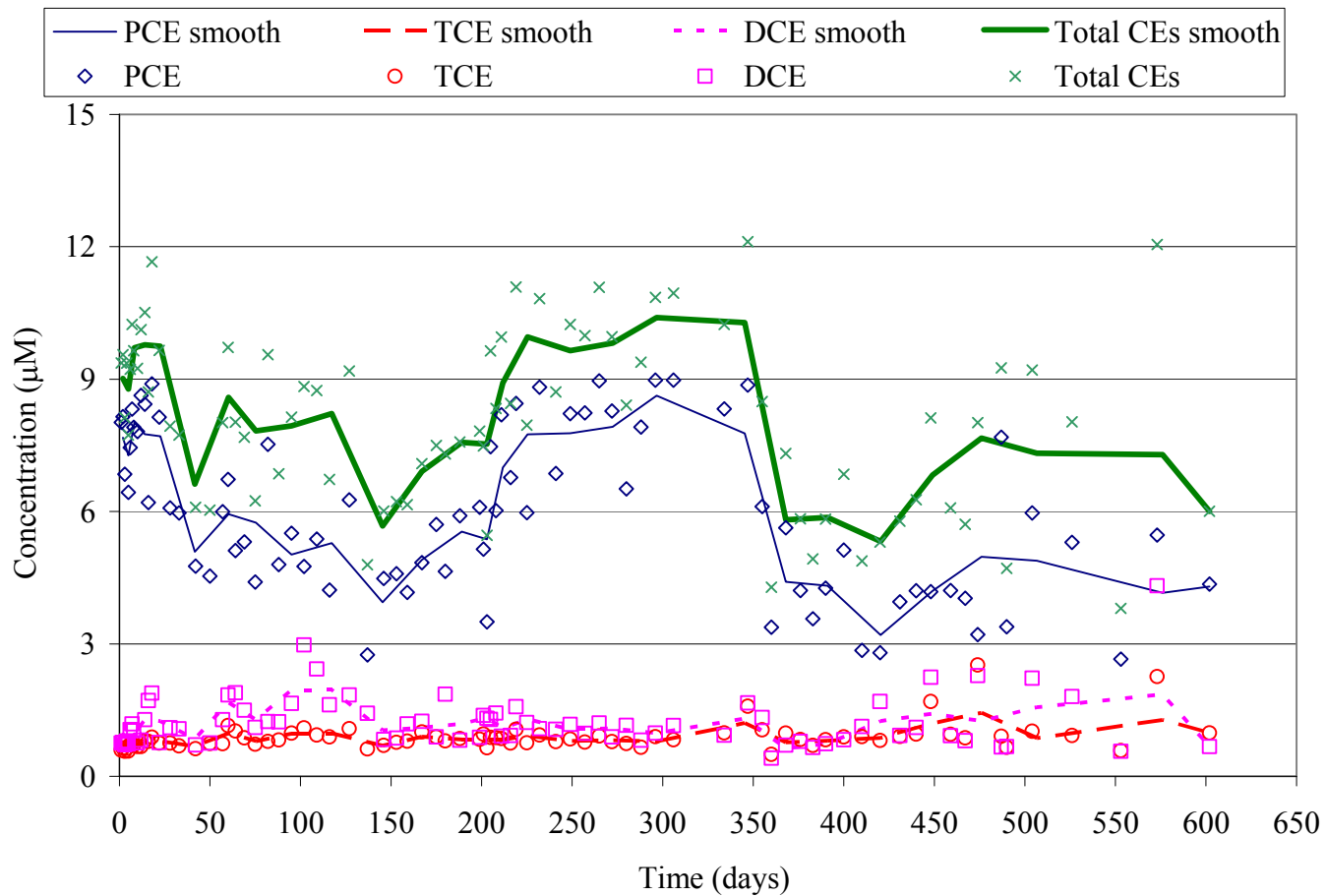
Figure 5.10b. Data points are mean concentrations (avg for three depths) of CEs in MW-9S. Lines were obtained by averaging three consecutive points from both X and Y axes data. Injection of nutrients was carried out on days 0, 56, 197, and 348.
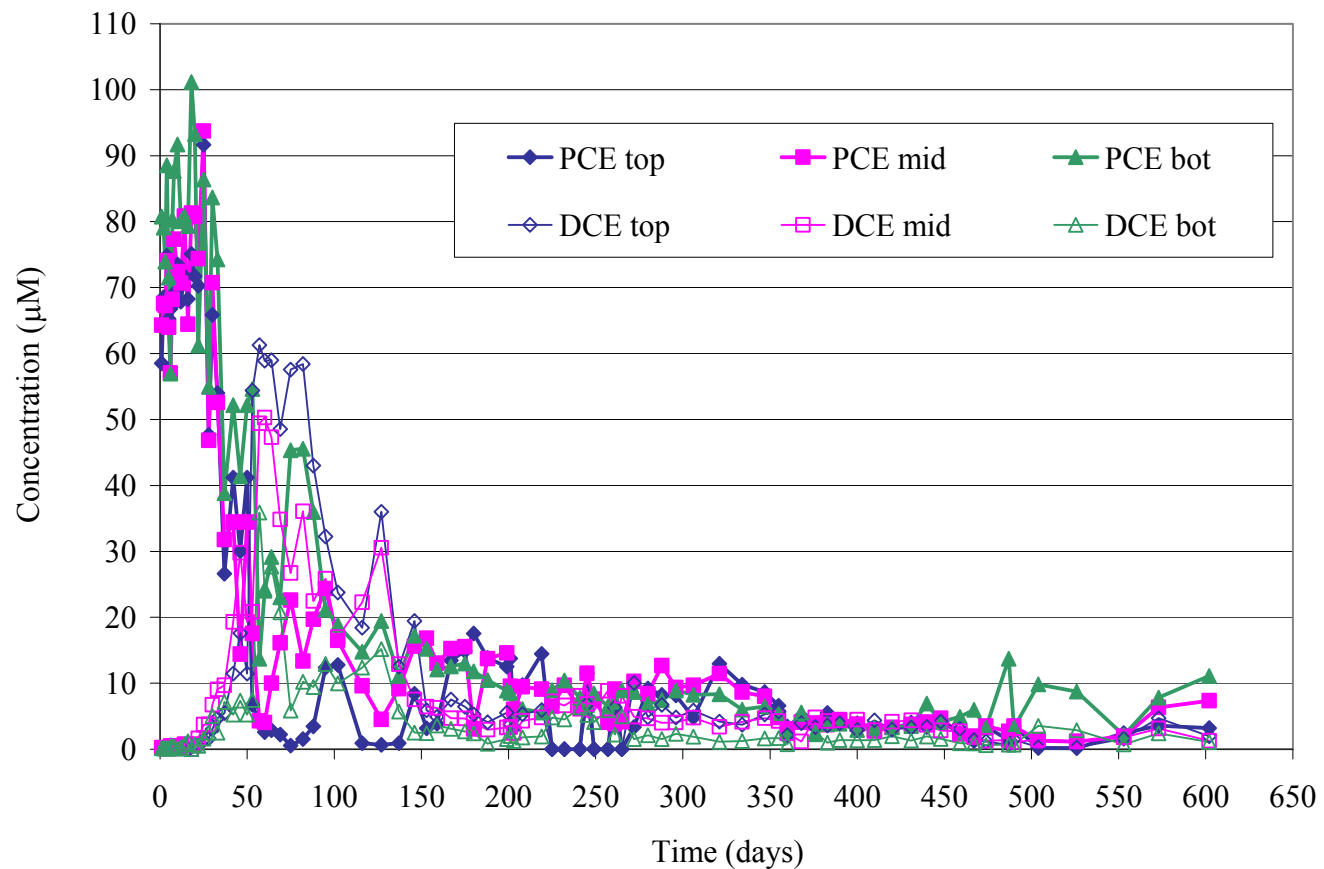
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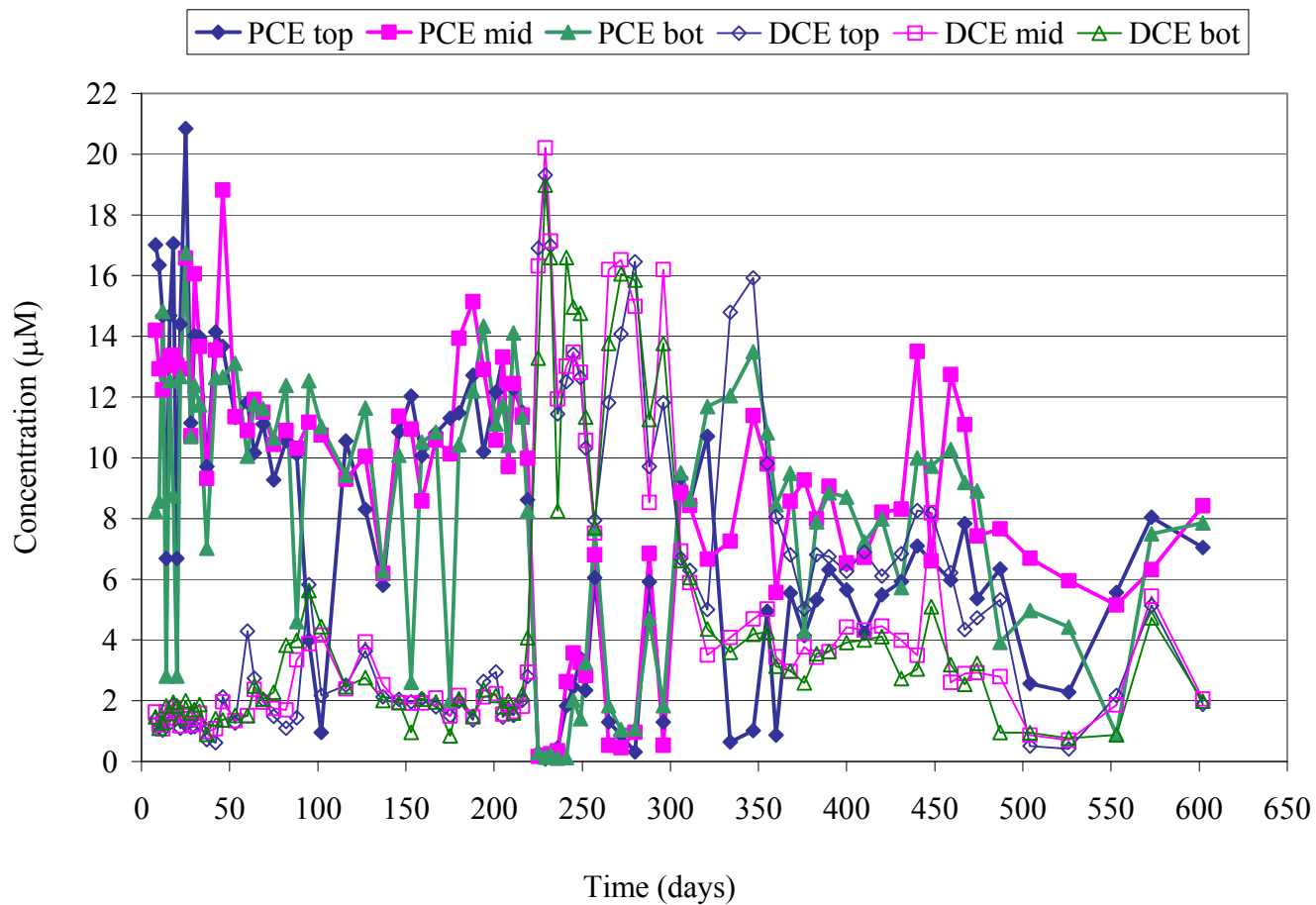
Figure 5.11. Concentrations of PCE and DCE in MW-10S. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348, (Aug 1, 2006).
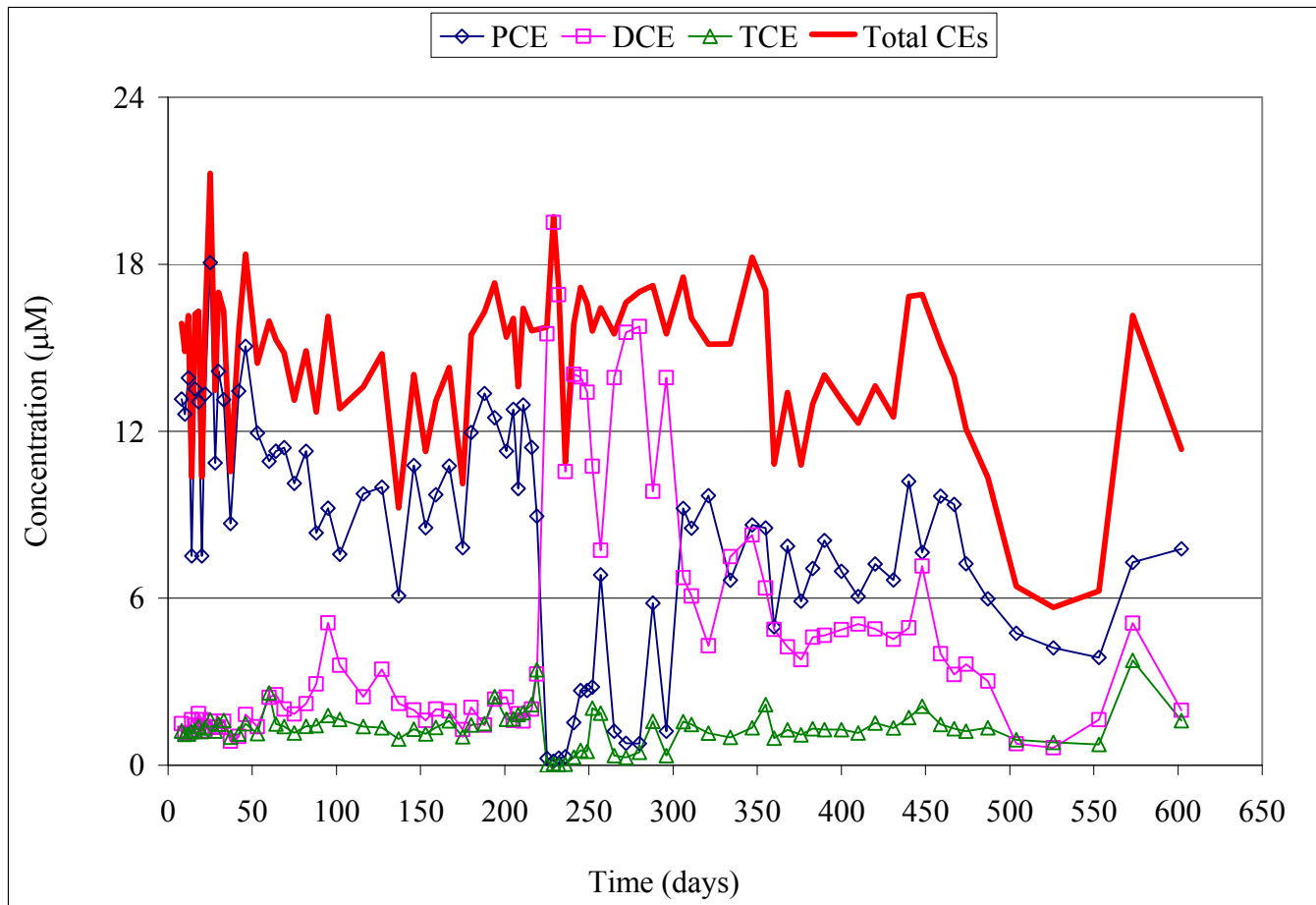
Figure 5.11a. Mean Concentrations (avg for three depths) of CEs in MW-10S. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
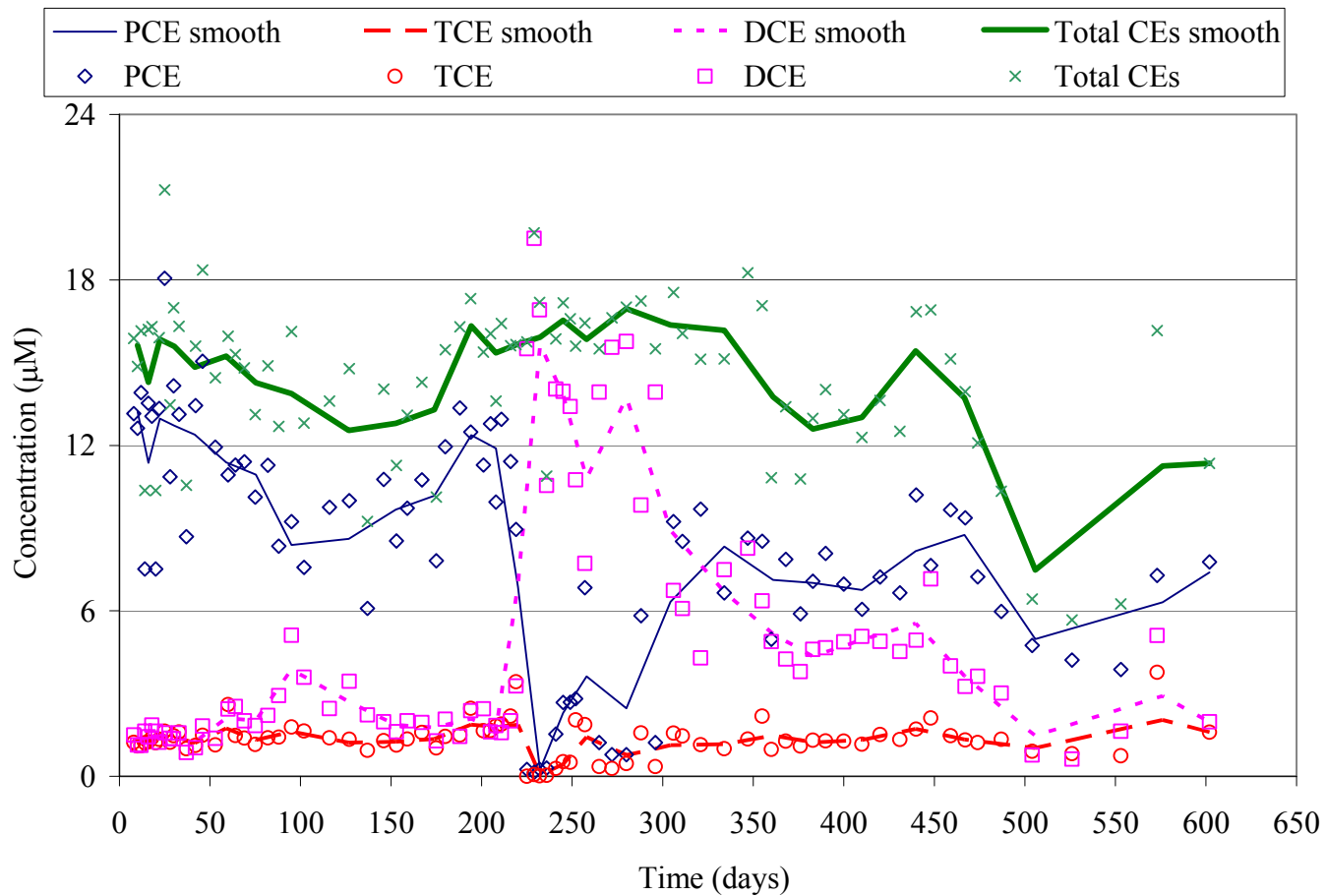
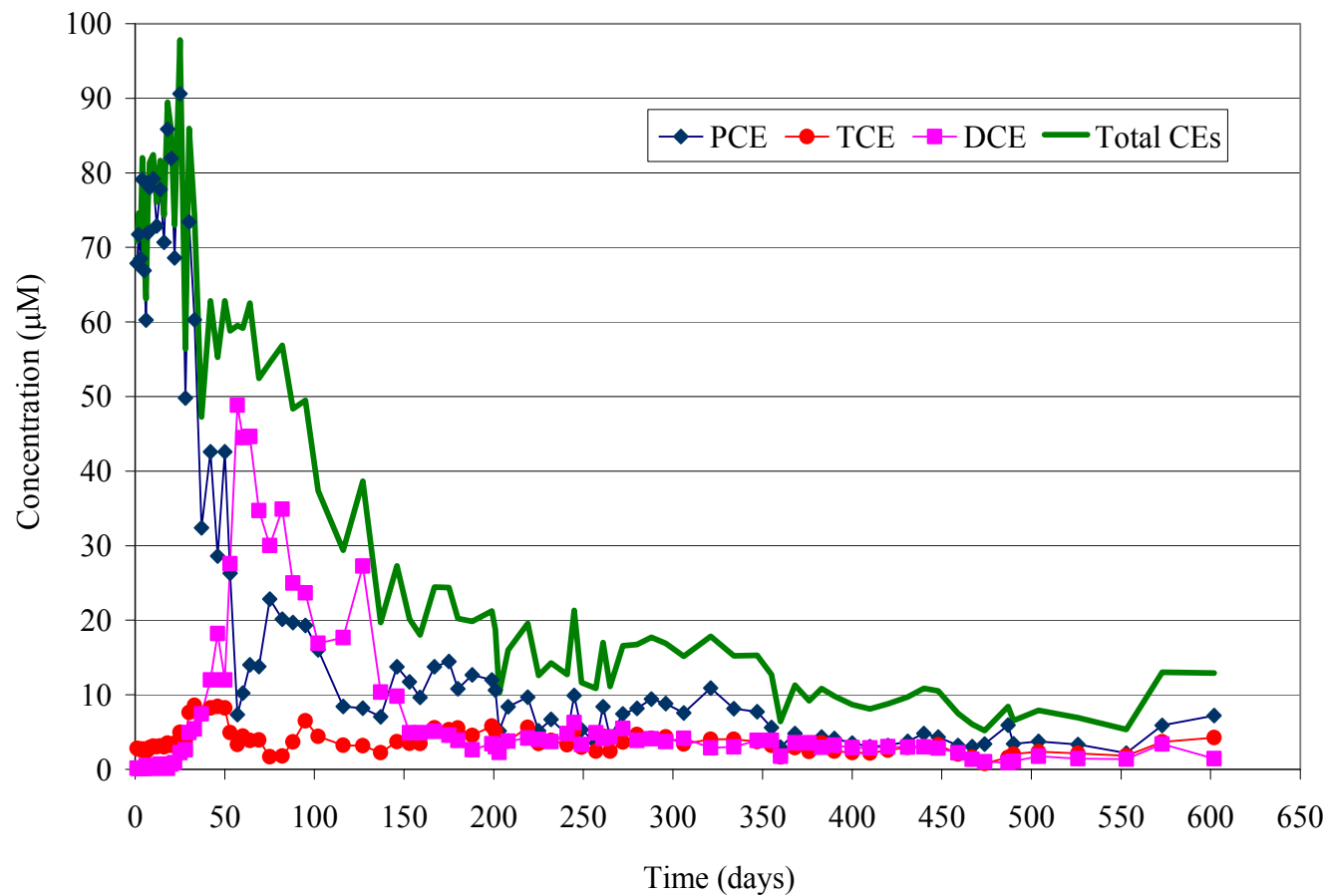Figure 5.11b. Data points are mean concentrations (avg for three depths) of CEs in MW-10S. Lines were obtained by averaging three consecutive points from both X and Y axes data. Injection of nutrients was carried out on days 0, 56, 197, and 348.
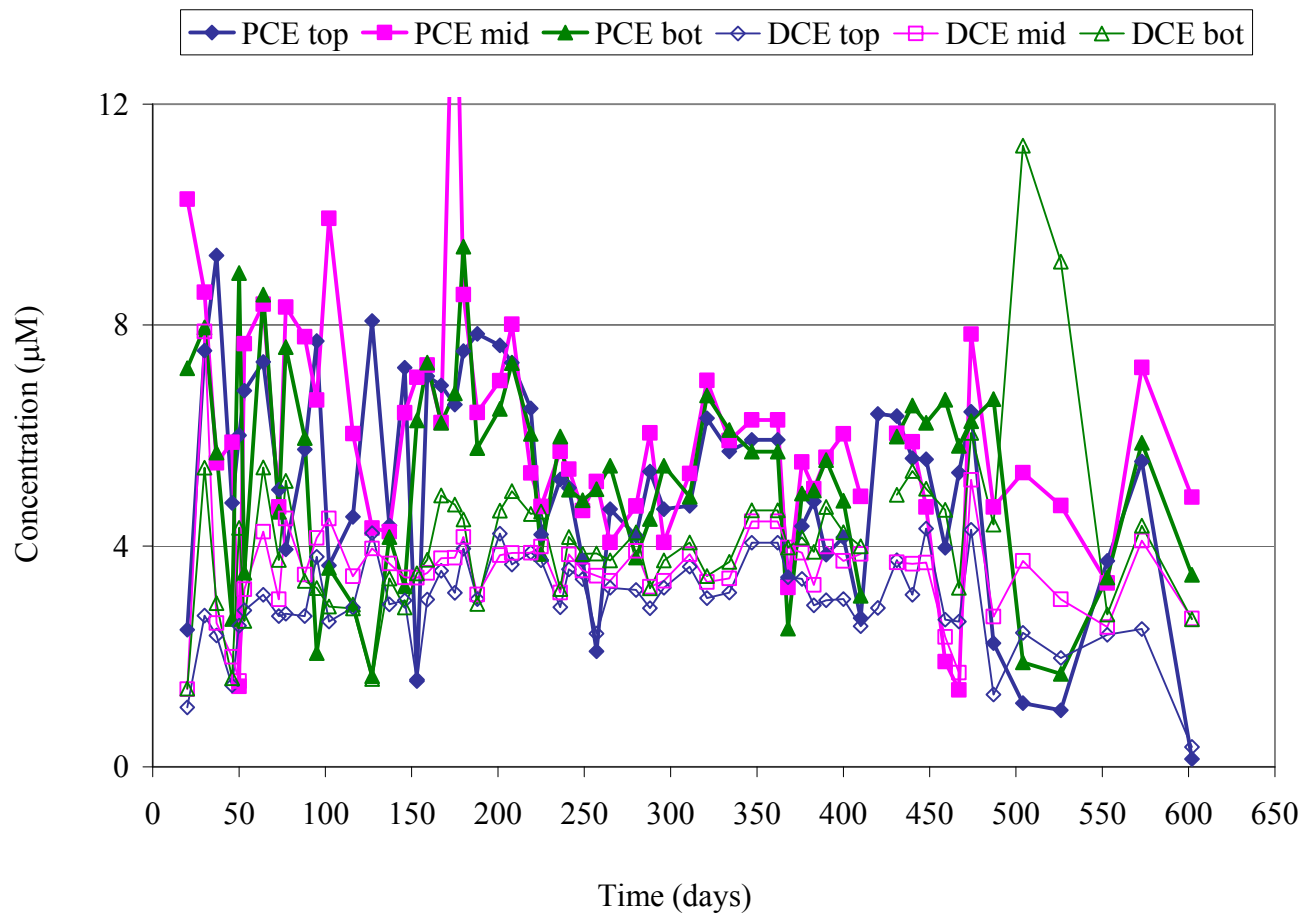
Figure 5.12. Concentrations of PCE and DCE in MW-12S. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
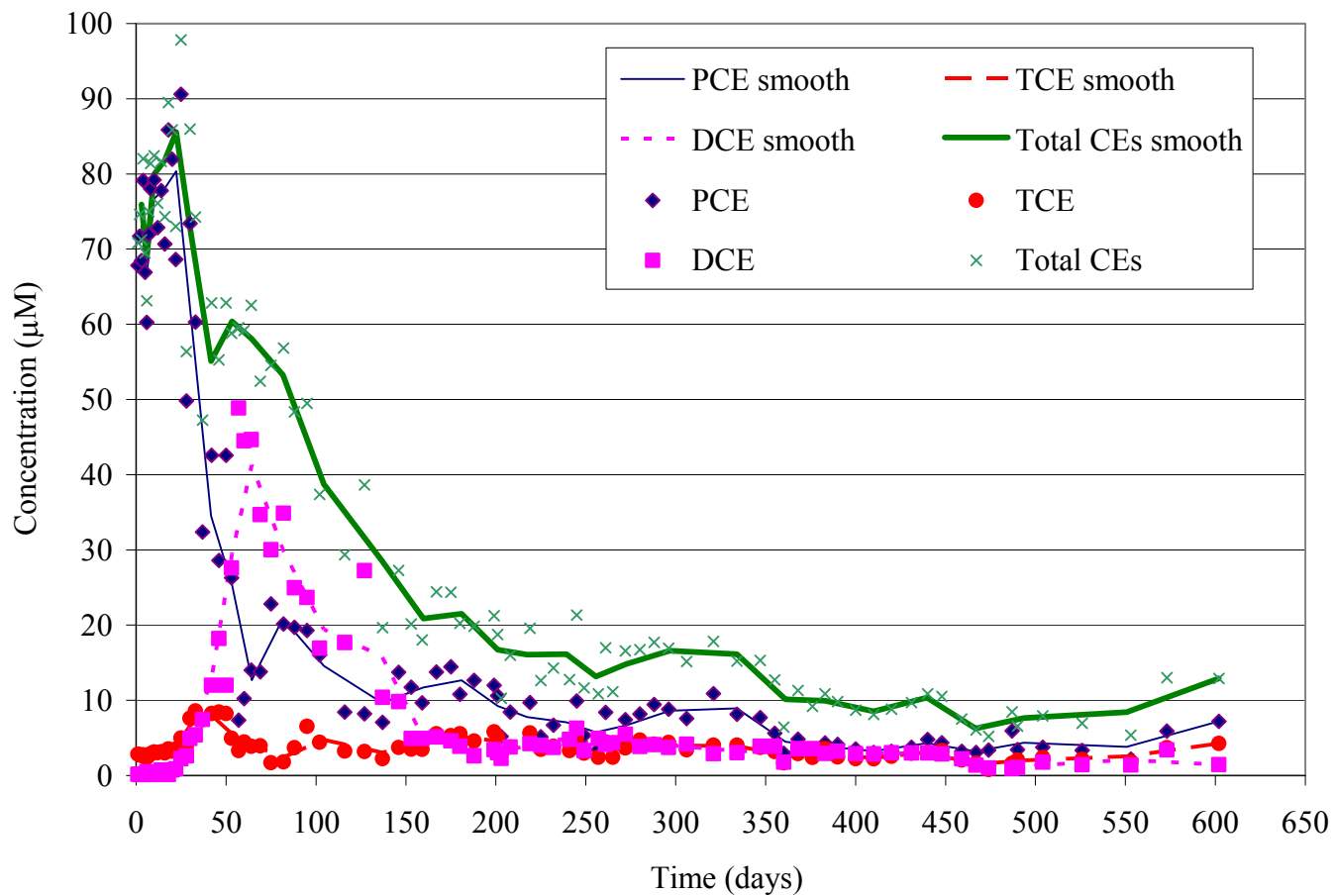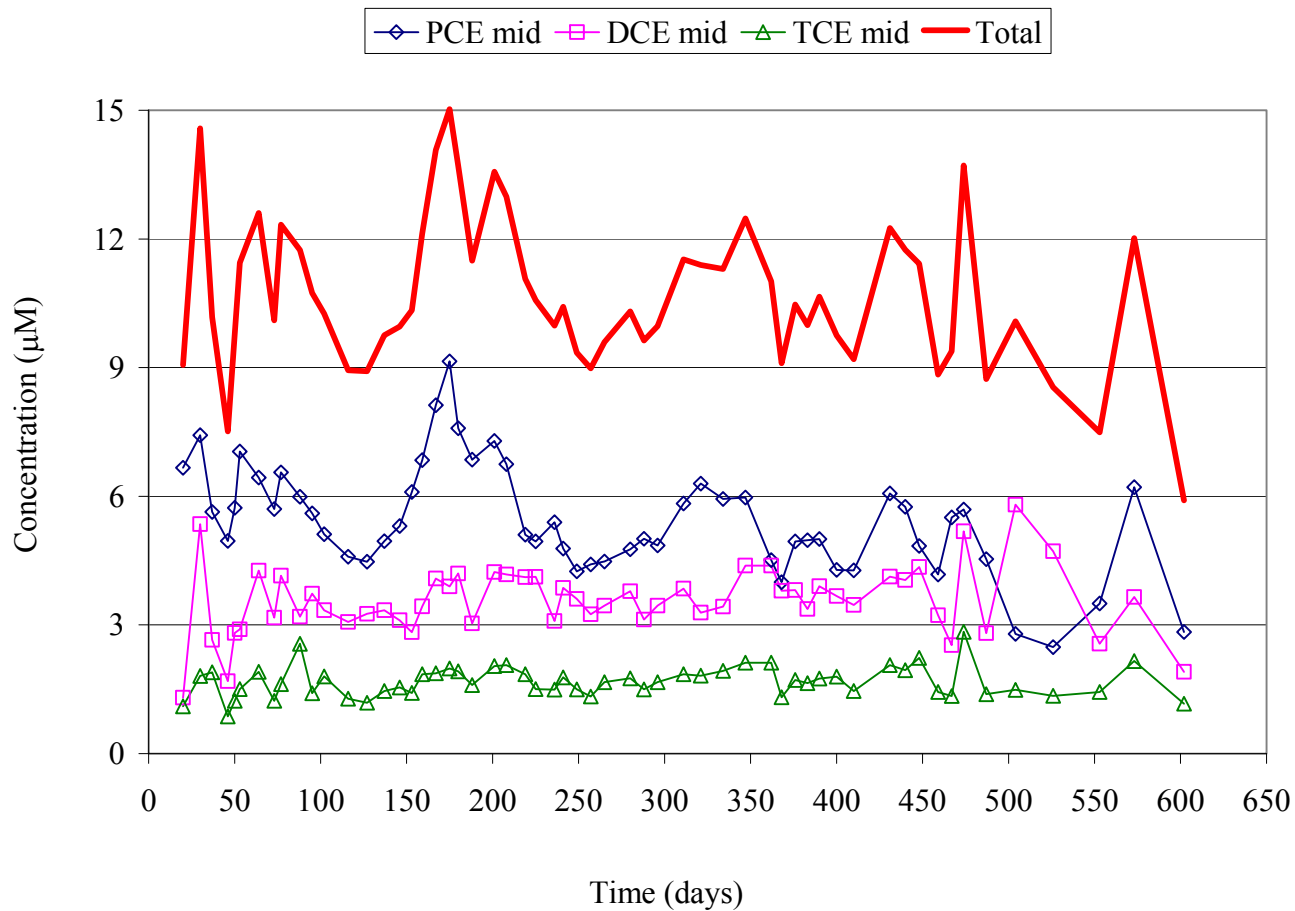
Figure 5.12a. Mean Concentrations (avg for three depths) of CEs in MW-12S. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
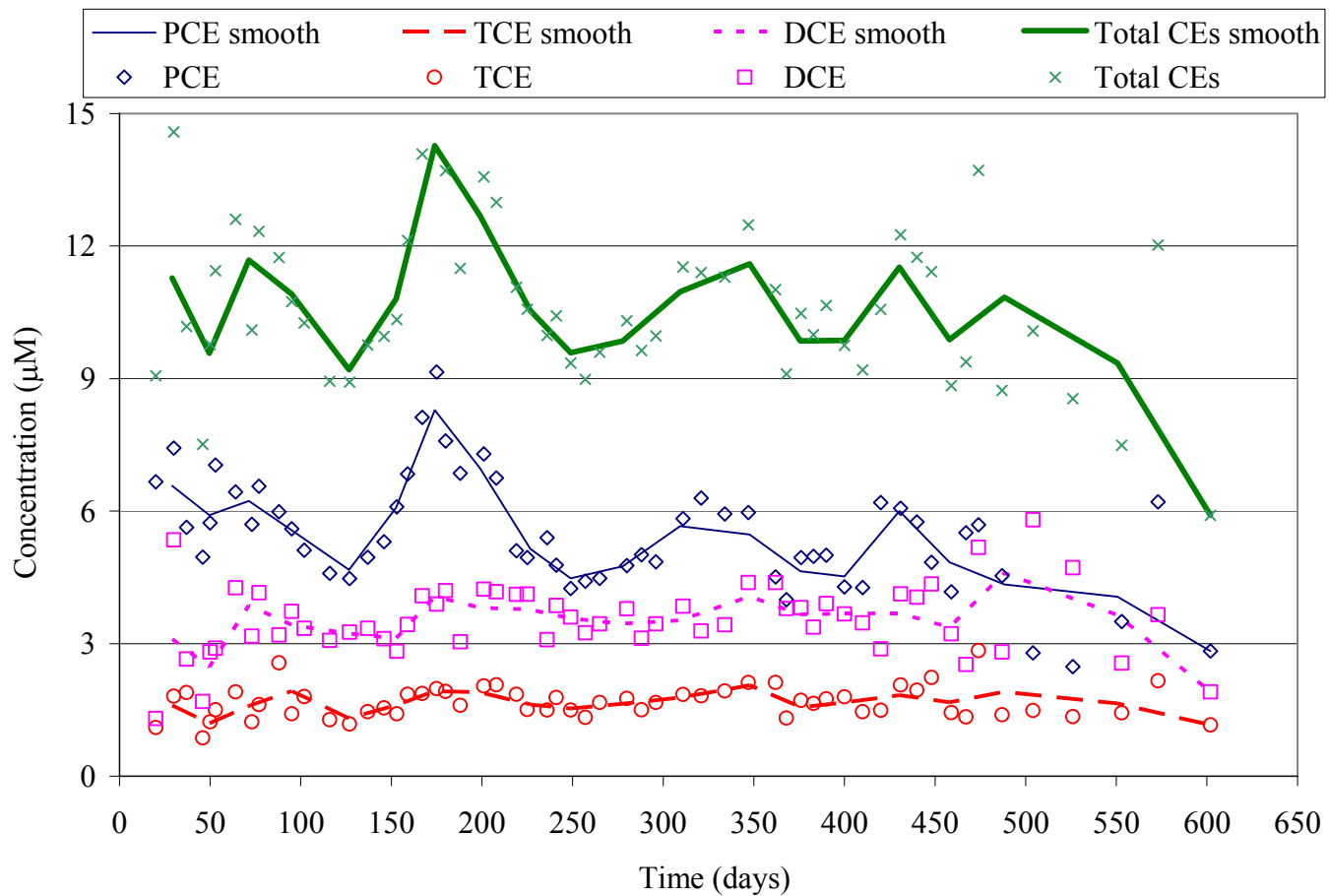
Figure 5.12b. Data points are mean concentrations (avg for three depths) of CEs in MW-12S. Lines were obtained by averaging three consecutive points from both X and Y axes data. Injection of nutrients was carried out on days 0, 56, 197, and 348.
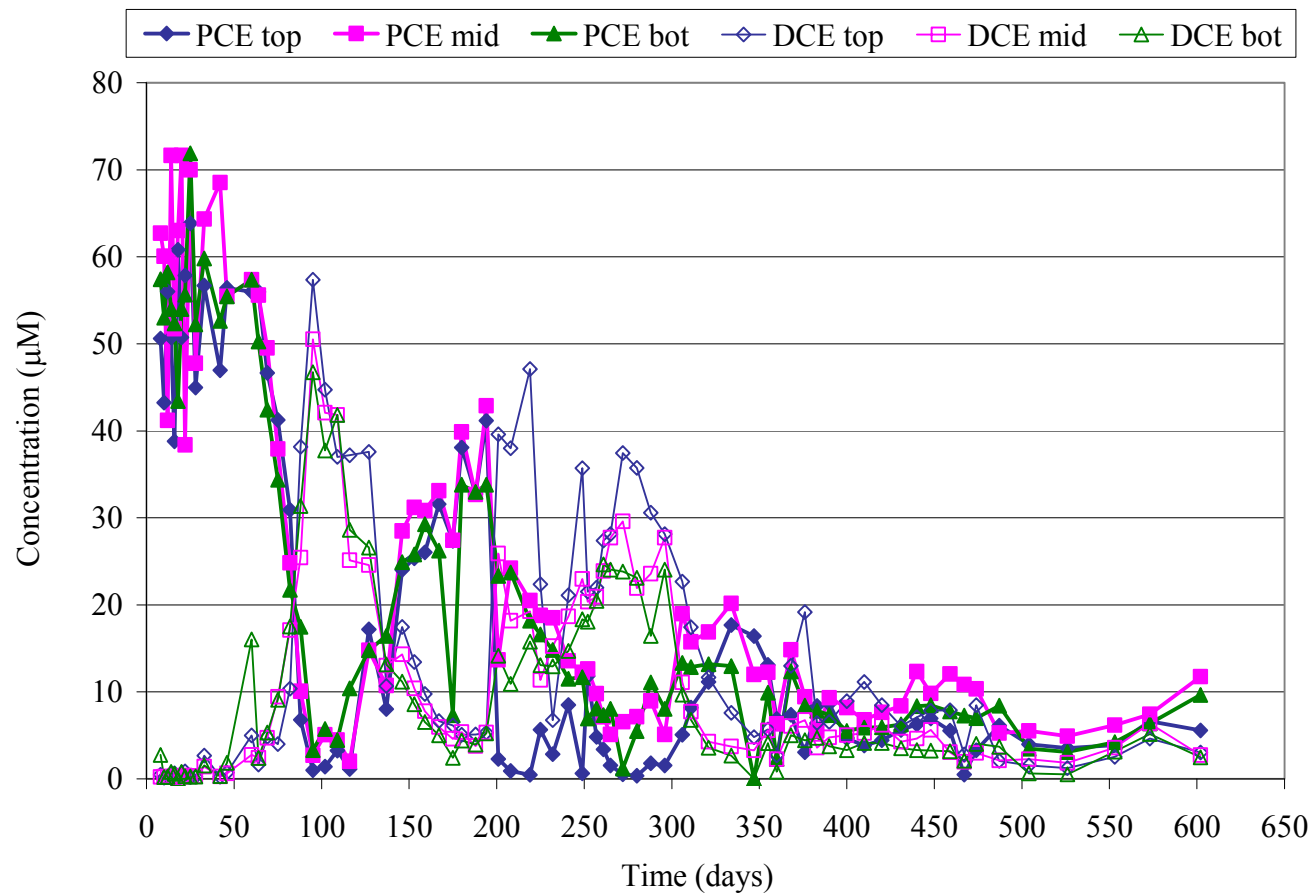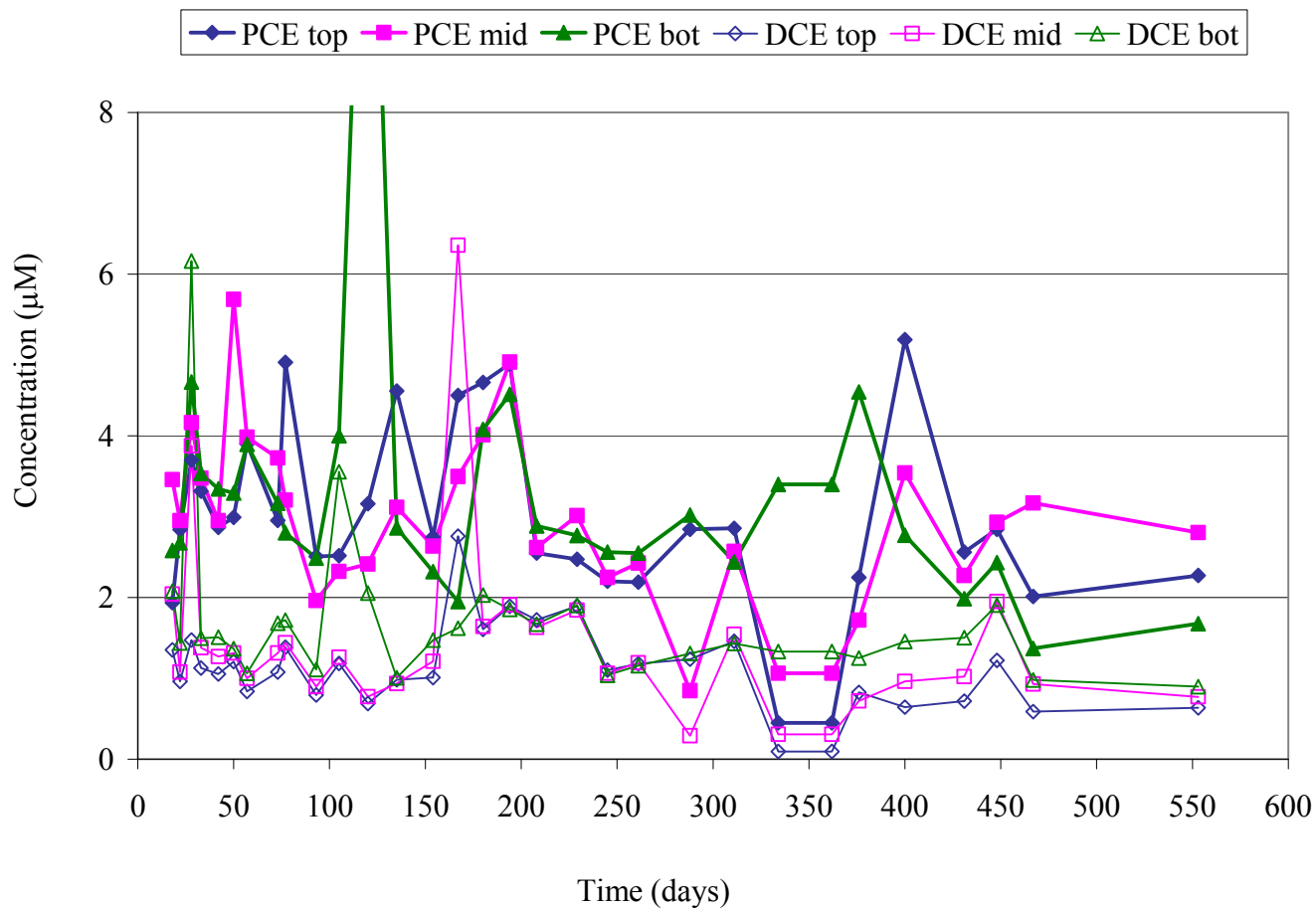
Figure 5.13. Concentrations of PCE and DCE in MW-7S. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
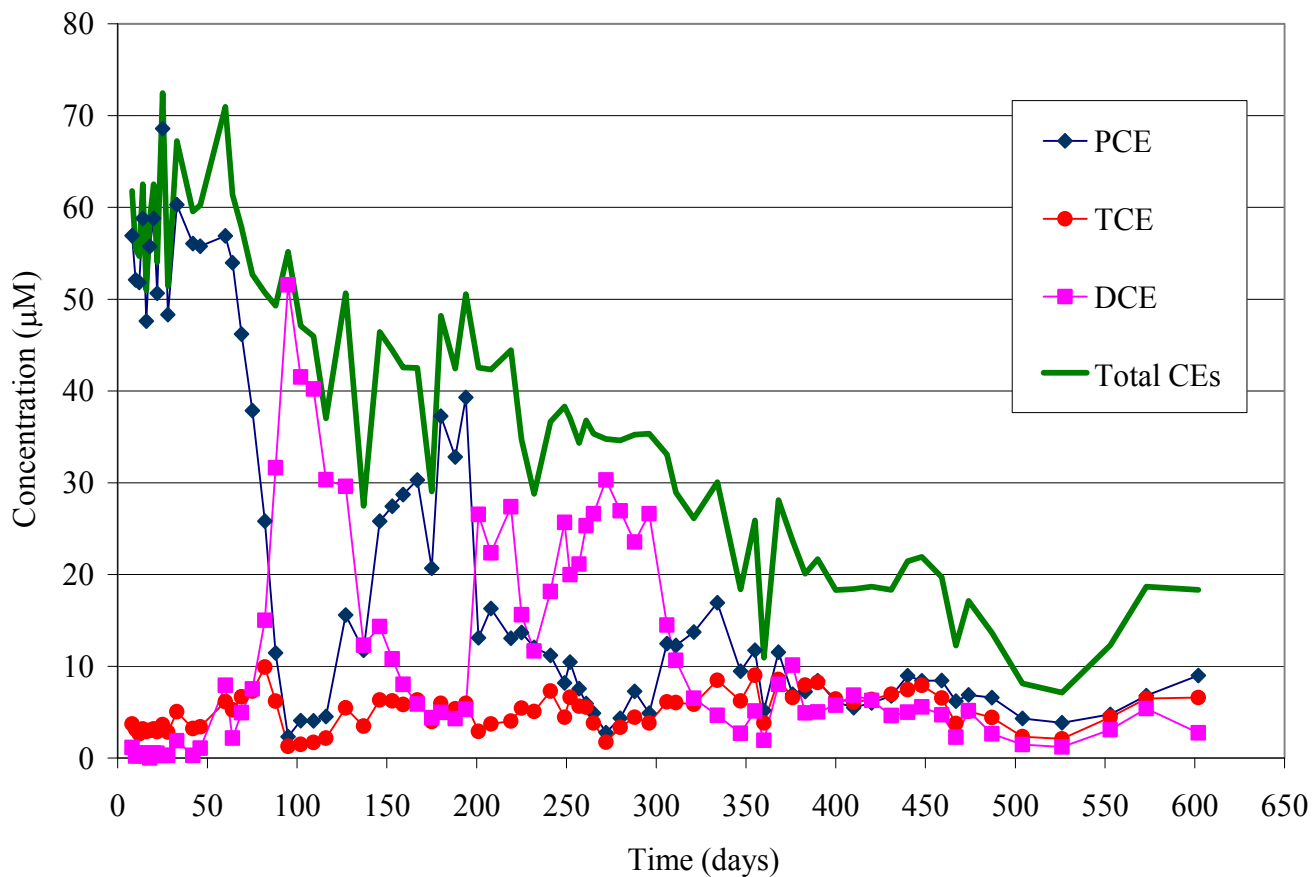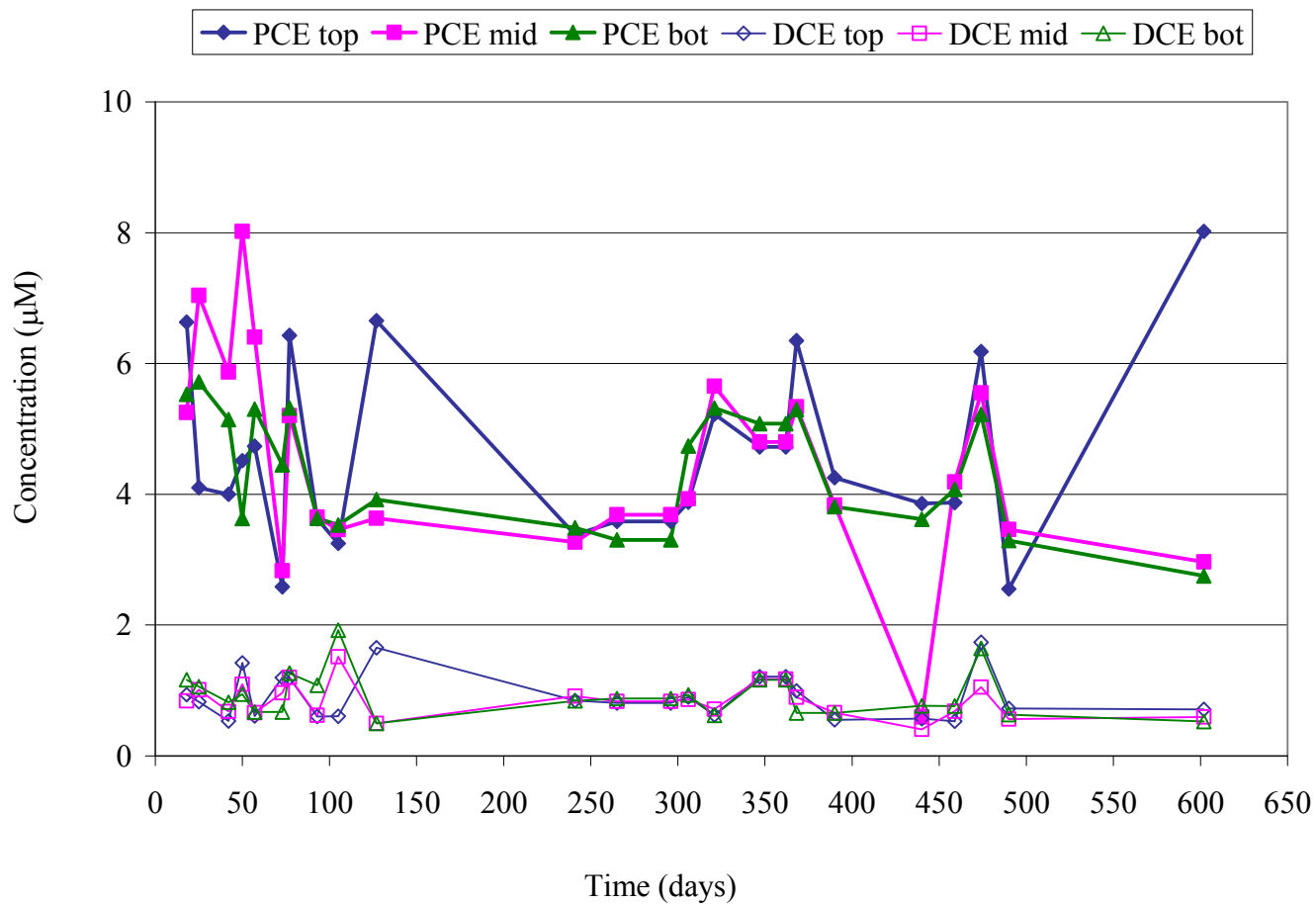
Figure 5.14. Concentrations of PCE and DCE in MW-11S. Injection of nutrients and bromide between MW-8 and MW-9 on day 0 (Aug 18, 2005); nutrients and KB-1 on day 56 (Oct 13, 2005); nutrients and bromide on day 197 (Mar 3, 2006); nutrients on day 348 (Aug 1, 2006).
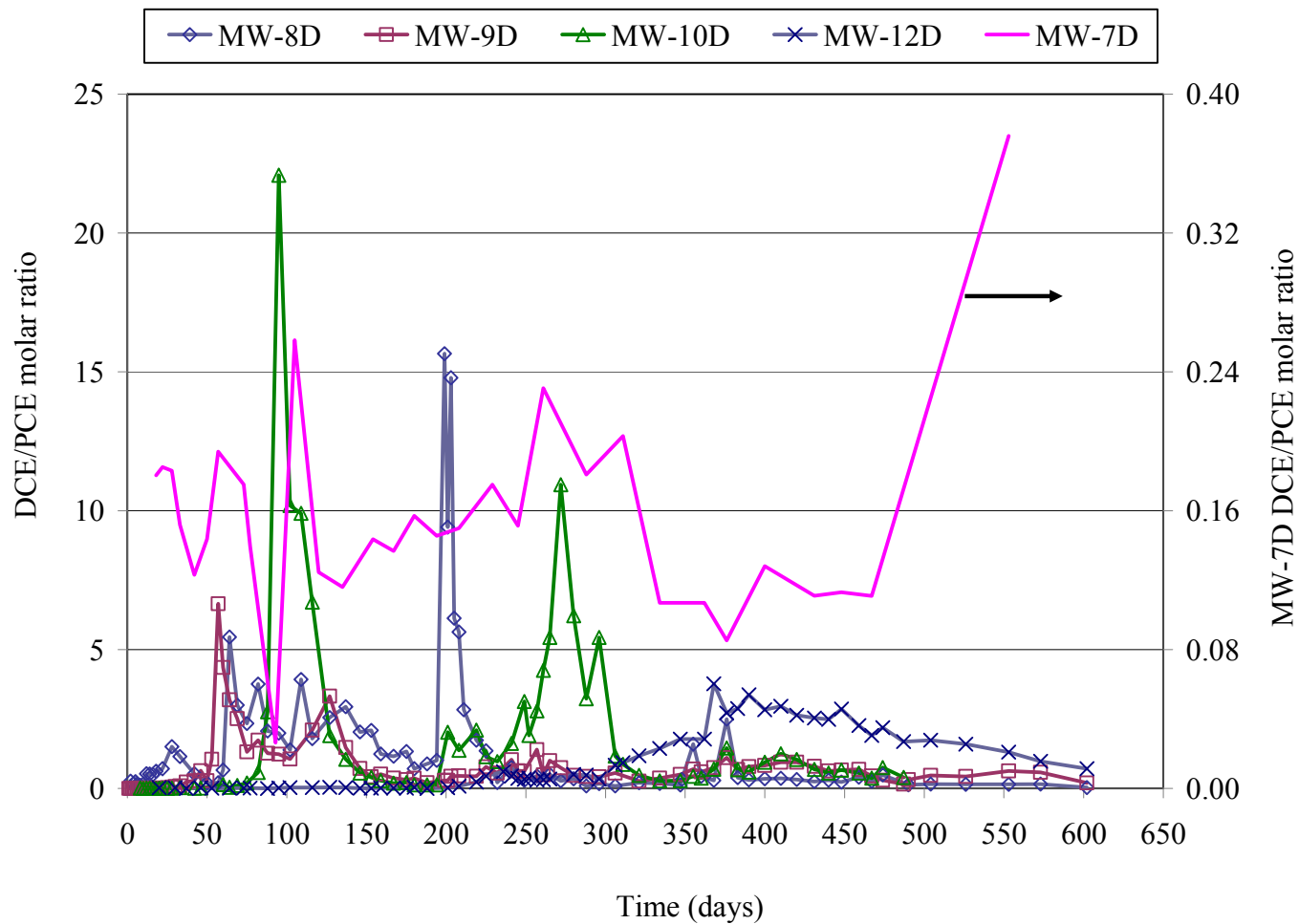
Figure 5.15. Ratio of molar concentrations (mean of values at three depths) of DCE over PCE in the deep zone of the pilot study area (PSA) (MW-8D, MW-9D, MW-10D), a location down-gradient (MW-12D) of PSA, and a location outside the influence (MW-7D) of PSA. MW-7D values are on right hand (secondary) y-axis.
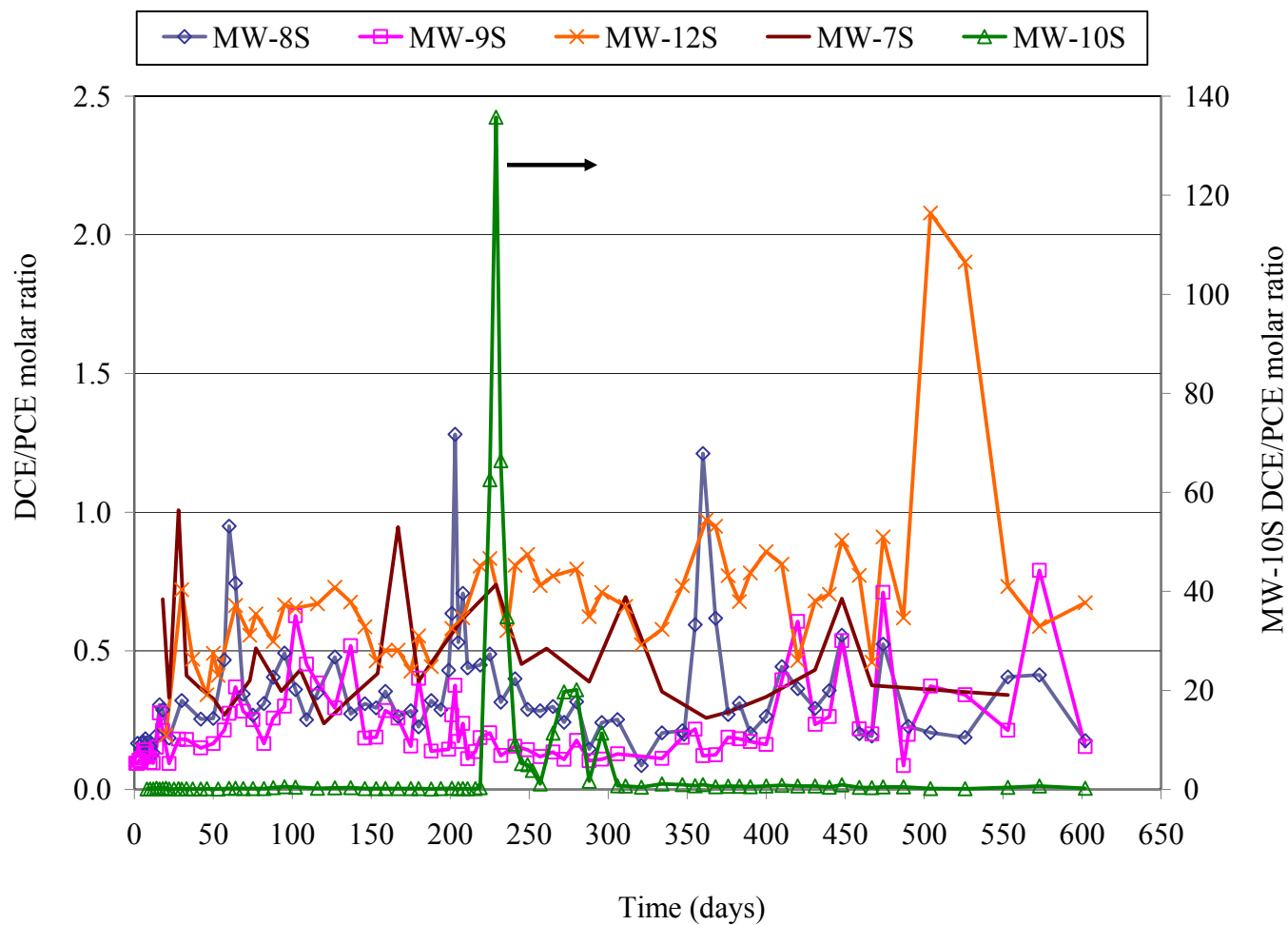
Figure 5.16. Ratio of molar concentrations (mean of values at three depths) of DCE over PCE in the shallow zone of the pilot study area (PSA) (MW-8S, MW-9S, MW-10S), a location down-gradient (MW-12S) of PSA, and a location outside the influence (MW-7S) of PSA. MW-10S values are on right hand (secondary) y-axis.

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Table 5.1. Amount of SOME, lactate, yeast extract and KBr added in the nutrient solution for injection on 8/18/2005.

	Deep Zone		Shallow Zone	
Water (L)	188		197	
Nutrient/Tracer	Mass (kg)	Concentration (%)	Mass (kg)	Concentration (%)
SOME	8	4	2	1
Lactate	0.8	0.4	0.8	0.4
YE	2	1	0.2	0.1
KBr	0.2	0.1	0.2	0.1

Table 5.2. Amount of glucose and yeast extract added in the anaerobic chase water for injection on 8/18/2005.

	Deep Zone		Shallow Zone	
Water (L)	200		200	
Nutrient	**Mass (gm)**	**Concentration (%)**	**Mass (gm)**	**Concentration (%)**
Glucose	40	0.02	40	0.02
YE	10	0.005	10	0.005

Table 5.3. Amount of SOME, lactate, yeast extract and KBr added in the nutrient solution for injection on 3/3/2006.

	Deep Zone		Shallow Zone	
Water (L)	197		197	
Nutrient/Tracer	**Mass (kg)**	**Concentration (%)**	**Mass (kg)**	**Concentration (%)**
SOME (L)	2	1	2	1
Glucose	0.02	0.01	0.02	0.01
YE	0.5	0.25	0.5	0.25
KBr	0.4	0.2	0.4	0.2

Table 5.4. Amount of glucose and yeast extract added in the anaerobic chase water for injection on 3/3/2006.

	Deep Zone		Shallow Zone	
Water (L)	200		200	
Nutrient	**Mass (gm)**	**Concentration (%)**	**Mass (gm)**	**Concentration (%)**
Glucose	20	0.01	20	0.01
YE	10	0.005	10	0.005

Appendix 5.A. Estimation of contaminant mass present in the aquifer

In this site, the width of the chlorinated ethenes (CEs) contaminated plume is about 95 ft (29.0 m). The width of the plume is defined by MW-11D on the south and MW-2 on the north (see Figure 4.2). As shown in Figures 5.8 and 5.A.1, the concentrations of PCE and other CEs are very low at MW-11D (less than 6 μM) and MW-2 (less than 1 μM during 2 out of 10 sampling events by KDHE's contractor; the concentrations were in the range 0.3 to 6.6 μM during the period from 2000 to 2006 compared to about 200 μM at the source). The distance from MW-2 to MW-7D is about 50 ft (15.3 m). MW-7D to MW-11D is about 45 ft (13.7 m).

The approximate thickness or height of the shallow zone (15 ft (4.57 m) bgs to 35 ft (10.67 m) bgs) = 20 ft (6.1 m). The approximate thickness or height of the deep zone (35 (10.67 m) to 55 ft (16.76 m) bgs) = 20 ft (6.1 m).

The subsurface soil is heterogeneous and the concentrations of the contaminants vary at each location in the saturated zone. To estimate the total mass of PCE present in the aqueous phase in the plume some simplifying assumptions are made.

1. Groundwater (GW) PCE concentration remains constant along the plume width. Actually the PCE concentration decreases as we go to the fringes of the plume and therefore, this assumption is not true; however, it helps to simplify the calculations and overestimates the mass of PCE, which can be considered as a safety factor in the remediation design. Only the mass of PCE is estimated and not the degradation compounds TCE and DCE due to their relatively low concentrations observed in the plume. The omission of TCE and DCE in contaminant mass computation is also another justification for overestimating the PCE mass.

2. GW PCE concentration decreases linearly along axis of the plume.

For the purpose of estimation of PCE mass in the aquifer, the plume is divided into three sections as shown in Figure 5.A.1. The deep aquifer is divided into three sections D1, D2 and D3; the shallow aquifer as S1, S2 and S3. The sections are chosen based on the location of the monitoring wells 5, 8, 12, and 14.

The widths of all sections are 95 ft (291.0 m), the width of the plume. The lengths are approximately 100 ft (30.5 m), 80 ft (24.4 m) and 600 ft (182.9 m) for section 1, section 2 and section 3 respectively. Based on the above assumptions, the mass of PCE is estimated as shown in Table 5.A.1.

From Table 5.A.1, the total PCE present in the aqueous phase is 113 kg. The amount of PCE present in the deep zone is 101 kg (about 90 %) and the amount of PCE present in the shallow zone it is 11.7 kg (about 10 % of the total). The "Slope" column in Table 5.A.1 indicates the gradient of PCE concentration with distance. For example in section D1, the concentration of PCE decreases from 30 mg/L to 15 mg/L at the rate of 0.5 mg/L/ft.

5.A.1. Estimation of sorbed phase PCE mass

From the aqueous phase PCE, we can estimate the mass of PCE adsorbed to the organic carbon in soil, using the partition coefficient for PCE to organic carbon.

$$Log[Koc] = 2.5; Koc = 316 \text{ (U.S. EPA, 1994)}$$

The extent to which sorption occurs in aquifers is expected to be limited compared to topsoil, primarily due to the lower organic carbon content. However, sorption mechanisms other than the hydrophobic partitioning process (which is dominating in topsoil) and which are not related to the organic carbon content may occur. Pedersen et al. (1991) and Christensen et al. (1996) reported that in pristine sandy aquifers the organic carbon content is usually low, with total organic carbon concentrations of about 1-4 mg/L in the groundwater and organic carbon fractions in the sediment of 0.01-0.48 % (Pedersen et al., 1991; Christensen et al., 1996). Sediment samples collected from the Columbia Aquifer in the Atlantic Coastal Plain of Virginia contained organic carbon in the range 0.02 % to 0.12 %. Using the extracted carbon as the sole electron donor source, tetrachloroethene was transformed to cis-1,2-dichloroethene and vinyl chloride in anaerobic enrichment culture experiments (Rectanus et al., 2007).

The soil samples collected during drilling of MW-9D were analysed at the Kansas State University soil testing laboratory, for minerals, nitrogen and carbon content. The total carbon fraction, for soil samples collected at five feet (1.5 m) intervals, varied from 0.03 % at 47.5 ft (14.5 m) bgs to 0.72 % at 7.5 ft (2.3 m) bgs.

The soil samples collected at different depths during the installation of monitoring wells were analysed for organic carbon fraction at the soil analysis laboratory at KSU. The organic

carbon fraction, in the shallow zone, reported for depths 17.5 ft, 22.5 ft, 27.5 ft and 32.5 ft are 0.36 %, 0.17 %, 5.3 % and 0.49 %, respectively. The last two values are anomalous; however, a repeated analysis yielded the same results. The high organic carbon content may just be a local effect and this value may not be uniform at different locations. The organic carbon fraction reported for depths 37.5 ft, 42.5 ft and 47.5 ft are 0.08 %, 0.06 % and 0.03 %, respectively. Assuming organic carbon is 0.1 % in the deep zone soil in the site, which can be considered as an upper limit and thus a safety factor to prevent underestimation of the amount of PCE, the effective partition coefficient of PCE to soil is

$K_d = K_{oc} * f_{oc} = 316 * 0.001 = 0.316$ (mg PCE/kg dry soil)/(mg PCE/L water)

For porosity of 0.3, the volume ratio of soil to water is 0.7/0.3.

If the soil density is assumed to be about 2.65 g/cc, the mass ratio, therefore is,

$$\frac{2.65 * 0.7g}{0.3g} = 6.2 \frac{g \text{ soil}}{g \text{ water}}$$

Therefore, the sorbed phase PCE $= 113*0.316*6.2 = 221$ kg

5.A.2. PCE in NAPL phase or adsorbed phase in vadose zone soil

During April through October 1997, the soil PCE concentration analysis was performed at the source, below the former dry cleaner facility, by the contractor BE & K/Terranext. Four boreholes were drilled to a depth of 5 ft (1.5 m) bgs and the soil was analysed for PCE concentration. Mean concentration of the four values is 1041.8 mg/kg. To estimate the mass of PCE present in the soil, this concentration is assumed to be uniformly distributed over a volume with following dimensions:

Depth of contamination = 10 ft (3.05 m)

Approximate area of the contaminated site (former dry cleaner facility) = 90 ft x 50 ft (27.4 m x 15.2 m)

Density of bulk soil = 1700 kg/m^3

Amount of PCE present in the vadose zone

$$= 90*50*10 ft^3 * 0.0283 \frac{m^3}{ft^3} * 1700 \frac{kg}{m^3} * 1041.8 \frac{mg}{kg} * 10^{-6} \frac{kg}{mg}$$

$$= 2255 \text{ kg}$$

The soil analysis was completed in 1997. After that a restaurant was established on the site and it is still in operation. It has not been possible to repeat the analysis. Some of the PCE has leached to groundwater since 1997, and therefore, that amount has to be deducted to obtain the mass of PCE in the soil at present.

Assuming that the groundwater PCE concentration was steady at 30 mg/L (actually it was in the range 25-36 mg/L during 2003 to 2006 analyses by KDHE's contractors), the mass of PCE that had leached over the period from September 1997 to December 2006 (3350 days) can be estimated.

Assuming a plume width of 50 ft (15.2 m) over which the PCE is leaching at a uniform concentration of 30 mg/L, the discharge is

$$= 30\frac{mgPCE}{L} * 2.55\frac{m^3}{d} * 1000\frac{L}{m^3} * 3350\,d * 10^{-6}\frac{kg}{mg}$$

$= 257$ kg

Therefore, the mass of PCE that is still present in the vadose zone

$= 2255 - 257 = 1998$ kg.

The total amount of PCE is the sum of PCE in the aqueous phase and sorbed phase in the aquifer as wells as the unsaturated zone. Therefore the total amount is

$= 113 + 221 + 1998 = 2332$ kg

In addition, PCE could be in other unidentified locations such as in the bottom of the deep aquifer; however, there is no evidence to support any other source location.
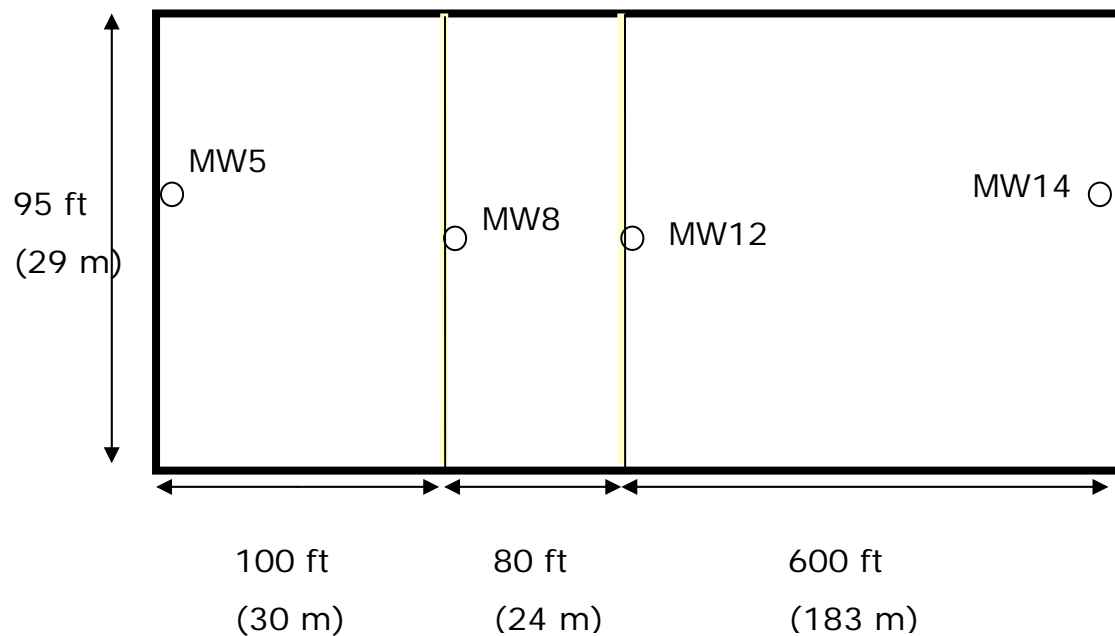
Figure 5.A.1. Schematic (not to scale) of the contaminated site divided into three sections, for estimating the mass of PCE.

Table 5.A.1. Estimation of aqueous phase PCE mass at the site; typical value of PCE concentration at each well is used based on the data during the period 2003 to 2005.

Sections	Upstream concentration of PCE (mg/L)	Downstream concentration of PCE (mg/L)	Distance (m)	Slope (Concentration/ Distance) (mg/L/m)	Groundwater Thickness (m)	Mass (kg)
Deep zone						
D1	30	15	30.5	-0.5	6.1	36.3
D2	15	10	24.4	-0.2	6.1	16.1
D3	10	0.005	182.9	-0.05	6.1	48.5
Shallow zone						
S1	5	1.5	30.5	-0.11	6.1	5.2
S2	1.5	1	24.4	-0.02	6.1	1.6
S3	1	0.005	182.9	-0.01	6.1	4.9
Total amount of PCE in aqueous phase						113
PCE in sorbed phase (assuming 0.1 % organic fraction)						221

Appendix 5.B. Estimation of the appropriate amount of substrates to be injected in the deep zone of the aquifer in the pilot study area

The electron donors employed in the pilot scale bioremediation were soy oil methyl esters (SOME), lactic acid and glucose. SOME acts as a slow electron donor while glucose and lactate act as fast electron donors to reduce the redox potential of groundwater. Yeast extract was added as a source of vitamins and minerals.

5. B. 1. Soy oil methyl esters (SOME)

The hydrogen source necessary for dechlorination can be emulsified soy oil methyl esters (SOME). Clapp et al. (2004) reported the average value for the number of moles of hydrogen required per mole of chlorinated ethene (PCE and daughter compounds TCE, DCE and VC) as 1.27, and the range of values reported in the literature are 1.03 to 1.85. If we consider the average value, the number of moles of hydrogen required for complete dehalogenation of PCE to ethene is, 1.27*4 = 5.08. Theoretically, only 4 molecules of hydrogen are needed for complete dehalogenation of PCE. The additional hydrogen may be utilized by methanogens and other competing microorganisms. The aqueous phase concentration of PCE entering the pilot study area is assumed to remain constant at 15 mg/L or 0.09 mmol/L, throughout the bioremediation process. Therefore, the stoichiometric H_2 concentration required = 0.0904 * 5.08 = 0.46 mmol H_2/L.

One mole of SOME can theoretically release 17 moles of hydrogen. Fermentation of a hydrocarbon typically does not release the stoichiometric amount of hydrogen molecules. Since 2 moles of H_2 as electron pairs are released from a hydrocarbon with 3 moles of H_2 during fermentation (Wood et al., 2006), the effective number of hydrogen molecules released are 17 * (2/3) = 11.4 moles H_2/moles SOME. For 0.09 mM of PCE, the required SOME = 0.46/11.4 = 0.04 mM = 11.8 mg/L or 0.00118%.

SOME added in the first injection = 8 kg

The injected SOME can reduce (15/11.8) * 8000 = 10.197 kg PCE.

If we consider a 12 ft (3.66 m) width, the distance between the farthest injection wells, and 10 ft (3.05 m) depth, the screening zone of a monitoring well in the deep zone, the velocity of groundwater as 0.3 ft/d and the porosity of the soil as 0.3, the volume of water passing through the deep zone is 12 ft x 10 ft x 0.3 ft/day * 0.3 = 10.8 ft^3/day = 302 L/day.

Mass of PCE flowing in one day in the chosen zone = 0.015 * 302 = 4.53 g.

Therefore, SOME added should, theoretically, last for 10197/4.53 = 2251 days = 6.2 years. However, the entire SOME added in the sub-surface may not be available for PCE degradation.

5. B. 2. Lactic acid/lactate

The chemical formula of lactic acid is $C_3H_6O_3$ and its formula weight is 90.08. Density of lactic acid (80 % purity) is 1.2 g/cc at 20°C (http://www.epa.gov/chemrtk/lactacid/c13462rs.pdf, 2002). Based on the stoichiometry of dechlorination of PCE by hydrogen, we can estimate the amount of lactic acid needed for bioremediation by indigenous microflora.One mole of PCE requires 4 moles of hydrogen for complete dechlorination to ethene.

The aqueous phase concentration of PCE entering the pilot study area is assumed to remain constant at 15 mg/L or 0.09 mmol/L, throughout the bioremediation process. Clapp et al. (2004) reported the average value for the number of moles of hydrogen required per mole of chlorinated ethene (PCE and daughter compounds TCE, DCE and VC) as 1.27, and the range of values reported in the literature are 1.03 to 1.85. If we consider the average value, the number of moles of hydrogen required for complete dehalogenation of PCE to ethene is, 1.27*4 = 5.08. Theoretically, only 4 molecules of hydrogen are needed for complete dehalogenation of PCE. The additional hydrogen may be utilized by methanogens and other competing microorganisms. Therefore, the H$_2$ concentration required = 0.0904 * 5.08 = 0.46 mmol H$_2$/L.

Lactic acid injected in groundwater is metabolized, thereby releasing molecular hydrogen (H$_2$) (Koenigsberg and Farone, 2000). The molecular hydrogen serves as an electron donor to stimulate reductive dehalogenation of chlorinated contaminants by indigenous bacteria. Since 2 moles of H$_2$ as electron pairs are released per mole of lactic acid during fermentation (Wood et al., 2006), we can calculate the amount of lactic acid to be injected: 0.46/2 = 0.23 mmol lactic acid/L = 20.7 mg/L. Concentration of lactic acid to be present in the plume is 20.7 mg/L, i.e., 0.00207 %. Since lactic acid is available at 85 % purity, the required concentration of the

commercial lactic acid to be applied in the field is 24.3 mg/L (0.00207/0.85 =0.00243 % or 0.0243 g/L) for 15 mg/L aqueous phase PCE concentration.

Therefore, the lactic acid added during the first injection (0.8 kg) should be able to degrade (15 * 800)/ 24.3 = 494 g of PCE. Therefore, the added lactic acid should last for 494/4.53 = 152 days.

Hence the nutrients added during the first injection, should theoretically, last for 109 + 2251 = 5450 days = 6.5 years. However, the entire SOME added in the sub-surface may not be available for PCE degradation. Moreover, groundwater with PCE flows from depth 35 ft bgs to 55 ft bgs and also outside the 12 ft width considered. Hence the SOME added may be utilized for PCE degradation outside the assumed PSA. Since lactate is soluble in water (10 g/L at 25°C), some of the lactate may be washed away from the pilot study area.

Similar calculations can be performed for the shallow zone of the aquifer.

CHAPTER 6 - Conclusions and Recommendations for Future Work

6.1. Conclusions

6.1.1. Laboratory Study

Supplements such as glucose, corn starch, and SOME stimulated the indigenous microbes to carry out the degradation of both tetrachloroethene (PCE) and carbon tetrachloride (CT). The pattern and rate of degradation of PCE and CT were different for different supplements. As a result, the degradation compound ratios were not the same in the glucose/corn starch/cheese whey and SOME amended channels. In both glucose and SOME fed channels, the degradation continued many days after stopping the feeding of supplements. The effect of SOME on degradation of PCE to DCE lasted for 246 days after stopping the feeding of SOME. The effect of glucose on degradation of PCE to DCE lasted for 68 days after stopping the feeding of glucose. Even though PCE concentration did not decrease to very low values (< 2 % of the inlet concentration) after 246 days in SOME fed channel and 68 days in glucose fed channel, the effect of the substrates were present for a long time by partial conversion of PCE. In the case of SOME fed channel, the outlet PCE concentrations were maintained below 1.6 µM (10 % of inlet value) from 246 days to 390 days after stopping the feeding of SOME. In the case of glucose fed channel, the outlet PCE concentrations were maintained below 5.7 µM (40 % of inlet value) from 68 days to 194 days after stopping the feeding of glucose. The effect of the substrates would have continued for a long time until the outlet PCE concentration reached the value of inlet PCE concentration. However, to maintain the reducing conditions in the channel, the substrates were added again.

Glucose was found to be a better supplement than cheese whey for CT degradation; however, further study is necessary to determine if the concentration and frequency of cheese whey addition can improve the CT degradation process. The concentration of methylene chloride (MC), an intermediate compound in CT degradation, was generally higher in the channel with SOME compared to the channel with glucose. In the soil sample analysis, CT was not detected in the vadose zone but found in the saturated zone in appreciable concentrations, in the control channel and the SOME fed channel.

The relative concentrations of degradation products of PCE were not the same in the glucose/corn starch and SOME amended channels. Glucose was found to be a better supplement than SOME with respect to the onset time for PCE degradation. Because glucose is soluble in water, it is distributed more rapidly.

In both PCE and CT channels, most of the degradation process took place in the initial portion of the SOME fed channel, since SOME likely stayed near the inlet of the channel, due to sorption and retarded flow. SOME is present as a non-aqueous phase liquid (NAPL) which does not flow freely like an aqueous solution. It may also sorb to soil organic matter in the initial portion of the channel. In the soil sample analysis, PCE concentration was low in the vadose zone, but it was present in the saturated zone in appreciable concentrations.

This study demonstrated that the supplements glucose and SOME are effective substrates that can be added to PCE or CT contaminated groundwater to promote degradation at contaminated sites.

The soil matrix and the microorganisms were able to store the supplements/degradation products of supplements and provide a long-term source of carbon and hydrogen. This finding is very important in the design of remediation systems in field sites to determine the frequency of supplement addition. The mesocosm studies have shown that there is a significant residual effect of introduced carbon supplements. In the soil system, the microbes which are present as a biofilm assimilate glucose and SOME, store polysaccharides, and have a reservoir of carbohydrates to use to reduce the chlorinated organic compounds. The process of endogenous decay may also provide a source of food. There are also the effects of distributed flow and channeling. These phenomena influence the length of time the system operates effectively and degrades the chlorinated organic compounds after food is added. Because SOME has very low solubility in water, it is retained near the point of injection, and it is able to provide needed substrate for a relatively long time compared to a soluble substrate such as glucose.

Addition of KB-1 at well 3, located approximately in the middle of the channel, had significant impact in the degradation of DCE, in both glucose and SOME amended channels, compared to addition at the inlet. The KB-1 culture added to the channel was active even 155 days later, suggesting that there is sustainable growth of KB-1 when provided with suitable conditions and substrates.

The results impart an idea of how SOME and KB-1 should be applied in the field: they should be injected at several points down-gradient rather than at a single point.

The native microorganisms from the six-channel system were not capable of reducing the DCE further even if provided sufficiently long time in microcosms, prepared from the outlet solution of SOME and glucose fed channel contaminated with PCE. DCE remained steady in the microcosms and it is concluded that microorganisms capable of reducing DCE to ethene must be added to degrade DCE.

6.1.2. Field Study

The tracer study showed that the groundwater flow in the aquifer is predominantly towards the east in both the shallow zone and the deep zone. The groundwater velocity in the shallow zone is greater than in the deep zone. The hydraulic conductivity is larger in the deep zone; however, the static head gradient is about one order of magnitude larger in the shallow zone. Based on the observed static head gradient and the estimated hydraulic conductivities from pumping tests, the velocity is estimated to be larger in the shallow zone as was observed. Lenses of higher permeability regions may be present in the shallow zone.

The time duration for the bromide to reach MW-9D, MW-10D and MW-12D are consistent indicating that the hydraulic conductivity of the soil does not vary significantly in the down-gradient area. The velocity in the pilot study area decreased from the 2004 study to the later studies in 2005 and 2006. This appears to be due to biofilms associated with the biodegradation process. The nitrate concentration variations associated with nitrate being used as an electron acceptor are consistent with the bromide data; the organic substrates flowing with the bromide provide food for the organisms to consume with nitrate as electron acceptor.

Biodegradation of PCE is an electron donor limited process in the study site. Addition of electron donors such as SOME, glucose and lactic acid stimulated the growth of native microbes that degraded PCE to DCE. Injection of KB-1 enhanced conversion of DCE to end products such as methane or ethene. The results indicate that the KB-1 cultures may have been distributed downgradient by fluid motion to MW-9D, MW-10D, and MW-12D. Since higher concentrations of CEs were found in MW-10D compared to 9D, a significant portion of the groundwater at 10D may not have passed through the area near MW-9D.

The arrival time of the tracer precisely coincided with the appearance time of DCE and

the front of PCE disappearance, indicating that there is no appreciable retardation of PCE and DCE within the aquifer. It is possible that nutrients may have traveled with the tracer to the downgradient wells and that biodegradation of PCE to DCE took place at or near these wells. It is also possible that a portion of the groundwater from the upgradient wells may be reaching the downgradient wells. If either DCE or PCE were appreciably sorbed to aquifer materials, there would be a measurable retardation of their arrival times relative to the bromide tracer.

In the batch studies conducted by Ibbini et al (2007), the lag time for biodegradation of PCE was observed to be approximately 10 days and 20 days, respectively, for the microcosms with KB-1 and the microcosms with native microorganisms.

PCE present at the site of contamination provides a long term source for the plume. The data for MW-5D indicate that the upper portion of the deep zone of the aquifer is impacted by the source of contamination more than the lower portion of the deep zone. This suggests that the source of contamination may be in the unsaturated zone above the deep aquifer. A different approach such as chemical oxidation using potassium permanganate or soil vapor extraction should be considered to remediate the source area of contamination.

The measured concentrations of PCE and DCE in the deep zone of the aquifer provide supporting evidence that flow in the aquifer near the points of nutrient addition was altered because of microbial growth and microbial product formation which reduced the hydraulic conductivity.

The general results of the pilot study show that both nutrient addition and KB-1 addition were beneficial. The resulting concentrations of PCE, TCE, and DCE were much lower because of the added nutrients and cultures. While the resulting concentrations were well above the desired end point (MCLs), this may be because of the continuous mixing of treated water with other water that is untreated or partially treated. In the laboratory channel studies, the PCE concentration decreased to below MCL in the glucose fed channel (0.02 μM) and the minimum value in the SOME fed channel was 0.074 μM, almost 2.5 times the value of MCL. This is possible in the channels since this is a closed system with only one inlet for PCE, whereas in the field only a part of the plume width was injected with nutrients.

6.2. Future Work

Devlin and Muller (1999) reported inhibition of degradation of carbon tetrachloride (CT) in the presence of metals such as Fe, Cu, Co, Mo etc. Even though CT was degraded completely in the mesocosm in our study, further research is needed to determine the conditions and compounds that inhibit CT degradation.

The laboratory and field work demonstrated that the concentration of CEs (PCE, TCE, DCE, and VC) can be decreased to very low levels by biostimulation and bioaugmentation. Being a pilot study, the injection wells were installed over a distance of 12 ft. For the design of the full remediation plan, more injection wells can be installed between MW-8 and MW-7. Nutrients and KB-1 can be injected through these injection wells in sufficient amounts. One additional injection well may be sufficient between MW-8 and MW-11 since the concentration of CEs is very low at MW-11, in both the deep and shallow zone.

The optimal amount of nutrients to be added and the concentration and the frequency of injection can be estimated based on the present study. The optimal amount of nutrients to be added may vary based on the goal of the remediation. If the target is to reach MCLs at the remediation zone the amount of nutrients and the injection frequency must be increased. If the nutrients and KB-1 cultures are injected over a wider distance in the North-South direction and also over a greater vertical distance, the concentrations of PCE, TCE and DCE can be decreased further and MCLs can most likely be achieved.

Based on the vertical distribution of PCE, the screening of the injection wells can be varied, such that more nutrients are injected at a location where PCE concentration is higher.

Monitoring wells can be installed 5 ft to the North and 5 ft to the South of MW-9 or MW-10 to determine if biofouling resulted in a change of the groundwater flow path. These lateral MWs also aid in determining the lateral distribution of tracer and nutrients and to delineate the width of the remediation zone.

The dispersion coefficient, groundwater velocity and hydraulic conductivity estimated from the experimental data can be incorporated into the advection-dispersion-reaction equation (ADRE) for contaminant fate and transport modeling and simulation.

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