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Laboratory Characterization of Phyto-transformation Products of Perchloroethylene (PCE), Trichloroethylene (TCE) and Perchlorate

Final Report

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Abstract.

Bench scale tests were conducted to investigate the uptake and metabolism of TCE by eastern cottonwoods and willows. The initial rapid removal of the parent compounds from solution (first two days) due to the combined effects of partitioning and uptake was followed by a slow uptake and transformation step. The metabolites identified in the plant tissues over the course of this study suggest that the phyto-transformation pathway of TCE and PCE by woody plants is more complex than previously reported in the published literature. The identification of polar and nonpolar TCE and PCE metabolites in the plants suggested that both oxidative and reductive enzymes mediated the reactions. Based on the distribution of the identified metabolites in the different plant tissues, it appears that the reductive enzyme (activity) is highest in and around the roots and oxidative activity is highest in the leaves. No evidence was obtained to support the possible phytoaccumulation of TCE and PCE in the tree leaves.

Selected woody plants were exposed to perchlorate dosed nutrient medium and found to be effective in the remediation of the perchlorate contaminated water. The initial or prolonged exposure of Eucalyptus and willow trees to perchlorate dosed medium enhanced their ability to degrade perchlorate in the root zone (rhizosphere). The reduction of perchlorate was enhanced at lower nitrate concentration in the medium (<100-ppm) and described by zero-order kinetics. Homogeneous transformation studies conducted with autoclaved, filtered and untreated medium taken from the root-zone of willow plants confirmed that rhizosphere associated microorganisms mediated the degradation of perchlorate to chloride. The latter studies also confirmed that at higher nitrate concentrations of >200 ppm the reduction of perchlorate to chloride was inhibited. The three phytoprocesses identified by this study to be important in the remediation of perchlorate-contaminated water and soils include phytoextraction (accumulation in the branches and leaves), phytodegradation and rhizotransformation. Since perchlorate does not volatilize from water, we envision a remediation scheme of intensively cultivated plantations of plants with phraetophytic characteristics, such as willows and *Eucalyptus* or other suitable vegetation irrigated with the contaminated water.

Introduction

A two-phase laboratory phytoremediation project was initiated in April 1998. The first task was to investigate the phytotransformation kinetics and pathway(s) of cottonwood plants currently used in the remediation of trichloroethylene (TCE) contaminated groundwater at the Carswell Naval Air Station near White Settlement, TX. A second set of studies involved the screening of woody plants and herbs to identify plants suitable for the remediation of perchlorate contaminated groundwater.

Two *Populus* plantations were started in 1996 at Carswell AFB for the purpose of remediating a shallow groundwater plume of TCE. Cuttings of Eastern cottonwood and willows trees growing on the impacted site were harvested and used in bench-scale tests to study the phytotransformation of TCE and perchloroethylene (PCE). In the perchlorate study, one cottonwood, two willows (*Salix nigra and Carolianana*), *Eucalyptus cineria*, and *Myriophyllum*

aquaticum harvested from a local wetland, were used in the screen tests. French tarragon (*Artemisia dracunculus*) and spinach (purchased from a local supermarket and nursery) were minced and used in a number of studies. In total, seven plant species and one mixed species microbial mat were screened.

Most of the results presented in this report were obtained with plants grown under natural conditions in the Summer of 1998. Since this is the growing season for most plants in Georgia, these results may not reflect the true remediation potential of these plants under other seasonal or environmental conditions. The specific tasks investigated over the course of this study are listed below.

Project Tasks

- Phytotransformation rates of PCE and TCE.
- Phytotransformation products of PCE and TCE.
- Phytotransformation pathway(s).
- Screen tests to provide evidence of phytoremediation of perchlorate contaminated water.
- Extracted and quantified perchlorate in the plants used in screen tests.
- Dosed willow bioreactor multiple times to simulate the long-term effects of perchlorate on woody plants.
- Verified the complete reduction of perchlorate to chloride in rhizosphere of willow trees.
- Determined the role of bacteria in rhizodegradation of perchlorate by willow trees.
- Preliminary chloride mass balance estimation.

Methods

TCE and PCE Studies.

Tree cuttings of the woody plants listed below (willows and cottonwoods) were obtained from Carswell Air Force Base (AFB) in Texas. The trees were grown hydroponically in 25% of full strength Hoagland's solution (Carolina Biological Supply Company) for three months. The trees with a good root system were used for the different studies. Analytical grade TCE, PCE, MTBE (t-butyl-methyl-ether), and other chlorinated reagents were obtained from Aldrich Chemical Company. TCE or PCE saturated aqueous solution was used to dose the planted reactors to obtain an initial concentration of about 50 mg/L in each bioreactor. High concentrations of the parent compounds were used to enable for the formation of detectable concentrations of the different metabolites. HPLC grade hexane was from T. J. Baker. All other reagents were of analytical grade.

Plants used in PCE and TCE phytotransformation studies
Eastern Cottonwood ..... Carswell AFB, Texas
Willow (Salix ssp) ..... Carswell AFB, Texas
Willow (Carolianana) ..... Tampa, FL

Bioreactor Studies - TCE and PCE.

A 2.2-liter bioreactor flask with two side ports was used in the experiment (Figure 1). One port was connected with a valve and a reservoir for water replenishment and the other port was equipped with a sampling-port. The growth solution was 25% of full strength Hoagland's solution and was dosed with either saturated TCE or PCE aqueous solution. Each tree was fastened into the screwed cap and sealed with dental cement and acrylic glue. All roots were completely submersed in the aqueous nutrient media and leaves were outside of the reactor. The final solution volume was 2000 - 2100 mL and the headspace was about 10 mL. The roots were shielded from direct light by shielding the medium-containing portion of the reactors with aluminum foil. The studies were conducted outside under natural summer conditions in Georgia. The volume of medium was kept constant by adding water through the replenishment port. A 1-mL aliquot was withdrawn everyday through the sampling-port and extracted into 6-18 mL of hexane. The volatile organic compounds (VOCs) in the hexane phase were measured by GC/ECD. When the PCE concentration was one quarter of the initial concentration, the experiment was terminated. The static headspace samples (1-mL) were taken at the beginning and at the end of the study, and analyzed on both GC/FID and GC/MS.

The aqueous phase remaining after the hexane extraction was acidified with a couple of drops of concentrated sulfuric acid, and re-extracted with t-butyl-methyl ether (MTBE). Sample trees were sectioned into roots, lower stem (below the water surface), upper stem (above water surface), and leaves. The different tree portions were cut into fine pieces and ground. The ground plant tissues were first extracted multiple times with hexane in an ultrasonic bath and the extracts were analyzed on GC/ECD for chlorinated hydrocarbons. Following the hexane extraction, the pellets were treated with 10-40 mL of 1 M H_2SO_4 aqueous solution overnight, depending on the sample size. The mixture was extracted with MTBE. The MTBE extracts were derivertized with diazomethane for the measurement of monochloro-, dichloro-, and trichloro-acetic acids. The concentration of PCE and its identified metabolites were expressed in mg per kg of samples.

Headspace Measurement of VOCs

A Hewlett-Packard 5890A Gas Chromatograph with FID was used for VOCs analysis. Exactly 0.5 mL headspace was manually injected into the injection port maintained at a temperature of 200 °C. A capillary column DB-VRX, 30 m X 0.25 mm I.D., 1.4 um film (J & W Scientific) was used in the separation of the different VOC components. The measurement was under helium carrier gas at the following temperature program: 30 °C for 5 minutes; followed by temperature increase of 5 °C/minute to 60 °C and held for 2 minutes, then increased at 25 °C/minute to 200 °C and held for 2 minutes. Data acquisition was performed on HP 3365 ChemStation. Further VOC quantification was done on a Shimadzu GC-17A Gas Chromatograph with QP-5000 Mass Spectrometer with a DB-VRX column.

Measurement of Chlorinated Hydrocarbons

The parent compounds and their transformation products (TCE, PCE, trichloroethanol, dichloroethanol) in different parts of the trees and aqueous phase were quantitatively measured following the complete extraction of the crushed plant tissue with hexane. The hexane mixture was diluted to the linear range (0.5 - 2.5 ppm for TCE and PCE). Other chlorinated hydrocarbons, such as DCE, TCA were qualitatively identified with individual standard compounds because of their low sensitivity. All samples were injected (0.5 μL) by Shimadzu AOC-17 Autosampler, and measured on Shimadzu GC-14A GC/ECD. The temperature was programmed at 35°C for 2 minutes, followed by temperature increase of 5°C min^{-1} to 90°C min^{-1} and ramped up to 120°C at a rate of 15°C min^{-1}. The injection and detection temperatures were 200 and 300°C, respectively. The separation column was a DB-VRX, 30 m X 0.25 mm I.D., 1.4 um film). The carrier gas was nitrogen. The data were integrated on Shimadzu CR501 Chromatopac.

Measurement of Chlorinated Acetic Acids

Following the hexane extraction, solid and aqueous samples were treated with 1-M sulfuric acid and placed in an ultrasonic bath for several hours. Then, the mixture solution was extracted using MTBE. 2 mL MTBE extract was mixed with 150 μL diazomethane saturated MTBE solution prepared in a Wheaton Generator with 1 gram 1-methyl-3-nitro-1-nitrosoguanidine and 5 mL of 5 M NaOH. The mixture was placed in an ice bath for 5 minutes, and then at room temperature for at least 15 minutes (Hales et al., 1973). Chlorinated acetate methyl esters were measured on a Shimadzu GC-14A/ECD. The standard solutions of chloroacetic acid, dichloroacetic acid, and trichloroacetic acid were derivertized and measured under the same condition.

RESULTS AND DISCUSSION

Uptake of PCE

In studies with both cottonwood and willow, the initial PCE concentration of approximately 40-ppm in the aqueous nutrient medium decreased with increasing reaction time (Fig 2 and 3). In the first 1-2 days the initial rapid decrease in PCE concentration was attributed to multiple processes including evaporation into the limited headspace of 10 mL, sorption to the roots and lower stem and uptake into the plant. After partitioning equilibrium was reached between the solution/plant and medium/headspace phases further decrease in PCE concentration in the medium was linear to time and attributed to uptake and metabolism by the trees. Thus, the PCE removal from solution was described by zero-order kinetics. The zero-order rate constants for the cottonwood and willow trees were 0.48 ppm/day and 0.66 ppm/day, respectively. The estimated squared correlation coefficients for cottonwood and willow trees were 0.96 and 0.97, respectively. In the cottonwood tree studies, ethene, TCE, 1,1,1,2-tetrachloroethane (PCA) and PCE were detected in the headspace above the nutrient medium (Table 1). In the latter study, ethene was the major component in the headspace mixture. Meanwhile, in the willow tree

experiment, DCE was the major component in the headspace. Only trace amounts of ethene, TCE and 1,1,1,2-tetrachloroethane were observed in the headspace (Table 2).

When the PCE concentration in the medium decreased to 10 ppm, the experiments were terminated and the plants sacrificed for analysis of PCE transformation products. The highest accumulation of the parent compound (PCE) in cottonwoods and willows was in the roots and lower stem and no PCE was detected in the leaves. After 2 weeks, the TCE concentration remained constant at 1.7 ppm in the medium before increasing to 2.6 ppm. This indicated that the formation and removal rates of TCE were nearly same. The highest concentration of TCE detected in the medium was 2.6 ppm while the lower stem had the highest TCE concentration of 1 mg/Kg. TCE was not the terminal PCE degradation product in cottonwood trees. On the other hand, there was no significant TCE released into aqueous phase from willow tree. There is preliminary indication that in the rhizosphere, which is anaerobic, all chlorinated products and PCE are eventually reduced into ethane or ethene. This needs to be confirmed by using deuterated water in further studies.

Trichloro- and dichloroacetic acids (TCAA) were the major polar products detected in extracts of both plants. The highest concentrations of the polar metabolites were detected in the leaves. The concentration of the polar metabolites in the willow leaves were higher than those of cottonwood (Tables 1 and 2). Trichloroethanol was the first polar transformation product detected in the leaves, usually after one week of dosing the planted bioreactor. The identification of both polar and nonpolar products in the plant tissues (Tables 1 and 2) suggests that more than one pathway may be involved in phytotransformation of TCE and PCE by the cottonwood and willow trees (Fig 4).

The uptake and metabolism of TCE by willow was described by first-order kinetics (Figure 5). The calculated half-life was 11 days. After two weeks of dosing willow trees with TCE, trichloroethanol was detected in the leaves and branches and none in solution. Trace concentrations of DCE, VC, ethene and ethanol were detected in the headspace. This again confirms that more than one pathway may be involved in the phytodegradation of TCE and PCE by woody plants. Direct confirmation that TCE and PCE are evapotranspired by these trees was not available at the time this report was prepared.

Summary of Results – TCE and PCE Studies

- Cottonwood and Willow plants take up PCE and TCE from Hoagland's nutrient solution.
- The phytotransformation pathway of chlorinated compounds appears to be more complex than previously reported in the literature.
- Oxidizing and reducing enzymes (factors) in these plants transform the phytoextracted TCE and PCE.
- The distribution of PCE and TCE transformation products suggests that the highest reducing activity is present in the roots and the highest oxidizing activity is in the leaves.
- We have not found any evidence that PCE and TCE are phyto-accumulated in leaves of the woody plants we tested.

Perchlorate Studies.

Plants used in study

* Eastern Cottonwood ..... Carswell AFB, Texas
* Willow (Salix ssp.) ..... Carswell AFB, Texas
* Willow (Carolianana) ..... Tampa, FL
* *Eucalyptus cineria* ..... Local Nursery, Athens, GA
* Myriophyllum aquaticum ..... *Local Wetland, Athens, GA*
* *Artemisia dracunculus* ..... Local Nursery, Athens, GA (French tarragon)
* Spinach
* Constructed Mixed-species microbial mats

Perchlorate ion uptake by woody plants was investigated in 2-liter glass reactors with side sampling ports. One cutting was grown in each sand bioreactor. Pristine sand was placed at the bottom of each reactor to support the tree roots so that the branches and leaves were outside of the reactor. The sand occupied about half of the reactor volume. The growth solution was 50% of full strength Hoagland's solution and was spiked with a stock perchlorate solution to make an initial perchlorate concentration of 20 and 100 ppm, respectively, as ClO_4^-. This concentration is about two orders of magnitude higher than that reported for most perchlorate contaminated sites. Total volume of liquid media in each reactor was 600 - 900 mL. The reactor was wrapped in a sheet of PARAFILM® and the solution portion of the reactor was shielded from the light by aluminum foil. For a few initial experiments conducted in early Spring 1998, 65 watts plant growth lights illuminated the trees for 12/12 hours day and night cycle in the laboratory. Experiments conducted in summer months were located outside where the plants were exposed to natural environmental conditions. The water in the reactor was kept at a constant level by replenishing the medium lost by evapotranspiration with a known volume of half-strength Hoagland's solution. This necessitated adding diluted Hoagland's solution twice or thrice a day to each reactor. A daily record of the water uptake by each plant was maintained over the course of each study. A 1-mL aliquot was taken every day from the sampling port and was replaced with an equivalent volume of half strength medium. The sample was diluted with deionized water to the IC measurement range of the perchlorate ion.

The distribution of perchlorate in different plant fractions was determined by sacrificing some of the study plants for extraction and analysis. At the end of each study the plant was removed from the medium, rinsed with deionized water and sectioned into roots, lower stem, upper stem, branches and leaves. Each fraction was extracted several times by blending for 30 - 60 min with a solution of 1 mM NaOH (pH = 11). The extract was separated from the aqueous-plant phase by centrifugation. The number of extractions needed to ensure complete removal of the extractable perchlorate and its analogs was dependent on the plant fraction and type of plant. On average three extractions were needed to completely extract the extractable perchlorate from the respective ground plant organs. The extract was analyzed for perchlorate, chlorate, chlorite and chloride ions. All experiments with each plant species were replicated.

Perchlorate ion uptake by herbs and microbial mats was investigated in 60-mL serum bottles. French tarragon and spinach were each minced before used in the perchlorate studies.

One gram of the minced plant was added to 20-mL vials. The remaining space in the sampling vials were filled with deionized water and dosed with perchlorate to obtain an initial solution concentration of about 10 mg/L. The vial were mixed on a rotary shaker and sacrificed for analysis at predetermined intervals. Pieces of the wet mats were weighed into the vials and the headspace filled with deionized water, dosed with perchlorate to obtain an initial solution concentration of 10 mg/L and mixed on a rotary shaker. The pellets were separated from solution by centrifugation, and the liquid-phase was analyzed by IC. A total of 9 sample vials were used in each of the replicate experiments. Controls contained no plant matter and were dosed with the same concentration of perchlorate as the samples.

Sorption of Perchlorate to Sand.

The sorption of perchlorate to sand used in the bioreactors was determined in separate sorption equilibrium studies. The same mass of sand (10 g) was weighed into 50-mL Pyrex glass centrifuge tubes. Then each tube was filled with half-strength Hoagland's solution containing the appropriate amount of perchlorate. The concentration range used in the experiment ranged from 0 to 100 ppm. Corresponding controls containing dosed medium without sand were prepared and handled in parallel with the samples. Duplicate vials were prepared for each control and sample concentration point.

Homogeneous Perchlorate Transformation Studies.

Four sets of batch experiments were conducted to verify the effect of nitrate and acetate on perchlorate degradation in the willow bioreactor. A total of 400 ml of medium was withdrawn from the rhizosphere of the willow reactor after the fifth perchlorate spike had been completely degraded. 50 mL of the medium was placed in eight serum bottles. Sodium nitrate was added to a pair of the vials to obtain a total nitrate concentration of 200 ppm. Acetate was added to a second pair of vials to obtain an initial concentration of 400 ppm. To the third pair of vials, both nitrate and acetate were added to obtain initial concentrations of 200 and 400 ppm, respectively. One pair of vials contained the unamended medium. Corresponding controls consisted of deionized water only or deionized water with the same concentrations of nitrate, acetate, or both compounds. The samples and controls were each dosed with perchlorate to obtain an initial solution concentration of 100 ppm and sealed with serum wrap and incubated at room temperature. One ml of solution was taken from sample and control every 24 hours, diluted and analyzed for perchlorate, nitrate, acetate and chloride.

Ion Chromatography.

A Dionex DX-100 Ion Chromatograph with SRS control was used in all analysis. The IC was equipped with a Dionex AI-450 Chromatography Automation System and the Advanced Computer Interface Module (ACI). An autosampler with a holding capacity of sixty 5-mL vials

was used. Sample injection volume was 25 μL for high perchlorate concentrations (ppm) or 500 μL for low concentrations (ppb). Both IONPAC® AG11 guard column (2 x 50 mm) and IONPAC® AS 11 analytical column (4 x 250 mm) were used. The analytical conditions developed by Dionex Corporation for analysis of low concentrations of perchlorate in drinking water and ground water by Ion Chromatography were followed. Flow rate of eluent was 1 mL/min. 5 mM NaOH solution was used as eluent for analysis of chloride, chlorite, chlorate, nitrate and acetate ions while 100 mM NaOH solution was used for the perchlorate ion measurement. The working perchlorate concentration range was 80 -1000 ppb and the conductivity was less than 0.3 μS. The detection limit for perchlorate for the above method was 2 ppb. The run time was 15 minutes. Deionized water (resistance of 18.0 - 18.2 MΩ-cm) was used as a system blank sample to establish the baseline and to confirm the presence of or lack of contamination in the system. Low and/or high concentration calibration curves were determined each day of sample analysis to ensure accurate quantification of perchlorate (Figs 6A,B).

Results and Discussion – Perchlorate Studies

Rooted cuttings of three woody plants (willow [Salix spp.], Eastern Cottonwood [poplar] *Eucalyptus cineria*) were observed to take up perchlorate ions from aqueous solution (Fig 7). When the plants were initially exposed to the perchlorate medium, the rate of perchlorate uptake was proportional to the water uptake rate of each plant. Figure 7 and Tables 4 – 6 show that the one-year-old Eucalyptus plant had the largest fraction root and leaf of 6.6 and 46%; respectively, and took up perchlorate from the nutrient medium at the fastest rate. The evapotranspiration rate of the Eucalyptus was also higher than that of the cottonwood and willow plants used in the latter study. The perchlorate concentration in the unplanted reactor did not change over the course of this study.

The results presented in Fig 7 also show that when each of the three plants was first exposed to perchlorate in the planted bioreactors, three distinct reaction phases could be distinguished. Phase 1 was described by a rapid decrease in perchlorate concentration in the medium, which was proportional to the volume of water transpired by each plant. In Phase 2, the progressive increase in water uptake was not accompanied by any significant loss of perchlorate from solution. Phase 2 persisted in studies with cottonwoods up till the conclusion of the experiment when the plants were sacrificed for extraction and analysis. Meanwhile, in Phase 3, a very rapid decrease in perchlorate concentration in the medium was observed, but not proportional to water uptake by the plants. The disappearance of perchlorate from solution was initially described by first-order kinetics (i.e. data represented by Phase 1 and 2), which changed to zero order (Phase 3) towards the end of the run. The plateau in the data (Phase 2) was apparently attributed to higher ionic strength of nitrate in the medium. Since the half-strength Hoagland's solution was continuously added to the reactor to make up for the transpired water the rate of nutrient addition probably exceeded its utilization rate and nitrate out-competed perchlorate as the dominant terminal electron acceptor.

When the rhizosphere nitrate concentration in the willow bioreactor was diluted from 262 ppm to below 100 ppm a rapid decrease in perchlorate concentration in the medium was observed. This suggested that the remediation of perchlorate by plants could be affected by the

ionic strength of nitrate in the nutrient. It is also possible that as the concentration of perchlorate increased in leafs to toxic levels the plants developed mechanisms to resist further uptake of perchlorate until the accumulated fraction had been detoxified. It is not clear from this study which of the latter processes caused the plateau observed in studies with all three plants. Because the control and sample plants continued to grow at the same rate, there is no clear evidence that the perchlorate concentration used in this study was toxic to the cottonwood, Eucalyptus or willow.

The distribution of perchlorate in the pore water in the sand layer, the liquid medium, and in various organs of the cottonwood, Eucalyptus and willow sacrificed for analysis at the termination of the kinetic studies shown in Fig 7 is presented in Tables 4 – 6. The aqueous perchlorate concentration is higher because of the higher concentration recovered from the sand layer which was not in direct contact with the plant roots. Sorption studies confirmed that perchlorate was not sorbed by the sand. This means that the higher perchlorate concentration measured in the sand layer was due to dissolved perchlorate trapped in the pore water. If uptake was the predominant mechanism responsible for perchlorate loss from the bioreactor at that point, then the slow diffusion of perchlorate ions to the root zone should be the limiting process. Additional studies were performed in bioreactors with perchlorate-dosed medium and no sand (Fig 8). Two phases of perchlorate removal from solution were observed in the latter study: a slower initial step attributed mostly to uptake followed by a rapid decrease attributed to both uptake and rhizodegradation.

Comparing the perchlorate concentration (mass/mass) extracted from the different plant organs, it is evident that the perchlorate taken up by all three woody plants was mostly accumulated in leafs and branches. The relatively small amount of perchlorate measured in the roots and lower stem (Tables 4 - 6) suggests that the perchlorate was not accumulated in these parts of the woody plants. The mass balance data in Table 5 shows that the Eucalyptus tree was relatively more effective in the uptake, accumulation and transformation of perchlorate than the cottonwood and willow. The 42% of unrecovered perchlorate in studies with Eucalyptus plants was assumed degraded to chloride or irreversibly bound to the plant tissue. The concentration of perchlorate detected in extracted fresh leafs of a willow tree dosed five times with perchlorate was 261 μg/g and 755 μg/g in leafs showing senile properties whereas the chloride concentration was 226 and 803 μg/g, respectively. We speculate that once in the tree leafs, the perchlorate ions were very slowly transformed by deoxygenase or reducing plant enzymes.

Direct evidence of phytodegradation was obtained from experiments in which the crude extracts and minced French tarragon and spinach were used to degrade perchlorate. Figure 9 shows representative results of perchlorate degradation by minced French tarragon. The degradation of perchlorate by the crude extract and minced herbs provided additional evidence the reactions were enzymatically catalyzed.

In a further study, a cottonwood tree exposed to perchlorate for about one month was removed from the perchlorate dosed medium, rinsed with deionized water and transferred to a perchlorate-free half-strength Hoagland's solution. The purpose of this investigation was to verify if the perchlorate accumulated in the tree leafs and branches can be released back into the remediated water by osmosis. After 7 days no detectable perchlorate was measured in the clean medium and analysis of the extracted plants confirmed that the perchlorate remained unchanged (641 ug/L) in the cottonwood leafs and branches. This means that if the perchlorate taken up by

the woody plants is not transformed but simply accumulated, it is not likely to be released back into the remediated water by osmosis.

Figure 10 shows that following an initial exposure of the woody plants to perchlorate in a hydroponic reactor the predominant phytoremediation mechanism changed from uptake to degradation and the rate of perchlorate reduction in the medium increased by several orders of magnitude. For example, the rate of degradation of the second dose of perchlorate added to the willow bioreactor was much faster than that of the initial spike. In less than 72 hours 109 ppm of perchlorate was degraded to below the method detection limit of 2 ppb. When this reactor was dosed for a third time (initial concentration 86 ppm), the perchlorate was degraded to below the method detection limit in 43 hours. The rate of decrease of perchlorate in the bioreactor was described by zero order kinetics. After dosing the same reactor for a total of five times, the rate of perchlorate reduction by the willow showed no decrease (Fig 10). The estimated zero-order rate constants for the 2nd, 3rd, 4th, and 5th spikes were 15.3, 20.5, 32.6, and 25.9 μM/h. The average water uptake rate by the willow during the latter experiment was 64.5, 129, 93, 95, 80 mL/day for the 1st, 2nd, 3rd, 4th, and 5th spikes, respectively. The very fast kinetics observed after the initial spike shown in Fig 10 suggested that the predominant reaction mechanism was degradation in the root zone (rhizotransformation) and not phytoextraction. The latter was confirmed in subsequent studies by monitoring the chloride concentration in the rhizosphere (Fig 11). Further evidence from studies conducted with radiolabeled chloride is needed to confirm the latter observation.

Possible transformation products of perchlorate such as chlorate, chlorite, hypochlorite and dichlorooxide were analyzed for but none was identified in the medium or plant extract. Complete transformation of perchlorate by the willow was confirmed by monitoring the change in chloride concentration over the course the study. The chloride concentration in the full strength Hoagland's solution was below the IC detection limit in the diluted sample. The chloride concentration in the medium was measured and observed to increase as each dose of perchlorate was completely degraded to below the IC detection limit. A chloride mass balance of 86% was obtained for the study whose results are presented in Fig 11A (6th spike of the willow bioreactor). The stoichiometric reduction of perchlorate to chloride cannot be inferred from this data since, some chloride was taken up by the willow tree (Figs 11A,B). Studies with the radiolabeled chemical should reveal whether the unrecovered perchlorate is completely transformed to chloride or simply bound to the plant tissue. It was observed that both nitrate and perchlorate utilization rates in the medium were similar and described by zero-order kinetics. Acetate was the only plant exudate identified in the medium at pH of 6.5. We inferred from the latter results that bacteria with perchlorate and nitrate acting as terminal electron acceptors oxidized acetate.

Effect of Nitrate and Acetate on Perchlorate Degradation

The concentration of nitrate in the medium spiked multiple times with perchlorate was maintained below 100 ppm because the results of preliminary studies described earlier suggested that perchlorate degradation was inhibited at a higher nitrate concentration of >200 ppm. Homogeneous transformation experiments conducted with medium taken from the root zone of a willow plant confirmed that at a nitrate concentration of about 200-ppm the reduction of perchlorate to chloride (Fig 12) was inhibited. The results in Fig 12 also indicate that amending

the medium with high concentrations of acetate or both acetate and nitrate did not inhibit the reduction of perchlorate to chloride. Because perchlorate was degraded in hydroponic medium withdrawn from the rhizosphere of a willow plant that previously showed activity in its root zone, we have concluded that the reaction was microbially mediated. The acetate exuded into the medium by the willow roots apparently stimulated the growth of bacteria consortia that utilized perchlorate and nitrate as terminal electron acceptors. The simultaneous utilization of nitrate and perchlorate in the presence of acetate in the medium as observed by this study point to a transformation reaction mediated by nitrate-reducing microorganisms harbored in the rhizosphere of the woody plants.

Overall, the results of this study suggest that in the presence of nitrate and root exudates, such as acetate, the rhizotransformation of perchlorate may be enhanced. The two phytoprocesses identified by this study to be important in the remediation of perchlorate-contaminated water and soils include hyperaccumulation of perchlorate in the tree branches and leafs and rhizotransformation. We have shown that phytoremediation is a potentially promising approach for the remediation of perchlorate. Pilot testing of the approach should provide data needed to validate the feasibility of phytoremediation of perchlorate contaminated sites. Since perchlorate does not volatilize from water easily, such a remediation scheme may involve an intensively cultivated plantation of plants with phraetophytic characteristics, such as willows and *Eucalyptus* or other suitable vegetation and irrigation with the contaminated water.

Summary of Results – Perchlorate Studies

- Selected woody plants have been shown to be potentially effective in the remediation of perchlorate contaminated water.
- Minced French tarragon, spinach and their crude extracts, *Myriophyllum aquaticum* and mixed-species microbial mats degraded perchlorate to chloride at different rates.
- Perchlorate is both phyto-accumulated and degraded by woody plants.
- Minced French tarragon and spinach degraded perchlorate to chloride.
- In Eucalyptus plants up to 40% of the initial 100-ppm Perchlorate was degraded in 25 days.
- Prolonged exposure of Eucalyptus and willow plants to perchlorate dosed medium enhanced rhizodegradation.
- Homogeneous perchlorate transformation studies with medium taken from the root zone of a willow confirmed that perchlorate is completely reduced in the rhizosphere to chloride.
- Plant exudates (for example acetate) enhanced the rhizodegradation of perchlorate.
- Remediation of perchlorate is impeded at high nitrate concentrations in solution.
- Bacteria associated with the rhizodegradation of perchlorate have been isolated from medium taken from the rhizosphere of a willow plant.
- Phytoremediation mechanisms of perchlorate include
    - Phytoextraction and accumulation in Plant leaves, branches and upper stem
    - Rhizostimulation
    - Phytodegradation

The results of this study present strong evidence that selected plants are effective in the remediation of TCE, PCE and perchlorate contaminated water and soils. Future work should include the isolation of rhizosphere bacteria and the plant enzyme(s) that catalyze the degradation of perchlorate. Also, further studies are needed to verify if one plant species could be effective in the remediation of mixed-wastes of chlorinated solvents and perchlorate.

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(1) Hales, H.M.; Jaouni, T.M. and Babashak, J.F., 1973, Simple device for preparing ethereal diazomethane without resorting to codistillation. Analytical Chemistry, 45(13), 2302.

Table 1. The Distribution of Identified PCE Metabolites in Solution and Cottonwood Tissues

Products	Aqueous Phase	Root	Lower Stem	Upper Stem	Leaf	Headspace
PCE (mg/kg)	10	38.64	33.99	4.32	0	trace
TCE (mg/kg)	2.16	0.6	1	0.09	0	trace
TCAA (mg/kg)	0.009	0.589	1.424	6.411	21.66	
DCAA (mg/kg)	0	0.219	0.111	0.884	1.703	
MCAA (mg/kg)	0	0	0.265	0.666	0.24	
Trichloroethanol (ug/kg)	trace	0	0.24	0.337	0.096	
1,1,1,2-tetrachloroethane	trace			trace	0	
1,1,2-trichkroethane	trace			trace	trace	trace
Ethene						trace
Trans-DCE (mg/kg)	0	0	0	0	0	0

* TCAA: trichloroacetic acid, DCAA: dichloroacetic acid, MCAA: monochloroacetic acid.

Appendix A

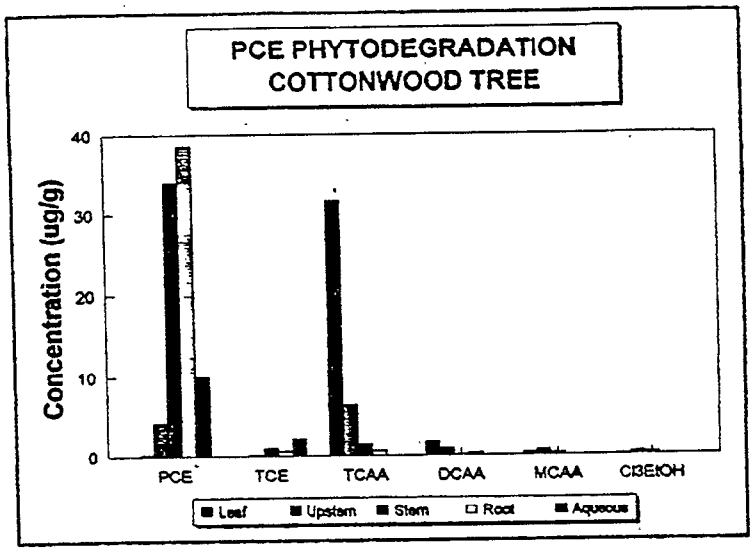

PCE PHYTODEGRADATION
COTTONWOOD TREE
Concentration (ug/g)
40
30
20
10
0
PCE
TCE
TCAA
DCAA
MCAA
CBEtOH
Leaf
Upstem
Stem
Root
Aqueous

TABLE 2: The Distribution of PCE Metabolites in Solution and Willow Tissues

Products	Aqueous Phase	Root	Lower Stem	Upper Stem	Leaf	Headspace
PCE (mg/kg)	10.72	38.75	19.5	3.11	0	trace
TCE (mg/kg)	0.069	0	0	0	0	0
TCAA (mg/kg)	0	1.61	0.109	0.158	2899.5	
DCAA (mg/kg)	0	2.64	0.82	0.495	1684	
MCAA (mg/kg)	0	trace	0	0	0	
Trichloroethanol (ug/kg)	trace	0	68.8	33.4	41	
1,1,1,2-tetrachloroethane	trace			trace	0	
1,1,2-trichloroethane	trace			trace	trace	trace
Dichloroethanol	trace			trace	trace	0
Ethene						trace
Trans-DCE (mg/kg)	trace	trace	trace	trace	trace	trace

* TCAA: trichloroacetic acid, DCAA: dichloroacetic acid, MCAA: monochloroacetic acid.

Table 3. TCE Metabolites Identified in Willow Tree-Within 2 Weeks

Date	TCE(ppm)	Trichloroethanol(ppm)	TCAA	DCAA	MCAA	Ethene	Vinyl Chloride	DCE	Ethanol
07/17/98									
Solution:									
Initial	55.16	0	ND	ND	ND				
07/30/98									
Solution:	23.04	0	ND	ND	ND				
Branch:									
Hexane	trace	0.36	NA	NA	NA				
MTBE	trace	2.02	0	0	0				
Total	trace	2.38	0	0	0				
Leaves:									
Hexane	trace	0.18	NA	NA	NA				
MTBE	trace	0.7	0	0	0				
Total	trace	0.78	0	0	0				
Headspace:	Yes	ND	NA	NA	NA	Yes	Yes	Yes	Yes

* NA : Method does not apply; ND: Not detected.

Table 4. The Distribution of Perchlorate in Willow Tree and Solution Phase after 26 Days

Portion	Fraction (%)	Perchlorate Accumulated (mg/kg)	Mass of Perchlorate Ion (mg)	Fraction of Initial Mass (%)
Root	1.56	94.66	0.043	0.05
Lower Stem	22.61	28.11	0.184	0.21
Upper Stem	73.79	16.11	0.344	0.40
Leaf	2.04	813.0	0.481	0.55
Sand Layer		17.86	25.82	31.77
Aqueous		55.95	50.36	56.03

Perchlorate Mass Balance:

Initial	86.8 (mg)
Recovered	77.2 (mg)
Degraded	11.0 %

* The volume of aqueous phase was 900 mL.

Table 5. The Distribution of Perchlorate in Eucalyptus and Solution Phase after 24 Days

Portion	Fraction %	Perchlorate Accumulated mg/kg	Mass of Perchlorate Ion mg	Fraction of Initial Mass %
Root	6.63	0.4	0.002	0.003
Lower Stem	16.38	0.54	0.005	0.006
Branch	31.33	351.6	6.031	7.82
Leaf	45.78	640.9	16.78	21.74
Sand Layer		11.04	22.08	28.61
Aqueous		1.39	1.25	1.62

Perchlorate Mass Balance:

Initial	77.2 (mg)
Recovered	46.2 (mg)
Degraded	40.2 %

* The volume of aqueous phase was 900 mL.

Table 6. The Distribution of Perchlorate in Cottonwood and Solution Phase after 42 Days.

Portion	Fraction %	Perchlorate Accumulated mg/kg	Mass of Perchlorate Ion mg	Fraction of Initial Mass %
Root	1.22	0	0	0
Lower Stem	38.33	0	0	0
Upper stem	58.07	7.80	0.128	0.15
Branch	2.25	239.4	0.153	0.18
Leaf	3.99	3536	4.016	4.73
Sand Layer		19.83	44.22	52.10
Aqueous		56.95	34.17	40.26

Perchlorate Mass Balance:

Initial	84.9 (mg)
Recovered	82.7(mg)
Degraded	2. 6 %

* The volume of aqueous phase was 600 mL.

Figure Captions

Figure 1: Reactor Schematic used in TCE and PCE Phytotransformation Studies.

Figure 2: Representative Plot of Uptake and Transformation of PCE by Cottonwood Trees.

Figure 3: Representative Plot of Uptake and Transformation of PCE by Willow Trees.

Figure 4: Proposed Phytotransformation Pathways for PCE Based Only on Positively Identified Products.

Figure 5: Representative Plot of Uptake and Transformation of TCE by Willow Trees.

Figure 6: Calibration Curve of Low (A) and High (B) Concentrations of Perchlorate By Ion Chromatography Using a Dionex method.

Figure 7: Representative Plots of Uptake and Transformation of Perchlorate by Cottonwood, Willows and Eucalyptus Trees. (Sand Bioreactor)

Figure 8: Uptake and Transformation of Low Concentrations (20 ppm) of Perchlorate by Willow Trees. (No sand in Bioreactor)

Figure 9: Phytodegradation of Perchlorate by Minced French Tarragon (*Artemisia dracunculus*).

Figure 10: Long-term Study on Uptake and Transformation Including Rhizodegradation of Perchlorate by Willow Trees Dosed Multiple Times.

Figure 11 (A, B): Net increase of Chloride formed in the Root-zone of a Willow Tree Dosed Multiple Times with Perchlorate (A) and Chloride Mass Balance (B) in the Rhizosphere.

Figure 12: Homogeneous Degradation of Perchlorate in the Medium Withdrawn from Rhizosphere of Willow Trees. Effects of Amending Medium with Nitrate, Acetate, and both Acetate and Nitrate.

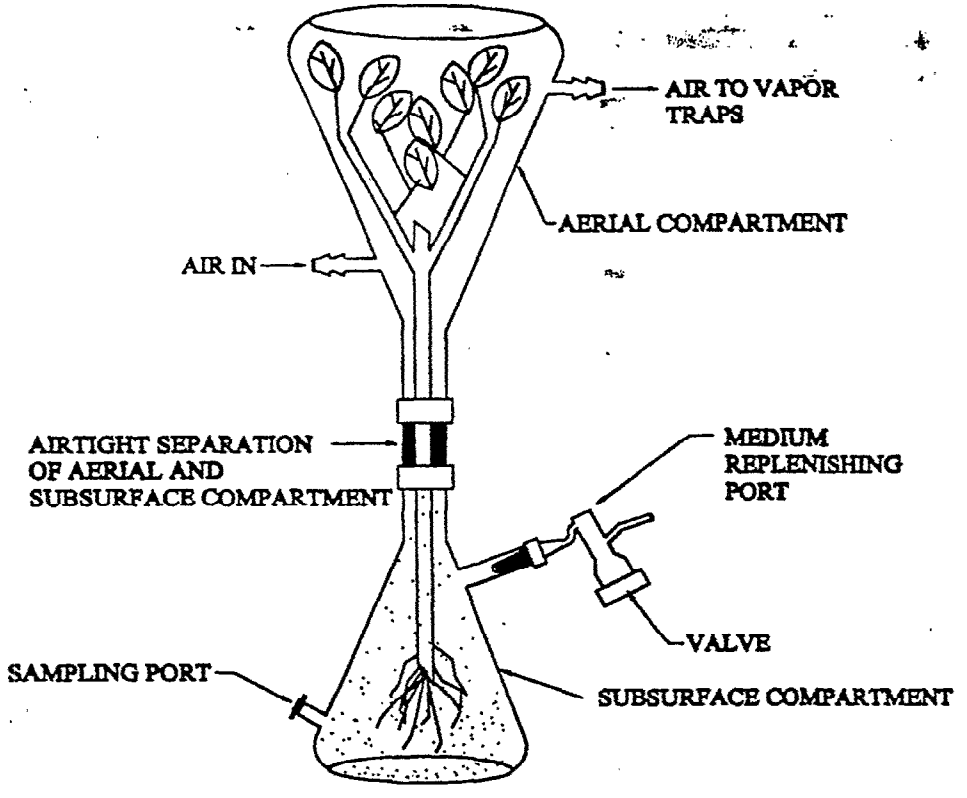

AIR TO VAPOR TRAPS
AERIAL COMPARTMENT
AIR IN
AIRTIGHT SEPARATION OF AERIAL AND SUBSURFACE COMPARTMENT
MEDIUM REPLENISHING PORT
VALVE
SAMPLING PORT
SUBSURFACE COMPARTMENT

Figure 1

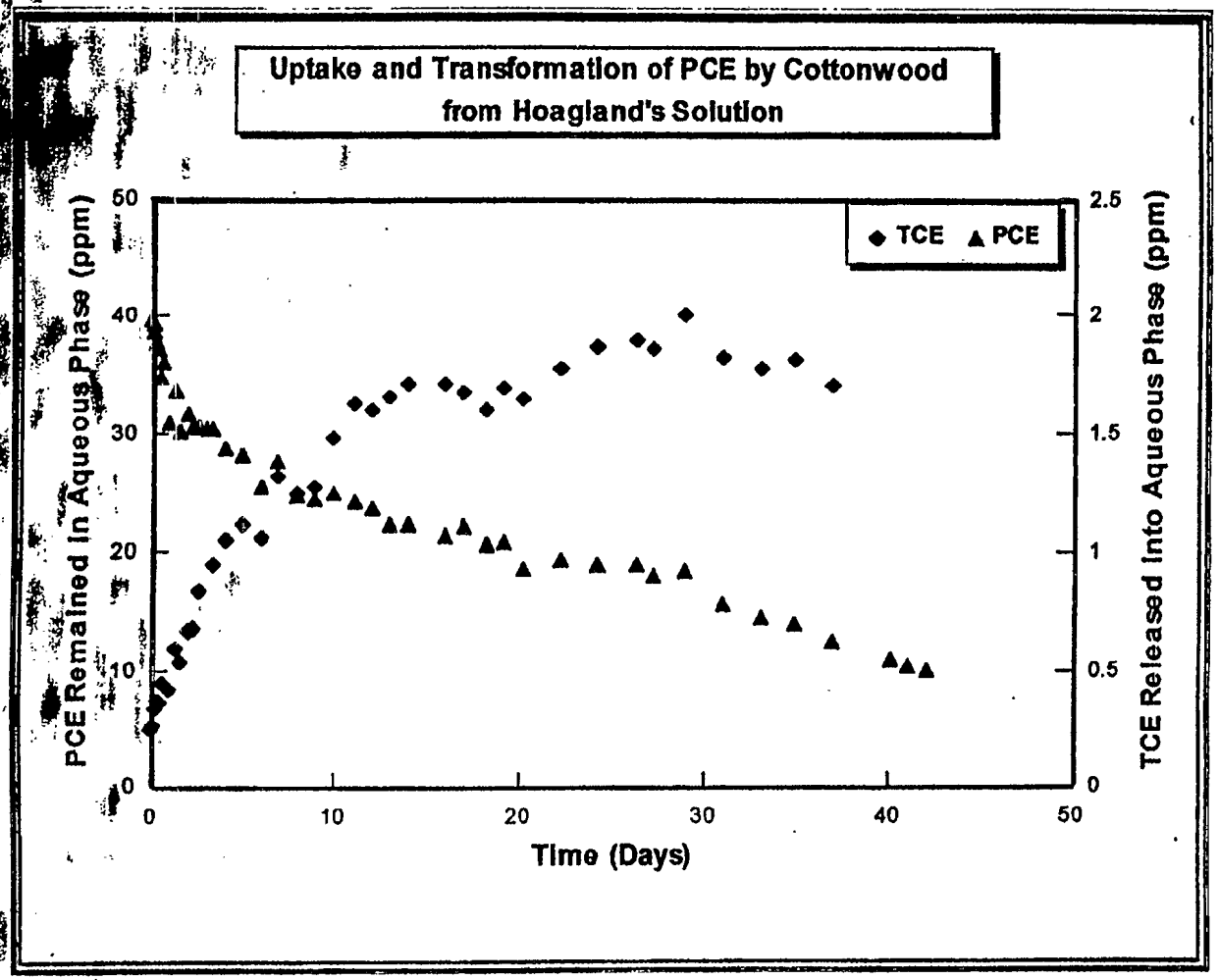

Uptake and Transformation of PCE by Cottonwood
from Hoagland's Solution
TCE
PCE
PCE Remained In Aqueous Phase (ppm)
TCE Released Into Aqueous Phase (ppm)
Time (Days)
0
10
20
30
40
50
0.5
1
1.5
2
2.5

Figure 2

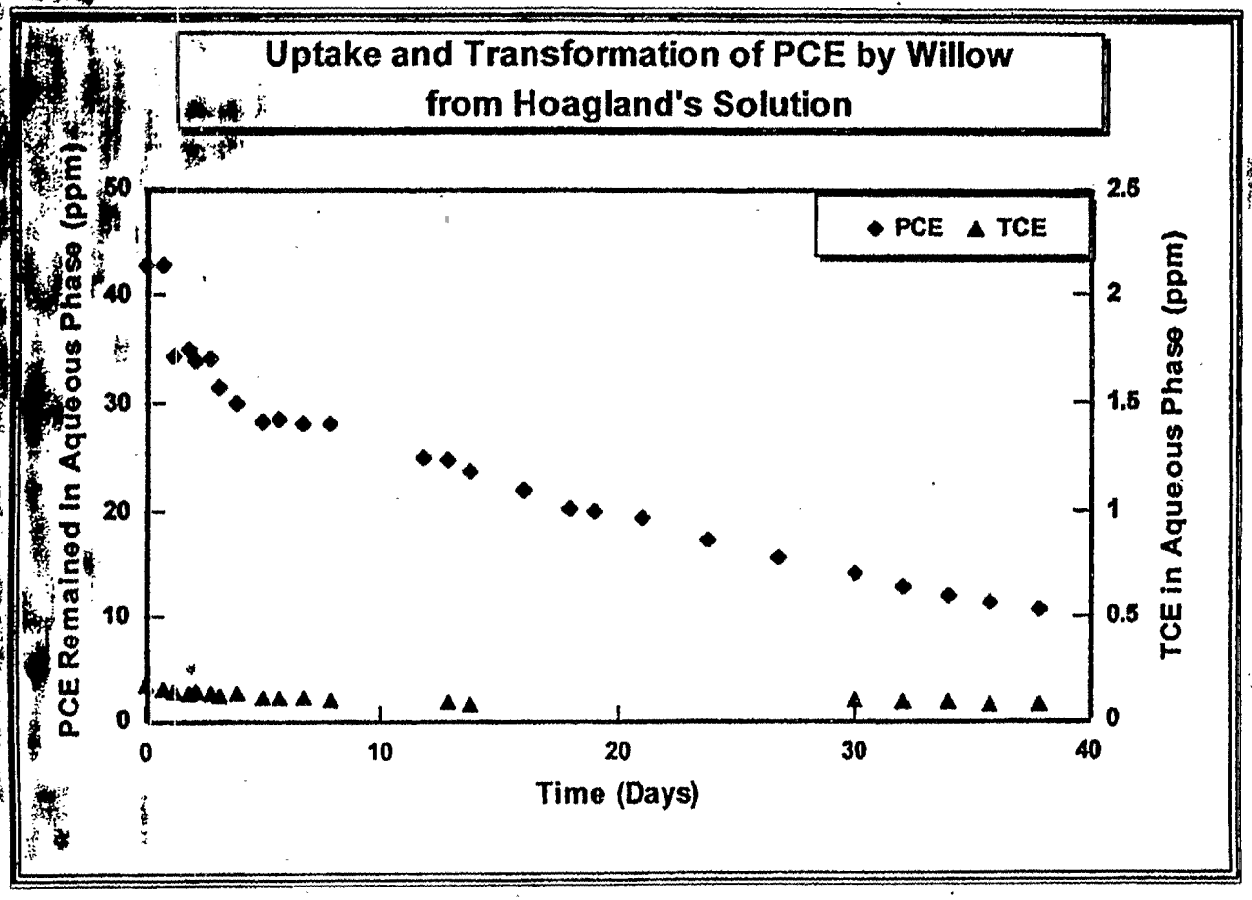
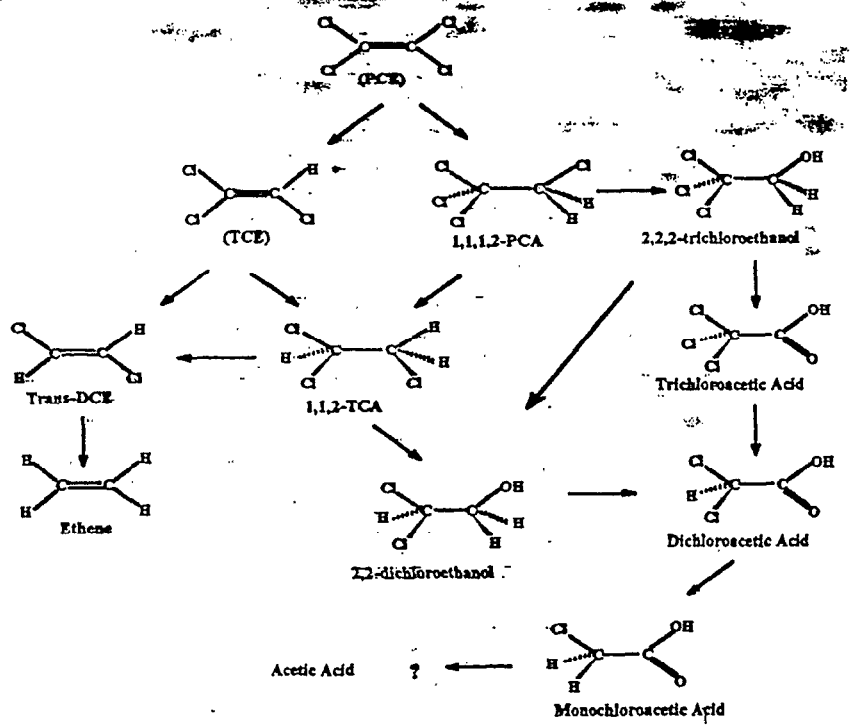

Uptake and Transformation of PCE by Willow
from Hoagland's Solution
PCE Remained In Aqueous Phase (ppm)
TCE in Aqueous Phase (ppm)
Time (Days)
PCE
TCE
0
10
20
30
40
50
0.5
1
1.5
2
2.5

Figure 3



The Pathway of PCE Degradation by Woody Plants
*** Not detected with Willows**

Figure 4

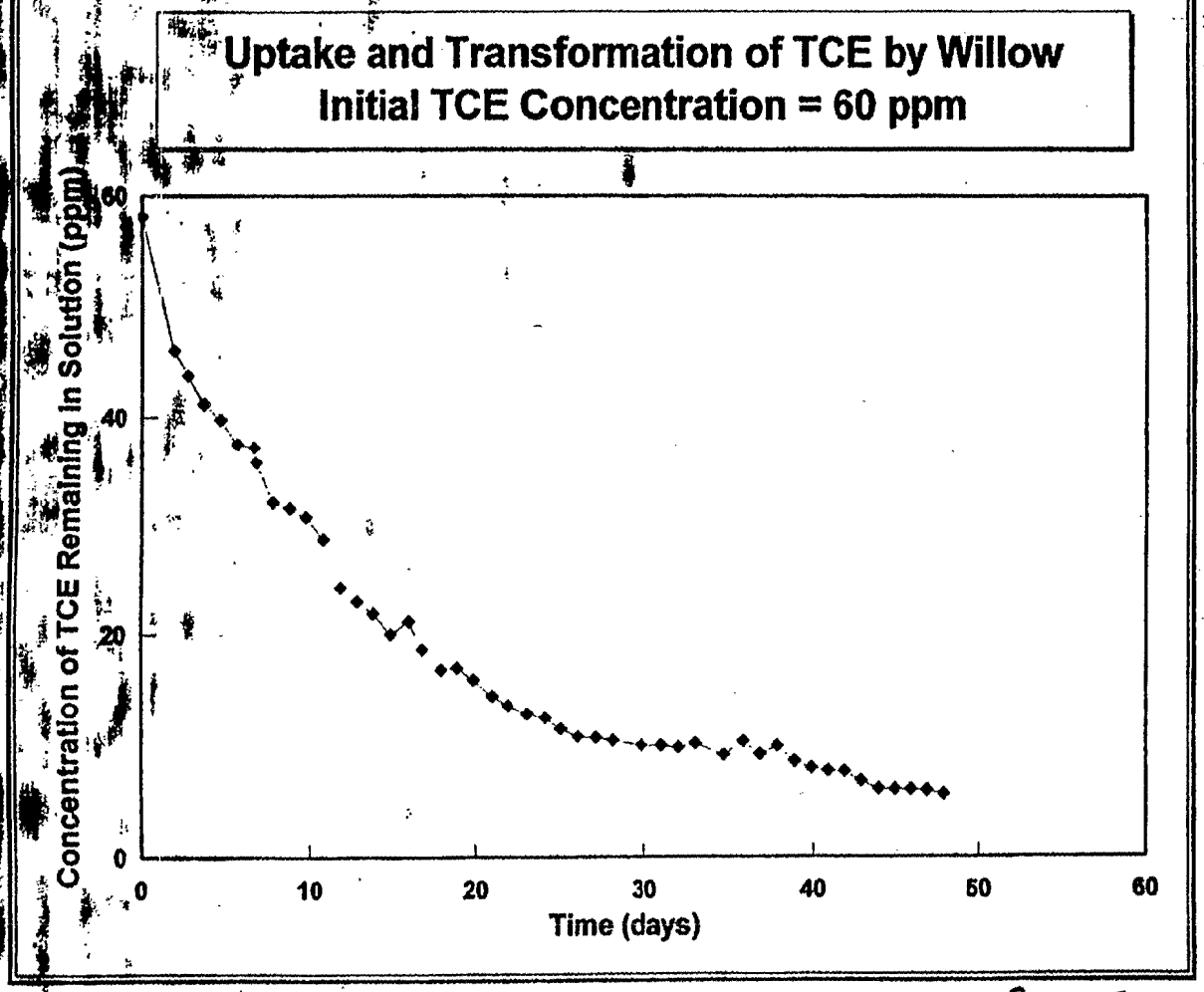

Uptake and Transformation of TCE by Willow
Initial TCE Concentration = 60 ppm
Concentration of TCE Remaining In Solution (ppm)
0
20
40
60
0
10
20
30
40
50
60
Time (days)

Figure 5

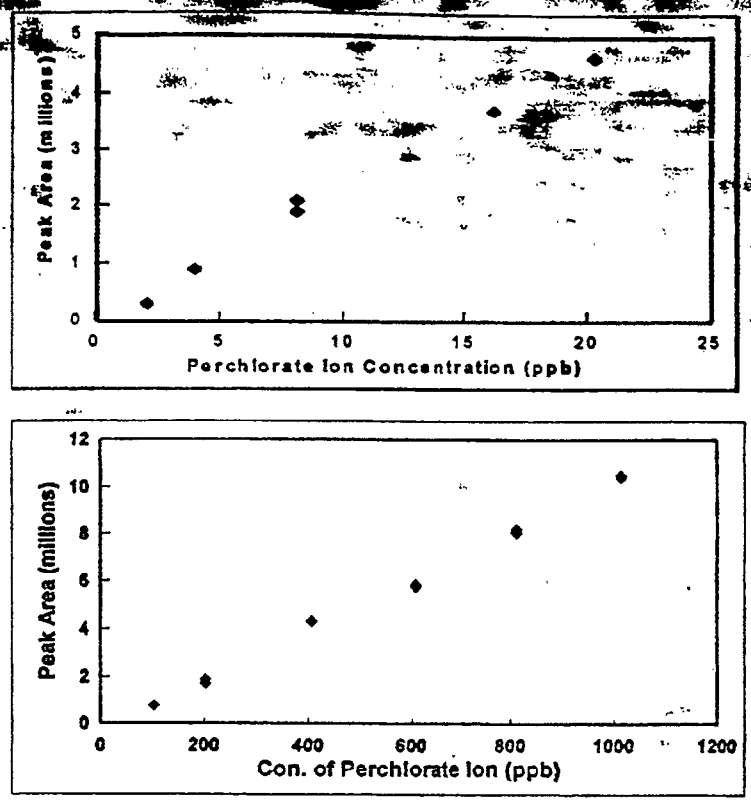


Figure 6. Calibration Curve for Perchlorate Measurement by Ion Chromatography

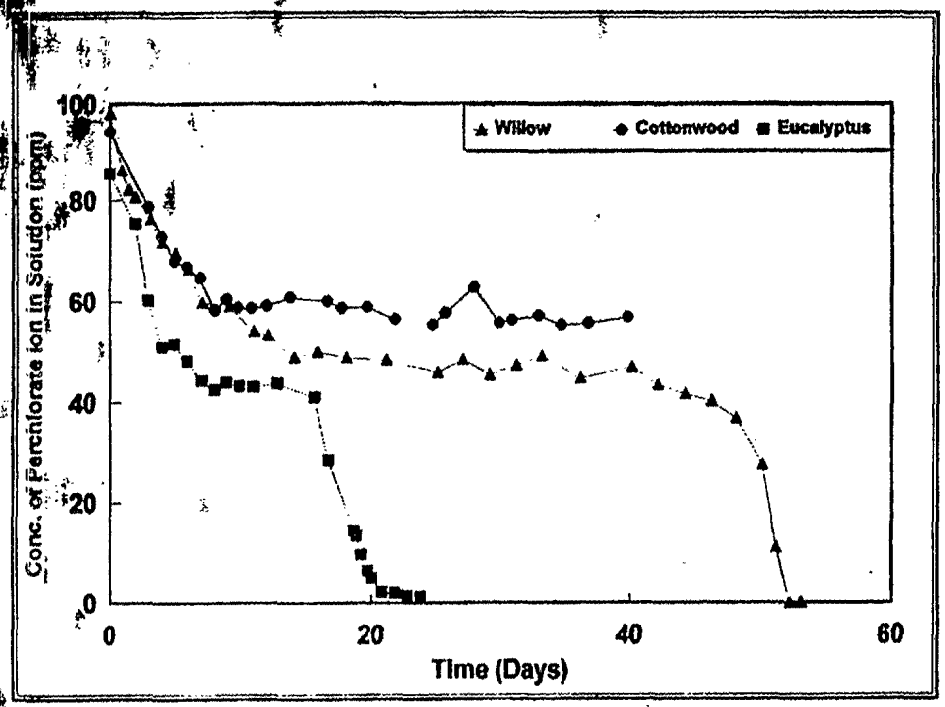

Willow
Cottonwood
Eucalyptus
Conc. of Perchlorate ion in Solution (ppm)
100
80
60
40
20
0
0
20
40
60
Time (Days)

Figure 7

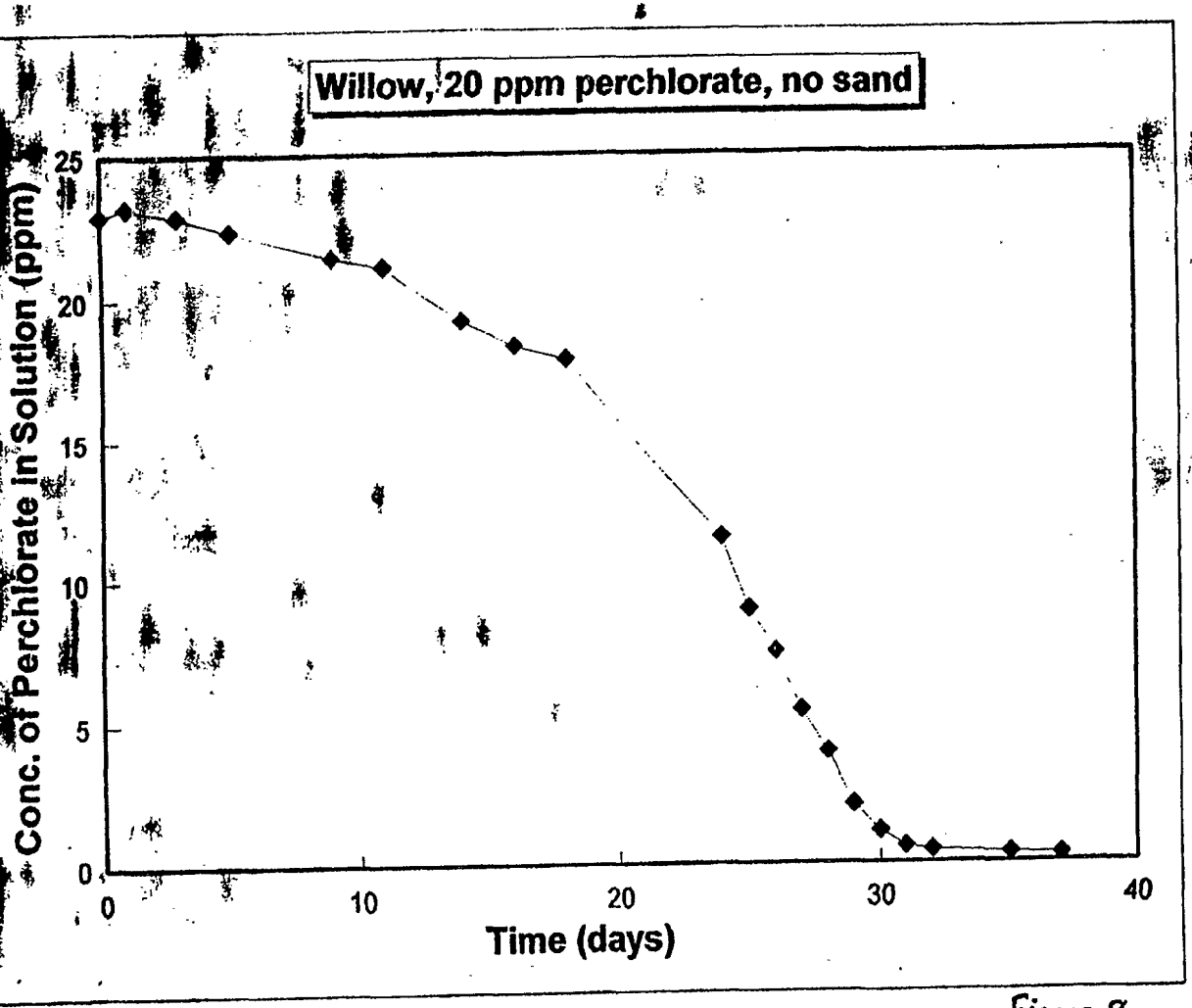

Willow, 20 ppm perchlorate, no sand
Conc. of Perchlorate in Solution (ppm)
25
20
15
10
5
0
0
10
20
30
40
Time (days)

Figure 8

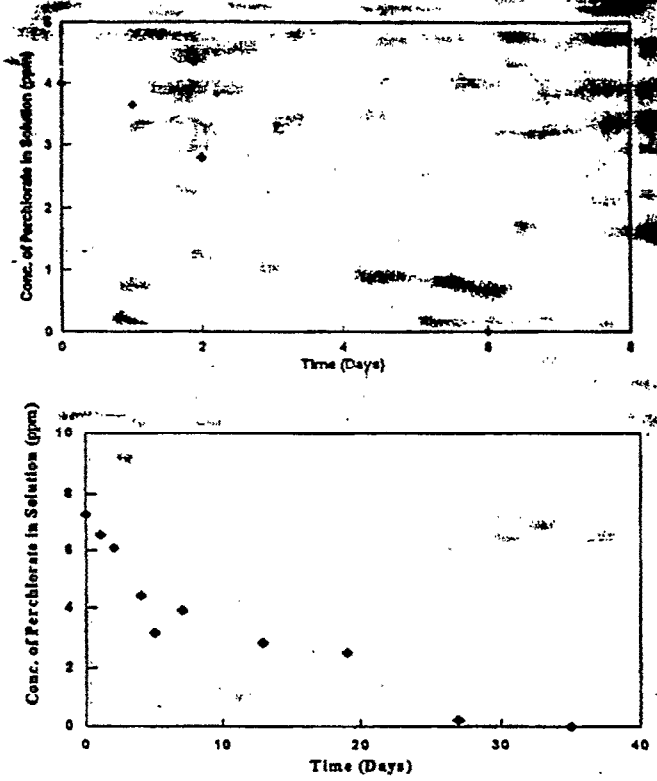


Figure 9. Perchlorate Degradation by Taragon Leaves.

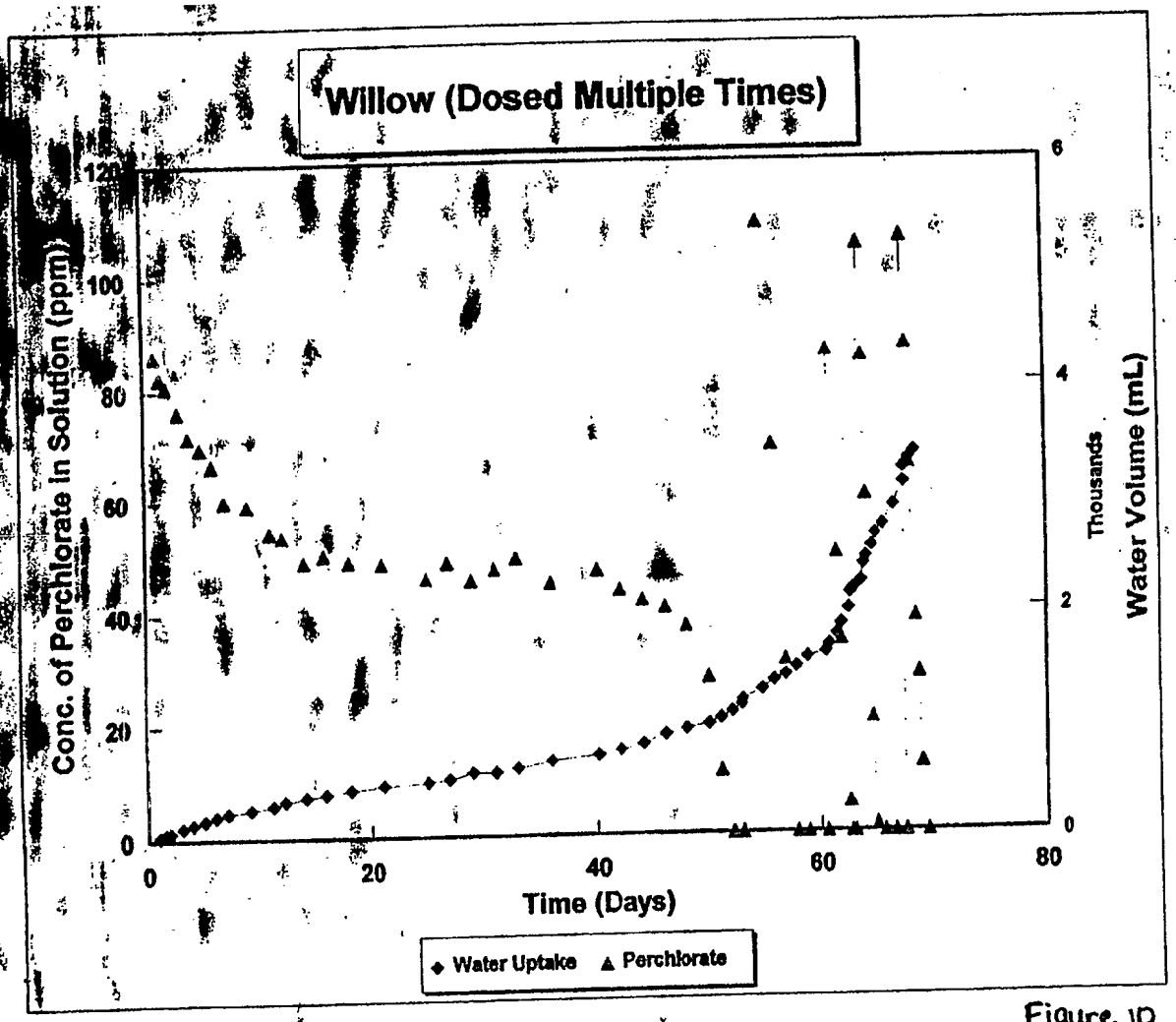

Willow (Dosed Multiple Times)
Conc. of Perchlorate in Solution (ppm)
0
20
40
60
80
100
120
Time (Days)
0
20
40
60
80
Water Volume (mL)
Thousands
0
2
4
6
Water Uptake
Perchlorate

Figure 10

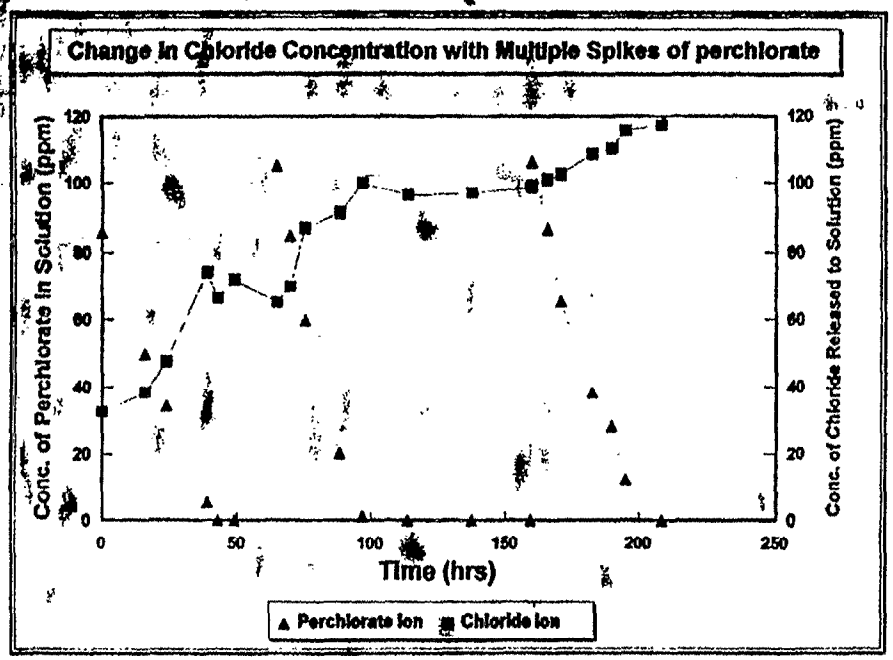

Change in Chloride Concentration with Multiple Spikes of perchlorate
Conc. of Perchlorate in Solution (ppm)
Conc. of Chloride Released to Solution (ppm)
Time (hrs)
0
20
40
60
80
100
120
0
50
100
150
200
250
Perchlorate Ion
Chloride Ion

Figure 11A

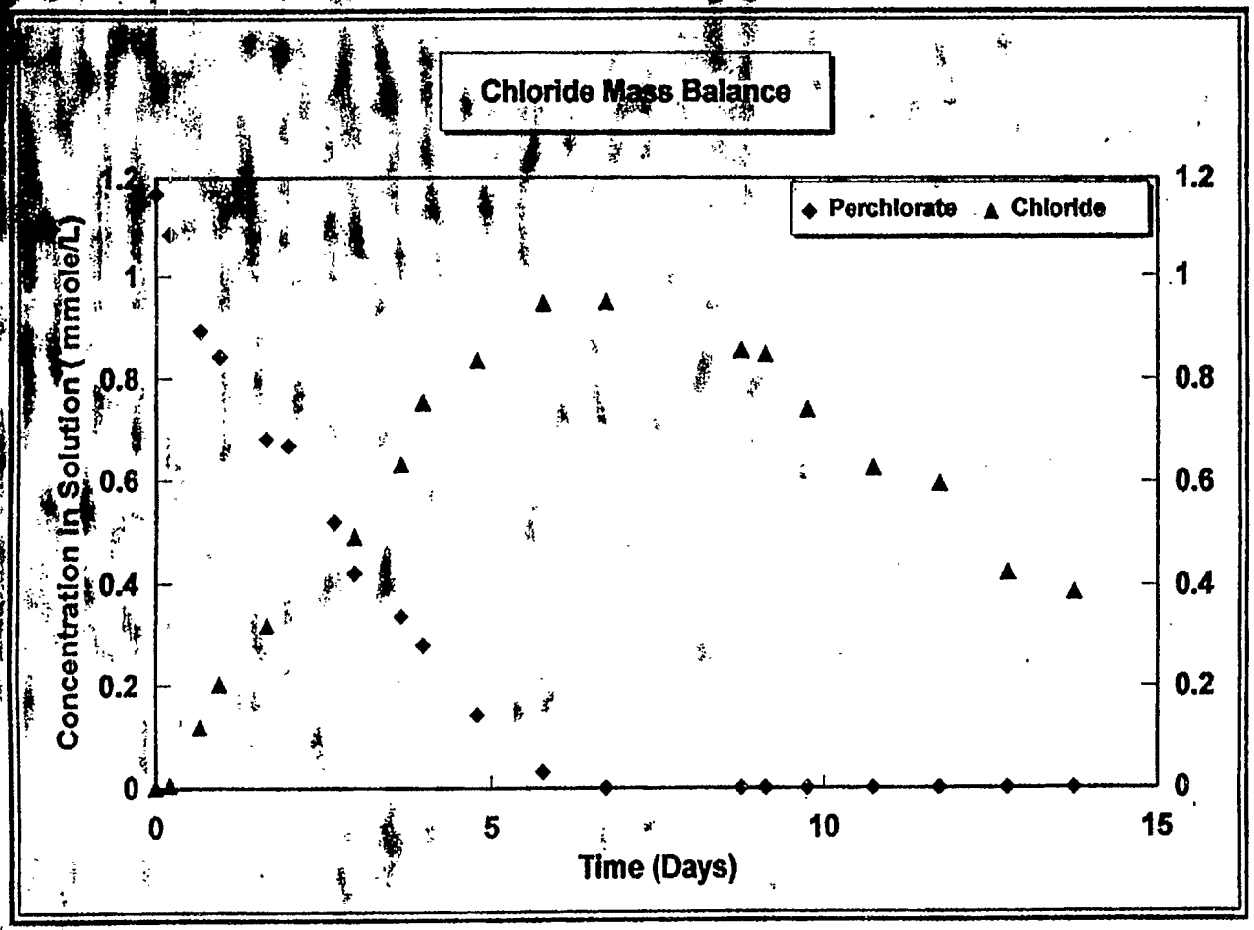

Chloride Mass Balance
Perchlorate
Chloride
Concentration in Solution (mmole/L)
Time (Days)
0
0.2
0.4
0.6
0.8
1
1.2
0
5
10
15

Figure 11B

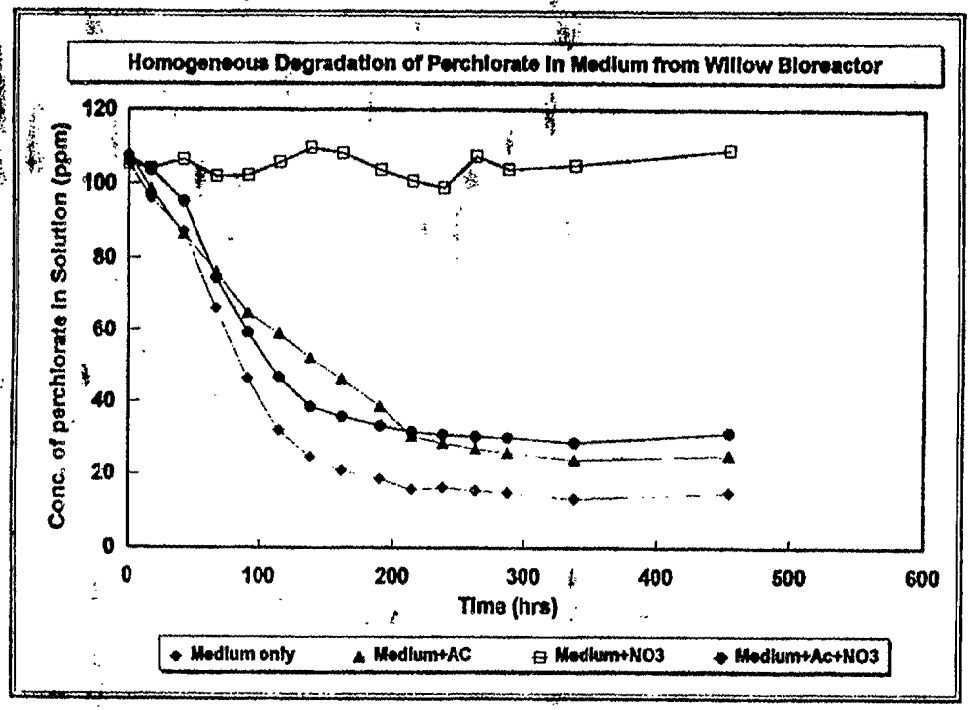

Homogeneous Degradation of Perchlorate in Medium from Willow Bioreactor
Conc. of perchlorate in Solution (ppm)
120
100
80
60
40
20
0
0
100
200
300
400
500
600
Time (hrs)
Medium only
Medium+AC
Medium+NO3
Medium+Ac+NO3

Figure 12