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Is this an abstract?

RHIZOSPHERE MICROBIOLOGY OF CHLORINATED ETHENE-CONTAMINATED SOILS: EFFECTS ON PHOSPHOLIPID FATTY ACID CONTENT

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Microbial degradation of chlorinated ethenes (CE) in rhizosphere soils was investigated at seepline areas impacted by CE plumes. Successful bioremediation of CE in rhizosphere soils is dependant on microbial activity, soil types, plant species, and groundwater CE concentrations. Seepline soils were exposed to trichloroethylene (TCE) and perchloroethylene (PCE) in the 10-50 ppb range. Greenhouse soils were exposed to 2-10 ppm TCE. Plants at the seepline were poplar and pine while the greenhouse contained sweet gum, willow, pine, and poplar. Phospholipid fatty acid (PLFA) analyses were performed to assess the microbial activity in rhizosphere soils. Biomass content was lowest in the nonvegetated control soil and highest in the Sweet Gum soil. Bacterial rhizosphere densities, as measured by PLFA, were similar in different vegetated soils while fungi biomass was highly variable. The PLFA soil profiles showed diverse microbial communities primarily composed of Gram-negative bacteria. Adaptation of the microbial community to CE was determined by the ratio of ω7t/ω7c fatty acids. Ratios (16:1ω7t/16:1ω7c and 18:1ω7t/18:1ω7c) greater than 0.1 were demonstrated in soils exposed to higher CE concentrations (10-50ppm), indicating an adaptation to CE resulting in decreased membrane permeability. Ratios of cyclopropyl fatty acids showed that the vegetated control soil sample contained the fastest microbial turnover rate and least amount of environmental stress. PLFA results provide evidence that sulfate reducing bacteria (SRB) are active in these soils. Microcosm studies with these soils showed CE dechlorinating activity was occurring. This study demonstrates microbial adaptation to environmental contamination and supports the application of natural soil rhizosphere activity as a remedial strategy.

Introduction

Groundwater monitoring at the Department of Energy, Savannah River site (SRS) in Aiken, SC has revealed that there is groundwater contamination in the A/M area of the SRS including the Lost Lake aquifer. A plume containing tetrachloroethylene (PCE) and trichloroethylene (TCE) has migrated towards a seepline associated with the Lost Lake aquifer. Phytoremediation is an emerging technology that has proven to be environmentally friendly, cost effective and capable of remediating contaminants *in situ*. Phytoremediation relies on microbial activity in the rhizosphere for processing nutrients as well as potential contaminants. Organic contamiants including chlorinated ethenes (CE) may be degraded by specific microbial activity in rhizosphere soils and the underlying sediments.

This study was undertaken to characterize microbial communities in the rhizosphere soils of four tree species, Hybrid Poplar (*Trichocarpa deltoids*), Coyote Willow (*Salix exigua*), Sweet Gum (*Liquidambar stryaciflua*) and Loblolly Pine (*Pinus taeda*). These soils are exposed to PCE and TCE and the impact of these contaminants on their respective rhizosphere communities is unclear. A greenhouse study was conducted using the above trees to test exposure to water contaminated with 10 ppm TCE and soils. A field project utilizing pines and poplars continually irrigated with groundwater contaminated with 48 ppb PCE and 46 ppb TCE. In addition sediment cores were taken from a 'clean' seepline area as well as a 'contaminated' area for evaluation. Phospholipid fatty acid analysis was used to directly characterize rhizosphere soils and site sediments.

At harvest, measurements revealed that trees in the vegetated control were slightly taller and weighed more than the trees fed with the contaminated groundwater. Soil analysis from GC-MS showed that the PCE and TCE concentrations were much higher in the non-vegetated control than the vegetated soils. The pine rhizosphere soils demonstrated the presence of cis-dichloroethylene (cDCE), indicating some microbial dechlorination activity.

The observance of PCE and TCE in the tissues of the tree species confirms the potential for their viable phytoremediation option combined with soil monitored natural attenuation (MNA) at the PCE/TCE contaminated area at the SRS.

MATERIALS and METHODS

Soils and Sediments

Rhizosphere samples were collected from cells containing rhizosphere soils with TCE and PCE contaminated water flowing though them (Figure 1). The cells contained Pine, Poplar, and a soil control. The plants developed a large rhizosphere/root complex before harvest (Figure 2). For microbial analysis rhizosphere samples were taken from the cell with a stainless steel auger at depths of .5 m and 1 m (Figure 3).

A rhizosphere sample was taken at a contaminated area of the seepline (SSL-31 top) and a sediment sample taken 3 m deeper (SSL-31 3m). Similarly, a rhizosphere sample was taken at a non-contaminated area of the seepline (SSL-32 top) and a sediment sample taken 3 m deeper (SSL-32 3m) for evaluation.

A greenhouse study with Hybrid Poplar (*T. deltoids*), Coyote Willow (*S. exigua*), Sweet Gum (*L. stryaciflua*), Loblolly Pine (*P. taeda*), and a soil control was used to test the influence of exposure to water containing up to 10 ppm TCE on rhizosphere microbial communities.

Rhizosphere Soil and Sediment Samples: Microbial Analysis

Soil samples were collected at discrete depths from each of the soil and sediment cores. Samples were collected aseptically from the core and placed in sterile polyethylene bags (WhirlPack® Type). Following collection samples were refrigerated at 4°C for subsequent microbiological processing.

Acridine Orange Direct Counts: Total microbial population densities in the sediment samples were determined by the Acridine Orange Direct Count (AODC) Method (Balkwill 1989). Dry weights were determined, and density results were reported in cells/gram dry weight.

Total Plate Counts - Viable: Aerobic heterotrophic plate counts provide an estimate of the total number of viable aerobic and facultatively anaerobic bacteria in the soil. 5 gram

aliquots of sediment were collected from depth-discrete intervals from the seepline core and were aseptically mixed with 45mL Difco's FA Buffer, vortexed for 4 minutes, and plated onto media of 1% peptone, trypticase, yeast extract and glucose (PTYG) with 0.1% cycloheximide to inhibit fungal growth (Balkwill, 1989). The plates were incubated at room temperature (25°C) and read after 7 days on the Leica Quebec Darkfield Colony Counter. Dry weights were determined on the soil being tested and density determinations are reported in colony forming units (CFU)/gram dry weight.

PLFA. The microbial communities in soil and sediment samples were further characterized by phospholipid fatty acid content (PLFA Analysis) as described by Tunlid and White (Tunlid and White 1991) by Microbial Insights (Knoxville, TN).

Microscopy. Further direct characterization of rhizosphere microbiology was accomplished by epifluorescent examination of root material. Roots from Pine and Willow were aseptically dissected and fixed for 30 min in PBS with 4% formaldehyde in microcentrifuge tubes (1.5 ml). The fixation was followed by a 3X wash with PBS and then incubated for 10 min with 1 ml of FITC or PBS. Samples were washed once more and then examined and photographed on slides at a magnification of 1300X with a Zeiss Model 510 Laser Scanning Confocal Microscope (LSCM) (Carl Zeiss Inc., Thornwood, NY).

Figures:

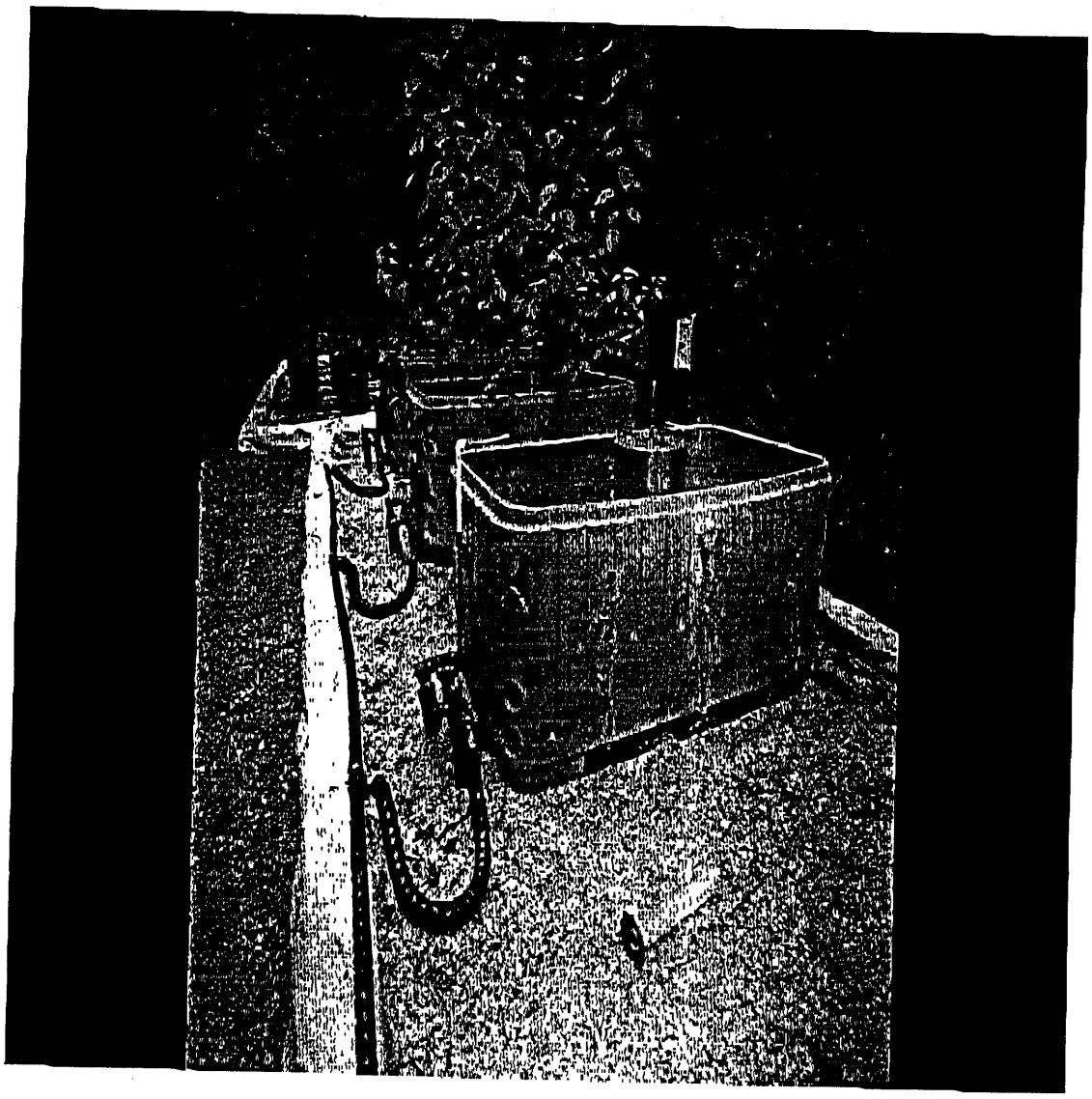

Figure 1. Poplar trees and soil control soils in cells supplied with PCE/TCE contaminated groundwater.

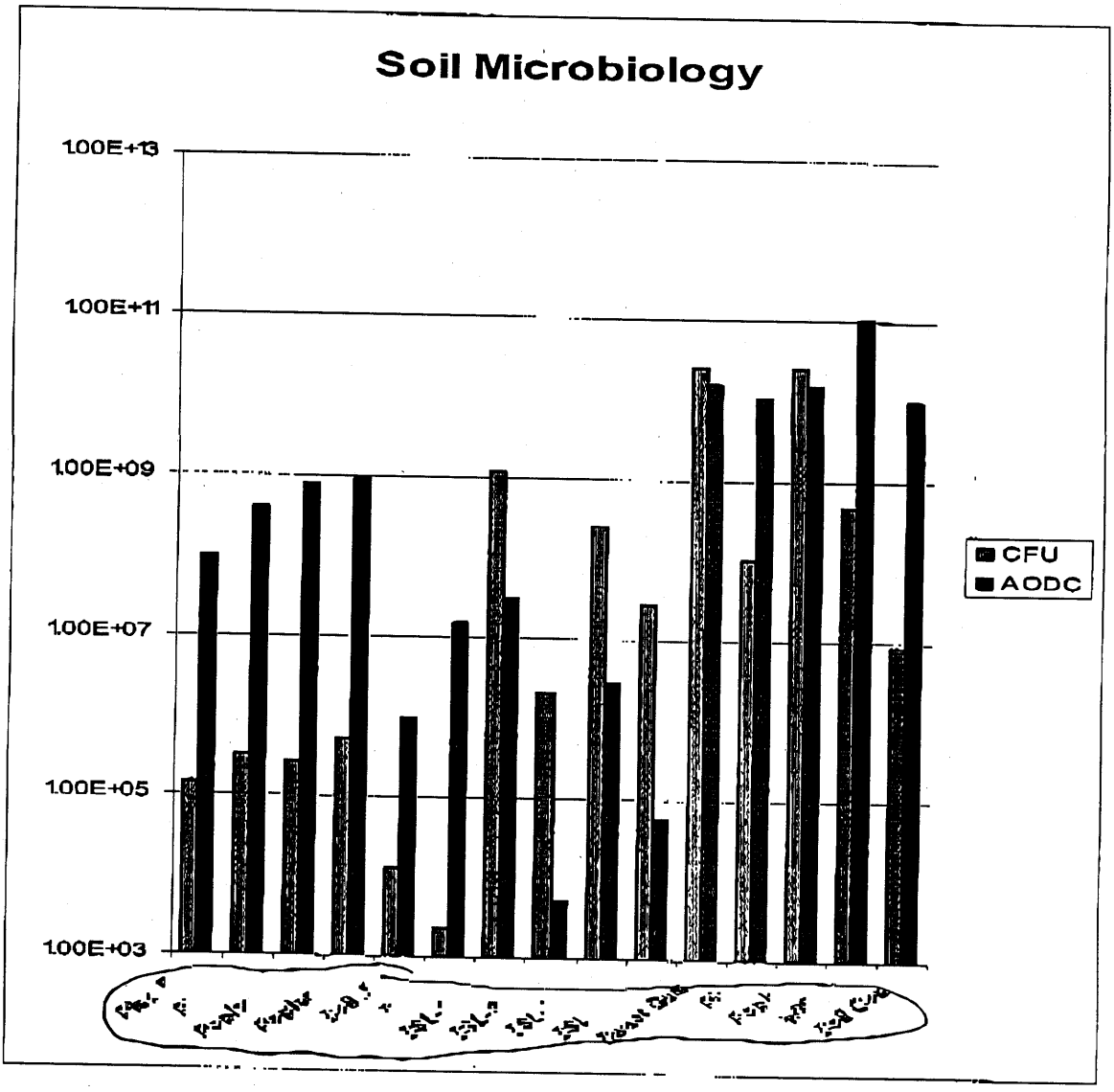

Figure 5. Soil and sediment microbial densities are presented as total counts/gram dry soil as measured by counting cells stained w/AODC. Heterotrophic cell concentrations were determined by plate counts w/Colony Forming Units (CFU).

- The microbial communities from those rhizosphere soils containing ppb levels of PCE and TCE (field cells) showed little difference from non-contaminated sites with similar soil types.

Laser Confocal Microscopy allows direct epifluorescent examination of FITC labeled material without disrupting the integrity of the sample.

- Figure 10A demonstrates methanotrophic bacteria labeled with FITC on the surface of Willow roots.
- Figure 10B demonstrates rhizosphere bacteria labeled within sectioned Willow root associated with internal plant cellular material.
- Figure 10C demonstrates bacteria labeled with FITC closely associated with Pine root hairs. These FITC labeled cells remained attached after several washes and rinses in processing.
- Rhizosphere bacteria labeled with FITC were observed in association with mycorrhiza in Pine root samples (10D). Extensive growth of mycorrhiza were observed in the pine rhizosphere soils.

Where are Figures?