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1999 Bioremediation Research Program Review

Foreword

In July 1995, the Biotechnology Research Subcommittee of the Committee on Fundamental Science (now the Committee on Science) of the National Science and Technology Council published a report, *Biotechnology for the 21st Century: New Horizons.* The chapter on Environmental Biotechnology in the report stated, "the successful development and application of bioremediation technologies depends on field-based research to verify the efficacy of planned approaches under natural conditions."

In Fiscal Years 1996, 1997, and 1998, four federal members of the Subcommittee - the U.S. Environmental Protection Agency, the National Science Foundation, U.S. Department of Energy, and the Office of Naval Research - joined together to initiate research at the field level that would address some of the scientific gaps and augment our understanding of basic chemical and physical principles as applied to the use of bioremediation technologies for the cleanup of hazardous wastes. The interagency committee focused the three solicitations for research grants on the fundamental issue of the bioavailability of chemicals for bioremediation processes in complex mixtures under field conditions. In the three years of the program, 29 grants were awarded for research on various aspects of bioavailability.

Bioavailability, defined for the purpose of this program as the availability of contaminants to an organism (including microorganisms, plants, and animals) that might degrade or otherwise transform them, is one of the principal factors controlling bioremediation processes. The future of this technology will depend on our ability to facilitate the interaction between the organisms and the chemicals in their environment so that they can be utilized as carbon sources or be otherwise co-metabolized and thus degraded. The physical, chemical, and biological aspects of these interactions are the subjects of the research grants that have been awarded.

This second program review is held in conjunction with the EPA-sponsored workshop on *Innovative Clean-up Approaches: Investments in Technology Development, Results and Outlook for the Future* held in Bloomingdale, Illinois, November 2-4, 1999. Presenting the results obtained by grantees in this program to workshop attendees will facilitate communication with the bioremediation community and provide useful information directly to users. In addition, the opportunity for feed-back from users to researchers will be useful in influencing future research directions. The results presented at the program review will also inform the participating agencies about research questions that need their future support.

Contents

BIOREMEDIATION PROGRAM REVIEW MEETING
EPA/DOE/NSF/ONR
NOVEMBER 3, 4, & 5, 1999
INDIAN LAKES RESORT, BLOMMINGDALE, IL

Wednesday, November 3,

10:00-10:30 Welcome and Introduction (Paul Bayer, DOE)

10:30 Clyde L. Munster, Malcolm Drew, M. Yavuz Corapcioglu,
Texas A&M University.
Phytoremediation and Modeling of Land Contaminated by Hydrocarbons

11:00 Richard F. Lee, Marc E. Frischer, Herbert L. Windom,
Skidaway Institute of Oceanography.
Controls on Plant Bioavailability in Salt Marsh Environments Which Can be Manipulated for Contaminated Sediment Remediation

11:30 Karl Rockne, Gary Taghon, Lily Young, David Kosson, Wenhsin Liang, Leslie Shor, Rutgers University.
Bioavailability of Organic Contaminants in Estuarine Sediments to Microbes and Benthic Animals

11:30 to 1:15 Lunch

1:15 Marc E. Frischer, Keith A. Maruya, Joel E. Kostka, Herbert L. Windom,
Skidaway Institute of Oceanography.
Biogeochemical Factors Limiting Transformation of Co-Occurring Contaminants in Salt Marsh Sediments.

1:45 Peddrick Weis, Judith Weis, Rutgers Unversity.
Wetland Plants' Roles in Uptake and Transport of Heavy Metals and Remediation

2:15 to 2:30 Break

2:30 John Sanseverino, Alice Layton, Betsy Gregory, James Easter, Fu-Min Menn,
T. Wayne Schultz, Gary S. Sayler, University of Tennessee.
Bioavailability of PCBs in Surfactant-Washed Soils With and Without UV-Irradation

3:00 Gina S. Shreve, William Finnerty, Wayne State University.
Biosurfactant Specificity and Influence on Microbial Degradation of Hydrocarbons by Microbial Consortia in the Field.

3:30 Todd Sandrin, Raina M. Maier, Ivan L. Pepper, University of Arizona.
Role of Metal Bioavailability in In Situ Bioremediation of Metal and Organic Co-Contaminated Sites

4:00 Teresa W-M. Fan, Fabienne Baraud, Richard Higashi, University of California, Davis.
Interaction of Heavy Metal Sequestration and Production of Metal Ion Ligands in Wheat Under Fe Deficient, CD and Soil Humic Treatments

4:30 Anne O. Summers, Keith Pitts, Andreas Heltzel, University of Georgia.
Enzymology of the Degradation of Organometallic Compounds

5:00 Speaker's corner Exhibits/Poster Sessions

Thursday, November 4, 1999

Recommend Session "A" on Metals - Bioavailability for early birds

9:15 - 9:30 Comments by Linda Chrisey (ONR)

9:30 Lisa Strong, Lawrence P. Wackett, Michael J. Sadowsky
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10:00 Tom M.Young, K.M. Scow, R.M. Higashi, T.W-M. Fan, E.Schwartz, L.F. Schultz, N. Watanabe, S. Loebmann, University of California, Davis.
Influence of Soil Organic Matter Composition on Desorption and Biodegradation of Aromatic Pollutants.

10:30 Morton A. Barlaz, Detlef R.U. Knappe, Bingyan Wu, Matthew Pelton, Caleb Taylor, North Carolina State University.
The Effects of Aging and Sorbent Decomposition on the Bioavailability of Toluene and Xylene in Solid Waste

11:00 - 12:00 Lunch

1:00 James D. Bryers, University of Connecticut.
Interaction Between Substrata Surface Chemistry, Conformation of Contaminant Upon Adsorption and Availability of Bacterial Degradation

1:30 Michael H. Huesemann, Tom Hausmann, Tim Fortman, Ann Drum, Battelle Pacific Northwest Division, Marine Science Laboratory.
The Influence of Soil Characteristics and Molecular Properties of Hydrophobic Contaminants on Bioavailability in Aged Soils

2:00 S.B. Soderstrom, A.D. Lueking, M. Johnson, M. Kim, W. Huang, W.J. Weber Jr., University of Michigan.
The Effects of Soil Organic Matter on Mineralization, Desorption, and Sequestration/Transformation of Phenanthrene

2:30-3:00 Break

3:00 Pedro Alvarez, E. Sawvel, M. Wildman, B. Eberle, K. Gregory, G.F. Parkin, J.L. Schnoor, University of Iowa.
Biogeochemical Interactions in Reactive Zero-Valent Iron Barriers

3:30 Jon D. Chorover, Patrick G. Hatcher, William D. Burgos, Pennsylvania State University.
Bioavailability of Aromatic Contaminants Bound to Solid and Aqueous Phase Natural Organic Matter

4:00 Sean W. McNamara, Richard G. Luthy, Carnegie Mellon University.
Bioavailability and Biostabilization of PCBs in Soils

4:30 Mark Brusseau

5:00 Adjourn for the day

Friday 5, 1999

8:30 - 9:30 Check Out

9:30 Anu Ramaswami, Tissa Illangasekare, Angela Bielefeldt, Kendra Morrison, Timothy J. Donahue, Eric Vestal, Allison Riffel, University of Colorado.
Bioavailability and Biostabilization of Multicomponent Non-Aqueous Phase Liquids (NAPLs) in the Subsurface

10:00 - 10:15 Break

10:15 P. A. Holden, A. Paranjpye, B. Bierwagen, S. Sirivithayapakorn, A.A. Keller, University of California, Santa Barbara.
Understanding Seasonal Variation of Bioavailability of Residual NAPL in the Vadose Zone

10:45 David M. Ward, E.A. Kern, G.M. Colores, W.P. Inskeep, Montana State University.
Population Biology of Bacteria Involved in Contaminant Bioremediation

11:15 Paul M. Bertsch, P.J. Morris, A.G. Sowder, T.V. Khijniak, University of Georgia.
Microbial Ecology and the Potential of Apatite Amendments for Reducing Metal Availability and Toxicity

11:45 - 1:00 Lunch

1:00 A. P. Schwab, M.K. Banks, J. Scott Smith, Purdue University.
The Effect of Plants on the Bioavailability and Toxicity of Contaminants in Soil

1:30 J.J. Pignatello, J.C. White, P.I. Ravikovitch, R. Russo, A. Neimark, Connecticut Agricultural Experiment Station.
The Influence of Nanoporosity in Soils From Contaminated Sites on Hydrocarbon Desorption Kinetics and Bioavailability

2:00 Sara J. MacNaughton, J.R. Stephen, Y.J. Chang, Y.D. Gan, J. Bownas, K.R. Carman, R. Millward, M. Barcelona, D.C. White, University of Tennessee.
Bioavailibility of Toxicants as Reflected in the In-Situ Microbial Community Ecology and Relationship to Defensible End-Points

2:30 Peter Adriaens, Kim F. Hayes, Michael J. Barcelona, University of Michigan.
Assessment of Biotic and Abiotic Processes Controlling the Fate of Chlorinated Solvents in Mixed-Waste Under Iron and Sulfate-Reducing Conditions Using Laboratory and In-Situ Microcosms

3:00 - 3:30 Wrap-up R.E. Menzer, (EPA)

Interaction of Heavy Metal Sequestration and Production of Metal ION Ligands in Wheat Under FE Deficient, CD, and Soil Humic Treatments

Teresa W-M Fan, Fabienne Baraud, and Richard Higashi
University of California, Davis, CA

Root exudation of metal ion ligands (MIL) such as phytosiderophoresis vital to nutritional acquisition of Fe and Zn, and may also be important to metal contaminant mobilization by plants. We have developed a broad chemical analysis of unfractionated exudates and plant tissue extracts for MIL by multidimensional NMR and GC-MS. Organic anions including amino and organic acids and mugineic acid (MA) phytosiderophores were identified and quantified. SH-rich peptides were also analyzed using fluorescent tag and SDS-PAGE. The MIL profile differed among plants and genotypes. Cd treatments of wheat caused a large reduction in the exudation of 2'-deoxymugineic acid (2'-DMA) and other MIL and yet a substantial increase in Fe, Zn, Cu, and Ni sequestration into roots, regardless of the Fe sufficient or deficient status. This suggests a higher efficiency of MIL or a different mechanism in facilitating transition metal (TM) uptake in Cd-contaminated rhizosphere. A large increase in the production of tissue SH-rich peptide and other MIL in Cd-treated wheat tissues may be related to the intracellular immobilization of excess Cd and transition metal ions.

In addition, we investigated the interaction of soil humic substance (HS, an important extant rhizosphere ligand) and Cd on metal ion uptake by wheat. The Cd treatment resulted in a large increase of metal ion content of Fe, Ni, Cu, Zn, and Mn in roots but not in shoots (except for Zn). Zn translocation to shoots was enhanced by the Cd treatment. The co-treatment of soil HS induced a biomass increase and a higher exudation of 2'-DMA, acetate, lactate, glycinebetaine, Ala, and g-aminobutyrate. This was, in turn correlated with a higher sequestration of Cd, Mn, Zn, and Ni into roots. The HS alone treatment also stimulated biomass production but a significant decrease in the exudation of major ligands 2'-DMA, malate, and acetate. The latter was related to the decreased content of Fe and Zn in roots and that of Mn, Fe, Zn, Cu, and Ni in shoots. It is likely that the exudation of some of all of these components were involved in the metal ion mobilization.

The concentrations of several MIL (malate, citrate, lactate, Asn, and glyceraldehyde-3-phosphate) in wheat roots were positively correlated with the root content of Fe, Ni, Cu, Mn, Zn, and Cd in the HS/Cd treatment series. It is likely that these compounds were involved in the intracellular chelation of excess metal ions, in addition to that by SH-rich peptides.

The Influence of Nanoporosity in Soils from Contaminated Sites on Hydrocarbon Desorption Kinetics and Bioavailability

W. Braida, J.C. White, J.J.Pignatello, The Connecticut Agricultural Experiment Station, New Haven; and
P. I. Ravikovitch, R. Russo, and A. Neimark, TRI/Princeton, Princeton, NJ

Biodegradation of contaminants in soil and sediment is often rate limited by contaminant mass transfer from remote to more accessible microregions of soil particles. An understanding of the mechanisms responsible for desorption resistance is the subject of this research. We hypothesize that desorption resistance is due to hindered diffusion in nanopores of molecular scale (0.2-2 nm) existing within the interstices of soil organic matter (SOM) and mineral aggregates. The objectives of the work are to develop methodology to determine soil nanoporosity based on CO_2 adsorption linked with theoretical models, and then to assess the relationship between nanoporosity and physical-chemical and biological availability parameters for aromatic hydrocarbons. In addition, we suggest it is possible to alter SOM nanoporosity by changing its glassy/rubbery properties via certain treatments or addition of co-solutes.

Phenanthrene sorption rates were measured for soils spanning a wide range of organic carbon (OC) content (Wurtsmith AFB 1AB, 0.18 %; Mount Pleasant Silty Loam, 4.45%; Pahokee Peat soil, an IHSS reference sample, 43.9%). Characteristic diffusion times (D/L^2, where D is the effective diffusion coefficient and L is the diffusion path length) were estimated for 'infinite bath' conditions (i.e., constant phenanthrene concentration) by fitting a radial diffusion model to sorbed concentration data estimated from experimental Freundlich isotherms measured at different times in batch experiments. The value of D/L^2 (Figure 1) is seen to depend on phenanthrene concentration and on soil (Braida and Pignatello, submitted for publication). The concentration dependence is a consequence of the thermodynamic nonlinearity of sorption and may be related to the glassy character of the SOM.

Additional work has focussed on competitive effects among PAHs. The existence of competition supports a heterogenous 'polymer' model of soil organic matter and is relevant to contaminated sites where mixtures are commonplace. Work in the first year (White et al., *Environ. Toxicol. Chem.*, 18:1728-32) showed that competitive displacement of phenanthrene by pyrene increased the bioavailability of phenanthrene. Current research sought to determine whether competition was expressed thermodynamically or kinetically, or both. Pyrene significantly reduced the sorption of phenanthrene and increased the linearity of its isotherm. Moreover, normalized *rates* of phenanthrene desorption at constant pyrene concentration increased significantly with increasing pyrene concentrations (e.g., Fig 2). This effect was observed in two soils of widely different OC contents, and even at low and equimolar phenanthrene and pyrene (closed circles). Competition may be due to the plugging of pores in glassy organic matter by the co-solute.

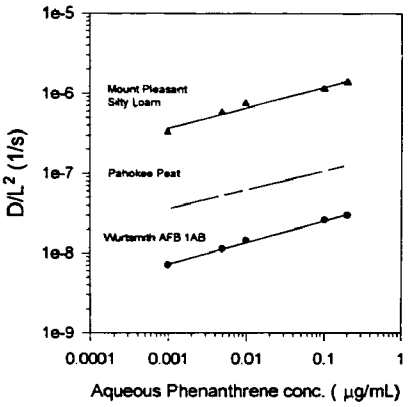
We have developed and verified a new experimental protocol and molecular level theoretical models for assessing nanoporosity in soils. The micropore size distributions are calculated from comparison of the experimental gas adsorption isotherms and the theoretical isotherms in model pores predicted by means of density functional theory (DFT) and grand canonical Monte Carlo (GCMC) simulations. The DFT model, theoretical foundations of which were formulated in our earlier work, has been modified by taking into account specifics of CO_2 interaction with carbonaceous matrixes. A new set of intermolecular parameters for CO_2/solid potentials was defined and verified against literature data. The MC simulation model has been developed and is being verified against reference samples. Preliminary results demonstrate consistency of the results of DFT and MC models.

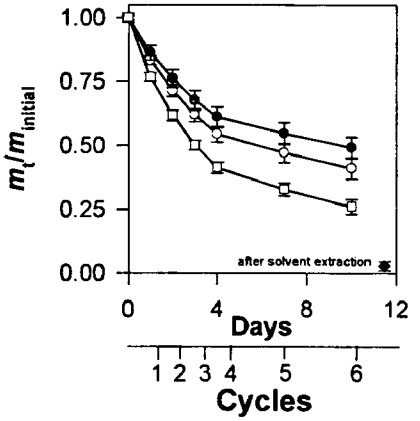
We have designed and fabricated a new electric thermostat allowing us to maintain temperature in the adsorption cell in the range -20° - +40 °C with a precision of 0.1 °C. Isotherms at different temperatures allow us to estimate the heat of sorption (see, for example, Figure 3).

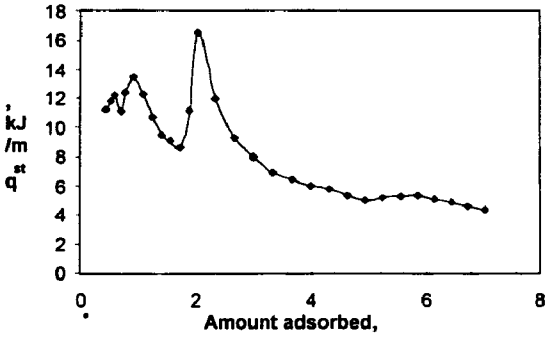
The CO_2 adsorption isotherms at 0°C and N_2 adsorption isotherms at 77K on 20+ soils samples and reference sorbents were measured and the pore size distributions were constructed. The CO_2 adsorption-desorption isotherms showed hysteresis that presumably indicates irreversibility of sorption in SOM nanopores. Hysteresis was especially strong in samples having high OC content: peat and its purified humin and humic acid fractions (Figure 4). CO_2 desorption was hindered until the vapor pressure was reduced tenfold. The adsorption isotherm on peat and humic acid revealed a bend not observed in humin. This behavior probably indicates a phase transformation in the humic acid fraction upon CO_2 sorption. The hysteresis phenomenon requires further study. We are currently developing

molecular models of sorption hysteresis that can be tested against capillary condensation isotherms collected on reference adsorbents such as mesoporous molecular sieves.

We hypothesize that the nanopores of 0.3 - 1 nm revealed in CO_2 sorption measurements are responsible for a delayed desorption kinetics of organic contaminants sequestered in pores of soil particles. The results obtained justify that CO_2 is a suitable molecular probe to study structural and sorption-desorption properties of soil particles containing SOM.







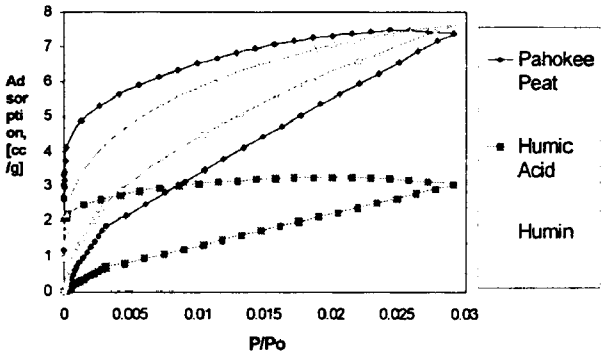


Figure 1. (upper left). Characteristic diffusion time in three soils as a function of phenanthrene concentration.

Figure 2. (upper right). Effect of pyrene on desorption of phenanthrene aged for 42 days in Cheshire fine sandy laom using a sequential dilution technique in which the supernatant in each cycle was replaced with water at constant pyrene concentration. The $m_{initial}$ is the initial mass present on the solids. Pyrene was added 14 days before phenanthrene at: 0.0 (•), 114 (O), and 1010 (□) µg (g OC)$^{-1}$.

Figure 3. (lower left). Isosteric heats of CO_2 sorption on Pahokee peat derived from the adsorption branches of the isotherms at three temperatures using Clausius-Clapeyron equation.

Figure 4. (lower right). CO_2 adsorption-desorption isotherms at 0 °C on Pahokee peat soil (93% SOM) and its humin and humic acid fractions. Extreme low-pressure hysteresis between sorption and desorption isotherms is observed. This suggests that sorption in the organic domain (SOM) is responsible for contaminant retention by soils.

EPA Grant: R82-5958

Assessment of Biotic and Abiotic Processes Controlling the Fate of Chlorinated Solvents in Mixed-Waste Under Iron- and Sulfate-Reducin Conditions Using Laboratory and *In-Situ* Microcosms

Kim F. Hayes, Peter Adriaens, and Michael J. Barcelona
The University of Michigan, Ann Arbor, MI

The major objective of this research is to evaluate the relative importance of biotic and abiotic reductive dechlorination processes under iron- and sulfate-reducing conditions in both simple and mixed-waste systems. In this project period, we performed abiotic reductive dechlorination using synthetic Fe(II) solids as well as purel biological studies aimed at quantifying reductive dechlorination in the absence of solids under sulfate and iron reducing conditions. A comparison of rates and reaction products provides one means by which biotic and abiotic transformation might be distinguished. We obtained a quantitative description of the kinetics of the abiotic transformation of trichloroethylene (TCE) and tetrachloroethylene (PCE) b FeS. Rates of hexachloroethane (HCA) reductive dechlorination by FeS in the presence of metals and oxyacids were also measured to simulate the impacts of mixed-waste composition. In our biological studies, the kinetics of cell mediated carbon tetrachloride (CT) dechlorination were characterized for the iron reducing bacteria *Geobacter metallireducens*. Two sulfate reducing bacteria, *Desulforeducens autotrophica* and *Desulfovibrio vulgaris* subsp. *vulgaris* have been cultured during this project period and are currently being used to generate FeS for reductive dechlorination in sulfidogenic environments.

Abiotic experiments were performed to determine the rates and products of transformation of TCE and PCE b FeS. The principal reaction product for TCE transformation by FeS was acetylene, while *cis*-1,2-dichloroethylene (*cis*-DCE) and vinyl chloride (VC) were minor products. Data were interpreted assuming parallel transformation of TCE to these two products, with VC most likely forming from slow hydrogenolysis of *cis*-DCE. The solution to the differential equations describing such a reaction scheme indicated that TCE was transformed to acetylene 11.8 ± 1.1 times faster than to *cis*-DCE. Detection of acetylene as the principal TCE reductive dechlorination product contrasts with the sequential hydrogenolysis commonly observed in the transformation of TCE in microbiological systems, which can result in the accumulation of significant quantities of the harmful intermediates *cis*-DCE and VC. Similar results were obtained for the transformation of PCE b FeS. In addition, the impact of the metals Ag(I) Cu(II), and Cr(III) and oxyacids chromate, arsenite, and selenite on HCA reductive dechlorination by FeS was investigated. T date, the results indicate the softer metal acids Ag(I), Cu(II) tend to increase reductive rates while hard acid Cr(III) and the oxyacids tend to reduce the rate. We hypothesize that the former interact with the Fermi level of FeS(s), a known conductor, while the later reduce reactivity by undergoing redox reactions with FeS(s). These studies reveal that metals and oxyacids present in mixed wastes may impact reductive dechlorination.

Biological experiments have been conducted using cell suspensions of the dissimilative iron reducing bacteria *Geobacter metallireducens* At carbon tetrachloride (CT) concentrations less than 100 μM, cell mediated dechlorination rates were found to follow first order kinetics. The only volatile product detected was chloroform (CF) which accounted for approximately 15% of the transformed CT. Approximately 85% of transformed C was recovered as either a cell bound product (68%) or as a non-volatile aqueous product presumed to be cell lysate (17%). By contrast in magnetite mediated dechlorination, we have observed a similar amount of CF formation (~20-30%) but also a minor amount of methane (~4-5%). This suggests methane may be a positive indicator for Fe_3O_4 mediated CT dechlorination in systems containing both magnetite and iron reducing bacteria.

Extending the biological investigations to sulfidogenic environments, two pure cultures of sulfate-reducing bacteria, *Desulforeducens autotrophica* and *Desulfovibrio vulgaris* subsp. *vulgaris*, were grown in liquid media under a range of environmental conditions to determine the effect of solution chemistry on bacterial activity and iron solids production. Preliminary results show that *D. vulgaris*, a freshwater species, is the more robust strain for generating biologically-modified iron solids.

In the future, abiotic reductive dechlorination by biogenically produced FeS will be investigated. A more extensive evaluation of abiotic transformations of HCA, PCE and TCE by magnetite as a function of solution conditions will also be conducted, as well as reductive dechlorination by FeS and magnetite in the presence of metal cations and oxyacids. Future biological work will include examination of the impact of temperature on biological and abiotic

reductive dechlorination. We also anticipate setting up several *in-situ* microcosm (ISMs) this Fall at the FT2 training site at the Wurtsmith Air Force Base in Oscoda, MI. Once the ISMs are in place, sulfate reducing conditions will be stimulated and HCA will be injected and reductive dechlorination activity monitored for up to 120 days.

Publications and Presentations

Jeong, H. and K. F. Hayes (1999), "Impact of Transition Metals and Oxyacids on Reductive Dechlorination of HCA by Iron Sulfide," 22nd Midwest Environmental Chemistry Workshop, Michigan Technological University, Houghton, Michigan, October 1-3, 1999.

Butler, E. C. and K. F. Hayes (1999), "Kinetics of the Transformation of Trichloroethylene and Tetrachloroethylene by Iron Sulfide,"*Environ. Sci. Technol.*, 33, 2021-2027.

Adriaens, P., McCormick, M.L., Butler, E.C., and Hayes, K.F. (1999), "Biotic and Abiotic Dechlorination of Alkyl Halides under Iron and Sulfate Reducing Conditions," Swiss Federal Institute for Environmental Science and Technology, Dubendorf, Switzerland.

McCormick, M.L., and P. Adriaens (1999), "Biotic and Abiotic Reductive Transformation of Chlorinated Solvents in Iron Reducing Sediments," Fourth Annual EPA STAR Graduate Fellowship Conference , July 17-20, Arlington, VA.

McCormick, M.L. and P. Adriaens (1999), "Reductive Transformation of Carbon Tetrachloride by the Dissimilative Iron Reducing Bacteria Geobacter metallireducens," 99th General meeting of the American Society for Microbiology, May 30-June 3, Chicago, IL.

Butler, E. C. and K. F. Hayes (1999), "Correlation Analysis in the Reductive Transformation of Halogenated Organic Compounds by Iron Sulfide," 217th American Chemical Society National Meeting, March 21-25, 1999, Anaheim, California.

Butler, E. C. and K. F. Hayes (1998), "Transformation of Trichloroethylene and Tetrachloroethylene by Iron Sulfide" 21st Midwest Environmental Chemistry Workshop, University of Michigan, Ann Arbor, Michigan, October 16-18, 1998.

Danielsen, K. M. and K. F. Hayes (1998), "Abiotic Reductive Dechlorination of HCA by Reduced Iron" 21st Midwest Environmental Chemistry Workshop, University of Michigan, Ann Arbor, Michigan, October 16-18, 1998.

McCormick, M. L., H. S. Kim, and P. Adriaens (1998), "Reductive Transformation of Chlorinated Organic compounds by Biologically Generated Magnetite" 21st Midwest Environmental Chemistry Workshop, University of Michigan, Ann Arbor, Michigan, October 16-18, 1998.

McCormick, M.L., H.S. Kim, E.J. Bouwer, and P. Adriaens (1998), "Abiotic transformation of chlorinated solvents as a consequence of microbial iron reduction: An investigation of the role of biogenic magnetite in mediating reductive dechlorination," Proceedings of the 30th Mid-Atlantic Industrial and Hazardous Waste Conference, p. 339-349, Technomic Publishing, Lancaster, PA.

McCormick, M.L., and P. Adriaens (1998), "Tetrachloroethylene Transformation in an Iron Reducing Enrichment Culture," 98th General meeting of the American Society for Microbiology, May 17-21, Atlanta, GA.

Phytoremediation and Modeling of Land Contaminated by Hydrocarbons

Clyde Munster, Associate Professor, Agricultural Engineering Dept., Malcolm Drew, Professor, Horticultural Sciences Dept., M. Yavuz Corapcioglu, A. P. and Florence Wiley Professor, Civil Engineering Dept., Texas A&M University.

Plants may assist in the remediation of recalcitrant chemicals at contaminated sites by various processes. The possible use of deep-rooted plants for phytoremediation of soil contamination has been offered as a potential alternative for waste management, particularly for in-situ remediation of large volumes of contaminated soils. In addition, a phytoremediation computer model has been recently developed for predicting the fate of recalcitrant hydrocarbons in soil. However, the model requires extensive testing with field data for validation and calibration. Therefore, the objective of this research was to undertake the principles, as well as the practices, of dealing with the persistence of recalcitrant contaminants in the soil and their simultaneous removal by plants, as well as the collection of field data for model testing.

For this research project, a warm season grass (Johnsongrass) and a cool season grass (Canada wild-rye) were chosen to evaluate the effectiveness of phytoremediation of soil contaminated with a recalcitrant mix of a polybrominated biphenyl (PBB, 2,2',5,5'-tetrabromobiphenyl), a polycyclic aromatic hydrocarbon (PAH), chrysene, and 2,4,6-trinitrotoluene (TNT). Two types of lysimeters were developed for field-testing. Twelve metal box lysimeters, 1.5 m x 1.5 m x 0.75 m (length, width, height) and 72 polyvinyl chloride (PVC) column lysimeters, 0.1 m diameter x 1.5 m in height were fabricated. A translucent, corrugated PVC panel roof that provided shelter from the rain covered both sets of lysimeters. A leachate collection system was installed in each lysimeter to obtain leachate for chemical analysis. The box and column lysimeters were placed in the ground to maintain an in-situ temperature gradient throughout the soil profile. The lysimeters were filled with virgin Weswood silt loam soil (23 % sand, 47, % silt, 30 % clay, 0.8 % organic carbon, and 7.9 pH). That was mixed with chrysene, TNT, and PBB to a target concentration of 10 mg of each contaminant per kg of soil. As the soil was added to the lysimeters, time domain reflectance (TDR) probes, for soil moisture determination, were placed at depths of 0.125, 0.375, and 0.625m. The soil was packed to a bulk density of 1400 kg m^{-3} to match field values.

Chemical losses during the initial 360 days of this experiment were similar for both box and column lysimeters, at all depths, and between vegetated and unvegetated soils (Figure 1). The largest and most rapid loss in soil-chemical concentration was TNT, which decreased to < 10 μg kg^{-1} after 360 days. Contaminant detection in plant herbage and leachate has been insignificant. Enumeration of soil microorganisms reveals a robust population in both the bulk soil and root rhizosphere, but no significant differences. Field data are being used to calibrate and validate the phytoremediation computer model. Simulations with TNT demonstrate that the model can predict decreases in contaminant concentration as observed from actual field conditions (Figure 2). Simulation for PAH and PBB are currently being evaluated. Additional model parameters are being examined and samples will continue to be collected and analyzed during a two-year period. The validated and calibrated computer model may provide insight into the selection and optimization of phytoremediation at contaminated sites.

Publications and Presentations

Colville, C. J., R. L. Rhykerd, C. L. Munster, M. C. Drew and M. Y. Corapcioglu. 1999. Phytoremediation of soils contaminated by TNT, PBBs and PAHs. The 1999 ASAE Annual International Meeting, Toronto, Canada, July 18-21, 1999. ASAE Paper No. 992183. St. Joseph, MI, ASAE.

Corapcioglu, Y.M., C.L. Munster, M.C. Drew, R.L. Rhykerd, K. Sung, and Y.Y. Chang. 1999. Phytoremediation and modeling of land contaminated by hydrocarbons. Proceedings, 5th International In Situ On-Site Bioremediation Symposium. San Diego, CA.

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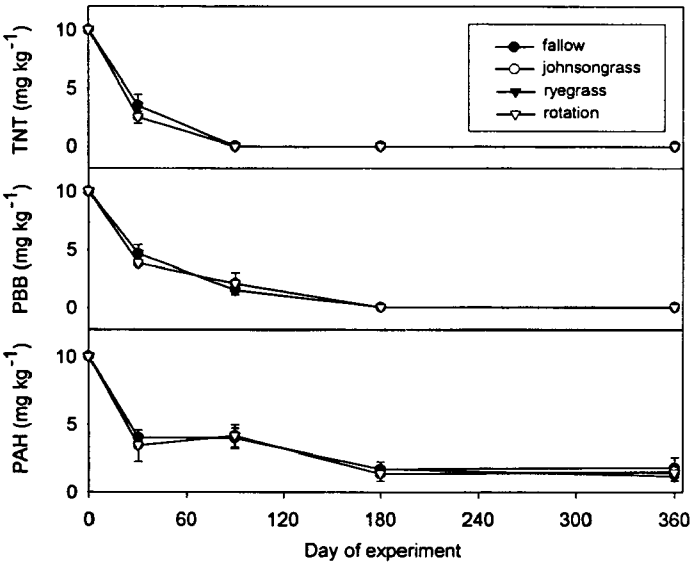

Figure 1. Soil concentrations of TNT, PBB, and PAH from column lysimeters under Johnsongrass, Canada wild-rye, a Johnsongrass/Canadian wild-rye rotation or fallow.

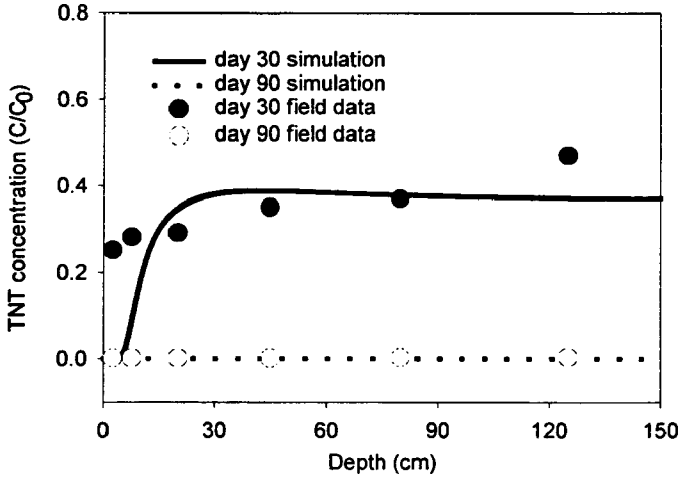

Figure 2. Loss of TNT from a contaminated Weswood soil determined by immunoassay analysis of field soil (symbols) and predicted by computer model simulations (lines). Computer simulations were based upon soil parameters from the field study. Analyses and simulations were made 30 and 90 days after the soil was contaminated with 10 mg kg^{-1} TNT.

EPA Grant: R82-5365

In-Situ Assessment of PCB Availability in Unsaturated Soils

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The objective of the current study is to develop understanding of the relationship between bioavailability and biostabilization of polychlorinated biphenyls (PCBs) in aged soils that have undergone active land biotreatment, but which still show a residual concentration. At the study's conception we recognized a deficiency in field sampling techniques that could assess the bioavailable fraction of PCBs, and other hydrophobic organic compounds (HOCs), in impacted soils. Here, the bioavailable fraction is considered that present in the aqueous phase – i.e., available for active or passive diffusion across a microbial membrane. Consequently, the research direction under this assistance grant has been focused primarily on the development of *field* sampling devices and procedure to measure the mobility and availability of PCBs in unsaturated soils.

The new sampling device consists of a cylindrical porous stainless steel interface, a sorbent packing media (granular activated carbon), and a fiberglass wick. The interface details are shown in Figure 1. The device can be installed in field soils by direct-push or a soil auger pilot-hole, thus minimizing installation requirements. The device uses the capillarity of a fiberglass wick and elevation potential to provide the necessary driving force to slowly sample water from unsaturated soils, thereby eliminating the need for an external power source. Water is drawn through the cylindrical interface, into and along the wick, and is collected to quantify the amount of water sampled (maintains a water balance). The annular space of the cylinder is packed with a sorbent media, so water that passes through the interface must pass through the sorbent media before entering the wick. Target constituents are retained on the sorbent media for chemical analysis (allows for a chemical mass balance with minimal losses) at the end of the sampling interval (weeks or months). In this way, the new sampling device is designed to provide an integrated *field* estimate of the time-averaged mass and volumetric flux rates of HOCs in the soil. A patent application has been submitted for the new device and sampling technique.

The new sampling device has been optimized for its hydraulic performance when installed in a horizontal configuration (Figure 2). This configuration would be applicable for shallow soils in lined land treatment units, biopiles, confined disposal facilities, etc. (McNamara, 1999). Laboratory and field evidence indicate that the device can withstand extended periods of drought and recover hydraulically when water becomes available in the surrounding soil pore-water. A hydraulic model has been conceived to predict the sampler's performance in soil under a range of moisture conditions.

The device is currently being tested for its chemical capture potential in PCB-impacted soils. Mass balance calculations based on the analysis of the sampler's sorbent material and concurrent leaching studies should provide insight into the sampler's ability to accurately predict the flux of PCBs in the subsurface. A new pressurized-solvent extraction procedure has been developed to recover low-level PCBs from the sampler's sorbent material - granular activated carbon. The new procedure allows for higher recoveries and smaller RSDs than is possible with the typical extraction procedures for soil (i.e., Soxhlet or sonication). Similar procedures for additional HOCs (e.g. PAHs and pesticides) are currently being evaluated.

The presentation will overview the hydraulic performance of the device in lab and field trials, discuss work on the chemical evaluation of the sampling device, and present preliminary information on the field work scheduled for the Spring of 2000 in PCB-impacted soils.

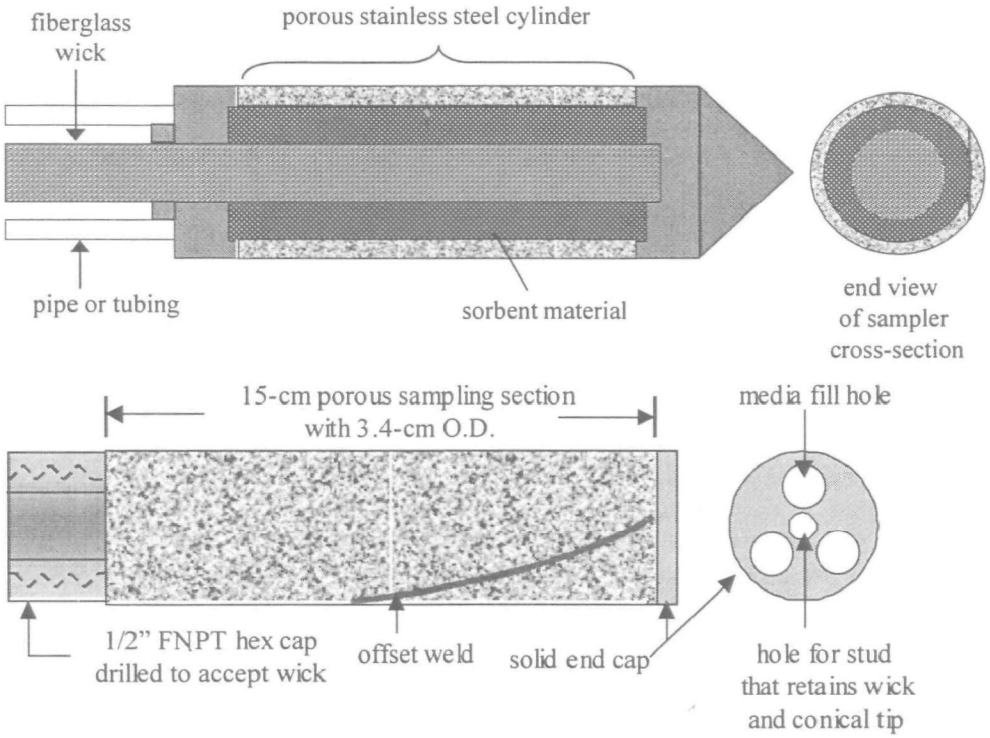

Figure 1. Cross-section and plan view of new sampling device design

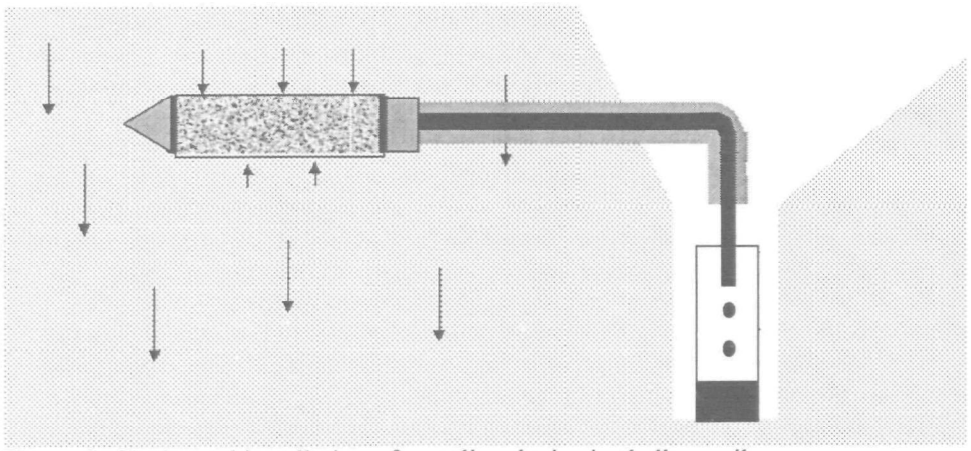

Figure 2. Horizontal installation of sampling device in shallow soil.

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Population Biology of Bacteria Involved in Contaminant Bioremediation

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Molecular approaches allow us to investigate in detail the microbial populations that can potentially be involved in bioremediation of contaminants and to assess which populations are likely to be most important under given sets of environmental conditions. We have used denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene segments, and sequencing of the resultant DGGE bands, combined with cultivation and molecular characterization of isolates, to assay microbial populations that might occupy hypothesized specialized niches in contaminant microenvironments. During the past year we have focused on three areas of research related to this theme. Progress in each area is summarized separately below.

Adaptations of Phenanthrene-Degrading Bacteria along a Bioavailability Gradient. In previous work we demonstrated that enrichment of soil bacteria on phenanthrene presorbed to an acrylate resin, BioBead SM7, resulted in recovery of mycobacteria, whereas enrichments on a less strongly sorptive organic solid, the polystyrene resin Amberlite IRC-50, or on sand or without a solid phase, led to recovery of *Burkholderia* sp. These mycobacteria exhibited 5- to 7.5-fold greater relative rates of mineralization of SM7-associated phenanthrene than *Burkholderia* from control enrichments, suggesting that the former may be adapted to low-bioavailability microenvironments. Examination of near full-length 16S rRNA sequence data of isolates suggests that closely related *Burkholderia* strains (differing by only 1.5 to 3.5%) that exhibit different distributions in Amberlite, sand and control enrichments may also be adapted to high and moderately reduced bioavailability settings. A current objective is to examine the relative fitness of these mycobacteria and *Burkholderia* isolates in competition with each other across the bioavailability gradient.

Succession of Bacteria Associated With Chemical Changes During Biodegradation of Natural and Synthetic Contaminant Mixtures. Contaminant spills often result in the introduction of mixtures of compounds to the natural environment. Using crude oil and simpler hydrocarbon mixtures as models, we seek to understand relationships between natural contaminant mixtures and niche diversity of contaminant-degrading microorganisms. Batch enrichments inoculated with soil were used to study microbial population changes associated with the changing composition of weathered crude oil and a simple mixture of normal- and isoprenoid-aliphatic hydrocarbons that are among the most predominant oil components. A similar approach was used to determine populations that were enriched during degradation of pure components of this mixture. Parallel samples were taken for gas chromatography/mass spectrometry analysis of column-separated aliphatic and aromatic compounds and for DGGE. DGGE band profiles for crude oil and the synthetic mixture displayed identical successional patterns, documenting that different bacterial populations appear to be associated with degradation of n- and isoprenoid-alkanes. Band sequences suggest that an *Acinetobacter* population is likely to be responsible for n-alkane biodegradation, whereas a *Rhodococcus* population may responsible for isoprenoid-alkane degradation. Liquid enrichments on pure compounds yielded the same bands and band seqeunces for n- and isoprenoid-alkanes, respectively. However, direct isolation from soil on solidified medium containing n- or isoprenoid-alkanes permitted recovery of only the isoprenoid-degrading *Rhodococcus* population. Since *Rhodococcus* isolates matching the DGGE band associated with branched hydrocarbon metabolism were obtained on plates containing either n- or isoprenoid alkanes, our results suggest that the fitness of the *Rhodococcus* population to degrade n-alkanes seems lower than that of the *Acinetobacter* population under our enrichment conditions.

Effects of Hydrocarbon and Surfactant Amendments on Soil Microbial Community Composition. We have also continued research in more realistic soil environments studying changes in microbial populations associated with surfactant (Witconol SN70, a nonionic alcohol ethoxylate) amendments to hydrocarbon contaminated soils. Effects of surfactant and hydrocarbon amendment on catabolism were studied by monitoring conversion of ^{14}C-labeled hydrocarbons to $^{14}CO_2$, while effects on populations were monitored using DGGE and cultivation. Additions of 15 mg hexadecane gm^{-1} soil with or without 0.25 mg phenanthrene gm^{-1} soil caused an increase in the intensity of one DGGE band whose sequence indicated it was contributed by a *Nocardia* species. Plating on solidified media with added hexadecane failed to recover an isolate matching this sequence. However, we did isolate a hexadecane-utilizing *Rhodococcus* sp. with 100% sequence identity to a band of lesser intensity observed in treatments where hexadecane mineralization was observed. Amendment with Witconol below the CMC' (2 mg gm^{-1} soil) did not affect hydrocarbon metabolism or microbial populations with the exception of increased intensity of the *Rhodococcus* sp. band. Additional amendment of Witconol at nearly the CMC' (10 mg gm^{-1} soil) delayed

hexadecane mineralization and completely inhibited phenanthrene mineralization; addition above the CMC' (40 mg gm^{-1} soil) inhibited all hydrocarbon mineralization. At near and above CMC' there was a decline in the band intensity of the *Nocardia* population and a rise in intensity of two DGGE bands that were contributed by relatives of *Pseudomonas putida* and *Alcaligenes xylosoxidans*. Isolates matching these bands were able to degrade both Witconol and hexadecane. Addition of high levels of Witconol thus appears to have caused a shift in species with the ability to degrade hydrocarbons; the basis for inhibition of hydrocarbon metabolism above CMC' remains unknown.

The Influence of Soil Characteristics and Molecular Properties of Hydrophobic Contaminants on Bioavailability in Aged Soils

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It is the objective of the currently conducted research to provide answers to the following key questions: 1) How do the properties of soil solids and the molecular structure of hydrophobic model pollutants such as petroleum hydrocarbons affect bioavailability in aged soils? 2) How does aging affect bioavailability? 3) What are the factors that determine whether bioremediation is reaction-rate or mass-transfer rate limited? 4) How does soil toxicity as measured by solid-phase MicrotoxTM change during bioremediation treatment? 5) What mechanisms control the leaching of soluble hydrocarbons from the aged, non-bioremediated soils? 6) What is the distribution of hydrocarbons on external particle surfaces before and after bioremediation?

In an effort to address these questions, eight model solids/soils (2 quartz sands, 2 silica gels, 2 clays, peat, Richland topsoil) were spiked with crude oil (5-10% wt) and aged for more than 2 years in the laboratory. These 8 aged soil solids plus one weathered NAVY field soil, one freshly spiked Richland topsoil (not aged), and non-porous glass beads spiked with crude oil or solvent extract from the NAVY field soil have been subjected to bioremediation treatment in well-mixed, aerated slurries in the laboratory (i.e., a total of 12 treatments) for more than 1.5 years.

Slurry samples were periodically analyzed for parent and alkylated PAHs and biomarkers (e.g., hopane) to determine the bioremediation kinetics of selected hydrocarbons. Abiotic release rates of PAHs from the soil slurries were measured using XAD-2 resin as a sorbent. The changes in soil toxicity during bioremediation were periodically measured using the solid-phase MictrotoxTM assay. Finally, continuous flow column experiments were performed to evaluate the leaching behavior of soluble hydrocarbons (BTEX) in the different aged, non-bioremediated model soils.

Preliminary findings indicate that:

- for most PAHs, the rate and extent of biodegradation is NOT significantly affected by soil properties, including organic matter content.
- after ca. 1.5 years of slurry bioremediation, only a few PAHs such as benzo(e)pyrene, perylene, and C4 chrysenes have not been completely biodegraded. The extent of biodegradation varies with soil type. The reasons for this variability are not clear.
- the effects of aging appear to be more pronounced with increasing molecular weight of the respective PAHs. However, in terms of total PAHs, the effects of aging were not very significant.
- PAHs with similar Kow's (e.g., 1M-phenanthrene and pyrene) have similar desorption/dissolution rates but different biodegradation rates indicating that
- differences in biodegradation kinetics are due to microbial factors.
- based on modeling results, leaching of BTEX from all soils with the exception of montmorrilonite clay is NOT affected by soil properties or aging. Instead, it appears that equilibrium dissolution of BTEX from the crude oil phase controls the leaching kinetics.
- soil toxicity as measured by MicrotoxTM decreases with increasing bioremediation time.
- the conservative biomarker C30 17",21$ (H) -hopane disappeared after more than 6 months of bioremediation in two different slurry bioreactors. Current research is underway to detect potential biotransformation products.
- based on data from fluorescence photography, the interior of aged soil particles may contain significant levels of PAHs (NAPLs in mineral pore space?).

Based on the above findings, it appears that aging and soil properties do not significantly affect the rate and extent of biodegradation in soils contaminated with crude oils at levels commonly found in the environment (i.e., ca. 5% or 50,000 mg/kg). It is likely that at these concentrations, most hydrocarbons are present in a NAPL-phase in which they are readily bioavailable for microbial degradation.

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Biogeochemical Interactions in Reactive Zero-Valent Iron Barriers

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Permeable reactive barriers are receiving a great deal of attention as an innovative, cost-effective technology for in-situ cleanup of groundwater contamination. Fe(0) barriers promote favorable conditions for treating mixtures of redox-sensitive pollutants. In addition to direct contaminant reduction by Fe(0), such *in situ* treatment systems could induce anaerobic biodegradation through the depletion of O_2 and the production of water-derived H_2 during anaerobic Fe(0) corrosion.

$$Fe^0 + 2H_2O \rightarrow Fe^{+2} + 2OH^- + H_2 \quad [1]$$

Batch and column experiments performed to date suggest (1) that an integrated microbial-Fe(0) system holds great promise for treating recalcitrant, redox-sensitive contaminants (e.g., RDX) and chlorinated solvent-heavy metal mixtures, (2) that indigenous microorganisms can colonize the Fe(0) surface, and (3) that the Fe(0) surface area concentration is an important design variable to optimize microbial activity and prevent inhibitory effects when multiple contaminants compete for sites on the Fe(0) surface.

RDX was rapidly reduced in aquifer microcosms amended with Fe(0) and in flow-through columns packed with steel wool. Adding anaerobic mixed cultures enhanced the rate and extent of RDX degradation. Apparently, H_2-consuming bacteria exploited Fe(0) corrosion as a metabolic niche participated in the further degradation of heterocyclic intermediates produced by the reaction of RDX with Fe(0). Reductive treatment of RDX with Fe(0) also reduced its toxicity to microorganisms and enhanced its subsequent biodegradability under either aerobic or anaerobic conditions. Therefore, a combined or sequential Fe(0)-biological treatment approach might improve treatment efficiency.

Contaminant interactions were studied in small reactors containing Fe(0) and various combinations of CCl_4, Cr(VI), and NO_3^-. The preferential degradation order for contaminants in these abiotic reactors was: Cr(VI) > CCl_4 > NO_3^-. Results show that at low Fe(0) surface area concentrations (11 m^2/L) significant competitive effects are observed (Figure 1). Yet, no inhibition was observed at high concentrations (1140 m^2/L). We hypothesize that inhibition was due to competition for a limited number of reactive surface sites at a low Fe(0) dose.

The effect of Fe(0) surface area concentration on microbial activity was studied using a mixed culture of H_2-consuming sulfate reducers. Fe(0) did not react with sulfate within the time frame of the experiment. Yet, H_2 production during Fe(0) corrosion stimulates microbial sulfate reduction. Thus, this experimental system isolated the effect of Fe(0) surface area concentration on microbial activity. Increasing the Fe(0) dose initially stimulated sulfate reduction, possibly due to a higher production of H_2. Nevertheless, high Fe(0) doses had an inhibitory effect due to a corrosion-induced increase in pH beyond the optimum range of the bacteria (equation [1]). An optimum Fe(0) surface area concentration occurred at 570 m^2/L for this combined microbial-Fe(0) treatment system (Figure 2). This optimum, however, is probably system specific and depends on the buffering capacity of the system.

Scanning electron microscopy of samples from an Fe(0) barrier that is treating a chlorinated solvents plume in Kansas City showed microbial colonization of the Fe(0) surface (Figure 3). Samples from an Fe(0) barrier treating an uranium plume at Fry Canyon, CO, were also analyzed for the presence of bacteria. Fluorescent in situ hybridization with 16S rRNA probes showed that more eubacteria cells were present within the barrier than in upgradient or downgradient aquifer samples. Interestingly, *Archea* cells (e.g., methanogens) were only detected in Fe(0) barrier samples. Apparently, indigenous microorganisms colonize Fe(0) barriers to exploit cathodic depolarization and bioremediation as metabolic niches.

In conclusion, the performance of Fe(0) barriers might be enhanced by the concurrent or subsequent participation of indigenous microorganisms that increase the rate and extent of contaminant degradation. Nevertheless, the effect of such biogeochemical interactions on the long-term performance and permeability of Fe(0) barriers remains to be determined.

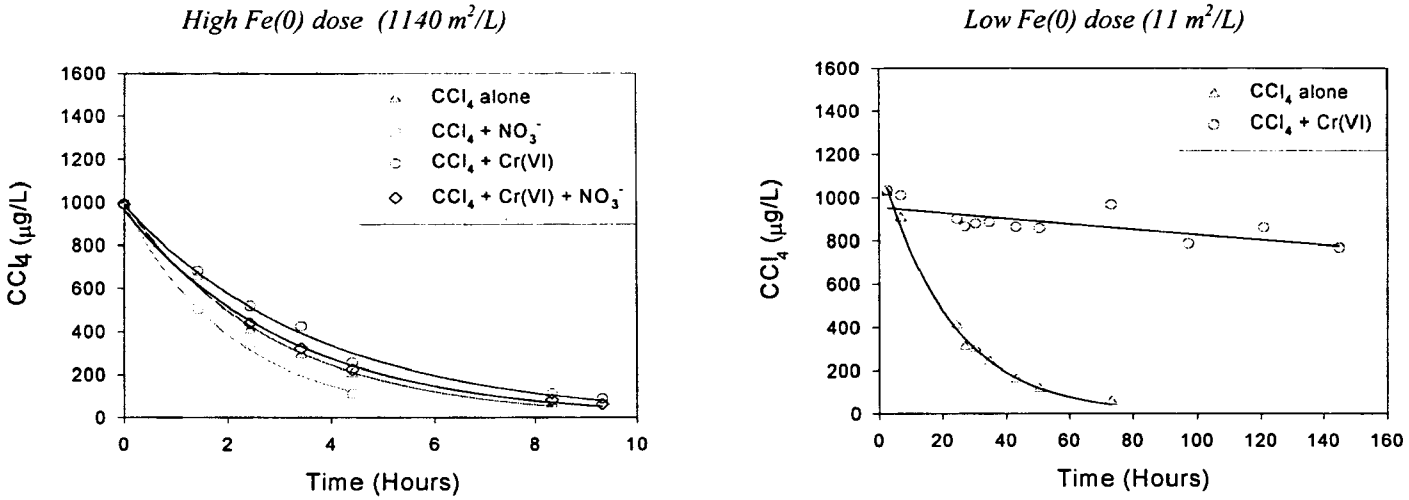

Figure 1. CCl_4 Degradation in the presence of other contaminants with different Fe(0) doses.
These results suggest that contaminants will compete for reactive sites on the Fe(0) surface. Such competitive interactions should not significantly affect specific degradation rates when the available Fe(0) surface area is relatively high (left panel). However, some contaminants will degrade slower when present in mixtures (relative to when present alone) when the Fe(0) surface area concentration is low (right panel). These observations illustrate the importance of the Fe(0) surface area concentration as a design variable

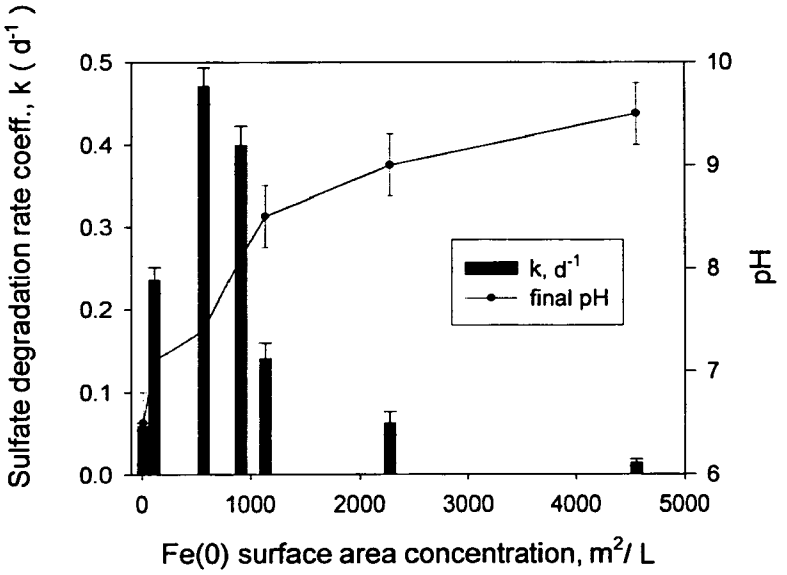

Figure 2. Effect of Fe(0) surface area concentration on sulfate reduction activity.
A maximum rate occurred when the Fe(0) surface area concentration was 570 m^2 l^{-1}, which resulted in circum neutral pH. Apparently, an Fe(0) dose lower than 570 m^2/L resulted in limited H_2 production via Fe(0) corrosion, and hence a limiting supply of electron donor to respire sulfate. While an Fe(0) dose higher than 570 m^2 l^{-1} produced much more H_2, this had an inhibitory effect due to a corrosion-induced increase in pH beyond the optimum range of the bacteria.

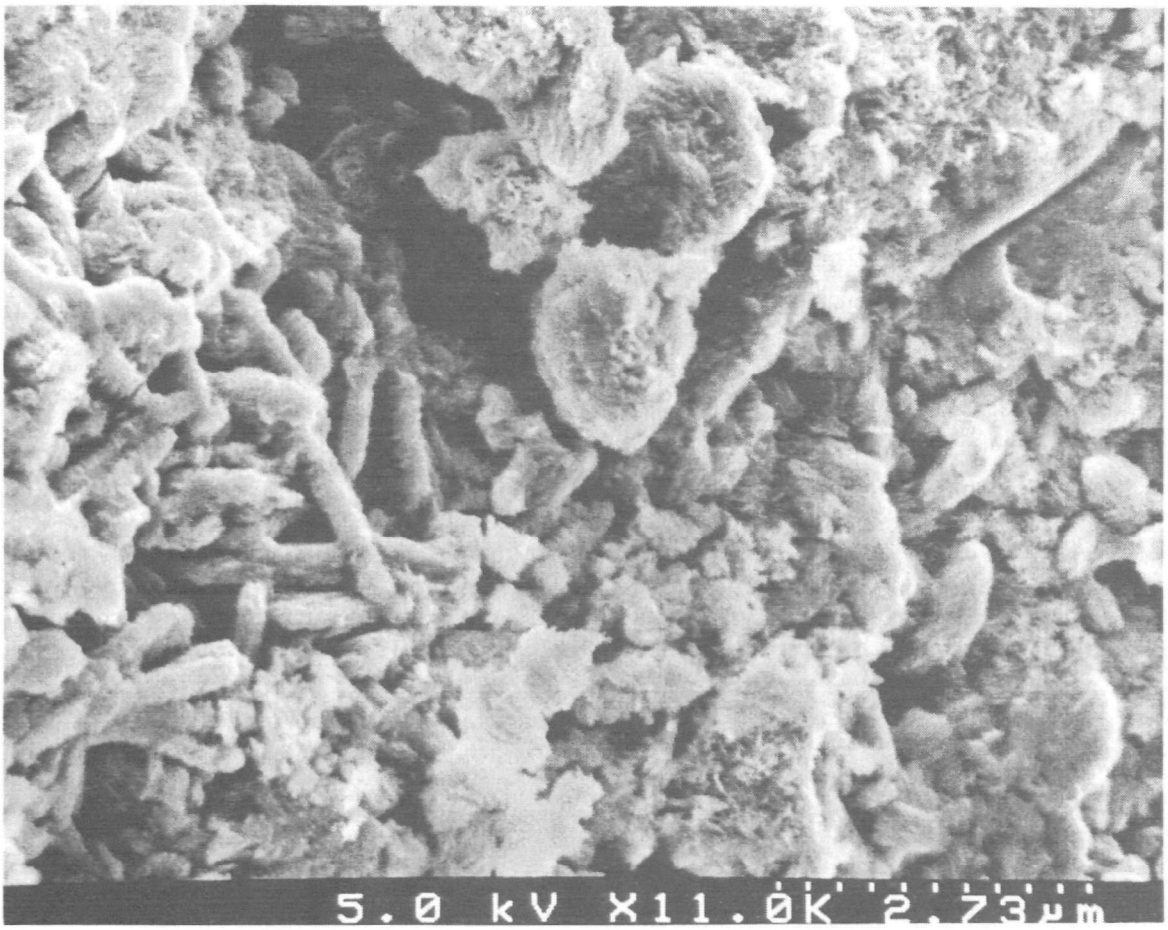

Figure 3. SEM Picture of Fe(0) Sample from a Barrier Treating a Chlorinated Solvent Plume. Picture shows colonization of the iron surface by rod-shaped microorganisms, about 2 µm long.

Controls on Plant Bioavailability in Salt Marsh Environments Which Can be Manipulated for Contaminated Sediment Remediation

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The sediments used in this study came from a former chloro-alkali plant (LCP site) near Brunswick,GA. Marsh sediments at this site showed a concentration gradient of total mercury (100 to 1 ppm) and PCBs (8000 to 2 ppm) going from the high tide marsh(nearest the plant) to the low tide marsh. The roots and stem/leaves of two marsh plants (*Spartina alterniflora* and *Juncus roemaricus*), sediments from different depths, sediment pore water, microbial mats and benthic invertebrates from the site were analyzed for PCBs, total mercury and methyl mercury. The original PCB mixture (Arochlor 1268) used at the site have been modified over time with increasing amounts of 3-,4- and 5-ringed congeners due to reductive dechlorination which is carried out by microbial systems in the rhizosphere layer of the sediment. The subrhizosphere layer had low concentrations of total mercury, methyl mercury and PCBs.

Sediments from the most contaminated stations were transferred into mesocosms at the Bioremediation and Environmental Mesocosm (BERM) facility located at the Skidaway Institute of Oceanography. The mesocosms are one meter by three meters deep. Water levels are tidally simulated using ambient estuarine water of intermediate salinity (ca. 20 ppt) Four mesocosms are being used: (1) reference sediments without marsh plants; (2) reference sediments with marsh plants; (3) contaminated sediments without marsh plants; (4) contaminated sediments with marsh plants. Several observations suggest that the mesocosms with transferred sediments support the same estuarine ecosystem found at the LCP site. For example, sediment porewater ion distribution and sulfate reduction rate depth profiles resemble those found at the LCP site. In addition, *Spartina* has gone through two growth seasons, microbial mats are established, and the mesocosms are colonized by fiddler crabs (*Uca* sp.) periwinkle snails (*Littorina* sp.) and small fish. The identification of microbial communities in the contaminated sediments from the LCP sites and in transferred sediments from this site are being done using 16S rRNA probes to determine the important microbial groups in the sediments and rhizosphere community.

For the past 1.5 years we have followed changes in the concentrations of total mercury, methyl mercury and PCB congener profiles in the sediments in the BERM mesocosms. For example the PCB 206 congener decreased from 48 to 0.6 g/g in the surface (0-3cm) sediments and from 98 to 5.8 g/g in the deeper (9-12cm) non-vegetated sediments. In the vegetated sediments PCB congener concentrations were much higher in the sediments after 500 days compared with the non-vegetated sediments. Changes in total mercury in sediment cores over the 500 day period are shown in Table 1. Mercury concentrations decreased in all depths sampled. The nonvegetated sediments showed a marked decreased in total mercury in the surface sediments (112 to 0.1 g/g) but little decrease in the subsurface (3-6cm). In contrast, the vegetated sediments showed a marked decrease in total mercury concentrations in the 3 to 6 cm depth. One possible explanation may be that microbial mats, which were very abundant in the nonvegetated sediments, formed volatile mercury. Earlier studies showed high production of methyl mercury by microbial mats exposed to mercury contaminated sediments. Methyl mercury can be acted on by methyl mercury lyase to form mercuric ions which can then be reduced by mercuric reductase to elemental mercury which volatilizes from the sediment. Thus, microbial mats may be making mercury more available to the action of bacterial lyases and reductases. The decrease in total mercury in the rhizosphere of the vegetated sediment suggests the importance of the plants in combination with rhizosphere microbes in volatilizing mercury.

To pursue the possible importance of plants as an avenue for the volatilization of mercury from the sediments we are measuring elemental mercury in the air above the BERM mesocosms using a plexiglass enclosure to allow periodic air samples from vegetated and non-vegetated mesocosms. An outlet and inlet to the enclosure allow for periodic air sampling. Volatile mercury is collected on gold-coated quartz sand. The concentrations of elemental mercury in the ambient air of the BERM facility ranges from 2 to 4ng/m^3.

Studies on the effects of added nitrogen to the mesocosms on attenuation of PCBs and mercury are presently underway.

Table 1: Changes in Total Mercury in BERM Sediments Due to Natural Attenuation

I. Sediments from contaminated BERM mesocosm B vegetated

Depth (cm)	Total Mercury (g / g sediment)	
	300 days	500 days
0-3	95	6.1
3-6	22	4.6
6-9	14	5.4
9-12	10	3.3

II. Sediments from contaminated BERM mesocosm B nonvegetated

0-3	112	0.1
3-6	24	22.2
6-9	16	8.0
9-12	10	0.6

Publications based on support by NSF DEB-9706317

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Bioavailability of Aromatic Contaminants Bound to Solid and Aqueous Phase Natural Organic Matter

Jon Chorover, Department of Agronomy, Patrick G. Hatcher, Department of Chemistry, Newman and Wolfrom Lab, William D. Burgos, Department of Civil Engineering, Penn State University

The overall goals of this project are (a) to examine the effects of solution chemistry on interaction of contrasting polycyclic aromatic compounds (PACs) with dissolved and mineral-sorbed humic acid (HA) and (b) to determine the consequences for PAC bioavailability to microbial degraders. Naphthalene, naphthol and quinoline were selected to represent neutral, acidic and basic PACs, respectively. PAC-HA sorption was studied using equilibrium dialysis, fluorescence spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. The PACs exhibit different affinities for humic acid as a function of solution chemistry, as can be seen from Figure 1 (Karthikeyan and Chorover, in prep.). These differences can be attributed to functional group chemistry. The neutral compound (naphthalene) shows relatively low but positive sorption to HA, and the extent was unaffected by pH or ionic strength [both are known to affect the conformation of humic acid] (Fig. 1a). Hydroxylation of the C-1 carbon, as occurs during naphthalene biodegradation in the presence of monooxygenases, produces 1-naphthol. Sorption to HA of this weakly acidic PAC exhibits strong time, pH and ionic strength dependencies that were found to result from (a) weak initial complexation of 1-naphthol by HA and (b) oxidative (abiotic) transformation of 1-naphthol [slow reaction] resulting in the formation of strongly bound secondary products detectable by HPLC/MS analyses (Karthikeyan and Chorover, in review). Data on 1-naphthol sorption after 7 d equilibration time are shown in Fig. 1b.

Quinoline is an N-heterocyclic compound that becomes protonated (cationic) below pH 4.9. Sorption to humic acid was dominantly via cation exchange and was highly dependent on protonation and competition with H^+ and background cation. Biodegradation experiments (Pisutpaisal et al., in prep.) were conducted under conditions of maximum sorption, as dictated by dialysis and fluorescence data. Degradation by isolated pure cultures of PAC degraders and consortia were compared using biomass additions corresponding to a known degrader activity (Tuntoolavest and Burgos, in prep.). Figure 2 provides a partial summary of degradation *extent* as a function of PAC and HA concentration. Napthalene degradation was slightly reduced in the presence of HA (65 mg L^{-1} as DOC, Fig. 2a) because of positive – but low – napthalene affinity for HA. Quinoline degradation was slightly reduced (Fig. 2c), despite the fact that it forms only weak complexes with HA via cation exchange (Chorover et al., 1999). The effect of humic sorption on 1-naphthol biodegradation was dependent on the concentration ratio of PAC to HA. In the absence of HA, a decrease in biodegradation resulted from the production of toxic intermediates (naphthoquinones). Since HA was shown to be an effective sequestering agent for naphthoquinones (Karthikeyan and Chorover, in review), the presence of this sorbent in solution nullified the toxic effect (Fig. 2b). These results underscore the importance of considering the reactivity of intermediate compounds in bioremediation of organic contaminants.

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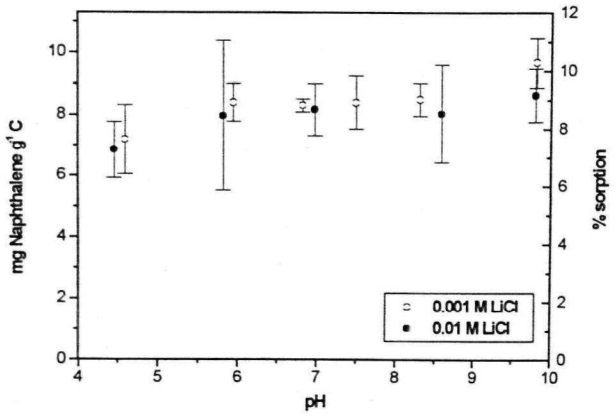

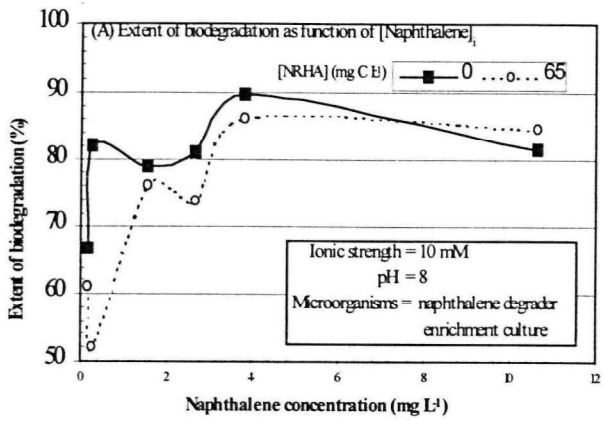

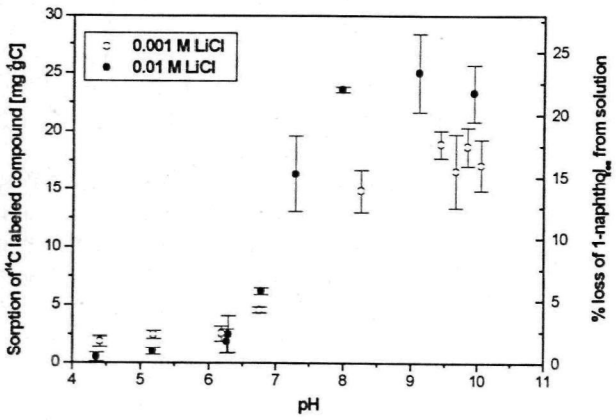

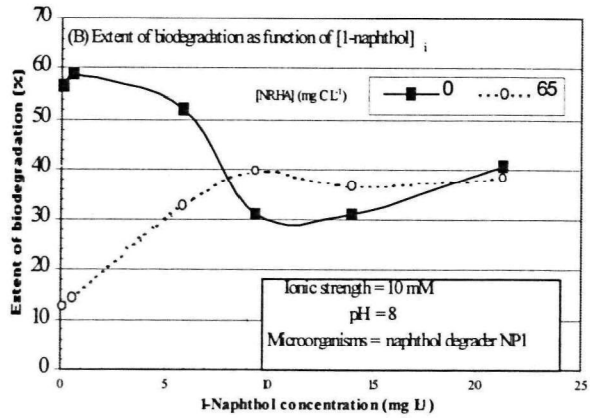

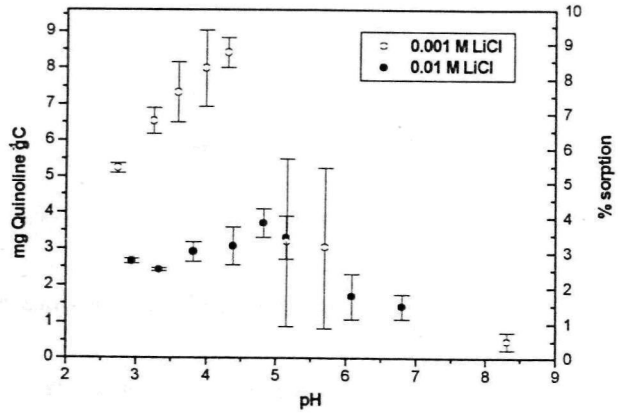

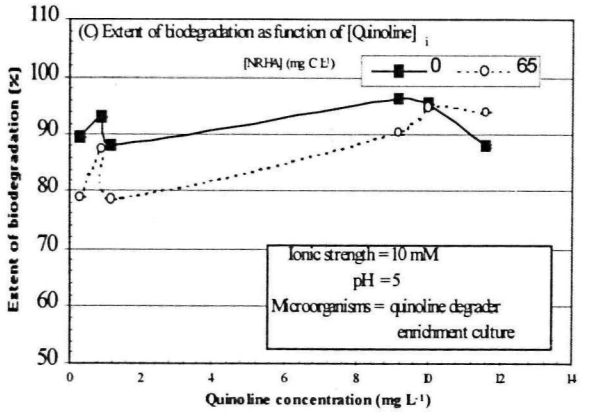

The Effects of Soil Organic Matter on Mineralization, Desorption, and Sequestration/Transformation of Phenanthrene

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Sorption, bioavailability, and sequestration are interrelated phenomena affecting the transport and ultimate environmental fate of organic contaminants in subsurface systems. An important, yet poorly defined, condition that influences these phenomena is the physicochemical character of the sorbent, particularly that of its associated natural organic matter. Previous studies in our laboratories have shown that soil/sediment organic matter (SOM) can be modeled by two separate domains. Older soils and sediments that have undergone significant diagenetic alteration typically have "hard-carbon" dominated-SOMs that are physically condensed and chemically reduced. These soils exhibit more nonlinear, slower, and only partially reversible sorption of hydrophobic organic compounds (HOCs) and have greater organic-carbon-normalized sorption capacities for such contaminants. Conversely, younger soils that have undergone little or no diagenetic alteration have "soft carbon" dominated-SOMs that are more physically amorphous and chemically oxidized, and typically exhibit nearly linear, faster, and reversible HOC sorption, and lower organic-carbon-normalized sorption capacities.

By using three geosorbents that exhibit different degrees of diagenetic alteration, the desorption rate, mineralization extent, and degree of sequestration/transformation were shown to vary with the degree of diagenesis. The chemical nature and relative degree of diagenetic alteration of the organic matter associated with each geosorbent were characterized using solid state ^{13}C-NMR spectrometry. 50°C water column flow-through extraction of the sorbed phenanthrene results in a desorption profile similar to that using an infinite-sink method within a shorter time period. This suggests that 50°C water extraction can be used as an assay to predict long-term desorption.

Mineralization profiles in aqueous suspensions, using sorbed ^{14}C- phenanthrene as a representative HOC, illustrated that initial degradation rates were much faster for the younger geosorbents (Michigan peat and Chelsea soil) when compared to the older geosorbent (Lachine shale). For the younger geosorbents, mineralization slowed after the initial degradation period; mineralization in the older geosorbent-water system continued at a nearly constant rate after the initial degradation period. Abiotic desorption experiments using the infinite-sink method provided similar trends suggesting that desorption is the rate-limiting step in the system. The mineralization and desorption profiles are shown in Figures 1 and 2. After completion of the mineralization experiments, both combustion and methanol Soxhlet extraction were used to recover ^{14}C-organics from the geosorbents.

The amount of extractable ^{14}C-organic material varied with the degree of diagenetic alteration of the soil: relatively small amounts of ^{14}C-organics were extractable from the younger geosorbents while virtually all ^{14}C-organics were extractable from the older geosorbents. The extraction results imply that biological activity alters the SOM and changes the nature of sequestration/transformation, and that this alteration is more pronounced for geosorbents that are younger, more chemically oxidized, and more biologically active.

Figure 1. Mineralization profile for three geosorbents

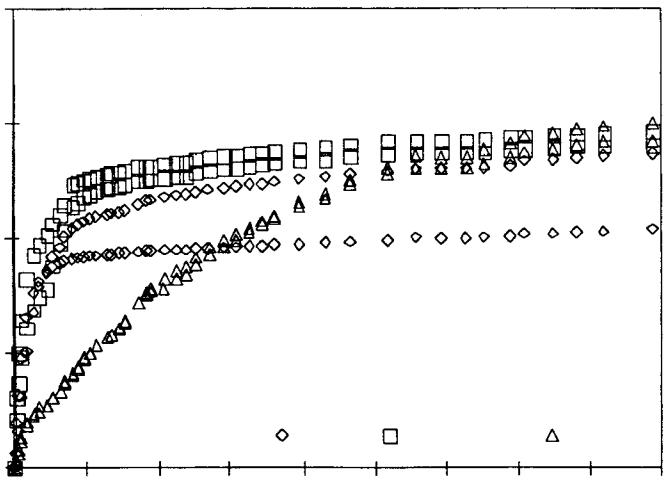
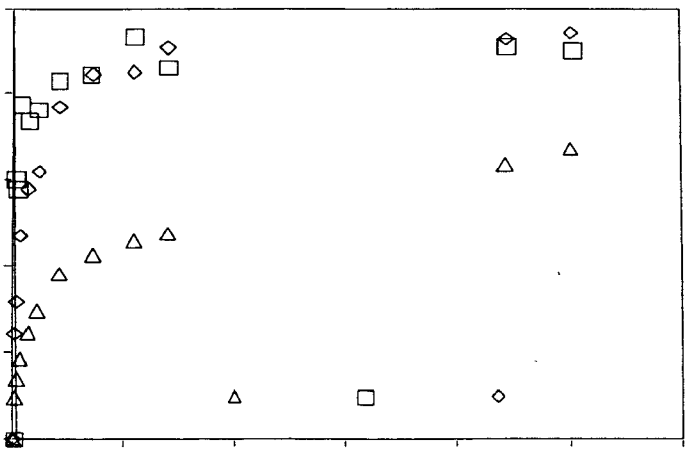


Figure 2. Desorption profile for three geosorbents



Bioavailability of Toxicants as Reflected in the in-situ Microbial Community Ecology and Relationship to Defensible End-Points

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Pollution of the subsurface with bioavailable toxins has been shown to induce marked shifts in the viable biomass, community structure and nutritional/physiological status of the microbial community. Combined lipid biomarker analysis and PCR-DGGE of the bacterial 16S rDNA shows the impact of pollutants on the *in-situ* soil microbiota. Recovery of communities from perturbation may provide quantitative definition of defensible endpoints for natural and attenuated bioremediation.

Two ongoing projects: First, a constructed plume at the Michigan Integrated Remediation Laboratory (MIRTL), shows the impact of MTBE (methyl-t-butyl-ether) and BTEX on the microbiota within the plume. To simulate the impact of fuel constituents that were not present in this experiment, a reducing barrier was placed ahead of the test lane to stimulate suboxic redox conditions within the aquifer prior to the injection of the MTBE, BTEX and an aqueous tracer solution. An oxidizing barrier utilizing ORC (Regenesis Bioremediation Products, Inc.) was placed within the test lane to evaluate its potential for use in the in-situ treatment of MTBE. Samples were taken at bi-monthly intervals from transects located along the constructed plume, with the ORC located between Transects at 145' and 155' from the injection well. Herein we present lipid biomarker and PCR-DGGE 16S rDNA data from the first 6 months of this ongoing study. The presence of the reducing barrier resulted in the expected shift in the redox potential to a suboxic conditions immediately down gradient, and resulted in an increased relative abundance *Flavobacteria, Pseudomonas* and *Clostridia.* Independent of sampling time, higher biomass levels were detected in samples obtained from closer to the center of the plume. The shift in biomass/community structure generally took the form of increased biomass compared to background samples and as yet unexposed areas of the plume, as well as an increase in the proportion of PLFA indicative of Gram-negative bacteria, anaerobic biomass and sulfate reducing bacteria. The presence of the ORC resulted in an increase in viable biomass with substantially more PLFA indicative of Gram negative biomass at Transect 155. Phylogenetic characterization of the dominant bacteria in the plume region has consisted of the excision and sequence analysis of 125 PCR-DGGE 16S rDNA fragments. The great majority of these are related to proteobacteria (α–, β– and γ-subgroups; including methanotrophs, caulobacters and pseudomonads), *Flavobacteriaceae*, and the *Geobacteraceae* (Gram-), and Gram+ bacteria of the *Bacillus/Staphylococcus* group and *Clostridiaceae.*

The second project concerns the impact of co-contaminants (toxic metal and diesel-fuel) on the microbiota of coastal salt marshes. Previous studies have focused on both these contaminants separately, but essentially nothing is known about the microbial response to co-contamination. Herein we present the results of the first of two preliminary microcosm studies. This study was designed to determine the concentration of toxic metals required to elicit a response from the benthic community. Salt marsh sediments were contaminated with known concentrations of a mixture of Navy-relevant toxic metals including Cu, Cr, Cd, Pb and Hg. Metal concentrations were chosen to simulate the range of concentrations typical of San Diego Harbor (SDH), with samples analyzed on days 0, 12 and 30 following contamination. Metal addition had no significant impact ($P>0.05$) on the total biomass content (PLFA) of the sediments, however, at the higher metal concentrations (x 1, and x 10 SDH levels) changes were detected in the community structure. Principal components analysis of the PLFA profiles showed clustering of the samples from days 12 and 30 of the x 10 SDH metal concentration samples. The PLFA with the greatest influence on the clustering were indicative of certain eukaryote biomass, increased metabolic stress and anaerobic biomass. The PLFA indicative of bacterial response to metabolic stress were shown to increase in samples from day 12 and 30 from both the x 1 and x 10 SDH metal concentrations. PCR-DGGE detected changes in the bacterial population structure of samples from day 30 at both x10 SDH and, too a lesser extent, at x1 SDH. The primers used also detect chloroplast rDNA, and indicated that the increased eukaryotic biomass was in part due to the growth of the oxygenic phototrophic eukaryote *Amphora delicatissima* or a related species. Subsequent microcosms will use the data obtained regarding metal concentrations to examine the impact of the metal/diesel on the microbiota and bioavailability of the co-contaminants. Diesel and metals will be used at known-effect concentrations and at elevated but sub toxic concentrations.

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The Effects of Aging and Sorbent Decompostion on the Bioavailability of Toluene and Xylene in Solid Waste

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Approximately 25% of the sites on the National Priority List of Superfund are municipal landfills that accepted hazardous waste. Unlined landfills usually result in groundwater contamination, and priority pollutants such as alkylbenzenes are typically present. Ultimately, the EPA must develop strategies to manage these sites in a manner that is both cost-effective and protective of the environment. To select cost-effective risk management alternatives, better information on factors controlling the fate of contaminants in landfills is required.

In this research, we focus on the importance of sorption/desorption, contaminant aging, leachate composition, sorbent decomposition, and humification on the bioavailability and fate of organic contaminants in municipal solid waste (MSW). Results from prior studies suggest that sorbed contaminants are less bioavailable than dissolved contaminants, and that aged contaminants are less bioavailable than freshly sorbed contaminants. However, most studies focusing on bioavailability have been conducted in soils that had lower organic carbon contents than MSW and in aqueous phases that had lower organic carbon concentrations than landfill leachate.

The sorption capacity of ^{14}C-labeled toluene and o-xylene on major organic MSW components [polyvinyl-chloride (PVC), high density polyethylene (HDPE), newsprint, office paper, and model food and yard waste (rabbit food)] was determined. Batch isotherm data were collected in phosphate-buffered organic-free water and in acidogenic leachate. Flame-sealed glass ampules were used to minimize volatilization losses, and sodium azide was added to the liquid phase to prevent aerobic sorbate degradation. Acidogenic leachate was prepared by recirculating water through fresh residential municipal waste. Generation of methanogenic leachate from decomposed refuse is ongoing. In addition, anaerobically degraded office paper and newsprint have been prepared, and generation of anaerobically degraded rabbit food is ongoing.

Initial tests were conducted to determine the time required to reach short-term sorption equilibria. Short-term sorption equilibria for toluene on HDPE, newsprint, office paper, and rabbit food were reached within 2 days in organic-free water and acidogenic leachate while 20 days were required with PVC. Sorption kinetics of toluene on PVC exhibited a rapid initial rate of toluene uptake followed by a slower phase. A slower approach to equilibrium for the glassy polymer PVC was expected given that diffusion in glassy polymers is orders of magnitude slower than in rubbery polymers such as HDPE.

The Freundlich model ($q = K_f C^N$) was employed to describe toluene isotherm data. **Table 1** summarizes the parameters K_f and N with 95% confidence limits that describe single-solute toluene sorption on each material. In addition, Table 1 depicts the correlation coefficients (R^2), the number of data points for each isotherm (n), and the studied equilibrium liquid-phase concentration ranges (C). A comparison of the K_F values in Table 1 shows that PVC exhibited the largest sorptive capacity for toluene. In contrast, the sorption capacity of office paper for toluene was about 100 times smaller than that of PVC. The Freundlich N values for the studied materials were generally close to 1, indicating that partitioning dominated toluene uptake. The largest deviations from linearity were observed for office paper and PVC.

Table 2 summarizes the Freundlich isotherm parameters describing toluene sorption from acidogenic leachate. A comparison of Freundlich K_F values in Tables 1 and 2 shows that the single-solute toluene sorption capacities of HDPE, rabbit food, and newsprint were statistically similar to those obtained in acidogenic leachate. In contrast, the sorptive capacity of office paper for toluene from acidogenic leachate was approximately 20% of that from organic-free water. Given that the single-solute toluene isotherm on office paper was not quite linear (N=0.87), it is possible that components in acidogenic leachate competed with toluene for adsorption sites on office paper. The single-solute toluene sorption capacity of PVC was also greater than that determined in acidogenic leachate; however, it is unclear at this point whether we attained true equilibrium with PVC. Longer-term isotherm tests with PVC are ongoing.

Table 3 summarizes single-solute isotherm parameters describing o-xylene sorption on HDPE and rabbit food. A comparison of K_F values in Tables 1 and 3 indicates that the sorption capacity of the tested MSW components for o-

xylene was about 2 to 3 times larger than that for toluene, a result that is consistent with the greater hydrophobicity of o-xylene.

Table 1. Single-solute Freundlich isotherm parameters for toluene sorption on MSW components. The 95% confidence intervals for K_f and N are shown in parentheses

Material	K_f (μg/kg)(μg/L)$^{-N}$	N	R^2	n	C (μg/L)
PVC	804.1 (751.8, 860.0)	0.93 (0.91, 0.95)	0.99	93	1-958
HDPE	66.9 (59.9, 74.8)	1.01 (0.99, 1.03)	0.99	54	4-1078
Rabbit food	28.2 (27.0, 29.4)	1.00 (0.99, 1.01)	0.99	46	2-684
Newsprint	16.6 (15.6, 17.8)	0.96 (0.94, 0.98)	0.99	44	5-740
Office paper	8.9 (6.9, 11.5)	0.87 (0.82, 0.92)	0.97	48	7-1014

Table 2. Freundlich isotherm parameters for toluene sorption on MSW components from acidogenic leachate. The 95% confidence intervals for K_f and N are shown in parentheses

Material	K_f (μg/kg)(μg/L)$^{-N}$	N	R^2	n	C (μg/L)
PVC	487.4 (452.9, 528.7)	0.98 (0.96, 1.00)	0.99	60	2-840
HDPE	57.8 (50.0, 66.8)	1.04 (1.01, 1.07)	0.99	44	6-900
Rabbit food	26.1 (25.1, 27.1)	1.02 (1.01, 1.03)	0.99	47	6-848
Newsprint	15.2 (13.2, 17.4)	0.97 (0.93, 1.00)	0.99	38	4-640
Office paper	1. 8 (1.5, 2.1)	1.02 (0.99, 1.05)	0.98	98	6-840

Table 3. Single-solute Freundlich isotherm parameters for o-xylene sorption on MSW components. The 95% confidence intervals for K_f and N are shown in parentheses

Material	K_f (μg/kg)(μg/L)$^{-N}$	N	R^2	n	C (μg/L)
HDPE	212.7 (197.9, 228.5)	1.02 (1.01, 1.04)	0.99	48	1-670
Rabbit food	65.8 (60.4, 71.7)	1.01 (1.00, 1.03)	0.99	44	5-730

Preliminary Investigation of U and Ni in Riparian and Wetland Sediments: Microbial Ecology and the Potential of Apatite Amendments for Reducing Metal Availability and Toxicity

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The direct discharge of metallurgical process effluents to the environment on the Department of Energy's Savannah River Site (SRS) has led to extensive contamination of the vadose zone and groundwater with trichloroethylene (TCE) and tetrachloroethylene (PCE) and stream sediments and wetlands with uranium (U) and nickel (Ni). Riparian and wetland systems are recognized for their natural attenuation capacity due to high organic matter content, diverse microbial populations, and range of geochemical conditions conducive to the biodegradation of organics and the biotransformation of metals. However, metal toxicity is a potential obstacle for natural attentuation of organic plumes in critical SRS riparian and wetland areas, where U and Ni sediment concentrations can exceed 1000 $mg \cdot kg^{-1}$. It is critical, therefore, to understand the effect of metals on *in situ* biodegradation of organics and to design strategies for eliminating metal toxicity in support of intrinsic and enhanced biodegradation. In situ chemical stabilization of metals using apatite and other phosphate media offers a low-cost and minimally invasive alternative to traditional metal remediation methods such as excavation. The efficacy of hydroxyapatite (HA) for stabilizing a large assortment of metals, metalloids, and radionuclides has been demonstrated in a number of studies.[1,2,3] In our initial work, we have found HA amendments to be effective for application rates as low as 1% by weight.[3] Accordingly, research was initiated to:

- Determine the chemical speciation of U and Ni in wetland sediments and establish linkages between contaminant speciation and availability, bioavailability, and microbial toxicity.
- Assess the impact of U and Ni on native microbial communities, especially with respect to their capacity for degrading TCE in riparian and wetland sediments on the SRS.
- Evaluate the effectiveness of *in situ* apatite treatments for reducing U and Ni availability and toxicity and for facilitating TCE degradation.

Denaturing gradient gel electrophoresis (DGGE) profiles of sediment DNA amplified using a universal bacterial primer indicate no significant differences in bacterial diversity between contaminated and uncontaminated sites. However, overall diversity is not the only important indicator of metal stress. High U and Ni levels may adversely impact specific groups of microorganisms (e.g. methanotrophs, sulfate reducers, nitrifiers, and ammonia oxidizers), and it is this diversity of metabolic activity in systems that supports natural attenuation capacity. Ongoing research using selective bacterial primers focuses on the effect of U and Ni sediment contamination on specific functional groups of bacteria. Microorganisms isolated from highly contaminated sediments (pH 4.55; 2140 $mg \cdot kg^{-1}$ U; 581 $mg \cdot kg^{-1}$ Ni) displayed no enhanced U resistance but did exhibit unusually high Ni tolerance with respect to cultures isolated from unimpacted sediments (Figure 1). In preliminary assessments of metal availability (extractions in 10 mM $CaCl_2$ solutions), dissolved U and Ni concentrations on the order of 0.1 and 10 $mg \cdot L^{-1}$, respectively, were observed for the most contaminated sediments. These results explain the dissimilar responses of sediment bacteria to U and Ni. While total U sediment concentrations are over 3.5 times greater than Ni, Ni appears to be 100 times more available. Consequently, the adaptive response of sediment bacteria to Ni and not U is a reflection of metal lability and bioavailability in their environment. A metal specific toxicity assay, MetPLATE™, indicated metal toxicity for dissolved concentrations of 3 $mg \cdot L^{-1}$ Ni (EC_{50} = 10 – 20 $mg \cdot L^{-1}$) and 10 $mg \cdot L^{-1}$ U (EC_{50} > 50 $mg \cdot L^{-1}$), which is consistent with the expression of an adaptive response of sediment bacteria for Ni only as observed U concentrations fall well below toxic levels. Apatite amendments (5% w/w) were able to reduce metal availability by one order of magnitude, and therefore, appear to be effective for maintaining both U and Ni below dissolved concentrations of concern.

Preliminary results from sediment extractions and microbial metal resistance studies indicate that Ni is of greater concern than U in terms of environmental availability and toxicity to native microorganisms. Consequently, minimizing ecological and human risk and maximizing natural attenuation capacity in the Tims Branch system require careful consideration of U and Ni speciation and environmental availability.

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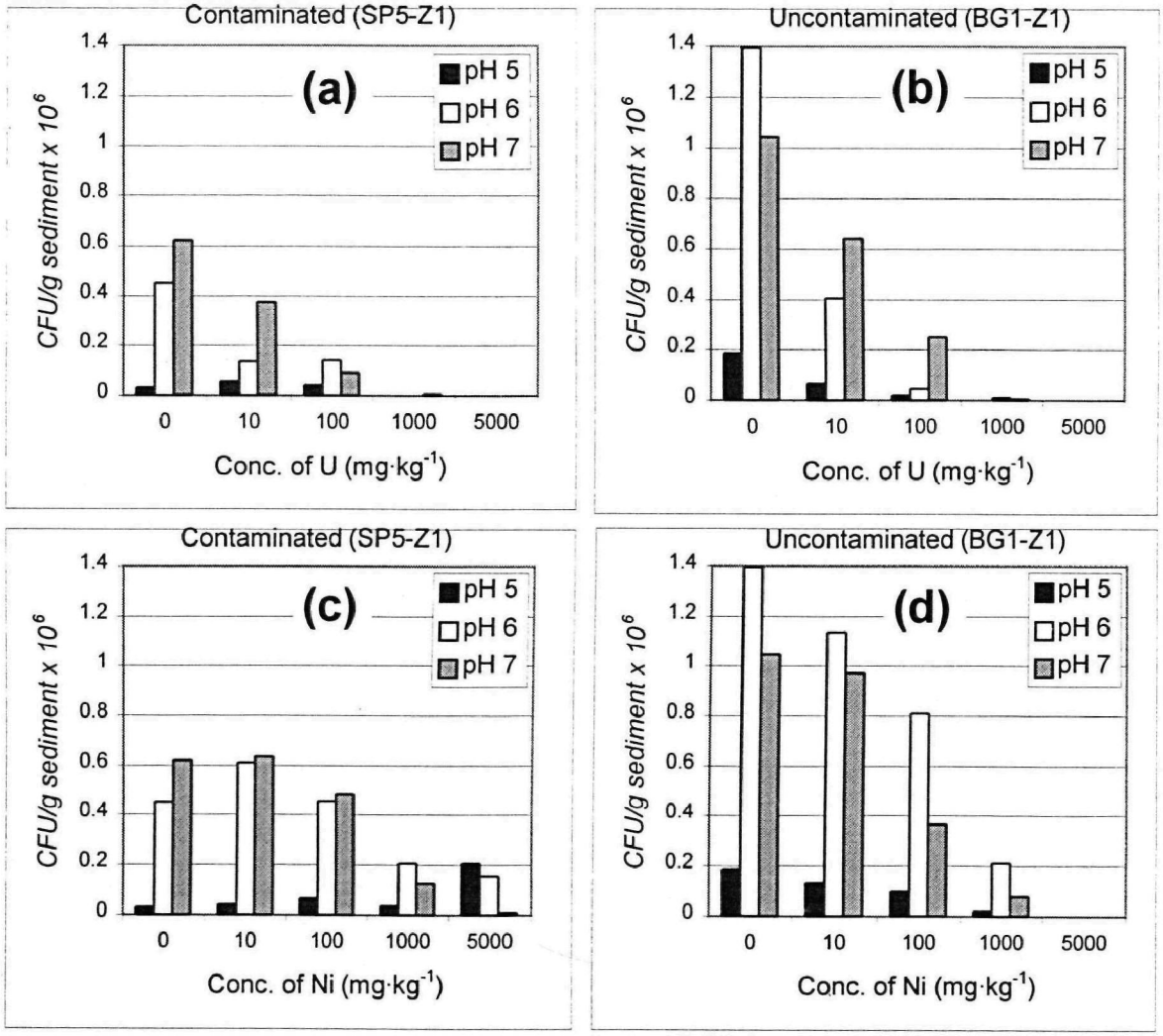

Figure 1. Culturing of sediment bacteria on U and Ni loaded media indicates the metal tolerance of organisms. (a) Cultures from a contaminated sediment (SP5-Z1) exhibited the same tolerance for U as those (b) from an uncontaminated sediment (BG1-Z1). In contrast, (c) organisms from the same contaminated sediment displayed a much greater resistance to Ni than those (d) from the reference sediment. (SP5-Z1: pH 4.55; 2140 mg•kg^{-1} U; 581 mg•kg^{-1} Ni. BG1-Z1: pH 4.97; 2 mg•kg^{-1} U; 4 mg•kg^{-1} Ni.)

Publications Associated with Project

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Sowder, A. G., Khijniak, T. V., Novak, M. T., Morris, P. J., Bertsch, P. M. Impact of U and Ni on the Microbial Ecology of Aged-Contaminated Sediments and the Potential of Apatite Amendments for Reducing Metal Availability and Toxicity. *Radiochimica Acta.* (Submitted).

Understanding Seasonal Variation of Bioavailability of Residual NAPL in the Vadose Zone

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Natural attenuation of non-aqueous phase liquid (NAPL) hydrocarbon pollutants proceeds in the vadose zone at unknown rates and as a function of uncertain mechanisms. While we know that mass transfer and microbial physiology both play a role in bioattenuation of pollutants *in-situ*, we don't know the relative role of these processes or the operative environmental factors. Here, we are investigating how bioavailability and natural bioattenuation of NAPL hydrocarbon pollutants are affected by natural cycles of wetting (precipitation) and evaporative drying. We are performing this work at three scales: the pore or microscale, the core or mesoscale, and the field or macroscale. The specific objectives are to:

- determine the effects of wetting and drying cycles on NAPL distribution in pore spaces for specific environmentally-significant pollutants with differing physicochemical properties;
- determine the mass transfer and biological responses to sequential wetting and drying;
- determine the influence of the microbial exopolymeric substances (EPS) on spreading and partitioning characteristics of selected pollutants;
- relate the temporal patterns of microbial growth and biodegradation to spatial locations of biodegrading microbes and residual NAPL;
- relate the effects of wetting and drying cycles to field-scale observations of natural attenuation; and
- mathematically describe bioavailability as a function of drying and wetting cycles.

We are conducting pore scale studies using glass micromodels operated under unsaturated conditions and visualized with fluorescent and visible light microscopy. Using micromodels, our abiotic goals are to determine spreading and distribution characteristics of study compounds (toluene and hexadecane) under conditions of water-wet and mixed wettability surfaces. Thus far, we have studied the imbibition of NAPLs into a dry matrix in the micromodel, or the drainage of a water-filled matrix using NAPLs. We have also recorded the dissolution and volatilization processes, to determine the rate of mass transfer at the pore scale. We are next determining the spatial response of NAPLs to changes in moisture under abiotic conditions.

In separate micromodel studies, we are studying bacterial colonization of micromodel surfaces when either non-toxic and pollutant substrates are provided for growth. We have developed a method for applying *Pseudomonas aeruginosa* to the model, and have acquired images of the distribution of fluorescently-stained inocula. Through a tri-parental mating procedure, we have developed a mutant of our *P. aeruginosa* strain that overproduces alginate, the primary constituent of EPS for this organism. Our current work is aimed at confirming expression of alginate overproduction in liquid and in sand culture. We will use the wild-type and mutant strains in the micromodel to study spreading and distribution of pollutants with microbial biofilms to determine the influence of the EPS matrix on mass transfer and bioavailability in response to sequential wetting and drying events. Water potential will be varied across a range that represents wet to very dry conditions (0.0 MPa to 1.5 MPa) by equilibrating the micromodel with a polyethylene glycol solution (PEG-8000). We are in progress with engineering a mutant of our *P. aeruginosa* strain that produces green fluorescent protein (GFP) in response to hexadecane and our plan is to use this organism to estimate the location of biodegradation activity within the micromodel.

Core studies using silica sand as the substratum for growth and mass transfer will be conducted with the same pollutants, water potential controls and biodegradation measurements used in micromodel studies. Thus far, we have begun development of prototype reactors for the core experiments. NAPL saturation distribution in cores as a function of wetting and drying will be determined using X-ray imaging. Currently we are developing the best conditions for X-ray imaging.

Results from the pore and core scale experiments will be coupled to develop a mechanistic explanation of seasonal variation in bioavailability. Field test data from well-monitored sites of intrinsic or managed bioremediation will be used to evaluate the effect that drying and wetting cycles have on biodegradation rates. The results from these three scales will be modeled to provide an empirical and a mechanistic interpretation of the functional relationship between bioavailability and soil moisture. Overall, we expect to gain an understanding of the physicochemical and biological mechanisms that determine bioavailability under realistic climatic conditions. With an understanding of bioavailability / biodegradation seasonality, it is increasingly feasible to predict the efficacy of natural biological attenuation and the necessity of engineered or managed remediation for specific vadose zone pollutants.

Biosurfactant Specificity and Influence on Microbial Degradation of Hydrocarbons by Microbial Consortia in the Field

Gina S. Shreve, Department of Chemical Engineering, Wayne State University, Detroit, MI and William Finnerty, Dynazyme Inc., Athens, GA

A comprehensive research program involving basic and applied field investigations is defined to establish the efficacy of various classes of biosurfactants in the remediation of soils contaminated with mixed hydrocarbon wastes. The proposed research objectives are (1) to determine the basis for the hydrocarbon specificity of biosurfactants in terms of micelle size, micelle dielectric constant, and targeting of minimal interfacial tension values for mixed micelle solutions to mixed wastes; (2) elucidation of the influence of pollutant mixtures on the effectiveness of pure and mixed biosurfactant micelles upon solubilization of hydrocarbons; (3) the assessment of hydrocarbon solubilization on the microbial degradation of target pollutants; and (4) field studies to determine the role and influence of biosurfactants in the remediation of polluted target sites.

The research objectives addressed to date include: (1) examining the basis of the hydrocarbon specificity of biosurfactants based on structural and chemical properties of the biosurfactants, and, (2) determining the influence of contaminant mixtures on the effectiveness of pure and mixed micelle preparations of microbial biosurfactants for solubilization of specific classes of hydrocarbons. The properties of three biosurfactants are currently under investigation for a number of contaminants that are present or structurally similar to those present in the contaminated soils from the field site. Two biosurfactants under investigation, Dyna 270 and Dyna 200, are provided by Dynazyme Incorporated. A mixture of rhamnolipids R1 & R3 is also being used in these studies. Dyna 270 and the rhamnolipids have been characterized with respect to their solubilization, interfacial tension, and micelle structural properties against target hydrocarbons from various hydrocarbon classes, including: straight chain alkanes, branched alkanes, polyaromatic hydrocarbons, monoaromatic hydrocarbons, and a light weight petroleum mixture. Various other physical-chemical properties such as the pH optima, the most effective alkane carbon number (EACN) were also determined. The pH optimas are 5.0 for Dyna 270, 11.0 for Dyna 200 and 6.8 for rhamnolipid. The EACN for Dyna 270 is 6, the EACN for Dyna 200 is 5, and the EACN determined for rhamnolipid is dodecane. Data on the structural characterization of Dyna 270, the biosurfactant solubilization of target hydrocarbons, and the physical-chemical characterization of the biosurfactant micelles are contained in the Annual Progress report and will be presented.

Purification procedures have been developed and mass spectrometry has been performed to determine the structure of the previously uncharacterized biosurfactant Dyna 270. The molecule is currently identified as a mannosyl ester fatty acyl compound using carbohydrate chemistry and subsequent TLC analysis. The remaining linkages and functional groups are being determined currently through LC-MS analysis at the University of Georgia Center for Complex Carbohydrates.

Rhamnolipid and the mannosyl ester 3-hydroxydecanoic acid biosurfactant (Dyna 270) have been evaluated with respect to their physical properties and their ability to solubilize several classes of hydrocarbons (Table 1 of Technical Report/Summary). Both biosurfactants were similar in demonstrating the ability to solubilize alkane hydrocarbons, branched alkane hydrocarbons and monoaromatic hydrocarbons in order of decreasing effectiveness. Their effectiveness is currently under investigation for polyaromatic and chlorinated hydrocarbons as well as mixtures of the target hydcarbons of each structural class. While the surfactant show similar packing properties at the interface as represented by similar area per surfactant monomer head group, Dyna 270 consistantly reduces the interfacial tension to a greater extent. The critical micelle concentration determined for Dyna 270 is also much lower than that of the rhamnolipid R1 and R3 mixture. This results in a much higher measured solubilization of the target hydrocarbon on a milligram of hydrocarbon solubilized per milligram of biosurfactant basis. Hydrocarbon mixture results are currently under examination to determine if these observed trends continue for complex mixtures of hydrocarbons. Data on the structural characterization of Dyna 270, the biosurfactant solubilization of target hydrocarbons, and the physical-chemical characterization of the biosurfactant micelles will be presented.

The direct solubilization of various structural classes of hydrocarbons was measured by equilibrium partitioning experiments followed by extraction of the aqueous phase and gas chromatography analysis for the hydrocarbon species. These results were graphed for each hydrocarbon species and biosurfactant mixture and the slope of the line was calculated for each target contaminant and is reported in Table 1 as the solubilization in units of moles of

target hydrocarbon per mole of rhamnolipid and moles of target hydrocarbon per microliter of Dyna 270 in buffer solution. Both Dyna 270 and rhamnolipid solubilized linear alkane hydrocarbons effectively. Rhamnolipid also solubilized branched alkanes and to a lesser extent monoaromatic hydrocarbons. Rhamnolipid solubilization of the PAH's (napthalene and phenanthrene) appear low, therefore, these experiments are being repeated. Dyna 270 is also being reexamined to determine it's solubilization of the branched hydrocarbons and PAHs.

Mixtures of these same hydrocarbons are now being examined to determine if these trends also occur for the solubilization of these same hydrocarbon species from mixed organic phases or whether the solubilization of a target contaminant from a matrix presented by complex mixtures of contaminants differs from the results observed with pure hydrocarbons.

Interfacial properties of the biosurfactants, Dyna 270 and rhamnolipid, were investigated at 25 C in a buffer/hydrocarbon solution (for Dyna 270) or in a hydrocarbon/water system for rhanmnolipid against specific hydrocarbons from various structural classes of hydrcarbons. Other parameters of importance that were obtained from these experiments are the critical micelle concentration (CMC) in the hydrocarbon/biosurfactant system and the interfacial tension at the CMC.

Dyna 270 is more effective in lowering the interfacial tension against all hydrocarbons except hexadecane. For rhamnolipid, lower interfacial tension appears to correlate somewhat with the solubilization trends observed.

Soil cores from the LNAPL plum near FT-2 at the former Wurtsmith AFB have been obtained and column studies are currently being set up to examine the properties of each biosurfactant for microscopic displacement of the target hydrocarbons from the soil matrix. We intend to model the results using a combined transport/equilibrium partitioning model. Batch solution phase experiments will be conducted to examine the biodegradability of the micellar phase hydrocarbon and the applicability of an organic/aqueous solution phase biodegradation model (Sekelsky and Shreve, 1999) to describe this. The microbial consortia showing optimal growth and degradation of the target hydrocarbons will then be applied to the column experiments. Mathematical models will be used to describe the effect of biosurfactnat mediated microbial degradation of sorbed contaminant prior to the beginning of the field studies at the former Wurtsmith AFB.

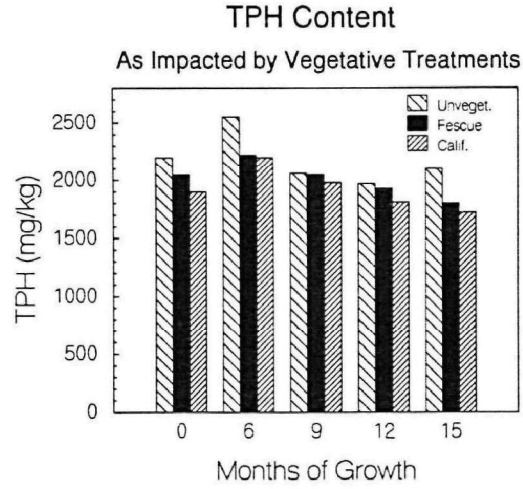
THE EFFECT OF PLANTS ON THE BIOAVAILABILITY AND TOXICITY OF CONTAMINANTS IN SOIL

M.K Banks, Civil Engineering, Purdue University, A.P. Schwab, Agronomy, Purdue University, and J. Scott Smith, Animal Sciences, Kansas State University

The objectives of the research are to: (1) evaluate the impact of plants on contaminant toxicity and bioremediation/phytoremediation efficiency, (2) investigate the fate of contaminants in plant/soil systems, and (3) determine the impact of plants on leaching of contaminants.

Remediation of Petroleum Contaminated Soil: Port Hueneme, California

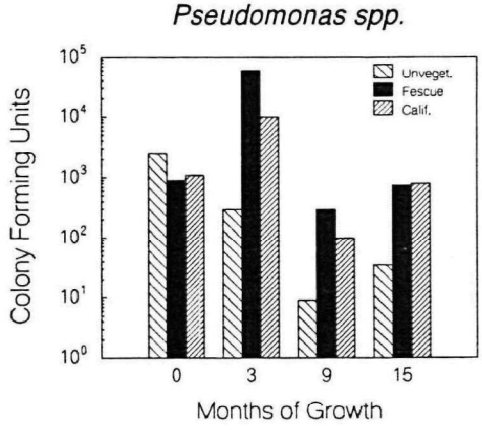
In 1997, we established a site at the Port Hueneme Naval Station near Oxnard, California in cooperation with the Naval Facilities Engineering Service Center. The soil was contaminated with bunker oil with an average total petroleum hydrocarbon (TPH) contamination concentration of 3,000 mg/kg. A small phytoremediation cell was constructed (30 feet by 60 feet by 3.5 feet deep) and filled with the contaminated soil. Vegetative treatments were imposed in a randomized complete block design with four replications and treatments of unvegetated, a mix of native grass and legume species, and a typical mixture of plant species used in roadside revegetation. An automatic irrigation system keeps the plots moist, and fertilizer N and P are added quarterly. The soil is sampled quarterly and monitored for changes in TPH, petroleum degrading bacteria, and toxicity as determined by lettuce germination, earthworm response, and Microtox©.



The standard TPH determination reveals that the vegetated and unvegetated treatments are proceding at the same, slow rates (see figure at right). The lack of difference between the vegetated and unvegetated treatments in due in part to the fact that the contaminant residues have been heavily aged. The TPH as determined by standard protocols is only a small fraction of the total extractable hydrocarbons; the largest fraction consitsts of weathered, polar organic materials that are beginning to resemble soil humus and are very slowly degraded. The combination of low TPH levels, low polyaromatic hydrocarbon concentrations, and the weathering stage of the residues suggests that the soils probably represent a very small environmental threat.

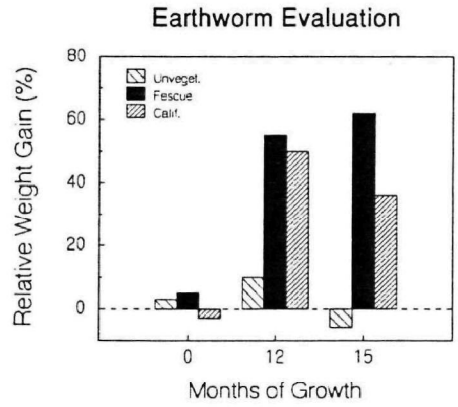
Lettuce germination was restricted only in soil collected at the time of seedbed establishment; germination at all other times was statistically equal to 100% (data not shown). The initial, low germination was likely because of the high salinity of the soil, which was reduced by management of the application of irrigation water. The Microtox© results were quite similar.

Several species of the *Pseudomonas* bacteria are capable of degrading PAHs and other petroleum hydrocarbons. Therefore, we quantified the populations of these micro-organisms by plate counts. As expected, there were no treatment differences at the time of seeding, but the vegetated plots had signifi-cantly higher *Pseudomonas spp* by the end of only three months, and the trend continued through at least 15 months of plant growth (see figure at left).



The US EPA standard protocol for earthworm toxicity is based upon mortality, and these soils did not induce mortality regardless of the time of sampling or treatment. This result is consistent with lettuce germination and Microtox©, but appears to be an insensitive measure of overall toxicity. Therefore, a modified procedure was used in which worms were added to the soil, incubated for two weeks, and change in mass quantified. This approach proved to be much more sensitive to sampling time and

treatment. In the figure to the right, 0% growth would mean that the worms had the same mass before and after incubation. Growth of 100% would indicate mass gain equal that of worms incubated in an uncontaminated, control soil. Worms in soils from the vegetative treatments had significantly higher mass gain than those in unvegetated soils. Thus, even though the soils were not toxic, the presence of plants enhanced the overall quality of the soil and clearly improved the ability of the worms to thrive.



The Port Hueneme study is scheduled to be completed in January, 2000 at which time we have final measures of TPH, specific PAHs, and toxicity determinations.

The Impact of Aging and Phytoremediation on Contaminant Toxicity

In an on-going study, soils have been contaminated with petroleum products (diesel fuel, motor oil, and selected PAHs). The soils are allowed to age for 0, 3, 6, 12, and 18 months followed by 12 months of phytoremediation. Degradation of contaminants and toxicity will be monitored as a function of time and remediation treatments. We also are engaged in a study of the impact of the rhizosphere on the mineralization of benzo[a]pyrene in which plants are grown in chambers that isolate the roots from shoots and allow us to evaluate CO_2(g) evolution from soil and aerial portions of the system.

Submitted Publications

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DOE contract No: DE-FG02-97ER62350

Bioavailability of PCBs in Surfactant-Washed Soils With and Without UV-Irradation

John Sanseverino, Alice Layton, Betsy Gregory, James Easter, Fu-Min Menn, T. Wayne Schultz, and Gary S. Sayler. Center for Environmental Biotechnology, University of Tennessee, Knoxville, Tennessee.

A major factor in developing PCB bioremediation technology and gauging endpoints of effective treatment is overcoming the relative insolubility of PCB and its sorptive properties, hence low bioavailability in many soils and sediments. The problem of poor bioavailability (defined as availability of substrate to the microorganism) has been overcome by soil washing with nonionic surfactants and feeding the surfactant/PCB mixture to 2 previously developed genetically-engineered organisms, *Pseudomonas putida* IPL5::TnPCB and *Ralstonia eutrophus* B30P4::TnPCB. These organisms degrade surfactant and constitutively express a biphenyl operon for degradation of lessor chlorinated PCB congeners.

Bioavailability (defined now as availability to human and ecological receptors) and measuring associated toxicity is fundamental to risk assessment and defining environmentally acceptable endpoints. After treatment, PCBs removed and remaining on contaminated soil should not pose an unacceptable risk to human health and the environment. The long-range goal of this project is to develop an alternative, low-cost treatment process for the *in situ/ex situ* remediation of PCBs. This goal is being accomplished by (i) increasing PCB bioavailability by surfactant washing, (ii) increasing the extent of PCB biodegradation by photolytic dechlorination of highly chlorinated congeners, and (iii) testing the surfactant/PCB solutions and the washed soil for environmental estrogens and toxicity.

A PCB-contaminated soil (designated PR3-1; 1,100 mg Aroclor 1248/kg soil and 90 mg Aroclor 1260/kg soil) was washed with 2% polyoxyethylene 10 lauryl ether (POL(10)). Approximately 86% of soxhlet-extractable PCBs were removed after 3 wash cycles. Surfactant/PCB solutions were divided with half subjected to biodegradation and the other half subjected to photolysis followed by biodegradation. In all cases, approximately 95% of the surfactant was degraded in 2-3 days. The combination of photolysis and biodegradation significantly enhanced the total removal of PCBs (approximately 80%; Fig. 1) than biodegradation alone (approximately 59%; Fig. 1). The purpose of photolysis was to dechlorinate the heavily chlorinated congeners economically and shift the congener profile towards the more biodegradable congeners. Either method used alone is not practical for 2 reasons: (1) Photolysis as a means to completely dechlorinate PCBs is too expensive, and (2) biodegradation is not effective with the highly chlorinated congeners. The combination of both methods provides an efficient, economical means to treat PCB-contaminated soil.

Three molecular-based assays are being utilized to assess bioavailability of PCBs and by-products: a bacterial bioluminescent reporter assay, the presence of environmental estrogens using a yeast-based human estrogen assay, and the presence of potentially toxic aromatic compounds using a yeast-based human aryl hydrocarbon receptor (AHR) assay. The bioluminescence assay detects biphenyl and mono-chlorinated biphenyls. These compounds were present in pure Aroclor 1242 and photolyzed surfactant/PCB solutions (Table 1). Nonphotolyzed solutions were not reactive in this assay implying that photolysis produced some biphenyl and monodichlorinated by-products.

Nonphotolyzed surfactant/PCB solutions were reactive with the human estrogen receptor (Table 1) indicating that environmental estrogens were present. However, tests with pure aroclors did not react with this assay further confirming that unmodified PCBs are not estrogenic. Photolyzed solutions however, were not estrogenic indicating that photolysis had the dual effect of dechlorinating highly chlorinated congeners as well as preferentially degrading potentially estrogenic compounds. Hydroxylated PCBs have been previously identified as environmental estrogens. Work is on-going to determine if bacterial metabolism can generate hydroxylated congeners that may act as environmental estrogens and if they were present in the soil.

The AHR binds aromatic compounds and enhances transcription of cytochrome P450 genes. Cytochrome P450 oxidizes these aromatic compounds which may result in serious health effects. A yeast-based reporter system has been developed with a human AHR enabling testing of the bioavailability of PCBs in soil. At this time, the assay is being validated for pure PCBs. Future work will test the washed soil directly to determine if soil washing reduced

the bioavailability (to human receptors) of remaining contaminants and environmental estrogens. This data will be used to develop environmentally acceptable endpoints for remediation of this soil.

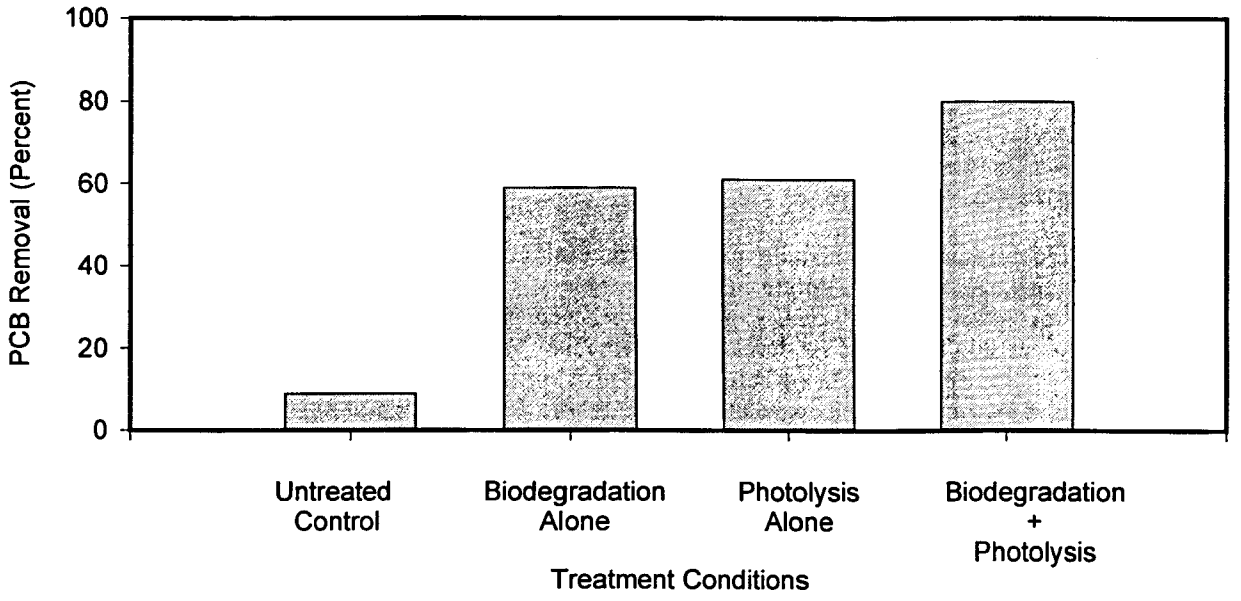

Figure 1. Percent PCB removal from surfactant/PCB micelles undergoing treatment by biodegradation, photolysis, and biodegradation combined with photolysis

Table 2. Summary of bioluminescence, estrogenic activity and aryl hydrocarbon receptor assays

Sample	Bioluminescence	hER Activity	**AHR Activity**
Aroclor 1242	+	-	+
Aroclor 1248	-	-	+
Aroclor 1260	-	-	+
POL (10)	-	-	Not tested
Nonphotolyzed Sol'n	-	+	Not tested
Photolyzed Sol'n	+	-	Not tested

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Biogeochemical Factors Limiting Transformation of Co-Occurring Contaminants in Salt Marsh Sediments

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A common difficulty associated with devising bioremediation strategies for many contaminated sites is the co-occurrence of several classes of toxic chemicals. In the case where mixed contaminants are present, the likelihood of interactions between candidate transformation pathways is high and greatly complicates remediation processes. Because these pathways are believed to involve complex microbial consortia, this program seeks to investigate the linkages among biogeochemical parameters, microbial activity, microbial diversity and community structure, as they apply to the biotransformation of co-occurring contaminants. It is hypothesized that a fundamental understanding of these linkages will lead to the development of enhanced *in situ* remediation strategies. To address this thesis we have assembled a multi-disciplinary research team with expertise in biogeochemistry, microbial ecology, molecular biology, and environmental organic chemistry/engineering to focus on saltmarsh sediments in which co-occurring polychlorinated biphenyls (PCBs-principally as Aroclor 1268) and mercury dominate a complex assemblage of contaminants. Studies are focusing on a saltmarsh Superfund site ("LCP") in southeastern coastal Georgia (USA) that has been active since the 1920's.

Surprisingly, during the first project year observations made at the LCP site suggested that PCB dechlorination are likely occurring under sulfidogenic conditions. Therefore, one of the primary objectives of the current phase of the project has been to study the linkage between sulfate reduction and PCB dechlorination. Using native contaminated sediment from the LCP site, controlled microcosm studies indicated that PCB dechlorination activity could be induced under both methanogenic and sulfidogenic conditions in the presence of high mercury concentrations. Microcosms that were primed with 2,3,4,5,6-pentachlorobiphenyl (a "meta" primer) exhibited the greatest decrease in Aroclor 1268 (18% by mass after 3 months). Furthermore, the distribution of PCB dechlorination products differed under sulfidogenic vs. methanogenic conditions suggesting that different microbial dechlorination activities were inducible. In experiments supported by our AASERT augmentation project, temperature and/or pH were found to influence the extent and rate of primer dechlorination in sulfate-amended sediment slurry incubations, suggesting that specific dechlorination activities can be induced under varying environmental conditions.

To identify potentially important microbial consortia involved in contaminant transformation, studies were undertaken to correlate the structure of microbial communities associated with pristine and contaminated sediments. Characterization of microbial community structure was determined using several molecular approaches including direct sequencing of the 16S rRNA gene, cell blot hybridization with suites of phylogenetic broad group-specific 16S rRNA targeted oligonucleotide probes, and Amplified Ribosomal DNA Restriction Analysis (ARDRA). Characterization of 16S rDNA clone collections by ARDRA and sequence analysis suggests that microbial diversity is at least as high in contaminated sediments as those found in other environments. Although sulfate reducing bacteria (SRB) accounted for the majority of activity and probe hybridization signal, respectively, the delta proteobacteria did not numerically dominate the microbial consortium as determined by sequence analysis of clones obtained from pristine or contaminated sites. Within the SRB populations, the abundance of *Desulfobacterium* was positively correlated to the presence of contaminants. These studies suggest the role of sulfate reducing populations of bacteria and indicate the importance of diverse microbial assemblages for the tolerance and transformation of complex contaminant mixtures in saltmarsh sediments.

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Enzymology of the Degradation of Organometallic Compounds

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For decades the form of mercury of greatest public health concern has been the neurotoxic organomercurial monomethylmercury (MMM). This simple but potent compound is biomagnified in the food chain and, apart from accidental exposure to now-outlawed MMM fungicides, the most common route of exposure is through freshwater fish and seafood. While precise routes of accumulation in fish are not well-defined, the origin of the initial methylation process has been increasingly well detailed in freshwater settings in just the last 5 years.

Industrially produced organotin compounds are also of considerable environmental concern. They have been used for decades as marine antifouling agents, agricultural fungicides, and plasticizers on the assumption that they are relatively benign to higher organisms. However, recent work makes it clear that compounds such as relatively stable tributyltin (TBT) exert potent estrogenic effects.

Early work on our project established that another enzymatic process, Hg(0) oxidation by catalase, takes place in bacteria at rates sufficiently high to constitute a potentially important source of methylatable Hg(II) in nature (1). Our present work focusses on a bacterial enzyme capable of effecting the direct degradation of both organomercury and organotin compounds, the organomercurial lyase, product of the plasmid-encoded merB gene. MerB catalyzes protonolysis of a large variety of organomercurials with the alkyl mercurials being poorer substrates than the aryl mercurials. In collaboration with Genetics Dept. colleague, Rich Meagher, we have recently shown that this novel enzyme in combination with another bacterial enzyme, mercuric reductase, can confer resistance to organomercurials in plants (2). Interestingly, MerB also effects degradation of certain organotin compounds including tetravinyltin, triethylvinyltin, tetramethyltin, and trimethyltin fluoride, although various other organotin compounds including the widely used anti-fouling agent TBT oxide are not substrates for the existing enzyme.

The remaining goals of our work are: (1) to determine the 3 dimensional solution NMR structure of the MerB enzyme and (2) to derive variants of MerB optimized for degradation of the most environmentally important organometals, MMM and TBT.

In respect of the first sub-goal, we have demonstrated that merB is a cytosolic enzyme, over-expressed the protein, devised a facile spectrophotometric assay, and optimized its purification (3). The protein is a monomer of 24 kDa, well within the range of solution structure determination by highfield NMR. In collaboration with Biochemistry Dept. colleagues Jim Omichinski and Pascale Legault we have determined a 1-H, 15-N HSQC spectrum in which nearly 200 of the protein's 220 amide protons are well-resolved. On the basis of these very promising results we have obtained supplemental funding from DOE and the University of Georgia for determination of the entire solution structure of MerB, and anticipate completion of the structure determination during 2000.

In respect of the second sub-goal of the project, we have used high efficiency PCR and mutator strain random mutagenesis of merB to derive several dozen variants (detected by various screening and selection strategies) which have lost or gained the ability to metabolize organometal compounds. DNA sequence determination currently underway will, for the first time, reveal residues involved in the novel functions of this enzyme. Detailed biochemical and biophysical characterization of key mutant proteins will follow during 2000. In addition to being incorporated into living bacterial cells for remediative purposes, with enhanced ability to degrade MMM or TBT could be used alone on a solid support or introduced into plants or animals (including insects) for contained or field treatment.

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Bioavailability and Biostabilization of Multicomponent Non-Aqueous Phase Liquids (NAPLs) in the Subsurface

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The objective of this project is to understand key factors that control the bioavailability and biostabilization of high molecular weight organic contaminants (PAHs and PCBs) sequestered within multi-component DNAPLs entrapped in heterogeneous soil systems. The main hypothesis of this project is that slow dissolution of contaminants released from DNAPL pools entrapped in the subsurface, when combined with low-level microbial activity in the vicinity of the DNAPL source region, can result in stabilization of contamination with diminished plume formation and associated risk reduction.

The project consists of four phases:

Phase 1: Biostabilization Screening Tests and Biokinetic Studies. Bench-scale biostabilization screening tests are developed to examine the potential for biostabilization of various DNAPLs. The protocols are tested with DNAPL coal tar (composed of a mix of PAHs), and an Aroclor DNAPL (composed of a mix of PCBs). Phase 1 research also examines the biodegradation kinetics of individual PAH and PCBs in the absence of mass transfer constraints.

Phase 2: Mass Transfer and Bioavailability Tests measure the equilibrium partitioning and kinetics of release of multiple organic compounds from DNAPL in two abiotic tank systems: a small-scale, bench-top system and a larger, pilot-scale system. The goal is to determine key factors that control the availability of organic contaminants released from DNAPL pools, at two different spatial scales.

Phase 3: Biostabilization Tests examine the combined effect of contaminant dissolution from DNAPLs and microbial degradation on stabilization and risk reduction at two spatial scales.

Phase 4: Modeling and Scale-up of Laboratory Data: The goal is to develop mathematical models and engineering protocols that would enable scale-up of biostabilization processes (mass transfer and biokinetics) from the bench-scale to the tank-scale, ultimately to the field.

The work to-date in Years 1 and 2 has resulted in completion of **Phase 1** activities, and steady progress in **Phase 2** and **Phase 4** research. As part of **Phase 1** research, the biostabilization potential of coal tar and Aroclor 1242 has been examined, and the biodegradation kinetics of bi-phenyl, styrene, naphthalene, methyl-naphthalene and phenanthrene have been studied and modeled. As part of **Phase 2** work, bench-scale and tank-scale systems have been designed and tested to examine the kinetics of release of contaminants from DNAPL pools at different water flow rates. Diagnostic tests with octanol and coal tar revealed problems in localizing and immobilizing the DNAPL pool, due to which a smaller pilot-scale tank was designed and constructed with glass and metal walls. A bench-scale cell has also been developed and tested with solid naphthalene. Both systems are currently being operated to assess mass transfer from DNAPL pools at the small-scale and the intermediate-scale. We are evaluating current methods used to model NAPL pool dissolution as they may place limitations on modeling dissolution under biomass at the pool-water interface. A new modeling methodology that uses a local dispersivity that gets modified due to biomass growth is proposed and tested in our ongoing research. As part of **Phase 4** work, a finite element groundwater contamination modeling software package, SUTRA, is under modification to incorporate biostabilization phenomena. These modifications involve the incorporation of multiple species in the transport equations, incorporating bio-kinetics and bio-growth, modeling of oxygen supply, modification of hydraulic conductivity field due to biomass growth and biomass transport.

Results from **Phase 1** research are presented in this section. This work has resulted in successful completion of an M.S thesis and an M.S. report by two students, Ms. Allison Riffel (University of Colorado, Boulder) and Ms. Kendra Morrison (University of Colorado, Denver) who examined biodegradation of PCBs and PAHs, respectively. The Monod model was found to describe the biodegradation kinetics of biphenyl, naphthalene and methyl naphthalene, with the following average estimated parameter values: biphenyl: Ks=22 mg/L, k= 0.3 mg biphenyl/ mg VSS-hr, Y=1 g VSS/g biphenyl; naphthalene: Ks=0.5 mg/L, k= 1.4 mg naph./mg VSS-hr, Y=0.83; m-

naphthalene: Ks=0.006 mg/L, k=4.3 mg m-naph./mg VSS-hr, Y=0.83. Styrene exhibited toxicity to the microbes at a concentration > ~15 mg/L. Phenanthrene exhibited first order kinetics with a degradation rate constant of 1.8 L/mg VSS-hr. The biostabilization screening tests indicated potential for biostabilization of both coal tar and Aroclor. Unlike coal tar, which demonstrated stabilization of aggregate aqueous phase toxicity, DNAPL composition (depletion of more soluble PAHs), as well as microbial counts after a 100 day period, Aroclor studies revealed stabilization of two parameters: DNAPL composition (depletion of higher solubility congeners) and microbial counts. Aqueous phase PCB concentrations were not significantly different from controls and aggregate aqueous-phase toxicity is currently being re-evaluated.

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Bioavailability of Organic Contaminants in Estuarine Sediments to Microbes and Benthic Animals

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The goal of this project is to determine the bioavailability of sediment-associated contaminants to microbes and benthic animals. Most often bioavailability refers to the fraction of a contaminant that may enter biological processes. Alternatively, bioavailability can refer to the flux of contaminants. When defined as a flux, bioavailability will be a function of the local environmental conditions, including contaminant interactions with the solid matrix, concentration gradients, pH, redox potential, and solution composition. If, under a given set of conditions, the resulting flux is below the minimum flux required by the organism for uptake or utilization, then the contaminant would not be bioavailable. Understanding and quantifying the relationship between the physical and chemical characteristics of sediments and fluxes of contaminants to microbial and animal communities is essential for prudent risk-based decision making.

Our approach has focused on characterizing contaminant partitioning, biodegradation, and uptake behavior in field-aged estuarine sediments. The test sediments come from two locations in the heavily industrialized New York/New Jersey Harbor. Piles Creek is a tidal creek located within an industrial park consisting of petroleum refineries and oil storage tanks. Newtown Creek is a small channel off the East River in New York City with a long history of contamination from industrial and municipal waste sources. Sediments from both locations contain a complex assortment of organic and inorganic contaminants. Our emphasis is on the polycyclic aromatic hydrocarbons (PAHs). The physical and chemical properties of these sediments have been extensively characterized, and many differences in these properties which may affect the bioavailability of contaminants have been observed. Newtown Creek sediment is very fine-grained, with 94% (by mass) silt and clay, while Piles Creek sediment is relatively coarser at 55% silt and clay. The sediments have been fractionated on the basis of particle size and particle density. Large (>500 Fm), low-density (<1.9 g/cm3) particles from Piles Creek have the highest PAH concentrations, approaching 3000 ppm. PAH concentrations in Newtown Creek sediment are lower, peaking at 800 ppm in the 125-300 Fm sized low-density fraction. For both sediments, the majority of the total PAHs are found in the low-density fraction, although this fraction makes up only a minor part of the total sediment mass (4% for Piles Creek, 15% for Newtown Creek). When PAH concentrations are normalized to the carbon content of the size- and density-fractionated particles, both sediments show deviations from the predictions of equilibrium partitioning theory, especially for Piles Creek sediment.

Characterization of the physical structure of the sediments demonstrated that Newtown creek sediment had much greater macropore (pores > 50 nm) and mesopore (pores 2-50 nm) volume and surface area. This resulted in much higher PAH concentrations (normalized to surface area) in Piles Creek sediment and over twice the pore volume (and three times the pore surface area) of pores accessible to bacteria (>0.5 Fm) in Newtown Creek sediment.

We investigated the abiotic desorption flux of field-aged PAHs (PAHs already present in the sediment) from the various sediment fractions to determine whether the flux rates were independent of size- or density-fraction. We found substantial differences in desorption kinetic behavior between the sediments. For example, the desorption rate of benzo[a]pyrene was an order of magnitude higher in the low density fraction than in the high density fraction, and had a very different flux curve.

A bacterium isolated from Piles Creek sediment has been partially characterized and used in biodegradation rate studies. The strain, designated PC01, is weakly motile, Gram positive, and capable of growing on phenanthrene and pyrene. The degradation of phenanthrene by PC01 varies depending on sediment type. For freshly spiked Piles Creek sediment, phenanthrene degradation rate is 67 Fm/h/mg protein, whereas the rate on Newtown Creek sediment is much lower, at 25 Fm/h/mg protein. PC01 can degrade pyrene and phenanthrene simultaneously, but pyrene degradation proceeds more rapidly in the presence of phenanthrene. Degradation of freshly added pyrene also proceeds faster in Piles Creek sediment than in Newtown Creek sediment. These biodegradation rates were similar to initial PAH desorption flux measurements in the field-aged sediments, suggesting that initial biodegradation rate is biologically-limited. However, the rapid decrease in desorption flux after approximately 24 hours observed in the abiotic desorption experiments suggests that biodegradation will rapidly become desorption rate-limited. Degradation by PC01 of field-aged pyrene shows a different pattern from the freshly spiked sediment

experiments, with biodegradation rates approximately six times faster in Newtown Creek sediment. This difference may be due to the greater pore volume and pore surface area of Newtown Creek sediment, resulting in increased surficial contact area for bacteria. Not surprisingly, the biodegradation rates were much lower than those observed with the freshly spiked sediment, demonstrating the effect of field aging on biodegradation rates.

Our data demonstrate the effect sediment physical and chemical structure has on sequestration, biodegradation, and abiotic desorption behavior. These results led us to hypothesize that sediment detritivory (sediment feeding by benthic animals), which can change the physical and chemical structure of sediment, will have a substantial effect on partitioning behavior. To test this hypothesis, we first quantified the changes in physical structure caused by sediment detritivory using *Capitella* sp. I as our model polychaete. We characterized the pore structure in our test sediments before and after feeding, and found substantial differences in particle size selectivity. In addition, in both sediments (and a non-contaminated control) there were substantial changes in the macropore structure caused by feeding. This suggests that PAH transport processes affected by macropore structure may be affected significantly by detritivory.

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Role of Metal Bioavailability in *In-Situ* Bioremediation of Metal and Organic Co-Contaminated Sites

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A large proportion of hazardous waste sites are co-contaminated with organics and various metals. Such co-contaminated sites are difficult to bioremediate due to the nature of the mixed contaminants. Specifically, the presence of a co-contaminating metal imposes increased stress on indigenous populations already impacted by organic contaminant stress. As a result, rates of biodegradation of organics may be reduced significantly in the presence of a co-contaminating metal. The overall objective of this research is to investigate the effect of varying metal bioavailability on microbial populations and biodegradation of organics to allow a better understanding of how to optimize remediation of co-contaminated sites.

One of the first steps in understanding the response of indigenous microbial populations to metal stress is to examine different mechanisms of metal resistance. We have chosen cadmium as a model metal for this study and have obtained samples of cadmium-contaminated soil as well as similar uncontaminated soil for comparison. Cadmium-resistant isolates resistant were isolated, discriminated by ERIC DNA fingerprinting, and identified at the genus level by metabolic fingerprinting (BIOLOG). Isolates obtained include *Arthrobacter, Bacillus, Corynebacterium* and *Pseudomonas* spp. Six isolates were studied further to determine the specific mechanism of metal resistance. Three different mechanisms were observed; biosurfactant production (1 isolate), exopolysaccharide complexation of metal (2 isolates), intracellular accumulation of cadmium (2 isolates), and an unidentified mechanism (1 isolate). These results suggest that there is much greater diversity in cadmium-resistance mechanisms within environmental isolates than was previously thought. These isolates were then used to evaluate the impact of different resistance mechanisms in a co-contaminated soil system. The organic contaminant was 2,4-dichlorophenoxyacetic acid (2,4-D) and the co-contaminating metal was cadmium. Initial experiments were performed in pure culture using one of the cadmium-resistant isolates obtained as a part of this study and a cadmium-sensitive bacterium *Alcaligenes eutrophus* JMP134 that carries the pJP4 plasmid for 2,4-D degradation. While none of the cadmium-resistant isolates could degrade 2,4-D, four of the resistant isolates supported the degradation of 500 ug/ml 2,4-D by the cadmium sensitive 2,4-D degrader, *Alcaligenes eutrophus* JMP134. Degradation occurred in the presence of up to 24 ug/ml cadmium in pure culture and up to 60 ug/g cadmium in amended soil microcosms. In a pilot intermediate-scale field study conducted in five-gallon soil bioreactors, one cadmium resistant isolate, *Pseudomonas* H1, enhanced 2,4-D degradation in the presence of 60 ug/g cadmium.

A second approach being evaluated for stimulation of biodegradation within co-contaminated sites is to alter the bioavailability of the toxic metal through the addition of a biosurfactant. Previous work has shown that a rhamnolipid biosurfactant can complex metals such as cadmium, lead, and zinc. However, it was not known whether this complexation affected metal bioavailability. Therefore, a series of experiments were conducted to investigate the effect of rhamnolipid on biodegradation in a model co-contaminated system containing cadmium and naphthalene. In this system, cadmium was found to inhibit naphthalene degradation at 45 and 89 μM. When rhamnolipid was added at a 10-fold greater concentration than cadmium, complete protection against metal toxicity was observed. Additions of equimolar concentrations of rhamnolipid reduced cadmium toxicity. Rhamnolipid added at a 10-fold smaller concentration than cadmium had no effect on cadmium toxicity. The degrader did not utilize rhamnolipid as a carbon source under the conditions of this study, suggesting that the observed effect of rhamnolipid on cadmium toxicity was protective rather than nutritive. A series of soil experiments was then performed to determine whether rhamnolipid could mitigate the toxic effect of cadmium on an indigenous soil population during the degradation of phenanthrene. A Brazito sandy loam with an indigenous population of phenanthrene degraders was tested. Cadmium (866.2 mg/Kg) was added to the soil to achieve a bioavailable cadmium concentration of 20 mg/L which inhibited phenanthrene (500 mg/kg) degradation for over two weeks. Rhamnolipid was added at two concentrations, 100 and 1,000 mg/kg. Results indicate that rhamnolipid added at 1,000 mg/kg protected against cadmium toxicity while rhamnolipid added at 100 mg/kg had no effect. Further studies indicated that rhamnolipid is degraded by the indigenous soil population. This led us to try a strategy of pulsed addition of rhamnolipid. The results indicate that at a concentration of 20 mg/L bioavailable cadmium, phenanthrene mineralization levels achieved in the absence of cadmium could be attained with an initial rhamnolipid concentration of 1,000 mg/Kg and a second pulse of that concentration at 336 hours. This strategy was tested on a second soil, a Gila loam, with similar results. These results suggest that rhamnolipid may be useful in enhancing

bioremediation of organic contaminants in sites that are co-contaminated with organics and metals and that a pulsed application strategy may be necessary to maximal complete degradation.

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Wetland Plants' Roles in Uptake and Transport of Heavy Metals and Remediation

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Wetlands can be used to mitigate pollution runoff and to bioremediate contaminated soils. However, wetland plants can subsequently act as sources, as well as sinks of contaminants in an ecosystem. We are testing the hypothesis that the saltmarsh cordgrass, *Spartina alterniflora*, while yielding a more productive marsh by putting more detritus into its ecosystem than the reed *Phragmites australis*, also transports more metals from the sediment into the water column and into higher trophic levels than does *Phragmites*. In our first year, we have examined the nutritive value of the detritus, contaminant flux and uptake/trophic transfer of metals, both *in situ* and in plants and detritus brought into the laboratory from natural and restored *Spartina* marshes and from *Phragmites* marshes.

The two species of plants, both raised in a greenhouse and studied in the field (the metal-contaminated Hackensack Meadowlands (HM)), were analyzed for metals in leaf tissue and for excretion of metals. Utilizing natural and restored *Spartina* and natural *Phragmites*, all from HM, the nutritive value of the detritus was evaluated by raising juvenile fiddler crabs and grass shrimp on the three sources of detritus and comparing their growth, mortality and regenerative ability. Parallel studies were performed with the three types of detritus from a relatively clean salt marsh for comparison. The crabs and shrimp were analyzed for metal uptake, focusing on Hg, Cu, Zn, Cr and Pb.

Our hypothesis that *Spartina* would be a more nutritious diet than *Phragmites*, but at the same time a more effective source of transfer of toxicants to fiddler crab and grass shrimp consumers via detritus, was not proven. These animals grew, regenerated and took up metals similarly from the several diets, despite substantial differences in metal levels among the several detritus diets (Table 1). Our hypothesis that *Spartina* would export more metals into the water column via excretion through salt glands was proven. *Spartina* was found to excrete significantly more of all metals through leaf tissue than *Phragmites* under both field and laboratory conditions (Figure 1). *Spartina* was also found to accumulate significantly more Cr and Pb in leaves than *Phragmites*. Therefore, *Spartina* both removes more of certain metals from sediments and excretes more of all analyzed metals into the water column.

Experiments are currently under way to determine which plant species breaks down sooner as detritus and releases its metal burden into the environment. The results of the experiments completed thus far suggest that *Phragmites*, rather than *Spartina*, may be the more appropriate species to plant in a wetland designated for bioremediation.

Table 1. Metal contents (μg/g ± SD) in detritus. HM = Hackensack Meadowlands, AC = Accabonac Harbor, NS = natural *Spartina*, RS = restored *Spartina*, P = *Phragmites*. For each metal, groups with the same superscript are not significantly different from one another.

Site	Plant	Cu	Zn	Cr	Pb	Hg
HM	NS	48.0 ± 0.5^{b}	124 ± 2.6^{a}	43.5 ± 5.9^{b}	4.7 ± 0.4^{c}	0.30 ± 0.37^{b}
	RS	104 ± 8.5^{a}	42.1 ± 2.0^{c}	138 ± 10^{a}	9.8 ± 2.3^{a}	2.22 ± 1.13^{a}
	P	107 ± 9.4^{a}	34.8 ± 9.2^{b}	108 ± 24.5^{a}	9.2 ± 2.4^{ab}	1.63 ± 1.27^{a}
AC	NS	23.4 ± 3.6^{c}	54.8 ± 7.7^{bc}	16.2 ± 4.8^{b}	4.7 ± 0.4^{c}	0.09 ± 0.04^{b}
	RS	$25.1 \pm 1.9b^{c}$	67.4 ± 3.3^{b}	13.0 ± 1.6^{b}	5.3 ± 0.2^{bc}	0.04 ± 0.03^{b}
	P	28.2 ± 14.7^{bc}	62.5 ± 8.1^{b}	17.2 ± 5.2^{b}	5.3 ± 0.7^{bc}	0.04 ± 0.02^{b}
SRM		4.02 ± 0.31	17.7 ± 0.6	0.97 ± 0.02	0.42 ± 0.02	$2.05 + 0.12$
(expected)		3.7 ± 0.4	17.9 ± 0.4	1.0 (n.c.) *	0.87 ± 0.03	$1.99 + 0.10$

* not certified

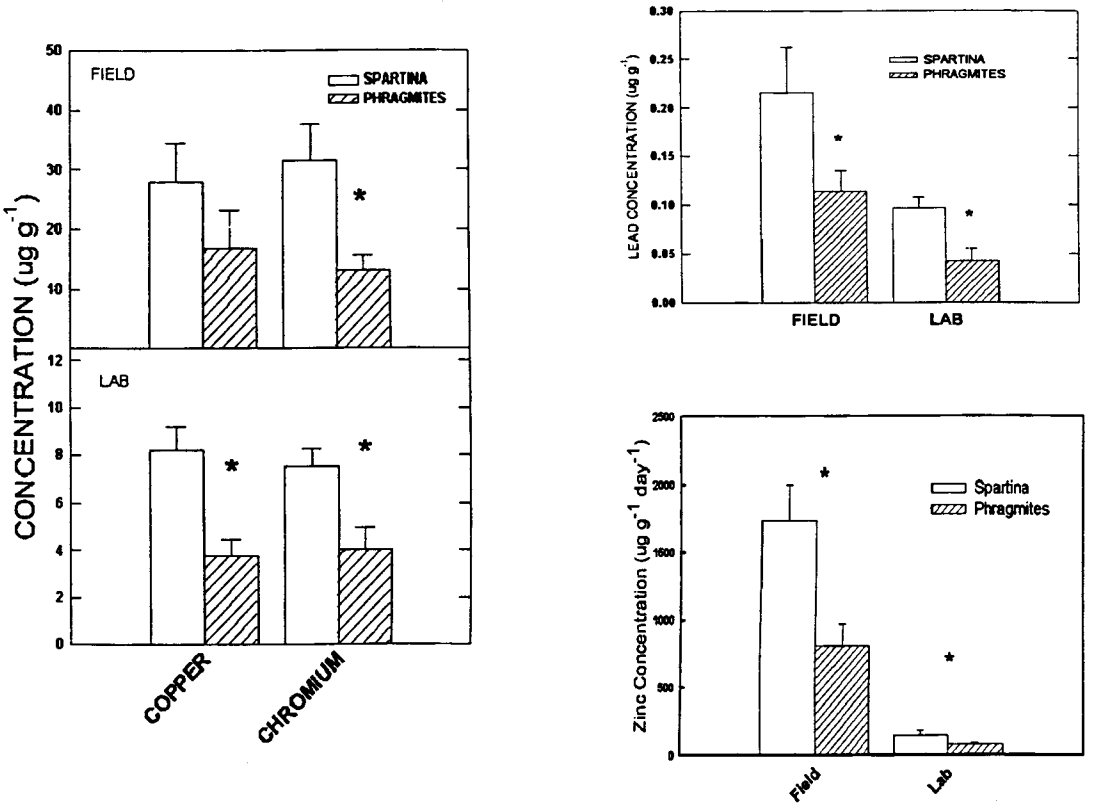

Figure 1. Metal excretion by *Spartina* and *Phragmites* leaves under field and lab conditions over a 48-hour period. Differences between field and lab probably relate to differences in evapo-transpiration rates.

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Interaction Between Substrata Surface Chemistry, Conformation of Contaminant Upon Adsorption and Availability for Bacterial Degradation

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We hypothesize that the bioavailability of organic contaminants adsorbed to subsurface materials is determined by the contaminants' conformation upon adsorption and that this conformation is affected by the chemical properties of the solid support and the contaminant. Concomitant adsorption of mixed organics or metals will also affect the molecule's conformation upon a surface. Research will quantify the surface chemistry effects on adsorption conformation and subsequent availability of adsorbed contaminants for bacterial degradation.

We have experimentally determined the bacterial degradation kinetics of three bacterial species, each individually degrading a specific type of hazardous waste organics. The three organic contaminants considered are a PAH (naphthalene-NPH), an *s*-Triazine (cyanuric acid-CN), and a chlorinated solvent (trichloroethylene; TCE). The respective bacterial species degrading these organics are *Pseudomonas aeruginosa* 19SJ (naphthalene) , *Klebsiella pneumonia* 99 (cyanuric acid), and *Burkholdia cepacia* 17616-pTOM31c (TCE). *Suspended* batch and continuous culture aerobic degradation studies have been carried out on the three contaminant:bacterial species combinations to develop a data base for degradation rates and bacterial growth kinetic constants. Currently underway are similar experiments to evaluate degradation rates of mixtures of organics by combinations of defined mixed cultures of all species.

Bacterial adhesion rates, biofilm formation kinetics, and the rates of extracellular polysaccharide synthesis were determined under flowing fluid conditions (to minimize mass transfer limitations) for each species. Growth limiting substrate in these control experiments was glucose. Silicon 1,1 crystal, pre-treated with an oxygen beam to affect a smooth surface (surface topography characterized by atomic force spectroscopy), served as the substratum. Rates of bacterial cell adhesion and biofilm formation were measured non-invasively using fluorescent microscopy and digital image analysis and verified from destructive samples of the surface, followed by conventional cell enumeration.

We have employed self assembled monolayers (SAMs) of akyl-silanes as molecular "tethers", to bind desired functional groups to the substrata; thus manipulating the spatial chemistry of the substratum while maintaining its topography constant (Figure 1). Oxygen beam pre-treated silicon 1,1 crystal substrata (topographic distortions $< \pm$ 10 nm), were coated with a self-assembly monolayer of vinyl terminated alkyl silane . The alkyl silane binds covalently to the surface at the oxygen terminal end, creating a structured layer of molecules that presents vinyl end groups to the environment. Using a spatial template, a pattern of desired chemical functional groups can be fabricated across a substratum (Figure 2). In the first series of fabrications, the resultant SAMs were left alone, affecting a layer of vinyl end groups exposed to the environment. Time-of-Flight Secondary Ion Mass Spectroscopy (**TOF SIMS**), was used to confirm both the spatial location and chemical composition of each substratum pre-treatment layer (Figure 3). AFM was used to quantify the apparent "topography" of the chemically modified surfaces.

Currently, we are refining these techniques to sulfonate the vinyl groups, thus providing a reactive group for the deposition of goethite (FeOOH) from a supersaturated ferric salts solution. The resulting surface will be a thin, nonporous, iron oxide film, providing a surface that is an analog to a soil mineral. In Year three, a series of these SAM-coated substrata will be exposed to single organic contaminants (NPH, CN or TCE) or various combinations of the three organics and the spatial content and conformation of organics determined using a combination of AFM (Figure 4 A&B), XPS, and TOF-SIMS. Each contaminant-coated substratum above will be exposed to suspended cultures of the appropriate degrading bacterial species. Rates of bacterial cell adhesion and biofilm formation will be measured non-invasively using fluorescent microscopy and digital image analysis. Degradation of adsorbed contaminants will be qualitatively monitored using fluorescent reporter genes under the control of the contaminant degradation gene promoter. Rates of contaminant degradation and adherent cell growth will also be determined from invasive sampling and destructive analysis of the substratum surface.

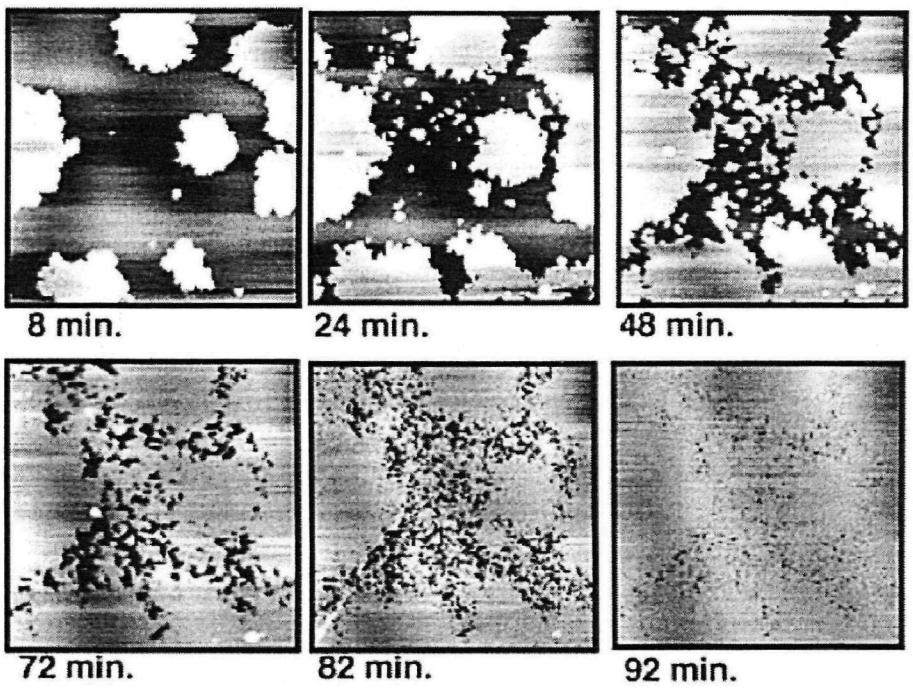

Figure 1. Evolution of a alkyl silane self-assembling monolayer as seen via atomic force microscopy.

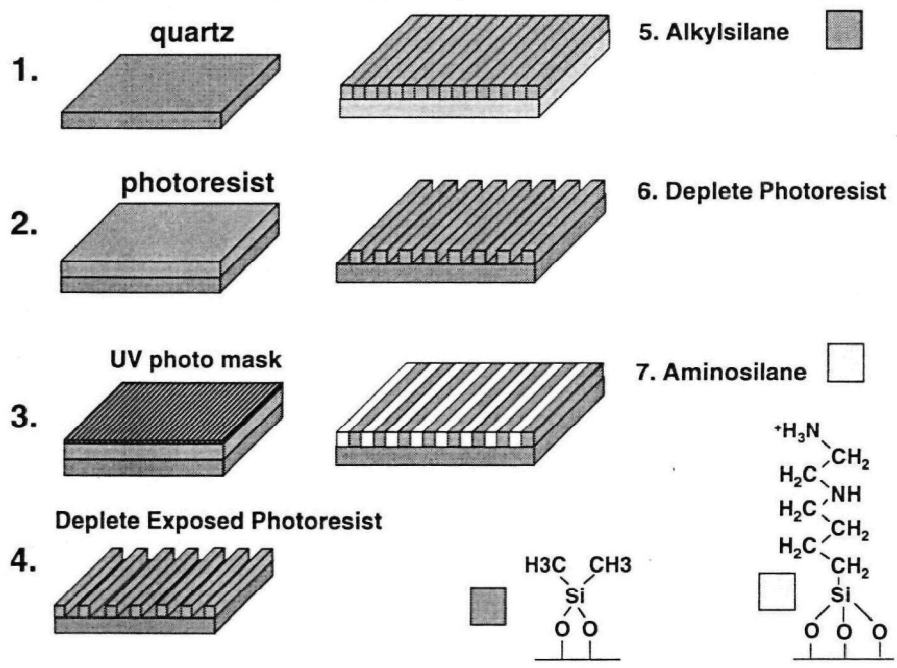

Figure 2. Schematic of template process to affect surfaces of known spatial chemistry.

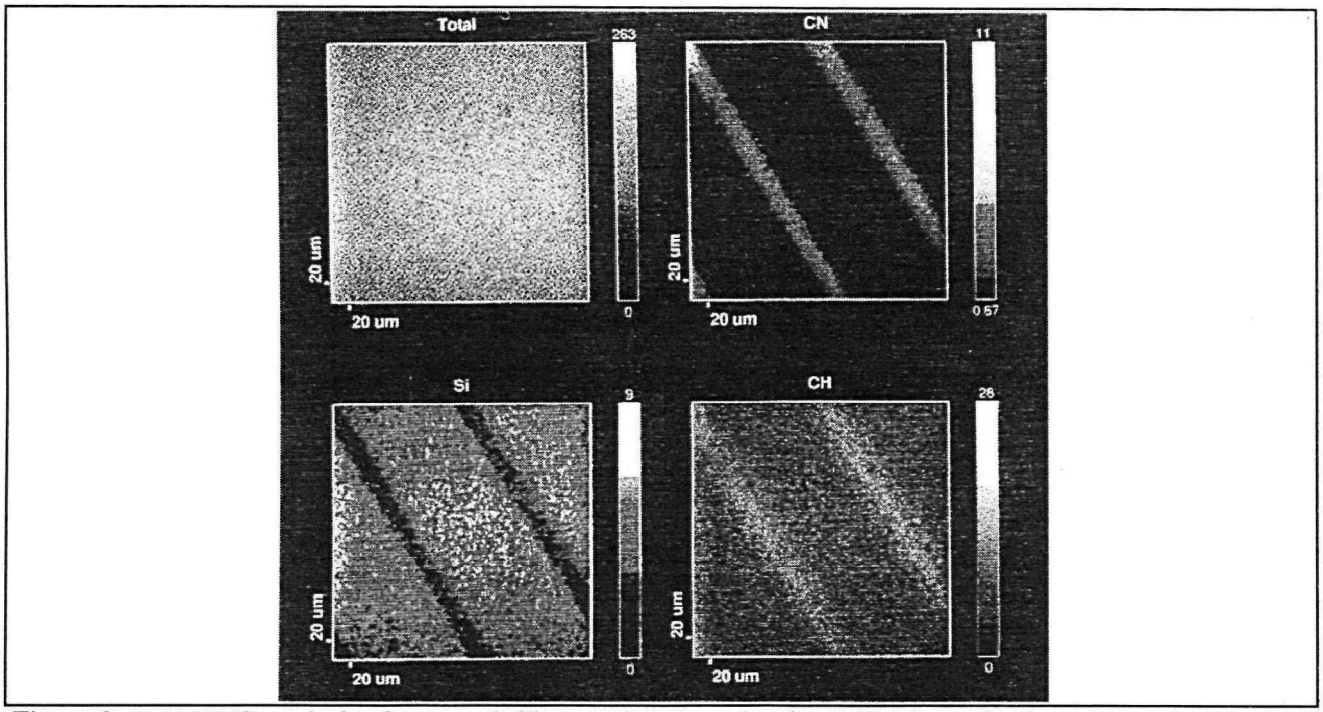

Figure 3. ToF-SIMS analysis of patterned silicone substratum chemistry. Total negative ion map (top-L); location of the CN^- fragment related to the amino-silane (top-R); location of Si^- related to methyl-silane (lower-L); location of C_2H^- related to amino-silane.

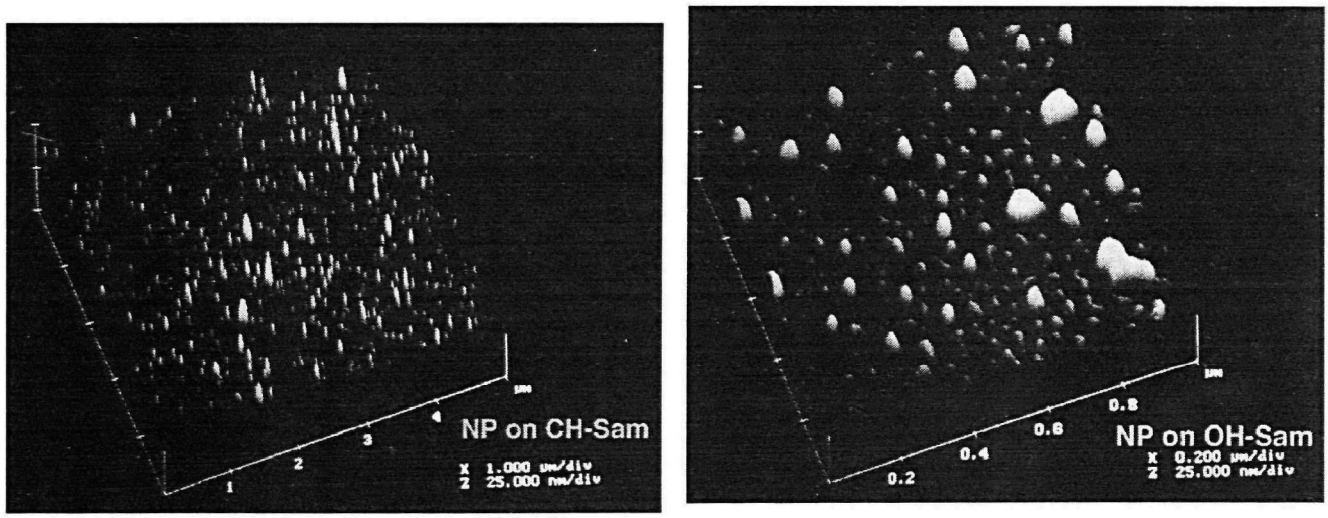

Figure 4. **A**. AFM images of naphthalene adsorbed to silicone 1,1 crystal with a methyl-silane SAM. **B.** Naphthalene on a hydroxyl-silane SAM (on the right).

Bioluminescent Sensors for Measuring Pollutant Bioavailability and Validity of Environmentally Acceptable Endpoints in Bioremediation

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Research is being conducted to construct and characterize a panel of whole-cell based bioluminescent sensors for determination of biological availability of the major classes of pollutants. The biosensors are useful to determine changes in bioavailability of the organic pollutants. The biosensors are constructed by cloning specific regulatory regions, derived from bacterial DNA conferring the ability to catabolize xenobiotic compounds, upstream of a promoterless *luxCDABE* operon (encoding for bacterial luciferase) in plasmid pUCD615. Cells exposed to certain pollutant classes respond by emittting light. Light emission is readily quantitated by scintillation counting. A *camR-o/p:lux CDABE* plasmid was constructed and expressed in *Escherichia coli* [strain HMS174 (designated OS30)]. This strain responded to a variety of monocyclic, bicyclic and tricyclic alkanes. It was a surprise, but also very useful, that the strain emits light in the presence of highly chlorinated aliphatic compounds such as pentachloroethane, hexachloroethane and 1,1,1,2-tetrachloropropane. Another strain constructed, *E. coli* (*ipbR o/p::luxCDABE*), improves on a previously constructed strain strain OS25. Although OS25 responds well to aromatic hydrocarbons such as BTEX and to chlorinated solvents such as TCE and perchloroethylene (PCE), light emission from OS25 gradually became constitutive due to an unstable insertion sequence element present in the cloned region of the *ipb* gene region. The destabilizing insertion sequence element has now been removed. We are investigating the potential to detect pesticides by biosensors. Atrazine is being used as a model herbicide, building on our extensive knowledge of microbial atrazine degradation. The approach is to use cells which constitutively express *lux*. Upon exposure to chemicals that inhibit photosystem II electron transfer reactions, bioluminescence will be inhibited. We have supplied our colleagues at Minnesota with a *mer-lux* strain for sensitively sensing mercury and they have developed immobilized containing *E. coli* cells in patches within a 30 mm latex polymer film (1). The copolymer patches could be stored at –20°C for at least 3 months with minimal loss of activity. This offers a practical method for mercury detection in the field with only inexpensive equipment brought on site.

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Influence of Soil Organic Matter Composition on Desorption and Biodegradation of Aromatic Pollutants

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The availability of sorbed organic chemicals to organisms appears to be controlled in many cases by desorption rates at the grain scale. Adequate risk assessment or bioremediation system design will therefore depend on a thorough understanding of the desorption process, particularly the role of sorbent structure and contact time in determining desorption rates. Previous efforts to understand how soil organic matter (SOM) composition affects both desorption and biodegradation rates have relied upon elemental analysis (carbon, hydrogen, nitrogen, oxygen) of SOM for characterizing the sorbing medium. The goal of this research is to elucidate linkages between the rate and extent of desorption and biodegradation with SOM composition determined by pyrolysis GC-MS, diffuse reflectance Fourier transform infrared (DRIFT) microscopy, and solution-phase ^{13}C-NMR. The study design includes determining (i) biodegradation behavior and active microbial communities, (ii) SOM composition, (iii) sorption-desorption characteristics, and (iv) supercritical CO_2 extractability of aromatic organic pollutants in soils with different types and amounts of organic matter.

Pyrolysis-gas chromatography/mass spectrometry was combined with standard degradative chemical methods and DRIFT to characterize nine soils varying in organic matter content, litter input, and degree of humification. Thirty-five pyrolysis fragments were quantified and grouped into pools based on the structures they most likely originated from in soil. Structural hypotheses based on these pyrolysis pools were consistent with findings by degradative chemical methods and DRIFT.

Linear correlations were determined between structural properties, including pyrolysis pools, degradative chemical parameters, and ratios of DRIFT peak heights, and functional properties, including Freundlich parameters from phenanthrene aqueous and supercritical CO_2 sorption and desorption experiments, thermal desorption experiments, and phenanthrene microbial mineralization studies. Results suggest that the organic carbon-normalized Freundlich coefficient is negatively correlated with polar features in SOM and positively correlated with hydrophobic features. Conversely, measurements of non-ideal sorption behavior, including sorption-desorption hysteresis and slow uptake, as well as decreased microbial mineralization appear to be correlated with hydrophobic features in soils. This supports the hypothesis that hydrophobic regions in SOM, particularly aromatic structures, sequester hydrophobic contaminants contributing to non-ideal behavior. Correlations with isotherm nonlinearity were less definitive.

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Genetic Expression During Biofilm Growth And Development

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Despite the acknowledged importance of biofilms, little is known about gene expression once bacterial cells are attached to a surface. This is partly because there are relatively few cells present, and partly because true biofilms are mixed cultures, making it difficult to study any single species.

We have used bioreporters of specific contaminants to determine when genes are active in biofilms. These bioreporter strains produce bioluminescence when active, and sensitive photodetecting equipment is able to visualize the response. In addition, we have used the confocal microscope to determine the three-dimensional structure and development of the biofilms under investigation. The convergence of these techniques has revealed unexpected patterns of gene expression for genes involved in metal reduction and for biodegradation of toluene. These results have been conformed using reverse transcriptase-polymerase chain reaction and with in situ hybridization to specific gene probes, demonstrating that the system does not generate artefacts.

It was found that biofilms aged 48 hours showed the most gene activity, although the presence of casamino acids severely reduced gene expression. This was unexpected, since there was no immediate reason for this level of control and the effect had not been observed in bulk phase cultures. However, this phenomenon points up the differences in gene expression between attached and free bacteria. Experiments with flowcells demonstrated that gene expression could be greatly influenced by medium components that were not classical inducers of the system.

Subsequent experiments showed that precursors of homoserine lactones were able to affect gene expression, suggesting that a quorum sensing effect is important for gene expression in attached microcolonies. This was directly observed using the confocal microscope, and showed that the cells of a microcolony (less than 100 cells) were influenced to different degrees by a change in medium composition. This suggests that some cells are sentinels of environmental changes, and influence the gene expression of other cells in the colony. Whether this represents a true differentiation of cells is open to debate.

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