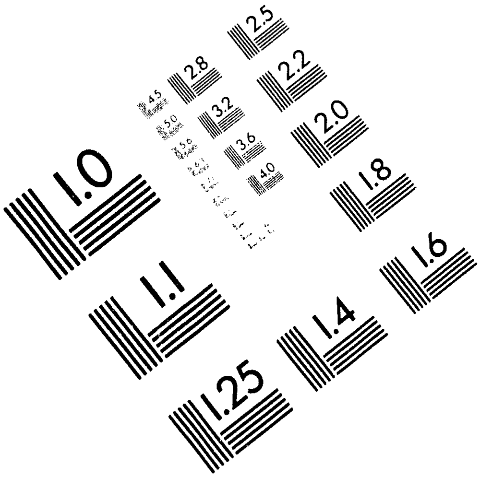

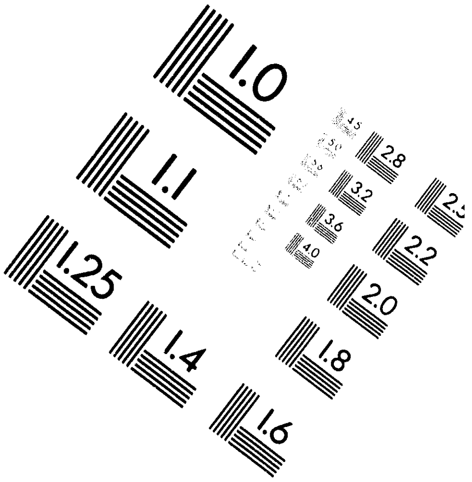

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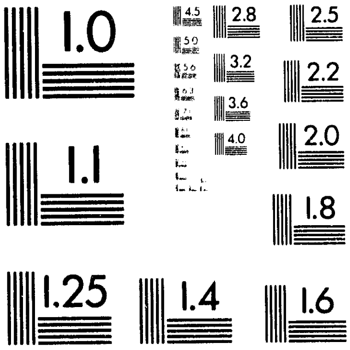

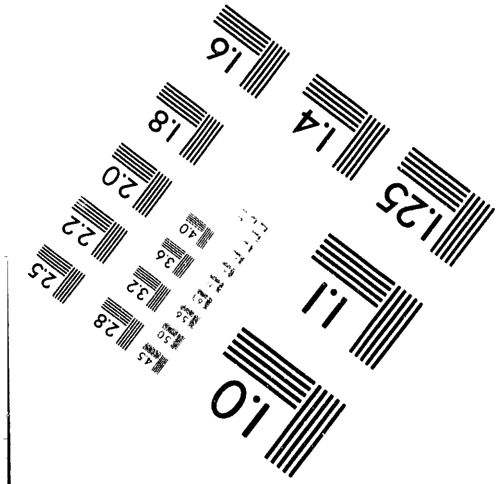

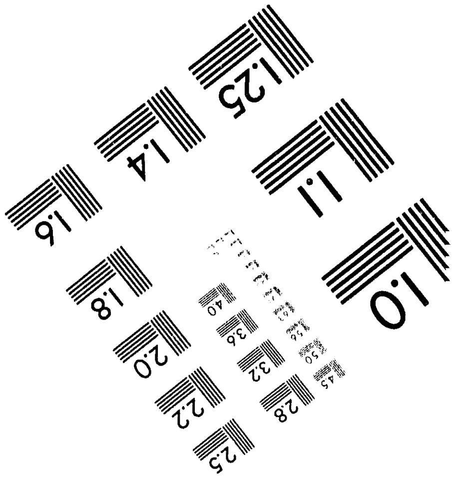

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PNL-9000 Pt. 2
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Pacific Northwest Laboratory Annual Report for 1993 to the DOE Office of Energy Research

Part 2: Environmental Sciences

Staff Members
of Pacific Northwest Laboratory

April 1994

Prepared for
the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
Richland, Washington 99352

Preface

This 1993 Annual Report from Pacific Northwest Laboratory (PNL) to the U.S. Department of Energy (DOE) describes research in environment and health conducted during fiscal year (FY) 1993. This year, the report consists of four parts, each in a separate volume.

The four parts of the report are oriented to particular segments of the PNL program, describing research performed for the DOE Office of Health and Environmental Research (OHER) within the Office of Energy Research. In some cases, the volumes also report on research funded by other DOE components or by other governmental entities under interagency agreements. Each part consists of project reports authored by scientists from several PNL research departments, reflecting the multidisciplinary nature of the research effort.

The parts of the 1993 Annual Report are as follow:

Part 1: Biomedical Sciences	J. F. Park, Program Manager A. L. Brooks, Report Coordinator C. C. Lumetta, Editor
Part 2: Environmental Sciences	R. E. Wildung, Program Manager L. K. Grove, Editor
Part 3: Atmospheric Sciences	W. R. Barchet, Program Manager B. V. Johnston, Editor
Part 4: Physical Sciences	L. A. Braby, Program Manager S. L. Downs, Editor

Activities of the scientists whose work is described in this annual report are even broader in scope than the articles indicate. Throughout the year, PNL staff have responded to numerous requests from DOE for planning, for service on various task groups, and for special assistance.

Credit for this annual report goes to the many scientists who performed the research and wrote the individual project reports, to the program managers who directed the research and coordinated the technical progress reports, to the editors who edited the individual project reports and assembled the four parts, and to Ray Baalman, editor in chief, who directed the total effort.

T. S. Tenforde
Manager, Health and Environmental Research Program

Previous annual reports in this series:

1951	HW-25021, HW-25709
1952	HW-27814, HW-28636
1953	HW-30437, HW-30464
1954	HW-30306, HW-33128, HW-35905, HW-35917
1955	HW-39558, HW-41315, HW-41500
1956	HW-47500
1957	HW-53500
1958	HW-59500
1959	HW-63824, HW-65500
1960	HW-69500, HW-70050
1961	HW-72500, HW-73337
1962	HW-76000, HW-77609
1963	HW-80500, HW-81746
1964	BNWL-122
1965	BNWL-280, BNWL-235, Vol. 1-4; BNWL-361
1966	BNWL-480, Vol. 1; BNWL-481, Vol. 2, Pt. 1-4
1967	BNWL-714, Vol. 1; BNWL-715, Vol. 2, Pt. 1-4
1968	BNWL-1050, Vol. 1, Pt. 1-2; BNWL-1051, Vol. 2, Pt. 1-3
1969	BNWL-1306, Vol. 1, Pt. 1-2; BNWL-1307, Vol. 2, Pt. 1-3
1970	BNWL-1550, Vol. 1, Pt. 1-2; BNWL-1551, Vol. 2, Pt. 1-2
1971	BNWL-1650, Vol. 1, Pt. 1-2; BNWL-1651, Vol. 2, Pt. 1-2
1972	BNWL-1750, Vol. 1, Pt. 1-2; BNWL-1751, Vol. 2, Pt. 1-2
1973	BNWL-1850, Pt. 1-4
1974	BNWL-1950, Pt. 1-4
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1980	PNL-3700, Pt. 1-5
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1982	PNL-4600, Pt. 1-5
1983	PNL-5000, Pt. 1-5
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1985	PNL-5750, Pt. 1-5
1986	PNL-6100, Pt. 1-5
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1988	PNL-6800, Pt. 1-5
1989	PNL-7200, Pt. 1-5
1990	PNL-7600, Pt. 1-5
1991	PNL-8000, Pt. 1-5
1992	PNL-8500, Pt. 1-4

Foreword

This report summarizes progress in environmental sciences research conducted in FY 1993 by PNL for the DOE's Office of Health and Environmental Research. The research is directed toward developing a fundamental understanding of subsurface and terrestrial systems as a basis for both managing these critical resources and addressing such formidable environmental problems as environmental restoration and global change. These studies are making outstanding contributions in a number of scientific disciplines and in the interdisciplinary research so critical to obtaining a system-level perspective. The Technology Transfer section of this report describes examples in which fundamental research is laying the groundwork for the technology needed to resolve important environmental problems. The Interactions with Educational Institutions section of the report illustrates the results of a long-term, proactive program to make PNL facilities available for university and pre-university education and to involve educational institutions in research programs. Other sections of the report summarize Laboratory-Directed Research and Development projects intended to nurture new cutting-edge scientific capabilities, identify PNL-university collaborations, and summarize publications and presentations, which provide an important measure of the success of these efforts.

Each project in the PNL research program is also a component of an integrated approach conducted at the laboratory, intermediate, and field scales, and designed to examine multiple phenomena at increasing levels of complexity. This approach is being implemented with a strong emphasis on multidisciplinary teaming. The complex areas under investigation include the effect of fundamental geochemical and physical phenomena on the diversity and function of microorganisms in deep subsurface environments, new ways to address subsurface heterogeneity, and new ways to determine the key biochemical and physiological pathways (and corresponding DNA markers) that control nutrient, water, and energy dynamics in arid ecosystems and, consequently, the response of these systems to disturbance and climatic change. University liaisons continue to expand, so that more than 30 universities nationwide are now included. These relationships both strengthen the research and help train the scientists who will address long-term environmental problems in the future.

The Environmental Sciences Research Center has enabled PNL to enlarge on fundamental knowledge of subsurface science to develop new concepts and tools for understanding natural systems, and also to reach out to the universities and transfer this knowledge for use by government and industry. Contributions have extended well beyond environmental restoration, with new efforts to make industrial use of subsurface microorganisms and to take advantage of deep microbiological exploration to help understand how petroleum hydrocarbons form in deep basins. New PNL investments have been made to develop advanced concepts for addressing chemical desorption kinetics, redesigning enzymes, scaling subsurface properties to account for heterogeneity in natural systems, and using tools from molecular biology to help understand fundamental ecological processes. This report reflects the results of past investments and the growth of PNL's technical strengths, strong multidisciplinary interactions, and rapidly developing facilities for simulating and quantifying environmental phenomena.

Raymond E. Wildung
Program Manager
Environmental Sciences

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Terrestrial Science

Subsurface
Science

Subsurface Science

The contamination of the subsurface environment by mixed radioactive and hazardous waste is a major concern for DOE lands and for many other waste sites across the nation. To aid in the assessment and management of environmental and health risks and to provide a fundamental basis for subsurface research, the scientific community is now being asked to 1) predict the behavior of contaminants in subsurface environments and 2) develop more effective techniques for degrading, mobilizing, or immobilizing contaminants *in situ*. It is especially difficult to predict contaminant behavior and to design and evaluate the results at contaminant remediation on DOE lands, where the codisposal of radionuclides, metal ions, salts, organic solutes, and organic liquids into the ground has created unique environmental situations. The difficulties are exacerbated by the superimposition of these materials on highly complex subsurface environments representing a wide range of natural heterogeneities nationwide.

The basic scientific studies described in this report address the fundamental chemical, microbiological, and hydrologic processes that control the behavior of contaminants in subsurface environments. The focus is on DOE-specific contaminants, but the broader scientific benefits being realized include better predictions of the subsurface behavior of organic/inorganic mixtures and organic liquids generally, under a variety of geochemical and hydrogeologic conditions. Subsurface chemistry studies are investigating multisolute sorption, the effects of cosolvents and complexation, and the relationship between surface sorption and microbial degradation on subsoil and aquifer materials. Microbiological investigations concern the nature of microorganisms in different deep hydrogeologic environments; the physical, geochemical, and hydrologic factors that govern the distribution and function of these microorganisms; and the ability of these microorganisms to alter radionuclide chemistry and degrade complexing ligands. Hydrologic research is defining the flow physics of multiphase mixtures of organic liquids and water in saturated and unsaturated porous media and systematically examining how subsurface heterogeneities affect transport.

Multidisciplinary studies of chemical transport are being performed both in laboratory columns and in a unique Subsurface Environmental Research Facility, where the combined effects of microbiology, chemistry, and hydrology can be assessed at a scale that is intermediate between those of the laboratory and the field. In field research, new research is examining microbial heterogeneity at scales necessary to relate microbial processes to physical and chemical conditions in the subsurface environment. Overall, this research is playing an important role in improving contaminant transport models and in providing the fundamental basis needed for developing effective strategies to reduce contaminant migration rates and remove contaminants from groundwater.

Subsurface Chemistry of Organic-Radionuclide Mixtures

John M. Zachara

Contributors

James K. Fredrickson and Yuri A. Gorby

Because the anionic character of organic complexes of radionuclides reduces sorptive interactions with subsurface mineral phases that generally carry net negative charge, these complexes have been found as the mobile form of radionuclides in subsurface environments. There is, however, little direct experimental evidence for sorptive interactions between complexed radionuclides and subsurface materials that could be used to predict migration velocities in subsurface environments. Uncertainties exist with respect to 1) the identity of the responsible surface complexation reactions, 2) the surface chemical properties of the natural mineral sorbents that control reactive site concentrations and their binding energies, and 3) modeling techniques for describing heterogeneous surfaces. Furthermore, such organic complexes are also subject to other reaction vectors involving dissolution and microbiology that, when combined with interfacial reactivity, produce highly complex time- and distance-variant retardation behavior in subsurface systems.

This project is investigating linked geochemical and microbiological phenomena that control the stability, aqueous concentrations, and migration velocities of organically complexed radionuclides in subsurface environments. The research focuses on surface complexation reactions that regulate subsurface concentrations of complexed radionuclides via adsorption and on the role of dissolution of iron and aluminum oxide in destabilizing complexed radionuclides via ligand competition. The microbiological phenomena being investigated either influence the surface chemistry of reactive subsurface mineral phases or strongly impact the aqueous speciation of organic-radionuclide complexes via solid-phase dissolution.

FY 1993 Research Highlights

In FY 1993, research focused on two multi-investigator series of experiments that form the basis for DOE's Subsurface Science Program's Co-Contaminant Chemistry research: 1) interfacial chemistry experiments with uranium and 2) transport experiments with cobalt-ethylenediaminetetraacetic acid (Co-EDTA). The goals, objectives, and overall scope of these experiments have been described in the Co-Contaminant Chemistry Subprogram Plan, which was written in FY 1993 as part of this project. Additional collaborative research was initiated with Deep Microbiology investigators in the Subsurface Science Program to investigate the influence of iron-reducing bacteria on the content and surface chemistry of reactive iron oxides in subsurface sediments.

Uranium Interfacial Experiments

The objective of this research was to identify the surface-complexation reactions that control the adsorption behavior of uranium(VI) and its hydrolyzed species on the surfaces of smectites. These experiments are a precursor to more complex ones to investigate the sorption behavior of U-citrate complexes in FY 1994. The complex aqueous speciation of uranium mandates that the sorption chemistry of ionic uranium(VI) be well understood before the effects of organic ligands are investigated. It was hypothesized that smectite crystallite morphology would impact uranium(VI) adsorption behavior by controlling the concentration of strongly reactive edge sites.

Sorption experiments with UO_2^{2+} were performed at PNL using specimen and natural smectites whose surface properties were known in detail. Experimental variables included pH, ionic strength, electrolyte cation valence, and carbon dioxide partial pressure, which were varied to establish key factors controlling the binding of uranium to smectite. A surface chemical/surface-complexation model containing three distinct populations of surface sites was developed to describe the experimental data and provide predictions of surface speciation as a function of experimental variables.

Suspensions of uranium(VI)-containing smectite were carefully synthesized at PNL under conditions of pH and ionic strength that were expected to yield different distributions of uranium(VI) species on the surface of smectite. These suspensions were relayed to other investigators at Los Alamos National Laboratory, who performed spectroscopic analyses on the clay suspensions to ascertain the chemical nature (structure and bonding) and concentrations of different uranium(VI) surface species.

The major findings of this research were as follow:

- The sorption behavior of uranium was complex, but could be described by accounting for the effects of aqueous speciation, ion exchange to fixed-charge sites, and surface complexation to edge sites on the clay.
- The subsurface smectites exhibited greater sorptivity for uranium(VI) than did specimen smectites because of their smaller size and greater concentration of edge sites.
- A sophisticated new technique called time-gating was applied by researchers at Los Alamos National Laboratory to resolve different populations of uranium(VI) species on the smectite surface using fluorescence spectroscopy.
- Spectroscopic measurements generally confirmed the hypothesized surface speciation of uranium(VI) based on macroscopic measurements, but also suggested the presence of unexpected minor species as well.

The experiment successfully resolved the dominant adsorption mechanisms of ionic uranium(VI) on smectite, paving the way for interdisciplinary research on the coupled effects of adsorption and microbiologic degradation on the subsurface behavior of uranium-citrate complexes, which will be initiated in FY 1994.

Co-EDTA Transport Experiments

The long-term goal of this series of experiments is to evaluate field-scale issues involving mineralogic and geochemical heterogeneity that influence the reactive transport of Co-EDTA in subsurface environments. Planned intermediate-scale experiments containing 1) random inclusions of reactive iron and manganese oxides and 2) undisturbed blocks of subsurface sediments with iron oxide banding and inclusions will test a series of hypotheses focused on important *in situ* mineralogic features of subsurface materials.

In this project, research was performed on two experimental subjects relevant to the collaborative transport experiment: 1) Co-EDTA geochemical interaction with laboratory-synthesized iron oxide-coated sands proposed for use in the first intermediate-scale experiment and 2) Co-EDTA geochemical interaction with a series of natural iron oxide-coated sands, with the iron oxide phases varying in crystallinity, surface area, morphology, and genesis mechanism. A central hypothesis of this research was that the Co(II)-EDTA complex would be adsorbed by the iron oxide fraction but would dissociate on its surface and dissolve the adsorbing phase, liberating both cobalt(II) and iron(III)-EDTA [Fe(III)-EDTA]. We suspect that this heterogeneous reaction sequence will impart complex kinetic behavior to Co-EDTA retardation in subsurface environments.

Synthetic iron oxide-coated sand. An iron oxide-coated sand with 0.6% iron was synthesized by contacting amorphous iron oxide gel with a micaceous sand at pH 7 and air-drying the aggregated material. This procedure yields a stable ferrihydrite coating on the sand grains that is representative of many subsurface materials. The first intermediate-scale experiment will use inclusions of this material as well as the same sand coated with manganese oxide (pyrolusite). Batch and column experiments at PNL investigated the thermodynamics and kinetics of Co(II)-EDTA interaction with the iron oxide-coated sand as a basis for refining the experimental design and interpreting the results of the intermediate-scale experiment. These studies targeted 1) the adsorption of Co(II,III)-EDTA to the coated sand and 2) the rate of iron oxide dissolution promoted by dissociation of the adsorbed Co(II)-EDTA complex. Oak Ridge

National Laboratory has developed parallel basic information on a Co-EDTA/manganese oxide-coated sand system, with emphasis on the rate of Co(II)-EDTA oxidation to Co(III)-EDTA.

The PNL research produced the following significant findings:

- Co(II)-EDTA adsorbs to the iron oxide-coated sand as an anion, with the greatest sorption observed at lower pH. The adsorption behavior can be readily modeled using surface-complexation models and the assumption that an outer-sphere surface complex forms.
- The adsorbed Co(II)-EDTA dissociates with time and dissolves the sorbing iron oxide phase. The dissolution rate increases with decreasing pH, and the rate-controlling step is the surface detachment of an Fe(III)-EDTA complex. The dissociation/dissolution reaction is rather slow but is important at the time scale of groundwater flow.
- Readsorption of Fe(III)-EDTA complexes causes the dissolution kinetics to be complex and nonlinear.

Natural iron oxide-coated sands. A series of natural iron oxide-coated sands from the Subsurface Science Program's subsurface materials collection was used in batch interaction experiments with Co(II)-EDTA over ranges in pH and sorbate concentration. The purpose of these experiments was to establish the generality of the findings with the synthetic iron oxide-coated sand and to obtain additional background information for a proposed intermediate-scale transport experiment with undisturbed subsurface sediment blocks. The natural iron oxide-coated sands contained iron oxides in various forms (i.e., ferrihydrite, goethite, hematite) and morphologic states (i.e., discrete phase, patch-like precipitates, grain coatings, and interparticle cements). The natural materials were subject to microscopic and detailed surface chemical characterization to facilitate interpretation and surface chemical modeling of results. We had hypothesized that surface-coating iron oxides would be more reactive then other morphologic forms because of their high effective surface area and microporosity.

This research made the following significant findings:

- The adsorption/dissolution behavior of Co(II)-EDTA complexes showed marked similarity on 12 natural materials. Like the synthetic material, the complex adsorbed as an anion, with adsorption increasing with decreasing pH. Adsorption of Co(II)-EDTA was significant, with distribution ratios (K_d) ranging from 2 to over 50, depending on pH. The sorptivity of Co(III)-EDTA, in contrast, was low and barely measurable.
- The dissociation/dissolution rate at near-neutral pH was slow and comparable to that of the synthetically coated material. At lower pH, however, the rate was orders of magnitude more rapid, as a result of the release of aluminum as an exchangeable ion from clays and by dissolution of poorly ordered aluminum oxide grain coatings.
- Oxidation of Co(II)-EDTA to Co(III)-EDTA was found to occur almost instantaneously in those materials containing managanese oxide. The Co(III)-EDTA complex is highly stable, does not dissociate to dissolve iron oxide, and is poorly sorbed and highly mobile ($K_d \approx 1$).

Collectively, the experiments with the synthetic and natural iron oxide-coated sands have generated a sufficient understanding of the identity, thermodynamics, and kinetics of controlling adsorption, dissociation, and dissolution reactions to allow initiation of intermediate-scale transport experiments in FY 1994.

Additional Collaborative Research

Under this project, collaborative research was initiated with researchers in the Subsurface Science Program's Deep Microbiology subprogram to evaluate whether iron-reducing bacteria, which are often found in anoxic groundwater zones, can dissolve crystalline iron oxides and the extent to which their activity changes the surface properties and reactivity of natural iron oxide surfaces. Although past research indicated that only amorphous iron oxides can be used as electron acceptors by dissimilatory iron-reducing bacteria, we hypothesized that crystalline iron oxides in subsurface sediments would also be available to microorganisms because of their poorly ordered nature and large surface area.

Suspensions of synthetic goethite, hematite, and ferrihydrite and eight subsurface materials containing iron oxides of varying forms and morphology were inoculated with several strains of iron-reducing bacteria, with lactate as a carbon source. The suspensions were incubated for more than two months; the extent and rate of iron reduction were tracked by the evolution of Fe(II)(aq). One specific strain, BrY, was found to grow on all forms of iron oxide and reduced the total iron(III) in the suspensions of discrete mineral solids and the subsurface materials by 10-35%. Various experiments were performed to investigate the mineralogic and chemical factors controlling the bacterial reduction rate in the subsurface materials.

Preliminary results of this research are as follow:

- Approximately 20% of the total mass of crystalline iron oxides in subsurface sediments was bacterially reducible, including natural goethite and hematite.
- Certain microbial strains can grow on iron oxides in the presence of a suitable carbon source, indicating that their presence and function are likely in subsurface environments containing such phases.
- The bacterial reduction of iron oxide phases in subsurface sediments markedly changes material surface properties and the aqueous speciation of associated waters. Major impacts on contaminant behavior can therefore be expected.

Research in FY 1994 will continue to investigate this topic, with emphasis on the impacts of bacterial iron oxide reduction on the adsorption and speciation of organic ligand-radionuclide complexes [beginning with Co(II)EDTA].

Chemistry/Microbiology Controlling Chelated Radionuclide Transport

Donald C. Girvin and Harvey Bolton, Jr.

Synthetic chelating agents, such as ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA), form strong water-soluble complexes with a wide range of radionuclide and metal ions and have therefore been used to decontaminate nuclear reactors and for various nuclear waste processing procedures. The co-disposal of these synthetic chelating agents with radionuclides has resulted in an increase in radionuclide transport in the subsurface environment. Both chelated radionuclide adsorption and chelate degradation can impact radionuclide transport. However, the current limited scientific understanding of chelated radionuclide adsorption and biodegradation and of the physicochemical and microbiological parameters that influence these processes prevents assessment of their role in controlling chelated radionuclide transport at DOE mixed waste sites. This lack of knowledge precludes manipulating the geochemical and microbiological conditions in the subsurface environment to limit radionuclide migration when synthetic chelating agents are present.

The objective of this project is to develop an understanding of 1) the mutual interactions between chelated radionuclide adsorption and chelate biodegradation and 2) the effect of these chemical and microbial processes on radionuclide mobility and transport. Background information on adsorption of chelated radionuclides and the physicochemical conditions influencing microbial degradation and transformation of synthetic chelates is being developed within this project and in collaboration with other investigators in the Co-Contaminant Chemistry Subprogram of DOE's Subsurface Science Program. The modeling of chelate aqueous speciation, adsorption, and biodegradation is an integral part of the design and interpretation of experiments in this project.

FY 1993 Research Highlights

Sorption-Degradation Experiments with NTA, Gibbsite, and an NTA-Degrading Bacterium, ATCC Strain 29600

Mineralization of NTA was significantly slower in systems where the NTA was initially all adsorbed to gibbsite than in systems where the NTA was initially all in the solution phase. This difference was observed at pH 6 and 8 with NTA only and at pH 7 with NTA and equal-molar cobalt. A three-step first-order model describing NTA desorption, readsorption, and subsequent mineralization has been successfully used to describe observations at pH 8 in the NTA-gibbsite-strain 29600 system. The model is constrained by independent determination of the NTA mineralization rate, the NTA desorption rate, and the NTA equilibrium adsorption coefficient. Experiments and modeling suggest that adsorbed NTA is not degraded by strain 29600 and that desorption is the rate-limiting step, regardless of whether equal-molar cobalt is present.

In separate experiments, the location of the cobalt after degradation of the NTA in equal-molar Co-NTA systems without gibbsite was examined. It was found that after NTA degradation, the cobalt was present in solution rather than associated with the cell of the NTA-degrader. These findings imply that adsorption of the NTA chelate will limit its microbial degradation, and that degradation of a radionuclide-NTA chelate will release the radionuclide to solution rather than incorporating it into the microbial biomass. These observations apply for the NTA and strain 29600 system only, and the behavior of other chelates and organisms may differ. To address this issue, we will examine the sorption-degradation behavior of EDTA and an EDTA-degrading organism in FY 1994.

NTA Adsorption by Gibbsite

The investigation of NTA, cobalt, and Co-NTA adsorption by gibbsite as a function of pH, adsorbate, adsorbent concentration, and ionic strength was completed in FY 1993. The major findings are as follows: The adsorption of cobalt (Co-only) and NTA (NTA-only) exhibits cation-like and anion-like adsorption edges, respectively, as a function of pH. When equal-molar Co-NTA is added to gibbsite suspensions, the cobalt and NTA adsorb relatively independently of one another. However, some intact Co-NTA chelate is adsorbed, causing the cobalt and NTA edges to deviate from those observed when only cobalt or only NTA is present. The adsorption behavior of the equal-molar Co-NTA has been described in terms of competitive equilibria between the dissolved aluminum, cobalt, and NTA and the surface hydroxyl groups of gibbsite. The ionic-strength dependence of NTA adsorption is consistent with the formation of inner-sphere surface NTA complexes with the surface hydroxyl groups of gibbsite. The observed shifts in ionic strength that occur with adsorption of cobalt and Co-NTA by gibbsite are not consistent with the current models of surface coordination: cobalt adsorption increases significantly with increasing ionic strength. It appears that increases in ionic strength may increase the number of gibbsite surface hydroxyl groups available for cobalt adsorption. This behavior has not been reported in the literature for other aluminum or iron oxides. Thus, at DOE waste disposal sites where cobalt-60 and NTA have been disposed of together, the Co-NTA adsorption behavior reported here means that NTA would not significantly alter the mobility of cobalt-60 in soil pore waters and groundwaters where gibbsite or clay minerals with gibbsite-like edges are important mineral sorbents.

EDTA Degradation

Investigations were started on EDTA degradation by a single EDTA-degrading bacterium. In FY 1993, an optimal medium for growth on EDTA was identified. The hydrogen form of EDTA could be degraded, and preliminary experiments showed that EDTA degradation is optimal at pH 7, with limited degradation at pH 6 or 8. Degradation of EDTA is a function of EDTA concentration from 0.01 to 1 μM. This work will continue in FY 1994 in collaboration with the Subsurface Science Program's Microbial Physiology project (Fred Brockman, PNL). Continued investigation of this EDTA-degrading bacterium could lead to a major application in intermediate-scale experiments and possibly be of practical use at locations where ^{60}Co-EDTA transport in groundwater is suspected.

Enzymatic Degradation of Specific Metal-NTA Complexes

Collaborative experiments with Fred Brockman (PNL) and Luying Xun (Washington State University, Tri-Cities) on the degradation of specific metal-NTA complexes by NTA monooxygenase were conducted in FY 1993. Because the use of whole cells involves a complex system for studying degradation of metal-NTA complexes, this study was conducted using partially purified NTA monooxygenase enzyme, the first enzyme used in the degradation of NTA. Results indicate that Mg-NTA is probably the form of NTA being degraded.

Future Research

Experiments on Pu-NTA degradation (FY 1994), Pu-NTA degradation in mixed-metal systems (FY 1994-1995), and the interaction of Pu-NTA, mixed metals, and sorption (FY 1996) have been planned with Don Reed (Argonne National Laboratory). The purpose of these experiments is to determine how degradation of the NTA influences plutonium valence and mobility. We hypothesize that degradation of the NTA in the Pu-NTA systems will release the plutonium to the aqueous phase and cause the formation of plutonium polymer, which in turn will reduce plutonium mobility.

Work in FY 1994 will focus on completion of the adsorption-degradation studies with the NTA-gibbsite-strain 29600 system and modeling of results for NTA at both pH 6 and 8 and for Co-NTA at pH 7. Important supporting information for this modeling will be obtained in FY 1994, including measurement of NTA desorption rates and gibbsite adsorption site densities. Finally, collaboration with Fred Brockman (PNL) will provide background information on EDTA degradation that will allow adsorption-degradation experiments with EDTA and iron or aluminum oxide to be conducted in late FY 1994 and FY 1995. These experiments will be the basis for an intermediate-scale flow-cell experiment on the sorption-degradation of Co(II, III)-EDTA with heterogeneous sorbents.

Microbial Sequestration and Bioaccumulation of Radionuclides and Metals

Harvey Bolton, Jr.

Contributors

Nancy Valentine (EMCORE) and Mark T. Kingsley (AWU-NW)

In the past, many inorganic contaminants, including radionuclides and toxic metals, have been disposed of at DOE sites. In some cases, these inorganic contaminants have migrated into the subsurface environment, and they may migrate farther to contaminate domestic groundwater supplies. Currently, economical approaches to remediate or stabilize deep contaminated zones are limited by a lack of understanding of the geochemical and biological factors that affect these wastes. One process for immobilizing radionuclides and metals is bioimmobilization, or the biosorption of radionuclides and metals by subsurface microorganisms. Because most microorganisms in the subsurface environment are associated with stationary strata, bioaccumulation of mobile radionuclides and metals would initially decrease inorganic waste transport. However, information on this microbially mediated process is currently limited. How long the inorganic wastes would remain immobilized, the selectivity of the bioaccumulation process for specific inorganic wastes, the mechanisms involved, and how the geochemistry and growth conditions of the subsurface environment influence bioaccumulation are all currently unknown. The objective of this research is to determine 1) the effect of long-term exposure to inorganic contaminants on the microbial ecology and the ability of microorganisms to biosorb metals, 2) the selectivity of the bioaccumulation process for specific metals, and 3) the mechanisms and kinetics of metal bioaccumulation.

FY 1993 Research Highlights

Microbiology of a Radionuclide- and Metal-Contaminated Waste Site

Microorganisms isolated from metal- and radionuclide-contaminated sediments may differ from isolates from more pristine environments in their abilities to bioaccumulate metals. The presence of radionuclides and metals may also have lowered or altered microbial populations. To determine the impact of mixed inorganic contaminants on microbial communities in sediments, we sampled a processing pond that was used from 1948 to 1975 for the disposal of radioactive and metal-contaminated wastewaters from laboratories and nuclear fuel fabrication facilities on the Hanford Site in south-central Washington State. The environment is semiarid, with an average rainfall of 159 millimeters per year; after wastewater input into the pond ceased in 1975, the pond dried, but a settling basin remains. To obtain sediments with varying concentrations of inorganic contaminants, six sediments were sampled from two locations in the basin and at various depths.

The inorganic contaminants and their concentration ranges in micrograms per gram of sediment (from upper to lower depths) were silver (347 - <4), copper (32,400 - 353), chromium (1,456 - <32), nickel (623 - 35), uranium (3,305 - 45), and zirconium (11,190 - 234). Radioactive contamination was discernible in the three most contaminated sediments on sampling with a hand-held β,γ-counter. The primary radiological contaminants in the sediments were uranium-238 and uranium-235, at activities ranging from 46,250 to 825 and from 1,658 to 28 becquerels per kilogram, respectively. Viable populations of microorganisms were present in all six sediments assayed, ranging from 4.2 to 6.6 log colony-forming units per gram of sediment. Microbes able to grow on agar plates containing 10 mM copper, nickel, or chromium were found only in the three most contaminated sediments, suggesting a selection for microorganisms that were resistant to the metals present in their environment. Even in the three most contaminated sediments, there was no decrease in viable counts. These results indicate that microorganisms were able to survive in sediments with high concentrations of total inorganic contaminants and radiation. However, there was no increase of radiation-resistant microorganisms in the radioactive sediments. Similar radiation dose-response curves were observed when the six sediments were exposed to a cobalt-60 source (maximum of 0-25 Mrad). Adenosine triphosphate concentrations were highest in the most contaminated sediments, indicating a larger total microbial biomass than in the uncontaminated sediments. However, microbial activity, as determined by carbon-14-glucose mineralization, was lowest. Thus, although the contaminated sediments had a larger biomass, it was less active than in the uncontaminated sediments. There was no difference in the abilities of microorganisms from the contaminated versus uncontaminated sediments to biosorb cadmium, cobalt, nickel, or strontium. The presence of inorganic contaminants over long periods did not select for microorganisms with cellular binding sites different from those of isolates from less contaminated sediments.

Biosorption of Radionuclides by a Subsurface Bacterium

Subsurface microorganisms may not have the same abilities to biosorb metals from solution as surface isolates. Because there have been few studies on sorption of metals and radionuclides by subsurface isolates, it is unclear what potential subsurface microorganisms have to decrease cationic radionuclide transport. For this reason, an endospore-forming bacterium isolated from the vadose (unsaturated) zone at the Hanford Site was used in a study of biosorption of radiolabeled divalent metals. The metals included cadmium-109, cobalt-57, nickel-63, and strontium-85, which have different specificities for ligand-binding sites on the cell surface. The subsurface bacterium formed spores resistant to heat (80°C for 1 hour), ethanol (90% for 1 hour), and cobalt-60 γ-irradiation (0.25 Mrad); under the same conditions, the vegetative cells decreased in viability. Metal uptake was significantly greater for resting vegetative cells than for spores. Subsequently, log-phase cells that had been grown in a complex medium, then washed and resuspended in pH 6 buffer [0.001 M Piperazine-N,N'-bis[2-ethane-sulfonic acid] (PIPES), 0.01 M KNO_3] were used for metal uptake studies. Selectivity for divalent metal uptake at 1 μM concentration was in the order cadmium > nickel > cobalt > strontium. Metal uptake was a passive process that did not require energy, as evidenced by the lack of effect of sodium azide or 2,4-dinitrophenol on metal biosorption. Also, heat-killed cells removed more metal from solution than viable cells did. The optimum pH for metal uptake was 6 and above, indicating that variable-charged protonation sites (e.g., carboxylate, phosphate, amino groups) were responsible for metal uptake. Biosorption followed a Freundlich isotherm with metal concentrations from 10^{-7} to 10^{-4} M. Metal uptake by the subsurface bacterium was significantly higher than that by the surface bacteria *Escherichia coli* or *Bacillus subtilis*, the model Gram-negative and Gram-positive bacteria used in the past for metal-uptake studies. Metal biosorbed by the subsurface bacterium was readily removed from the cell by suspending the metal-laden cells in an ethylenediaminetetraacetic acid (EDTA) solution, indicating that cell ligand-binding sites cannot compete with synthetic ligands for the radiolabeled metals.

Microbiological Phenomena Controlling Contaminant Transport

Fred Brockman

Contributors

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Studies of contaminant degradation in the laboratory have shown that indigenous microorganisms can degrade many contaminants to innocuous by-products in the subsurface environment. These laboratory studies have typically involved the use of a single test contaminant. In reality, however, at DOE sites and elsewhere, contaminants usually occur as mixtures of aromatic compounds, chlorinated aromatic and aliphatic compounds, organic chelating agents, metals, and radionuclides. These mixtures are often more toxic to microorganisms than are the individual components, and they are therefore more recalcitrant to microbial degradation/transformation.

One of the objectives of our research is to better understand the interactions between bacteria and co-contaminants that are of importance to DOE. Two contaminants, quinoline and nitrilotriacetic acid (NTA), were selected to represent classes of compounds that are commonly co-disposed of or that co-occur at DOE sites. Quinoline is a bicyclic aromatic compound and NTA is a chelating agent that complexes metals and radionuclides. Chelating compounds are particularly important contaminants because they can greatly enhance the transport of radionuclides in groundwater. Two bacteria were selected that degrade either quinoline or NTA, but not both, as their sole source of carbon, nitrogen, and energy. Cells that had been starved for 60 days were used to simulate the physiological state of bacteria in the subsurface environment. The interactions that were studied included the induction of degradation by each bacterium in the presence of the other, in the presence of the other organism's target contaminant, and in the combined of both the other organism and its target contaminant.

The quinoline-degrader, *Pseudomonas cepacia* strain 866A, was isolated from subsurface sediments at the Savannah River Site. The NTA-degrader, *Chelatobacter* (ATCC strain 29600), was isolated from soil; no subsurface microorganisms capable of degrading NTA and other chelates have been isolated as yet. Four replicate continuous-flow column experiments were performed for each combination of bacteria and substrates. The column feed solution contained a minimal salt medium containing one or more model contaminants at the following concentrations: 106 μM NTA, 39 μM quinoline, and 34 μM 2-hydroxyquinoline.

The time required for induction of quinoline degradation by strain 866A was unaffected by the presence of strain 29600, the presence of NTA, or the presence of both strain 29600 and NTA (Table 1).

In contrast, the time required for induction of NTA degradation by strain 29600 was affected by the presence of quinoline, the presence of the first metabolite of quinoline degradation (2-hydroxyquinoline; 2OHQ), and the presence of strain 866A (Table 2). The presence of strain 866A without quinoline resulted in more rapid induction of NTA degradation, indicating a synergistic relationship between the two microorganisms. The presence of quinoline without strain 866A delayed induction of NTA degradation. The delay (termed inhibition) was even greater in the presence of strain 866A and quinoline. The presence of metabolites of quinoline degradation, 2-hydroxyquinoline and

Table 1. Time required for induction of quinoline degradation by strain 866A. Values are means of four column experiments. Values followed by different letters are statistically different at p = 0.05.

Strain(s)/ Contaminant(s)	Hours to Induction
866A/quinoline (control)	12a
866A + 29600/quinoline	9a
866A/quinoline + NTA	13a
866A + 29600/quinoline + NTA	11a

Table 2. Time required for induction of NTA degradation by strain 29600. Values are means of four column experiments. Values followed by different letters are statistically different at p = 0.05.

Strain(s)/ Contaminant(s)	Hours to Induction
29600/NTA (control)	44b
29600 + 866A/NTA	27a
29600/NTA + quinoline	83d
29600 + 866A/NTA + quinoline	>100e
29600 + 866A/NTA + 2OHQ	67c

its subsequent metabolites, also inhibited induction of NTA degradation. The inhibition was greatest in the presence of strain 866A and quinoline because strain 29600 is simultaneously exposed to quinoline, 2-hydroxyquinoline, and other metabolites produced by the degradation of quinoline by strain 866A.

Additional experiments were conducted to determine whether quinoline was a specific inhibitor of NTA utilization or a general inhibitor of energy generation and growth. Strain 29600 grew at the same rate in the presence of NTA and glucose as in the presence of NTA, quinoline, and glucose. On the other hand, in the presence of NTA, quinoline, and glucose, induction of NTA degradation was delayed compared to controls (NTA and glucose; NTA only). These data indicate that quinoline does not inhibit the primary metabolic reactions involved in energy generation and growth on glucose, but that it is a specific inhibitor of NTA utilization by strain 29600.

Both quinoline and NTA are known to be toxic to microorganisms at concentrations of 1 to 10 mM. The toxicity of quinoline is probably related to its hydrophobicity and tendency to interact with and disrupt cell membrane functions. Because the first reaction in NTA degradation by strain 29600 is cytoplasmic rather than membrane-associated, quinoline may interfere with NTA degradation by interfering with NTA receptors on the cell surface or proteins involved in transport of NTA into the cell.

The success or failure of bioremediation in environments containing multiple contaminants and mixed wastes is a complex interplay that is poorly understood. Further laboratory studies that simultaneously examine contaminant toxicity, microbial degradation, and the effect of contaminants and degradation products on other microorganisms will advance understanding of this complexity.

In other efforts in this project, we are investigating the specificity of transport of different metal/radionuclide-NTA complexes into the microbial cell, the specificity of the NTA monooxygenase enzyme to degrade different metal/radionuclide-NTA complexes that may gain entry into the cell (in collaboration with other investigators in the Co-Contaminant Chemistry subprogram of the Subsurface Science Program, who are focusing on the chemistry and microbiology controlling chelated radionuclide transport), and the microbial degradation of another chelating compound, ethylenediaminetetraacetic acid (EDTA). These investigations will improve knowledge about limitations to microbial degradation of metal/radionuclide-chelate complexes and about how the enhanced transport of radionuclide-chelate complexes may be retarded.

Intermediate-Scale Investigations of Interactive Subsurface Processes

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Contributors

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The natural subsurface environment is often highly heterogeneous in its physical, chemical, and biologic properties, and the simultaneous interaction of chemical, microbiological, and hydrologic phenomena makes subsurface processes complex. These interacting phenomena determine the distribution and structure of the subsurface microbial community and the migration and fate of solutes. Ultimately, the effectiveness of remediation strategies depends on our ability to accurately predict the subsurface transport and transformation of contaminants in these complex systems. Field studies have shown that our predictive ability is currently limited by 1) a lack of understanding of interacting processes on a mechanistic level, and 2) an inability to scale mechanistic processes from observations in the laboratory to the field. Improving our understanding of mechanistic processes and our ability to scale these processes to relevant field problems can best be addressed by controlled laboratory experimentation in intermediate-scale flow cells. The goals of this project are to identify and test our understanding of interactive mechanisms and to develop and test approaches to scaling mechanistic processes to the field. Intermediate-scale experiments currently under way will improve our understanding of how physical heterogeneities in the subsurface environment control solute transport and consequently the distribution of microorganisms in subsurface sediments. Competitive interactions between aerobic and anaerobic microorganisms also influence the structure of subsurface microbial communities and will be investigated in future laboratory intermediate-scale experimentation.

FY 1993 Research Highlights

During the course of this project, intermediate-scale experiments have grown from simple two-layer systems to complex heterogeneous fields containing low-conductivity inclusions (binary inclusive field). Evolving scales of physical and microbial heterogeneity can be depicted conceptually on a classical measurement/observation scale diagram (Figure 1). The value of a physical parameter, such as porosity, may be relatively constant for certain sample size ranges between the scales of the heterogeneity but exhibit noisy fluctuations at the scale of the heterogeneity (e.g., at the pore scale porosity would be 0 if measured on a sand grain and 1 if measured in the pore space) (Figure 1a). Natural subsurface ecosystems typically reflect such multiscale heterogeneity; accounting for the consequences of chemical and microbial subsurface dynamics, a process known as upscaling, is required for understanding (and predicting) transport processes on the field scale. New work in upscaling has shown that, under certain patterns of heterogeneity, the classical equations of transport may not even be suitable frameworks for simulating transport processes on large scales.

Like the scales of physical heterogeneity, microbial heterogeneity also evolves over time frames relevant to transport (Figure 1b). Microbial kinetics have generally been determined in experiments in homogeneous columns containing homogeneous microbial populations; however, transport induces microbial heterogeneity on scales above that of the column. For example, high-resolution modeling has shown that low-conductivity inclusions are associated with increased microbial growth (Figure 2). Substrate that diffuses and disperses into low-conductivity regions has a longer effective residence time than it would have in the high-conductivity material, allowing organisms to metabolize a larger fraction of the substrate pulse in the low-conductivity region. Thus, low-conductivity inclusions cause both a scale dependence for physical transport processes and the localization of microbial growth, which in turn leads to spatial microbial heterogeneity.

Premodeling was used in this project to validate two crucial scale issues in the design of experiments to study transport/degradation in heterogeneous systems:

- the scale of heterogeneity must be sufficient to allow observation of the scale dependence of transport processes within the size of the flow cell; such observations usually require larger experiments and/or simpler heterogeneities.
- the exact placement of the low-conductivity inclusions need not be known, a fact that enables application of this scaling theory to the field.

A "control measure" for nonreactive scale-dependent transport in binary inclusive systems has also been identified. Preliminary results show that the shape of tracer breakthrough curves is highly dependent on the separation distance between inclusions as measured along streamlines of flow. This dependence has implications for viable scaling methods; for instance, periodic methods, which rely on a uniform spatial distribution of heterogeneities, cannot capture this aspect of the pattern. Another interesting ramification is that uniform packings of inclusive heterogeneities, which have commonly been used in past work, may largely misrepresent transport in natural systems.

Parallel theoretical work on microbial kinetics and transport physics has shown that under

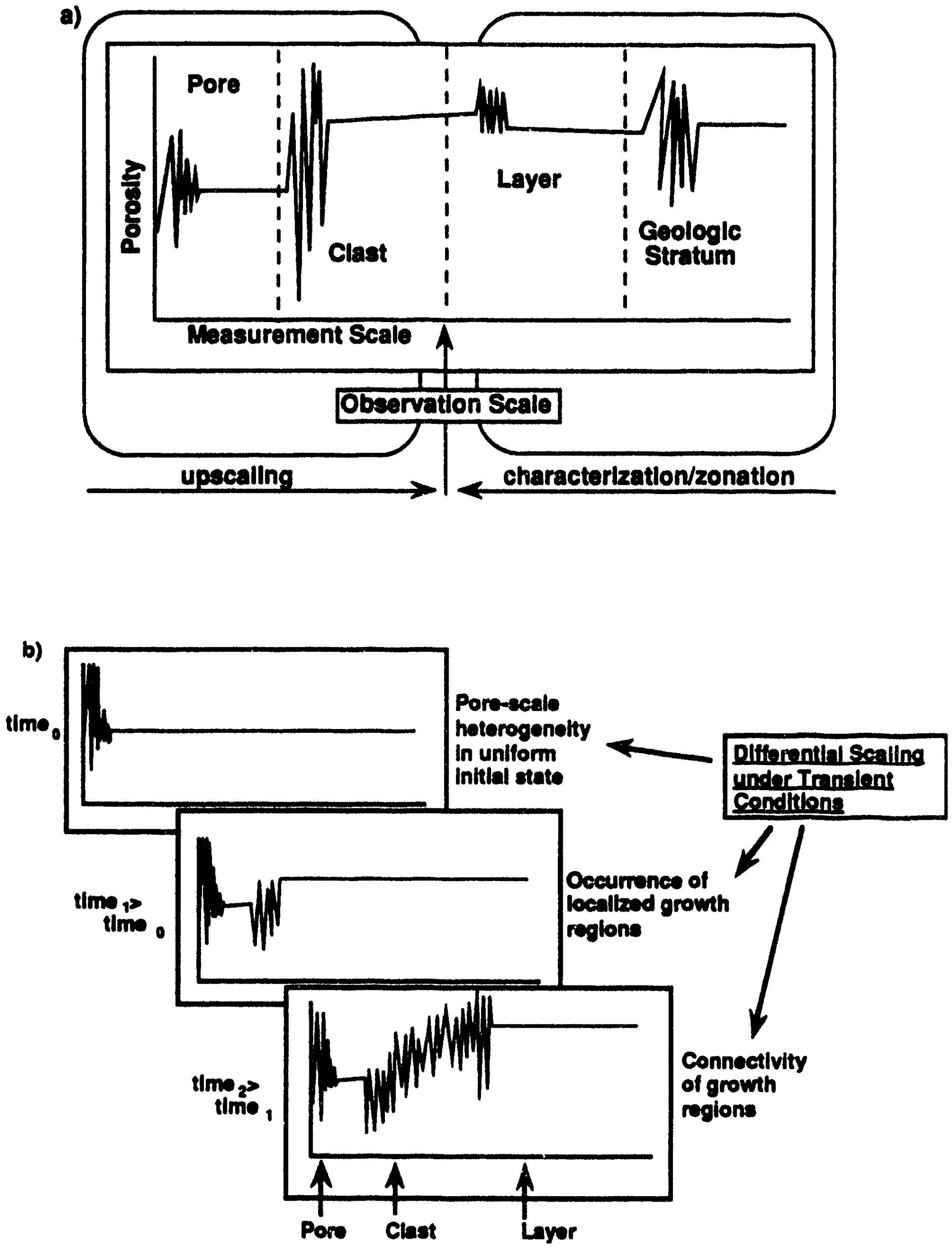

Figure 1. Evolving scales of physical and microbial heterogeneity: a) conceptual diagram of heterogeneity dependence on the measurement or observation scale; b) evolution of microbial heterogeneity under transient conditions.

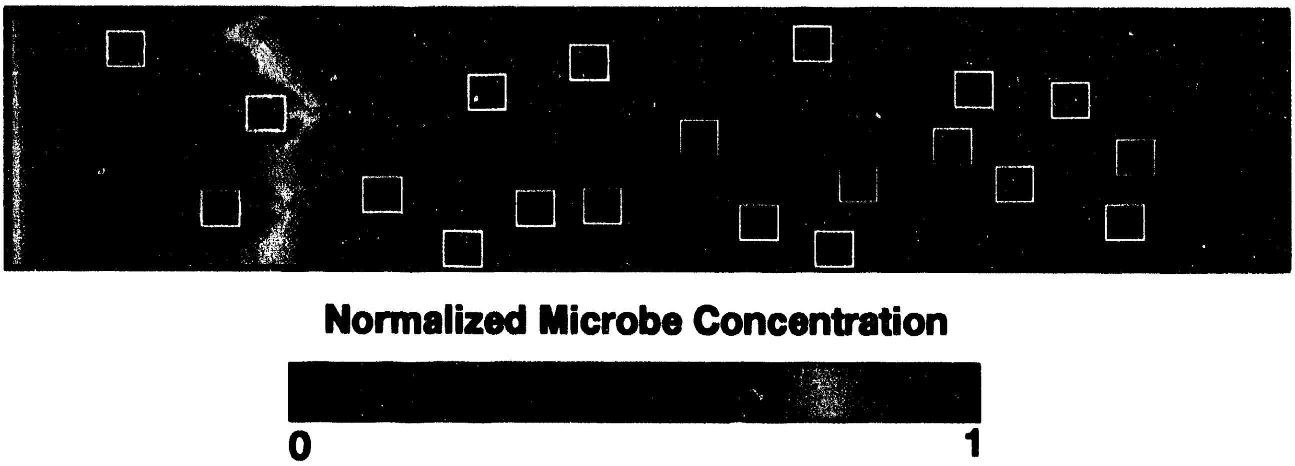

Figure 2. Microbial growth with solute flux in a binary heterogeneous field. Microbial growth is normalized, with 0 = no growth and 1 = highest growth. White boxes show locations of the low-conductivity inclusions.

relatively general conditions, use of the stochastic-convective theory makes it possible to separate the scaling of the transport process from the scaling of the microbial degradation process, and thus allows the problem to be broken down into distinct transport and microbial kinetic scaling characterizations. Without this ability, coupled transport/biodegradation processes on the field scale could be understood only through prohibitively expensive site-specific field-scale experimentation involving all interactive processes. The following characteristic model has been developed for the stochastic-convective reaction transport process based on streamline dynamics:

Transport Equation:

$$\frac{\delta c}{\delta t} = V(x)\frac{\delta c}{\delta x} - \mu\left(\frac{M}{qy}\right)V(x)\left(\frac{C}{c+k}\right)$$

Microbial Growth Kinetics:

$$\frac{\delta M}{\delta t} = \mu M\left(\frac{c}{c+k}\right)$$

where c = normalized concentration
V = fluid velocity within a streamtube
q = flow rate
y = yield coefficient
k = half-saturation constant
μ = specific growth rate.

What distinguishes this approach from classical (volume-averaged) methods is the fact that the streamline velocity, *V*(*x*), is treated as a stochastic process. This approach makes the transport equation for a specific streamline much simpler (as written above) but still accounts for dispersive and diffusive physical spreading through averaging of the characteristic solution over the random velocity field. This simple form of the transport equation makes it possible to derive characteristic solutions of the reactive transport problem in dimensionless form. For instance, the surface in Figure 3a shows the evolution of a substrate pulse over time and in one spatial dimension. Transects of the surface at fixed times give profiles ("snapshots") of the pulse in a one-dimensional system, and transects at fixed distances provide breakthrough curves of the pulse at different locations downstream (Figure 3b). Under certain general conditions, the averaged (upscaled) system behavior may be represented by simply scaling Figure 3 for each realization of velocity and averaging the results.

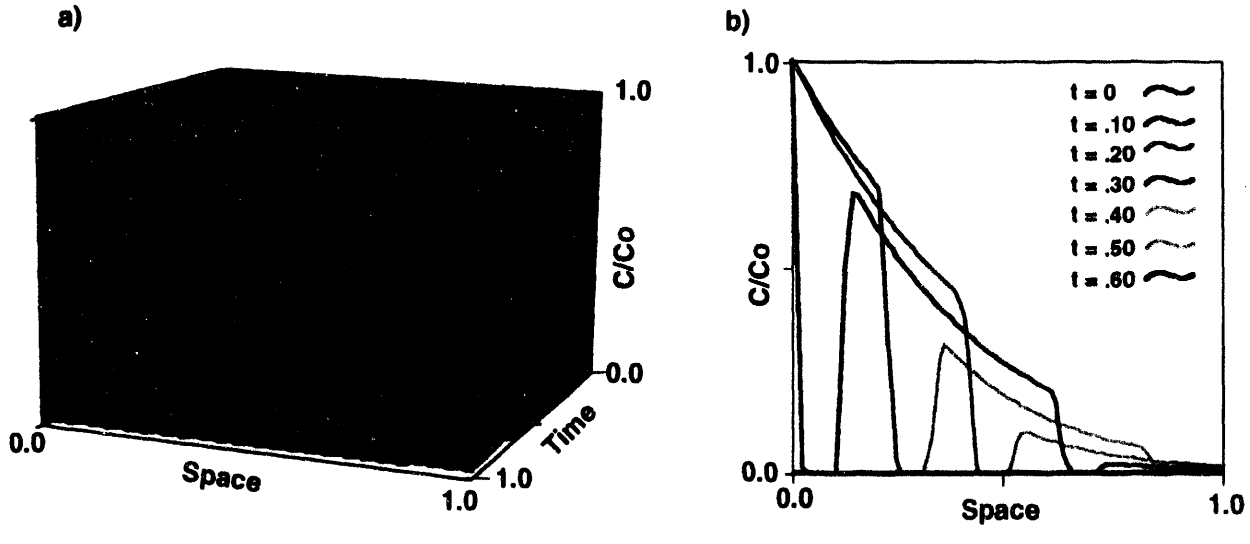

Figure 3. Characteristic model for the stochastic-convective reaction transport process: a) three-dimensional depiction of dimensionless solution with unit pulse input; b) breakthrough curves of the same pulse at different locations downstream.

Subsurface Microbial Processes

James K. Fredrickson, Fred J. Brockman, and Todd O. Stevens

During FY 1993, the focus of this project was on a geochemical, microbiological, and hydrological experiment (GEMHEX), which was conducted using intact core samples obtained from the unconfined aquifer at the Hanford Site in south-central Washington. The GEMHEX focused on several key hypotheses pertaining to microbial ecology as described in the next article in this report. The hypotheses related to phosphorous as a limiting nutrient in subsurface environments, iron biogeochemistry, and physical controls on microbial activities and population densities.

A key question for subsurface microbiology is the origin of the microbial communities. Were the organisms transported to their current location recently, by groundwater flow from the surface? Or have they been living in the subsurface strata since the time of deposition, when those formations were exposed to the surface? Exploratory research to address the origins of bacteria in deep subsurface environments was therefore initiated as part of DOE's Subsurface Science Program's new research in Subsurface Microbial Origins. An opportunity to obtain core samples for microbiological and geochemical analysis arose as the result of exploratory hydrocarbon drilling in eastern Virginia.

FY 1993 Research Highlights

Results of FY 1993 research in three areas is summarized below. The first two summaries focus on the GEMHEX, and the third describes the exploratory research conducted at the Thorn Hill, Virginia, site.

Microbial and Geochemical Interactions within and between Adjacent Subsurface Strata of Lacustrine, Paleosol, and Fluvial Origins

How geochemical and hydrologic properties affect the composition and function of microorganisms in a deep sedimentary sequence was investigated by collecting and analyzing 29 separate core samples. Core depths ranged from 173 to 197 meters, and the age of the sediments is approximately 6 to 8 million years. The sediments consisted of lacustrine, paleosol, and fluvial sequences (all in the Ringold Formation). These fine-textured sediments act as a confining layer between highly permeable sediments that transmit O_2-bearing groundwaters. The investigation focused on the relationships between organic carbon, dissolved O_2, reduction potential, and the abundance and activities of aerobic and anaerobic microorganisms.

Geochemical analyses of core samples included total, dissolved, and microbial respirable organic carbon; carbonate; pore-water dissolved inorganic carbon; reduction potential (by vanadate titration); extractable iron; pore-water cations and anions (including nutrient and electron acceptor species); pH; and Eh. Characterization of the microbial community included estimates of total culturable aerobic heterotrophic bacteria, culturable anaerobic bacteria (including fermentors), iron-reducing bacteria, sulfate-reducing bacteria, methanogens, acridine orange direct counts, and total phospholipids. Microbial activity was estimated using incorporation of ^{14}C-acetate into lipids, phosphatase activity, and mineralization of ^{14}C-glucose and ^{14}C-succinate.

The top 1 meter of the lacustrine sediment, near the contact with the transmissive overlying fluvial sands and gravels, contained banded iron-oxyhydroxide precipitates and was low in both total organic carbon (<300 milligrams per kilogram of soil) and carbonate (<20 milligrams per kilogram). In contrast, the bottom 10 meters of the lacustrine sediment had higher concentrations of total organic carbon (5,370 to 12,180 milligrams per kilogram) and carbonate (40 to 2,060 milligrams per kilogram), and iron-oxyhydroxide precipitates were not evident. These observations, combined with platinum electrode measurements on displaced pore waters and reduction potential measurements in the solids, indicated a decrease in the redox potential with depth into the lacustrine sediments. Densities of culturable aerobic and anaerobic bacteria generally did not exceed 10^3 per gram but were consistently higher in the unoxidized portions of the fine-grained lacustrine sediments than in associated paleosols or fluvial sands. Concentrations of microbial lipids (1-5 picomoles total phospholipids per gram) and activities as measured by ^{14}C-glucose mineralization (3-10% evolved as $^{14}CO_2$ after 7 days) were highest in the lower section of the lacustrine sediments. In general, both the density of microorganisms and total organic carbon (160 to 7,410 milligrams per kilogram) were very low or below detection in the paleosol and fluvial sediments.

The results suggest that O_2 availability in the lacustrine sediments, either from groundwaters in the overlying fluvial sediments (4 milligrams of dissolved O_2 per liter) or during subaerial exposure in the past, caused oxidation of organic matter in the top meter. The lack of sediment oxidation below this zone suggests that there has been minimal transport of O_2 from the overlying fluvial sediments into the fine-textured, organic carbon-rich lacustrine sediments. Hydraulic conductivities measured on sediment cores from both the top and bottom of this section were less than 10^{-9} centimeters per second, confirming that transport of electron acceptors would be diffusion-limited.

Phospholipid community structure analysis indicated that lipids characteristic of sulfate-reducing bacteria were present. In addition, concentrations of pore-water sulfate were depressed in the lacustrine sediments. The abundance of total organic carbon in the lower lacustrine layer is likely to be responsible for the long-term maintenance of microorganisms in this layer, but because microbial metabolism is electron-acceptor-limited, relatively high concentrations of microbially metabolizable organic carbon remain.

Nutrient Stimulation of Subsurface Microbial Communities

To assess the potential response of the community to *in situ* bioremediation, this study examined stimulation of the microbial community in seven GEMHEX samples after addition of low levels of nutrients and (in separate experiments) after storage of samples in the presence of increased oxygen concentrations. The subsurface sediments were obtained 175 to 210 meters below the surface of the Hanford Site and included fine-grained lacustrine sediments, paleosols, and sandy fluvial sediments.

Five-gram samples of sediment were amended with either 100 microliters of water, 5 milliliters of water plus 20 nanomoles of phosphorous as K_2HPO_4, 5 milliliters of water plus 50 nanomoles of nitrogen as NH_4NO_3, or 5 milliliters of water plus 3.0 micrograms of sheared salmon sperm DNA (162, 62.3, and 16.6 nanomoles of carbon, nitrogen, and phosphorous). The amended samples also contained 83.5 nanomoles of ^{14}C-glucose or 4.15 nanomoles of ^{14}C-succinate. Mineralization of the radiolabeled substrates was monitored over a period of 40 to 60 days. To study ^3H-acetate incorporation into lipids, 2 grams of sediment was amended as above, and 1 milliliter of water containing 2.48 nanomoles of ^3H-acetate was added to assay microbial activity. Incorporation of ^3H-acetate into lipids was monitored over a period of 3 days. With the addition of 5 milliliters of water, six of the seven sediments showed stimulation, which they did not on the addition of 0.1 milliliter of water. This stimulation was not enhanced by the presence of nitrogen, phosphorous, or DNA in any of the sediments. Thus, addition of 5 milliliters of water apparently makes either the radiolabel or the sediment-associated nutrients (or both) more available to microorganisms. These results suggest that the added nutrients (at the concentrations used) were largely unavailable to microorganisms, or that available sediment-associated nutrients were preferred.

In separate experiments, disaggregated samples of four sediments were stored at 17°C for up to 22 weeks. Two samples of the same sediments (both paleosols) were also stored as intact cores. After approximately 1, 3, 10, and 20 weeks of storage, plate counts were performed and microbiological measurements made. In the disaggregated sediments, rate constants for ^{14}C-glucose mineralization increased by about 4, 6, 20, and 30 times over those measured immediately following sample acquisition. For the two paleosols, rate constants were about 6 times greater in the disaggregated samples than in the intact samples. Dramatic increases in the numbers of culturable aerobic heterotrophs were evident after 1 week of storage, and after 20 weeks of storage populations had reached 10^5-10^6 colony-forming units per gram of sediment in all sediment samples. Although increases in the intact samples were slower than in the disaggregated samples, all reached similar endpoint populations. These results demonstrate that microorganisms in these sediments can readily use sediment-associated nutrients for growth or, alternatively, can regain culturability after recovering from a dormant or injured state.

Thermophilic Anaerobic Bacteria in Samples from 2,800 meters below the Surface

PNL recently participated in a multilaboratory, multidisciplinary project, as part of DOE's Subsurface Microbial Origins Program, to begin to explore the question of microbial origins by investigating samples from a formation so deep that recent transport is very unlikely.

Researchers assayed 200,000,000-year-old shale samples retrieved from 2,800 meters beneath the land surface in eastern Virginia for the presence of a variety of anaerobic bacterial types. DOE received the samples courtesy of Texaco, Inc., and the Eastern Virginia Gas Company. The samples were processed in an inert atmosphere in the field, then transported overnight under argon gas to various laboratories for further investigation; some of the PNL experiments were done in collaboration with David Boone of the Oregon Graduate Institute.

In the PNL experiments, all samples and enrichments were handled under strictly anaerobic conditions at all times. Despite the limited sample size, relative numbers of organisms were determined by single-decimal-dilution series. Samples that were pebbles and could not be diluted were incubated whole. The samples were placed in selective media that would enrich for different physiological groups. Culture conditions simulated the expected ambient conditions of the formation from which the original samples were retrieved: 50°C or 60°C, 1% or 5% NaCl, and reducing conditions. The electron donors provided were mixtures of organic acids, carbohydrates, trimethylamine (for methanogens), or hydrogen gas. The electron acceptors were NO_3^-, iron(III), manganese(IV), S^o, SO_4^{2-}, or HCO_3^-.

After about 60 days of incubation, enrichments became slightly turbid, and bacterial growth in pared core samples was confirmed by microscopy. However, no growth occurred in any of the samples in 5% NaCl. Denitrifying bacteria were observed in enrichments receiving 1 gram of sample, but not in any other dilution. Iron(III)-reducing bacteria were observed in the 1-gram enrichments (and thus were growing on H_2) but not in those on mixed organics. Manganese(IV)-reducing bacteria were observed in 50-milligram enrichments growing on mixed organics. Fermentative bacteria were observed in 10^0 enrichments. No sulfate reduction, sulfur reduction, or methane production was detected in pared inner core samples.

More of the pared samples contained manganese- and iron-reducing organisms and H_2-oxidizing bacteria than contained bacteria utilizing other electron acceptors and donors. This suggests that these may be the energy-yielding nutrients that are most available in this deep subsurface environment.

The results from this exploratory research are promising in that a variety of anaerobic bacteria were obtained from the subsurface core samples that were obtained from an isolated deep subsurface environment. Thus, this is preliminary evidence that bacteria can survive for hundreds of millions of years in subsurface environments, independent of contact with the surface. However, the specific origin of these microorganisms remains equivocal and the subject of future research.

Physical Constraints on Nutrient Flux in Saturated Sediments--Results of An Integrated Geochemical, Microbiological, and Hydrological Experiment (GEMHEX)

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Contributors

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The principal scientific objectives of this project are to 1) understand how such diagenetic processes as mineral dissolution/precipitation and physical compaction affect the *in situ* metabolic activities of subsurface microorganisms; 2) determine the geochemical and geophysical processes that most influence the subsurface flux of electron donors, electron acceptors, and inorganic nutrients required to sustain subsurface microbial communities; 3) quantify the effects of physical and chemical heterogeneity on *in situ* microbial activity; and 4) evaluate how physical structure and mineralogy (of secondary phases and grain coatings) affect bacterial transport in shallow aquifer systems. The following research questions are being addressed:

- What mineral phases serve as sources/sinks of electron acceptors and inorganic nutrients in sedimentary aquifer systems?
- How do diagenetic changes in pore structure and permeability affect nutrient flux through different lithologic units?
- What physical and mineralogical characteristics limit bacterial transport in subsurface sediments?

Project investigators also provide geological expertise for drilling and sampling conducted under DOE's Subsurface Science Program. This ensures that the subsurface samples collected are free of microbial cross-contamination, because aseptic drilling and sampling protocols and multiple downhole tracers are used. The project also ensures that research questions related to subsurface microbial ecology can be addressed through subsampling schemes appropriate for conducting integrated geochemical, microbiological, and hydrological experiments. As part of the Subsurface Science Program's Deep Microbiology Subprogram, research under this project contributes directly to subprogram goals by identifying the critical geochemical and hydrological processes that promote the survival or transport of microorganisms in subsurface environments.

In the past three years, several extensive field experiments related to this project have been conducted. In FY 1990 - FY 1991, samples were acquired from deep unsaturated zones of arid western sites, specifically the Idaho National Engineering Laboratory and the Hanford Site, for comparison with earlier results from the Savannah River Site. Generally, microorganisms were less abundant in both the unsaturated zones and the aquifers at the western sites than at the Savannah River Site; moisture content and inorganic nutrients (such as phosphorous) appeared to be critical factors in the maintenance of subsurface microbial activity in these environments (McKinley et al. 1993). In FY 1992, an integrated geochemical, microbiological, and hydrological experiment (GEMHEX) was conducted in a semiconfined aquifer at Hanford to test hypotheses developed based on previous findings. In the Yakima Barricade Borehole, a 20-meter zone of sediments was sampled continuously to assess the vertical spatial variability of microbial activity, and a comprehensive set of chemical, physical, and microbial analyses and experiments was conducted on the cores.

In FY 1993, emphasis was placed on using the data from the GEMHEX interval to determine how diagenesis and its associated physical and chemical changes affected subsurface nutrient flux through the sampled interval.

Project investigators also participated in research on microbial origins conducted at the Thorn Hill borehole drilled by Texaco in Virginia. The researchers are collaborating with T. C. Onstott (Princeton University) in interpreting the evolution of the sedimentary basin from which anaerobic, thermophilic bacteria were isolated. Finally, in conjunction with researchers from other national laboratories and universities, the investigators have developed a comprehensive set of aseptic drilling and sampling protocols to ensure that subsurface samples are not compromised by microbial cross-contamination during sample acquisition.

Sample Acquisition for GEMHEX

The relationships between sediment physical properties, selected geochemical properties, and the occurrence of different types of subsurface microorganisms were assessed for twenty-nine samples collected from fine-grained sediments and paleosols from depths of 173-197 meters in the Ringold Formation at the Hanford Site. Sampled sediments ranged in age from 6 to 8.5 million years. Sediment cores (1 meter long by 10 centimeters in diameter) were acquired aseptically and analyzed to elucidate the interactions between subsurface geophysical, geochemical, and microbial processes. The sampling interval included fine-grained laminated lacustrine sediments, well-developed paleosols, and relatively coarse-grained fluvial sands and gravels. This lithologic sequence was selected for detailed study because it provided a range of physical and geochemical properties that made it possible to test hypotheses that have broad relevance to the understanding of subsurface microbial ecology. The FY 1993 investigation focused on interactions between organic carbon and dissolved oxygen gradients, iron mineralogy/valence, abundance of aerobic and anaerobic microorganisms, and observed microbial activities across lithologic units (Long et al. 1993b; Rawson et al. 1993; Fredrickson et al. 1993).

Sample Quality Ensured by Multiple Tracers

Quantifying the extent to which samples collected from the subsurface are representative of subsurface microbial and geochemical conditions has been an important part of the Subsurface Science Program (Long et al. 1993a). A threefold approach was used to ensure the quality of the GEMHEX samples: 1) procedural controls minimized the exposure of samples to microbial contaminants, 2) drilling and sampling procedures minimized the physical introduction of potential contaminants to the sample, and 3) chemical and proxy microbial tracers permitted estimation of the extent to which samples may have been microbially or chemically contaminated during the drilling and sampling process.

Cable-tool drilling was used in the Yakima Barricade Borehole, obviating the need for circulating drilling fluids. Carbon steel casing was inserted during drilling to maintain the integrity of the borehole in poorly consolidated sediments. Samples were collected in split-spoon samplers containing polycarbonate liners. The liners protected sample cores from contamination after removal from the split-spoon sampler and before processing. Sample collection for GEMHEX required consideration of possible mixing of sample pore water with the standing water column. A bromide tracer was added to the water column and maintained at a concentration of approximately 200 milligrams per liter. Concentrations of bromide in the fluid were monitored at the sediment/water interface using a down-hole sampler. In addition, the split-spoon sampler was equipped with a particulate tracer to trace potential microbial contaminants and a second solute tracer (perchlorate) to trace mixing of water during coring. These tracers were released by breaking plastic bags and piston displacement of fluid from the polycarbonate liner of the split-spoon sampler during coring.

Tracer concentrations dropped by four to six orders of magnitude in the fine-grained, low-permeability sediment samples, indicating a very low probability that the samples were contaminated with either exogeneous microorganisms or wellbore fluids. In sandy gravels, however, the reduction in tracer concentrations was only one to three orders of magnitude. These results may indicate contamination of the sample due to rotation of the larger clasts during driving of the split-spoon sampler. These samples are also assumed to be contaminated with microorganisms from the wellbore fluid. However, the demonstrated reduction of particulate and solute tracer concentrations suggests that samples of fine-grained sediments from the GEMHEX interval represent very low probabilities for cross-contamination. Project investigators continue to provide their colleagues with similar tracer technologies that are customized to site-specific geological, geochemical, and hydrological characteristics and to the research questions being addressed by other projects under the Subsurface Science Program.

Each sediment core was subsampled to minimize physical and chemical heterogeneity; approximately 2 kilograms of sample were homogenized for analysis of grain-size distribution, moisture content, bulk and particle density, microbial assays, and selected geochemical parameters. Intact cores paired with the homogenized samples were analyzed for permeability, hydraulic conductivity, porosity, grain-size distribution, and clay mineralogy. Geochemical analyses included solid-phase total, organic, and inorganic carbon as calcium carbonate; solid phase extractable iron and solid phase iron(II)/iron(III); and pore-water solutes. The pore-water solutes included dissolved organic and inorganic carbon, pH, and cations and anions including nutrient and electron acceptor species. Microbial assays included total culturable aerobic heterotrophic bacteria, culturable anaerobic bacteria (fermentors, iron-reducing and sulfate-reducing bacteria, and methanogens), acridine orange direct counts, total phospholipid fatty acids, and microbial activity based on mineralization of ^{14}C-glucose and ^{14}C-succinate, incorporation of ^{14}C-acetate into lipids, and phosphatase activity (Fredrickson et al. 1993; Rawson et al. 1993).

Microbial and Geochemical Variation Defined by Lithologic Differences

As part of the research, the interactions between geochemical and geophysical properties and the composition and function of microbial communities in vertically adjacent subsurface strata of contrasting depositional environments were investigated.

The sampled interval occurred between two fluvial sequences of muddy sandy gravel, the overlying Middle Ringold Formation and the underlying Lower Ringold Formation. The lowest section of the sampled interval consisted of 3.8 meters of well-sorted silty sand to sand in graded beds, which was overlain by a 7.8-meter-thick overbank/paleosol sequence of bioturbated clay, silt, and sand exhibiting calcic soil development. The paleosol was directly overlain by 5 centimeters of gray volcanic tuff, which in turn was overlain by 12.4 meters of laminated lacustrine sediments exhibiting clayey to silty clay grain size. The top 1.5 meters of the lacustrine sediments were yellow-brown and contained concretions of oxidized minerals, predominantly iron phases. The lower 10.9 meters of the lacustrine sediments were dark gray and exhibited no observable oxidized zones. At the top of the GEMHEX interval, the lacustrine sediments were overlain unconformably by orange-stained muddy gravel with a bimodal distribution of well-rounded clasts in a poorly sorted matrix. Hydrologically, the lacustrine sediments and the paleosol apparently function as an aquitard.

The top 1.5 meters of the lacustrine sediment, which was in contact with the fluvial gravels, had inclusions of oxidized iron phases and was low in total organic carbon (<300 milligrams per kilogram) and solid phase carbonate (<20 milligrams per kilogram). In contrast, the lower 10 meters of the lacustrine sediment had high total organic carbon (5,370 - 12,180 milligrams per kilogram) and carbonate (40 - 2,060 milligrams per kilogram). Although densities of culturable aerobic and anaerobic bacteria generally did not exceed 10^3 per gram, they were consistently higher in the unoxidized portions of the fine-grained lacustrine sediments. In addition, the highest concentrations of microbial lipids (1-5 picomoles total phospholipid fatty acid per gram) and highest activities as measured by ^{14}C-glucose mineralization (3-10% evolved as $^{14}CO_2$ after 7 days) were present in the lower section of the lacustrine sediments. In general, the density of microorganisms was very low or below detection in those paleosol and fluvial sediments that were also low in organic carbon (160-7,410 milligrams per kilogram).

The results suggest that O_2 transport into the lacustrine sediments, either from the overlying fluvial sediments or during past subaerial exposure, resulted in oxidation of organic carbon in the upper meter. The current rate of transport of O_2 from the overlying fluvial sediments into the lacustrine layer appears to limit the aerobic respiration of total organic carbon in the lacustrine sediments. Consequently, the abundance of organic carbon in the lacustrine layer is likely to be responsible for the long-term maintenance of microorganisms in the layer.

Physical Influences on Solute Flux

The focus of this research was to determine how changes in sediment physical properties within sampled lithologies and across lithologic boundaries affected the distribution of aerobic heterotrophic bacteria and the transport of related electron donors and acceptors (e.g., dissolved organic carbon and oxygen).

Low levels of microbial activity were observed in fine-grained lacustrine sediments; the pore structure of the sediment suggests that less than 5% of the pore throats are big enough (>0.2 microns) to allow microbial transport either into or out of the unit. Microorganisms are hypothesized to have survived in these sediments due to the presence of organic carbon in the unit and the limited electron acceptor flux (oxygen and potentially sulfate) caused by the decreased permeability at interfaces with more transmissive lithologic units. Diagenetic precipitation of silicates was one factor in permeability reduction.

Sampled sediments were deposited in a fluvial-lacustrine environment that included subaerial exposure and intermittent soil development. The sampled sequence contained fluvial sands that graded upward into calcic to argillic paleosols, which were in turn overlain by lacustrine sediments. Sandy gravels of the Middle Ringold unit were deposited unconformably on the sampled interval. Evidence for microbial occurrence (culturable counts, phospholipid fatty acids, acridine orange direct counts) and activity (^{14}C-glucose mineralization) was greatest for lacustrine sediments, second greatest for paleosols, and least for fluvial sands; total organic carbon contents showed similar trends. The proportion of silt- and clay-sized particles (<0.063 millimeters in diameter) ranged from more than 90% in lacustrine sediments to 80 to 85% in paleosols to 40% in fluvial sands. Although average hydraulic conductivity varied in the order fluvial sand (4.4×10^{-7} centimeters per second) > paleosols (2×10^{-7} centimeters per second) > lacustrine sediments (6.5×10^{-8} centimeters per second), it was much lower at the contact between the Middle Ringold gravels and the underlying lacustrine sediments ($<1\times10^{-9}$ centimeters per second), and between the base of the paleosol and the top of the fluvial sands (also $<1\times10^{-9}$ centimeters per second). The hydraulic conductivity of the gravels ranged from 10^{-4} to 10^{-3} centimeters per second.

Although absolute numbers of aerobic heterotrophs were low ($<10^3$ colony-forming units per gram) throughout the sampled zone, the greatest abundances were associated with the finest-grained lacustrine sediments. The results suggest that observed differences in microbial occurrence within the sampled interval are related to physical constraints on solute movement through the low-permeability sediments. Current research is examining the possibility that the occurrence of below-average hydraulic conductivities and sediment permeabilities at the boundaries of the fine-grained lithologies may be related to secondary mineral precipitation during sediment diagenesis. The physical and hydraulic properties observed in the sediments support an interpretation of limited solute mixing from overlying oxygenated groundwaters in the Middle Ringold unit, and probably contribute to the persistence of microorganisms and organic carbon within the lacustrine sediments. The association of higher microbial activities and higher absolute numbers of heterotrophic bacteria with the finer-grained, less permeable sediments contradicts the earlier findings in southeastern coastal plain sediments and suggests the importance of nutrient flux for long-term maintenance of subsurface microbial populations.

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Pore-Water Chemistry within the Saturated Zone at the Yakima Barricade Borehole

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Contributors

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As described in the two preceding articles, the Yakima Barricade Borehole (YBB) at the Hanford Site in south-central Washington was drilled to sample strata that had not been impacted by human activities, for the purpose of sampling and analyzing the microbial population and its relationship to the enclosing chemical and physical environment. Vadose zone sampling was completed in past years; drilling and sampling within the water-saturated zone were completed in FY 1993. The water table was located at a depth of 100 meters, and the unconfined aquifer terminated at the contact between sedimentary rocks of the Ringold Formation and the underlying Columbia River basalts at a depth of 228 meters. Drilling and sampling within the saturated zone targeted a sequence of lacustrine and paleosol sediments that are both over- and underlain by coarser sands and gravels, all within the Ringold Formation (Figure 1). These sediments, designated the GEMHEX interval, were at a depth of 173 to 198 meters below the surface. Thus this study investigated a deep, saturated sequence of variable lithology overlain by an thick unsaturated zone, at an arid location experiencing little surface recharge.

Drilling was by the cable-tool method, with continuous core samples being removed from the target interval by split-spoon sampler. During drilling and coring, bromide was maintained at a concentration of 200 milligrams per liter in the borehole slurry to serve as an aqueous chemical tracer of sample contamination by the slurry. Sampling on a fine scale (approximately 0.6 vertical meters between sample points) was intended to reveal coupled differences in chemical, physical, and microbial properties across lithologic boundaries, differences that could not be resolved in studies in which the sampling scale was coarser. Cores were transferred immediately to an inert-atmosphere glovebox, where they were removed from the core liner, dissected, and subsampled.

Investigation of samples was undertaken collaboratively with other investigators working within DOE's Subsurface Science Program, including the Deep Subsurface Microbial Investigations and Deep Microbial Geosciences Testing projects at PNL and projects at New Mexico Institute of Mining and Technology, Florida State University, Idaho National Engineering Laboratory, Oak Ridge National Laboratory/University of Tennessee, and Rensselaer Polytechnic Institute. The Pore-Water Chemistry project was responsible for sampling and for analyzing chemical data obtained from pore-water extracts and sediments. These data were needed to address hypotheses pertaining to the biogeochemistry of iron and to whether phosphorous is a limiting nutrient. Pore waters were extracted from mixed sediments representing each cored interval. Sediments were packed into a titanium centrifuge apparatus and centrifuged for periods up to 14 hours to remove pore water, which was collected in a liquid-receiving cup within the apparatus. All sample handling and splitting was done in an inert-atmosphere glovebox. Extracted water volumes were limited (typically 10 milliliters per sampled interval) and were carefully split for analysis of pH, dissolved oxygen, Eh, major dissolved cations and anions, organic and inorganic carbon, iron(II/III), NO_3/NO_4, NH_4,

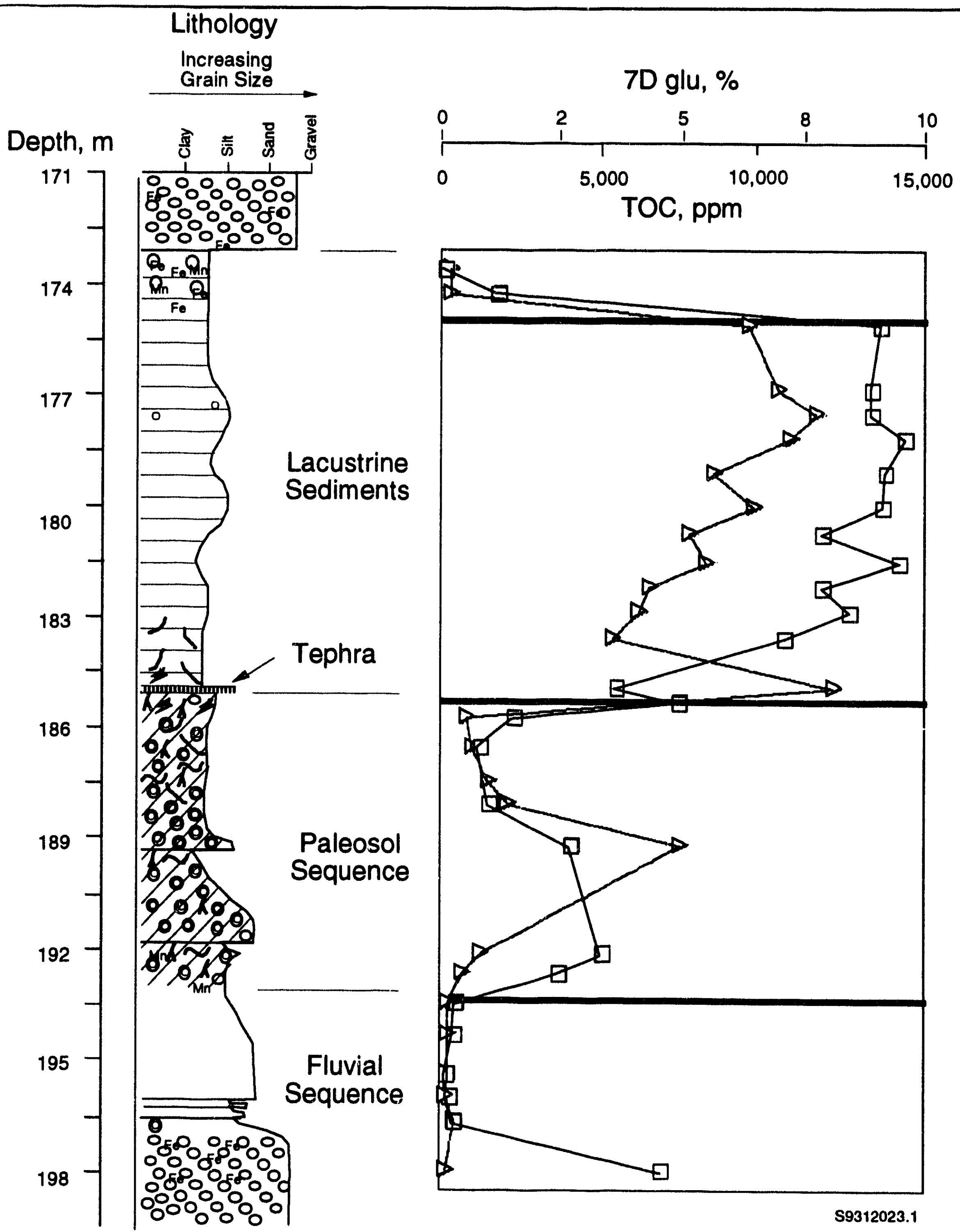

Figure 1. Plot of 7-day glucose (squares) and total organic carbon (triangles) against stratigraphy within the GEMHEX interval. Root casts and iron and manganese staining are indicated within the lithologic column (labeled Fe and Mn); grain sizes are represented as C, clay; Z, silt; S, sand; G, gravel.

and PO_4. Slurries of sediments (1:1 with water) prepared before and after extractions were analyzed for pH and Eh using a platinum electrode. Sediment samples were analyzed for bulk composition by proton-induced X-ray emission (PIXE) spectroscopy; for trace total organic carbon by combustion; for total inorganic carbon, nitrogen, acid-volatile sulfide, and phosphorous by acid digestion; and for hydroxyl amine-hydrochloride leachable iron. Results were shared with other participating projects for collaborative interpretation.

An overall description and discussion of the results of this research is being prepared; selected results are presented here to illustrate the utility of finer-scale sampling in investigations of interdependent microbial and chemical properties within aquifer environments. Tracer bromide analyses indicated that the samples obtained were essentially contaminant-free: measured bromide levels were within the range found in the unconfined aquifer (approximately 1 milligram per liter or less) or slightly above that level, indicating a maximum volumetric contamination of approximately 2% by mixing with borehole slurry.

Major lithologic divisions within the GEMHEX interval were distinct both in composition and in microbiology. The lacustrine sequence, with the exception of the two uppermost core samples, was richer in organic carbon (Figure 1) than the underlying paleosol sequence. Measurements of aerobic mineralization of radiolabeled glucose by *in situ* bacteria correlated positively with organic carbon concentration, suggesting a dependence of microbial numbers on nutrient supply. Similarly, pore-water chemistry also varied across lithologic boundaries. Dissolved inorganic carbon was more abundant within lacustrine sediments than in paleosols, paralleling the differences noted for the solid phases. Sulfate and nitrate, the most abundant anions other than carbonate, were less abundant in the lacustrine sequence than in the paleosol sequence (Figure 2), suggesting that *in situ* microbial respiration of organic carbon is electron-acceptor limited.

Microbial abundance and activity also varied within individual strata. For example, the uppermost portion of the lacustrine sequence (delineated by a line at approximately 175 meters in Figures 1 and 2) was lighter in color than the underlying sediment and contained less organic carbon; the leachable iron content of this sediment was approximately 1300 milligrams per kilogram, compared to 6,000 to 12,000 milligrams per kilogram in the remainder of the lacustrine sequence. These results suggest that the uppermost portion of the lacustrine sediments was oxidized by either diffusion or advection of oxygen from the overlying oxygen-rich formation water (6 milligrams per liter of O_2).

A full interpretation of chemical results with respect to specific hypotheses is limited by the lack of some data. Dissolved oxygen in pore waters, for example, could not be accurately measured because the samples were exposed to glovebox gases during processing. The determination of dissolved organic carbon was also compromised, as a result of unforeseen contamination by the extraction procedure.

Future Research

Plans for future work in this project include the deployment of a passive multilevel sampler to sample pore water across lithologic boundaries and within strata in the GEMHEX interval. This will provide data at a spacing of 6 to 10 centimeters for dissolved species, including O_2, dissolved organic carbon, and iron(II/III), without sample manipulation being required prior to analysis. Once in place, the sampler will provide a means of directly examining geochemical conditions as the well equilibrates with the subsurface environment. A complete and detailed determination of aqueous geochemistry within and adjacent to the GEMHEX interval will result from this research.

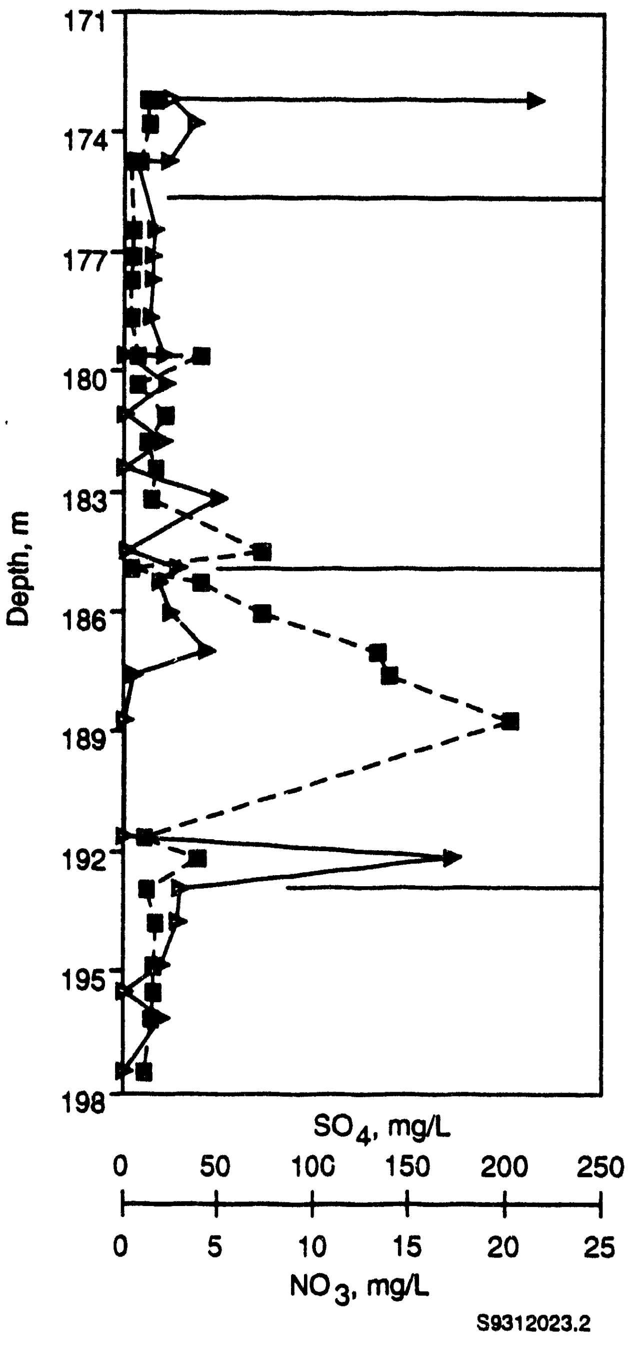

Figure 2. Sulfate and nitrate in GEMHEX pore waters vs. depth. Both ions (SO_4: squares, NO_3: triangles) are generally less abundant in the lacustrine sequence than in the paleosol sequence.

Microbial Origin/Transport in Subsurface Systems

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Contributors

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Resolving hypotheses about microbial origins requires an understanding of microbial transport phenomena at the regional scale. Regional scales imply time and distance scales that are considerably longer than what can be addressed by traditional tracer experiments (that is, hundreds to thousands of years and kilometer distances). In this project, a combination of geochemical modeling and stable isotope evaluation are used to reconstruct the changes that have occurred in the subsurface chemical environment that would have affected the transport and survival of microorganisms over regional time scales (established by groundwater dating). Measurements of groundwater age and of unsaturated and saturated transport rates give the most accurate information on the minimum amount of time that the microorganisms have evolved *in situ*. Reaction-path modeling of the geochemistry along groundwater flow paths provides detailed information on subsurface biogeochemical processes, leading to a better understanding of the distribution, diversity, and activity of microorganisms in groundwater. The fractionation of stable isotopes (isotopes of carbon, sulfur, nitrogen, and possibly iron) can be incorporated in reaction-path models to simulate microbial geochemical reactions. The overall goals of this research are 1) to determine if microbial communities were transported to where they are now found in deep subsurface systems or if they are relics of the original depositional environment, and 2) to investigate complex interactions between microbial communities and the subsurface geochemical environment.

Evidence of microbial transport over regional time and distance scales is necessarily indirect. One approach is to evaluate biological relatedness of microbial populations along a well-defined flow path. These well-defined flow paths are established by physical or hydraulic measurements and by chemical modeling of the groundwater evolution. It has been postulated that molecular or physiologic techniques could be used to establish the relationships between the microbial communities along a flow path and to gain an understanding of how the microbial communities evolved or adapted during transport, or indeed to establish evidence for whether the microbial community was transported along the flow path. However, the application of molecular/physiologic techniques to derive evidence of microbial transport in natural environmental systems has never been demonstrated. Initial studies were therefore designed to test the applicability of molecular techniques for determining microbial transport in a well-defined and well-controlled field situation.

To maintain control on the flow path, a flow path over a short distance (6-10 meters) was selected, so that water transport could be documented using physical measurements and natural chemical and isotopic tracers. Two types of flow paths in the vadose zone were selected: 1) a vertical profile where natural recharge is occurring in the vadose zone, and 2) an adjacent vertical profile where natural recharge has not occurred over the length of the flow path; this second profile serves as an experimental control. Topographic highs and lows in semiarid environments provide this type of spatial variation in recharge within uniform paleoenvironments.

The relative recharge rates of the field sites were determined by applying a mass balance to the chloride concentration in the soil pore waters. In areas of high recharge, the chloride is carried down through the soil profile. In areas of low recharge, chloride accumulates in the soil profile. At the Yakima Barricade Borehole, chloride in the first 5 meters represented atmospheric accumulation during the last 13,000 years (i.e., the time elapsed since the last catastrophic flood) and an average recharge rate of less than 0.02 millimeter per year. At the Cold Creek alluvium site, a topographic low area where runoff water infiltrates, there is no accumulation of chloride in the soil profile, indicating rapid recharge.

Relatedness of the microorganisms along flow paths is being sequentially evaluated at the

- community level - total culturable population and characterization, and phospholipid fatty acid analyses (PLFA)
- organism level - lipid analysis (MIDI, Microbial Identification, Inc.)
- genome level - DNA fingerprinting and 16S rRNA/DNA.

The community level provides information on microbial characteristics necessary for transport, leading to insight into mechanisms that control transport. Organism- and genome-level information, however, are also necessary to document relatedness and establish *a priori* evidence of transport. Microbiologists at PNL, University of Tennessee, and Florida State University are participating in the investigation.

The community-level analyses indicate a decrease in microbial biomass with depth at both sites, as determined by plate counts and by PLFA. Initial analyses of the community structure using PLFA showed that the community structure changed with depth in the low-recharge control site (Yakima Barricade Borehole), but remained relatively constant with depth at the high-recharge site (Cold Creek alluvium). This corresponds to the expectation that if microorganisms are quickly transported through the vadose zone in high-recharge areas, the microbial communities along a depth profile should have similar community structures. However, if the microbial communities have been isolated for long periods of time, the communities along a depth profile will show little relatedness, as was found at the Yakima Barricade Borehole. These initial studies at the community level are promising and indicate that molecular techniques may be a valuable tool for unraveling regional microbial transport.

Subsurface Organic Fluid Flow

Robert J. Lenhard and C. Steven Simmons

The movement of nonaqueous phase organic liquids (NAPLs) through subsurface environments is poorly understood. A fundamental scientific understanding of NAPL flow and retention in subsurface environments is essential before NAPL-contaminated sites at DOE facilities can be cleaned up effectively. The accurate prediction of NAPL flow and transport is critical to designing effective remediation strategies. This project focuses on investigating mechanisms of NAPL flow and transport in complex, heterogeneous subsurface environments in order to develop a scientific foundation on which to predict field-scale NAPL flow and transport. The long-term goal of the project is to scientifically investigate the physical, chemical, and microbiological processes that govern subsurface NAPL behavior so that the knowledge can be employed in the risk assessment and cleanup of contaminated DOE sites.

FY 1993 Research Highlights

Research in FY 1993 focused on 1) corroborating FY 1992 experimental investigations of density-driven vapor flow using trichloroethylene (TCE), 2) investigating differences in retention between spreading and nonspreading NAPLs, and 3) evaluating a predictive relative permeability-saturation-pressure model, which is needed to forecast transient NAPL flow.

Density-Driven Vapor Flow

In FY 1993, a TCE-vapor migration experiment was conducted in a 1-meter by 2-meter rectangular cell that is approximately 8 centimeters thick. Experimental procedures were developed to extract very small quantities of gas (i.e., 10^{-6} cubic meters) in which TCE concentrations could be accurately measured. Quantities of gas extracted from the cell must be very small so that a vapor migration pattern is not induced by the sampling procedures. The experimental results are currently being analyzed, and a scientific paper that evaluates density-driven vapor flow as a significant subsurface transport mechanism will be submitted for publication in FY 1994.

NAPL Retention Characteristics

Previous investigations in this project showed that nonspreading NAPLs behave differently from spreading NAPLs in porous media. Nonspreading NAPLs are those that are immiscible with water, and the sum of the air-NAPL and NAPL-water interfacial tensions is greater than the air-water interfacial tension. Nonspreading NAPLs may form discrete droplets on water surfaces, whereas spreading NAPLs form a continuous layer. To investigate whether this difference in behavior yields distinctly different NAPL-retention characteristics, hysteretic fluid pressure-saturation measurements were conducted with a sandy porous medium. In addition, analyses were conducted to determine whether the retention characteristics for the nonspreading NAPLs can be predicted from measured fluid properties. Initial results suggest that the scaling of fluid retention measurements of air-water systems to predict NAPL-retention behavior of three-phase air-NAPL-water systems in porous media may not yield accurate predictions for nonspreading NAPLs. In contrast, such scaling for spreading NAPLs yielded accurate predictions of three-phase fluid retention characteristics.

Transient NAPL Flow

Relations among fluid relative permeabilities (k), saturations (S), and pressures (P) must be known to predict the transient movement of multiple fluid phases in subsurface environments. These relations are known to be hysteretic; that is, the relations differ depending on whether the fluid contents are decreasing or increasing. To test the ability of the hysteretic k-S-P model to predict NAPL flow and retention, the model was incorporated in a numerical multiphase flow computer code by a project of the Volatile Organic Compounds-Arid Integrated Demonstration (VOC-Arid ID) in DOE's Office of Technology Development. The computer code will be used by the VOC-Arid ID to assist in the development and evaluation of remediation technologies and strategies. In simulations of a one-dimensional NAPL flow experiment, a fluctuating water-table scenario was imposed to measure NAPL movement and retention in the subsurface as a result of a rising or falling water table. The code predictions were compared to the experimental measurements, and close agreement was obtained (Figure 1). These results suggest that the computer code can accurately predict subsurface NAPL behavior.

Future Research

During prior years, this project focused on clarifying the physical processes and mechanisms that govern subsurface NAPL behavior. Such phenomena as NAPL flow and retention were investigated experimentally and theoretically. The scientific knowledge obtained from these investigations, together with new ability to experimentally measure complex multiphase flow phenomena, will be used in the coming years to investigate the interdependence of physical, chemical, and microbiological processes and NAPL migration in the subsurface. This complex approach is necessary to understand how these processes are embodied in field-scale movement and retention of NAPLs. Specific phenomena to be investigated in FY 1994 include 1) the effects of microbiological growth (i.e., pore clogging and biosurfactant production) on NAPL flow, and 2) physical and chemical mechanisms governing NAPL flow (i.e., relations among relative permeabilities, fluid contents, and fluid pressures).

Radon Transport Modeling: Soil to Structure

Diana J. Holford, Glendon W. Gee, and Harley D. Freeman

Radon movement from soil to structure is the dominant natural cause of health risks from radon in the United States. Modeling of this transport is an ongoing effort at PNL.

In FY 1993, a user's manual was completed for the Rn3D finite-element code, version 1.0 (Holford 1993). The Rn3D model simulates gas flow and radon transport in variably saturated, nonisothermal porous media. The model is applicable to a wide range of problems involving radon transport in soil because it can simulate either steady-state or transient flow and transport in one, two, or three dimensions (including radially symmetric two-dimensional problems).

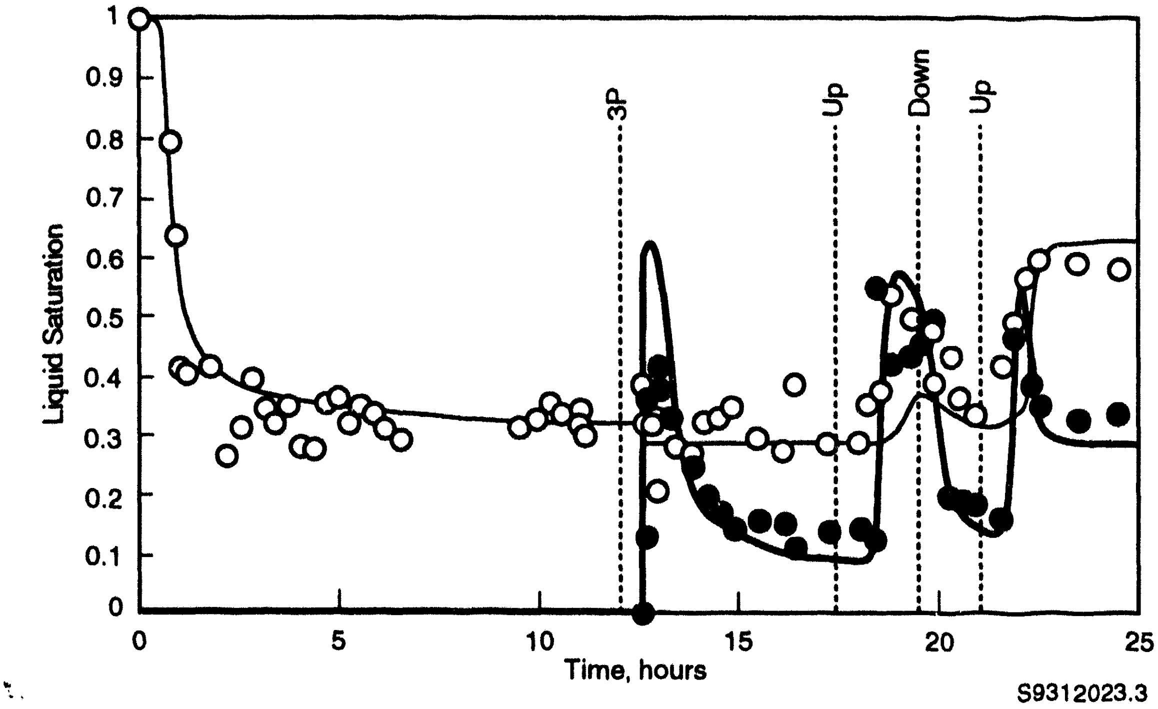

Figure 1. Measured and predicted water and NAPL contents for a measurement location of a controlled laboratory experiment. Hollow symbols are measured water contents, solid symbols are measured NAPL contents, and lines are predicted fluid contents (the thinner line is for water contents and the thicker line is for NAPL contents). The vertical broken lines indicate times at which the experimental boundary conditions were changed; 3P indicates the infiltration of NAPL from the surface, Up indicates raising the water-table elevation, and Down indicates lowering the water-table elevation.

The porous materials can be heterogeneous and anisotropic. The user's manual describes all mathematics related to the governing, boundary, and constitutive equations of the model, as well as the development of the finite-element equations used in the code. The manual also gives instructions for constructing Rn3D input files and executing the code, and describes all output files generated by the code. Five verification problems test various aspects of code operation, and example input files, FORTRAN programs for the analytical solutions, and plots of model results are provided. In addition, an example simulation illustrates the type of problem Rn3D is designed to solve. Finally, the manual provides instructions on how to convert Rn3D to simulate systems other than radon, air, and water.

The Rn3D finite-element model simulates pressure gradients in the soil and cracks caused by pressure changes at the ground surface. The resulting air flow drives the advective transport of radon gas, affecting the flux density of radon across the soil/air interface. The soil in the model may be heterogeneous and anisotropic. The pores in the soil may be completely dry or partially or completely filled with water. A uniform water pressure and temperature gradient can be specified in the model input. The cracks simulated by the model are evenly spaced and of a uniform width and depth.

In the model, molecular diffusion of radon-222 is assumed to be governed by Fick's law. The advective flow of gas is assumed to be laminar and governed by Darcy's law. A source of radon-producing radium-226 is distributed in the soil; the radium content may be distributed homogeneously or heterogeneously, and the actual radon emanation may be made a function of water content. Advection and diffusion are multidimensional; cracks do not extend down to the water table, and pressure changes will result in a complex, nonuniform flow field.

The Rn3D computer code simulates the transport of radon through various porous media, such as soil, rock, gravel, or concrete. This has enabled realistic simulation of radon transport from radium-bearing soils into buildings and determination of the soil properties that must be measured to predict radon entry rates into buildings (Owczarski et al. 1990). Among the most important parameters are gas permeability, porosity, and water content. The permeability of the soil affects the response of the soil to transient variations in pressure differentials between the soil and the atmosphere or buildings. The water content of the soil's void space greatly affects the rate of radon diffusion in soil, because the diffusion coefficient of radon in water is one-fifth that in air.

Natural variations in meteorological factors and soil properties contribute to uncertainty in subsurface model predictions of radon flux. When coupled with a building transport model, this will add further uncertainty to predictions of radon concentrations in homes. To assess the relative importance of these meteorological factors and the properties of the soil affecting the transport of radon, a statistical uncertainty analysis using the Rn3D finite-element numerical model was conducted (Holford et al. 1992a). The results indicate that important field measurements for more accurate predictions of radon fluxes are the presence of cracks (or other macropores), radium content, gas pressure gradient, and capillary pressure (which affects soil saturation and, ultimately, the strength of radon diffusion).

The Rn3D model is applicable to a wide range of problems involving radon transport in soil. For instance, the model has been used to simulate the relationship between barometric pressure variations and radon fluxes from a dry, cracked, gravelly sandy loam at a site near Socorro, New Mexico (Holford et al. 1992b). The Rn3D computer code has also been used in a two-dimensional study of the effects of winds on radon concentration profiles beneath a slab-on-grade house with dry gravel of various thicknesses surrounding the outer surfaces of the slab (Owczarski et al. 1991, 1992). This study confirmed that, in slabs underlain by gravel, winds can effectively dilute sub-slab radon concentrations. Results show that for a gravel depth of 0.1 meter, radon concentrations are reduced by up to 50% when the underlying soil is sand or loam, 60% when it is silt, and 90% when it is clay. With thicker gravel layers, reductions are even larger.

Future Research Issues

Development of the Rn3D model is now at a stage where validation is essential, requiring pertinent, high-quality field data. PNL is working with the Bonneville Power Administration (BPA), the Washington State Department of Health (WDOH), and the U.S. Environmental Protection Agency (EPA), which are funding Thomas J. Gerard and Associates, Inc., and Faytek, Inc., to investigate the effectiveness of passive radon vent stacks. This study provides an opportunity for PNL to obtain field data to validate the radon transport model. These additional data will enhance the overall study by providing a more fundamental understanding of the basic radon transport mechanisms through field and modeling studies. PNL will work with BPA, EPA, and the state to assist in interpreting the data obtained from the study. Data obtained from sensors installed for our purpose will be shared with the study sponsors, thus increasing the database of information on the parameters affecting passive stack performance.

The primary objective of the study is to investigate the effectiveness of using passive stacks to vent radon from beneath the foundations of houses. Passive stacks take advantage of the differences in density of air at different temperatures. Air beneath a house is cooler than the inside temperature of the house, but the air in the passive stack is heated, resulting in an upward flow of air that creates a positive pressure differential between the bottom and the top of the stack. This pressure differential will provide the driving force to move radon from beneath the structure.

Current data available from around the country are not sufficient for judging the effectiveness of the passive stack concept under the conditions found in Spokane, and so BPA, EPA, and WDOH have initiated this study to evaluate the effectiveness of the technique. Because many of the fundamental soil parameters required for input into Rn3D are being obtained as part of the study, this was an excellent opportunity for PNL to obtain field data for model validation.

In this study, each house site will be evaluated with respect to soil, geologic, hydrologic, and geographic characteristics. Soil samples from up to 40 sites will be stored and tested for particle size distribution, radium content, emanation fraction, air permeability, and bulk and specific density. The soil samples will be taken mostly from within the foundation perimeter of the homes to be built. Selected soil samples will be taken outside of the perimeter, and *in situ* air permeability measurements will be made at a number of locations at each house site. After the house is built, temperature, differential pressure, and radon sensors will be installed at a number of locations in the house. These sensors will allow monitoring of stack differential pressure and temperature, indoor and outdoor temperatures, differential pressure between the house and subslab floor, and times and durations of woodstove or fireplace use. Limited meteorological data (wind speed and direction and barometric pressure) will also be obtained at each house. The data from the sensors will be automatically logged on a datalogger, and the data will be periodically transmitted via phone line to a computer at PNL. The data will then be used as input and comparison data for modeling exercises to validate the Rn3D code.

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Technical Assistance to Coordination of Groundwater Research

Shirley A. Rawson

Under this project, PNL provides program management assistance to DOE's Subsurface Science Program. The project was initiated in FY 1992 to provide geochemical and hydrogeological information and advice on groundwater research to the Subsurface Science Program. Specific activities in FY 1993 included 1) technical representation of the Subsurface Science Program to intra- and interagency research committees and task forces; 2) participation in workshops and conferences where relevant scientific issues were addressed; 3) coordination of the physical, chemical, and microbiological aspects of groundwater research between subprograms of the Subsurface Science Program; and 4) technical coordination of hydrogeological and geochemical research performed as part of the Subsurface Science Program's Bacterial Transport Working Group.

Intra- and Interagency Committees and Task Forces

Major committee interactions involved those subcommittees that address the groundwater interests of the Committee on Earth and Environmental Sciences (CEES) of the Federal Coordinating Council on Science and Engineering Technology (FCCSET) and the Core Planning Group of DOE's Office of Technology Development *In Situ* Remediation Integrated Program. During FY 1993, the strategy document "Water Resources: A Research Strategy for Critical National Needs" underwent several reviews across DOE programs and this project contributed to reviews as requested by DOE's Office of Health and Environmental Research. Although in previous years, the Subsurface Science Program was represented at the Core Planning Group of the *In Situ* Remediation Integrated Program, this group did not meet in FY 1993.

By request of DOE's Office of Health and Environmental Research, the Subsurface Science Program was represented in the Office of Environmental Restoration and Waste Management's (EM's) standing working group to improve the process by which environmental restoration research and development activities are conducted within EM.

Subsurface Science Program Workshops

In March 1993 in Santa Fe, New Mexico, an overview of the scientific scope of the Subsurface Science Program and its Deep Microbiology subprogram was presented at the Subsurface Science Program's technology transfer workshop, "Aseptic Drilling, Sampling, and Sample Handling."

Coordination across Subsurface Science Program Subprograms

In early FY 1993, the review of proposals was coordinated for the Small Business Innovative Research (SBIR) Program. Reviews were solicited for several proposals addressing the deployment of chemical sensors in field pilot demonstrations.

Shirley Rawson (PNL) and a small working group of Subsurface Science Program investigators who adapted the research plan for the Strategic Environmental Research and Development Program (SERDP) to a draft subprogram plan for a Subsurface Science Program subprogram in subsurface heterogeneity. The group included Shirley Rawson (PNL), subprogram coordinators Ernie Majer (Lawrence Berkeley Laboratory) and Fred Brockman (PNL), and scientists John Cushman (Purdue University), Tim Ginn (PNL), Tony Palumbo (Oak Ridge National Laboratory), and Stephen Silliman

(Notre Dame University) and met several times in 1993 to define the scientific scope of a subprogram in subsurface microbial heterogeneity.

In March 1993, the Subsurface Science Program's Subprogram Coordinators convened to discuss program-wide research plans for FY 1993-FY 1994. At the request of the Office of Health and Environmental Research, an evaluation of scientific linkages across subprograms was presented to guide the development of multidisciplinary experiments at field sites in differing hydrogeologic regimes. The intent of the presentation was to alert subprogram coordinators to opportunities for collaborative research across subprogram boundaries.

During FY 1993, the project also supported Brian Shaw, who contributed to the planning and development of field research sites for Subsurface Science Program research into *in situ* microbial origins. Site-selection criteria were developed and scientific questions were formulated to guide field research to determine how long microorganisms might be able to survive in subsurface environments.

Bacterial Transport Working Group

The Subsurface Science Program initiated planning for a new subprogram in bacterial transport by establishing a Bacterial Transport Working Group in FY 1992. Shirley Rawson is co-coordinator with Aaron Mills, University of Virginia, of the working group, which includes current Subsurface Science Program investigators with theoretical and experimental interests in bacterial and biocolloid transport in natural aquifers. A draft plan for research in bacterial transport was presented in October 1992 for review and comment by participants in a DOE workshop entitled "Transport of Bacteria through Porous Media: Field-Scale Experiments." The small workshop included scientists with backgrounds in microbiology, hydrology, geochemistry, and geology. The consensus of the workshop participants was to emphasize forced-gradient transport experiments in a well-characterized hydrogeologic setting, probably on the east coast.

In 1993, a draft research plan for the bacterial transport subprogram was completed. The draft plan describes a subprogram in bacterial transport experimentation at the intermediate and field scales, with the goal of providing the fundamental science for understanding both field bioremediation and the transport aspects of microbial origins in the subsurface. The plan incorporates the details of an experimental plan for initial field experiments in bacterial injection at a site in Oyster, Virginia. Shirley Rawson visited the University of Virginia several times in 1993 to work with Aaron Mills, Janet Herman, and George Hornberger on the scientific scope of the subprogram plan and to develop the detailed field experimental plan. The first experiments are aimed at answering research questions on the mobility of several bacterial strains with different microbial characteristics in a chemically heterogeneous aquifer.

Other activities conducted as part of this project included peer review of research proposals for the Microbial Origins subprogram and other subprograms within the Subsurface Science Program, technical support in long-range program planning, and planning and coordination of individual subprogram meetings.

Terrestrial
Science

Terrestrial Science

The response of arid and semiarid landscapes to both natural and human-related stresses is a major concern for DOE lands, as well as for the nation. PNL has been conducting studies focused on understanding the fundamental mechanisms that control ecosystem functioning as a basis for evaluating the effects of ecosystem stress.

The dynamics of soil nutrients, water, gas flux across interfaces, and energy are being evaluated to understand how stress influences the efficiency with which arid land ecosystems process essential resources. This information will help provide an appropriate theoretical basis for understanding how a variety of factors, including global climate change, contamination, and environmental restoration activities affect DOE sites and activities.

Spatial and Temporal Variability of N_2O Flux Rates from a Shrub-Steppe Ecosystem

Daniel L. Mummey (Washington State University), Jeffrey L. Smith (U.S. Department of Agriculture - Agricultural Research Service), and Harvey Bolton, Jr.

Nitrous oxide (N_2O) influences the climate as a greenhouse gas, participates in the formation and destruction of ozone, and is a significant vector for the loss of nitrogen from terrestrial ecosystems. Interest in quantifying the rates of N_2O flux from terrestrial ecosystems has been stimulated by the finding that atmospheric N_2O concentrations have increased since monitoring began in the 1970s. However, quantifying N_2O emissions from terrestrial ecosystems is problematic because there is no methodology for effectively dealing with the high spatial and temporal variability associated with the processes responsible for N_2O formation. Quantification is further complicated by differences in nitrogen-cycling associated with different plant species and with interplant areas in ecosystems having spatially heterogeneous plant cover.

Recently, geostatistical methods have been found useful for analysis and interpretation of spatial relationships in ecological studies, including heterogeneous landscapes such as the shrub-steppe (Halvorson et al. 1994). Geostatistics provides a means of detecting and modeling spatial dependence (the degree to which samples collected close to one another are more similar than samples collected farther apart). In addition, geostatistics offers a means of producing unbiased estimates for unsampled locations across a study site when spatial dependence occurs, thus enabling the development of maps of spatial characteristics.

The current study was designed to determine how differences in nutrient cycling in soil around shrub-steppe vegetation interact with soil moisture dynamics to influence the spatial and temporal variability of N_2O flux. The specific objectives of this study were to 1) examine the spatial variability of the N_2O flux from soil and of soil NO_3-N and NH_4-N concentrations around the dominant vegetation in an undisturbed shrub-steppe ecosystem; 2) examine the yearly temporal variability of soil N_2O flux and soil inorganic nitrogen; and 3) estimate the total annual N_2O flux to the atmosphere from this shrub-steppe ecosystem, taking into account spatial variability.

The study site is located on the Arid Lands Ecology Reserve (ALE) within DOE's Hanford Site in south-central Washington. Between March 1992 and February 1993, N_2O samples were collected at 44 locations within three 2.4-meter x 2.4-meter plots centered on individual *Artemisia tridentata* shrubs. Parameters measured included N_2O flux, soil NO_3-N and NH_4-N concentrations, and soil moisture content.

FY 1993 Research Highlights

The N_2O flux for the March sampling (5% soil moisture) was typical of the spatial distribution of N_2O flux when soil moisture contents were low. The spatial distribution for N_2O flux was relatively random; no strong spatial relationships were visually detectable. Nitrous oxide flux for the 44 measurements taken in March averaged 0.17 kilogram of N_2O-N per hectare per year. The average soil NO_3-N and NH_4-N contents of 2 and 1.6 micrograms per gram of soil, respectively, were also typical for dry warm-season sample dates, which were not influenced by precipitation.

The spatial distribution of N_2O flux for the June sampling (9.5% soil moisture) was less random than that of the drier March sampling; high values are generally spatially associated with other high values. The average N_2O flux rate was 0.22 kilogram of N_2O-N per hectare per year. Soil NO_3-N and NH_4-N contents averaged 1.8 and 2.2 micrograms per gram of soil, respectively.

For the August sampling (13.5% soil moisture), recent precipitation onto dry soil was found to have resulted in distinct spatial patterns for N_2O flux. The flux of N_2O averaged 2.67 kilograms of N_2O-N per hectare per year, with average soil NO_3-N and NH_4-N concentrations of 19.8 and 13.5 micrograms per gram of soil, respectively.

The N_2O flux from plant-associated soil was significantly greater than that from interplant soil for the June sampling, but not for the March sampling (Table 1). Correlation between N_2O flux and plant-associated soil generally increased with soil water content. This may be due to an increase in microbial access to inorganic nitrogen when water contents are greater. There was more N_2O flux from plant-associated soil, which also had higher concentrations of inorganic nitrogen than interplant soil (Table 1).

At the August sampling, N_2O flux and soil NO_3-N and NH_4-N were significantly greater for plant-associated soil (Table 1). It is likely that after precipitation, plant-associated soil, with its

Table 1. Mean values and significant differences (determined using t test) of N_2O-N, CO_2-C flux, and soil NO_3-N and NH_4-N concentrations for soil samples associated with plants versus bare-soil-associated samples. Units are kilograms per hectare per year for N_2O-N and CO_2-C and micrograms of nitrogen per gram of soil for NO_3-N and NH_4-N.

	March			June			August		
	Plant		Bare	Plant		Bare	Plant		Bare
N_2O-N									
Mean	0.19		0.15	0.22		0.12	3.18		1.80
t		0.13			3.23**			3.87***	
CO_2-C									
Mean	1,220		980	8,900		5,900	38,000		36,400
t		1.31			2.62*			1.93	
NO_3-N									
Mean	2.30		1.60	1.64		1.29	25.90		9.37
t		1.44			1.34			4.20**	
NH_4-N									
Mean	1.67		1.41	2.50		1.80	15.80		9.66
t		1.00			2.17*			3.75**	

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*, ** , and *** signify significant differences between values at the 0.05, 0.01, and 0.001 levels of probability, respectively.

larger microbial biomass concentrations, releases more readily decomposable organic matter into the soil environment than interplant soil does, resulting in greater increases in inorganic nitrogen and subsequently in N_2O production (Table 1). This hypothesis is supported by the change in the ratio of soil inorganic nitrogen (i.e., extractable NO_3-N and NH_4-N) from plant-associated soil to soil inorganic nitrogen from samples farther away from plants. This ratio was 1.7 for the dry March sampling and 1.2 for the wetter June sampling. Twelve hours after precipitation in August, this ratio was 2.7.

Spatial covariance plots of N_2O flux for the March, June, and August sample dates are presented in Figure 1. The spatial covariance plot for the relatively dry March sampling (Figure 1a) suggests weak spatial dependence to a distance of about 1 meter. The Y-intercept suggests that over 80% of the variability is random and cannot be explained by spatial dependence between samples. The plot for the wetter June sampling (Figure 1b) suggests spatial continuity to a distance of 0.6 meter, with 50% of the variability being random and not explained by spatial dependence. These results indicate that N_2O flux from this ecosystem is partially spatially dependent at the scale of this study and that the relative variability that can be accounted for by spatial dependence increases with soil water content.

The spatial covariance plot for the August sampling date (Figure 1c) suggests that the pulse of N_2O flux that occurs after warm-season precipitation is highly spatially dependent, as is evidenced by spatial continuity to 0.6 meter and by more than 90% of the variability being accounted for by spatial dependence (indicated by the Y-intercept).

The highest N_2O flux rates occurred after precipitation events (Figure 2); however, the enhancement of N_2O flux and nitrogen mineralization after wetting dry soil is short-lived. A laboratory study using surface soil from this ecosystem (Mummey et al. 1994) showed that after wetting of dry soil, the spike of N_2O flux decreases rapidly after 24 hours and soil inorganic nitrogen concentrations return to pre-wet levels within days. This suggests that intense microbial competition for inorganic nitrogen rapidly reduces NH_4-N availability for nitrification. Nitrous oxide flux from this ecosystem is therefore limited by nitrogen availability, except immediately after precipitation onto dry soil.

Knowledge of the magnitude and duration of N_2O flux following warm-season precipitation has important implications for determining N_2O fluxes that are representative of seasonal conditions. Measurements taken soon after precipitation events would give inflated seasonal estimates of N_2O emissions. Conversely, failure to account for the pulses of N_2O flux that occur directly after precipitation events would result in underestimation of annual N_2O flux.

Measurements of N_2O flux obtained at times not following precipitation were highest early in the growing season, when soil moisture contents were high. During the late spring and summer, N_2O flux rates were relatively low and constant, except after precipitation (Figure 2). During the cold winter months, N_2O flux was below detection limits, even though soil moisture contents were relatively high and soil NO_3-N and NH_4-N averaged as high as 3.6 and 5.3 micrograms per gram of soil, respectively. This low value for N_2O flux indicates that N_2O production is severely limited by low temperatures during the winter months. Only soil water content was significantly correlated with N_2O flux ($r^2 = 0.81$, $p \leq 0.05$) at times other than the winter when sample times within 48 hours after precipitation were excluded.

Nitrous oxide flux rates were significantly correlated with soil NO_3-N ($r^2 = 0.81$, $p \leq 0.001$), NH_4-N ($r^2 = 0.98$, $p < 0.001$), and water content ($r^2 = 0.73$, $p < 0.05$) over the entire warm season (defined as the period when soil temperature at the 5-centimeter depth was greater than 15°C). Precipitation events with more than 0.4 centimeter over a 6-hour period were estimated to contribute an additional 2.74×10^{-3} kilograms of N_2O-N per hectare to the annual N_2O flux estimate. There were eleven such precipitation events during the year of this study. Integration of N_2O flux estimates for sample dates not following precipitation events

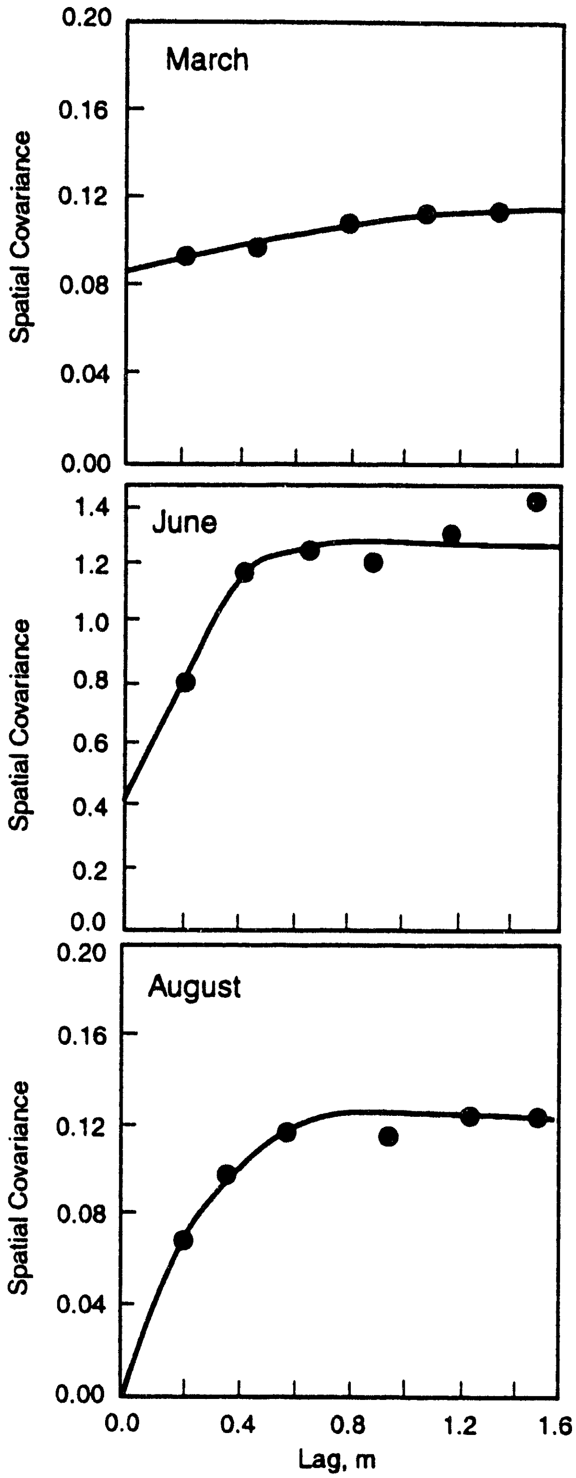

Figure 1. Spatial covariance plots of N_2O data from the a) March, b) June, and c) August sampling dates.

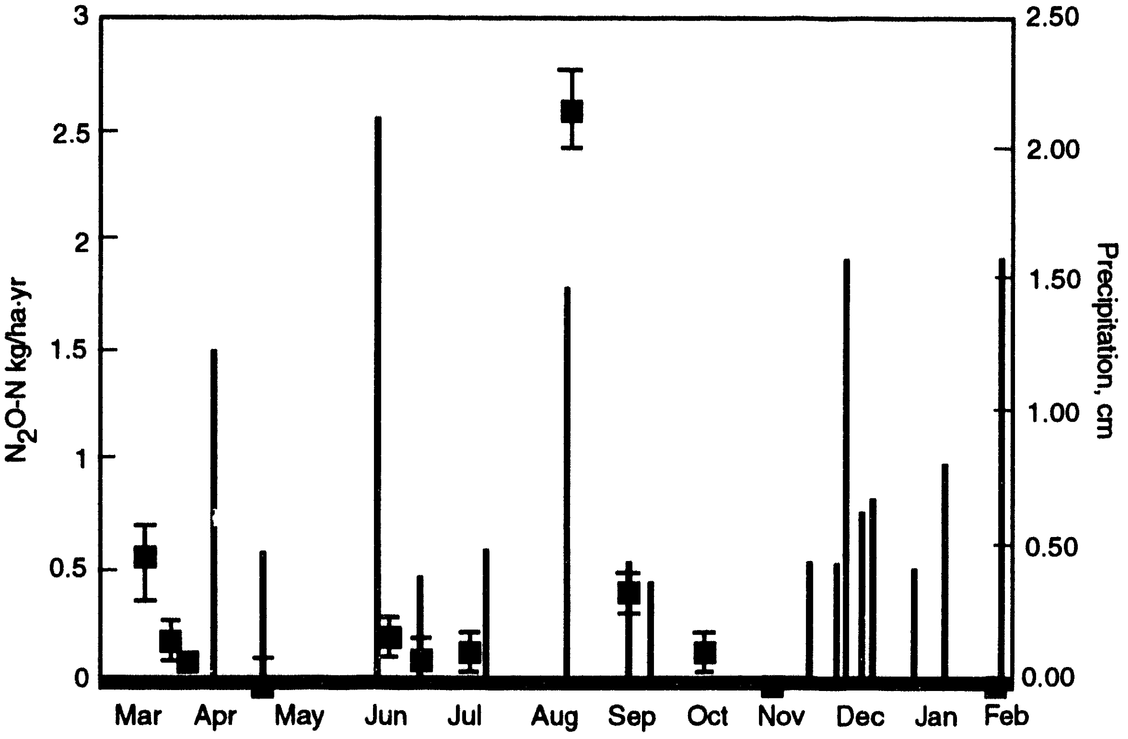

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Figure 2. Precipitation events during 1992-1993 study year (vertical lines) and periodic landscape estimates of N_2O flux from this shrub-steppe ecosystem (solid squares; bars indicate standard deviations).

yielded an annual estimate of 0.12 kilogram of N_2O-N per hectare for the study area. Addition of the estimated N_2O-N flux after all warm-season precipitation events to the annual estimate yielded a total estimate of 0.15 kilogram of N_2O-N per hectare per year. If these values are correct, N_2O flux occurring within 48 hours of precipitation events is responsible for 21% of the total N_2O flux to the atmosphere from this shrub-steppe ecosystem.

This N_2O flux estimate is less than the average 0.21 kilogram of N_2O-N per hectare per year estimated for a wetter Wyoming shrub-steppe ecosystem (Matson et al. 1991), and more than the 0.10 kilogram of N_2O-N per hectare per year simulated for a shortgrass steppe (Parton et al. 1988) and the 0.10 kilogram of N_2O-N per hectare per year estimated for Wisconsin prairies (Goodroad and Keeney 1984).

If estimates are representative of all the U.S. shrub-steppe (covering 6.45 x 10^7 hectares), a total N_2O flux of about 9.7 x 10^9 grams per year would occur. Total annual N_2O emissions from all undisturbed terrestrial ecosystems are reported to range from 7 to 16 x 10^{12} grams per year (Bowden 1986). Although the estimate resulting from the current effort is based on limited data from a small undisturbed region of the shrub-steppe, it does suggest that undisturbed shrub-steppe ecosystems make relatively small contributions to global N_2O atmospheric contributions when compared to wetland ecosystems or temperate or tropical forests (Bowden 1986; Matson et al. 1990). Nonetheless, this study also demonstrates the importance of considering spatial variability and warm-season precipitation events when estimating annual N_2O flux from shrub-steppe ecosystems.

Our study was conducted on an undisturbed shrub-steppe ecosystem. Large segments of shrub-steppe ecosystems have been subjected to human-induced disturbances, including invasion by alien species, farming, livestock grazing, mining, and urbanization (Beedlow et al. 1988; Bolton et al. 1990). Shrub-steppe ecosystems will also be subjected to changes in temperature and precipitation as a result of global climate change. Further research is needed to understand and quantify the effects of disturbance and global climate change on N_2O flux and nutrient cycling processes in shrub-steppe ecosystems.

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Remnant Resource Islands in a Burned Shrub-Steppe Site

Jonathan J. Halvorson (AWU-NW), Harvey Bolton, Jr., and Jeffrey L. Smith (U.S. Department of Agriculture - Agricultural Research Service)

Artemisia tridentata (big sagebrush) is a prominent shrub of the cool desert environment encompassing the Hanford Site and is known to affect the soil in which it grows. For example, we have measured higher concentrations of total carbon, total nitrogen, and inorganic nitrogen and higher rates of nitrogen cycling in soil beneath *A. tridentata* than in the soil between plants (Bolton et al. 1990, 1993; Halvorson et al. 1994). These zones of comparatively high concentrations of nutrients such as carbon and nitrogen and other elements are termed resource islands. To date, most measurements of resource islands have concentrated on live plants, and little is known about the fate of resource islands following the death or removal of the overlying vegetation.

The specific consequences of removing the overlying plant will be influenced by the particular plant-soil system involved, the method of plant removal, and the nature of the existing resource island patterns. Three scenarios are possible: Resource islands may diminish once the influence of the plant ceases. This is likely given that heterotrophic soil microorganisms consume easily utilizable carbon substrates in the soil, leaving the less available components of plant litter and soil organic matter. Furthermore, nitrogen and other nutrients would be lost from the zones of high concentration by such physical and biochemical processes as leaching and denitrification. Alternatively, if there is a large pool of resources stored in the soil or if soil microbial populations are stable, the resource island may persist unchanged for a relatively long period of time. Finally, if the relatively high concentrations of elements in the soil perpetuate the growth of other plants or nitrogen-fixing microorganisms, a resource island may become self-perpetuating.

The objective of this study was to detect remnant or "ghost" resource islands in the shrub-steppe environment. Ghost islands are likely to occur in the shrub-steppe for several reasons: Resource islands are known to exist for several soil parameters under individual *A. tridentata* and can be clearly delineated (Halvorson et al. 1994). The removal of overstory *A. tridentata* by wildfire is a natural episodic phenomenon in the shrub-steppe that removes the aboveground biomass without damaging the belowground portions of the plant (Beedlow et al. 1988). Finally, precipitation is comparatively low, which may serve to preserve ghost island patterns in the soil.

This study was conducted at the Arid Lands Ecology (ALE) Reserve on the Hanford Site in south-central Washington. We concentrated on a location where burned and unburned sites abut, separated only by a narrow unpaved road that had acted as a firebreak. An unburned stand of mature *A. tridentata* was located on the western side of the road. To the east was an area where all shrubs had been removed by fire in 1984 (Link et al. 1990). On April 16, 1993, we sampled surface soil (0-5 centimeters) under 10 mature *A. tridentata* and recorded average canopy radius and height. Samples were collected from locations directly beneath each plant (0), and at 20, 40, 60, 80, 100, and 120 centimeters along four transects oriented toward the cardinal directions. On the burned site, samples were collected in a similar fashion, except that transects originated from ten burned *A. tridentata* stems (stumps). For each living plant or stump, the four samples collected at each distance away from central axis (one for each of the cardinal directions) were combined. In the lab, these bulked samples were sieved (through 4-millimeter mesh) and mixed ($n = 140$). Plant biomass, separated from each sample during sieving, was recorded for all locations except the transect origin (0).

Each sample was analyzed for biological and chemical properties. Total nitrogen was measured using mass spectroscopy. Total inorganic nitrogen in soil was extracted with 2 M KCl and analyzed colorimetrically. Soil pH and electrical conductivity were measured in a 1:1 slurry of soil:deionized water. Soil microbial biomass carbon was estimated from the 3-hour respiratory response of soil to glucose amendment, as measured by gas chromatography and equations developed by Anderson and Domsch (1978). During measurement for soil microbial biomass carbon, control samples (no glucose added) were prepared, incubated, and measured for cumulative soil respiration at 3 and 24 hours. The 3-hour values were used together with the estimates of soil microbial biomass carbon to estimate metabolic quotient (qCO_2) or the amount of CO_2-C produced per unit of soil microbial biomass carbon (Anderson and Domsch 1986, 1990; Insam and Haselwandter 1989). The 24-hour values were used as an estimate of soil respiration. In a separate incubation, cumulative net soil respiration and nitrogen mineralization were measured after a 7-day anaerobic incubation (40° C, in dark), as described by Keeney (1982).

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Overall, samples from the unburned side of the road contained significantly more total nitrogen, total inorganic nitrogen, and soil microbial carbon; had significantly higher electrical conductivity, a higher rate of soil microbial respiration, and higher anaerobic carbon and nitrogen mineralization; and respired more carbon per unit biomass (Table 1). However, identical soil pH values were observed in burned and unburned sites, and plant biomass was about the same, suggesting that grass species have not been favored by the removal of *A. tridentata*. This interpretation, however, does not take into account the unrecorded luxuriant growth of grass observed overlying the location of some burnt stumps, which did not occur under mature *A. tridentata*.

More detailed analysis revealed that the differences between mean burned and unburned samples were greatest in samples collected near the plant (e.g., Figure 1). By contrast, samples of burned or unburned soil were not distinguishable at locations more than about 50 centimeters away from the transect origin. This distance corresponds to the average radius of a living *A. tridentata* (51.5 ± 1.8 centimeters), suggesting that the resource island effect was affected more by the removal of the plant than by the fire.

Resource islands were observed under living *A. tridentata* for all soil properties. For example, significantly higher total nitrogen concentrations were measured close to the plant

Table 1. Mean (standard error) values and the probability that these means are statistically different for soil parameters measured in adjacent burned and unburned sites (n = 70).

Property	Unburned	Burned	Probability
Total N[(a)]	1,190 (±88)	882 (±26)	<0.01
Total Inorganic N[(a)]	2.5 (±0.3)	1.4 (±0.1)	<0.01
pH	7.17 (±0.04)	7.17 (±0.04)	NS[(b)]
Conductivity[(c)]	1.34 (±0.09)	1.03 (±0.02)	<0.01
Soil Respiration[(d)]	1.53 (±0.11)	1.00 (±0.03)	<0.01
Soil Biomass C[(a)]	815 (±50)	577 (±14)	<0.01
qCO_2[(e)]	4.51 (±0.16)	3.91 (±0.11)	<0.01
Net anaerobic N[(f)]	58.8 (±3.4)	45.3 (±1.7)	<0.01
Net anaerobic CO_2-C[(f)]	79.9 (±3.9)	56.4 (±1.5)	<0.01
Plant biomass[(g)]	31.3 (±2.8)	28.1 (±2.7)	NS[(b)]

(a) milligrams per kilogram.
(b) not significant.
(c) x 100 µS per centimeter.
(d) milligrams CO_2-C per kilogram dry soil per hour.
(e) CO_2-C per gram soil microbial biomass C per hour.
(f) milligrams per kilogram per week.
(g) grams.

(Figure 1a), probably because of the accumulation of nitrogen by the plant and inputs into the soil through litter fall, which ranges from 10 to 20 grams per square meter per year (Rickard and Vaughan 1988). The significantly higher soil microbial biomass carbon under living *A. tridentata* (Figure 1b) suggests that other resources, such as carbon, are available there that can support higher or more active populations of heterotrophic microorganisms. High concentrations of soil microbial biomass carbon were significantly and positively correlated ($p \leq 0.001$) with soil respiration and anaerobic carbon and nitrogen mineralization, indicating that these locations are capable of higher rates of mineralization. Soil pH was lowest directly beneath living *A. tridentata* but was significantly higher only 20 centimeters away (Figure 1c). Comparatively low pH, or alternatively, a higher concentration of H^+ ions, could result from nitrification of soil organic material accumulated under *A. tridentata* (Mummey et al. 1994), the actions of roots (Stumm 1992), or the action of stem flow. The significantly higher pH observed at 20 centimeters may be the net result of these processes balanced against wind deposition and the accumulation of base cations in the soil caused by the decomposition of leaf litter. *A. tridentata* leaves contain a concentration of base cations (Rickard and Vaughan 1988). The significantly higher electrical conductivity observed near the living *A. tridentata* (Fig. 1d) is probably due to accumulations of H^+ ions in the soil and base cations from the decay of leaf litter.

Data taken from the burned site show that resource islands have persisted in locations of *A. tridentata* that were burned by the fire in 1984, but these resource islands are not expressed to as great a degree as those under live *A. tridentata*. The concentrations of soil variables near the locations of burned *A. tridentata* (as indicated by the presence of a charred stump) were smaller than under live *A. tridentata* and did not vary as much with distance from the plant axis. However, the patterns of soil variables in these remnant or ghost resource islands were distinctive. For example, significantly higher concentrations of total nitrogen and soil microbial carbon and significantly higher electrical conductivity were observed in soil collected from the location of a stump than at distances farther away (e.g., Figures 1a, b, d). These patterns indicate that resources are sequestered in soil organic matter and the soil microbial biomass. Although it did not change significantly with distance from a stump, the pattern of pH associated with burned plants was identical to that observed for live *A. tridentata* (Figure 1c).

Assuming that resource islands under *A. tridentata* were similar on both sides of the road before the fire, it can be concluded that resource islands at the ALE site are not self-perpetuating, but are closely coupled to the influence of a living plant. However, even without the inputs and influence of a live *A. tridentata*, ghost resource islands may persist for several years around the former locations of plants, resulting in zones of significantly higher concentration of total nitrogen, electrical conductivity, and microbial activity. These patterns for different soil variables or microbial processes would not be expected to diminish at the same rates.

Ghost islands may be a significant source of variability in soil properties that may be difficult to account for, because they may not be readily detectable. The recognition of the ghost islands may be important for determining the ecological or environmental consequences of the removal of *A. tridentata* or for understanding the scale of variability of soil properties on sites that have been denuded by fire or cutting.

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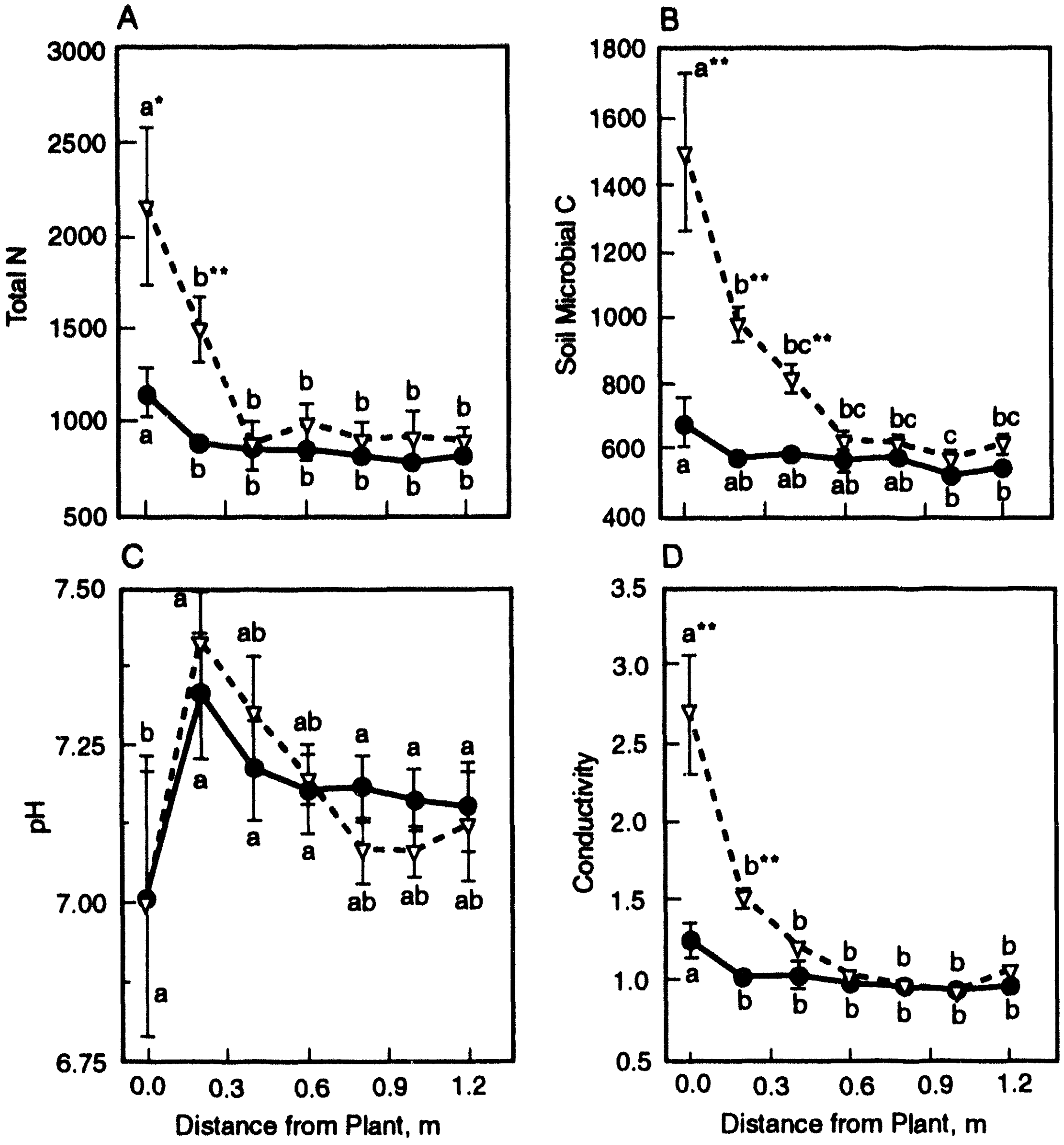

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Figure 1. Concentrations of a) total nitrogen (milligrams per kilogram soil); b) soil microbial biomass carbon (milligrams per kilogram soil); c) soil pH (1:1 in deionized water) and d) electrical conductivity (x 100 μS per cm) for soil samples collected under unburned *Artemisia tridentata* (open triangle) or around a burned *Artemisia tridentata* stump (closed circle). Each value shown is the mean calculated from 10 samples (± the standard error). Significant differences between burned and unburned locations were determined with a t-test and are denoted with asterisks (* indicates $p < 0.5$, ** indicates $p < 0.01$). Significant differences between locations within a site were determined with Tukey's least-significant difference test and are denoted by letter.

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The Role of Water in Competition Between Deep- and Shallow-Rooted Grasses

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In arid environments, the availability of water is a major factor in determining plant community composition and characteristics. The limited absolute quantity of water, the pattern of precipitation events, and the resulting distribution of water in the soil all influence which plants will prosper on a site and how competition will occur. At the same time, plant activity largely determines the depletion and distribution of water in the soil.

In the Columbia Basin, plants must compete for water that falls predominantly during the fall and winter. Two growth patterns may aid plants in competing for water: 1) early growth, as typified by the winter growth and very early flowering of *Bromus tectorum* L. (cheatgrass) and *Poa* *sandbergii* Vasey (Sandberg's bluegrass), and 2) deep rooting, which allows access to water not available to shallower-rooted plants, as seen in the deep-rooted *Artemisia tridentata* Nutt. (big sagebrush) and *Pseudoroegneria spicata* (Pursh) Scribn. & Smith (bluebunch wheatgrass) (Harris 1967). On the Hanford Site, shallower-rooted plants tend to show early growth and flowering in the spring, while deep-rooted plants extend growth into the summer months (Rickard and Vaughan 1988). During the spring, as plant activity increases and precipitation decreases, the soil dries from the surface down. As a result, shallow-rooted plants often complete flowering while competing for water in soils that have water potentials as low as -4.0 MPa at a depth of 25 centimeters in early May (Link et al. 1990). Recently, hydraulic lift has been observed in many plants, including sagebrush and bluebunch wheatgrass (Caldwell 1990). Hydraulic lift is a phenomenon in which, at night, water absorbed by deep roots in moist soils is leaked back into the drier shallow soils by surface roots. The subsequent appearance of that leaked water in the shoots of nearby plants indicates that the competitive atmosphere between shallow-rooted and deep-rooted neighbors may change as the soil dries near the surface (Caldwell and Richards 1989; Corak et al. 1987). Late in their life cycles, shallow-rooted plants such as *P. sandbergii* may actually benefit from hydraulically lifted water leaking from deeper-rooted plants.

Underground competition between the deep-rooted perennial grass *P. spicata* and the shallow-rooted perennial grass *P. sandbergii* was examined on the Hanford Site's Arid Lands Ecology (ALE) Reserve using root separation techniques. Both short-term and long-term effects of competition for water on *P. sandbergii* were observed. In addition, hydraulic lift was specifically investigated through measurements of soil water content and plant water stress.

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In a stand dominated by *P. spicata* and *P. sandbergii*, large polyvinyl chloride (PVC) pipes or tubes, with an inside diameter of 25 centimeters, were driven 50 centimeters into the ground around *P. sandbergii* individuals to isolate the *P. sandbergii* roots and prevent their interaction with surrounding *P. spicata* roots without damaging the *P. sandbergii* root system. Twenty-four sites were chosen; eight were randomly assigned as controls, tubes were inserted at eight on March 29, and tubes were inserted at the last eight on May 11.

Immediate effects of tube insertion on *P. sandbergii* plant water status were examined by comparing control and treatment predawn xylem pressure potentials both before and after tube insertion. Plant water status was measured using the pressure-bomb technique. Soil cores were also taken near the subject plants before and after tube insertion to measure any detectable differences in soil water levels after hydraulic lift had been cut off by tube insertion. Gravimetric soil water content and soil water potential were measured at six depths (2.5, 7.5, 15, 25, 35 and 45 cm). Soil water content was calculated after drying soil samples for 48 hours at 105°C. Soil water potential was measured using a ten-chambered ceramic bead soil psychrometer.

The long-term effects of releasing the *P. sandbergii* plants from competition with *P. spicata* was measured by observing phenology over time. From March 29 until the completion of flowering and vegetative growth in mid-May, phenological stage was recorded every other week. The number of flowering stems per individual and the heights of flowering stems and vegetative canopy were also measured. The rate of vegetative senescence was analyzed by taking a random sample of individuals from four control and four early-tube sites on May 12. After drying for 48 hours at 55°C, samples were analyzed for proportion of vegetative biomass 1) still green, 2) senescent and turning red, and 3) completely senescent and brown.

After tube insertion, predawn xylem water potential was significantly ($p=0.0464$) lower in the treatment condition than in controls, and controls showed no change in measures on consecutive days (Figure 1). Soil water content was

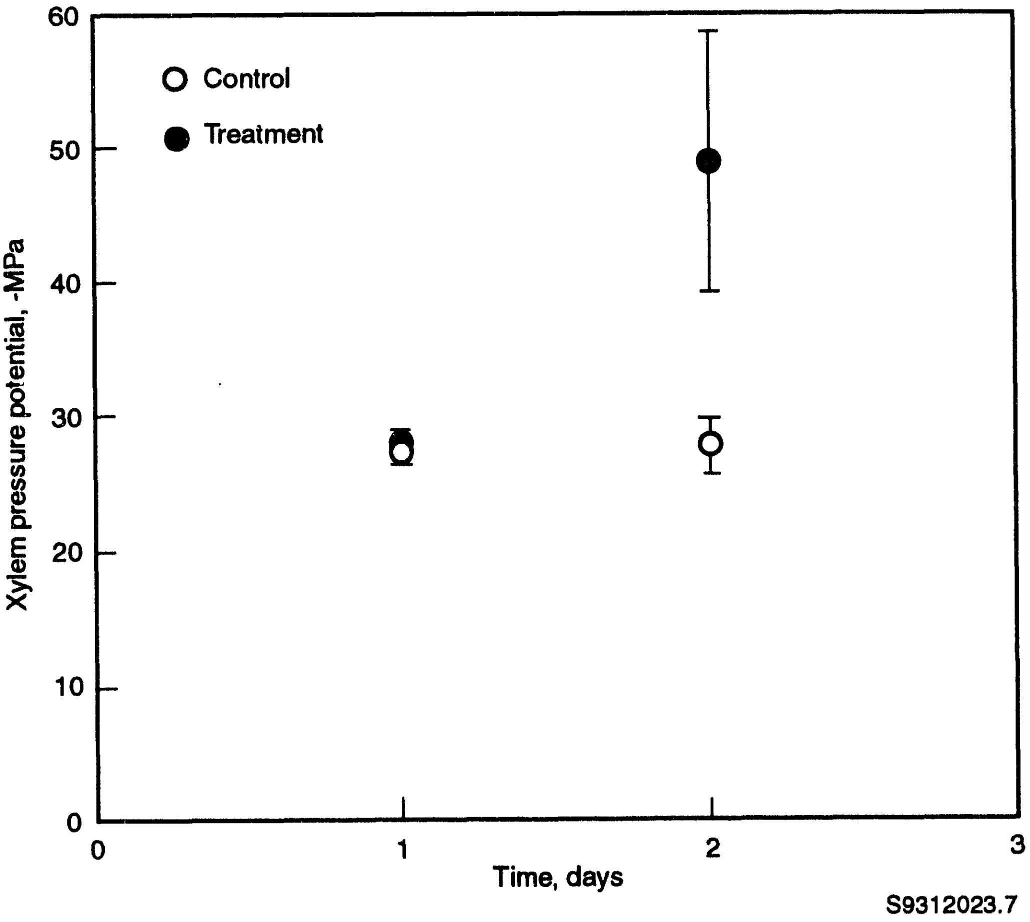

Figure 1. Xylem pressure potential of floral stem material of *Poa sandbergii*. Error bars represent one standard error around the mean.

measurably less (the soil was drier) on consecutive days over all depths for the late set of tubes compared to controls (Figure 2). The treatment effect was assessed by estimating the intercept term of a linear regression against depth through the treatment data (Figure 2). If the intercept term is different from 0, it can be concluded that the effect is significant. The intercept term, $b_0 = -0.00508 \pm 0.00198$, was significantly ($p = 0.016$) different from 0. Furthermore, all *P. sandbergii* plants within tubes inserted late in the growing season became inactive within four days of insertion, although controls were still succulent. These results indicate that hydraulic lift by *P. spicata* may release significant quantities of water to upper soils and that *P. sandbergii* plants may develop a dependence on this water supply.

In contrast, *P. sandbergii* plants within tubes inserted early in the growing season showed extended vegetative growth compared to untreated plants. On May 12, plants within early tubes still had an average of 35.4% of dry vegetative biomass classed as green material, while controls had only 3.6% green biomass. At that time, the plants from early tubes had 10.3% senescent red and 54.3% brown material, compared to 29.9% red and 66.5% brown in control plots. All of the early-treated sites maintained green material for at least another three weeks after the May 12

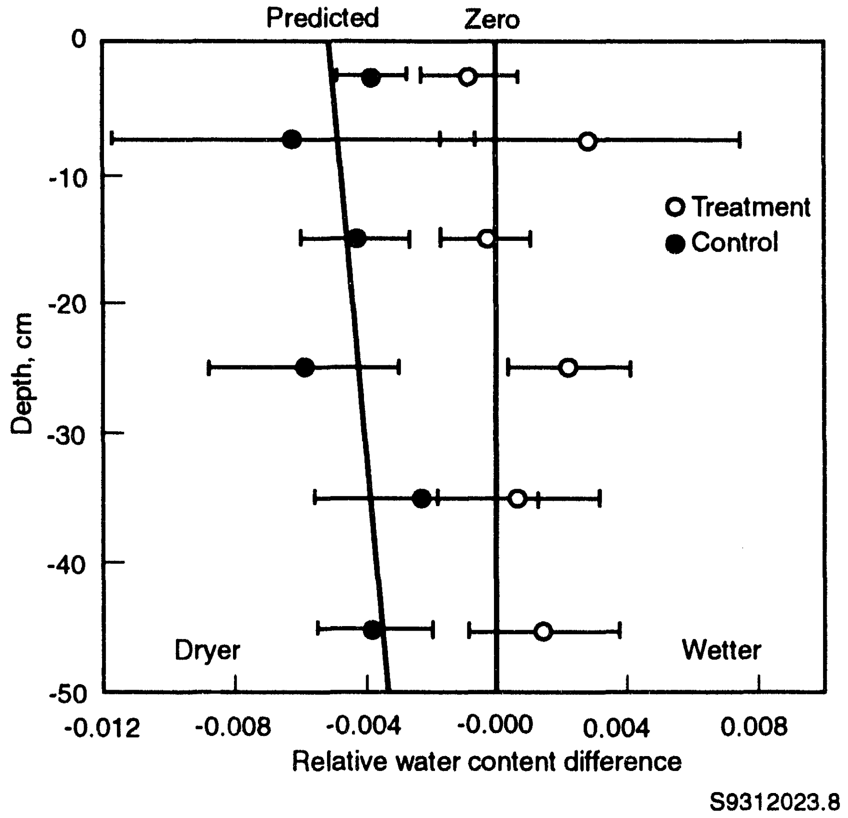

Figure 2. The difference between gravimetric soil water contents gathered on day 1 and day 2 after tube insertion around *Poa sandbergii* plants. Error bars represent one standard error around the mean.

sampling. However, no differences in flowering and canopy height were observed, and flowering phenology was not changed for the treated plants. Soil water content was significantly higher in sites with early tubes at all depths except 2.5 centimeters. Exclusion of *P. spicata* roots from soils around *P. sandbergii* apparently reduces total water use within the top 50 centimeters. This reduction in competition for water as well as other soil resources may allow the extended vegetative growth of *P. sandbergii* individuals.

Future Research

Underground competition between *P. sandbergii* and *P. spicata* appears to significantly shorten the vegetative activity of *P. sandbergii* in the spring. However, evidence also suggests that, as upper soils dry out, hydraulic lift by *P. spicata* may be an important source of water for *P. sandbergii* individuals. In 1994, similar root exclusion studies will be conducted to examine effects on soil water content, *P. sandbergii* water status, and gas exchange.

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Designs for Environmental Field Studies

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Contributors

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Because FY 1993 was the final year for this project, efforts were devoted to completing long-term studies of the relevance of sampling to ecological experimentation and some aspects of designing ecological experiments. The research on sampling (Eberhardt and Thomas 1991) was concluded by demonstrating the use of observational sampling as a means of studying the population dynamics of large mammals. Experimental design research was carried out to describe the philosophical basis for and relevance of some newer mathematical and statistical methods applied to such problems.

For ecosystem-level experiments to succeed, two major limitations must be addressed. The first is that replication is difficult or even impossible (replicates are expensive or unavailable), which means that statistical power is insufficient to detect meaningful ecological differences. Pseudoreplication, an approach that is sometimes used, results in a faulty field experiment, because subsamples are mistakenly substituted for true replicates. This substitution results in 1) error variances that are too small and 2) the detection of too many significant treatment effects. Second, experiments on intact, functioning ecosystems are not logistically practicable when testing multiple variables. Instead, parts of systems or model ecosystems, such as microcosms of various kinds, must be used. However, most inferences about ecosystems based on these approaches are very weak. This project therefore examined the available quantitative statistical methodology that could be modified or adapted to strengthen such inferences.

Design of Ecosystem Experiments

Because ecosystem experiments must not only detect change but also assign cause, a literature review was conducted to identify causal inference and statistical methods that are specific to experiments when replication is impossible or impractical. To provide the independence between treatments and units that is needed for the assignment of cause, the scientific method applies physical randomization of units to causes (or treatments). Holland (1986) restricted "causes" to only those things that could, in principle, be treatments in experiments. This restriction eliminated from being a cause anything that cannot be randomly applied to units within the defined population, including such attributes as gender, economic status, and climate. So-called natural experiments (e.g., the Yellowstone fires of 1988 and the 1980 eruption of Mount St. Helens), for which the observer has no control over the application of the "treatment," have more uncertainty associated with the ability to make a causal inference than controlled experiments have. This uncertainty results because there is the chance that the difference already existed in the various unit subsets and that it was not due to the "treatment." This interpretation involves a strict definition of cause and is not necessarily the interpretation used by philosophers, economists, and social scientists. Use of this definition of cause greatly impacts the development of statistical methods for causal inference associated with landscape, regional, or ecosystem change.

The inability to manipulate and replicate experiments at the level of landscapes or ecosystems has frustrated the development of ecosystem sciences beyond the level of description and association. Hargrove and Pickering (1992) suggest several alternative approaches to analysis when treatment replication is impossible, as in natural field experiments. They rely heavily on the differences observed before and after the natural event occurs. However, the differences observed cannot be attributed solely to the "treatment" without making strong untestable assumptions. In view of this, Hargrove and Pickering (1992) suggest that by combining the information from smaller-scale controlled experiments with the observations from the natural experiments, competing causes can be eliminated and the strength of the causal inference and its relation to the natural event increased.

Other approaches have also been considered when treatment replication is not feasible. Again, these approaches have a strong reliance on pre- and post-manipulation observations. For instance, time-series models can be useful in detecting nonrandom change, which can then be used to implicate the cause. Because data for long time series are required to fit these models, simpler randomization tests may be more appropriate for ecological data. Unfortunately, with either of these methods, the final determination of cause must be made nonstatistically: the inference is left to a subjective weighing of evidence and untestable assumptions.

An intriguing analytical approach to causal inference for landscapes or ecosystems is the estimation of the probability that a particular hypothesis or model is more likely than all the competitors. This methodology employs Bayesian statistics to calculate the likelihood of obtaining the observed data for a given set of distinct alternative explanations (or models). However, problems arise when more than one alternative model provides likely explanations. Combining information from several manipulations and small-scale controlled experiments within the likelihood calculation may provide a more robust evaluation.

The Bayesian calculation also relies on the assignment of prior probabilities that the model is correct. The effect of these prior probabilities, including the sensitivity of the posterior probabilities to slight changes in the prior probabilities, must still be evaluated. Another problem is that one can never be certain that all possible explanations have been evaluated. Indeed, the determination of the set of distinct alternative models to evaluate ecosystem adjustment could be the greatest challenge the researcher faces.

Bayesian concepts can also be used to choose among competing sampling designs for assessing ecosystem transport processes (Skalski and Thomas 1984). For example, when no information about a transport process is available, a grid design with a random starting point on the landscape or surface allows estimation of spatial variability and identification of possible flow routes. However, if the process is already known to flow from each source to another via a fairly direct path, a grid sampling design would be inefficient and costly. In the case of a transport process entailing outward migration from a source for which the boundaries of the flow are of interest, a grid sampling design would again be inefficient. In both the second and third cases, designs specific to the needs of the research can be evaluated based on a loss function to maximize the information gained against the cost of missing the boundary or channel.

Sampling designs required for defining and understanding ecosystem transport processes must also take into account prior information about the process under study. Often the direction of energy exchange is known, but the impact of changes to either the quality or the quantity of the energy or information flow to each component of the system is not known. Sampling at multiple scales of resolution using designs incorporating knowledge of the transport pathways would enhance understanding while minimizing the loss of spatial information and cost of ineffective sampling.

Methods of Evaluating Ecosystem Experiments

The quantitative methods and ideas that have appeared in the literature over the past few years can be categorized as theory and models, ecosystem design, and analysis. Hierarchical methods, cellular automata, metapopulations, and fractals come into the category of theory and models. Several forms of intervention analysis and a stochastic modeling approach that is based on superpopulation statistics are also categorized under theory and models. Meta-analysis is classed as an analysis technique.

Theory provides a framework within which experiments can be designed. For instance, postulating how a system is controlled allows the design of experiments to test hypotheses. One of the theories that postulates how complex systems are organized is hierarchical theory. In hierarchical theory, each complex system is viewed as a collection of levels occurring at different scales in space and time; the theory recognizes that the controlling processes are regulated simultaneously at several spatial and temporal scales. This organizational framework can be used to design ecosystem experiments by focusing on the relationships and weaknesses associated with experiments at various levels, such as space and time constraints at higher levels of organization and higher variability at lower levels.

Metapopulations, cellular automata, and fractals are used to analyze how systems are controlled (Taylor 1990; Hogeweg 1988; Milne 1992). The basis of cellular automata encompasses metapopulations and patch-occupancy models. The use of cellular automata involves theorizing how a system works through a set of rules; the system is visualized as a grid of cells, with the behavior of any one cell dependent on the state of the surrounding neighborhood of cells. The effect of these rules can then be simulated and compared to actual populations. Like cellular automata, fractals are used to model space-oriented ecological processes. Fractals are mathematical representations that provide measures of spatial dependence at several scales. Analysis of the fractal dimensions has been used to identify the sources and importance of variation in systems.

The various possible designs for ecosystem experiments involve a form of time-series analysis called intervention analysis. The basic design in ecosystem research is called Before-After-Control-Impact-Pairs (BACIP) and involves a paired design that is monitored before and after a perturbation. Modifications of the design include randomization intervention analysis, in which the distribution of the before/after differences is estimated by random permutations of the sequence of interecosystem differences. Stewart-Oaten et al. (1992) have pointed out many of the flaws in this design and suggest that many technical problems still exist. In addition, the BACIP design does not address time-treatment interactions. To resolve that problem, a "staircase" design, in which the treatment is initiated at different times on the treated areas, could be used. However, this method still incorporates many of the design features of BACIP that are troublesome, including pseudoreplication.

One of the assumptions of intervention analysis is that the control and treatment ecosystems are in similar successional states, that is, if no intervention occurred, the rate of change in the variable of interest would be the same for both systems. Loehle and Smith (1990) believe that this assumption is usually false, that "the path taken by a successional system has a stochastic element," and that succession is sensitive to initial conditions. Information about the rate of change occurring in the control should be collected before constructing a model for characterizing the behavior of the control through time. By adding a stochastic element, the result is a "stochastic envelope" with which the treatment system can be compared.

The term meta-analysis describes the statistical procedures for retrieving, selecting, and combining results from separate, independent studies. The method has only recently begun to be used in ecology (Gurevitch et al. 1992), but it has been used extensively in medical research. In meta-analysis, the outcomes of different studies are examined to test whether they are consistent with one another and whether together they demonstrate an effect that is large, moderate, small, or not significantly different from zero.

Field Studies (Observational Sampling)

In connection with long-term studies of the relevance of sampling to ecological experimentation, several research areas concerning sampling of wild populations were investigated. One of the studies involved sampling for reproductive rates for species that do not reproduce on a fixed annual schedule. Sea otters (*Enhydra lutris*) are a good example of such a species. Monte Carlo simulation studies were conducted to determine whether direct measurement of the interval between births provided a useful measure of birth rate in lieu of direct calculation from a record of "births per otter-year". Results of extensive simulations indicated that this interval method is biased and therefore should not be used to determine birth rate. Such data may nonetheless be useful for other research that is not concerned with population dynamics. The study results are relevant to problems in assessing the recovery of sea otter populations following the Exxon Valdez oil spill in Prince William Sound, Alaska.

Another aspect of sampling that was investigated concerns the issue of detecting whether a population is approaching an asymptotic (maximum, or "K") level. Extensive Monte Carlo simulations were used to investigate possible tests for determining whether populations were past the inflection point in a growth curve. Results have been reported in *Marine Mammal Science* (Eberhardt 1992). Another sampling investigation involved the issue of using recent data to aid in determining whether a much-reduced whale population is below its growth inflection point. Simulation studies showed that the issue appears resolvable when additional data are available.

A complex ecological problem of substantial importance in ecosystem studies is the analysis of predator-prey interactions. Nearly all of the theoretical work in the literature is based on studies of insects, and very little involves actual populations at higher trophic levels. In our study of these problems with data on wolves and caribou, we succeeded in reconstructing population trends for one case history (Eberhardt and Pitcher 1992).

Another sampling study concerned the integration of reproductive and survival data to determine whether an endangered species population is increasing or decreasing. Reproductive data from 22 individual female grizzly bears was used in conjunction with survival rates estimated from 400 female bear-years of observation. The calculations were submitted to a bootstrap analysis to provide confidence intervals and evidence of a significant rate of increase. The study results have been submitted for journal publication.

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Laboratory-Directed Research and Development

Laboratory-Directed Research and Development

Laboratory-directed research and development is conducted to develop new and promising scientific concepts that have a high potential for advancing the state of knowledge in environmental sciences. This section describes innovative research on the forefront of science that offers opportunities for future important contributions.

Chemical Desorption/Dissolution

Calvin C. Ainsworth

Because two phases are present, it is difficult to probe kinetic processes associated with solid surface-aqueous systems typical of mineral/water interactions that occur in subsurface environments. The present studies used two techniques [stop-flow and pressure-jump (p-jump)] to investigate sorption processes *in situ* at millisecond time scales. The results of these studies have extended the basic understanding of the adsorption/desorption process at the surface of environmentally important minerals for a number of cationic and anionic metal species.

In FY 1991, a stop-flow system was combined with an electron paramagnetic resonance spectrometer (EPR) to measure reactant species *in situ* on millisecond time scales in aqueous suspensions. This technique was used to determine the rates of adsorption and desorption of Mn^{2+} on the surface of birnessite (MnO_2) as part of a study of Cr^{3+} oxidation at the surface of MnO_2. The Mn^{2+} sorption reaction was complete within 1 second, with more than 80% of the Mn^{2+} sorbed within 200 milliseconds. A first-order rate dependence was observed; by using an integrated reversible first-order rate expression, the desorption reaction could be determined. The calculated Mn^{2+} desorption rate was smaller than the adsorption rate by more than an order of magnitude, suggesting that the desorption process is considerably slower than the adsorption process. A variety of inorganic metals (e.g., manganese, copper, vanadium) and many organic free radicals and radical cations can be used as EPR probes. Through the use of appropriate probes, the reaction kinetics of important geochemical reactions can be discerned at time scales that allow a more complete understanding of mechanisms and pathways.

In late FY 1992 and FY 1993, investigations centered on the kinetics of adsorption/desorption of Cu^{2+}, $H_2AsO_4^-$, and CrO_4^{2-} on goethite (α-FeOOH). These geochemical processes were evaluated using the p-jump relaxation technique with conductivity detection. This technique allows mechanistic information to be obtained for elementary processes occurring at millisecond time scales. The technique is based on the dependence of chemical equilibria on pressure; a small pressure perturbation results in a shift in the chemical equilibrium, causing the system to shift (relax) to the new equilibrium conditions. The rate of this relaxation is determined by the rate of the chemical reactions that make up the equilibrium state.

In the case of Cu^{2+} sorbed to the surface of goethite, a double relaxation event was observed. The rate of these relaxations (τ) decreased with an increase in pH. The fast relaxation was ascribed to a reaction mechanism associated with the formation of a monodentate inner-sphere Cu^{2+}/goethite surface complex; it was speculated that both observed relaxations were the result of the same mechanism, with the difference between the relaxations resulting from differences in the adsorption energy of different sorption sites. A modified triple-layer surface complexation model was used to describe the adsorption of Cu^{2+} on goethite, and a pH-stat proton release study was conducted to confirm the involvement of this mechanism. The intrinsic rate constant for adsorption (k_1^{int}) was larger than that for desorption by about two orders of magnitude. Using results from this study and others, it was established that the rate of adsorption of divalent metal cations on goethite was directly related to the rate of removal of a water molecule from the primary hydration sphere of a particular metal ion.

Single relaxations observed for $H_2AsO_4^-$ and CrO_4^{2-} sorbed to the surface of goethite have been ascribed to a reaction mechanism that is associated with the formation of a monodentate inner-sphere $HAsO_4^-$ and CrO_4^{2-}/goethite surface complex, respectively. Although this model of the $HAsO_4$-surface species is not novel, the CrO_4-surface species has in the past been described as an outer-sphere surface complex. However, spectroscopic interrogation of the surface-sorbed $HAsO_4^-$ and CrO_4^{2-} by X-ray absorption spectroscopy (XAS) tends to confirm the identification of an inner-sphere surface complex. For both $H_2AsO_4^-$ and CrO_4^{2-}, adsorption is markedly faster than desorption (e.g., the rate constant for $HAsO_4$ adsorption is larger than that for $HAsO_4$ desorption by approximately four orders of magnitude).

Enzymatic Transformation of Inorganic Chemicals

Harvey Bolton, Jr., and Yuri A. Gorby

The purpose of this research is to investigate microbial immobilization of radionuclides and metals via oxidation-reduction- (redox-) induced precipitation or direct enzymatic changes in oxidation state. Some of the dominant mobile groundwater contaminants at DOE sites, including uranium, chromate, cobalt(III)-ethylenediaminetetraacetic acid [Co(III)-EDTA], and pertechnetate ($^{99}TcO_4^-$), are present in various oxidation states that are responsive to redox controls. In their reduced oxidation states, these metals have decreased mobility in the environment. This research uses iron-reducing bacteria, which can use iron(III) as a terminal electron acceptor for metabolism and reduce it to iron(II), to investigate the direct enzymatic reduction of Co(III)-EDTA.

Investigations in FY 1992 focused on the enzymatic transformation of the Co-EDTA complex. The synthetic chelate EDTA forms water-soluble complexes with many radionuclide and metal ions. For this reason it has commonly been used as a decontaminating agent in many nuclear facilities and in the processing of nuclear material. The co-disposal of cobalt-60 and EDTA has resulted in the far-field migration of cobalt-60 at DOE sites. Such migration presumably occurs because the cobalt is present as Co(III)-EDTA, which has a high stability constant ($\log K = 41$) and only weakly adsorbs to sediments at neutral pH. In comparison, the reduced Co(II)-EDTA is much less stable ($\log K = 17$) and adsorbs to a greater extent. Our results demonstrated that some bacteria, known as dissimilatory iron-reducing bacteria (DIRB), enzymatically reduce Co(III)-EDTA to Co(II)-EDTA under anoxic conditions. Theoretically, this reduction of cobalt(III) to cobalt(II) should decrease the mobility of cobalt-60 in the environment.

In FY 1993, efforts were focused on identifying and purifying the enzyme(s) that catalyzes metal reduction. Purification of the enzyme is a prerequisite to investigation of the mechanisms of electron transfer to the metal. By understanding how the enzyme operates, it will become possible to model and predict the reduction of multivalent metals in complex, heterogeneous matrices, such as those found in subsurface environments. Enzyme purification can also lead to the development of molecular probes to be used to detect microbial populations that contain genes for metal reduction. In addition, knowledge of the amino acid sequence and information from X-ray crystallography of the reductase enzyme could be used to develop computer models to assist in predicting the activity of a redesigned reductase. Use of this approach may make it possible to increase the selectivity of the protein for multivalent contaminant ions, such as chromate, uranium, or cobalt(III).

The objective of the FY 1993 study was to partially purify the enzyme(s) and electron transport components that are involved in microbial metal reduction by the DIRB strain BrY. Washed cells of BrY were ruptured under a stream of nitrogen using a French pressure cell. Unbroken cells were removed from broken cell materials by low-speed centrifugation. Membranes were separated from the soluble fraction. Iron(III)- and cobalt(III)-reductase activity were determined at each step of the fractionation. The results demonstrated that rupturing cells did not significantly affect the specific activity of iron(III)-reductase in BrY. The results also show that reductase activity is localized in the bacterial membrane. Separating the membranes from soluble proteins resulted in more than threefold purification of the enzyme. Soluble proteins from the periplasm and cytoplasm expressed no activity. Both Co(III)-EDTA and iron(III) reductase activity were partially purified, suggesting that either the same enzyme is responsible for reduction of cobalt(III) and iron(III) or that the responsible enzymes were co-purified.

Further purification of metal reductase activity from solubilized membrane proteins by diethylaminoethyl-sephadex (DEAE) cellulose ion exchange chromatograph was not successful. No activity was present in separate fractions, nor was it restored by mixing the collected fractions. Apparently DEAE deactivates the metal reductase or one of the cellular components involved in electron transport to the metal reductase (e.g., hydrogenase). Consequently, to develop a functional *in vitro* assay, it may be necessary to purify individual components of the electron transport chain.

Integration of Molecular Research with Environmental Phenomena

Raymond E. Wildung and Luying Xun

Capabilities currently being developed at the molecular level have great potential for contributing to the resolution of DOE's problems in environmental restoration. Important environmental processes that must be addressed include 1) enzyme-level phenomena governing microbial biodegradation and sequestration and 2) geochemical reactions occurring at mineral-solution interfaces. Together with hydrologic transport, these phenomena govern contaminant mobility in subsurface environments and offer a basis for development of new remediation concepts.

To address these needs, two new positions are being established at the Tri-Cities branch campus of Washington State University. In conjunction with other capabilities being developed at PNL, these positions provide a basis for new research to define contaminant behavior and develop remediation measures.

Positions in organic geochemistry and microbial physiology were initially scoped and planned in 1991, initially under the auspices of the Geology and Microbiology departments at Washington State University in Pullman. The Microbial Physiology/Biochemistry position was advertised late in FY 1991 and filled in FY 1992. The very strong response that was received indicated broad interest in developing a capability critical to meeting DOE's research needs in environmental restoration. Although recruiting for the geochemistry position also elicited a strong response, the candidates either could not be recruited or were not acceptable to both institutions. Therefore the position was redefined in FY 1993, in conjunction with the Chemistry Department at Pullman. Recruiting for this new position will be completed in FY 1994, allowing staff to begin development of new scientific concepts that will serve as the basis for future programmatic support.

FY 1993 Research Highlights

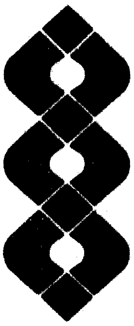
Research initiated in FY 1992 focused on the degradation of synthetic chelates that are responsible for mobilization of metals and radionuclides in subsurface systems. The first stage in the investigation was to isolate and purify the enzymes responsible for degradation of nitrilotriacetate (NTA). In addition, a graduate student was funded to clone the genes that regulate the expansion of these enzymes in subsurface microorganisms. In FY 1993, an NTA monooxygenase, NTA-Mo, was purified. The NTA-Mo has two protein components, and the N-terminal amino acid sequence of one component was determined. A gene probe was designed using the N-terminal amino acid sequence; this probe is specific to only one fragment of restriction-enzyme-digested DNA from *Chelatobacter* strain ATCC 29600. In addition, a summer student investigated the properties of NTA-Mo and found that the enzyme could degrade NTA only when NTA was complexed with the +2 forms of magnesium, manganese, nickel, cobalt, zinc, or iron, but not when it was complexed with the +2 forms of calcium, copper, chromium, tin, barium, cadmium, strontium, or palladium, the +3 forms of aluminum or chromium, or the +1 forms of potassium or sodium. A competitive research grant from the National Science Foundation is supporting investigation of the biochemistry of 2,4,5-trichlorophenoxyacetate (2,4,5-T) biodegradation by *Pseudomonas cepacia* strain AC1100. During FY 1993, an enzyme assay method was developed to detect 2,4,5-T monooxygenase, and the enzyme was purified. A post-doctoral fellow has now been recruited to characterize the enzyme.

Investigations were also initiated in FY 1993 to characterize a water-soluble blue pigment produced by a subsurface bacterium. Properties of the pigment suggest that it is closely related to bacterial anthocyanins.

Future Research

In FY 1994, the position in microbiology will play a key role in demonstrating advanced capabilities being developed in the Molecular Science Center and the Environmental Sciences Research Center for use in bioremediation. A tetrachloro-p-hydroquinone reductive halogenase will be purified and labeled for determination of structure by nuclear magnetic resonance (NMR). This will be the first application of the 750-MHz NMR equipment at PNL. New studies will also delineate mechanisms and isolate the key enzymes for 3-chlorobenzoate reductive dehalodegradation by *P. cepacia* strain AC1100. In addition, efforts will be undertaken to clone and sequence the gene encoding NTA-Mo.

Accomplishments in geochemistry in FY 1994 will depend in part on the research specialty of the person recruited to fill the position. However, the new investigator is expected to develop a joint capability in subsurface solute and colloid transport processes at multiple scales. The focus will be on 1) determining the mechanisms controlling subsurface transport of metallic hydroxide and aluminosilicate colloids and of biocolloids in physically heterogeneous porous media, and 2) developing kinetic models of colloidal attachment and deterministically modeling experimental results. Several colloids with well-characterized surface properties, including ferric hydroxide and bacterial strains in the resting state, are being selected for study. The mineralogy, surface chemistry, and physical properties of the porous medium will be determined using such methods as atomic force microscopy and X-ray photoelectron spectroscopy. Laboratory column experiments will be designed to determine the rates of colloidal attachment to aluminosilicates (quartz and montmorillonite) and ferric oxide-coated quartz. Hydraulic properties of the porous medium, surface structures, and the kinetic rates of attachment will be incorporated in deterministic reactive chemical transport models to aid in the design of pilot-scale experiments to test the effects of varying hydraulic properties on colloid transport.



Interactions with Educational Institutions

Interactions with Educational Institutions

A key part of PNL's mission is to contribute to strengthening and enhancing education in the areas of mathematics, science, and technology, and to developing strong outreach programs that serve the needs of the nation in research and education. These programs enhance national, regional, and local efforts to provide high-quality mathematics, science, and technology education for all students, from the elementary level through post-doctoral studies. Several programs focus specifically on students from groups that have traditionally been underrepresented in the fields of mathematics, science, and engineering. Through PNL's education programs, students and faculty are provided access to some of the Laboratory's unique resources, such as the Subsurface Environmental Research Facility, the Arid Lands Ecology facility, and the Aerosol Wind Tunnel Research Facility.

Pre-university programs are coordinated by the PNL Science Education Center. The Office of University Programs coordinates programs for minority institutions and universities. The Northwest College and University Association for Science (NORCUS) and Associated Western Universities (AWU) provided the mechanisms for student and faculty appointments to university programs in FY 1993.

Pre-University Interactions

PNL scientists take part in such pre-university education activities as 1) classroom instruction and demonstrations; 2) student apprenticeships, workshops, and field days in the laboratory; 3) teacher workshops and teacher participation in research; and 4) technical support and assistance to local and regional schools. The Arid Lands Ecology facility provides an important part of environmental education activities conducted for pre-university students and teachers.

Each summer, middle and secondary school teachers of mathematics, science, and technology are assigned to environmental research projects as part of the DOE Teacher Research Associates program. These eight-week assignments are made based on the educational background and teaching experience of the program participants. This year, 14 of the 50 national and regional appointees were assigned to projects in the areas of earth and environmental sciences.

Projects in 1993 included such activities as 1) evaluating chemical sensors for *in situ* monitoring of fuel components, 2) conducting laboratory experiments aimed at identifying abiotic and biotic pathways for reducing structural iron in soil minerals, and 3) assisting in gathering field data related to plant ecophysiology and plant population biology. In the program, teachers develop instructional strategies to use in their classrooms, based on their experiences at PNL.

As part of national efforts by DOE to improve the mathematical, scientific, and technological literacy of all students, PNL scientists worked with middle schools in southeastern Washington that have large populations of students from groups that have historically been underrepresented in these fields. Researchers teamed up with teachers to help enhance their technical knowledge and students' background in mathematics, science, and technology; researchers also assisted in developing and enriching instructional strategies and curricula.

In addition, researchers worked with elementary school teachers participating in Science Alive II, a three-week summer program in environmental sciences designed to develop a cadre of teacher leaders who act as "change agents" to improve science teaching in classrooms, school buildings, and school districts. Teachers participated in hands-on problem-solving field and laboratory experiences led by PNL scientists.

Through the Sharing Science with Schools program, researchers made presentations on current science and technology topics at local, regional, and community schools. These presentations covered such subjects as contamination in groundwater, remote sensing, and plant and wildlife ecology.

University Interactions

Through the NORCUS, AWU, and Science Engineering Research Semester (SERS) programs, PNL gives university undergraduate and graduate students and postdoctoral scientists and faculty an opportunity to work with staff in research activities and to participate in seminars, scientific meetings, and symposia.

During FY 1993, many undergraduate and graduate students and university faculty participated in environmental sciences programs. A total of 96 NORCUS students were assigned to environmental sciences this year. Through AWU, 10 more students and faculty worked with PNL researchers in environmental sciences, with seven in regular laboratory assignments and three taking part in the Environmental Management Career Opportunities Research Experience (EMCORE). Twenty undergraduate students also participated in environmental sciences under the Science Engineering Research Semester (SERS) program. Some of this student and faculty involvement is described below, and details are provided in research progress reports.

During the year, the Environmental Sciences Program also hosted many visiting scientists representing universities nationally and internationally for workshops, seminars, and DOE program reviews. New joint PNL-university partnerships are expected to be established in environmental sciences in FY 1994, as a result of new PNL-university partnerships with major U.S. universities, including Historically Black Colleges and Universities (HBCUs) and other minority institutions.

Subsurface Science

The project on subsurface chemistry of organic-radionuclide mixtures interacted with faculty members John Westall (Oregon State University) and George Yeh (Pennsylvania State University), as well as NORCUS students Eric Roden, Patrick Bruckhart, and Gaston Dolmans. Laura Kuzel, Montana State University, also participated in the project.

A SERS undergraduate student, Heather Lumppio, spent a semester and the summer participating in a project to determine the mechanism whereby microorganisms accumulate metals and radionuclides in contaminated subsurface environments. Another SERS undergraduate student, Nicole Flint, spent her fall semester working on the project. Also working on the project, Nancy L. Valentine completed her Master of Science degree in Environmental Science at Washington State University, Tri-Cities.

Luying Xun, assistant professor at Washington State University, Tri-Cities, is determining the specificity of the nitrilotriacetate (NTA) monooxygenase enzyme for degrading different metal/ radionuclide-NTA complexes. Kaiying Di, a Master's student at Washington State University, Pullman, also contributed to research on the interactions between bacteria and the model co-contaminants quinoline and NTA.

Research on subsurface microbiology provided educational experiences for a number of undergraduate and graduate students over the past year. This research also involved collaboration with faculty at a number of academic institutions, including

David Balkwill, Florida State University, who examined the phylogenetic and physiological properties of deep subsurface bacteria;

David Boone, Oregon Graduate Institute, who assisted in defining the community structure of the deep subsurface environment revealed through a joint DOE/Texaco exploratory drilling project. The research led to the detection and isolation of several strains of unique thermophilic manganese(IV)-reducing bacteria;

Thomas Kieft, New Mexico Institute of Mining and Technology, who participated in microbial analysis of GEMHEX samples from the Hanford confined aquifer;

Andy Ogram, Washington State University, who extracted and analyzed bacterial DNA in GEMHEX samples from the unconfined aquifer;

Wai Mun Huang and Ari Ferro, University of Utah, who investigated the use of DNA gyrase gene sequence as a tool to differentiate subsurface bacteria at the genus and species levels;

David Stahl and Norman Fry, University of Illinois, who characterized microbial communities in samples from the Hanford confined aquifer by extracting and hybridizing community RNA and DNA with DNA probes targeted for specific phylogenetic groups; and

David White and David Ringelberg, University of Tennessee, who collaborated in characterization of microbial phospholipids in GEMHEX samples using gas chromatography with mass spectrometry.

Collaborating geologists include T. C. Onstott (Princeton University). Research on physical and mineralogical influences on bacterial transport is being conducted in collaboration with A. L. Mills, G. Hornberger, and J. Herman (all of the University of Virginia).

The Microbial Transport and Origins project collaborated with John Cushman (Purdue University) and with Craig Arola (University of Virginia) and Norca L. Torres-Velázquez (University of Puerto Rico) under NORCUS and AWU. In addition, the project interacted with Paul Dixon, Los Alamos National Laboratory; David Balkwill, Florida State University; and David Ringelberg, University of Tennessee. Close collaboration was also maintained with Ethan Grossman, Texas A&M University.

The Subsurface Organic Fluid Flow project conducted collaborative research with two institutions. Staff provided information to Dennis Rolston of the University of California at Davis concerning the measurement and analysis of multiphase fluid retention characteristics. This permitted the University of California researchers, under Rolston's direction, to construct a three-phase air-NAPL-water retention apparatus so that scientific investigations on the retention of NAPLs in subsurface environments could be conducted.

In addition, F.A.L. Dullien, author of a seminal text book on fluid flow in porous media and chairman of the University of Waterloo's Porous Media Research Institute, collaborated with PNL to study the movement of trichloroethylene vapor in a large-scale laboratory flow cell experiment. A NORCUS appointment supported his two-month sabbatical visit at PNL, where he interacted with project principal investigators daily to help understand the phenomenon of gravity-driven diffusion of denser-than-air gases in an unsaturated subsurface medium. Dullien wrote a draft theoretical manuscript, which he provided to PNL researchers to facilitate interpretation of future experiments on organic vapor migration in the subsurface. He also presented two seminars on his own research and completed a study of hydrodynamic coupling of liquid flow that occurs in two-phase flows. This latter work was presented at the Thirteenth Annual Hydrology Days, held by the American Geophysical Union at the Colorado State University.

Scientific outreach to the academic community has been greatly facilitated by the Subsurface Science Program's Environmental Science Research Center (ESRC), located at PNL. University researchers are invited to participate in national workshops, scientific meetings, and publications, and university involvement in Cooperative Research and Development Agreements is sought. Through this program, the academic community has participated in refining research directions for the Subsurface Science Program and in multi-institutional studies of complex subsurface processes and interdisciplinary field research, such as the geological, microbiological, and hydrological experiment (GEMHEX).

Terrestrial Science

The project dealing with N_2O flux and with resource islands in the shrub-steppe ecosystem interacts extensively with Jeffrey L. Smith, a U.S. Department of Agriculture - Agricultural Research Service Soil Scientist at Pullman, Washington, on nitrogen dynamics of arid ecosystems. Both a post-doctoral fellow (Jay Halvorson) and a graduate student (Dan Mummey) were funded by the project to aid in the research reported for this fiscal year.

Research on plant competition for water supported graduate student Joe Healy (under R. Alan Black, Botany Department) at Washington State University. His research relates to hydraulic lift and competition for water in the shrub-steppe.

Technology
Transfer

Technology Transfer

Technology and information transfer is integral to DOE, and particularly to the DOE Subsurface Science Program, which supports scientific research to obtain fundamental knowledge needed to address the environmental contamination resulting from more than 40 years of weapons manufacturing at DOE sites. Basic research under the program focuses on building an understanding of the interacting geological, chemical, and biological processes that influence contaminant fate and transfer in natural subsurface systems. This basic research has resulted in the development of new research tools, methods, and remediation concepts that have been transferred to other offices within DOE, to other federal and state agencies, to academia, and to industry; together these make up the user community. The Subsurface Science Program's technology transfer program, which began in 1987, ensures that findings from the Subsurface Science Program's scientific investigations are effectively communicated to the user community through publication in the open scientific literature, by licensing of intellectual property, and by sponsorship of regional and national workshops that focus on information exchange.

Within the Subsurface Science Program, the identification of new opportunities for technology transfer, together with programmatic responsibility for coordinating workshops and other transfer activities, resides within the PNL Environmental Science Research Center (ESRC). The ESRC was formed to support the Subsurface Science Program's basic scientific mission, to facilitate the use of fundamental knowledge for developing innovative *in situ* remediation concepts, and to aid in transferring information and technology to the user community. The objective of the support efforts of the ESRC is to better integrate the Subsurface Science Program's technology transfer program into its core scientific activities, so that new opportunities are acted on promptly. A key goal of the Subsurface Science Program is to encourage subprogram managers and principal investigators to incorporate consideration of technology and information transfer into research planning and implementation.

As part of the Subsurface Science Program, the ESRC facilitated a number of information and technology transfers in FY 1993. Representative examples are 1) development of a Cooperative Research and Development Agreement focused on the Subsurface Science Program's Subsurface Microbial Culture Collection, 2) transfer of field research protocols to the DOE Office of Technology Development and DOE site operators, and 3) transfer of aseptic drilling and subsurface sampling methods to DOE sites.

Subsurface Microbial Culture Collection

Deep subsurface microbiology research conducted by the Subsurface Science Program at the Savannah River Site, the Idaho National Engineering Laboratory, the Nevada Test Site, and the Hanford Site, as well as at a Texaco exploratory oil well in Virginia, resulted in the establishment of the Subsurface Microbial Culture Collection at Florida State University. The Subsurface Microbial Culture Collection now holds more than 5,000 isolates, many of which probably represent new species or bacterial genera. In FY 1993, the Subsurface Science Program established a second subsurface collection at the Oregon Graduate Institute to maintain isolates with special culture requirements that reflect their origin in subsurface environments with extreme conditions of temperature, salinity, pressure, or pH.

In FY 1993, the DOE Laboratory Technology Transfer Program within the Office of Energy Research agreed to support a proposed Cooperative Research and Development Agreement among PNL, Florida State University, the National Cancer Institute, and the biotechnology industry to determine the degree to which the Subsurface Microbial Culture Collection may be a unique genetic resource and to screen the collection for bioactivities that might be useful leads in drug discovery programs. The National

Cancer Institute will screen the collection for anti-AIDS and anti-cancer activities. Members of the biotechnology industry will apply various molecular screening methods to identify activities suitable for pharmaceutical development in, for example, the areas of bone biology, diabetes, and anti-inflammatory products. Under the agreement, other industrial sectors will be given access to the collection at a later stage to screen for compounds that could be used in waste management, environmental restoration, or production of novel pigments, specialty chemicals (e.g., flavors and fragrances), or enzymes with unique capabilities for industrial processes, such as conversion of biomass to chemical and energy feedstocks.

Field Research Protocols

Fundamental research in the Subsurface Science Program led to the development of an experimental approach for investigating the feasibility of manipulating coupled chemical and microbiological processes as a means of altering the oxidation-reduction conditions in an aquifer. This approach makes use of novel concepts, experimental designs, and research tools for investigating the interactive effects of subsurface physical, chemical, and microbial processes on oxidation-reduction conditions in the field. The design for a field manipulation experiment has been transferred to the Office of Environmental Restoration and Waste Management for implementation in the Office of Technology Development's *In Situ* Remediation Integrated Program. Continuing liaison will assist the Office of Technology Development in incorporating the experiment into its applied research programs.

Aseptic Drilling and Sampling Methods

Workshops were held at Santa Fe, New Mexico, and Rocky Flats, Colorado, in FY 1993 to transfer information and technology to DOE field sites. Subsurface Science Program researchers from PNL, the Idaho National Engineering Laboratory, Golder Associates, and Oak Ridge National Laboratory cooperated to transfer their information about and methods pertaining to aseptic drilling, sampling, tracers, and sample handling for subsurface characterization and research. Both workshops attracted personnel from DOE field offices (Albuquerque and Rocky Flats), Office of Energy Research contractors (EG&G Rocky Flats), national laboratories (Sandia National Laboratories, Los Alamos National Laboratory), universities (University of Colorado, Colorado School of Mines), and industry (drilling companies). The workshops focused on maintaining and documenting the integrity of representative samples from the subsurface using innovative tracer methods.

Publications and Presentations

Publications

Subsurface Science

Ainsworth, C. C., J. K. Fredrickson, and S. C. Smith. 1993. "Effect of Sorption on the Degradation of Aromatic Acids and Bases." In *Sorption and Degradation of Pesticides and Organic Chemicals in Soil*. SSSA Special Publication No. 32, Soil Science Society of America, Madison, Wisconsin.

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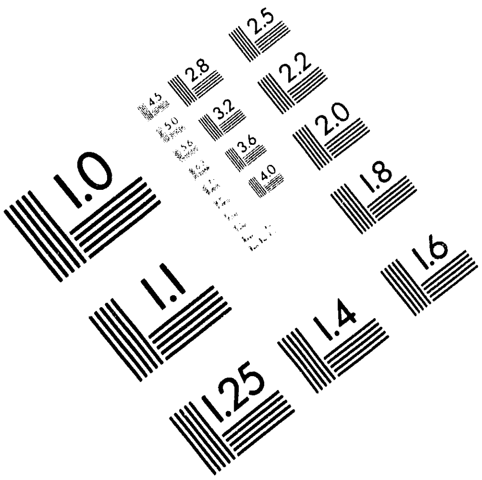

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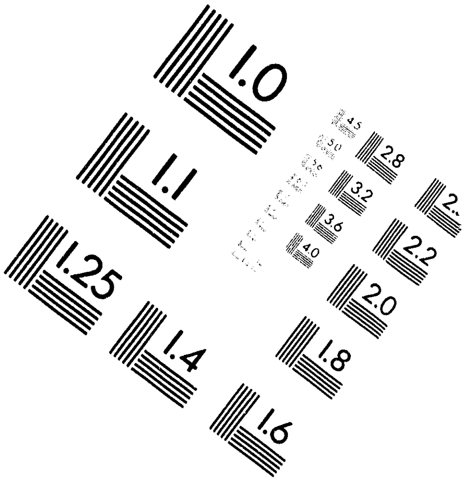

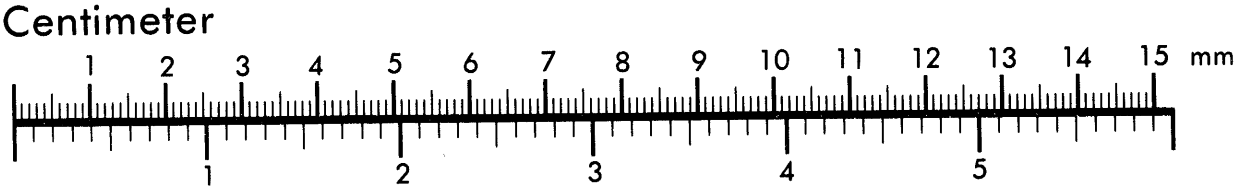

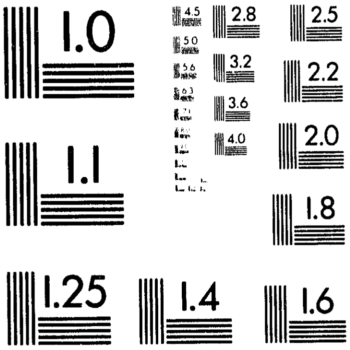

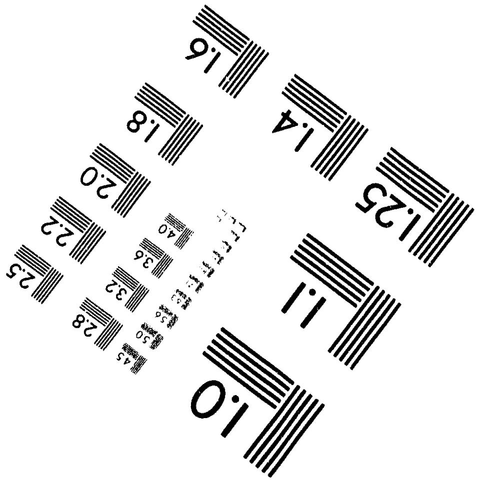

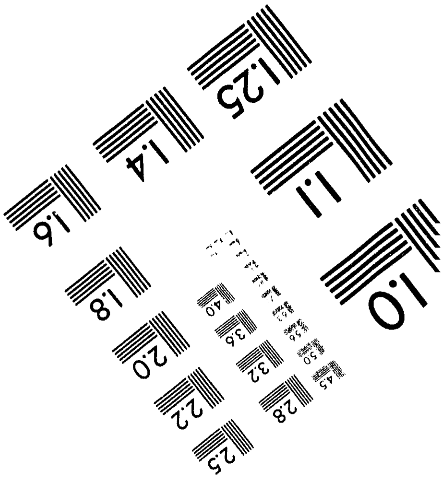

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