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In Situ Bioremediation and Efficacy Monitoring SERDP Project CU-030

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13. ABSTRACT (Maximum 200 words) The results of a three-year study funded by the Department of Defense under the Strategic Environmental Research and Development Program (SERDP) to couple modern geochemical techniques with biological and chemical methods to determine the efficacy of bioremediation strategies for fossil fuels are reported. The objective of this project was to directly measure the catabolic activity of indigenous soil microflora on the contaminants of interest. The combination of stable isotope measurements, contaminant concentration analysis and measurement of bacterial production rates in the different matrices accomplished the objective as follows. The monitoring of a bioremediation technology assessed the effectiveness of these strategies on diverse contamination problems. In addition, the suitability of $\delta^{13}\text{C}$ stable isotope measurements of multiple carbon pools as an indication of bioremediation was assessed.				
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IN SITU BIOREMEDIATION AND EFFICACY MONITORING

SERDP Project CU-030

Introduction

"*In situ* bioremediation" can be defined as a non-invasive degradation of organic materials by native microflora. Our ability to stimulate and manage that process on a large scale provides the potential for an effective, cost saving containment and remediation strategy for contaminated soil and groundwater. The placement of technologies, such as circulation wells, nutrient supplements, and bioreactors, which stimulate or facilitate microflora degradation is critical to this effort. This project, "*In situ* bioremediation and efficacy monitoring," is supported by the Department of Defense (DOD) under the Strategic Environmental Research and Development Program (SERDP). This project is a joint effort between two Federal agencies: the U.S. Naval Research Laboratory (NRL), and the Environmental Protection Agency (EPA-GBERL); the academic sector: Texas A&M University (TAMU); and the private sector: SBP Technologies, Inc. (SBP), IEG Technologies, Inc. (IEG), and Beazer East, Inc. The goal of this team is to conduct a laboratory and field demonstration of innovative *in situ* bioremediation technologies coupled to a monitoring strategy that demonstrates the efficacy of the system.

The objective of the project is to directly determine the catabolic activity of indigenous soil microflora on the contaminant of interest. Chemical mass balance calculations are difficult with *in situ* technologies. The use of stable isotope measurements as a monitoring tool is designed to overcome this limitation. Combining analysis of stable isotopes and contaminant concentrations of the different matrices accomplished the objective as follows. First, the monitoring of the various bioremediation technologies assessed the effectiveness of these strategies on two different contamination problems. Second, the suitability of respired CO₂ stable isotope measurement as an indication of bioremediation was demonstrated. And finally, information comparing volatile organic loss due to air stripping with loss due to bioremediation was derived. With the analyses, a pseudo-mass balance of co-reactants (O₂, CO₂, and inorganic nutrients), relatively direct calculations to describe biodegradation in relationship to abiotic removal was shown.

Two of the recognized limitations of *in situ* technologies are: 1) physicochemical restraints, such as bioavailability, desorption kinetics, and; 2) extended treatment time compared to *ex situ* biotreatment approaches. The unique hydrogeology of a site may also represent appreciable constraints. The presence of free product as light- or dense- non-aqueous liquid phase (LNAPL or DNAPL, respectively) represents probable chronic sources of contamination, but also concentrations of contaminant that will be predictively toxic to indigenous microflora. The greatest challenge is to deal effectively with heterogeneity, anisotropy and variability intrinsic to geologic formation.

Summary of Work Reported

We chose the Groundwater Circulation Well (GCW) technology for the *in situ* treatment of capillary fringes, phreatic and vadose zones contaminated with volatile organic compounds, including both LNAPLs and DNAPLs. The GCW is based on the vacuum vaporized well technology and has been shown to simultaneously remediate soils and groundwater contaminated with volatile and semi-volatile organic compounds. This technology is described in detail in the chapters entitled, "Field demonstration of in situ chemical containment technologies. Site study: Pt Hueneme CA" (Mueller *et al.* in "In Situ Bioremediation and Efficacy Monitoring," Edited by B.J. Spargo, Washington, DC (1996) NRL/PU/6115--96-317) and "Field demonstration of in situ chemical containment technologies. Site study: Gainesville, FL" (Mueller *et al.* in "In Situ Bioremediation and Efficacy Monitoring," Edited by B.J. Spargo, Washington, DC (1996) NRL/PU/6115--96-317).

The monitoring strategy is based on the stable isotope ratio of carbon ($^{13}\text{C}/^{12}\text{C}$ or $\delta^{13}\text{C}$), that has been used successfully to trace the flow of organic material through food webs. Measuring the carbon isotope ratios of the contaminant, indigenous organic matter, bacteria, and respired CO_2 provides information on the bacterial utilization of the available carbon sources. As the contaminant carbon is degraded and used by bacteria, the carbon isotope signature appears in the biomass and subsequently in the respired CO_2 . The effect of the GCW on stimulating microflora abundance, degradation of specific contaminants of interest, and contaminant migration and utilization was also monitored. An overview of this monitoring strategy can be found in the chapter entitled, "Validity of stable isotope analysis for monitoring bioremediation efficacy" (Trust *et al.* in "In Situ Bioremediation and Efficacy Monitoring," Edited by B.J. Spargo, Washington, DC (1996) NRL/PU/6115--96-317).

Two sites were identified to test the technology and the monitoring strategy. 1) A gasoline station located at the Naval Exchange (NEX) in the Naval Construction Battalion Command, Pt. Hueneme, CA., and; 2) Cabot Carbon/Kopper's, an EPA Superfund site located at the former Cabot Carbon Company site, a pine tar and charcoal facility, and Kopper's Industries, Inc., a wood treatment facility in Gainesville, FL. Detailed descriptions of these site is provided in the chapters entitled, "Field demonstration of in situ chemical containment technologies. Site study: Pt. Hueneme CA" (Mueller *et al.* in "In Situ Bioremediation and Efficacy Monitoring," Edited by B.J. Spargo, Washington, DC (1996) NRL/PU/6115--96-317) and "Field demonstration of in situ chemical containment technologies. Site study: Gainesville, FL" (Mueller *et al.* in "In Situ Bioremediation and Efficacy Monitoring," Edited by B.J. Spargo, Washington, DC (1996) NRL/PU/6115--96-317).

The bioremediation and monitoring effort was validated by using small and large scale laboratory models (microcosm and mesocosm), mathematical models, and multivariant analysis of the field data. These models explore the effect of variations in geophysical and geochemical parameters on bioremediation. Results of these efforts and discussions validating the field data

can be found the chapters entitled, "Validation of groundwater circulation cell models using fluorescent dye tracing" (Lakhwala in "In Situ Bioremediation and Efficacy Monitoring," Edited by B.J. Spargo, Washington, DC (1996) NRL/PU/6115--96-317), "Microcosm validation of model" (Morin *et al.* in "In Situ Bioremediation and Efficacy Monitoring," Edited by B.J. Spargo, Washington, DC (1996) NRL/PU/6115--96-317), "Mesocosm: Assessment of field processes in a controlled environment" (Borchert *et al.* in "In Situ Bioremediation and Efficacy Monitoring," Edited by B.J. Spargo, Washington, DC (1996) NRL/PU/6115--96-317), "Mathematical validation of model" (Connolly and Langley in "In Situ Bioremediation and Efficacy Monitoring," Edited by B.J. Spargo, Washington, DC (1996) NRL/PU/6115--96-317), and "Multivariate analysis of field data" (Campbell *et al.* in "In Situ Bioremediation and Efficacy Monitoring," Edited by B.J. Spargo, Washington, DC (1996) NRL/PU/6115--96-317).

Findings

- *In situ* groundwater circulation well strategy provided effective means of source management and containment of dissolved phase components.
- Cost/benefit analysis suggests a greater than 20% cost savings of this technology over other competitive technologies for PAH contamination.
- Combined chemical, physical and biological parameters for comprehensive evaluation of biodegradation/bioremediation processes.
- Recorded stimulation of biological activity due to groundwater circulation wells at Port Hueneme and Gainesville sites.
- Defined low inter-well variability for all analyses using the Port Hueneme biocurtain.
- $^{13}\text{CO}_2$ traced the source of carbon assimilated by bacteria in contaminated soils.
- $^{13}\text{CO}_2$ measurement of DIC allowed identification of the primary mode (aerobic, anaerobic) of degradation.
- Analysis of nutrient to contaminant ratios provided estimates of nitrogen and phosphorus concentrations required in groundwaters to support enhanced biodegradation rates.
- Isotopic fingerprinting identified multiple sources of BTEX contamination at the Port Hueneme site.
- Determined that ^{13}C values for contaminant and background organic carbon overlapped and made identification of specific compound degradation ambiguous.

- Found that high oxygen demand may have limited the ability of groundwater circulation wells to provide sufficient oxygen transfer to adjacent soils within the circulation zone.
- Identified geochemical conditions that promote chemical fouling of the groundwater circulation wells.
- The addition of nutrients affects bioremediation performance at Gainesville site (creosote) where inorganic nutrients are limiting.
- Anaerobic biodegradation is an important remediation component at the NCBC Port Hueneme site (BTEX).

Shortcomings

Several shortcomings of this technology and this study are apparent and should be considered as the development of these methods and technology are applied to other systems. Site characterization is critical to the use of groundwater circulation well technology. Hydrology, geology and plume characterization have a significant impact on the use and placement of circulation wells. For example, in Port Hueneme, the main unit was located in an area of free product and near several fully screened monitoring. The presence of a significant amount of free-product resulted in an unanticipated requirement for off-gas treatment (thermal destruction). The fully screened wells resulted in short-circuiting the circulation cell as it formed, therefore leaving a zone of influence at a fraction of the anticipated volume.

Background-Study Sites

Naval Construction Battalion Command, Port Hueneme, CA

In March 1985, the Public Works Department of the Naval Construction Battalion Center (NCBC) conducted leak tests at the Navy Exchange Gasoline Station. These tests indicated that gasoline had leaked from two delivery lines connecting the station's underground storage tanks and pumps. Fuel inventory records suggested that 15,142 L (4,000 gallons) of regular gasoline and 25,741 L (6,800 gallons) of premium unleaded gasoline were lost between September, 1984 and March, 1985.

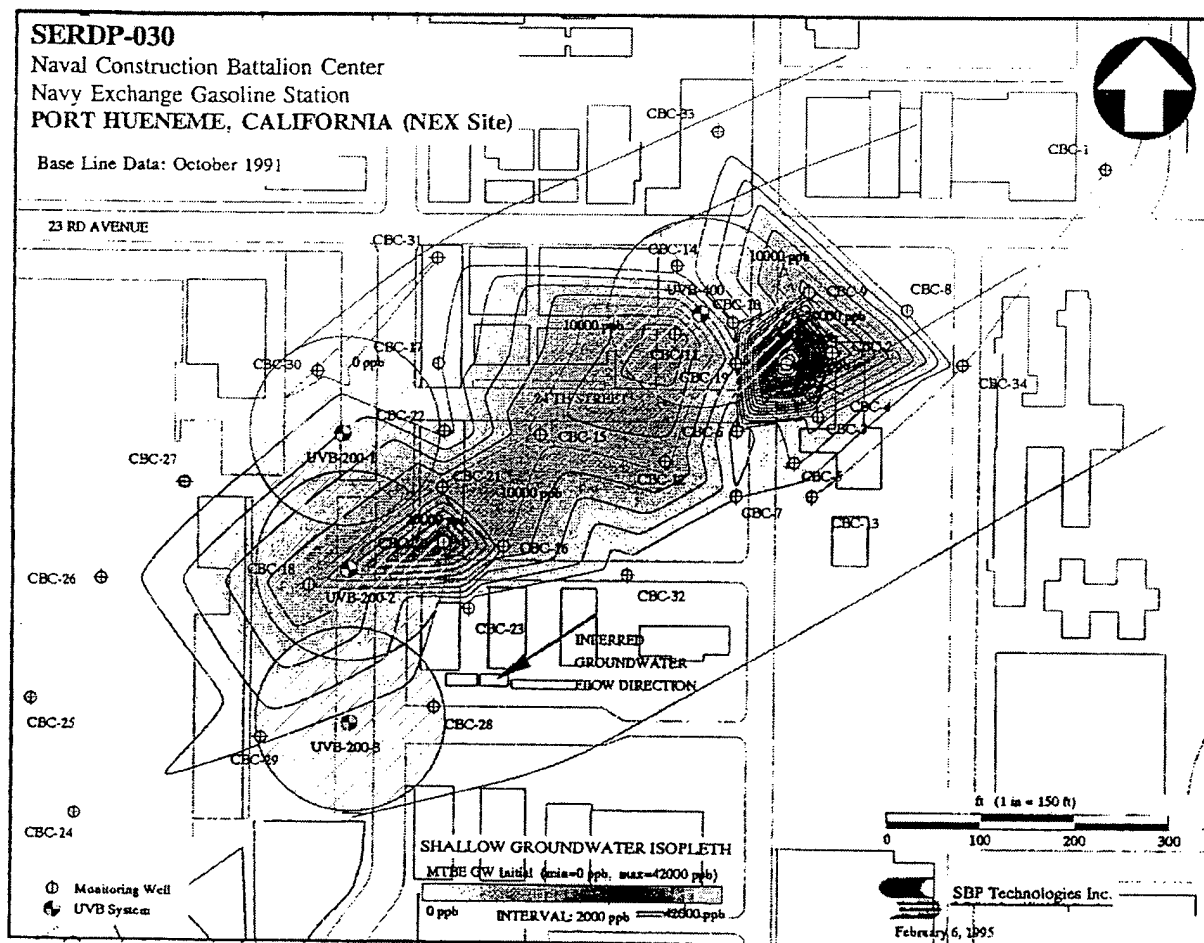
Geology

NCBC Port Hueneme is situated at the western margin of the Oxnard Plain in the western portion of the Ventura Basin, which is a relatively broad, level floodplain and river delta formed by the Santa Clara River. The Ventura Basin is bounded on the north and northwest by the Santa Ynez Mountains, on the south and east by the Santa Monica Mountains, and on the southwest by the Channel Islands. NCBC is underlain by approximately 100 m of unconsolidated clay, silt, sand, and gravel of Holocene age that overlie clay, shale, and sandstone deposits of Pleistocene and

older ages. The geology, within 10 m of the surface consists of unconsolidated sands, silts, and clays with minor amounts of gravel and fill material. The unconsolidated deposits are typically represented by three units: i) an upper fine-grained, silty sand unit, encountered from ground surface to a maximum depth of 1.7 m (5 ft); ii) an intermediate fine to coarse-grained sand unit, and iii) an underlying sandy to silty clay unit. The clay unit is encountered between 6.2 and 8 m (18.5 feet to 24 feet) bgs.

Hydrogeology

Groundwater beneath the Oxnard Plain is characterized by five significant aquifers contained in Holocene and Pleistocene deposits. The semi-perched aquifer is the uppermost groundwater unit present beneath NCBC Port Hueneme. The aquifer is contained within the first three depositional soil units that consist of an upper silty sand unit, an underlying fine- to coarse-grained sand unit, and a basal clay unit. It is this semi-perched aquifer that is impacted, hence the focus of the remedial efforts.



Results of numerous studies defined the following parameters:

Darcy velocity:	3.8×10^{-5} cm/sec
Soil type/texture:	gravel 25%, sand 58%, silt/clay 17%
Horizontal conductivity (Kh):	3.84×10^{-2} cm/sec
Vertical conductivity (Kv):	3.84×10^{-3} cm/sec (estimated)
Hydraulic gradient:	0.001 ft/ft
Thickness of saturated zone:	3.3 to 6.5 m (seasonal average 5.48 m)
Flow velocity:	231-548 m/yr (694 to 1,643 ft/yr)

Groundwater flows southwest which generally corresponds to the topographic gradient. Transmissivity values range from 71,923 to 170,344 L (19,000 to 45,000 gallons)/day/foot. Unconfined water table conditions typically characterize the semi-perched aquifer, with water levels ranging from 1 to 3.7 m (3 to 14 ft) bgs; however, semi-confined conditions have been observed at some locations.

Plume Delineation

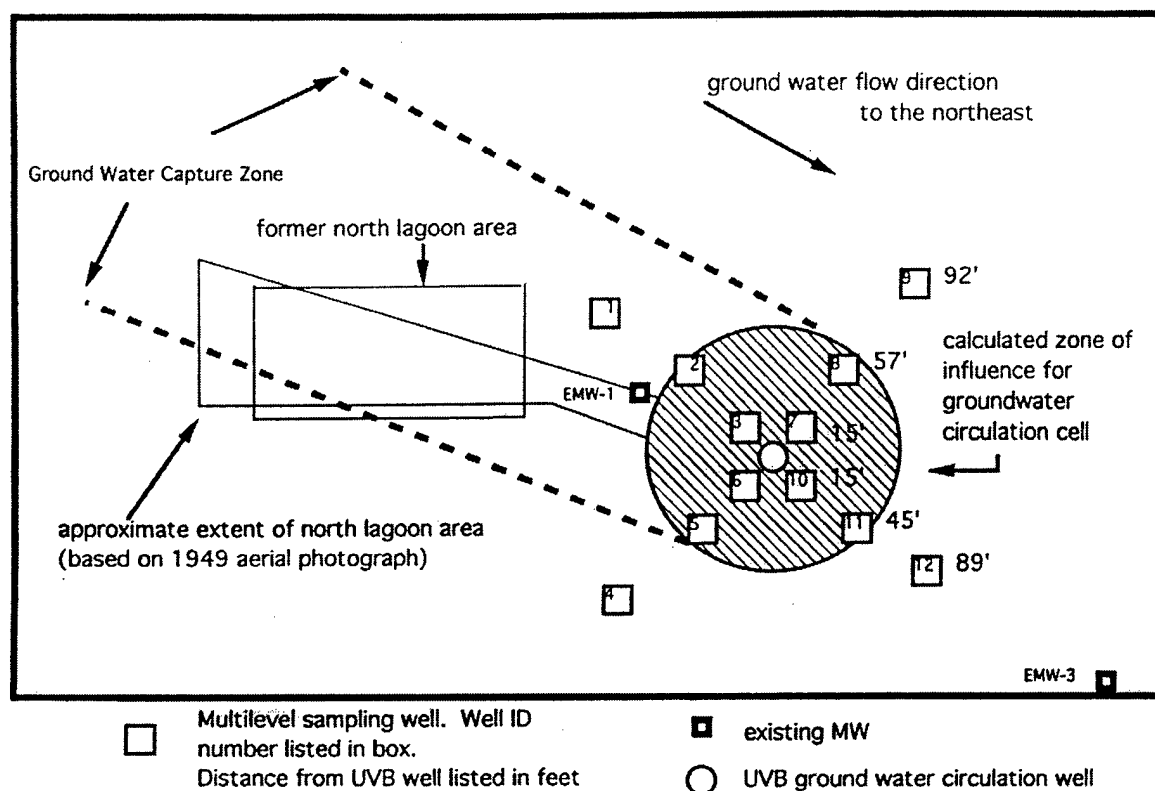
Results of a Phase II site characterization reported BTEX constituents in both the saturated and unsaturated zones. Within the saturated zone, the primary contamination consisted of free product situated within the capillary fringe with observed thicknesses between 0.18 and 0.33 m (0.54 and 1.0 ft). An estimated 41,640 L (11,000 gallons) of free product were reported in the capillary fringe.

Pre-design Studies compared product thickness and location in August 1987 with that measured in May, 1989. These data indicated that significant migration of product down gradient (180 m or 540 feet westward) had occurred over this time period at a rate of 0.3 m (0.89) ft/day, or 108 m (325 ft)/yr. The observed lobate nature of the free product plume suggested that either inputs of hydrocarbon into the aquifer had occurred over time, or that the hydrocarbons migrated through the aquifer at different rates due to observed heterogeneity of the formation. The scale of the dissolved phase plume (benzene) was estimated at 100 m x 833 m (300 ft x 2,500 ft). Revised estimates of groundwater contamination based on analytical data suggested that 113,562 L (30,000 gallons) of hydrocarbon were present in the formation.

Cabot Carbon/Kopper's Superfund Site, Gainesville, FL

The Cabot Carbon/Kopper's Superfund Site is located in the City of Gainesville, Florida. Since the mid-1920's, the former Cabot Carbon Company operated a 34 acre pine tar and charcoal generation facility, both which are now discontinued. During the same time, the Kopper's Industries, Inc. plant occupied about 90 acres as a wood treatment facility. Historically, the facility used creosote, pentachlorophenol (PCP), and chromated copper arsenate (CCA) to preserve wood utility poles and timbers. The facility continues to operate, but using only CCA on site.

Topographically, the site is relatively flat at an elevation between 175 to 185 feet above mean sea level. Adjacent to the west and northwest of the site is residential property consisting primarily of single-family housing. Commercial facilities are located to the south and east of the site, and an industrial park is located to the north. Stratigraphic formations encountered in the vicinity of the site consist of the Lake City Formation, the Avon Park limestone and the Suwannee limestone. Overlying these formations is the Hawthorn Formation (25 to 65 feet below ground surface [bgs]) which is marine clay interbedded with limestone and sandy phosphatic limestone, with a dense dolomite and limestone unit at the base. Overlying the Hawthorn Formation are Pleistocene Terrace deposits consisting of organic matter, clay and sand. There are three aquifers identified in the plant vicinity: 1) surficial unconfined aquifer in the Pleistocene sands (3 to 10 feet bgs); 2) intermediate aquifer of limestone and sandy beds of the Hawthorn Formation (25 to 175 feet bgs); and 3) Floridian Aquifer (>150 feet bgs).



Multiple process and storage areas associated with past facility operations have been identified as potential sources of organic wood preserving constituents. Results obtained from a Remedial Investigation conducted in 1989 indicated that groundwater in the shallow aquifer had been impacted by phenolic compounds, creosote, volatile organics and chromium; PCP was not detected. The former north lagoon area (which has since been filled and leveled) has been identified as a potential source of creosote constituents, with free product being detected as DNAPL and LNAPL. Analysis of soil and groundwater samples recovered during the installation of monitoring well 10 showed dissolved COI concentrations near this area.

Thus, this location was chosen for field demonstration of the *in situ* bioremediation and efficacy monitoring technologies. Strategic placement of the microbiologically enhanced UVB system at the front of the dissolved-phase COI plume supported the project's remedial objectives of demonstrating: 1) *In situ* chemical containment (*i.e.*, COI in the dissolved phase would be biodegraded/stripped within the area of ground water circulation cell thus inhibiting continued migration of the plume), and 2) collection of free product. Simultaneously, this location contained sufficient COI to support the evaluation of *in situ* monitoring approaches to assessing the role of biodegradation *per se* in the removal of monitored COI.

Results

Mineralization of contaminant. One of the major objectives of this research project was to measure and determine the extent to which biodegradation was occurring in this system. The use of stable isotopes to measure the evolution of CO_2 resulting from the biodegradation of contaminant organic matter was accomplished. The following three figures show the results from the Gainesville and Port Hueneme sites.

Figure 1: Soil CO_2 data from Gainesville, FL showing that isotope ratios in soil CO_2 from background site (BMW Gas) is easily distinguished from contaminated site (COI Gas). Data for particulates in groundwater also shown.

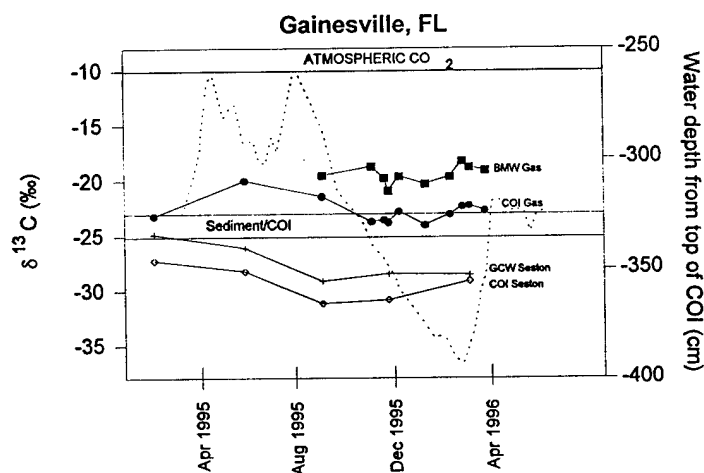


Figure 2: Soil CO_2 data from Pt. Hueneme, CA showing that isotope ratios in soil CO_2 from background site (BMW Gas) is easily distinguished from contaminated site (COI Gas). Data for particulates in groundwater also shown.

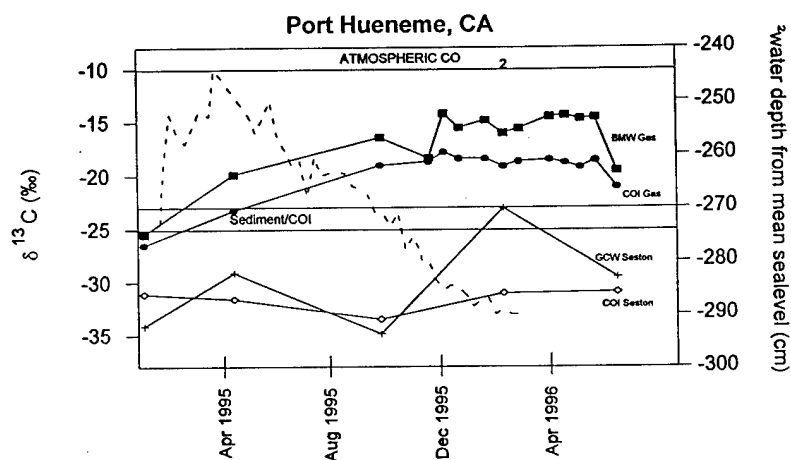


Figure 3: Soil CO₂ data from Gainesville, FL showing that isotopic data cluster along a mixing line between atm. CO₂ (-8 ‰) and degraded OM (-24 ‰), with some impact from methane oxidation (-60 ‰).

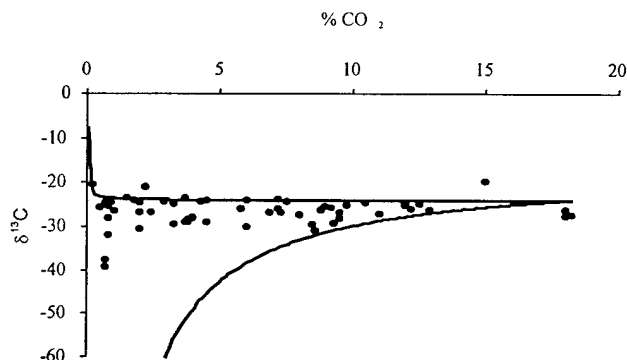
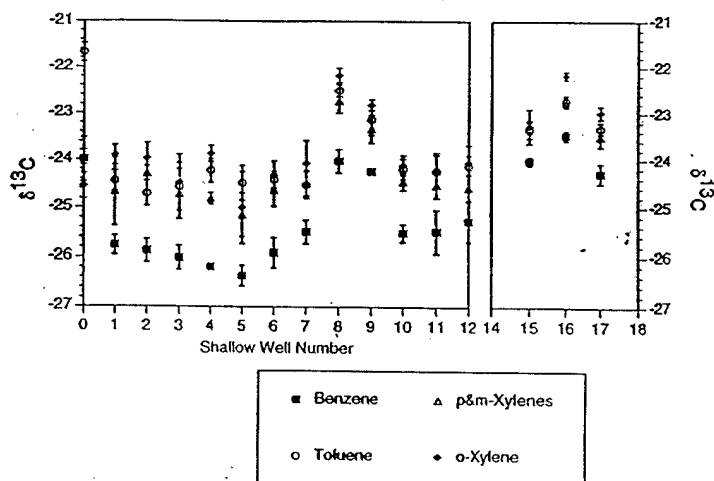


Figure 4: Stable carbon isotope ratios of benzene, toluene, ethylbenzene, and xylene indicate two distinct sources of petroleum contributed to the contaminant pool at Port Hueneme, CA.



The effects of inorganic nutrient supplements on bacterial production and mineralization.

Heterotrophic bacterial production (leucine incorporation) and mineralization of benzene and toluene (radiotracer addition) were measured on nutrient additions to a single monitoring well sample from the shallow and deep ports of MW-10. In addition, effect of nitrate additions on bacterial production was also measured.

Figure 5. Bacterial production was influenced by phosphate additions up to 10 μM in the deep monitoring well samples, whereas, 25 μM additions appear to be inhibitory to relative heterotrophic production.

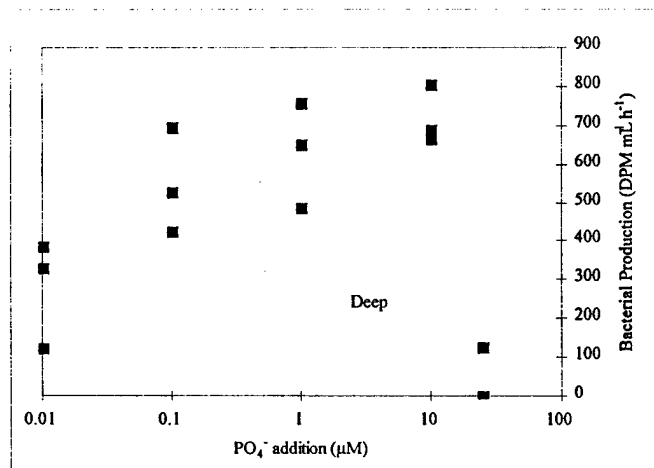


Figure 6. There was some stimulation with up to 1 μM additions of ammonium to the BTEX-impacted groundwater followed by inhibition of heterotrophic production.

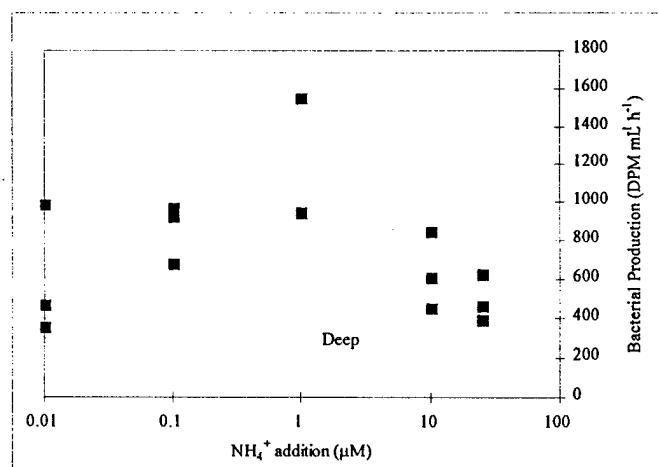


Figure 7. In groundwater from the shallow wells, heterotrophic production was stimulated by up to 0.1 μM addition of ammonium.

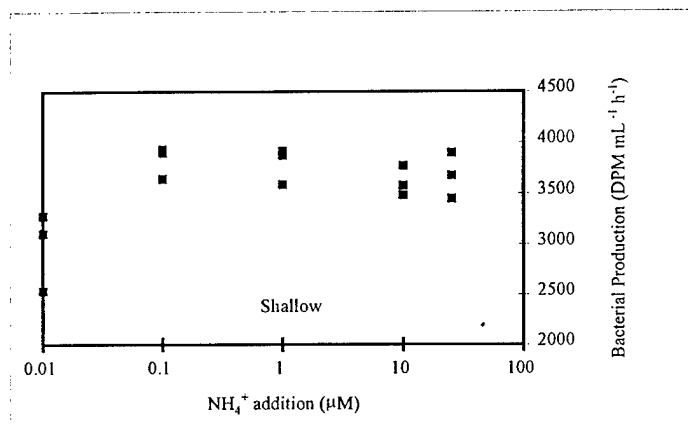
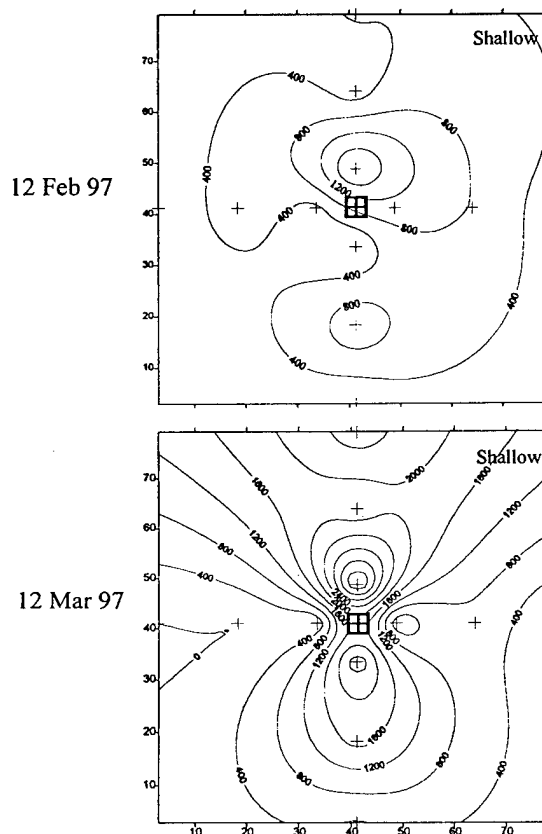
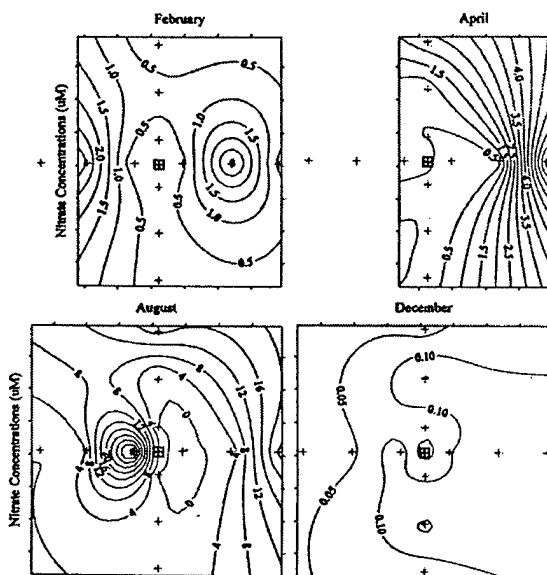
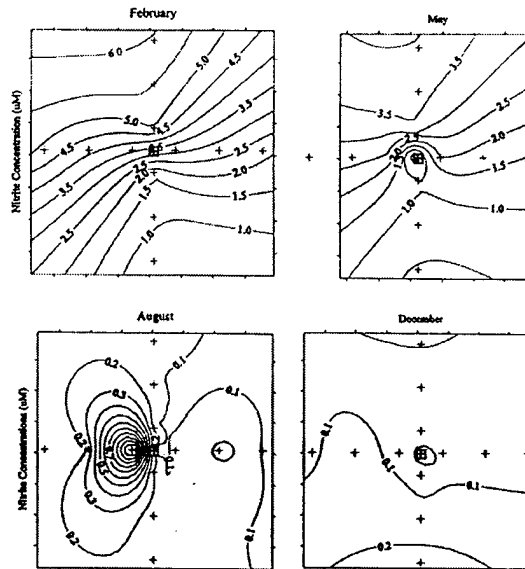
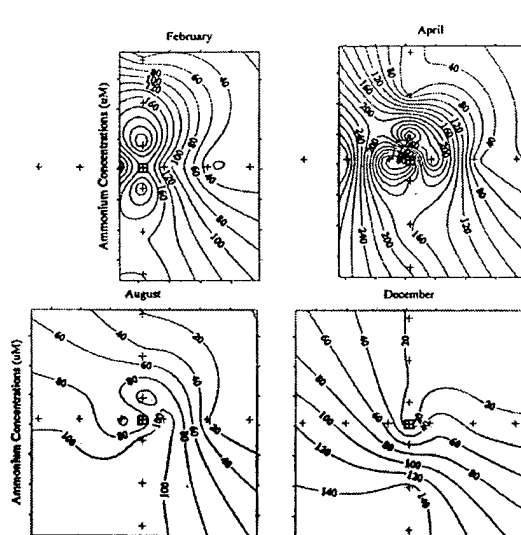
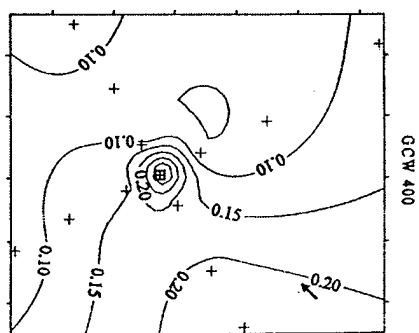
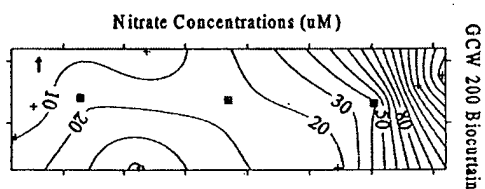
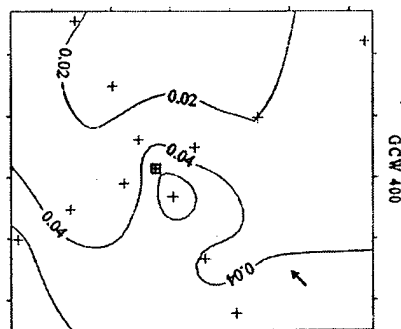
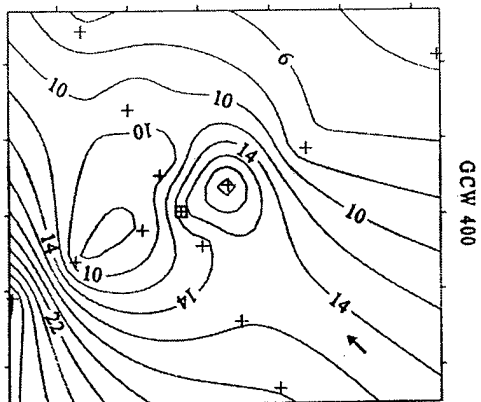
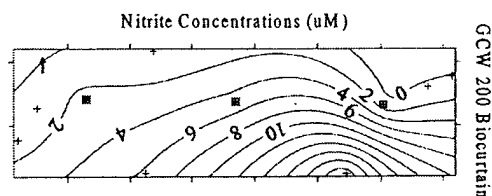
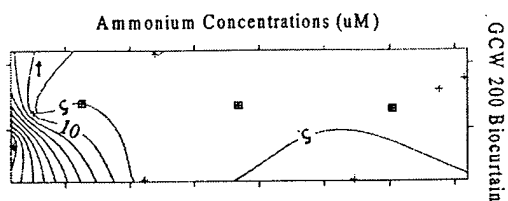


Figure 8. Bacterial production ($\text{cells mL}^{-1} \text{h}^{-1}$) increased after four weekly additions (starting 13 Feb) of diammonium phosphate in the monitoring wells adjacent to the GCW. Values for the GCW were removed for clarity.





Figures 9 A (upper left), B (upper right), C (lower left) present concentrations of ammonium, nitrite and nitrate during four sampling events in Gainesville. The temporal changes in nitrogen speciation and concentration indicate nitrification served part of the microbial community as an electron acceptor. This result could impede heterotrophic degradation of the contaminants. In this situation addition of the appropriate species of nitrogen to enhance bacterial degradation of the contaminant should be in the oxidized form.



Figures 10 A (upper left), B (upper right), C (lower left) present concentrations of ammonium, nitrite and nitrate at monitoring wells in Port Hueneme. Differences in inorganic nitrogen concentrations and speciation between contaminated and uncontaminated regions suggests that nitrate served as an electron acceptor for heterotrophic degradation of the BTEX.

Productivity

Peer-Reviewed Manuscripts

Salata, G. G., L. A. Roelke and L. A. Cifuentes. (submitted) A rapid and precise method for measuring stable carbon isotope ratios of dissolved inorganic carbon. *Mar. Chem.*

Coffin, R.B., Montgomery, M.T., Kelley, C.A., Hammer, B.T., Cifuentes, L.A., Mueller, J.G. (submitted) "Control of Nutrient Cycling by a Groundwater Circulation System" *Bioremediation Journal*.

Montgomery, M. T., Boyd, T.J., Spargo, B.J., Mueller, J.G., Coffin, R.B. and Smith, D.C. (submitted) "Bacterial production stimulated across the zone of influence of a groundwater circulation well in a BTEX-contaminated aquifer" *Environ. Sci. Technol.*

Kelley, C.A., Trust, B.A. and Coffin, R.B. "Tracing BTEX sources and transport in contaminated groundwater environments with GC/IRMS/ITMS" *Environ. Sci. Technol.* **31**, 2469-2472 (1997).

Pelz, O., Cifuentes, L.A., Kelley, C.A., Trust, B.A. and Coffin, R.B. "Tracing the assimilation of organic compounds using $\delta^{13}\text{C}$ analysis of unique amino acids in the bacterial cell wall" *FEMS Microbial Ecology* **25**, 229-240.

Pelz, O., Hesse, C., Tesar, M., Coffin, R.B. and Abraham, W.R. (in press) "Development of methods to measure carbon isotopic ratios of bacterial biomarkers in the environment" *Isotopes Environ. Health Stud.*

Trust, B.A., Hesse, D.L., Coffin, R.B., Mueller, J.G. and Cifuentes, L.A. (in press) "Tracing biodegradation with stable carbon isotope measurements of respired CO_2 " *Bioremediation*.

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