provectus
ENVIRONMENTAL PRODUCTS™

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STATEMENT OF QUALIFICATIONS – SEDIMENT ENVIRONMENTS

Provectus Environmental Products, Inc. has teamed with recognized experts to strategically manage sediment environments via specialized assessment, predictive analysis, and – where appropriate – remedial design support and implementation oversite. Each member of our Team has over 25 years' relevant experience and has documented scientific authority in their fields of expertise. Working in collaboration with RPs, environmental engineers, technical consultants, governmental regulators, and the wider academic community we provide scientifically valid, defensible, and cost-effective management strategies for even the most complex, challenging sites.

Personnel Resources

Members of Provectus' Sediment Management Team (**Appendix A**) and colleagues have worked together for almost 30 years. We have collaborated on dozens of sites around the world for both the public and private sectors. Our projects have included very large, very complex and exceedingly challenging sites involving multiple (international) parties. Projects and publications selected in **Appendices B and C** highlight this diverse expertise.

Services Offered

Using a variety of analytical tools (several of which we have developed over the past decades), our Project Team can provide defensible and highly accurate information on:

- **Fingerprinting Contaminant Source(s)**
- **Contaminant Transport & Residence Time**
- **Natural & Enhanced (Bio)degradation of Contaminants**
- **Spatial Variation of Contaminant Capacity for Accumulation vs. Degradation**
- **Differentiation from Background Concentrations**
- **Determination of Modern vs. Historic Anthropogenic Contributions**

We can support the development of Field Activity Plans by providing technical writing, sampling plans, analytical protocols, etc. We can assist with sample acquisition and related field work. We offer data interpretation and (statistical) analysis. When needed, we help present data in a clear and concise manner.

Version 2 – October 17, 2017. •

Remedial Technologies – Technical Resources

A range of remedial strategies have been designed and/or implemented in collaboration with site consultants. Where applicable, the Provectus line of environmental remediation products are available. These offer some truly unique chemistries focused on safety, tangible cost efficiencies, demonstrated effectiveness, distinguishable ease of use, and recognizable quality at the highest level.

- **Provect-CH4® Methanogen Inhibitor and ERD/ISCR Supplement**: Water-soluble amendment that can be used as a supplement to effectively control methane production.
- **Provect-IR® Solid, Antimethanogenic ISCR Reagent**: Unique combination of carbon + ZVI + AMR technology to treat halogenated compounds.
- **Provect-IRM® Solid Antimethanogenic ISCR Reagent / Metal Stabilization Reagent:** The Provect ISCR technology core makes this a more effective means of metal immobilization/ISCR that minimizes production of methylmetal(loids) for safer, more effective, long-term immobilization.
- **Provect-OX® Self-Activating ISCO/ Enhanced Bioremediation Reagent**: Persulfate-based ISCO reagent that is unique in terms of its safety (no extreme activators; no heat generated) and effectiveness, as it actively integrates enhanced bioremediation as part of the overall treatment process – only ISCO reagent designed to manage rebound;
- **AquaGate®-CH4™ Composite, Antimethanogenic Reactive Capping Technology:** Developed in collaboration with AquaBlok, LTD subaqueous caps can be constructed more effectively by minimizing gas ebullition and contaminant methylation.
- **Provect-GS™ NAPL Immobilization Technology:** A liquid reagent developed in collaboration Beazer East, Inc. for *in situ* geophysicochemical immobilization (ISGI) of DNAPL sources.
- **Compound-Specific Stable Isotope Analysis:** An accurate, cost effective method to identify organic and inorganic compound sources and fate.
- **Radiocarbon Isotope Analysis:** A forensic tool that confirms *in situ* organic compound degradation.
- **Mineralization and Microbial Production Assays:** Microbial contaminant utilization and overall microbial growth rate measurements using short incubations. Represents specific organic compound biodegradation in sediments or water column and its relevance to total microbial carbon and energy demand, providing thorough assessment of the compound of interest residence time.

Project Examples – Case Studies

Ambient polycyclic aromatic hydrocarbon (PAH) concentrations are often measured in estuarine sediments to evaluate risk of those chemicals to benthic infauna and adjacent ecosystems. Typical site investigations assume that PAHs are present in the sediment because of historical contamination despite overwhelming evidence of the dynamic nature of contaminants and associated sediment transport in estuaries. Although many processes may affect PAH concentration, we typically focus on two that are most likely to have to largest impact on PAH flux in the sediment; i) influx of PAH associated with settling water column particles, and ii) bacterial contaminant degradation in surface sediments. Our Team Members have developed several of the most advanced analytical tools and procedures to conclusively define and quantify these values. These evaluations equip site managers and regulators with valuable decision-making tools for ecological risk reduction due to intrinsic bioremediation relative to engineering solutions, such as remedial dredging. This focus has transitioned to capability to address chlorinated and nitroaromatic compounds.

Evaluating *in situ* biodegradation of organic contaminants requires an efficient, precise, and cost-effective monitoring strategy. Predicting environmental remediation time scales requires understanding contaminant turnover with respect to additional source introduction, contaminant transport and (bio) degradation. Multiple sources and their mixing may complicate remedial actions at many subsurface fuel-contaminated sites. Anthropogenic organic compound turnover depends on organic contaminant availability and inherent lability, nutrient availability, natural organic carbon concentrations, and seasonal physicochemical variability. Thus, defining the parameters necessary to substantiate natural attenuation is difficult and not without uncertainty. Stable carbon and radiocarbon isotope analysis ($\delta^{13}C$ and $\Delta^{14}C$, respectively) have been extensively used to assess basic biogeochemical roles in natural carbon cycling. More recently, stable carbon isotope analysis has been applied to identify contaminant carbon as well as biodegradation byproducts and residual contamination. Stable isotope techniques have also been applied to field settings by analyzing fractionation factors, addition of stable isotope labeled tracers, and evaluating efficacy of active bioremediation strategies through monitoring production of contaminant-derived CO_2. In addition, as a biomarker approach, bacterial nucleic acid stable carbon isotopes were analyzed from beaches contaminated with oil from the Valdez oil spill to confirm bacterial hydrocarbon biodegradation in Prince William Sound, Alaska. Aside from measuring fractionation factor(s) of residual contaminant pools, monitoring hydrocarbon biodegradation respiration products (e.g., CH_4 and CO_2) to confirm biodegradation may require the least analytical effort. This strategy has been applied to groundwater and vadose zone gases

A variety of relevant projects are outlined in **Appendix B**. These projects document our ability to save significant time and money by assisting with:

- Proper delineation of areas of interest
- Accurate assessment of transport processes for nonpoint-source contaminant loading
- Prediction of the natural in situ degradation of the contaminant
- Performance monitoring post remedial action

Representative example projects are summarized below:

- *NAVFAC, San Diego California:* Assessment of natural attenuation of PAH loading to sediments near San Diego Naval Air Station and through the urbanized water way.
- *NAVFAC, Charleston Navy Yard, South Carolina:* Sediment PAH source and natural attenuation within and adjacent to the Charleston Naval Ship Yard in the Cooper River.
- *NAVFAC, Philadelphia Navy Yard Reserve Basin:* PAH-impacted sediment transport and source assessment
- *Latvian Harbor Remediation Liepaja Latvia:* Harbor sediment contaminant source assessment and natural attenuation for economic free trade zone development.
- *NAVFAC, Norfolk, Virginia*: Hydrocarbon source discrimination using CSIA and multivariate statistics.
- *NAVFAC, Norfolk, Virginia*: Radiocarbon natural abundance application for confirmation of organic compound degradation.

Contact Information:

Dr. Jim Mueller, Provectus (815) 650-2230 or email jim.mueller@provectusenv.com

Dr. Richard Coffin, Strategic Carbon, llc (301) 404-2364 or email rcoffin@strategic-carbon.com

Case Study - NAVFAC, San Diego, California:

To help elucidate PAH fate in impacted sediments, a combination of sediment chemistry, modeling, and microbial ecology techniques measured the effects of bioirrigation on the natural microbial assemblage in coastal sediments from two stations in an urbanized waterway in San Diego Bay, CA. Rates of heterotrophic bacterial production and mineralization of naphthalene, phenanthrene and fluoranthene provided quantitative measure of intrinsic PAH bioremediation capacity. The microbial studies were accompanied by pore water chemical analyses to establish the redox conditions of the sediments.

All purported PAH fluxes were measured often (very expensive). It was ultimately observed that the difference between PAH settling and microbial PAH mineralization were responsible for the sediment concentration, not historical contamination. By disturbing the natural PAH attenuation processes via dredging, the concentration of PAHs in the incoming and settling particles was predicted to cause an actual increase in post-dredged sediment PAHs. In fact, this was observed with PAH concentrations increasing from 1-5 ppm pre-dredge to >20 ppm after dredging, thus questioning the value of the remedial action. Please see **Attachment 1** (Montgomery *et al*., 2008) for more details.

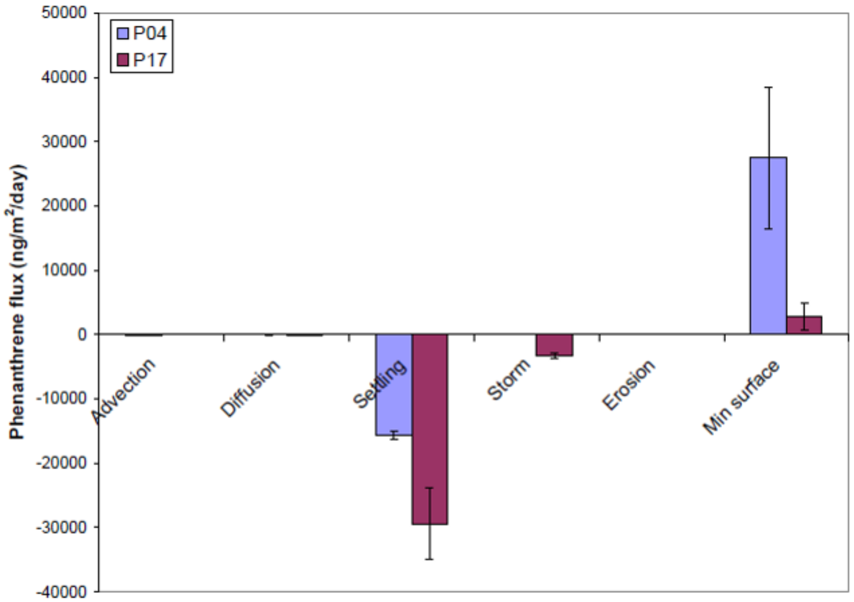


Figure 6-26. PRISM pathway fluxes for phenanthrene including comparison for depth-integrated biodegradation (top) and without it (bottom).

Case Study - NAVFAC, Charleston Navy Yard, South Carolina:

The Charleston Navy Yard (Figure 1) faced a proposal to dredge PAH-impacted sediments at an estimated cost of > $14M. Using a series of forensic assays, Team Members compared PAHs associated with water column seston, in the benthic boundary layer, and on settling particles with PAH biodegradation rates measured in the underlying sediments. These data demonstrated that PAH concentrations were an order of magnitude higher in the sediment trap material originating up-gradient and falling onto the targeted sediment itself.

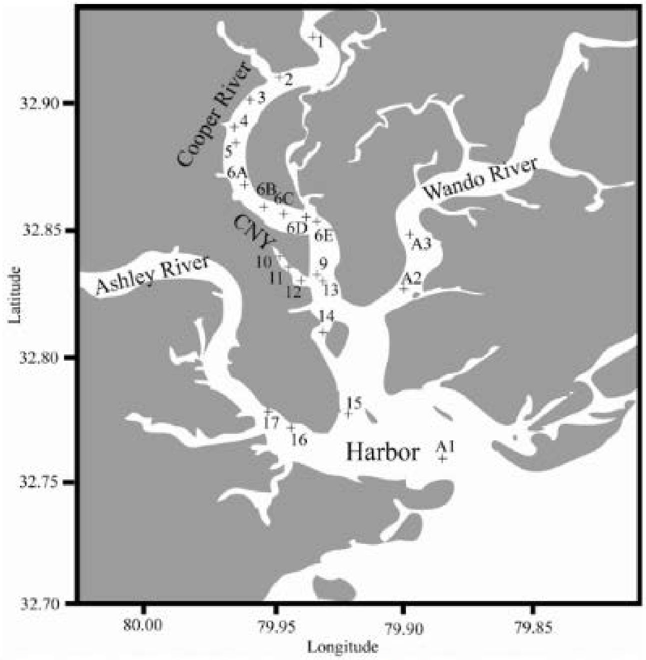
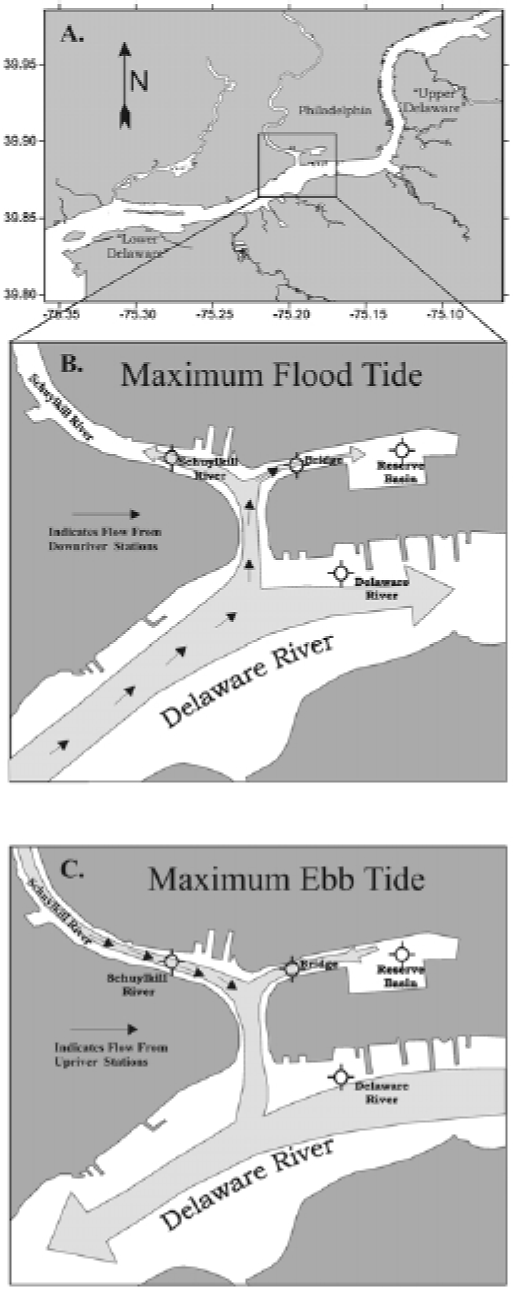


FIGURE 1. Sampling stations in the Charleston Harbor Estuary, SC, USA adjacent to the former Charleston Navy Yard (CNY).

Bacterial mineralization of PAH was similarly rapid between nepheloid and surface sediment samples with turnover times for phenanthrene in the sediments of around 100 days. Hence, the flux rates into the sediment matched the biodegradation rates normalized for the depth of the bioturbation layer thus explaining how the concentrations of PAHs in the sediments were lower than the settling particles. Carbon sourcing data also demonstrated that the PAHs originated off site (with the tidal sampling) and without control of the source(s), it was predicted that the PAH concentrations would increase to match the settling particle concentration post-dredging thus questioning the value of the effort. Please see **Attachment 2** (Montgomery *et al*., 2005) for more details.

Case Study - NAVFAC, Philadelphia Navy Yard:

The Philadelphia Naval Reserve Basin (RB) is a small semi-enclosed embayment near the confluence of the Schuylkill and Delaware Rivers in Pennsylvania (USA). It is the Navy's only freshwater reserve, and was once an area of active ship maintenance, storage and construction Because of past activities within the RB, it is considered a potential contaminant source to adjacent areas within the watershed, with tidal flux of particle-attached PAHs the primary mechanism of exchange between the RB and surrounding watershed. Because proximal sources of contamination (Naval activities) have been attenuated over the past 10 years (no releases) and the depositional accumulation of sedimentary material over time within the RB, we hypothesize that the RB may be a sink for contaminants in the watershed.

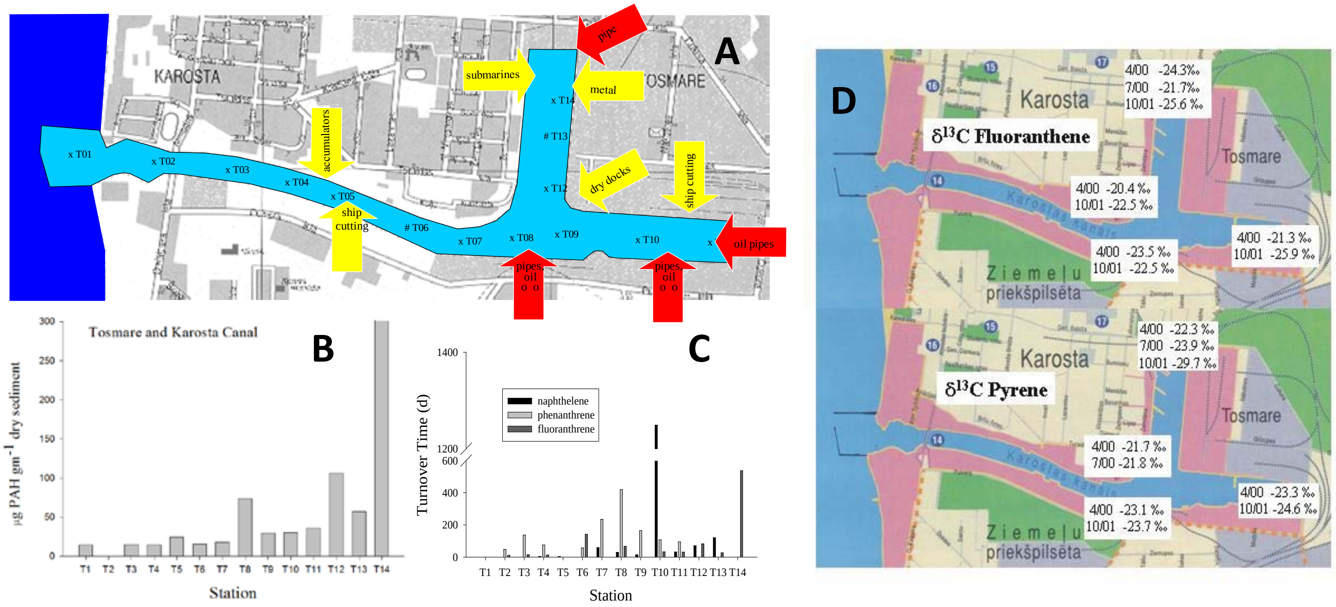


To validate whether the RB serves as a watershed source or sink for contaminants, we conducted a study at this site to determine the tidal flux of particles and particle-bound contaminants associated with the RB. Particle traps were placed at the mouth and inside the RB and in the Schuylkill and Delaware Rivers. There was net particle deposition into the RB, which was determined for three seasons. Spring and fall depositions were highest (1740 and 1230 kg of particles, respectively) while winter deposition was insignificant. PAH concentrations on settling particles indicated a net deposition of 12.7 g PAH in fall and 2.1 g PAH in spring over one tidal cycle. There was no significant PAH deposition in the winter. Biodegradation rates, calculated from C-labeled PAH substrate mineralization, could attenuate only about 0.25% of the PAH deposited during a tidal cycle in fall. However, in the spring, biodegradation could be responsible for degrading 50% of the settling PAHs. The RB appears to be a sink for PAHs in this watershed.

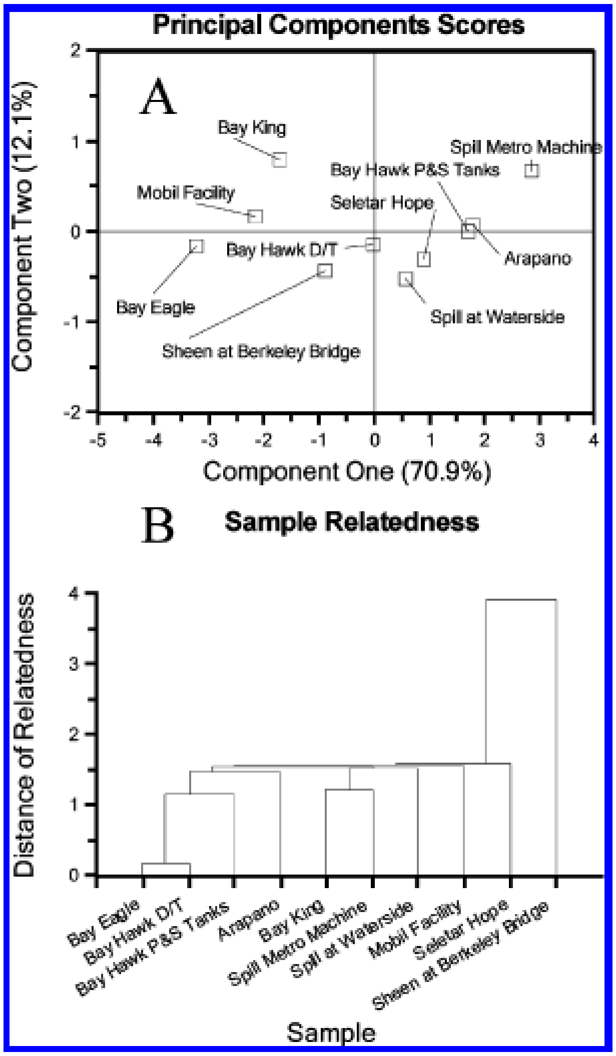
Please see **Attachment 3** (Pohlman *et al*., 2002) for more details.

Case Study: Latvian Harbor Remediation Liepaja Latvia.

Russian departure from Latvia during the late 1980's resulted a need for extensive environmental cleanup. The Russian military harbor in Liepaja was left with a large number of metal and petroleum wastes (panel A below). This region was considered as a key for development of an international port with a focus on economic free trade between Baltic, Scandinavian, and Western European nations. Success for development in this region required dredging of the harbor to allow access to large tanker and transport vessels. Initial assessment of this region showed high PAH loading through the harbor. Part of this focus was fuel contaminant source identification and prediction of the natural attenuation; technology developed by this Provectus team. Result of this study showed: 1) high PAH loading in the upper regions of the canal (panel B); active microbial degradation of PAHs (panel C); and 3) a range of stable carbon isotope values that suggests the presence of 4-5 anthropogenic sources (panel D). The variation in the isotope ratios at individual sites suggested multiple sources over time. The microbial assessment showed PAH concentrations at the end of the summer were low. Key learning from this assessment related to the harbor development included: 1) natural attenuation can provide a thorough cleanup of the ecosystem; 2) site variation of stable carbon isotope ratios indicated there were active leaking underground lines; and 3) selection of a dredging time for fall season, after active microbial degradation, would allow disposal of dredged harbor sediments offshore in the Baltic Sea. Note harbor dredging and deposition of the contaminated sediments was estimated to cost $26,000,000.



Case Study - NAVFAC, Hydrocarbon source discrimination using CSIA and multivariate statistics, NORFOLK:



Compound Specific Isotope Analysis (CSIA) has been shown to be a useful tool for assessing biodegradation, volatilization and hydrocarbon degradation. Attempts to use isotopic values for linking sources to spilled or otherwise unknown hydrocarbons have been hampered by the lack of a robust and rigorous statistical method for testing the hypothesis that two samples are or are not the same. The Provectus team developed a method by which CSIA information is projected in a simplified data-space, enabling multivariate analysis of variance (MANOVA) and highly precise testing of hypotheses between unknowns and putative sources. A Monte Carlo simulation of a 10 variable data set; tanks used to store, distribute and offload fuels from Navy vessels; and a series of spilled oil samples and local tug boats from Norfolk, VA (USA) were subjected to CSIA and the team-developed statistical methods. The technology was able to identify a leaked source tank among sampled ship's tanks at the Norfolk Navy Base. The method is amenable to any multicomponent organic contaminant source (such as PAHs). Samples from several areas can be directly compared to determine if the source(s) are the same or statistically different. For instance deeper sediments believed to have an historical contaminant source could be screened using this method against contemporately-deposited sediments to confirm or deny statistical differences. This method could be used to refute claims that a site's contaminants are being liberated and impacted an adjacent site's sediments.

Please see **Attachment 4** (Boyd *et al.*, 2006) for more details.

Case Study – NAVFAC, Norfolk, Virginia:

CO_2 and CH_4 radiocarbon and stable carbon isotope ratios were used to assess natural attenuation at a fuel-contaminated soil site at the Norfolk Navy Base, Norfolk, VA (Figure 1). Soil gas samples were collected spatially over a monitoring network in October 2002 and in March 2003. CO_2 and CH_4 from regions with high petroleum concentrations were ^{14}C-depleted relative to uncontaminated areas. ^{14}C-depleted methane suggested methanogenic hydrocarbon degradation.

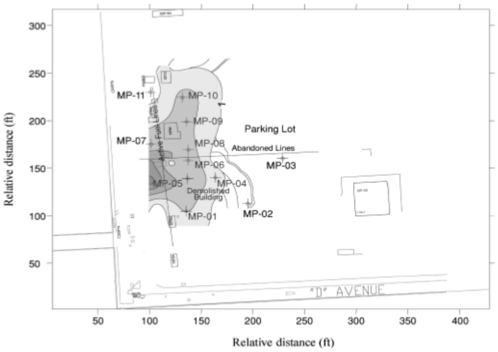


Figure 1: Overview of petroleum contamination from leaking underground pipelines at the Norfolk Navy Base. Data from monitoring wells are presented in the following figure.

The difference in CO_2 age between background and plume-influenced areas indicated that approximately 90% of the CO_2 at the latter was petroleum derived, making contaminant the primary source of carbon for the microbial assemblage. ^{14}C isotope analysis can be used to differentiate between contemporary carbon respiration relative to the petroleum-derived carbon respiration.The technique is very sensitive because there is considerable analytical resolution between end-members (approximately 1200‰). ^{14}C-depleted CO_2 occurred where CO_2 concentrations were elevated (Figure 2). For October 2002 and March 2003, CO_2 from the control MPs ranged from 8.6 to 27.8 pMC, which suggests isolation from atmospheric exchange and microbial degradation of aged plant material if one assumes there was no residual or lateral contributions from petroleum degradation. This study indicates that fuel hydrocarbons are a significant carbon source to in situ bacteria and that fuel hydrocarbon respiration resulted in the production of ^{14}C-depleted CO_2. Further analysis of data in this study shows a reduction of CO_2 to CH_4 with stable carbon isotope ratios, indicative of microbial methanogenesis.

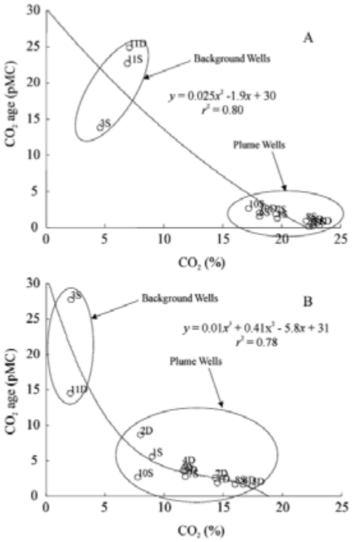


Figure 2: Percentage modern carbon (pMC) soil gas CO2 relative percent-age to CO2 for A) October and B) March.

Please see **Attachment 5** (Coffin *et al.,* 2008) for more details.

APPENDIX A

PROFESSIONAL PROFILES

Curriculum Vitae

Thomas J. Boyd
US Naval Research Laboratory
Marine Biogeochemistry, Code 6114
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EDUCATION

B.S. Biology, Washington and Lee University, Lexington, VA, 1987
B.A. Chemistry, Washington and Lee University, Lexington, VA, 1987
M.S. Marine Biology, Scripps Institution of Oceanography, La Jolla, CA, 1992
Ph.D. Marine Biology, Scripps Institution of Oceanography, La Jolla, CA, 1993

POSITIONS

Laboratory Assistant, Washington and Lee University, Lexington, VA, 1985-1987
Teaching Assistant, University of California, San Diego, La Jolla, CA, 1990
Research Assistant, Scripps Institution of Oceanography, La Jolla, CA, 1987-1994
Research Assistant, California Regional Marine Research Program, La Jolla, CA, 1993-1994
John Knauss Sea Grant Fellow, National Science Foundation, Arlington, VA, 1994-1995
Scientist, Geocenters, Inc., Naval Research Laboratory, Washington, DC, 1995
NRC Research Associate, Naval Research Laboratory, Washington, DC, 1995-1996
Research Microbiologist, Naval Research Laboratory, Washington, DC, 1996-present
Member, Vice-Chair, US NRL Radiation Safety Committee, Washington, DC, 1996-present
Member, Delaware Replacement Vessel Committee (to aid in science mission requirements for the University of Delaware *R/V Cape Henlopen* replacement vessel), Lewes, DE, 2002-2005
Network Technical Representative, Chemistry Division, US NRL, Washington, DC, 2006-present (assistant NTR, 2001-2005)
Program Officer, United States Arctic Military Cooperation Program. Program 2.3. Improving Conditions at Arctic Military Bases. (2003-2006) (concurrent assignment).
Program Officer, National Science Foundation. Earth Sciences Instrumentation & Facilities Program. Detail from NRL – April 2008 to March 2011.

TEACHING EXPERIENCE

High School Tutor, Biology, Lexington High, Lexington, Virginia, 1986-1987
Laboratory Assistant, Washington and Lee University, 1985-1987
Teaching Assistant, Microbiology, University of California, San Diego, 1990
SCUBA Diving Instructor, University of California, San Diego, 1992-1994
SCUBA Diving Instructor, SeaVentures, Fairfax, VA, 1994
Assistant Professor, Adjunct, University of Maryland, College Park, MD, 1999-2007.
Associate Professor, Adjunct, University of Maryland, College Park, MD, 2007-present.

AWARDS AND HONORS

Civilian

Phi Eta Sigma, 1984, Washington and Lee University
Alpha Epsilon Delta, 1985, Washington and Lee University
Phi Beta Kappa, 1986, Washington and Lee University
Biology Departmental Award, 1987, Washington and Lee University
Sigma Xi Grant-in-Aid for Research, 1989, Scripps Institution of Oceanography
Sea Grant Trainee, 1990, Scripps Institution of Oceanography
John Knauss Sea Grant Fellow, 1993, National Science Foundation
NRC Research Associate Award, 1995, Naval Research Laboratory
Alan Berman Basic Research Publication Award, 2005, Naval Research Laboratory
Alan Berman Basic Research Publication Award, 2007, Naval Research Laboratory
Nomination, Stanley Drazek Teaching Excellence Award, 2007, University of Maryland, University College
PMP, 2014, Project Management Institute.

RESEARCH INTERESTS

Microbial utilization of phenolic and other aromatic compounds in freshwater, marine, coastal and soil/groundwater environments. Microbial utilization of dilute organic compounds (amino acids, peptides, simple aromatic compounds and contaminants) in multiple environments. Biogeochemistry of recalcitrant organic matter (humic and aromatic compounds). Separation, characterization, and quantification of organic compounds (including contaminants) in seawater, wastewater, groundwater and freshwater. Specific bacterial attachment and induction of metabolic enzymes. Biochemistry and enzymology of contaminant biodegradation. Chemical identification techniques including stable- and radiocarbon analysis to assess biodegradation of aromatic compounds in contaminated and natural environments. Environmental forensics and the use of carbon isotope ratios to source-apportion chemical mixtures and track contaminant carbon into biogeochemical cycles. Chemometrics.

RESEARCH EXPERIENCE

Participation in 45+ oceanographic research cruises in the Northeast Pacific Ocean off California and Hawaii, Northeast Atlantic, Gulf of Mexico, South Pacific (New Zealand), Sulu Sea, Indian Ocean, and the Baltic Sea. Studies included determinations of microbial population dynamics in Benthic Boundary Layer (BBL), sea-surface microlayer environments, bulkwater and sediments. Sampling and determination of organic compounds and optical properties in estuaries, seawater, groundwater, and municipal wastewaters. Chief Scientist for over 25 cruises (Charleston, SC; Philadelphia, PA, Chesapeake Bay, Middle Atlantic Bight, San Francisco Bay, Gulf of Mexico, Puget Sound). Determination of microbial ecology parameters (contaminant mineralization, bacterial activities, bioremediation) in various contaminated groundwater, freshwater, marine and sediment systems. Training in and use of IR, UV-VIS, and NMR spectroscopy, HPLC and GC/MS, isotope ratio mass spectrometry, accelerator mass spectrometry, gel chromatography and radiolabeled tracers.

PUBLICATIONS (peer review)

1. **Boyd, T.J.**, Montgomery, M.T., Cuenca, R.H., Hagimoto, Y. 2015. Combined radiocarbon and CO_2 flux measurements used to determine *in situ* chlorinated solvent mineralization rate. Environmental Science: Process & Impacts. 17(3):683-692. DOI: 10.1039/C4EM00514G.

2. **Boyd, T.J.**, Pound, M.J., Lohr, D., and Coffin, R.B. 2013. Radiocarbon-depleted CO_2 evidence for fuel biodegradation at the Naval Air Station North Island (USA) fuel farm site. Environmental Science: Process & Impacts. 15(5):912-918. DOI: 10.1039/C3EM00008G.

3. Montgomery, M.T., Coffin, R.B., **Boyd, T.J.** and Osburn, C.L. 2013. Incorporation and mineralization of TNT and other anthropogenic organics by natural microbial assemblages from a small, tropical estuary. Environmental Pollution. 174:257-264.

4. Montgomery, M.T., Coffin, R.B., **Boyd, T.J.**, Smith, J.P., Plummer, R.E., Walker, S.E., and Osburn, C.L., 2011. Mineralization rates of 2,4,6-Trinitrotolene and bacterial production amongst natural microbial assemblages in coastal sediments. Environ. Poll. 159, 3673-3680. doi:10.1016/j.envpol.2011.07.018.

5. Montgomery, M.T., **Boyd, T.J.**, Osburn, C.L., and Smith, D.C. 2010. PAH mineralization and bacterial organotolerance in surface sediments of the Charleston Harbor estuary. Biodegrdation. 21(2):257-266.

6. Coffin, R.B., Pohlman, J.W., Grabowski, K.S., Knies, D.L., Plummer, R.E., Magee, R.W., and **T.J. Boyd**. 2008. Radiocarbon and Stable Carbon Isotope Analysis to Confirm Petroleum Natural Attenuation in the Vadose Zone. Environmental Forensics. 9(1):75-84.

7. **Boyd, T. J.**, Osburn, C. L., Birgl, K. B., and R. B. Coffin. 2006. Compound-Specific Isotope Analysis Coupled With Multivariate Statistics to Source-Apportion Hydrocarbon Mixtures. Environ. Sci. Technol. 40(6):1916-1924.

8. **Boyd, T.J.**, Montgomery, M.T., Steele, J.K., Pohlman, J.W., Reatherford, S.R., Spargo, B.J., and D.C. Smith. 2005. Dissolved oxygen saturation controls PAH biodegradation in freshwater estuary sediments. Microb. Ecol. 49(2):226-235.

Richard Banks Coffin

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EDUCATION

NSF funded Postdoctoral Fellow (Mar. 1986 - Dec. 1987) Gordon College, Wenham, MA. (Advisor: Richard T. Wright)
Ph.D., Oceanography (Sep. 1982 - June 1986), University of Delaware. (Advisor: Jonathan H. Sharp)
M.S., Microbiology (Sep. 1978 - May 1980), University of New Hampshire (Advisor: Galen Jones)
B.A., Microbiology (Sep. 1973 - June 1977), University of New Hampshire.

POSITIONS

Full Professor, Chair Department of Physical and Biological Sciences, Texas A&M University Corpus Christi, July 2014 – Present.

Founder-President Strategic-Carbon LLC 2011- Present.

Section Head Code 6114, Marine Biogeochemistry. Lead for international development of methane hydrate exploration and deep ocean sediment carbon sequestration. Naval Research Laboratory, DC, September 2003 to June 2014.

Adjunct Faculty Professor, Texas A&M University – Corpus Christi, October 2013 to Present.

Senior Research Microbiologist, Naval Research Laboratory, Washington DC, June 1996 to September 2003.

Research Microbial Ecologist, US Environmental Protection Agency, Gulf Breeze, FL. July 1990-June 1996.

Adjunct Faculty Member, Hawaii Natural Energy Institute, Univ. of Hawaii, Honolulu, Hawaii, August 2000- present.

Adjunct Faculty Member, College of Marine Studies, University of Delaware, Lewes DE September 1996 - 2005.

Adjunct Faculty Member at FSU and TAMU, September 1990 – 2000.

BACKGROUND

Isotope geochemistry is applied to determine key ecosystem cycles. Work has focused on soil groundwater systems, estuaries, coastal and deep ocean water column and sediment. Field work has been global with recent priorities on Navy harbors through the US, the Arctic Ocean and coastal regions off Chile, New Zealand, Canada, Norway, Japan and the US. During these field efforts contribution has been through working as leader or co-leader of planning, execution and interpretation. Work has addressed environmental assessment, energy exploration and field technology development. Examples of success include providing a 25 million dollar cost savings plan for harbor remediation in Liepaja Latvia and focusing methane hydrate energy exploration sites off the coasts New Zealand, Alaska, Texas and Chile providing 10 – 30 million dollar savings at each potential drill site.

KEY CAREER PROGRAMS

1996 – present, Adjunct Faculty Member – Universities of Delaware, Hawaii, Florida State, Texas A&M, Texas A&M Corpus Christi. Serving on 15 students Ph.D. and Masters committees and providing invited lectures to classes. Additional academic interactions have been with students from William and Mary University, the Catholic University of Valparaiso and University of Auckland.

2000 – present, Ocean Methane Hydrate Exploration - NRL and TAMU-CC lead for national and international methane hydrate research and development. Chief and Co-chief scientist off the coasts of US (Gulf of Mexico and Arctic), Canada, Chile, New Zealand and Norway on 16 expeditions. Approximate budget $25 mil.

2000 – present, International Methane Hydrate Research and Development Workshops. Co-organizer and developer of workshops to plan international methane hydrate research globally. Planners from the US, Norway and Japan have lead international workshops in Washington DC, Hawaii, Canada, New Zealand, Chile, Scotland, Norway and Japan. 25 countries participated. Approximate budget $2 mil.

1998 – 2008, Military Harbor Environmental Assessment. Lead and co-lead harbor remediation programs in 8 military harbors to assess contaminant sources and natural attenuation schedules. Approximate budget $12 mil.

2002 – 2004, Boron nanoparticle fuel enhancement. Approximate budget $1 mil.

2000 – 2004, Deep Ocean Carbon Sequestration - Chief scientist for field operations on an international deep ocean carbon sequestration involving participants from the US, Norway, Canada, Japan and Australia. Approximate budget $5 mil.

1992 – 1995, Florida Key Coral Reef Health. Chief Scientist on 6 expeditions through the Florida Keys to assess environmental impact on coral reefs. Approximate budget $ 2 mil.

1989 – 1996, Gulf of Mexico Hypoxia Assessment. Chief Scientist on 12 research expeditions to study Mississippi River impact on the Gulf of Mexico coastal hypoxia. Approximate budget $6 mil.

1990 -1995, National Biotechnology Risk Assessment Manager, US EPA Gulf Breeze. Planning and review of biotechnology risk assessment research conducted at EPA National Research Laboratories. Approximate budget $75 mil.

1990 – 1993, Ecuador Mangrove Deforestation/Shrimp Pond Development Environmental Impact. Assessment of environmental impact for mangrove deforestation and the resulting development of shrimp ponds resulted in a thorough ecosystem assessment. The data gathered resulted in Ecuadorian president requesting a legal testimony in an environmental impact law suit between shrimp and banana farmers. Approximate budget $1.5 mil.

RESEARCH EXPERIENCE

2017-2018, Chief scientist leading assessment of Texas Coastal oxygen demand. Research is supported through Texas OneGulf

2017-2018, CoPI for development of UAV and sensors to conduct airborne assessment of methane and carbon dioxide gas fluxes in the coastal ocean. Research is supported through NSF MRI.

2015 Co-chief scientist on an expedition off the Hikurangi Margin, New Zealand to provide geochemical assessment of a double BSR. Research is supported through NIWA, DOE, and ONRG.

2013-2014, Co-chief scientist of overflight assessment and ground truthing of Arctic methane flux from tundra, thermokrast lakes and coastal waters to the atmosphere. Researchers from NOAA, Harvard, USACE and NRL are supported with funding from NSF and Navy RRTO.

2011-2013, Co-chief scientist, New Zealand, paleo-geochemistry and methane hydrate exploration. 3 expeditions on the RV Polaris and RV Sonne. ONR, DOE, GEOMAR, and New Zealand government funding.

2009, Chief Scientist, Beaufort Sea, methane hydrate research, energy and climate. 4 nations, 34 scientists. ONR, DOE, NRL funding.

2007, Chief Scientist, Alaminos Canyon, Texas-Louisiana Shelf. Methane hydrate exploration. DOE, ONR funding.

2006, Co-chief scientist, Hikurangi Margin, New Zealand plate convergence contribution to vertical methane flux. ONR, NRL, ONRG, and DOE funding.

2000-2006, Military harbor remediation research, District of Columbia, Philadelphia, Charleston, Honolulu, Norfolk, San Diego, San Francsico. ONR funding.

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2003-2004, Co-chief scientist, two expeditions off the mid Chilean Margin. Methane hydrate exploration.

2000-2003, Co-chief scientist, three expeditions on the Cascadia Margin, Canada. Methane hydrate exploration.

2002-2005, Chief scientist, three expeditions of the Texas-Louisiana Shelf. Methane hydrate exploration. DOE, ONR funding.

1999-2004, Chief scientist - initiate preliminary survey for international experiment in carbon sequestration off Hawaii and Norway. Four cruises were for 7-10 days with scientists from Japan, Norway, Canada and the US. 6 sub dives on Loihi. DOE funding.

1999-2001, Co-Chief scientist -Five cruises in Russian Military Harbor Liepaja Latvia, working on ONR funded harbor remediation. Collaborative with University of Latvia. ONR, ONR Global funding.

1998, 2000, Co-Chief scientist, 14 submarine dives in the Gulf of Mexico, from the Edmund Link. Sample for $\Delta^{14}C$ and $\delta^{13}C$ analysis to initiate understanding of methane hydrate formation. NRL funding.

1992-1996, Co-Lead Scientist, Groundwater organic contaminant natural attenuation and source identification. Gainesville, FL and Port Hueneme CA. SERPD funding.

1995, Antarctica, Co-Chief Scientist, Ozone depletion. NSF funding.

1992-1995, Gulf of Mexico Hypoxia. Chief Scientist, 10 expeditions. EPA funding.

1990-1993, Florida Keys, coral health assessment. Chief Scientist 6 expeditions. EPA funding.

1990-1995, US EPA National Manager Biotechnology Risk Assessment, Gulf Breeze FL.

1990-1993, Ecuador Mangrove Research. World Bank and EPA funding.

1992, Ecuador shrimp pond toxicity. Ecuadorian government funding.

1989-1990, Valdez oil spill natural attenuation research. EPA and Exxon funding.

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Recent Presentations

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Coffin, R. 2016. Integration of Geochemistry and Geophysics Applied to Coastal Gas Hydrate Assessment. February 9, 2016. University of Tromsø. INVITED

Coffin, R., Rose, P., Pecher, I., Davy, B., Kvamme, B. 2016. Carbon Dioxide Vertical Migration on the Chatham Rise, New Zealand. Poster. Gordon Conference, Natural Gas Hydrate Systems, Galveston TX. 28 Feb. – 4 March 2016.

Osburn, C.L., Boyd, T.J., Montgomery, M.T., Bianchi, T.S., Coffin, R.B., Paerl, H.W. 2016. Optical Proxies for Dissolved Organic Matter in Estuaries and Coastal Waters. Ocean Sciences, New Orleans February, 2016.

Coffin, R.B. 2016. Integration of Geochemistry and Geophysics Applied to Coastal Gas Hydrate Assessment. Corpus Christi Geological Society & Coastal Bend Geophysical Society: Luncheon Meeting, February 17, 2016. INVITED.

Pecher, I., Davy, B., Rose, P., Coffin, R. 2015. Are seafloor pockmarks on the Chatham Rise, New Zealand, linked to CO2 hydrates: Gas hydrate stability considerations. AGU San Francisco OS22B-04.

Rose, P. Coffin, R., Yoza, B., Boyd, T., Crutchley, G., Mountjoy, J., Pecher, I. 2015. Gas and porewater composition of shallow sediments in the Tuaheni Basin, New Zealand. AGU San Francisco OS23B-191

Smith, A. Pecher, I., Davy, B., Coffin, R., Rose, P. 2015. Seismic Studies of Paleo-Pockmarks on the Chatham Rise, New Zealand. AGU San Francisco OS23B-1996

Coffin, R., Rose, P., Yoza, B., Boyd, T., Crutchley, G., Mountjoy, J. 2015. Vertical Methane Migration on the Hikurangi Margin off the Mahia Peninsula, New Zealand. AGU San Francisco OS23D-03

Coffin, R.B. 2015. An Overview of Gas-Hydrate Exploration Using Geo-Science Data. Invited Seminar, Univeristy of Auckland, Auckland New Zealand, April 11, 2015.

Coffin, R.B., Rose P.A. Klaucke, I., Pecher, I. 2014. Evaluation of shallow sediment methane cycling in a pockmark field on the Chatham Rise, New Zealand. AGU OS21A-1100.

Rose, P.A., Cochran, J., Heilbrun, C., Pecher, I., Coffin, R.B. 2014. Sediment Mixing in a Pockmark Field on the Chatham Rise, New Zealand – Implications for Paleoceanographic Reconstructions. AGU PP41C-1394.

Waghorn, K., Pecher, I., Strachan, L., Crutchley, G., Bialas, J., 2014 3-D seismic study into the origin of a large seafloor depression on the Chatham Rise, New Zealand. AGU OS21A-1102.

Murgulet, D., Rose, P., Hay, R., Coffin, R. 2014. Shallow Aquifer Methane Gas Source Assessment.

AGU H23C-0897.

Smith, J., Suriben, R., Coffin, R., Boyd, T. 2014. Late-Summer Tundra Methane Concentrations and Fluxes on the North Slope of Alaska. AGU GC11B-560.

MICHAEL THOMAS MONTGOMERY, Ph.D.

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Qualification Summary

Research Microbiologist with substantial background in marine biogeochemistry, benthic ecology, and intrinsic bioremediation, including specific knowledge of the marine biogeochemistry of lower Chesapeake Bay. Qualifications include direct expertise in:

- Evaluating contaminant biodegradation in estuarine waters and sediments
- Evaluating vendor technologies for organic contaminant remediation in sediments
- Explaining complex scientific ecosystem evaluation methods and principles to regulators and stakeholders
- Working with industry to develop remedial alternatives
- Transferring technology from basic science organizations to industrial sector
- Integrating measures of contaminant fate and transport within the context of complex physical and biological parameters of coastal waters

Professional Experience

Naval Research Laboratory, Marine Biogeochemistry Section, Washington, DC
1993-Present

Research Microbiologist NP-04, Federal	(September 2009–Present)
Acting Section Head, Federal	(December 2003–June 2004)
Research Microbiologist NP-03, Federal	(May 1998–September 2009)
Scientist III, On-Site Contractor	(March 1993–May 1998)

Foster the development of novel environmental research strategies involving contaminant degradation, transport, and source in coastal ecosystems, including tidally influenced groundwater, intertidal marshes, riverine, estuarine and marine ecosystems. Manage and partner with other government agencies, private industry and academic collaborators in multidisciplinary environmental research projects. Transfer technology for environmental cleanup and compliance strategies to DoD and private sector. Review US Navy guidance and policy draft documents for environmental installation, restoration and compliance for Naval Facilities Engineering Command Headquarters. Act as technology liaison with NAVFAC's Risk Assessment Workgroup, which is responsible for formulating US Navy environmental guidance. Performed over 140 environmental samplings in coastal waters, groundwater, saltwater marshes, and soil.

Texas A&M University, College of Science and Engineering, Corpus Christi, TX
Research Associate 2015—Present

Northern Virginia Community College, Biological Sciences, Annandale, VA
Adjunct Professor (56 credit hours taught) 2006—2014

Center of Marine Biotechnology, Baltimore, MD
Postdoctoral Research Associate 1991-1993

Education

- Ph.D. Marine Studies (Marine Biology/Biochemistry) – University of Delaware (1991)
- M.S. Marine Studies (Applied Ocean Sciences) – University of Delaware (1986)
- B.S. Biological Sciences – Old Dominion University (1983)

Professional Memberships

- American Society for Microbiology
- American Society of Limnology and Oceanography
- Association for Environmental Health and Sciences
- American Chemical Society

Scientific and Technical Reviewer

- Action Bioscience
- Applied and Environmental Microbiology
- Applied Microbiology and Biotechnology
- Biodegradation
- Chemosphere
- Department of Defense Advanced Technology Program
- Department of Energy NABIR Program
- Department of Energy Ocean Sciences Program
- Desalination and Water Treatment
- Desalination Journal
- Environmental Science and Pollution Research
- Environmental Science and Technology
- Environmental Toxicology and Chemistry
- International Journal of Environmental Research and Public Health
- Journal of Applied and Environmental Microbiology
- Journal of Environmental Quality
- Journal of Environmental Research and Public Health
- Journal of Hazardous Materials
- Marine Chemistry
- Naval Facilities Engineering Command Environmental Policy and Guidance
- North Carolina Sea Grant Funding Program
- Office of Naval Research (ONR) Marine Biotechnology Program
- ONR Small Business Innovative Research Program
- South Carolina Sea Grant Funding Program
- Virginia Academy of Science

Managed Funded Programs (>$6.5M)

- DoD’s SERDP Program (CU23-$800K; CU-30-$2100K ; CU1209-$146K; CU1431- $1350K; CU2123SEED-$147K; CL2123 $454K, New-$62K)
- Office of Naval Research 6.1 Marine Biotechnology Program ($605K)
- Naval Facilities Engineering Command (NAVFAC) N45 6.4 Pollution Prevention ($400K)
- NAVFAC South DIV, Environmental Restoration, Navy (ER,N) Program ($210K)
- NAVFAC EFA Northeast, ER,N Program ($60K)
- NAVFAC LANT DIV, ER,N Program ($150K)
- Beazer East, Inc. ($86K)

Peer-Reviewed Publications (28 total)

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Osburn, C. L., Boyd, T. J., **Montgomery, M. T**., Coffin, R. B., Bianchi, T. S., and H. W. Paerl. 2016. Optical proxies for terrestrial dissolved organic matter in estuaries and coastal waters. Frontiers in Marine Science 2:127. DOI:10.3389/fmars.2015.00127

Boyd, T. J., **Montgomery, M. T**., Cuenca, R. H., and Y. Hagimoto. 2015. Coupled radiocarbon and CO_2 flux measurements used to determine chlorinated solvent degradation rate. Environmental Science: processes and impacts, 17(3):683-692.

Coffin, R. B., Hamdan, L. J., Smith, J. P., Rose, P. S., Plummer, R. E., Yosa, B., Pecher, I. A., and **M. T. Montgomery**. 2014. Contribution of Vertical Methane Flux to Shallow Sediment Carbon Pools across the Porangahau Ridge, New Zealand. Energies 7(8):5332-5356.

Brym, A., Paerl, H. W., **Montgomery, M. T**., Handsel, L. T., Ziervogel, K., and C. L. Osburn. 2014. Optical and chemical characterization of base-extracted particulate organic matter in coastal marine environments. Marine Chemistry 162:96-113. DOI:10.1016/j.marchem.2014.03.006

Coffin, R. B., Smith, J. P., Plummer, R. E., Yosa, B., Millholland, L. C., and **M. T. Montgomery.** 2013. Spatial variation in shallow sediment methane sources and cycling on the Alaskan Beaufort Sea Shelf/Slope. Marine and Petroleum Geology 45:186-197.

Montgomery, M. T., Coffin, R. B., Boyd, T. J., and C. L. Osburn. 2013. Incorporation and mineralization of TNT and other anthropogenic organics by natural microbial assemblages from a small, tropical estuary. Environmental Pollution 174:257-264. DOI:10.1016/j.envpol.2012.11.036*

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Stamper, D. M., Morris, R. E., and **M. T. Montgomery**. 2012. Depletion of lubricity improvers from hydrotreated renewable and ultraslow sulfur petroleum diesels by marine microbiota. Energy and Fuels 26(11):6854–6862. DOI:10.1021/ef301158n

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Stamper, D. M., and **M. T. Montgomery**. 2008. Biological treatment and toxicity of low concentrations of oily wastewater (bilgewater). Canadian Journal of Microbiology 54(8):687-693.

Montgomery, M. T., Osburn, C. L., Furukawa, Y., and J. M. Gieskes. 2008. Increased capacity for PAH mineralization in biologically mixed marine sediments. Bioremediation 12(2):1-13.

Castle, D. M., **Montgomery, M. T.**, and D. L. Kirchman. 2006. Effects of naphthalene on microbial community composition in the Delaware Estuary. FEMS Microbial Ecology 56:55-63.

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Boyd, T. J., **Montgomery, M. T.,** Steele, J. K., Pohlman, J. W., Reatherford, S. R., Spargo, B. J., and D. C. Smith. 2005. Dissolved oxygen saturation controls PAH biodegradation in freshwater estuary sediments. Microbial Ecology 49(2): 226-235. DOI:10.1007/s00248-004-0279-0. PMID:15965718.

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Instructional Workshops and Classes

2006-2014. Introduction to Biology (BIO 101, 40 credit hours total; BIO 102, 8 credit hours total). Northern Virginia Community College, Annandale Campus, VA.

2004. Biological processes affecting remedial design and performance. USEPA/USACE/SMWG

Joint Sediment Conference Conference on Addressing Uncertainty and Managing Risk at Contaminated Sediment Sites., St. Louis, MO.

2001. Bacterial adaptation for intrinsic bioremediation of PAHs in sediments. Spring Workshop of the EPA Technical Support Project Engineering Forum. San Diego, CA.

2000. Intrinsic hydrocarbon bioremediation in the sediments of Charleston Harbor. Biocomplexity Series for the Graduate School of Oceanography at the University of Rhode Island, Narragansett, RI.

2000. Intrinsic hydrocarbon bioremediation in the sediments of Charleston Harbor. Spring Seminar Series for the Department of Marine Sciences at the University of Connecticut, Groton, CT.

1994. Monitoring bioremediation of BTEX and PAH at contaminated Naval sites. Convocation speaker at Bridgewater College, Bridgewater, VA.

Presentations (115 total)

Atar, J. N., Barnett, E. L., **Montgomery, M. T.,** Handsel, L. T., Boyd, T. J., Coffin, R. B., and C. L. Osburn, 2016. Dynamics of fluorescent organic matter compared between three contrasting estuarine environments. 2016 Ocean Sciences Meeting, New Orleans, LA, 21-26 February.

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Rose, P. S., Boyd, T. J., Millholland, L. C., Yoza, B., **Montgomery, M. T.,** Klaucke, I., Bialas, J., Pecher, I. A., Gorman, A. R., Coffin, R. B., and the SO226 Science Party. 2013. Carbon isotopic evidence of past and present CH_4 fluxes in a pockmark field on the Chatham Rise? Presentation at the Goldschmidt Conference on Earth Sciences, Florence, Italy, 25-30 August.

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Brym, A. J., Ziervogel, K., Paerl, H. W., **Montgomery, M. T.,** and C. L. Osburn. 2013. Characterization of particulate organic matter in three estuaries using parallel factor analysis (PARAFAC). Presentation at the ASLO 2013 Ocean Sciences Meeting, New Orleans, LA, 17-22 February.

Montgomery, M. T., Coffin, R. B., Boyd, T. J., Rose, P. S., Smith, J. P., Sachsenmaier, L., Mikan, M., and C. L. Osburn. 2011. 2,4,6-Trinitrotoluene (TNT) and aromatic organic matter metabolism by natural bacterial assemblages at estuarine transition zones (ER-2124). Presentation at the SERDP Partners in Environmental Technology Technical Symposium & Workshop, Washington, DC, November 30-December 2. (INVITED)

Luning Prak, D. J., O'Sullivan, D. W., Eisenberg, M. A., Osburn, C. L., **Montgomery, M. T.,** and J. P. Smith. 2011. Photolysis of dinitrotoluene: effects of salinity, nitrate, and humic substances. Presentation at the SERDP Partners in Environmental Technology Technical Symposium & Workshop, Washington, DC, November 30-December 2. (INVITED)

Stamper, D. M., Morris, R. E., and **M. T. Montgomery**. 2011. Biofouling of petroleum and bio-derived marine diesel fuels. Presentation at the 111th General Meeting of the American Society for Microbiology. New Orleans, LA, May 21-24.

Montgomery M. T., Coffin, R. B., Boyd, T. J., Smith, J. P., Walker, S. E., and C. L. Osburn. 2011. 2,4,6-Trinitrotoluene mineralization and bacterial production amongst natural microbial assemblages in coastal sediments. Presentation at the 6th International Conference on Remediation of Contaminated Sediments. New Orleans, LA, 7-10 February.

Boyd, T. J., **Montgomery, M. T.,** and S. D. Jarvela. 2009. Hydrocarbon source apportionment using compound-specific carbon isotope analysis at a multiple potentially-responsible party (PRP) UST site. International Network of Environmental Forensics Conference, Calgary, Alberta, Canada, August 31-September 2. (INVITED)

Montgomery, M. T., Boyd, T. J., Smith, J. P., Walker, S. E., and C. L. Osburn. 2009. 2,4,6-Trinitrotoluene mineralization and incorporation by natural bacterial assemblages in coastal ecosystems. 237th National Meeting of the American Chemical Society, Salt Lake City, UT, March 22-26. (INVITED)

O'Sullivan, D. W., **Montgomery, M. T.**, Denzel, J. R., and D. J. Luning Prak. 2008. Photolysis of 2,4,6-Trinitrotoluene in seawater: effects of salinity, nitrate, pH and dissolved organic matter. SERDP Partners in Environmental Technology Technical Symposium & Workshop, Washington, DC, December 2-4. (INVITED)

Montgomery, M. T., Boyd, T. J., Smith, J. P., Walker, S. E., and C. L. Osburn. 2008. Bacterial mineralization and incorporation of 2,4,6-Trinitrotoluene (TNT), RDX, and HMX in the coastal waters and sediments (ER-1431). SERDP Partners in Environmental Technology Technical Symposium and Workshop, Washington, DC, December 2-4. (INVITED)

Stamper, D. M., and **M. T. Montgomery**. 2008. Petroleum and synthetic oils biodegradation in a combined approach for treating oily and non-oily wastewaters. Presentation at the 108th American Society for Microbiology General Meeting, Boston, June 1-5.

Montgomery, M. T., Boyd, T. J., and C. L. Osburn. 2008. Bacterial mineralization and incorporation of 2,4,6-Trinitrotoluene (TNT) in the coastal waters of Hawaii, Gulf of Mexico and Chesapeake Bay. Presentation at the 18th Annual AEHS Meeting and West Coast Conference on Soils, Sediments, and Water. San Diego, March 10-13.

Montgomery, M. T., Boyd, T. J., and C. L. Osburn. 2007. Bacterial mineralization and incorporation of 2,4,6-Trinitrotoluene (TNT), RDX, and HMX in the coastal waters of Hawaii, Gulf of Mexico and Chesapeake Bay (ER-1431). Presentation at the SERDP Partners in Environmental Technology Technical Symposium and Workshop, Washington, DC, December 4-6. (INVITED)

Hilyard, E. J., **Montgomery, M. T.,** Hamdan, L. J., Spargo, B. J., and R. T. Hill. 2007. Multiple PAH-degrading bacteria isolated from estuarine sediment enrichment cultures. Presentation at the 107th American Society for Microbiology General Meeting, Toronto, Ontario, Canada, May 21-25.

Montgomery, M. T., Osburn, C. L., Walker, S. E., Boyd, T. J., Mueller, J. G., Li, Q. X., Paerl, H. W., Monteil-Rivera, F., and J. Hawari. 2007. Biodegradation of 2,4,6-Trinitrotoluene (TNT) in coastal waters and sediments. Presentation at the 17th Annual AEHS Meeting and West Coast Conference on Soils, Sediments, and Water. San Diego, March 19-22.

Boyd, T. J. and **M. T. Montgomery**. 2007. Hydrocarbon source apportionment using compound specific carbon isotope analyses and multivariate statistics. Presentation at the 17th Annual AEHS Meeting and West Coast Conference on Soils, Sediments, and Water. San Diego, March 19-22.

Montgomery, M. T., Osburn, C. L., Walker, S. E., Hamdan, L. J., Boyd, T. J., Furukawa, Y., O'Sullivan, D. W., Paerl, H. W., Li, Q. X., Monteil-Rivera, F., and J. Hawari. 2006. Rates of 2,4,6-Trinitrotoluene (TNT) mineralization and incorporation into biomass by estuarine microorganisms in coastal waters (ER-1431). Presentation at the SERDP Partners in Environmental Technology Technical Symposium and Workshop, Washington, DC, November 28-30. (INVITED)

O'Sullivan, D. W., Osburn, C. L., **Montgomery, M. T.,** and D. J. Lunning-Prak. 2006. Degradation of emergent and explosive contaminant compounds in natural waters. Presentation at the SERDP Partners in Environmental Technology Technical Symposium and Workshop, Washington, DC, November 28-30. (INVITED)

Montgomery, M. T., Monteil-Rivera, F., and J. Hawari. 2006. Contribution of hydrolysis in the natural attenuation of TNT, RDX, and HMX in coastal waters (ER-1431). Presentation at the SERDP Partners in Environmental Technology Technical Symposium and Workshop, Washington, DC, November 28-30. (INVITED)

Montgomery, M. T., Walker, S. W., Osburn, C. L., Hamdan, L. J., Boyd, T. J., Furukawa, Y., Hawari, J., Monteil-Rivera, F., O'Sullivan, D. W., Paerl, H. W., Li, Q. X., and J. G. Mueller. 2006. Biotic and abiotic attenuation of nitrogenous energetic compounds (NEC) in coastal waters and sediments: annual report. SERDP In Progress Review, February 7.

Osburn, C. L., Walker, S. W., Hamdan, L. J., Boyd, T. J., Furukawa, Y., O'Sullivan, D., Paerl, H. W., Li, Q., Monteil, F., Hawari, J., and **M. T. Montgomery.** 2006. Biotic and abiotic attenuation of nitrogenous energetic compounds (NEC) in coastal waters and sediments. NAVFAC 0817 program review, Vicksburg, MS, March 15.

Montgomery, M. T., Walker, S. W., Osburn, C. L., Hamdan, L. J., Boyd, T. J., Furukawa, Y., Hawari, J., Monteil-Rivera, F., O'Sullivan, D. W., Paerl, H. W., Li, Q. X., and J. G. Mueller. 2006. Biotic and abiotic attenuation of nitrogenous energetic compounds (NEC) in coastal waters and sediments. NAVFAC NW DIV/EPA site review, Bremerton, WA, July 26.

Montgomery, M. T., Walker, S. W., Hamdan, L. J., Osburn, C. L., Boyd, T. J., Furukawa, Y., Lunning-Prak, D., O'Sullivan, D., Paerl, H. W., Li, Q., Monteil, F., and J. Hawari. 2005. Biotic and abiotic attenuation of nitrogenous energetic compounds (NEC) in coastal waters and sediments. SERDP Partners in Environmental Technology Technical Symposium and Workshop, Washington, DC, November 29-December 1. (INVITED)

Montgomery, M. T., Osburn, C. L., Boyd, T. J., Hamdan, L. J., and S. E. Walker. 2005. Biodegradation of nitrogenous energetic compounds in coastal ecosystems. Presentation at the 26th Annual Meeting of SETAC North America, Baltimore, MD, November 13-16. (INVITED)

Montgomery, M. T., Boyd, T. J., Coffin, R. B., Pohlman, J. W., Osburn, C. L., and D. C. Smith. 2005. Relationship between PAH biodegradation and transport in estuarine sediments. Third International Conference on Remediation of Contaminated Sediments. New Orleans, LA, January 24-27. (INVITED)

Walker, S. E., Boyd, T. J., Osburn, C. L., Hamdan, L. J., Donowick, T. G., and **M. T. Montgomery**. 2004. Biodegradation of nitroaromatic energetic compounds in coastal systems. 9th FECS Conference on Chemistry and the Environment, Bordeaux, France, 29 August - 1 September.

Mueller, J. G., **Montgomery, M. T.,** and A. Seech. 2004. Bioremediation technologies for marine sediments. Presentation at the Baltic International Symposium on Advances in Marine Environmental Research, Monitoring and Technologies. Klaipeda, Lithuania, June 15-17. (INVITED)

Montgomery, M. T., Smith, J. P., Boyd T. J., and C. L. Osburn. 2004. The effect of benthic community change on bacterial PAH metabolism in anthropogenically-impacted estuarine sediments. Presentation at the ASLO 2004 Summer Meeting, Savannah, GA, June 13-18. (INVITED)

Montgomery, M. T., Smith, J. P., Boyd T. J., and C. L. Osburn. 2004. The effect of bioturbation on bacterial PAH and TNT metabolism in estuarine sediments. Presentation at the 104th General Meeting of the American Society for Microbiology, New Orleans, LA, May 23-27.

Montgomery, M. T, Boyd, T. J., Walker, S. E., Hamdan, L. J., and C. L. Osburn. 2004. TNT mineralization rates among natural bacterial assemblages in sediments from San Diego Bay, Norfolk, San Francisco Bay, and Pearl Harbor. ONR/NAVFAC Program Review, Vicksburg, MS, February 5-6. (INVITED)

Boyd, T. J., Coffin, R. B., Osburn, C. L., Pohlman, J. W., **Montgomery, M. T.**, Grabowski, K. S., and D. L. Knies. 2004. Carbon-based tools for confirming in situ biodegradation of fossil fuel-derived contaminants. Advance Remedial Technology Transfer Seminar Series to Naval Facilities Engineering Command, Washington, DC, January 14-17.

Montgomery, M. T., and C. L. Osburn. 2003. Bacterial PAH metabolism with depth in bioturbated and unbioturbated estuarine sediments. Presentation at the 17th Biennial Conference of the Estuarine Research Federation. Seattle, September 14-18.

Osburn, C. L., and **M. T. Montgomery**. 2003. Terriginous organic matter support of contaminant degradation in estuarine sediments. Presentation at the 17th Biennial Conference of the Estuarine Research Federation. Seattle, September 14-18.

Apitz, S. E., Chadwick, D. B., Germano, J., Geiskes, J., Maa, G., **Montgomery, M. T.,** Paulsen, R., Smith, C., and W. Ziebis. 2003. Integrated results of the pathway ranking for in-place sediment management (PRISM) program: critical assessment. Presentation at the 17th Biennial Conference of the Estuarine Research Federation, Seattle, September 14-18.

Boyd, T. J., Osburn, C. L., Pohlman, J. W., **Montgomery, M. T.,** and R. B. Coffin. 2003. Biogeochemistry of natural and anthropogenic organic matter at NRL. Chesapeake Bay Laboratory, University of Maryland Fall Seminar Series, Solomon's, MD, September 11.

Boyd, T. J., and **M. T. Montgomery.** 2003. Microbial mineralization of PAHs sorbed to allochthonous and autochthonous DOM determined in freshwater to marine transects. Presentation at the 103rd General Meeting of the American Society for Microbiology. Washington, DC, May 18-23.

Osburn, C. L., Donowick, T., and **M. T. Montgomery.** 2003. Lignin geochemistry and environmental remediation in estuarine sediments. Presentation at the Ohio University Seminar Series, Athens, OH, May 16.

Apitz, S. E., Chadwick, D. B., Germano, J., Gieskes, J. M., Kirtay, V.J., Maa, G., **Montgomery, M. T.,** Paulsen, R., Smith, C., and W. Zeibis. 2003. In situ evaluation of contaminant behaviour in nearshore sediments – results of the Pathway ranking for In-situ sediment management (PRISM) Programme. Presentation at the 6th Underwater Science Group Symposium, Aberdeen, Scotland, April 3-6. (INVITED)

Montgomery, M. T., Boyd, T. J., Osburn, C. L., Mueller, J. G., and D. C. Smith. 2003.

Preferential degradation of PAHs over natural organic matter by bacteria in intertidal sediments. Presentation at the 225th American Chemical Society National Meeting, New Orleans, LA, March 23-27. (INVITED)

Osburn, C. L., Donowick, T., and **M. T. Montgomery.** 2003. Cometabolism of natural and anthropogenic aromatics in estuarine sediments. Presentation at the 225th American Chemical Society National Meeting, New Orleans, LA, March 23-27. (INVITED)

Montgomery, M. T., Coffin, R. B., Beeson, K. E., Pohlman, J. W., Osburn, C. L., Boyd, T. J., and D. C. Smith. 2003. Tidal cycle transport of PAH verses biodegradation in surface sediments in Charleston Harbor. Presentation at the 13th Annual West Coast Conference on Contaminated Soils, Sediments and Water, San Diego, CA March 17-20. (INVITED)

Osburn, C. L., Donowick, T., and **M. T. Montgomery.** 2003. Microbial cometabolism of terriginous organic matter, PAHs, and nitroaromatics in estuarine sediments. Presentation at the 13th Annual West Coast Conference on Contaminated Soils, Sediments and Water, San Diego, CA March 17-20. (INVITED)

Montgomery, M. T., Chrisey, L. A., Osburn, C. L., and T. J. Boyd. 2003. Implementation of Harbor Processes Project #123. Presentation at the NAVFAC's 2003 Cleanup Conference, Port Hueneme, CA, February 11-13. (INVITED)

Apitz, S. E., Chadwick, D. B., Arias, E., Carlson, A., Germano, J., Gieskes, J. M., Kirtay, V.J., Maa, G., **Montgomery, M. T.,** Paulsen, R., Smith, C., and W. Zeibis. 2002. Pathway ranking for In-situ sediment management (PRISM) – Balancing risk and recovery. Presentation at the Partners Environmental Technology Technical Symposium, Washington, DC, December 3-5.

Apitz, S. E., Chadwick, D. B., Germano, J., Gieskes, J. M., Kirtay, V. J., Maa, G., **Montgomery, M. T.,** Paulsen, R., Smith, C., and W. Zeibis. 2002. Pathway ranking for In-situ sediment management (PRISM). Presentation at the 23rd Annual Meeting of the Society of Environmental Toxicology and Chemistry, Salt Lake City, UT, November 16-20. (INVITED)

Montgomery, M. T., Osburn, C. L., Chadwick, D. B., Germano, J., Mahn, C., Zeibis, W., and J. M. Gieskes. 2002. Depth profile of bacterial metabolism and PAH biodegradation in bioturbated and unbioturbated marine sediments. Presentation at the 23rd Annual Meeting of the Society of Environmental Toxicology and Chemistry, Salt Lake City, UT, November 16-20. (INVITED)

Montgomery, M. T., Reatherford, S. R., Smith, D. C., Osburn, C. L., and T. J. Boyd. 2002. Bacterial degradation of polycyclic aromatic hydrocarbons in surface sediments of coastal ecosystems. Presentation at the 17th AEHS Annual International Conference on Contaminated Soils, Sediments, and Water, Amherst, MA, October 21-24.

Montgomery, M. T., Reatherford, S. R., Smith, D. C., Osburn, C. L., and T. J. Boyd. 2002. Bacterial Degradation of Polycyclic Aromatic Hydrocarbons in Surface Sediments of Coastal Ecosystems. Presentation at the 17th AEHS Annual International Conference on Contaminated Soils, Sediments, and Water, Amherst, MA, October 21-24. (INVITED)

Coffin, R. B., **Montgomery, M. T.,** Boyd, T. J., and S. M. Masutani. 2002. Influence of ocean CO_2 sequestration on bacterial elemental cycling. Presentation at the Sixth International Conference on Greenhouse Gas Control Technologies, Kyoto, Japan, October 1-4.

Boyd, T. J., Osburn, C. L. Pohlman, J. W., and **M. T. Montgomery.** 2002. Natural and anthropogenic organic matter utilization in coastal waters. Navy Applied Research Program (PE 0602236N) Environmental Quality Technical Review, US Naval Academy, June 26. (INVITED)

Montgomery, M. T., Osburn, C. L., and T. J. Boyd. 2002. Bacterial degradation of PAHs in sediments of temperate and tropical coastal ecosystems. Navy Applied Research Program (PE

0602236N) Environmental Quality Technical Review, US Naval Academy, June 26. (INVITED)

Boyd, T. J., Osburn, C. L., Pohlman, J. W., and **M. T. Montgomery.** 2002. Biogeochemistry of natural and anthropogenic organic matter in coastal environments. Virginia Institute of Marine Science Spring Seminar Series. Gloucester Point, VA, April 12. (INVITED)

Montgomery, M. T., Smith, D. C., Osburn, C. L., Mueller, J. G., and T. J. Boyd. 2002. Seasonal succession of the PAH-mineralizing bacteria in creosote-impacted intertidal sediments. Presentation at the 12th Annual West Coast Conference on Contaminated Soils, Sediments and Water. San Diego, CA, March 18-21. (INVITED)

Montgomery, M. T., Smith, D. C., Osburn, C. L., and T. J. Boyd. 2002. Bacterial degradation of aromatic hydrocarbons in surface sediments of temperate and tropical coastal ecosystems. Presentation at the 2002 Ocean Sciences Meeting. Honolulu, HI, February 11-15.

Montgomery, M. T., Osburn, C. L., Steele, J. K., Badger, C. V., and T. J. Boyd. 2001. Bacterial adaptation to PAH degradation in the sediments of the Elizabeth River and lower Chesapeake Bay. Presentation at the 17th AEHS Annual International Conference on Contaminated Soils, Sediments, and Water, Amherst, MA, October 22-25. (INVITED)

Montgomery, M. T., Boyd, T. J., Coffin, R. B., and B. J. Spargo. 2001. Intrinsic bioremediation of PAHs in sediments around the former Charleston Navy Yard. Presentation at the 4th Tri-Service Environmental Technology. San Diego, CA, June 18-20.

Boyd, T. J., **Montgomery, M. T.,** Pohlman, J. W., and B. J. Spargo. 2001. Transport, fate and biodegradation in and around the Philadelphia Naval Complex Reserve Basin. Presentation at the 4th Tri-Service Environmental Technology, San Diego, CA, June 18-20.

Boyd, T. J., **Montgomery, M. T.,** Steele, J. K., Ward, D. M., Smith, D. C., Spargo, B. J., Coffin, R. B, Pohlman, J. W., Mueller, J. G., and D. C. Smith. 2001. PAH biodegradation in an intertidal salt marsh. Presentation for the Spring meeting of the EPA Technical Support Project Engineering Forum, San Diego, CA, May 10 (INVITED)

Montgomery, M. T., Boyd, T. J., Coffin, R. B., Spargo, B. J., Steele, J. K., Pohlman, J. P., Ward, D. M., and D. C. Smith. 2001. Bacterial adaptation for intrinsic bioremediation of PAHs in sediments. Presentation at the 11th Annual West Coast Conference on Contaminated Soils, Sediments and Water, San Diego, CA, March 19-22. (INVITED)

Coffin, R. B., Boyd, T. J., **Montgomery, M. T.,** and S. M. Masutani. 2001. Ocean carbon sequestration: CO_2 fate and environmental impact. Direct Ocean Sequestration Experts' Workshop, Monterey Bay Aquarium Research Institute, February 27- March 1. (INVITED)

Montgomery, M. T., Boyd, T. J., Steele, J. K., Ward, D. M., and D. C. Smith. 2001. Intrinsic bioremediation of PAHs in sediments of the Charleston Harbor. Presentation at the ONR Biodegradation of Pollutants Program Review. Bethesda, MD, February 6-7.

Ward, D. M., **Montgomery, M. T.,** and D. L. Kirchman. 2000. The effect of naphthalene additions on microbial communities in surface sediments. Presentation at the 21st Annual Meeting of the Society of Environmental Toxicology and Chemistry. Nashville, TN, November 12-16.

Montgomery, M. T., Boyd, T. J., Steele, J. K., Pohlman, J. P., Coffin, R. B., Ward, D. M., Spargo, B. J., and D. C. Smith. 2000. Intrinsic hydrocarbon bioremediation of sediments in the Charleston Harbor System. Presentation at the 21st Annual Meeting of the Society of Environmental Toxicology and Chemistry, Nashville, TN, November 12-16. (INVITED)

Bouknight, J. M., **Montgomery, M. T.,** Boyd, T. J., Slenska, M., and J. G. Mueller. 2000. Accelerated in situ bioremediation and phytoremediation of marsh sediments. Presentation at the

21st Annual Meeting of the Society of Environmental Toxicology and Chemistry, Nashville, TN, November 12-16.

Boyd, T. J. , **Montgomery, M. T.,** Mueller, J. G., Steele, J. K., Spargo, B. J., Coffin, R. B., Pohlman, J. W., Demetriades-Shah, T., and M. Slenska. 2000. Source reduction effect on creosote PAH bioremediation in marsh sediments. Presentation at the Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, May 22-25. (INVITED)

Boyd, T. J., Pohlman, J. W., Coffin, R. B., **Montgomery, M. T.,** Spargo, B. J., and J. K. Steele. 1999. Coupling contaminant fate and transport with biodegradation: is a small tidal basin a source or a sink for hydrocarbons? Presentation at 20th Annual Meeting of the Society of Environmental Toxicology And Chemistry, Philadelphia, PA, November 16-18. (INVITED)

Coffin, R. B., Boyd, T. J., **Montgomery, M. T.,** Pohlman, J. W., Mitchell, C. S., Steele, J. K., and B. J. Spargo. 1999. Transport and degradation of PAHs in the tidal region of the Anacostia River. Presentation at 20th Annual Meeting of the Society of Environmental Toxicology And Chemistry, Philadelphia, PA, November 16-18. (INVITED)

Montgomery, M. T., Boyd, T. J., Steele, J. K., Ward, D. M., Smith, D. C., Spargo, B. J.,. Coffin, R. B, Pohlman, J. W., Slenska, M., and J. G. Mueller. 1999. Measuring intrinsic bacterial degradation of PAHs in a salt marsh. Presentation at the International Conference on Wetlands and Remediation, Salt Lake City, UT, November 16-17. (INVITED)

Spargo, B. J., **Montgomery, M. T.,** Boyd, T. J., and J. K. Steele. 1999. Influence of the tidal cycle on petroleum biodegradation in groundwater. Presentation at the International Conference on Wetlands and Remediation, Salt Lake City, UT, November 16-17.

Montgomery, M. T., Boyd, T. J., Spargo, B. J., Coffin, R. B., Ward, D. M., Steele, J. K., and D. C. Smith. 1999. Bacterial assemblage changes as an ecological impact indicator on ecosystems. Presentation at the 5th International Symposium on In Situ and On-Site Bioremediation, San Diego, CA, April 19-22.

Mueller, J. G., Bold, J., **Montgomery, M. T.,** Boyd, T. J., and M. Slenska. 1999. Monitoring biodegradation of PAHs in wetlands using biogeochemical assays. Presentation at the 5th International Symposium on *In Situ* and On-Site Bioremediation, San Diego, CA, April 19-22.

Coffin, R. B., Pohlman, J. W., **Montgomery, M. T.,** Boyd, T. J., Jones-Meehan, J., Kolanko, C. J., and B. J. Spargo. 1999. Fate of PAHs in the Anacostia River tidal region. Presentation at the 5th International Symposium on In Situ and On-Site Bioremediation, San Diego, CA, April 19-22.

Boyd, T. J., **Montgomery, M. T.,** Spargo, B. J., and J. K. Steele. 1999. PAH distribution and biodegradation in the Delaware and Schuykill Rivers. Presentation at the 5th International Symposium on In Situ and On-Site Bioremediation, San Diego, CA, April 19-22.

Montgomery, M. T., Boyd, T. J., Spargo, B.J., Coffin, R. B., Ward, D. M., Steele, J. K., and D. C. Smith. 1998. Bacterial assemblage changes as an early indicator of ecological impact of military operations on industrialized ecosystems. Partners in Environmental Technology Symposium and Workshop, Arlington, VA, December 1-3.

Montgomery, M. T., Boyd, T. J., Coffin, R. B., Smith, D. C., and B. J. Spargo. 1998. Biodegradation of PAHs in the Cooper River Estuary, Charleston, SC (USA). Presentation at the Workshop on Contaminated Sediment Management: Office of Naval Research and Naval Facilities Engineering Command, San Diego, CA, October 14-16.

Boyd, T. J., **Montgomery, M. T.**, Spargo, B. J., Coffin, R. B., Ward, D. M., Steele, J. K., and

D.C. Smith. 1998. Bacterial assemblage changes as an early indicator of ecological impact of military operations on estuarine sediments. Presentation at the Workshop on Contaminated Sediment Management: Office of Naval Research and Naval Facilities Engineering Command, San Diego, CA, October 14-16.

Mueller, J. G., Lakhwala, F., Carter, J., Spargo, B., **Montgomery, M. T.,** and M. Brourman. 1998. Economics and performance of UVB Technology at a creosote site. Presentation at Battelle's First international Symposium on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, May 18-21.

Montgomery, M. T., Boyd, T. J., Spargo, B. J., and D. C. Smith. 1998. Heterotrophic bacterial production in the waters and sediments of two PAH-impacted ecosystems near Charleston, SC and Philadelphia, PA (USA). Presentation at the 98th General Meeting of the American Society for Microbiology, Atlanta, GA, May 16-20.

Boyd, T. J., Steele, J. K., **Montgomery, M. T.,** and B. J. Spargo. 1998. Biodegradation of PAHs in the Cooper River Estuary, Charleston, SC (USA). Presentation at the 98th general meeting of the American Society for Microbiology, Atlanta, GA, May 16-20.

Montgomery, M. T., Boyd, T. J., Spargo, B. J., Mueller, J. G., Cifuentes, L. A., Kelley, C. A., and R. B. Coffin. 1997. Effect of a groundwater circulation well on BTEX degradation in a gasoline contaminated aquifer. Presentation at the 97th General Meeting of the American Society for Microbiology, Miami, FL, May 11-14.

Boyd, T. J., **Montgomery, M. T.,** Spargo, B. J., Mueller, J. G., Kelley, C. A., Cifuentes, L. A., and R. B. Coffin. 1997. Effect of a groundwater circulation well on PAH degradation in a creosote contaminated aquifer. Presentation at the 97th General Meeting of the American Society for Microbiology, Miami, FL, May 11-14.

Montgomery, M. T., Boyd, T. J., Spargo, B. J., Mueller, J. G., and R. B. Coffin. 1997. Bacterial productivity in BTEX- and PAH-contaminated aquifers. Presentation at the 4th International Symposium on *In Situ* and On-Site Bioremediation, New Orleans, LA, April 28-May 1.

Boyd, T. J., **Montgomery, M. T.,** and B. J. Spargo. 1997. Utilization rates of benzene and toluene from a BTEX-contaminated aquifer. Presentation at the Fourth International Symposium on *In Situ* and On-Site Bioremediation, New Orleans, LA, April 28-May 1.

Coffin, R. B., **Montgomery, M. T.,** Kelley, C. A., and L. A. Cifuentes. 1997. Assessment of nutrient-contaminant carbon ratios for enhancing *in situ* bioremediation. Presentation at the Fourth International Symposium on *In Situ* and On-Site Bioremediation, New Orleans, LA, April 28-May 1.

Montgomery, M. T., and R. B. Coffin. 1996. *In situ* bioremediation and efficacy monitoring. Presentation at the 2nd Annual Strategic Environmental Research and Development Program Symposium, Arlington, VA, November 20-22.

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Montgomery, M. T., Boyd, T. J., Spargo, B. J., Mueller, J. G., Coffin, R. B., Owlett, R. A., Smith, D. C. 1996. Bacterial productivity in a BTEX-contaminated and a creosote-contaminated aquifer: a comparison of ^{3}H-leucine and ^{3}H-thymidine incorporation methods. Presentation at the 96th General Meeting of the American Society for Microbiology, New Orleans, LA, May 20-24.

Brower, J. B., Owlett, R. A., Kidd, J., Spargo, B. J., and **M. T. Montgomery.** 1996. The role of bacterial attachment in PAH degradation on surfaces. Presentation at the 96th General Meeting of the American Society for Microbiology, New Orleans, LA, May 20-24.

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Brower, J. B., Kidd, J., Chrisey, L. A., Rudolph, A. S., Spargo, B., and **M. T. Montgomery**. 1996. Induction of PAH-degrading activity and attachment of *Sphingomonas paucimobilis* EPA505. Presentation at ONR's Biofouling Meeting, Salt Lake City, UT.

Montgomery, M. T., Coffin, R. B., Cifuentes, L. A., Mueller, J. G., and B. J. Spargo. 1996. Encapsulated bacteria for *in situ* PAH bioremediation. Presentation of FY96 progress to the Strategic Environmental Research and Development Program, Fort Belvior, VA, May 28.

Montgomery, M. T., Coffin, R. B., Cifuentes, L. A., Mueller, J. G., and B. J. Spargo. 1996. *In situ* bioremediation and efficacy monitoring. Presentation of FY96 progress to the Strategic Environmental Research and Development Program, Fort Belvior, VA, May 28.

Mueller, J., Borchert, S., Heard, C., Lakhwala, F., Lantz, S., Klingel, E., Langley, W., Brourman, M., Coffin, R., Kelley, C., Trust, B., Cifuentes, L., **Montgomery, M.,** Boyd, T., and B. Spargo. 1996. Efficacy monitoring of fuel bioremediation. Presentation at the Sixth West Coast Conference on Contaminated Soils and Groundwater Association for the Environmental Health of Soils (AEHS), Newport Beach, CA, March 11-14.

Borchert, S. Mueller, J., Heard, C., Alesi, E., Klingel, E., Langley, W., Brourman, M., **Montgomery, M.,** Boyd, T., and B. Spargo. 1996. Multi-functional vertical groundwater circulation technology for in situ site bioremediation. Presentation at the Sixth West Coast Conference on Contaminated Soils and Groundwater Association for the Environmental Health of Soils (AEHS), Newport Beach, CA, March 11-14.

Mueller, J. G., Borchert, S., Heard, C., Lakhwala, F., Lantz, S. E., Klingel, E. J., Klingel, W., Brourman, M. D., Coffin, R. B., Kelley, C., Trust, B., Cifuentes, L. A., **Montgomery, M. T.,** Boyd, T. J., and W. W. Schultz. 1995. Management of PAH-impacted sites via *in situ* chemical containment and monitoring. IGT's 8th International Symposium on Gas, Oil, and Environmental Biotechnology, Colorado Springs, CO. December 11-13.

Mueller, J. G., Lakhwala, F., Lantz, S. E., Heard, C., Klingel, E. J., Brourman, M. D., Coffin, R. B., Trust, B., Kelley, C., Cifuentes, L. A., **Montgomery, M. T.,** and W. W. Schultz. 1995. Use of ground water circulation (UVB) technology and integrated bioreactors for chemical containment and *in situ* bioremediation of subsurface environments contaminated by coal tar creosote: full-scale field validation. Presentation at the International Symposium and Trade Fair on the Cleanup of MGP and Creosote Sites, Prague, Czech Republic, September 19-21.

Mueller, J. G., Coffin, R. B., Pritchard, P. H., Klingel, E. J., Alesi, A. J., Cifuentes, L. A., **Montgomery, M. T.,** and W. W. Schultz. 1995. Model studies of bioremediation strategies and monitoring technologies. Presentation at the Joint U.S.-E.C. Commission Workshop on Environmental Biotechnology, Brussels, Belgium, October 17-18.

Montgomery, M. T., Mueller, J. G., Spargo, B. J., Gaber, B. P., and W. W. Schultz. 1995. Encapsulated bacteria for *in situ* bioremediation. Presentation at the First Annual Strategic Environmental Research and Development Program Symposium, Washington, DC, April 12-14.

Coffin, R. B., Cifuentes, L. A., Mueller, J. G., Brourman, M., Klingel, E., **Montgomery, M. T.,** and W. W. Schultz. 1995. *In situ* bioremediation of fossil fuel and efficacy of monitoring. Presentation at the First Annual Strategic Environmental Research and Development Program

Symposium, Washington, DC, April 12-14.

Montgomery, M. T. 1994. Encapsulated bacteria for *in situ* PAH bioremediation. Presentation of FY94 progress to the Strategic Environmental Research and Development Program, Fort Belvior, VA, June 6.

Mueller, J. G., Lantz, S. E., Gatlin, C. V., **Montgomery, M. T.,** Schultz, W. W., Dalton, D. D., Cripe, C. R., and P. H. Pritchard. 1994. *In situ* treatment technologies for PAH-contaminated sediments. Presentation at the National Research Council's Committee on Contaminated Marine Sediments, Technology Review Workshop, Chicago, IL, April 21-23.

Flaherty, D. F., Mueller, J. G., Gaber, B. P., Schultz, W. W., and **M. T. Montgomery.** 1994. Application of transposon mutagenesis for the study of PAH degradation by soil pseudomonads. Presentation at the 94th General Meeting of the American Society of Microbiology, Las Vegas, NV, May 23-26.

Thompson, L. K., Gaber, B. P., Liss, H. L., and **M. T. Montgomery**. 1994. Use of transposon generated mutants for the study of polyurethane degradation by soil pseudomonads. Presentation at the 94th General Meeting of the American Society of Microbiology, Las Vegas, NV, May 23-26.

Lantz, S. E., Lin, J. E., Mueller, J. G., **Montgomery, M. T.,** and P. H. Pritchard. 1994. Influence of a surfactant on the mineralization of fluoranthene by Sphingomonas paucimobilis strain EPA505. Presentation at the 94th General Meeting of the American Society of Microbiology, Las Vegas, NV, May 23-26.

Kirchman, D. L., and **M. T. Montgomery.** 1993. Role of chitin-binding proteins in attachment by the marine bacterium Vibrio harveyi to chitin. Presentation at the 93rd General Meeting of the American Society of Microbiology, Atlanta, GA, May 8-13.

Montgomery, M. T., and M. R. Belas. 1993. Effect of chemotaxis mutations on swarmer cell differentiation in Vibrio parahaemolyticus. Presentation at the 2nd Conference on Bacterial Locomotion and Signal Transduction, Austin, TX, January 14-18.

Montgomery, M. T., and M. R. Belas. 1992. Relationship between chemotaxis behavior and swarmer cell differentiation in Vibrio parahaemolyticus. Presentation at the 92nd General Meeting of the American Society of Microbiology, New Orleans, LA, May 26-30.

Montgomery, M. T., and D. L. Kirchman. 1991. Specific attachment of the marine bacterium Vibrio harveyi to chitin. Presentation at the 91st General Meeting of the American Society of Microbiology, Dallas, TX, May 5-9.

Montgomery, M. T., and D. L. Kirchman. 1989. Importance of lectins in bacterial attachment to chitin and quantification of chitin using lectins. Presentation at the Bi-annual Meeting of the Estuarine Research Federation, Baltimore, MD, April 23-24.

Montgomery, M. T., and D. L. Kirchman. 1988. Use of FITC-labeled wheat germ agglutinin to quantify chitin in the marine environment. Presentation at the Annual Meeting of the American Society of Limnology and Oceanography, Boulder, CO, June 12-16.

Patents

Campbell, J. R., Crabbe, J. R., Thompson, L., **Montgomery, M. T.,** and S. E. Walz, "*Pseudomonas chloraraphis* Micro-organism Polyurethane Degrading Enzyme obtained therefrom and method of using enzyme", Patent No. 6180381 (2001).

Campbell, J. R., Crabbe, J. R., **Montgomery, M. T.,** Thompson, L., and S. E. Walz, "*Pseudomonas chlororaphis* Micro-organism Polyurethane Degrading Enzyme obtained therefrom and method of using enzyme ", Patent No. 5714378 (1998).

Academic Curriculum Vitae

Name:	James Gregory Mueller
Present Address:	Provectus Environmental Products, Inc. 2871 West Forest Road, Suite 2 Freeport, IL 61032 Office (815) 650-2230 Private (630) 309-1175

Education:

Northern Illinois University (1979-1980) General Studies

Southern Illinois University (1980-1983)
Degree: B.S., Plant and Soil Science
Responsibilities: course work; student worker in soil microbiology laboratory.

Southern Illinois University (1983-1985)
Degree: M.Sc., Soil Microbiology & Biochemistry
Responsibilities: course work and independent research; teaching assistant, 1) introductory soils, 2) soil microbiology lecture, 3) soil microbiology laboratory.

Clemson University (1985-1988)
Degree: Ph.D., Soil Microbiology & Biochemistry (Agronomy & Soils)
Responsibilities: course work and independent research; instructor advanced soil microbiology lecture and laboratory.

Postdoctoral Studies:

U. S. Environmental Protection Agency (1988-1990)
Position: U.S. EPA Postdoctoral Research Microbiologist, Microbial Ecology and Biotechnology Branch.
Responsibilities: define creosote and PAH biodegradation processes occurring *in situ*; assess the feasibility of various bioremediation technologies for effective site restoration.
Highlights:

Professional Experience:

Provectus Environmental Products, Inc. (May 1, 2014 – present)
Position: President
Responsibilities: Develop and commercialize internationally an acquired portfolio of patented/proprietary environmental biotechnologies. Manage private equity investment of new start-up company; Direct strategic initiatives for growth, expansion and development. Coordinate remedial designs and implementation internationally; Coordinate and manage all element of new business operations; Direct corporate R&D; Responsible for P&L.

PeroxyChem, LLC - Environmental Solutions Division (March 1, 2014 – April 15, 2014)
Position: Senior Technology Applications Manager
Responsibilities: Establish business operations in Latin America with an initial focus on Brazil and Colombia. Direct strategic account initiatives focused on industrial clients. Coordinate remedial designs and implementation internationally.

FMC Corporation (November 2011 – March 1, 2014 = Sold to PeroxyChem, LLC)
Position: Senior Technology Applications Manager
Responsibilities: Establish business operations in Latin America with an initial focus on Brazil and Colombia. Direct strategic account initiatives focused on industrial clients. Coordinate remedial designs and implementation internationally.

ADVENTUS AMERICAS INC (July, 2003 – November 11, 2011 = Sold to FMC)
Position: President / Director of Remedial Solutions and Strategies.
Responsibilities: Established business operations in the USA, South America and Europe for Adventus Remediation Technologies, Inc. (ART), a venture capital-based, Canadian company representing the former environmental biotechnology unit of W.R. Grace & Co. (GRA: NYSE). Direct new technology development, focused research and development, strategic alliances and international mergers / acquisitions. Adventus was sold to FMC Corporation in November, 2011.

MALCOLM PIRNIE, INC. (July 2002 – July 2003)
Position: Senior Consultant.
Responsibilities: One year special assignment to establish Midwest (Chicago) business operations for a 105-year old environmental wastewater firm headquartered in the NE USA. Successfully established profitable business operations and helped develop an office with 7 staff focused on environmental consulting and hazardous waste remediation - industrial applications.

URS/DAMES & MOORE (July 1997 – 2002)
Position: Senior Environmental Scientist / Director of International Advanced Remediation Technologies (ART) Program and Tier One Consultants Group
Responsibilities: Coordinate application of environmental biotechnology services for the Central and Eastern Divisions (including Latin America); develop new business based on application of advanced remedial technologies; implement by providing technical guidance, marketing support, and engineering and design assistance for the Company worldwide.

Labroides Dimidiatus, Inc. (LDD) - Israel (1999-present)
Position: Scientific Advisory Board
Responsibilities: Development of an environmental engineering and remediation company in Israel; focus on bioremediation operations.

Phytotech, Inc. (September, 1996 -1999)
Position: Scientific Advisory Board
Responsibilities: assist in the continued development, demonstration and commercialization of proprietary phytoremediation technologies by providing technical guidance, marketing support, and engineering/design assistance for full-scale field applications.

The EICON Group, Inc. (Environmental Infrastructure & Consultants) (Nov 1993 – July 1997)
Position: Chief Scientist, General Manager - Technology Applications Division
Responsibilities: SBP Technologies, Inc. (see below); Scientific advisor to the EICON Group which consisted of: 1) SBP Technologies, Inc., 2) SBP-NE, 3) Florida Groundwater Services, Inc., 4) Florida Remedial Services, Inc., 5) Westcott & Mapes, Inc., and 6) EICON Surveys, Inc.

Ver-Val Environmental Enterprises, Inc. (VVEE) (October 1993 - 1995)
Position: Co-founder, Senior Environmental Microbiologist
Responsibilities: Field bioremediation operations; U.S. DOD/DOE proposal writing (AFCEE, NAVFAC, Army Corp, *etcetera*).

University of West Florida - Center for Environmental Diagnostics and Bioremediation (Jan 1993-1995). *Position*: Adjunct Research Assistant Professor

Clemson University (May 1992-present)
Position: Adjunct Assistant Professor of Microbiology
SBP Technologies, Inc. (formerly Southern Bio Products) (May 1990-July, 1997)
Position: Co-founder, General Manager & Senior Environmental Microbiologist.

***Technical Highlights*:**

- Two Level Three Science and Technology Achievement Awards from U.S. EPA, 1995.
- Lead Project Scientist for U.S.DOD Technology Demonstration/Validation Project (multiple year, multiple site SERDP award of $2.45 million)
- Lead Project Scientist for 2 U.S. EPA SITE Demonstration Projects
- Acting Team Leader for Biodegradation Research, Microbial Ecology and Biotechnology Branch, U.S. EPA Environmental Research Laboratory at Gulf Breeze, FL (1992).
- Coordinate oil spill bioremediation research for U.S. EPA and SBP (1989-1991).
- Design and conduct of bench-, pilot-, and full-scale remediation projects; biotreatability studies.
- Develop protocols for evaluation of oil spill bioremediation products when applied to land and open waters (U.S. EPA, NETAC).

Corporate Development Highlights

- Liaison and Project Leader for International Technology Development Programs with private sector, U.S. and European Corporations and Governments.
- Achieved profitability during first or second year of commercial operations of all startup endeavors.
- Increased revenue from at least 50% to >500% annually for every commercial endeavor.
- Identified major acquisition targets and procured licenses or agreements for multiple (11) international joint business partners offering complementary technology and services.
- Identified and procured International Teaming Partners for joint business development and international technology applications.

Research Interests:

Basic and applied aspects of biological detoxification of pesticides and hazardous wastes; Isolation, characterization and management of native degraders as well as the design and construction of organisms potentially capable of more efficiently removing targeted compounds from contaminated environments; Developing protocol for proper evaluation and effective implementation of field-scale remediation technologies; Design, implementation and evaluation of advanced remediation technologies for laboratory-, pilot- and full-scale operations.

Honor Societies:
- Sigma Xi (not active)
- Gamma Sigma Delta (not active)

Certifications:
- OSHA Hazardous Waste Operations and Emergency Response Standard [29 CFR 1910.120(e)] - 40 hour HAZWOPER #CIH291032, 1991.
- 8-hour HAZWOPER refreshers
- Administrative Hearing and Trial Training Course

Recognitions:
- Two Scientific and Technological Achievement Awards, Level III recognition in the category of Control Systems and Technologies. U.S. EPA Office of Research and Development, Washington, D.C., 1995.
- Nominated for the State of Arkansas Scientific Advisory Board, 1994 -1996.
- Scientific and Technological Achievement Award, Level I (highest level; $5000.00), recognition in the category of Transport and Fate. U.S. EPA Office of Research and Development, Washington, D.C., 1990.
- Outstanding Service Award, Alaskan Oil Spill Bioremediation Efforts, U.S. EPA, Gulf Breeze Environmental Research Laboratory, 1990.
- Special Recognition Award for Excellence in Biodegradation Research, U.S. EPA, Gulf Breeze Environmental Research Laboratory, 1989.
- First Place, Oral Research Presentation Graduate Student Competition, Weed Science Society of America, 1988.
- Third Place, Oral Research Presentation Graduate Student Competition, American Society of Agronomy, 1986.
- Graduate Student Representative to the College of Agricultural Sciences Research Committee, Clemson (1987-1988).
- Graduate Student Representative to the College of Agriculture Advisory Board, SIU-C (1984-1985).

Professional Affiliations:
- American Society of Agronomy (inactive)
- American Society for Microbiology
- National Groundwater Association
- Soil Science Society of America (inactive)
- Weed Science Society of America (inactive)

Activities:

- Reviewer, Environmental Science & Technology (1991 - 1999)
- Reviewer, Applied Environmental Microbiology (1992 - 2005)
- Reviewer, Kluwer Academic Publishers (1995-2002)
- Editorial Board, Antonio Van Leeuwanhoek, January 1995-2000
- Scientific Advisory Board, Phytotech, Inc., Princeton, NJ 1996-1998
- Scientific Advisory Board, *Labroides Dimidiatus* Environmental (LDD), Israel
- Editorial Board, Applied & Environmental Microbiology, January 1999-2004
- Review Committee – Battelle's *In Situ* and On-Site Bioremediation Symposium, 1999
- Review Committee – Battelle's *In Situ* and On-Site Bioremediation Symposium, 2001
- Review Committee – Battelle's *In Situ* and On-Site Bioremediation Symposium, 2002
- Review Committee/Session Chair – Battelle's *In Situ* and On-Site Bioremediation Symposium, 2003
- Review Committee/Session Chair – Battelle's *In Situ* and On-Site Bioremediation Symposium, 2004
- Review Committee/Session Chair – Battelle's *In Situ* and On-Site Bioremediation Symposium, 2005
- Review Committee/Session Chair – Battelle's *In Situ* and On-Site Bioremediation Symposium, 2006
- Review Committee/Session Chair – Battelle's *In Situ* and On-Site Bioremediation Symposium, 2007
- Review Committee/Session Chair – Battelle's *In Situ* and On-Site Bioremediation Symposium, 2008
- Review Committee/Session Chair – Battelle's *In Situ* and On-Site Bioremediation Symposium, 2009
- Review Committee/Session Chair – Battelle's *In Situ* and On-Site Bioremediation Symposium, 2010 Review Committee/Session Chair – Battelle's *In Situ* and On-Site Bioremediation Symposium, 2011
- Review Committee/Session Chair – Battelle's *In Situ* and On-Site Bioremediation Symposium, 2012
- Review Committee – 21st Annual International Conference on Soils, Sediments and Water (UMASS Symposium), 2005
- Review Committee/Session Chair – Oxidation and Reduction Technologies (ORT's-1) for *In Situ* Treatment of Soils and Water, 2004.
- Review Committee/Session Chair – Oxidation and Reduction Technologies (ORT's-2) for *In Situ* Treatment of Soils and Water, 2005
- Review Committee/Session Chair – Challenges in Site Remediation (CSR-1) for *In Situ* Treatment of Soils and Water, 2005

Master's Thesis:

Mueller, J.G. 1985. Enhancement of soybean nodulation via multiple antibiotic resistant mutants of *Rhizobium japonicum* strain 110. Master Thesis. Southern Illinois University, Carbondale, Illinois. 59 pages.

Doctoral Dissertation:

Mueller, J.G. 1988. Biodegradation of carbamothioate herbicides. Doctoral Dissertation. Clemson University, Clemson, South Carolina. 111 pages.

Refereed Publications:

Mueller, J.G., H.D. Skipper, E.R. Shipe, L.W. Grimes and S.C. Wagner. 1988. Intrinsic antibiotic resistance in *Bradyrhizobium japonicum*. Soil Biol. Biochem. 20:879-882.

Mueller, J.G., H.D. Skipper and E.L. Kline. 1988. Loss of butylate-utilizing ability by a Flavobacterium sp.. Pestic. Biochem. Phys. 32:189-196.

Mueller, J.G., H.D. Skipper and E.L. Kline. 1989. Bacterial stimulation by carbamothioate herbicides. Weed Sci. 37:424-427.

Mueller, J.G., P.J. Chapman and P.H. Pritchard. 1989. Creosote-contaminated sites: Their potential for bioremediation. Environ. Sci. Technol. 23:1197-1201.

Mueller, J.G., P.J. Chapman and P.H. Pritchard. 1989. Action of a fluoranthene-utilizing bacterial community on polycyclic aromatic hydrocarbon components of creosote. Appl. Environ. Microbiol. 55:3085-3090. *1989 U.S. EPA Scientific and Technological Achievement Award, Level 1*.

Mueller, J.G., P.J. Chapman, B.O. Blattmann and P.H. Pritchard. 1990. Isolation and characterization of a fluoranthene-utilizing strain of *Pseudomonas paucimobilis*. Appl. Environ. Microbiol. 56:1079-1086.

Mueller, J.G., S.E. Lantz, B.O. Blattmann and P.J. Chapman. 1990. Alternative biological treatment processes for remediation of creosote-contaminated materials: bench-scale treatability studies. EPA/600/9-90/049. 89 p.

Mueller, J.G., S.E. Lantz, B.O. Blattmann and P.J. Chapman. 1991. Bench-scale evaluation of alternative biological treatment processes for the remediation of pentachlorophenol- and creosote-contaminated materials: solid-phase bioremediation. Environ. Sci. Technol. 25:1045-1055.

Mueller, J.G., S.E. Lantz, B.O. Blattmann and P.J. Chapman. 1991. Bench-scale evaluation of alternative biological treatment processes for the remediation of pentachlorophenol- and creosote-contaminated materials: slurry-phase bioremediation. Environ. Sci. Technol. 25:1055-1061.

Mueller, J.G., D.P. Middaugh, S.E. Lantz and P.J. Chapman. 1991. Biodegradation of creosote and pentachlorophenol in groundwater: chemical and biological assessment. Appl. Environ. Microbiol. 57:1277-1285.

Middaugh, D.P., J.G. Mueller, R.L. Thomas, S.E. Lantz, M.J.Hemmer, G.T. Brooks and P.J. Chapman. 1991. Detoxification of creosote- and PCP-contaminated groundwater by physical extraction: chemical and biological assessment. Arch. Environ. Contam. Toxicol. 21:233-244.

Mueller, J.G., S.M. Resnick, M.E. Shelton, and P.H. Pritchard. 1992. Effect of inoculation on the biodegradation of weathered Prudhoe Bay crude oil. J. Indust. Microbiol. 10:95-105.

Middaugh, D.P., S.M. Resnick, S.E. Lantz, C.S. Heard and J.G. Mueller. 1993. Toxicological assessment of biodegraded pentachlorophenol: Microtox and fish embryos. Arch. Environ. Contam. Toxicol. 24:165-172.

Mueller, J.G., S.E. Lantz, R.J. Colvin, D. Ross, D.P. Middaugh and P.H. Pritchard. 1993. Strategy using bioreactors and specially selected microorganisms for bioremediation of groundwater contaminated with creosote and pentachlorophenol. Environ. Sci. Technol. 27:691-698.

Mueller, J.G., J.-E. Lin, S.E. Lantz, and P.H. Pritchard. 1993. Recent Developments in Cleanup Technologies: Implementing Innovative Bioremediation Technologies. Remediation/ Summer Issue 369-381.

Pritchard, P.H., J.G. Mueller, J.C. Rogers, F.V. Kremer and J.A. Glaser. 1993. Oil spill bioremediation: Experiences, lessons and results from the *Exxon Valdez* oil spill in Alaska. Biodegradation 3:315-335.

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Middaugh, D.P., R. Thomas, C.S. Heard, S.E. Lantz and J.G. Mueller. 1994. Field-scale testing of a hyperfiltration unit for removal of creosote and pentachlorophenol from ground water: chemical and biological assessment. Arch. Environ. Contam. Toxicol. 26:309-319. *1995 U.S. EPA Scientific and Technological Achievement Award, Level III.*

Mueller, J.G., R. Devereux, D.S. Santavy, S.E. Lantz, S. Willis and P.H. Pritchard. 1997. Phylogenetic and physiological comparisons of PAH-degrading microorganisms from geographically diverse soils. Antonie Van Leeuwenhoek. 71:329-343.

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Borchert, S.M., F.S. Lakhwala and J.G. Mueller. 1997. UVB Technology invigorates microbes at a Superfund Site. Soil & Groundwater Cleanup. February/March Issue: pages 6-10.

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Kelley, C.A., R.B. Coffin, and J.G. Mueller. 1998. Stable Isotope Analyses – An Innovative Technique to Monitor Biodegradation of Petroleum Hydrocarbons. Geotechnical News September: 20-24.

Lakhwala, F., R.J. Desrosiers and J.G. Mueller. 1998. Demonstration of a microbiologically enhanced vertical groundwater circulation well technology at a Superfund site. Groundwater Monitoring and Remediation. Spring: 97-106.

Kelley, C.A., R.B. Coffin, and J.G. Mueller. 1998. Stable Isotope Analyses – An innovative technique to monitor biodegradation of petroleum hydrocarbons. GeoTechnical News, September, 1998, Pages 20-24.

Hammer, B.T., C.A. Kelley, R. B. Coffin, L.A. Cifuentes, J. G. Mueller. 1998. d13C values of polycyclic aromatic hydrocarbons collected from two creosote-contaminated sites. Chem. Geol. 152: 43-58.

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Ho, Y-F., M. Jackson, Y-Yang, J.G. Mueller, and P.H. Pritchard. 2000. Characterization of Fluoranthene- and Pyrene-Degrading bacteria isolated from PAH-Contaminated Soils and Sediments and Comparison of Several *Sphinogmonas* Sp.. J. Ind. Microbiol. Biotechnol. 24:100-112.

Story, S.P., J.D. Kline, S.H. Parker, T-R J. Tzeng, J. Mueller and E. Kline. 2001. Identification of Intermediate metabolites of fluoranthene and phenanthrene degradation by three classes of TN5 mutants of *S. paucimobilis* EPA505 deficient in polycyclic aromatic hydrocarbon utilization. Gene 378-393.

Story, S. P.; Parker, S. H.; Kline, J. D.; Tzeng, T.-R. J.; Mueller, J. G.; Kline, E. L. Identification of four structural genes and two putative promoters necessary for utilization of naphthalene, phenanthrene, and fluoranthene by *Sphingomonas paucimobilis* var. EPA 505. *Gene* **2000**, *260*, 155-169.

Boyd, T. J., Montgomery, M. T., Spargo, B. J., Smith, D.C., Coffin, R. B., Kelley, C. A., and J. G. Mueller. 2001. Effects of oxygenation on hydrocarbon biodegradation in a hypoxic environment. Bioremediation. J. 5(2):145-157.

Montgomery, M. T., Spargo, B. J., Mueller, J. G., Coffin, R. B., Smith, D. C., and T. J. Boyd. 2002. Bacterial production stimulated across the zone of influence of a groundwater circulation well in a BTEX-contaminated aquifer. Ground Water Monitoring and Remediation. Summer 2002:144-150.

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Patents:

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Mueller, J.G. and A.G. Seech. 2007. ISCR for Turf Grass Applications. Refused.

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Scalzi, M., A. Karachalios and J. Mueller. 2015. Inhibition of Methanogenesis to Control Wood Boring Insects and Pestilence – statins. US PTO filing 62/220,389

Mueller, J. 2016. Inhibition of Methanogenesis to Control Wood Boring Insects and Pestilence – synthetics. US PTO filing EL 538242711 US

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Scalzi, M., A. Karachalios and J. Mueller. 2016/2017. Inhibition of Methanogenesis during Environmental Applications - essential oils. US PTO filing 62/279,519; 15/408,154

Research Grants Awarded:

USDA Competitive Grant: Loss of herbicide efficacy by microbial mediated processes; $50,000.00

Milkhaus South Laboratories: Mineralization of alachlor by pure and mixed bacterial cultures; $3,500.00.

Southern Bio Products, Inc.: Laboratory and field evaluation of multi-stage creosote and PCB biotreatment processes; $250,000.00

Massey-Burch Investment Group: Bioremediation of hazardous wastes: Venture Capital for SBP Technologies, Inc. $3,500,000.00

Groundwater Pollution Prevention Fund, Office of Environmental Processes and Effects Research: Reduction of Pesticide Applications through Bioregulation; $700,000.00

U.S. EPA Innovative Research Program: Role of plasmid transfer in microbial adaptation processes; $50,000.00

Superfund Program, U.S. Environmental Protection Agency, Region IV: Biotreatability of creosote-contaminated soil and water: bench studies; $92,000.00

Superfund Innovative Technologies Evaluation (SITE) Program: Biological treatment for the remediation of creosote and creosote derivatives in an aqueous state: pilot-scale demonstration; $1,250,000.00

Norwegian State Railway: Microbial Ecology of PAH Biodegradation $10,000 joint with Aquateam, Inc. (Norway) and U.S. EPA ($60,000).

National Institute of Health, SBIR: Biological indicators for hazardous waste technologies; $50,000 Phase I.

Strategic Environmental Research and Development Program (SERDP), U.S. DOD/U.S.EPA*: In situ* bioremediation and efficacy monitoring for fossil fuel contamination. $4,800,000.00

U.S. Naval Research Laboratory: *In situ* bioremediation of PAH-contaminated sediments using encapsulated catalysts: $990,000.00

U.S. DOD Dem/Val: Field monitoring of *in situ* bioremediation using overlapping ground water circulation cells (UVB biocurtain): $250,000.00.

Superfund Innovative Technologies Evaluation (SITE) Program: *In situ* groundwater treatment using microbiologically enhanced groundwater circulation technology: full-scale field demonstration $250,000.00.

Countries Worked In:

United States of America, (Argentina), Australia, Austria, Belgium, Brazil, Canada, China, Colombia, Czech Republic, Denmark, France, Germany, Hungary, Israel, Italy, Jordan, Latvia, (Libya), Lithuania, Macedonia, Mexico, Norway, Poland, Slovakia, Spain, Sweden, Taiwan, Thailand, United Kingdom, (Vietnam) and Venezuela.

January 2017

APPENDIX B - RELEVANT PROJECTS

Project / Location	Date	Services Provided - Summary
US EPA / Pensacola, FL American Creosote Works	1988 to 2005	Isotopic analysis to monitor environmental fate & effect – creosote site / PAHs
US EPA / Valdez, AK Exxon Valdez Oil Spill	1989 to 1994	Isotopic analysis to monitor environmental fate & effect / bioremediation – crude oil release/ PAHs
SERDP / Port Hueneme, CA US Navy Base	1994 to 1996	Isotopic and microbial analyses to identify sources and monitor remedial efficiency – Fuel UST sites
SERDP / Gainesville, FL Former Koppers' Site	1994 to 1996	Isotopic analysis to monitor environmental fate & effect – creosote site / PAHs
Beazer East / Salisbury, MD Former Koppers' Site	1996 to 1998	Isotopic analysis to monitor environmental fate & effect – creosote site / PAHs
Beazer East / Charleston, SC Former Koppers' Site	1998 to 2002	Isotopic and microbial analyses to monitor remedial efficiency – creosote site / PAHs
Charleston, SC US Navy BRAC Site	1998 to 2002	PAH biodegradation and transport in sediment; BTEX/TCE biodegradation groundwater
US DOD – Latvia Liepaja Military Harbor	1997 to 2001	Isotopic analysis to identify contaminant sources – mixed contaminants/ PAHs
US DOD – Norfolk, VA US Navy Harbor	1997 to 2004	Isotopic and microbial analyses to identify contaminant sources, transport and monitor remedial efficiency – Fuels/PAHs
US DOD – Washington, DC Anacostia Navy Yard	1997 to 2000	Isotopic and microbial analyses to identify contaminant sources and transport – Fuels/PAHs
US DOD – Philadelphia, PA Philadelphia Navy Yard	1996 to 2000	Isotopic and microbial analyses to identify contaminant sources and transport – Fuels/PAHs
BP-Horizon Oil Spill	2010	Consulting on dispersant environmental impact
US DOD / San Diego, CA Active Navy Site	2016 to present	Isotopic and microbial analyses to monitor remedial efficiency and methanogenesis (source) – chlorinated solvent site
State of Michigan – Confidential Former dry cleaner site	2017 to present	Isotopic forensic analyses to monitor remedial efficiency and methanogenesis (source) – chlorinated solvent site
State of Indiana – Indianapolis Former dry cleaner site	2017 to present	Isotopic forensic analyses to monitor remedial efficiency and methanogenesis (source) – chlorinated solvent site
State of Indiana – Confidential Former industrial site	2017 to present	Isotopic forensic analyses to monitor remedial efficiency and methanogenesis (source) – chlorinated solvent site
State of New Jersey - Confidential Former Industrial Site	2017 to present	Isotopic and microbial analyses to identify contaminant sources, transport and monitor remedial efficiency – metals/PAHs
States of Texas and Louisiana - Confidential	2017 to present	Cost effective, efficient fracking and bilge water cleanup technology, Isotopic confirmation

APPENDIX C - REPRESENTATIVE PUBLICATIONS

Peer-Reviewed Publications (>30)

Boyd, T. J., Montgomery, M. T., Spargo, B. J., Smith, D. C., Coffin, R. B., Kelley, C. A., and J. G. Mueller. 2001. Effects of oxygenation on hydrocarbon biodegradation in a hypoxic environment. Bioremediation Journal 5(2):145-157 (doi:10.1080/20018891079258).

Coffin, R.B., Pohlman, J.W., Grabowski, K.S., Knies, D.L., Plummer, R.E., Magee, R.W., Boyd, T.J. 2008. Radiocarbon and stable carbon isotope analysis to confirm petroleum natural attenuation in the vadose zone. Environmental Forensics 9:75-84

Hammer, B.T., C. A. Kelley, R. B. Coffin, L. A. Cifuentes, J. Mueller. 1998. d^{13}C values of polycyclic aromatic hydrocarbon collected from two creosote-contaminated sites. Chem. Geol. 152:43-59.

Kelley, C. A., B. A. Trust and R. B. Coffin. 1997. Concentrations and stable isotope values of BTEX in gasoline-contaminated groundwater. Environ. Sci. Technol. 31:2469-2472.

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Lantz, S. E., Montgomery, M. T., Schultz, W. W., Pritchard, P. H., Spargo, B. J. and Mueller, J. G. 1997. Constituents of organic wood preservatives that inhibit the fluoranthene degrading activity of bacterial strain *Sphingomonas paucimobilis* Strain EPA505. Environmental Science and Technology 31:3573-3580.

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Mueller, J. G., Borchert, S., Heard, C., Lakhwala, F., Lantz, S. E., Klingel, E. J., Klingel, W., Brourman, M. D., Coffin, R. B., Kelley, C., Trust, B., Cifuentes, L. A., Montgomery, M. T., Boyd, T. J., and W. W. Schultz. 1995. Management of PAH-impacted sites via in situ chemical containment and monitoring. Land Contamination and Reclamation 3(6):11-16.

Spargo, B.J. (ed) (Boyd, Brourman, Coffin, Montgomery, Mueller) "*In Situ* Bioremediation and Efficacy Monitoring". US NAVY, Naval Research Laboratory, Washington D.C. NRL/PU/6115-96-317. October 1996. 323 pages.

Trust, B.A., D.L. Hesse, R.B. Coffin, J.G. Mueller, and L.A. Cifuentes. 1999. Tracing biodegradation with stable carbon isotope measurements of respired CO_2. Bioremediation Journal.

Technical Abstracts / Conference Proceedings (>75 total)

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Boyd, T. J., Montgomery, M. T., Spargo, B. J., Coffin, R. B., Mueller, J. G., Steele, J. K., Pohlman, J. W., Demetriades-Shah, T., and M. Slenska. 2000. Source Reduction Effect on Creosote PAH Bioremediation in Marsh Sediments. In: Case Studies in the Remediation of Chlorinated and Recalcitrant Compounds. G. B. Wickramanayake, A. R. Gavaskar, J. T. Gibbs, and J. L. Means (eds.), Battelle Press, Columbus, OH, pp. 189-195.

Coffin, R., A. Andrushaitis, T. Boyd, J. Pohlman, S. Walker, K. Graboski and D. Knies. June 15-17, 2004. Evaluation of Organic Compound Sources and Natural Attenuation, Liepaja Latvia. USA-Baltic International Symposium. CD Written. 1C Sediment Contamination I.

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Mueller, J.G., F. Lakhwala, S.E. Lantz, E.J. Klingel, M.D. Brourman, R.B. Coffin, C. Kelley, B. Trust, L.A. Cifuentes, M.T. Montgomery and W.W. Schultz. 1995. Use of UVB Technology and Integrated Bioreactors for Chemical Containment and *In Situ* Bioremediation of Subsurface Environments Contaminated by Coal Tar Creosote: Full-Scale Field Demonstration. International Symposium and Trade Fair on the Cleanup of Manufactured Gas Plants and Creosote Sites, September 19-21, 1995, Prague, Czech Republic.

Mueller, J., J. Bold, M. Montgomery, T. Boyd, and M. Slenska. 1999. Monitoring Biodegradation of PAHs in Wetlands Using Biogeochemical Assays. Proceedings Battelle's Fifth International Symposium on *In Situ* and on-Site Bioremediation April 19-22, San Diego, CA.

ATTACHMENT 1

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Increased Capacity for Polycyclic Aromatic Hydrocarbon Mineralization in Bioirrigated Coastal Marine Sediments

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ABSTRACT Bioirrigation of marine sediments by benthic infauna has the potential to increase both the rate and depth of bacterial mineralization of polycyclic aromatic hydrocarbons (PAHs) by recirculating oxygenated bottom water into sediment burrows. Rates of heterotrophic bacterial production and mineralization of PAHs (naphthalene, phenanthrene, and fluoranthene) were measured in sections of sediment cores sampled from stations in San Diego Bay. Data suggest that rates of PAH biodegradation and bacterial heterotrophy were influenced by bioirrigation by benthic infauna. PAH mineralization and heterotrophic production were higher in core sections where sulfide was not detected relative to core sections containing sulfide. Depth-integrated capacity of the upper 17 cm of sediment to mineralize PAHs was 4 to 10 times higher at the station with bioirrigation coefficients that increased with depth. Remedial dredging of sediments to remove contaminant mass (and presumable lower ecological risk) will also remove benthic infauna. Removal of infauna and the subsequent lowering of bioirrigation in surface sediments would be expected to lower the capacity of intrinsic PAH bioremediation. This could cause local increases in ambient PAH concentration and consequently increase the ecological risk at the site and potentially degrade the health of the ecosystem by removing a sink for PAHs.

KEYWORDS bacterial production, bioirrigation, intrinsic, marine, mineralization, PAH, radiotracers, sediment

Disclosure. The opinions and assertions contained herein are not to be construed as official or reflecting the views of the U.S. Navy or the Federal Government at large. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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INTRODUCTION

Polycyclic aromatic hydrocarbon (PAH)-degrading bacteria are ubiquitous in estuarine and marine sediments and are commonly found in areas that do not have substantial known PAH sources (Chung and King, 2001). Rapid PAH catabolism generally depends on molecular oxygen availability to sedimentary bacteria (Cerniglia, 1992; Chung and King, 1999; Leahy and Olsen, 1997), though recently, PAH mineralization has been coupled with other redox processes, such as sulfate reduction (Coates et al., 1998; Hayes and Lovely, 2002; Zhang and Young, 1997; Bedessem et al., 1997) and nitrification (Deni and

Penninckx, 1999; Bonin et al., 1994; Gilewicz et al., 1991). In unperturbed, organic carbon–enriched sediment, heterotrophic bacterial metabolism rapidly depletes oxygen, limiting its availability to the top few millimeters of sediment (Rasmussen and Jorgensen, 1992). Processes that mix the sediment with oxygenated bottom waters can increase the amount of oxygen available to bacteria that are deeper in the sediment. One of these processes involves the activities of benthic infauna that excavate and mix large portions of the sediment and then ventilate their burrows (Aller, 1988).

Bioirrigation has been linked to dramatic changes in both the composition and metabolic activity of the sedimentary bacterial assemblage (see review by Kogure and Wada, 2005; Cuny et al., 2007). Macrofaunal burrows harbor unique assemblages of bacteria that mineralize PAHs more rapidly than those from adjacent, nonburrowed sediment (Chung and King, 1999, 2001; Holmer et al., 1997; Madsen et al., 1997; Schaffner et al., 1997; Bauer et al., 1988; Granberg et al., 2005). Madsen et al. (1997) found that the depth-integrated removal of fluoranthene was twice as high when capitellids worms were present. Bauer et al. (1988) had similar findings with regards to anthracene degradation by capitellids. The activity of diverse macrofaunal communities has also been linked to seasonal removal of PAHs and polychlorinated biphenyls (Schaffner et al., 1997). These findings have led several researchers to postulate that the relative abundance and composition of benthic macroorganism communities control the rate of bacterial PAH degradation in marine sediment (Madsen et al., 1997). Chung and King (2001) concluded that the capacity for PAH biodegradation in hydrocarbon-impacted ecosystems depends on the composition of the natural bacterial assemblage and its response to environmental parameters, rather than on the introduction of new taxa (bioaugmentation). The activities of the benthic macrofauna may create an environment that preferentially selects PAH-degrading bacteria and these activities may increase the depth of the transition zones within the sediment that are important to enhancing bacterial metabolism (Bauer et al., 1988; Ghiorse et al., 1995; Polerecky et al., 2006).

Dredging, a commonly considered remedial alternative for reducing the ecological risk of PAH-impacted submerged sediments, is thought to be beneficial to the health of the ecosystem. However, in addition to the PAHs, benthic infauna are also removed and their pore water mixing activities (i.e., bioirrigation) are likewise disrupted. If bioirrigation enhances intrinsic PAH biodegradation by sedimentary bacteria, then the physical removal of infauna may negatively affect the capacity of the ecosystem to biologically remove PAHs from the watershed. Though there has been much work on the impact of PAH on macrofaunal communities (see review by Fleeger et al., 2003; Carman et al., 1995, 1996), there are few studies providing a quantitative measure of intrinsic PAH bioremediation capacity in coastal sediments. To study the latter, rates of heterotrophic bacterial production and mineralization of naphthalene, phenanthrene, and fluoranthene were measured in sections of sediment cores sampled from two stations in an urbanized waterway in San Diego Bay, CA. The microbial studies were accompanied by pore water chemical studies to establish the redox conditions of the sediments. Pore water chemistry was described by chemical analysis of pore water extracted by centrifugation. The combination of sediment chemistry, modeling, and microbial ecology techniques were used to examine the effects of bioirrigation on the capacity of the natural microbial assemblage to naturally attenuate PAHs.

MATERIAL AND METHODS

Site Description and Sampling

Paleta Creek is an urbanized waterway that receives surface runoff from the adjacent Naval Station and downtown San Diego. Station P17 (longitude: 117.1160° W; latitude: 32.6738° N; water depth: ~6 m) is near the headwaters of the creek and station P04 (longitude 117.1211°W; latitude: 32.6715° N; water depth: ~11 m) is closer to the confluence with San Diego Bay (Figure 1). Sediment at the study site was primarily fine grained (major mode $\geq 4\Phi$) (Germano, 2002), with organic carbon content ranging from 1.1% to 1.7% at station P04 and 1.1% to 2.9% at station P17.

In January 2002, four adjacent cores were taken from each station using a multicore sampler and transferred intact to the laboratory and at ambient temperature within 3 h of collection. Two 5-cm diameter cores from each station were sectioned (2 to 3 cm each) and subsampled for PAH concentration, bacterial production, and mineralization of PAH (e.g., naphthalene, phenanthrene, and fluoranthene). Two other adjacent cores were sectioned for measurement of pore water composition. Slurries for biological assays were made from filtered water overlying the respective cores.

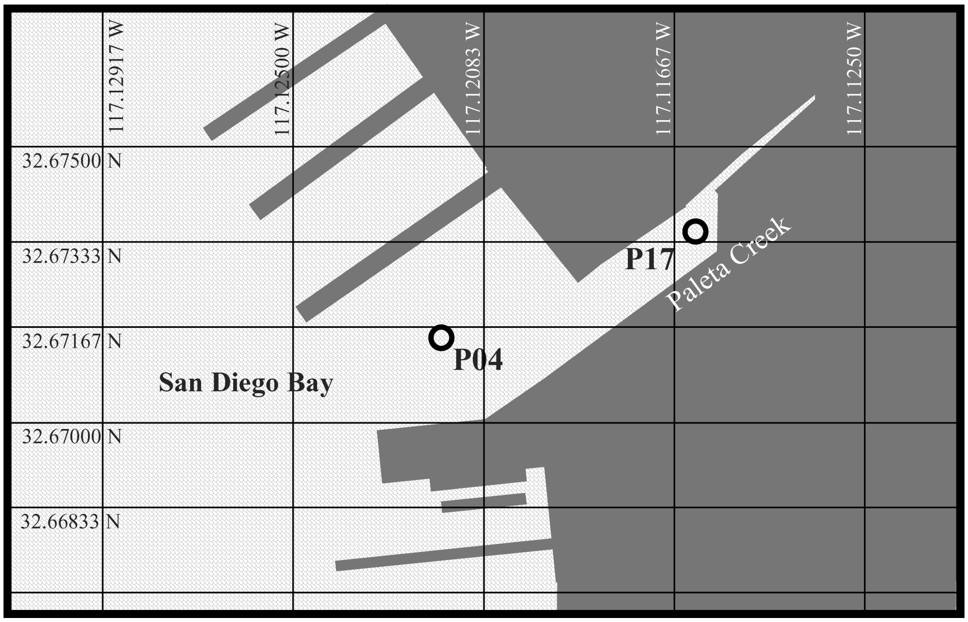

FIGURE 1 Sampling locations for stations P17 and P04 in Paleta Creek in San Diego Bay, CA.

Heterotrophic Bacterial Production

The leucine incorporation method (Kirchman et al., 1985; Kirchman, 1993; Smith and Azam, 1992) was used to measure bacterial production as adapted by Montgomery et al. (1999). A 50-μl sample of wet sediment from each core section was added to 2-ml microcentrifuge tubes sealed with screw caps with O-rings (Fisher Scientific; three experimental and one killed control), which were precharged with [^{3}H-4,5]-l-leucine (20 nM final concentration; specific activity: 154 mCi mmol^{-1}; Sigma). The sediment was extracted from the core section and added to the microfuge tube using a 1-cm^3 plastic syringe with the end cut off. One milliliter of filtered (0.45-μm nominal pore diameter; Acrodisk, Gelman) bottom water (collected from above the upper core section) was then added to each tube to form a sediment slurry. Samples were incubated for 2 h at in situ temperatures and subsequently processed by the method of Smith and Azam (1992). Incubations were ended by adding 57 μl of 100% trichloroacetic acid (TCA; 5% final concentration; Fisher Scientific). Killed controls had the TCA added prior to the addition of the sediment and their values were subtracted from those of the experimental samples. A constant isotope dilution factor of two was used for all samples. This was estimated from actual measurements of sediment dissolved free amino acids (Burdige and Martens, 1990) and saturation experiment estimates (Tuominen, 1995). Samples of wet sediment at 1 cm^3 were dried at 50°C to a constant mass to convert production values to dry weight. Leucine incorporation rate was converted to bacterial carbon using factors determined by Simon and Azam (1989) and the formula of Smith and Azam (1992).

Pore Water Chemistry

Pore water samples were obtained by centrifugation (Sorval; 10,000 × g) under nitrogen to prevent oxidation of labile components (e.g., Fe^{2+} and HS$^-$). Iron was determined by inductively coupled plasma–optical emission spectra (ICP-OES). Alkalinity was determined by acidimetric titration, whereas ammonium, sulfide, sulfate, and phosphate were determined by spectrophotometry (Gieskes et al., 1991).

PAH Degradation

Mineralization Rates

PAH mineralization assays were initiated within 3 h of sediment sample collection using a modification of Boyd et al. (2005) and Pohlman et al. (2002). Three sentinel PAHs (Sigma) were used as radiotracers: uL-^{14}C-naphthalene (specific activity: 18.6 mCi mmol^{-1}), 3-^{14}C-fluoranthene (45 mCi mmol^{-1}), and 9-^{14}C-phenanthrene (55.7 mCi mmol^{-1}). They were added in separate incubations (triplicate live samples and one killed control) to sediment samples (1 cm^3 wet volume) from each core section in 100 ×16-mm polystyrene test tubes to a final concentration of about 0.2 μg g^{-1} (depending on specific activity). Isotope dilution was calculated from the ambient PAH concentration in an attempt to keep additions to less than 10% of ambient concentrations so that it is a radiotracer of assemblage degradation rate and not a calculation of biodegradation potential (see also Deming, 1993). Samples were incubated for 24 h at in situ temperature to minimize bacterial assemblage change, and evolved $^{14}CO_2$ was captured on NaOH-soaked filter papers. H_2SO_4 (2 ml, 2 N) was added to end incubations, kill the control, and partition remaining CO_2 into headspace of the tube and ultimately to the filter paper trap. The filter paper traps containing metabolized $^{14}CO_2$ were removed, radioassayed and then used to calculate substrate mineralization.

PAH Concentration

Ambient PAH concentrations of 18 semivolatile United States Environmental Protection Agency (US EPA) priority pollutants were determined using the US EPA SW 8270 method (Fisher et al., 1997). Briefly, 10 to 15 g of sediment was dried with diatomaceous earth and then extracted in methanol and methylene chloride using standard accelerated solvent extraction. The extracts were concentrated under a N_2 stream (Speedvap) and analyzed by GC/MS (Fisher et al., 1997). *p*-Terphenyl-d_{14} and 2-fluorobiphenyl were used as surrogate standards, with modifications described in Pohlman et al. (2002).

Turnover Time

The turnover time was calculated by dividing the ambient concentration of phenanthrene or fluoranthene (μg kg^{-1}; naphthalene was below detection limits of 1.00×10^{-3} μg kg^{-1} day^{-1}) with the mineralization rate (μg kg^{-1} day^{-1}). This value (in days) is a measure of the average residence time of an individual PAH molecule in the ambient pool of PAH in the sediment. Rapid turnover times (days to weeks) are characteristic of transient compounds that are rapidly metabolized by the natural heterotrophic bacteria assemblage. Slow turnover times (years to decades) suggest that there are relatively low rates of intrinsic bioremediation of these compounds as molecules entering the pool have a long residence time within the sediment.

Capacity for Intrinsic Biodegradation

The intrinsic degradation capacity of a sediment column was calculated by multiplying the mineralization rate of 1 wet cm^3 (the actual unit of measure of the assay) by the depth of the core that was sampled (17 cm). The mineralization values for 0 to 1 cm and 1 to 2 cm were taken from the 0 to 2 cm mineralization measurement, whereas the values for 8 to 9, 9 to 10, and 10 to 11 cm were taken from the 8 to 11 cm mineralization value. These average values for each cm^3 were then summed for a sediment column of 1 cm × 1 cm × 17 cm for each of the three cores, giving a degradation capacity of each core. This capacity was then extrapolated to 1 m × 1 m × 17 cm (multiply by 10,000) to put the analyses in units that would be useful in management of contaminated sediments or an ecosystem level evaluation.

Modeling Approach

An inverse modeling approach (Berg et al., 1998; Meile et al., 2001; Furukawa et al., 2004) was used to quantify biological pore water mixing (i.e., bioirrigation) and consequential deep O_2 fluxes at stations P04 and P17. MatLab software (Mathworks, Natick, MA) was used for all numerical modeling.

Determination of α through Inverse Modeling

The objective of inverse modeling was to determine the irrigation coefficient, α, as a function of depth (x). The inverse model used in this study was based on the one-dimensional (1D) mass conservation equation for solute species in which molecular diffusion, bioirrigation, and production/consumption reactions were considered to dominate chemical mass transport:

$$\frac{\partial C}{\partial t} = D' \frac{\partial^2 C}{\partial x^2} + \alpha(C_0 - C) + \Sigma R \qquad (1)$$

(Berner, 1980), in which the time- (t) dependent concentration of a dissolved species (C) along the vertical axis (x) was determined by its diffusive transport (with D' being the tortuosity-corrected molecular diffusion coefficient (Boudreau, 1996)), transport by bioirrigation (with α being the bioirrigation coefficient [Emerson et al., 1984]), and net rate of production and consumption reactions (ΣR). The use of Equation (1) assumes chemical mass transfer due to sediment accumulation, erosion, and compaction to be negligible (Boudreau, 1996).

The partial discretization of Equation (1) for the i-th node on a vertical grid yields:

$$\frac{dC_i}{dt} = D'_i \frac{C_{i+1} - 2C_i + C_{i-1}}{\Delta x^2} + \alpha_i (C_0 - C_i) + \Sigma R_i \tag{2}$$

(Boudreau, 1996). C_i and D'_i denote the concentration and diffusion coefficient at the i-th node, respectively, and Δx is the distance between adjacent nodes.

By assuming that the geochemical profiles were at steady state, the left-hand side of Equation (2) was set to zero, and the right-hand side was reorganized as:

$$aa_i C_{i-1} + bb_i C_i + cc_i C_{i+1} + dd_i = 0 \tag{3}$$

where

$$aa_i = \frac{D'}{(\Delta x)^2} \tag{4-1}$$

$$bb_i = -\frac{2D'}{(\Delta x)^2} - \alpha_i \tag{4-2}$$

$$cc_i = \frac{D'}{(\Delta x)^2} \tag{4-3}$$

$$dd_i = \alpha_i C_0 + \Sigma R_i \tag{4-4}$$

It should be noted that the assumption of steady state means that there was a balance between the flux (due to accumulation or loss to the water column), intrasediment transport (due to molecular diffusion and bioirrigation), and reaction (either production or consumption). If known concentrations are used at WSI (i.e., $x = 0$, 1st node), C_0, and at the bottom of the modeled sediment column (i.e., $x = \mathrm{L}_b$, n-th node), C_b, as the boundary condition, the following equations can also be written:

$$C_{i=1} = C_0 \tag{5-1}$$

$$C_{i=n} = C_b \tag{5-2}$$

Consequently, the series of equations can be written as:

$$\begin{pmatrix} bb_1 & cc_1 & & & & & \\ aa_2 & bb_2 & cc_2 & & & & \\ & aa_3 & bb_3 & cc_3 & & & \\ & & \ddots & & & & \\ & & & \ddots & & & \\ & & & aa_i & bb_i & cc_i & \\ & & & & \ddots & & \\ & & & & & \ddots & \\ & & & & aa_{n-1} & bb_{n-1} & cc_{n-1} \\ & & & & & aa_n & bb_n \end{pmatrix} \begin{pmatrix} C_1 \\ C_2 \\ C_3 \\ \vdots \\ C_{i-1} \\ C_i \\ C_{i+1} \\ \vdots \\ C_{n-1} \\ C_n \end{pmatrix} + \begin{pmatrix} dd_1 \\ dd_2 \\ dd_3 \\ \vdots \\ dd_{i-1} \\ dd_i \\ dd_{i+1} \\ \vdots \\ dd_{n-1} \\ dd_n \end{pmatrix} = 0 \tag{6-1}$$

or

$$ABC \cdot C + DD = 0 \tag{6-2}$$

in which C and DD are vectors and ABC is a tridiagonal matrix.

Inverse Modeling Strategy for Rate Determination

Equations (2) through (6) show that α is the only unknown if depth dependent values of C and ΣR are known a priori. Depth-concentration profiles of dissolved inorganic carbon (DIC; as approximated by alkalinity) were available from P17 and P04, and the net production rate of DIC was estimated from the leucine incorporation rate, assuming a linear correlation between bacterial production and carbon metabolism with a 30 % growth efficiency to calculate CO_2 evolution (Benner et al., 1988). Thus, the modeling proceeds by: (1) calculating the concentration profiles (C_{calc}) for DIC by initially using randomly assigned values for the α profile; and (2) iteratively refining the α profile by seeking to minimize the difference between calculated (C_{calc}) and measured or interpolated ($C_{measured}$) profiles for DIC.

A simple matrix manipulation of equation (6-2) yields:

$$C = -ABC^{-1} \cdot DD \tag{7}$$

Consequently, the calculated concentration of DIC at i-th node can be expressed as a function of *a priori* parameters as well as α using:

$$C_{calc,i} = F_1(D', \alpha_i, \Sigma R_i) \tag{8}$$

In practice, the inverse determination of the α profile progresses as follows. First, a randomly generated set of initial values are assigned to $\alpha_1, \alpha_2, \ldots, \alpha_i, \ldots \alpha_n$. It is assumed that α remains constant within each 0.005 m vertical section. Then, using the set of α values in Equation (7), a DIC concentration profile is calculated (i.e., $C'_{calc,1} C'_{calc,2} \cdots, C'_{calc,i} \cdots, C_{calc,n}$). Next, the differences between measured/interpolated and calculated concentration profiles is evaluated as follows:

$$\Sigma F = \sum_{i=1}^{n} \frac{|C_{calc,i} - C_{measured,i}|}{C_{measured,i}} \tag{9}$$

Subsequently, α_i values are iteratively adjusted to find the optimized set of values until the value of ΣF is minimized according to the trust region methods for nonlinear minimization (Coleman and Li, 1996). The routine used is that supplied in the Matlab Optimization Toolbox. To ensure that the optimization process leads to the global minima rather than the local minima, the process is repeated up to 50 times, each with a new, randomly generated initial α values (Meile et al., 2001).

Forward Calculation of Depth-Concentration Profiles

Once the depth profile of α was determined through inverse modeling, it was used in the forward modeling to confirm that the calculated depth profiles DIC were in agreement with measured profiles. In the forward modeling, Equation (2), an ordinary differential equation (ODE) after the partial discretization, was written for DIC, and solved for C_i's by integrating *dC/dt* over time using the stiff ODE solver built in Matlab (ode23tb). The series of Equation (2) was written for each node and parameterized using the same D' and ΣR values used in the inverse modeling above.

RESULTS AND DISCUSSION

Heterotrophic Bacterial Production

Heterotrophic bacterial production (as measured by the leucine incorporation method) represents a standard measure of growth and carbon metabolism in aquatic systems. Rates are generally much higher in mixed aerobic systems than in static, anaerobic environments. In San Diego Bay, bacterial production was highest near the sediment-water interface, ranging from 297 to 11.9 μg C kg^{-1} day^{-1} with depth at P04 and from 198 to 6.00 μg C kg^{-1} day^{-1} with depth at P17 (Figure 2). Production was highest in the uppermost (0 to 2 cm below surface) section in all three core profiles.

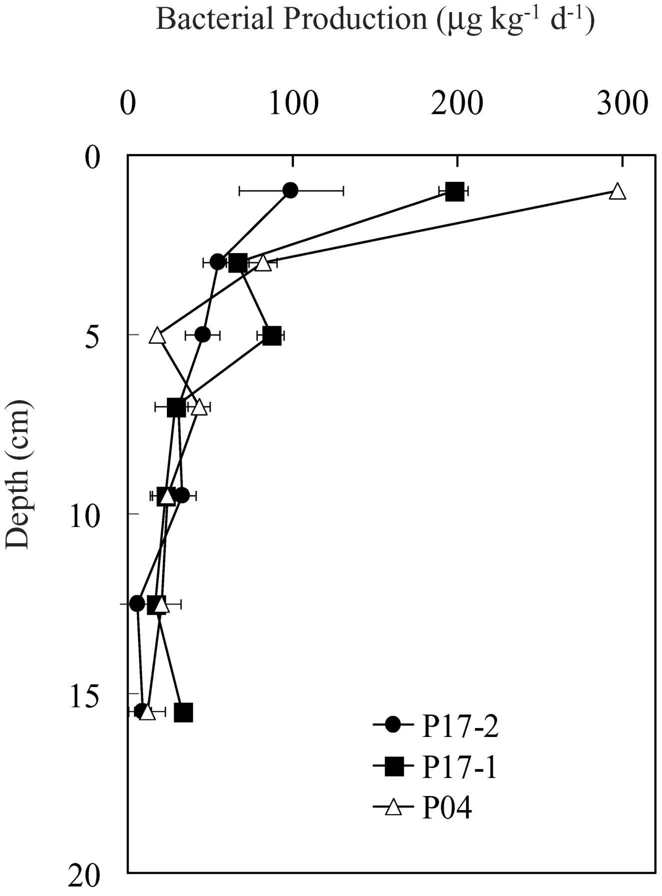

FIGURE 2 **Bacterial production (μg C kg^{-1} day^{-1}) with depth (cm) for cores from stations P17 and P04. Error bars on bacterial production are the standard deviation of triplicate samples.**

Pore Water Chemistry

With depth in the sediment from the sediment-water interface, manganese and iron oxides are often generated between the zones of dissolved oxygen (DO) and sulfate reduction. Based on chemical analyses of the pore water from San Diego cores, sulfide was not detected in the P04 cores using spectrophotometry but increased with depth in the P17 cores (Figure 3A), whereas sulfate decreased in concentration (Figure 3B). Alkalinity in the P04 cores showed little change with depth from the sediment-water interface, whereas there is an increase with depth in the P17 cores (Figure 3C). Manganese oxide reduction at station P17 occurs immediately at or below the sediment water interface, followed by a rapid decrease within the upper 1 to 2 cm

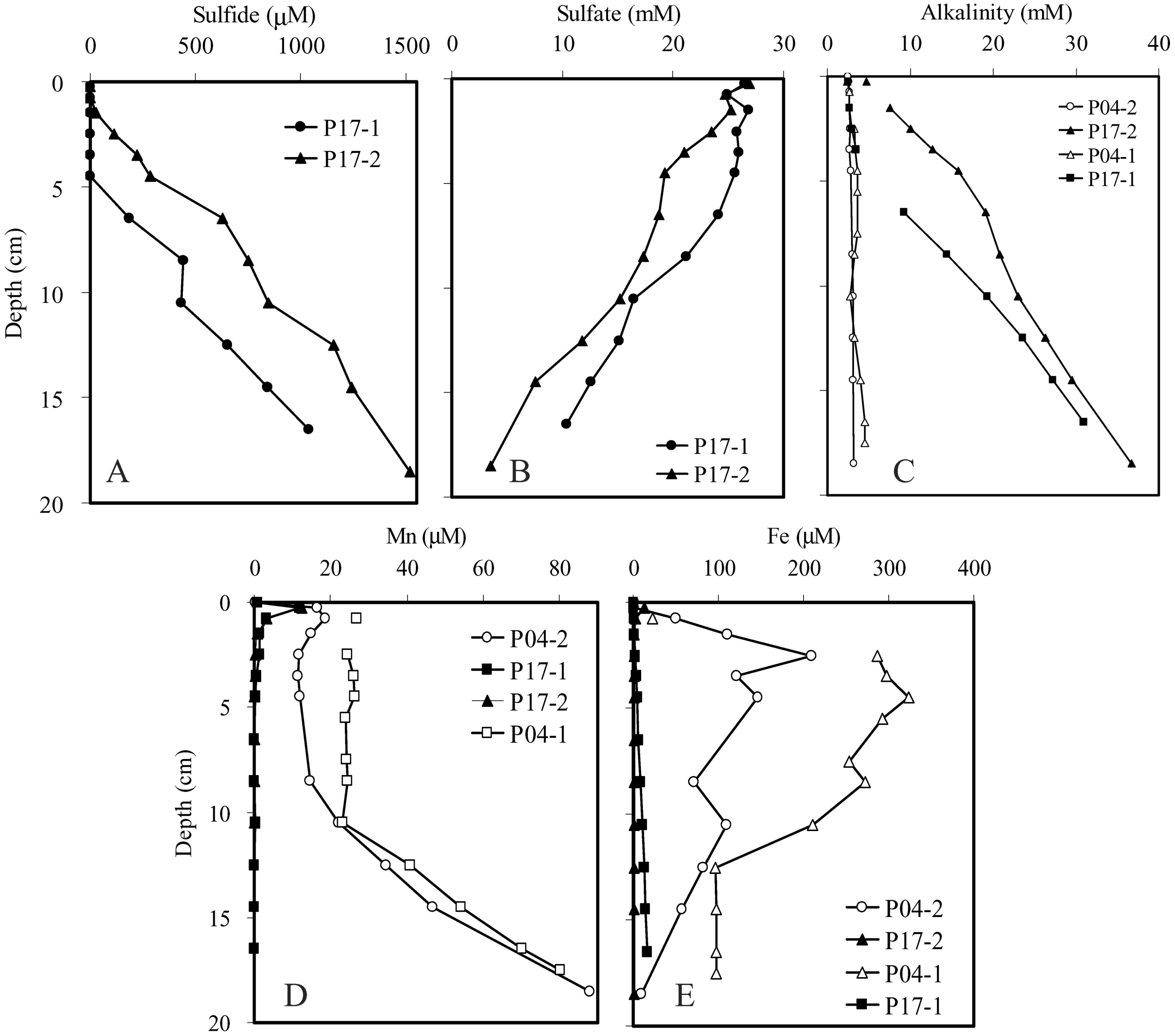

FIGURE 3 Pore water concentrations for sulfide (A), sulfate (B), alkalinity (C), manganese (D), and iron (E) for P17 (filled) and P04 (empty) cores.

(Figure 3D). Similarly, dissolved iron maxima occur at or very near the core surface, followed by a rapid decrease, partially controlled by iron sulfide solubility (Figure 3E). At station P04, dissolved manganese rapidly increases near the surface sediment, followed by a secondary increase below ~10 cm (Figure 3D), i.e., in the zone of initial sulfate reduction. These profiles suggest that the pore water from the P04 cores is more frequently mixed with bottom water at the sediment-water interface than is the pore water from P17.

PAH Mineralization and Turnover

Total PAH concentration in the P17 cores ranged from 1.19 to 3.18 μg g^{-1} (8 to 11 cm section), whereas PAH concentration at P04 ranged from 0.40 to 1.16 μg g^{-1} (14 to 17 cm section; Figure 4). Ambient naphthalene concentrations were below detection (0.01 ppm) in all core sections for the two stations, so turnover time could not be calculated. Naphthalene mineralization rates were also low with most sections below the detection level (1.00×10^{-3} μg kg^{-1} day^{-1}) ranging up to 2.65 ($\pm$0.19) μg kg^{-1} day^{-1} (average of replicate measurements ± 1 σ), but most values were not differentiable from the killed control (Table 1). Although naphthalene flux through the sediments cannot be determined based on ambient concentration, given the transient nature of this compound and the low mineralization rates, there does not appear to be rapid intrinsic bioremediation of this PAH in these

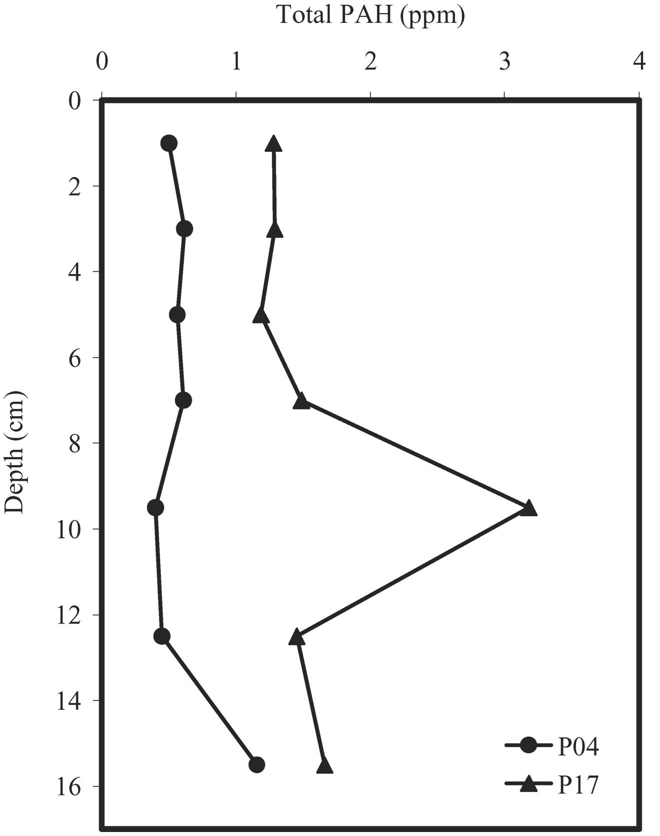

FIGURE 4 **Total PAH concentrations (ppm) with depth (cm) for core sections from P17 for P04 cores.**

sediments. This is expected given that the low ambient concentrations and the assumed low flux of naphthalene would not provide selective pressure for naphthalene degrading bacteria amongst the natural microbial assemblage.

The ambient phenanthrene concentrations were higher than those for naphthalene, ranging from 0.03 to 0.06 μg g^{-1}at P17 and 0.02 to 0.03 μg g^{-1}at P04, and mineralization rates were also higher ranging from below detection to 3.48 (±1.2) μg kg^{-1} day^{-1} at P17 and 2.69 (±0.73) to 8.57 (±5.5) μg kg^{-1} day^{-1} at P04. These phenanthrene mineralization rates are similar to those found for core slices in Eagle Harbor, WA sediments using longer incubation times (ca. 2 μg kg^{-1} day^{-1} calculated from Tang et al., 2006). Phenanthrene turnover time was similar between the two P17 cores, ranging from 14 to 119 days with P17-1 and 28 to 77 days with P17-2. Turnover time was much more rapid throughout the entire 17 cm at P04, ranging from 3 to 8 days. This suggests that the natural bacterial assemblage was rapidly processing phenanthrene molecules that were migrating into the top cm of sediment at P04. Biological mixing of the deeper sediments with bottom water particles or nepheloid layer material depositing onto the surface sediments from the water column could comprise such a flux of PAH to depth (Baker et al., 1991).

The ambient fluoranthene concentration in the sediments ranged from 0.09 to 0.38 μg g^{-1} at P17 and 0.05 to 0.10 μg g^{-1} at P04, whereas mineralization rates ranged from below detect to 1.09 (±0.54) μg kg^{-1} day^{-1} at P17 and 0.18 (±0.18) to 5.09 (±0.81) μg kg^{-1} day^{-1} at P04 (Table 1). Fluoranthene turnover time with the two P17 cores ranged from 236 to 1598 days with P17-1 and 193 to 1632 days with P17-2. The turnover time was much more rapid throughout the entire 17 cm at P04, ranging from 5 to 91 days. These PAH turnover times were within the seasonal range of 6 to 160 days (Pohlman et al., 2002) and 17 to 430 days (phenanthrene; Boyd et al., 2005) reported for surface sediments in the Delaware River estuary. Looking only at surface sediments (within 0.1 cm of the sediment-water interface), Boyd et al. (2005) found a relationship between fluoranthene mineralization rates and seasonal fluctuations in DO concentrations. The finding that elevated mineralization rates occur only when DO is above 70% saturation (Boyd et al., 2005) reinforces the connection between the biological mixing activities of benthic infauna and the rates of intrinsic hydrocarbon bioremediation by bacteria.

The presence of hydrogen sulfide in core sections would be indicative of marine sediments that were poorly bioirrigated or otherwise unmixed with the overlying aerobic water column. For each core, values for bacterial production and PAH mineralization were averaged for core sections where no sulfide was detected by spectroscopy (upper sections) and compared with those averaged among sections where sulfide was detected (deeper sediments). Bacterial production in upper sediments without sulfide was about three to five times the average of that in the lower sulfidic sediments (Table 2). Average PAH mineralization rates were always higher (up to ten times) in nonsulfidic sediment sections than in the poorly mixed sulfidic sediments (Table 2). This comparison of rates between the sulfidic and nonsulfidic regions within each core further supports a positive relationship between bioirrigation and PAH mineralization.

TABLE 1 **Average (AVG) and Standard Deviation (*SD*) of PAH Mineralization Rates and Turnover Time**

		Mineralization rate (μg C kg^{-1} day^{-1})							
		Naphthalene		Phenanthrene		Fluoranthene		Turnover time (days)	
Core	Depth (cm)	AVG	SD	AVG	SD	AVG	SD	Phenanthrene	Fluoranthene
P17-1	0–2	1.06	0.162	3.48	1.20	0.445	0.232	14	530
	2–4	0.271	0.0416	0.435	0.180	0.105	0.114	119	1598
	4–6	0	0	0.592	0.128	0.244	0.177	90	436
	6–8	0	0	0.990	0.812	0	0	59	ND
	8–11	0	0	1.42	0.700	1.09	0.543	35	345
	11–14	0	0	0.418	0.080	0	0	78	ND
	14–17	0	0	0.718	0.526	0.497	0.056	60	236
P17-2	0–2	0	0	1.20	1.05	0.848	0.275	42	278
	2–4	0.521	0.031	0.742	0.125	0	0	70	ND
	4–6	0.064	0.007	0	0	0.065	0.029	ND	1632
	6–8	1.05	0.126	0.758	0.392	0.615	0.291	77	250
	8–11	0	0	1.73	0.547	0.476	0.166	28	790
	11–14	0.049	0.002	1.37	0.484	0.608	0.215	31	193
	14–17	0.284	0.009	0.762	0.245	0.133	0.048	43	684
P04	0–2	0	0	7.86	1.07	5.09	0.807	3	20
	2–4	2.65	0.193	8.57	5.50	1.80	1.75	3	5
	4–6	0	0	3.53	0.508	2.38	1.85	7	27
	6–8	0	0	5.09	2.13	1.99	1.37	6	43
	8–11	0	0	2.69	0.726	0.790	0.491	8	67
	11–14	0.183	0.021	5.02	1.32	0.872	0.149	5	91
	14–17	0	0	3.51	0.602	3.07	2.52	4	15

Note. Mineralization rates and turnover time were measured for each of the three cores with depth (cm). ND = not determined.

Determination of Bioirrigation Coefficient, α

Net Rate (ΣR) of DIC Production as a Function of Depth

The net rate of DIC production was estimated from heterotrophic bacterial production measurements. Bacterial production rate profiles for P17-1, P17-2, and P04 were expressed in terms of the bacterial carbon produced (μg) per kg of dry sediments per day (Figure 2). With average sediment porosity of 60% and a dry sediment density (i.e., grain density) of 2.65, the bacterial carbon production rate can be expressed in terms of moles L^{-1} of pore water s^{-1} by multiplying by the factor of 1.70×10^{-12} (i.e., $= 10^{-6} \times \frac{1}{12} \times \frac{1}{\frac{1}{2.65} \times \frac{0.6}{1-0.6}} \times \frac{1}{24 \times 60 \times 60}$). The conversion is necessary because DIC is reported in terms of moles L^{-1}. Further, these values were converted to the rate of DIC production by using the bacterial growth efficiency of 30 % (Benner et al., 1988) by multiplying by 2.33 (i.e., = 0.7/0.3).

Other Parameters

The depth profiles of DIC concentrations, C_i's, were assumed to be equal to the measured alkalinity (i.e.,

TABLE 2 **Bacterial Production and PAH Mineralization Rate Averages**

		AVG rate of core section (μg C kg^{-1} day^{-1})	
Rate parameter	Station	No sulfide	Sulfide present
Bacterial production	P17	99.4	33.4
	P04	110.1	18.9
Naphthalene mineralization	P17	0.48	0.20
	P04	0.66	0.06
Phenanthrene mineralization	P17	0.68	0.33
	P04	2.53	1.54
Fluoranthene mineralization	P17	0.42	0.35
	P04	6.88	0.88

Note. Bacterial production and PAH mineralization rate averages were higher for core sections that did not have sulfide present compared with those that had measurable sulfide concentrations.

Figure C). The tortuosity-corrected diffusion coefficient, D', was estimated from the following relationship between porosity φ (= 0.6), bottom water temperature T (= 20°C), and infinite-dilution diffusion coefficient for bicarbonate ion HCO_3^-, $D^0_{HCO_3^-}$ (Boudreau, 1996):

$$D^0_{HCO_3^-} = (5.06 + 0.275 \times T) \times 10^{-6}\text{cm}^2\text{s}^{-1}$$

$$D' = \frac{D^0_{HCO_3^-}}{1 - \ln(\varphi^2)} = 5.22 \times 10^{-6}\text{cm}^2\text{s}^{-1}$$

Inversely Determined α

The depth profiles of α for cores P17-1, P17-2, and P04 were determined through inverse modeling (Figure 5). The model-derived irrigation coefficients indicated that (1) biological pore water mixing was focused within the upper ~4 cm of sediments at P17-1, whereas it intensified at a deeper portion of the sediments (~9 to 14 cm) at P04; and (2) biological pore water mixing was very heterogeneous since two cores from P17 exhibited very different α profiles (i.e., significant bioirrigation in P17-1 in the top 4 to 6 cm, and virtual lack of bioirrigation in P17-2). These high α values were similar to those reported from other heavily irrigated harbor sediments such as Buzzard Bay, Massachusetts (Martin and Banta, 1992) and New York Bay (Komada et al., 2004).

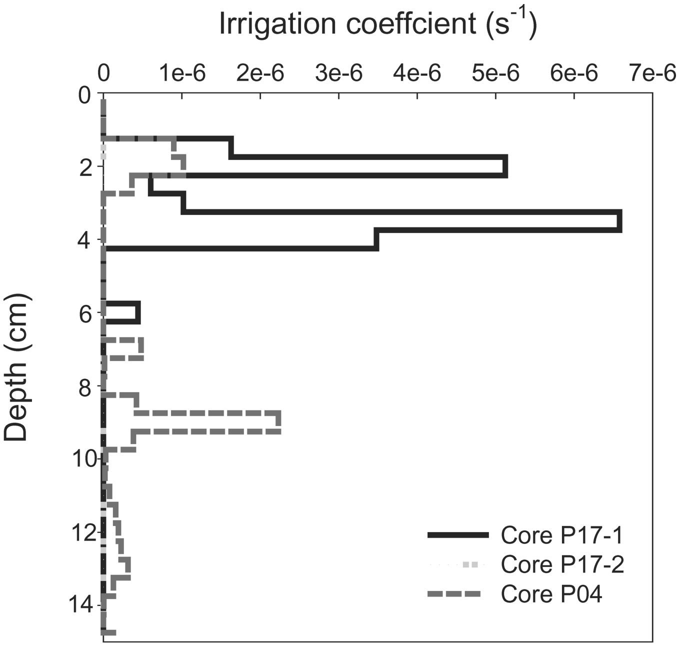

FIGURE 5 **Model-calculated irrigation coefficients are shown as functions of depth. The model results indicate that the DIC profile of core P17-1 was a result of strong bioirrigation near the sediment-water interface, and that of core P04 was a result of deep-penetrating bioirrigation as a significant bioirrigation was hindcasted for 13 to 14 cm below the interface. The DIC profile of core P17-2 indicates a lack of bioirrigation.**

There is a positive linear relationship between irrigation coefficients and the availability of an interface between the burrow wall and oxygenated burrow water (Koretsky et al., 2002). In turn, there is a positive correlation between the burrow wall surface area and diffusive oxygen flux (Wenzhöfer and Glud, 2004). Thus, the high α value in the deeper part of P04 directly indicates a high O_2 flux. Although irrigation coefficients are often thought to decrease with depth (Martin and Banta, 1992), subsurface maxima in profiles have been determined using a similar inverse model technique in Buzzards Bay and Washington Shelf (Meile et al., 2001). High PAH mineralization rates found in the deeper part of P04 corroborate the large values of α estimated for the deeper part of P04.

Intrinsic Biodegradation Capacity

Irrigation coefficients were greater than 10^{-7} cm^2 s^{-1} throughout the core at P04 but only in the top four cm of core P17-1 and in no section of core P17-2. The intrinsic biodegradation capacity for each station was determined based on the mineralization rates of each core section integrated to the 17 cm depth of each core. Though there was some difference in the modeled effect of bioirrigation in the top four cm between cores P17-1 and P17-2, there was little difference in calculated intrinsic biodegradation capacity for naphthalene (27 versus 43 μg C m^{-2} day^{-1}), phenanthrene (187 versus 170 μg C m^{-2} day^{-1}), and fluoranthene (64 versus 67 μg C m^{-2} day^{-1}) when integrated over the entire depth of the cores (Table 3). At station P04, the intrinsic PAH biodegradation capacity was slightly higher for naphthalene (58 μg C m^{-2} day^{-1}), and much higher for phenanthrene (837 μg C m^{-2} day^{-1}), and fluoranthene (692 μg C m^{-2} day^{-1}) (Table 3). Because most of the capacity for PAH mineralization of the station P17 cores was in the top several centimeters, making measurements on deeper cores would not be expected to substantially increase the calculated biodegradation capacity at the station. In the case of station P04, however, bioirrigation extended throughout the 17 cm depth of the core and may have occurred in deeper regions thus making this a conservative estimate of intrinsic PAH biodegradation capacity.

In site investigations of US coastal sediments, the ambient PAH concentration is typically considered

TABLE 3 **Irrigation Coefficient and Intrinsic Degradation Rates**

Core	Irrigation coefficient 10^{-7} cm^2 s^{-1} (depth, cm)	Intrinsic biodegradation capacity (μg C m^{-2} day^{-1} for top 17-cm sediment) Naphthalene	Phenanthrene	Fluoranthene
P17-1	0–4	27	187	64
P17-2	0	43	170	67
P04	0–17	58	837	692

Note. The irrigation coefficient was greater than 10^{-7} cm^2 s^{-1} in the top 0 to 4 cm at P17 but extended throughout the 17-cm depth of the core from P04. The intrinsic degradation rates for each PAH were used to calculate the degradation capacity of 1 m^2 of surface sediment integrated to the 17-cm depth of the core measurements.

to be the result of historical anthropogenic inputs as EPA guidance focuses on comparison with historical land use of areas adjacent to the site rather than on tracking contaminant flux to the sediment (e.g. via sediment traps; http://www.epa.gov/waterscience/cs/). However, the sediment-water interface in coastal ecosystems is frequently impacted by dynamic processes involving tides, rain events, ship traffic and bioturbation. As PAH concentrations increase in the sediment, so does the selective pressure for PAH degrading bacteria amongst the natural microbial assemblage (Roch and Alexander, 1997; Aelion et al., 1989). In dynamic environments, the ambient PAH concentration is more likely the result of a balance between PAH flux to the surface sediments (sedimentation of PAHs on particles) and removal processes (biodegradation, chemical transformation).

In a related study, Chadwick et al. (2006) estimated rates of particle-bound PAH flux to the sediment at stations P04 and P17 using data collected from sediment traps deployed during the two weeks prior to this study. They found that particle-associated deposition of naphthalene, phenanthrene and fluoranthene was 0.47, 31.2, and 54.0 μg m^2 day^{-1} (respectively) at station P17, and 0.74, 17.1, and 32.1 μg m^2 day^{-1} (respectively) at station P04 (Chadwick et al., 2006). Total PAH concentration in the sediment trap material ranged from 9.7 to 41.6 μg g^{-1} (Chadwick et al., 2006), which is much higher than that found in the underlying sediment (0.4 to 3.18 μg g^{-1}; Figure 5). This analysis does not include episodic PAH inputs from oil spills or rain events, nor are there measures of PAH flux to or from the station via lateral transport in the bottom boundary layer. Notwithstanding these potential errors, the intrinsic biodegradation capacity of the mixed surface sediments at station P04 exceeds the PAH flux due to sedimentation. The flux of naphthalene, phenanthrene and fluoranthene to the surface sediment at P04 is matched by the bacterial mineralization of those compounds in a column of sediment less than 1 cm deep (0.03, 0.54, and 0.63 cm, respectively). At P17, naphthalene and phenanthrene flux would be matched by the mineralization rate in a column of sediment 0.09 and 2.41 cm deep, respectively. However it would take a mixed sediment layer of 12.2 cm deep to match the fluoranthene flux, thus fluoranthene could be accumulating at station P17.

CONCLUSIONS

In the US federal and state regulatory community overseeing the management of contaminated sediment, there is a widespread belief that mass removal always lowers ecological risk at an impacted site. It is also widely held that PAH biodegradation only occurs in the top several mm of sediment due to rapid depletion of oxygen in marine sediments. However, the mixing activities of benthic infauna can dramatically change the composition of electron acceptors deep within sediment through reoxygenation. Within a sediment core, sections that did not contain sulfide exhibited higher rates of PAH mineralization than those that contained sulfide, even in core sections that were 17 cm below the sediment-water interface. Because sulfide is rapidly oxidized in the presence of oxygen, it was clear that the higher rates of PAH mineralization were due to the presence of oxygen, which can be attributed to bioirrigation. In addition, the more bioirrigated sediment station (based on biogeochemistry and modeling of irrigation coefficients) had a higher capacity for intrinsic biodegradation of phenanthrene and fluoranthene. Remedial dredging activities may have the unintended effect of disrupting the relationship between PAH degrading bacteria and benthic infauna, inadvertently reducing the intrinsic PAH bioremediation capacity of the ecosystem.

This work adds to the published literature demonstrating the association between the activity of benthic infauna and PAH degradation by natural bacterial assemblages in coastal sediments. More importantly, this represents the first attempt to provide a quantitative measure of intrinsic PAH biodegradation in submerged coastal sediments. The type of evaluation used here can give site managers and regulators a decision-making tool for evaluating ecological risk reduction due to intrinsic bioremediation relative to engineering solutions, such as remedial dredging.

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ATTACHMENT 2

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Relationship Between PAH Biodegradation and Transport in Estuarine Sediments

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ABSTRACT: Ambient polycyclic aromatic hydrocarbon (PAH) concentrations are often measured in estuarine sediments to evaluate risk of those chemicals to benthic infauna and adjacent ecosystems. Typical site investigations assume that PAHs are present in the sediment as a result of historical contamination despite overwhelming evidence of the dynamic nature of contaminants and associated sediment transport in estuaries. Although many processes may affect PAH concentration, we focused on two that were most likely to have to largest impact on PAH flux in the sediment; influx of PAH associated with settling water column particles and bacterial biodegradation within the surface sediments. We compared PAH associated with water column seston, in the benthic boundary layer and on settling particles with PAH biodegradation rates measured in the underlying sediments in the Cooper River in Charleston Harbor Estuary, USA. PAH concentration was generally higher in seston than in the benthic boundary particles and even lower in the underlying sediment for a given station. Bacterial mineralization of PAH was similarly rapid between nepheloid and surface sediment samples with turnover times for phenanthrene in the sediments of around 100 days. This data supports a site conceptual model where sediment in the lower Cooper River is a sink for PAH influx from particles from the water column and benthic boundary layer.

INTRODUCTION

Ambient PAH concentrations in estuarine sediment are the net result of many processes that can increase or decrease these values. Processes that can increase the ambient PAH concentration in the sediment include settling of particles from the water column, groundwater intrusion, and lateral particle transport in the benthic boundary layer. Processes that can deplete standing PAH mass include heterotrophic biodegradation, diffusion, and sediment resuspension. Because PAH compounds are hydrophobic, transport to the sediments would primarily occur on particles while degradation would occur in the water column, benthic boundary layer and within the aerobic portion of the sediments. There has been much recent interest in the benthic boundary layer with regards to transport and degradation of organic matter. Organic matter metabolism close to the seafloor may be as rapid as that which occurs in the euphotic zone (for review see Meyers, 2000).

Recently, Pohlman et al. (2002) examined independent measures of PAH fate in a tidally influenced riverine ecosystem including transport in the bottom boundary layer. They found that bacterial heterotrophy can seasonally degrade 50% of settling PAHs in a tidal basin that receives PAH from the adjacent Schuylkill River. During some portions of the year, the tidal basin and the associated sedimentary microflora acted as a sink for

PAH within this aquatic watershed. The flux of PAHs from the water column particles to the sediment supported a bacterial assemblage capable of relatively rapid PAH metabolism to carbon dioxide and bacterial biomass. By relying solely on ambient PAH concentration (up to 20 ppm) in the sediment, one may have mistakenly concluded that the tidal basin was a source of PAH to the water column or adjacent ecosystems. In a similar study, we compared PAH concentrations, and rates of deposition and degradation within the top cm of surface sediments in the Cooper River of Charleston Harbor to determine whether these sediments were PAH sources or sinks within the ecosystem.

Site Description. This study involved seasonal sampling of twenty-two stations through the Charleston Harbor and the three major rivers that are part of the watershed, the Ashley, Wando and Cooper (Figure 1). The former Charleston Navy Yard (CNY) is situated along the Cooper River about one mile upriver from the Charleston Harbor. Six of the stations were adjacent to the CNY in the Cooper River (6A, 6B, 6C, 6D, 6E, 9). Five stations were upriver of CNY (1-5). Two stations were downriver from CNY (13, 14). Three stations were in a tributary off the Cooper called Shipyard Creek (10, 11, 12). Two stations each were near the mouth of the Ashley River (16, 17), in the Wando River (A2

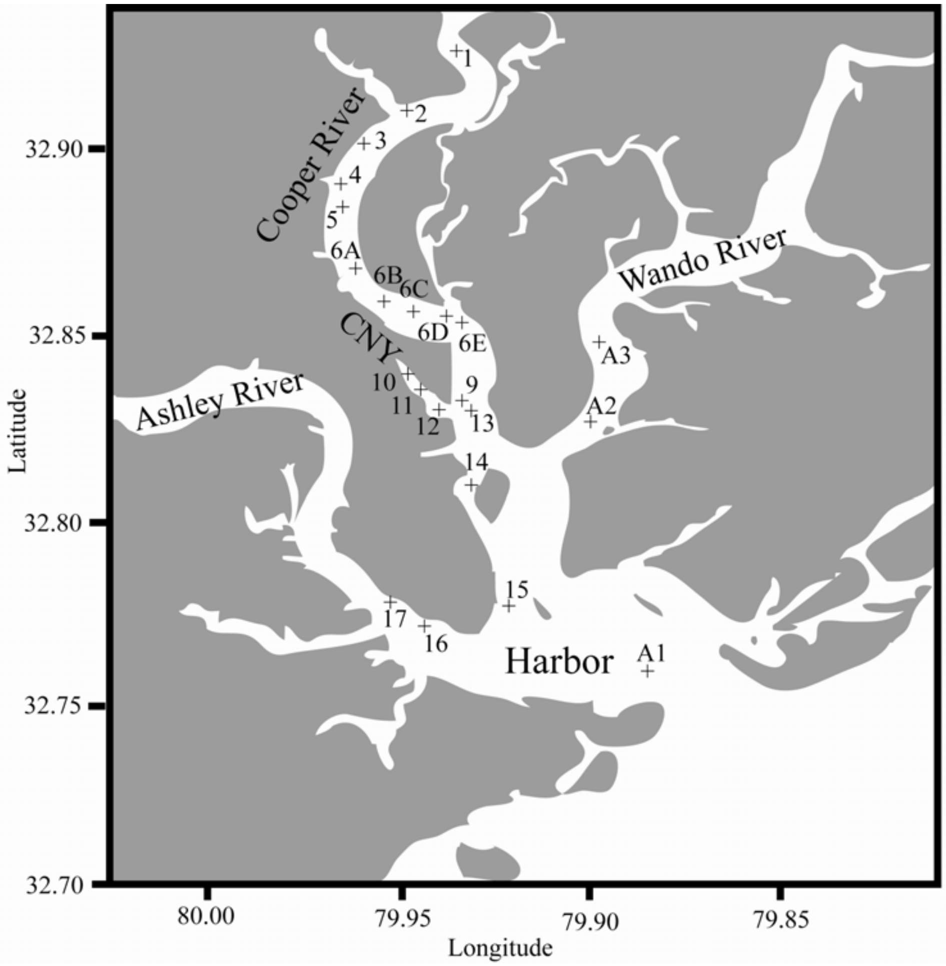

FIGURE 1. Sampling stations in the Charleston Harbor Estuary, SC, USA adjacent to the former Charleston Navy Yard (CNY).

and A3) and in the Charleston Harbor (15, A1). Surface sediment was collected with a Petite Ponor sediment grab. Seston was collected by filtration of 20 L of surface water onto 144-mm-diameter (0.22 μm nom. pore size) Nuclepore® polycarbonate filters. Benthic boundary particles were collected with a sampling device and methods described by Pohlman et al. (2002).

MATERIALS AND METHODS

Radiotracer mineralization assays were initiated within one hour of sediment sample collection using a modification of Boyd et al. (2004) and Pohlman et al. (2002). As radiotracers, we used three sentinel PAHs: UL-^{14}C-naphthalene (18.6 mCi mmol^{-1}), 3-^{14}C-fluoranthene (45 mCi mmol^{-1}), and 9-^{14}C-phenanthrene (47 mCi mmol^{-1}) that were purchased from Sigma Chemical. They were added in separate incubations to surface sediment samples (1 mL wet volume) in 100 × 16 mm test tubes to a final concentration of about 0.2 μg g^{-1} (depending on specific activity). Isotope dilution of PAH was calculated from the ambient PAH concentration and additions were intended to be <10% of ambient PAH concentration to minimize selective pressure on the natural bacterial assemblage. Samples were incubated no longer than 24 h at in situ temperature and evolved $^{14}CO_2$ was captured on NaOH-soaked filter papers suspended in the headspace of each tube. H_2SO_4 was added to end incubations and to partition any remaining CO_2 into headspace of the tube and to the filter paper trap. The filter paper traps containing metabolized $^{14}CO_2$ were removed, radioassayed and subsequently used to calculate substrate mineralization. Ambient PAH concentrations of 18 semi-volatile priority pollutants were determined. First, 10-15 g of sediment was dried with diatomaceous earth and then extracted in methanol using accelerated solvent extraction. The extracts were concentrated under a N_2 stream (Speedvap) and analyzed by GC/MS (Fisher et al., 1997). *p*-Terphenyl-d_{14} and 2-fluorobiphenyl were used as surrogate standards, following the method described in Pohlman et al. (2002).

The sediment trap construction and deployment strategy is similar to that described by Pohlman et al. (2002). Settling particles were collected in 5 to 72 h deployments of bands of three polycarbonate tubes sealed at the bottom and with 10 cm butyrate baffles at the top of their 6 cm-diameter mouth. Traps were attached to an anchor line and held upright with a mooring buoy and then deployed at the CNY stations 6A, 6C and 6E, upriver of CNY at station 5 and downriver at station 13.

RESULTS AND DISCUSSION

From November 1997 to October 1999, we examined PAH concentrations and degradation rates in sediment and on particles from the overlying waters in the Charleston Harbor Estuary, SC. In general, average PAH concentration was higher in suspended particles (seston, benthic boundary layer samples) than in sediments (Table 1). Differences in organic matter concentration between suspended particles in the Cooper River (nepheloid = 3.8-5.4%; seston = 4.9-5.1%) and sediments (4.9-5.3%) does not account for these differences in PAH concentration (see also Schneider et al., 2002). The two most likely explanations are that PAH mass on particles are being laterally transported out of the Cooper River or are degraded by bacteria in the benthic boundary layer and surface sediments.

TABLE 1. PAH concentration in water column particles verses sediments for the Cooper River in Charleston, SC, USA.

Total PAH Concentration (ppm)		
Cooper River (stations)	**Seston/Nepheloid**	**Sediment**
Upriver (1-5)	16.2 / 9.2	7.8
Navy Yard (6A-13)	20.2 / 5.0	4.2
Downriver (14-16)	17.7 / 4.6	1.8

During most of the year, there is no net flow out of the Cooper River though there is a large tidal movement of water (Van Dolah et al., 1990) and a well defined salt wedge with a vertical salinity gradient of 12 to 26‰ (based on shipboard CTD measurements). We measured changes in seston concentration from 45 mg L^{-1} upriver of the Navy Yard to 12-18 mg L^{-1} at the confluence of the Cooper River and the Charleston Harbor (downriver station 15). This change in seston concentration corresponded with an increase in transmissivity in the surface water from 3.9 to 24 RTU as measured with the shipboard CTD. These measurements appear to reflect the large scale settling of particles out of the surface water column in this area by the Navy Yard as has been documented by other studies (Van Dolah et al., 1990).

Tidal mixing can create conditions in the bottom water and bottom boundary layer that can accelerate bacterial degradation of organic matter. PAH degradation (based on wet volume) was similar between benthic boundary layer and sediment with phenanthrene mineralization being the highest relative to naphthalene and fluoranthene (Table 2). Without knowing the retention time for particles in the benthic boundary layer, we cannot determine the proportion of PAH degradation that is occurring as the particles are mixed tidally in this layer relative to that occurring in the surface sediments. The fact that turnover time for phenanthrene is rapid (ca. 100 days) and stable throughout the year in the sediment suggests that there is enough flux of PAH to the surface sediments to maintain selective pressure for PAH-degrading bacteria amongst the natural assemblage (Figure 2). Such rapid PAH turnover within the sediments decreases the likelihood that ambient PAH concentrations are the result of historical deposition.

PAH degradation with depth was not measured at Cooper River stations so one cannot necessarily extrapolate the rate measurement made in the top cm of aerobic sediment to depth unless the deeper sediments are aerated by benthic infauna (Montgomery et al., 2005). We calculated the bioturbation depth required to maintain aerobic rates of

TABLE 2. PAH degradation in the nepheloid and sediment samples (1 mL wet volume) at Cooper River station 6A.

PAH	**Nepheloid ($\mu g \times 10^{-4}$ mL^{-1} d^{-1})**		**Sediment ($\mu g \times 10^{-4}$ mL^{-1} d^{-1})**	
	AVG	**SD**	**AVG**	**SD**
Naphthalene	6.1	0.9	10.7	2.3
Phenanthrene	134	13	72	32
Fluoranthene	0.81	0.12	2.1	0.5

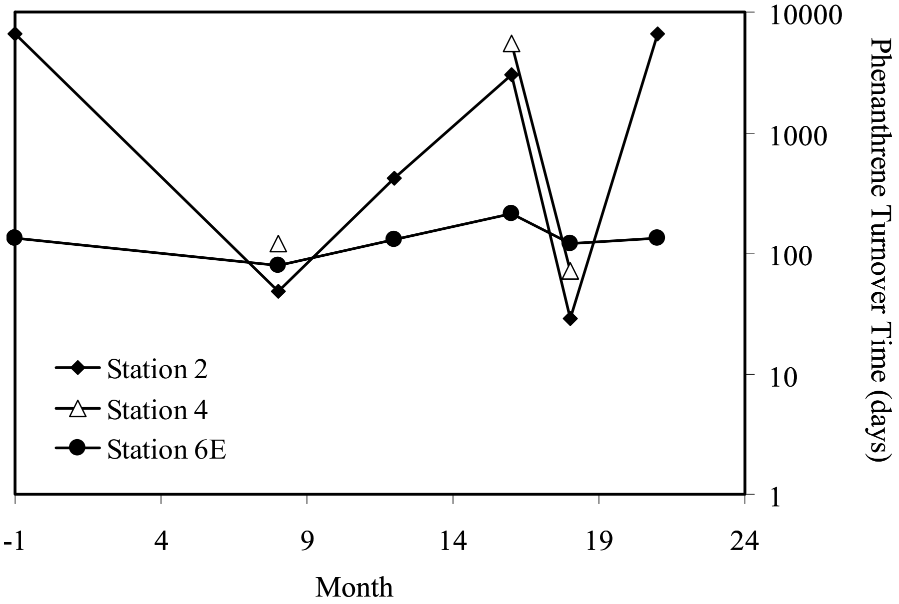

FIGURE 2. Phenanthrene turnover time in sediments of Cooper River, SC, USA from November 1997 to October 1999.

bacterial PAH degradation high enough to balance the PAH flux to the sediment from settling particles. In some cases, such as upriver of the Navy Yard, 367 cm of aerated (bioturbated) sediment would be needed to balance the PAH settling from the water column (Table 3). Such a deep bioturbation depth suggests that the settling PAH is either increasing the ambient PAH concentration in the local sediment (PAH concentration is several fold higher at this station) or the particle associated PAH is transporting laterally in the nepheloid layer. With the upriver sediments being only 3-4 ppm higher in ambient PAH concentration, the latter scenario is most likely. In contrast, the sediments adjacent to CNY (stations 6A-6E) would need only a 5 cm layer of bioturbation to increase the PAH degrading capacity enough to metabolize the PAH mass flux from settling particles. This does not take into account PAH flux transported in the benthic boundary layer from upriver so either a deeper layer of bioturbation is needed, PAH is degraded in the nepheloid layer, or PAH mass migrates past the CNY site to the Charleston Harbor. Rapid PAH turnover, low ambient concentration, and high flux suggest that the sediment adjacent to the CNY (stations 6A-6E) is acting as a sink for PAH in this estuarine system.

TABLE 3. Total PAH degradation verses deposition for Cooper River, SC, USA.

Location	Total PAH Depositing (mg PAH m^{-2} d^{-1})		PAH Degrading* (mg PAH m^{-2} d^{-1})	Bioturbation Depth Needed (cm)
	AVG	SD		
Upriver	7.35	1.62	0.02	367
CNY	6.09	0.35	1.30	5
Downriver	5.79	0.32	0.57	10

*1 cm deep

CONCLUSIONS

This paper used a strategy first described by Pohlman et al. (2002) for using independent measures of PAH transport, flux between media, and biodegradation, to determine the predominant source of PAH at an estuarine sediment site and its rate of intrinsic biodegradation. Pohlman et al. (2002) calculated that bacterial degradation in the top 10 mm could metabolize 25-50% of depositing PAHs annually. Bioturbation of just the top 4 cm would be enough to stimulate heterotrophic bacteria to degrade the entire mass of PAH depositing to the sediment at the Philadelphia Reserve Basin. In addition, Apitz and Chadwick (1999) compared various mechanisms of PAH transport and removal in San Diego Bay sediment and similarly concluded that ambient PAH concentration was a net result of particle settling and biodegradation by sedimentary bacteria (Montgomery et al., 2005). These studies demonstrate the importance of comparing independently derived measures of contaminant fate in estuarine systems.

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ATTACHMENT 3

TRANSPORT, DEPOSITION AND BIODEGRADATION OF PARTICLE BOUND POLYCYCLIC AROMATIC HYDROCARBONS IN A TIDAL BASIN OF AN INDUSTRIAL WATERSHED

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Abstract. Polycylic aromatic hydrocarbons (PAHs) are common contaminants in industrial watersheds. Their origin, transport and fate are important to scientists, environmental managers and citizens. The Philadelphia Naval Reserve Basin (RB) is a small semi-enclosed embayment near the confluence of the Schuylkill and Delaware Rivers in Pennsylvania (USA). We conducted a study at this site to determine the tidal flux of particles and particle-bound contaminants associated with the RB. Particle traps were placed at the mouth and inside the RB and in the Schuylkill and Delaware Rivers. There was net particle deposition into the RB, which was determined for three seasons. Spring and fall depositions were highest (1740 and 1230 kg of particles, respectively) while winter deposition was insignificant. PAH concentrations on settling particles indicated a net deposition of 12.7 g PAH in fall and 2.1 g PAH in spring over one tidal cycle. There was no significant PAH deposition in the winter. Biodegradation rates, calculated from ^{14}C-labeled PAH substrate mineralization, could attenuate only about 0.25% of the PAH deposited during a tidal cycle in fall. However, in the spring, biodegradation could be responsible for degrading 50% of the settling PAHs. The RB appears to be a sink for PAHs in this watershed.

Keywords: biodegradation, deposition, PAHs, particle-associated contaminants, sediment transport

1. Introduction

PAHs (Polycyclic Aromatic Hydrocabons) are common contaminants in aquatic ecosystems that, when present in significant quantities, pose a threat to human health and the environment (Fernandes *et al.*, 1997; Menzie *et al.*, 1992). Sources of PAHs are both natural and anthropogenic, with anthropogenic sources dominating environmental input (Wijayaratne and Means, 1984). PAHs are produced when hydrocarbon fragments from incomplete combustion of fossil fuels condense into multi-ringed aromatic structures (Witt, 1995). Industrial processes using coal or oil as a raw material and incidental oil releases at refineries produce effluents containing PAHs that enter streams, ground water and rivers. Atmospheric deposition, surface runoff and domestic wastes also deliver PAHs into the environment (Manoli

Environmental Monitoring and Assessment **75:** 155–167, 2002.

and Samara, 1999). Identifying PAH sources and understanding their impact on watersheds is matter of scientific and public concern.

Because of their hydrophobic nature, PAHs rapidly adsorb onto nonpolar surfaces of particles and colloidal material in the water column (Bouloubassi and Saliot, 1991; Wijayaratne and Means, 1984). Although colloidal material is an important pool for PAH sequestration, the depositional nature of particles makes them the dominant source of PAH to aquatic sediments (Ko and Baker, 1995). Particles can be transported great distances before being deposited (Bouloubassi and Saliot, 1991). Thus, determining how far contaminants travel from a point source or from where contamination at a particular site originated, can be difficult. Numerous physical, chemical and biological factors must be considered to make an accurate assessment of the sources and fate of contaminants at an impacted site (Jaffe, 1991).

This study focuses on the concentration, distribution, transport and fate (biodegradation and burial) of particle bound PAHs in the Philadelphia Naval Reserve Basin (RB) and portions of the Schuylkill and Delaware Rivers (Figure 1A). The main issue is whether the RB serves as a watershed source or sink for contaminants. The RB is a semi-enclosed embayment covering approximately 10 900 m^2. Its depth ranges between 6.4 to 9.4 m (mean low water) and is subject to semi-diurnal 2 m tides. It is the Navy's only freshwater reserve, and was once an area of active ship maintenance, storage and construction. Its semi-enclosed nature and location at the confluence of two rivers makes it an ideal study site for the modeling of contaminant and particle exchange, and biodegradation. The volume of water tidally exchanged each day is greater than 50% of the average volume of the entire basin. Runoff and stormwater discharge were determined to be a negligible source of contamants to the RB (EA Engineering, 1997). Because of past activities within the RB, it is considered a potential contaminant source to adjacent areas within the watershed, with tidal flux of particle-attached PAHs the primary mechanism of exchange between the RB and surrounding watershed (EA Engineering, 1997). Because promixal sources of contamination (Naval activities) have been attenuated over the past 10 years (no releases) and the depositional accumulation of sedimentary material over time within the RB, we hypothesize that the RB may be a sink for contaminants in the watershed.

2. Experimental

2.1. SAMPLING

Sampling took place 17–20 September 1998 (Sep98), 14–16 December 1998 (Dec98) and 2–5 May 1999 (May99). Samples from the Schuylkill and Delaware River were taken aboard the *R/V Cape Henlopen* and samples from the RB were collected from an inflatable *AVON* boat.

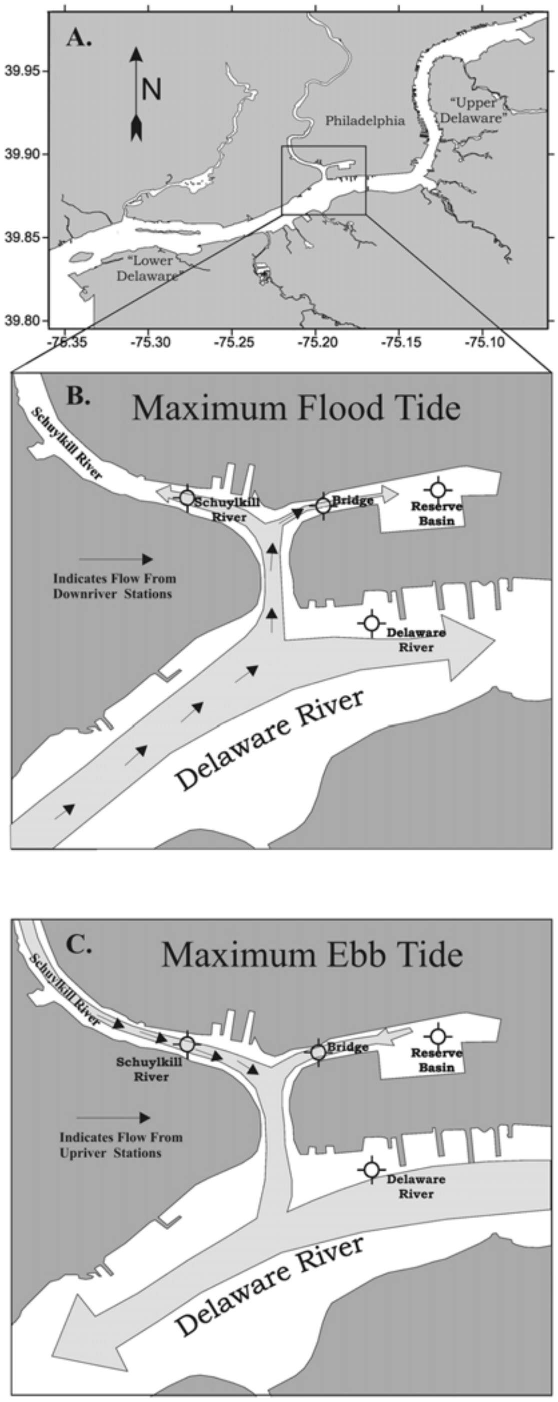

Figure 1. Study site (A), showing current directions during maximum tides (B,C).

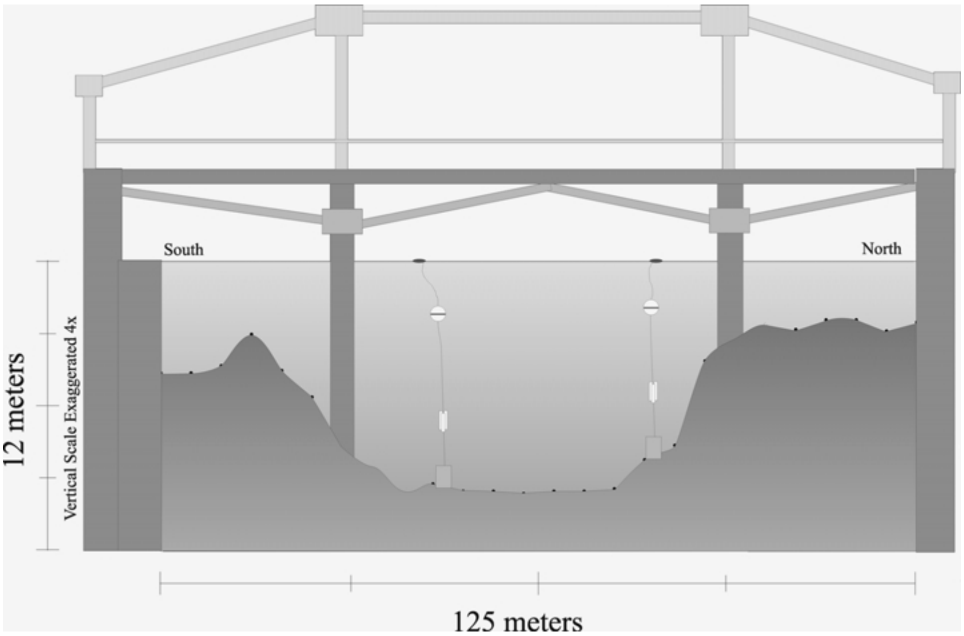

Figure 2. Reserve Basin lift-bridge pass showing particle trap locations.

2.2. PARTICLE COLLECTION

During each sampling, particle traps were deployed on both flood and ebb tides within the mouth region of RB and other portions of the watershed to quantify particle dynamics. Suspended particles were trapped in polycarbonate tubes packed at the top with 10 cm tenite butyrate baffles (US Plastics, Ocean NJ). The baffles reduced the velocity of water passing across the 6 cm-diameter mouth of the trap, which caused particles from the water to fall into the trap. Because of lateral water flow (2 m tidal range with maximum flow rates up to ~ 0.2 m s^{-1}), both actively sinking and suspended particles were likely trapped. In the context of this paper, this collected material is termed suspended particulate matter (SPM). A trap set (3 traps banded together) was attached to an anchored line and held upright by a mooring buoy set below the low tide level. A small surface float was tied to mooring buoys and was used to haul the traps into the boat. One trap was placed in the Delaware River above the Schuylkill River convergence, and one was placed in the Schuylkill River upriver of the RB (Figure 1B, C). Two traps were placed at the mouth of the RB (Figure 2), and one trap was placed in the center of the RB (Figure 1B, C). Upon recovery, samples were drained into acid cleaned Nalgene carboys through a tube at the bottom of each trap and fixed with sodium azide to a concentration of 0.1% (w/v). After 5–7 days of settling, the supernatant was siphoned from the carboys. Samples were stored at 4°C until they were processed.

In addition to collecting suspended sediments from the water column, nepheloid material, the buoyant floc suspended above the sediments, was pneumatically pumped to the surface through an intake funnel and silicone tubing. The funnel was attached to an aluminum plate set 2-15 cm above a perforated base plate. The nepheloid layer depth determined the height the intake funnel was set above the base plate. Samples were collected in acid cleaned mason jars and were fixed to a final concentration of 0.1% (w/v) sodium azide.

Surface sediment samples were collected with a Petite Ponar sediment grab from the *AVON* in the RB and with a Smith-Mack grab from the *R/V Cape Henlopen* in the Delaware and Schuylkill Rivers. Samples were transferred to 50 mL centrifuge tubes and stored at 4 °C until processed.

The concentration of SPM was measured gravimetrically in Sept98 and by nephelometry with a Hydrolab Datasonde 4 (Hydrolab, Austin, TX) in Dec98 and May99. Nepheloid units (NTUs) registered by the Datasonde 4 were converted to concentration (mg L^{-1}) using a standard curve created by comparing gravimetrically determined concentrations against the *in situ* NTU measurement from the water samples (Dec98, $r^2 = 0.99$; May99, $r^2 = 0.94$).

2.3. PAH ANALYSIS

Particle trap, sediment and nepheloid samples were processed for PAHs using Accelerated Solvent Extraction (Fisher *et al.*, 1997) and GC/MS analysis. Samples were spiked with a surrogate standard (2-bromobiphenyl and P-Terphenyl) and extracted for 20 min (100 °C @ 2400 psi) with 1:1 acetone:methylene chloride. Post extraction cleanup was performed using silica gel columns (Supelco, Bellefonte, PA). Extracts were reduced to 1 mL under a gentle stream of purified N_2 gas. A Hewlett-Packard 6890 gas chromatograph (GC) with a 60 m 5% phenyl 95% methyl (SPB-5) 0.250 μm ID capillary column, coupled to a 5973 mass spectrometer (MS) was used for PAH analysis. Sample extracts were injected using an autosampler into a splitless mode inlet maintained at 250 °C. Pressure pulse programming was used to increase inlet pressure from 16 to 25 psi prior to the septum purge at 2 min into the run. Overall column flow was 1.0 mL min^{-1} with helium as the carrier gas. The initial column temperature was 40 °C. The temperature was ramped as follows: 1) at 1 min run-time, ramp 4.0 °C min^{-1} to 180 °C, hold 5 min, 2) ramp 4.0 °C min^{-1} to 220, hold 5 min, 3) ramp 4.0 °C min^{-1} to 280 °C, hold 5 min, 4) ramp 4.0 °C min^{-1} to 300 °C, hold 10 min. The solvent delay was 7.0 min and the MS was set to scan from 35 to 300 amu. The MS was tuned with perfluortributlyamine (PFTBA). The MS temperature was set to 106 °C, and the source was set to 230 °C.

There was very low recovery of surrogate standards from small nepheloid samples collected in Sept98 and Dec98. We conducted an experiment using varying amounts of initial sample with equal additions of surrogate standards. It was found that below roughly one gram starting material there was a linear relationship between sample mass and surrogate standard recovery (Sep98, $r^2 = 0.89$; Dec98, $r^2 = 0.71$). This phenomenon artificially increased the calculation for PAH concentration in smaller samples. Smaller samples may not have had enough surface area and/or time to bind the surrogate with the same affinity as larger samples. Below one gram sample weight, the measured GC/MS responses were corrected to remove the variation caused by the loss of the surrogate standards during solvent extraction.

2.4. MINERALIZATION ANALYSES

PAH mineralization assays were initiated within 1 hour of sediment sample collection using a modification of Boyd *et al.* (1996). UL-^{14}C-naphthalene (Sigma Chemical, 18.6 mCi mmol^{-1}), 3-^{14}C-fluoranthene (Sigma Chemical, 45 mCi mmol^{-1}) and 9-^{14}C-phenanthrene (Sigma Chemical, 47 mCi mmol^{-1}) were added to surface sediment samples (1 mL wet weight) in 100 × 16 mm test tubes to a final concentration of about 500 ng g^{-1} (depending on specific activity) and, in all cases, below the ambient concentration. Isotope dilution was calculated from the ambient test PAH concentration. Samples were incubated no longer than 24 h at *in situ* temperature and evolved $^{14}CO_2$ was captured on NaOH-soaked filter papers. H_2SO_4 was added to end incubations and to force any remaining CO_2 into headspace of the tube to the filter paper trap. The filter papers containing metabolized $^{14}CO_2$ were removed, radioassayed and used to calculate substrate mineralization.

2.5. PAH AND PARTICLE TRANSPORT

The bathymetry across the RB pass was determined by taking depth soundings at 1 m intervals. The water column was divided into three equivalent depth bins (top, middle and bottom) and the relative area of each level determined. The top, middle and bottom levels, respectively, constituted 51.5%, 29% and 19.5% of the total cross sectional area of the pass (Figure 2, Bridge sampling point). Water transport through each bin was estimated by multiplying the fraction of each depth bin by the total transport (in L) coming in or going out of the RB during a tidal cycle. Total transport for the tidal cycle was calculated by multiplying the total area of the RB and tidal range (measured on the seawall). Suspended particulate matter (SPM) was measured hourly during flood and ebb tides and a time weighted averaged for total SPM coming in or going out of the RB per tide was calculated. In this way, any resuspension events within the sampling area would be detected and integrated into the net SPM flux. PAH concentrations were measured from particle trap samples collected during each phase of the tidal cycle. In Sep98 and Dec98, samples were collected only from the bottom layer. That concentration was assumed to be representative of the entire water column and was used in the PAH transport for each discreet level. In May99, samples were collected from each level to investigate the vertical variation of PAHs in the water column. PAH transport in each level was calculated using the PAH concentration measured at that depth.

The total transport of SPM into or out of the RB was calculated by the difference between ($\Sigma H_2O_{\text{flood tide}}$ * $SPM_{\text{flood tide}}$) and ($\Sigma H_2O_{\text{ebb tide}}$ * $SPM_{\text{ebb tide}}$). The PAH transport was calculated using the measured particle trap PAH concentrations and the following formula:

$$\begin{aligned}\Sigma PAH_{\text{trans}} = \;& \{\Sigma H_2O_{\text{top}} * [SPM_{\text{top}}] * [PAH_{\text{top}}]\} + \\ & \{\Sigma H_2O_{\text{mid}} * [SPM_{\text{mid}}] * [PAH_{\text{mid}}]\} + \\ & \{\Sigma H_2O_{\text{bot}} * [SPM_{\text{bot}}] * [PAH_{\text{bot}}]\}\end{aligned}$$

3. Results and Discussion

3.1. SPM-ATTACHED PAH DISTRIBUTION

The SPM-attached PAH concentrations (those found on the collected particles) in the Delaware and Schuylkill Rivers were slightly higher than the mouth or interior of the RB during each sampling event (paired *t*-test; $P = 0.03$). The Schuylkill River ($n = 3$) and Delaware River ($n = 3$) traps had almost identical average SPM-attached PAH concentrations of 5.12 and 5.15 μg PAH g^{-1} particles, respectively ($P = 0.98$). Average SPM-attached PAH concentrations collected over a complete tidal cycle at the mouth of the RB (3.69 μg PAH g^{-1} particle; $n = 6$) and interior of the basin (3.47 μg PAH g^{-1} particle; $n = 3$) were also similar ($P = 0.63$).

Particles collected coming into the RB during rising tide (3.77 ± 1.88 mg PAH g^{-1}) did not have a significantly different PAH concentration ($P = 0.08$) than particles collected in the adjacent Schuylkill River (5.12 ± 1.64 mg PAH g^{-1}). River traps collected particles during both phases of a tidal cycle that reverses the direction of flow in the Delaware and Schuylkill Rivers (NOAA on-line verified tide and current data). When the tide is at maximum flood, the major source of particles collected in river particle traps is the Delaware River below the convergence of the Delaware and Schuylkill Rivers (Figure 1B). When the tide is falling, the source of particles collected in the river traps is the upper Schuylkill River (see Figure 1C). Traps in the RB receive a higher proportion of particles from the Delaware River and lower Schuylkill River water, which is pushed into the RB by the rising tide. Particles from the upper Schuylkill River are partially excluded from the RB because it is draining when the upper Schuylkill River is flowing toward it. Particle traps in the rivers were deployed for an entire tidal cycle; thus their concentrations may represent a sum of a higher concentration period (when Schuylkill River is flowing downstream) and lower concentration period (when Delaware River water is flowing upstream). On the other hand, the particle traps at the mouth of the reserve basin only collected particles during the lower concentration period.

TABLE I

Average nepheloid PAH content (μg g^{-1}) from regions 'upstream' and 'downstream' of the RB

	'Downstream'	*n*	'Upstream'	*n*	*P*
Sep98 (μg g^{-1})	6.50 ± 4.87	6	11.40 ± 4.66	4	0.15
Dec98 (μg g^{-1})	5.08 ± 3.64	6	16.61 ± 4.08	4	<0.01
May99 (μg g^{-1})	1.61 ± 2.45	4	1.82 ± 0.57	4	0.87

TABLE II

Particle deposition in the RB (1 tidal cycle)

	SPM flood tide (mg L^{-1})	SPM ebb tide (mg L^{-1})	μgPAH g^{-1} particles flood tide	μgPAH g^{-1} particles ebb tide	Import (kg±6%)	Export (kg±6%)	Deposition (kg±6%)	Depth deposited (mm)
Sep98	8.59 ± 0.85	4.91 ± 0.31	4.17 ± 0.04	2.91 ± 0.62	6381	5150	1231	0.25
Dec98	0.65 ± 0.09	0.60 ± 0.09	5.42 ± 0.27	6.73 ± 0.79	500	460	N/A[a]	N/A[a]
May99	6.40 ± 0.64	4.90 ± 0.51	1.72 ± 1.05	2.12 ± 1.39	4512	2774	1738	0.36

[a] No significant difference.

Because the particle traps placed in the rivers collected material over a period of several tidal cycles, the particle-attached PAH concentrations measured from those traps represents the time averaged concentration of particles passing through that region of the river. To constrain the regional distribution of the particle-attached PAHs, we measured nepheloid PAH concentrations. Nepheloid material is mobile, which makes nepheloid PAH data a useful proxy for particle attached PAHs. For both Sep98 and Dec98, the highest nepheloid PAH concentrations were from the upper Schuylkill River (Table I). Higher PAH concentrations in this region explains why particle traps deployed in the river had higher particle attached PAH concentrations than those deployed in the RB. Particles from the region with the highest PAH abundance do no enter the RB during the falling tide, when the RB is ebbing water.

3.2. NET PARTICLE AND PAH TRANSPORT

Total PAH transport depends upon the concentration of both the SPM and the SPM-attached PAH concentration. During the Dec98 and May99 sampling events, there was no difference between the average particle-attached PAH concentrations from the ebb and flood tides (Dec: $P = 0.05$, May: $P = 0.71$) (see Table II). The SPM-attached PAH concentrations from samples collected in Sep98 were slightly higher during the flood tide than they were during the ebb tide ($P = 0.02$).

To determine the total PAH transport, it was necessary to consider the SPM concentrations during each season. Seasonally variable factors such as phytoplankton production, soil erosion and sediment resuspension influence particle concentrations (Ko and Baker, 1995; Witt, 1995) in rivers and estuaries. During Dec98, when rainfall was low (2.5 cm during three weeks before sampling) and surface primary productivity is traditionally minimal (Coffin and Sharp, 1987), the average particle concentration was 0.6 ± 0.1 mg L^{-1} (n = 18). In Sep98 and May99, when detrital inputs were higher due to increased rainfall (3.8 and 4.2 cm, respectively, during three weeks prior to sampling) and perhaps due to higher plankton primary production, the average suspended particle concentrations were also higher (5.7 ± 2.2 mg L^{-1} (n = 29) in Sep98 and 5.5 ± 3.9 (n = 52) mg L^{-1} in May99).

In addition to seasonal differences in SPM concentrations water leaving the RB had a lower SPM concentration than water entering the RB during every sampling event at every depth sampled (Table II). The difference between the mass of particulate matter carried into the basin and the mass of the particulate matter exported from the basin is the amount deposited in the basin during each tidal cycle. There was a net deposition of 11.7 g PAH in Sep98 and 2.1 g PAH in May99 in the RB during each of the tidal cycles studied. There was no significant (P = 0.32) net deposition of PAH in the basin for the tidal cycle studied in Dec98. PAH removal from the incoming water was 43.8% in Sep98 and 21.7% in May99 (Table II).

In the RB's associated watershed, SPM concentration was the dominant factor in determining PAH transport. During winter, when fossil fuel use at coal-fired power plants and private heating sources is higher and biodegradation may be lower, there may be a greater net input of PAHs into aquatic systems (Witt, 1995). Our data agree with this observation as particle attached PAH concentrations were highest in Dec98 (Table II). Nevertheless, because the SPM concentrations were so low, the net transport of PAHs into the RB at that time was the lowest of any sampling period.

3.3. BIODEGRADATION OF PAHS IN THE RB

Biodegradation of PAHs in settling particles was estimated by relating the PAH mineralization in the upper 10 mm of sediment surface to the amount of net sediment deposition over a given tidal cycle. Biodegradation rates were averaged for all RB stations for each sampling event. The mean biodegradation rate was multiplied by the SPM deposition (Table II) over a tidal cycle to arrive at a net PAH biodegradation (Table III). Because we only measured naphthalene, phenanthrene and fluoranthene mineralization, the total reflects only the sum of these PAHs. During the Sep98 sampling, PAH biodegradation could only account for a small percentage of potential PAH removal (0.25%). However, in May99, the average biodegradation rate may have been responsible for attenuating almost 50% of the PAH (naphthalene, phenanthrene and fluoranthene) deposition within the measured tidal cycle. Depending on *in situ* growth efficiencies, which have been shown to be

TABLE III

PAH Deposition, biodegradation, and turnover time in the RB

	Sep98	Dec98	May99
Net PAH deposition (g)	12.00 ± 17%	-----	2.1 ± 15%
Net particle PAH[a] biodegradation (g 24 h^{-1})	0.03 ± 60%	-----	1.0 ± 80%
Total PAH[a] in reserve basin[b] (g)	51.00 ± 50%	42.00 ± 60%	88.0 ± 48%
Total reserve basin PAH[a] biodegradation[b] (g 24 h^{-1})	0.63 ± 90%	0.26 ± 90%	14.0 ± 60%
PAH[a] turnover time in reserve basin (days)	77.00 ± 60%	160.00 ± 60%	6.1 ± 50%

[a] Naphthalene, phenanthrene and fluoranthene.

[b] (upper 10 mm).

significant (Herbes and Schwall, 1978), higher total biodegradation (50%) may have actually occurred. Total PAH mass and biodegradation within the upper 10 mm of the RB was calculated by multiplying the area of the RB by the PAH concentration and biodegradation in the upper 10 mm of sediment surface. A turnover time was calculated by dividing the rate into the total PAH concentration (Table III). These data indicate temporal separation in the deposition:biodegradation dynamics of PAHs within the RB. There appears to be a median turnover time in Sep98 when net PAH deposition is highest. In Dec98, deposition is low and turnover time is high (160 days). In May99, there is low deposition (2.1 g), yet short turnover (6.1 days). We postulate that PAHs accumulate in fall and winter months and are attenuated by biodegradation in the spring. From a previous sampling in Jun98, we observed low dissolved oxygen above the sediment surface within the RB. We also noted lower PAH biodegradation rates than in other seasons. Perhaps, rapid biological activity depletes dissolved oxygen to a level that inhibits PAH biodegradation. Thus, net accumulation of PAH may also occur in summer months.

3.4. THREE-LAYER – VS – BOTTOM LAYER MODEL

In Sep98 and Dec98, PAH transport into the basin was calculated by assuming the particles collected 2 meters above bottom were representative of the entire water column. Suspended particle concentrations used in the calculations, however, were measured at three distinct levels – 2, 4 and 7 meters above bottom. Bottom SPM concentrations were notably higher than those at the middle and near surface depths during May99 and Sep98. This distribution could have lead to variations in the particle PAH binding capacity (Ko and Baker, 1995). Consequently, we

TABLE IV

PAH concentrations in SPM collected in sediment traps, nepheloid material and sediment in the RB

	Sept98 (μg PAH g^{-1})	*n*	Dec98 (μg PAH g^{-1})	*n*	May99 (μg PAH g^{-1})	*n*
Particle Traps	3.90 ± 0.66	8	5.33 ± 1.22	8	2.09 ± 1.16	18
Nepheloid	10.91 ± 2.81	10	10.95 ± 3.48	9	1.72 ± 2.08	5
Sediments	16.83 ± 8.20	29	15.29 ± 6.17	19	15.48 ± 5.69	15

investigated the vertical stratification of the particle attached PAH concentrations in May99.

Three sets of traps were placed on the mooring line at the same levels suspended particle concentrations were measured to determine if the SPM-attached particle concentrations were vertically stratified. The shallowest samples (7 meters above bottom) for each treatment had the lowest SPM-attached PAH concentrations which ranged between 0.5 to 1.2 μg PAH g^{-1} particles. The middle level (4 meters above bottom; 2.5 to 3.7 μg PAH g^{-1} particle) and deepest level (2 meters above bottom; 2.1 to 2.7 μg PAH g^{-1} particle) concentrations were a factor of 2-3 times higher than the surface samples.

Using only the bottom layer model with the May99 data, deposition of PAHs into the RB was 20.4% greater than calculated using the three-layer model. The difference in the two model calculations can be attributed to the relatively low SPM PAH concentrations in the shallow traps (7 meters above bottom). Despite seasonal differences in SPM composition (Asper *et al.*, 1992; Thunell, 1997), it is reasonable to assume there may be similar variation in the net PAH transport values calculated in Sep98 and Dec98. Thus, the PAH deposition we have reported for each sampling event may be an overestimate of the actual deposition.

3.5. FATE OF PAHS IN THE RB

Model calculations for maximum current velocities within the RB are thought to be below those necessary to scour the sediment surface (EA Engineering, 1997). Settling particles carry watershed contaminants like PAHs into the RB, a relatively quiescent body of water, where they likely stay. The net deposition of particles in the RB makes it a sink for PAHs in the watershed. The fate of particles (i.e. remaining suspended in the water column, aggregating in a nepheloid layers, or accumulating as sediment) may provide insight into the inter-sample differences observed in PAH deposition. There were clear differences in PAH concentrations in each of the particle reservoirs in the RB (Table IV). The sediments had the highest concentration of PAHs during all samplings, while the nepheloid layer had intermediate concentrations in Sep98 and Dec98. Only during May99 did the nepheloid layer and water column suspended sediments have similar PAH concentrations ($P = 0.63$). The discrepancy between the PAH concentrations of the two mobile phases (nepheloid layer and water column) may explain why the sediment bound PAHs are more concentrated than the particle bound PAHs. Nepheloid material migrating into the reserve basin would elude capture by the traps deployed 2 meters above bottom.

Sediments are the likely reservoir for PAHs within the RB. Because biodegradation rates are too low to attenuate all of the settling SPM-associated PAHs (turnover times as long as 160 days), sediment PAHs will probably reach a steady state concentration (Table IV). The balancing of biodegradation (which is probably effective only during certain seasons) and burial into sediment most likely dictates the PAH concentration within an average RB sediment core. Evidence suggests that biodegradation of PAHs under anaerobic conditions within deeper sediments is very slow if significant at all (Coates *et al.*, 1996; Coates *et al.*, 1997). Because of low sulfate concentrations commonly found in freshwater sediments, one would not expect sulfate reduction, which has been identified as one of the major electron accepting processes associated with PAH biodegradation in marine sediments, to be the major factor in the freshwater RB.

4. Conclusions

Based on the results of this study, the RB appears to be a sink for particle-associated PAHs within its adjacent watershed. Although the PAH concentrations of settling particles were lowest within the RB, net particle deposition over tidal cycles indicates accumulation of PAHs within the RB. An estimate of biodegradation rates showed that during the spring season, PAH biodegradation may attenuate a significant fraction of the PAHs settling within the Basin. However, for other seasons sampled (fall and winter) PAH biodegradation was not rapid enough to attenuate PAH input. To validate our methods, we developed a three-layer model of transport that showed slightly lower accumulation of particles during incoming and outgoing tides. Because this error is most likely systematic, estimates of net particle deposition from Sep98 and Dec98 samplings were likely about 20% lower than reported.

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ATTACHMENT 4

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Compound-Specific Isotope Analysis Coupled with Multivariate Statistics to Source-Apportion Hydrocarbon Mixtures

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Compound Specific Isotope Analysis (CSIA) has been shown to be a useful tool for assessing biodegradation, volatilization, and hydrocarbon degradation. One major advantage of this technique is that it does not rely on determining absolute or relative abundances of individual components of a hydrocarbon mixture which may change considerably during weathering processes. However, attempts to use isotopic values for linking sources to spilled or otherwise unknown hydrocarbons have been hampered by the lack of a robust and rigorous statistical method for testing the hypothesis that two samples are or are not the same. Univariate tests are prone to Type I and Type II error, and current means of correcting error make hypothesis testing of CSIA source-apportionment data problematic. Multivariate statistical tests are more appropriate for use in CSIA data. However, many multivariate statistical tests require high numbers of replicate measurements. Due to the high precision of IRMS instruments and the high cost of CSIA analysis, it is impractical, and often unnecessary, to perform many replicate analyses. In this paper, a method is presented whereby triplicate CSIA information can be projected in a simplified data-space, enabling multivariate analysis of variance (MANOVA) and highly precise testing of hypotheses between unknowns and putative sources. The method relies on performing pairwise principal components analysis (PCA), then performing a MANOVA upon the principal component variables (for instance, three, using triplicate analyses) which capture most of the variability in the original data set. A probability value is obtained allowing the investigator to state whether there is a statistical difference between two individual samples. A protocol is also presented whereby results of the coupled pairwise PCA−MANOVA analysis are used to down-select putative sources for other analysis of variance methods (i.e., PCA on a subset of the original data) and hierarchical clustering to look for relationships among samples which are not significantly different. A Monte Carlo simulation of a 10 variable data set; tanks used to store, distribute, and offload fuels from Navy vessels; and a series of spilled oil samples and local tug boats from Norfolk, VA (U.S.A.) were subjected to CSIA and the statistical analyses described in this manuscript, and the results are presented. The analysis techniques described herein combined with traditional forensic analyses provide a collection of tools suitable for source-apportionment of hydrocarbons and any organic compound amenable to GC-combustion-IRMS.

Introduction

Release of hydrocarbon contaminants into the environment is a significant problem (cf., http://www.nrc.uscg.mil/incident97-02.html), not only from an environmental hazard and cleanup standpoint, but also in terms liability assessment. Releases often occur from multiple sources and, in the case of on-water spills, are subject to complex transport and sedimentation processes. From a forensics and legal perspective, there is a considerable need to distinguish spill or release hydrocarbons from generalized nonpoint source contamination. In practice, the assignment of liability for released hydrocarbons usually involves collecting multiple lines of evidence, then using different chemical analyses to test a given set of scenarios (e.g., ref *1*). Advances in instrumentation have allowed the collection of these multiple types of data, generally at ever-increasing resolution. However, an increase in the amount of available data has not always proved a boon because rigorous and valid interpretation becomes increasingly more complicated. Investigators have turned to the application of multivariate statistics and chemometric approaches to help understand complex chemical data (cf., ref *2*).

To source-apportion hydrocarbons, a number of methods have been reported over the past 10 years. Generalized approaches pinpointing individual constituents of spilled hydrocarbons using gas chromatography (GC) and GC−mass spectrometry (GCMS) have been employed by the US Coast Guard and other agencies in the U.S. (*3*, *4*). Refinements in both chemical techniques, such as comprehensive two-dimension gas chromatography (*5*), and data collection and processing, such as GCMS with multiple single ion monitoring (*6*) or biomarker analysis (*1*, *7*) have provided analytical methodologies for apportioning hydrocarbons to anthropogenic or natural sources. Real advances have been made recently in applying multivariate statistics to chemical data (*1*, *8*) culminating with recent work (*9*) in which statistical probabilities can be assigned to matches between spilled oils and potential sources, providing a defensible framework with which to assess liability.

One biogeochemical property of hydrocarbons that has had less chemometric attention is the application of stable carbon isotopes to environmental forensics. Stable isotope analyses have been developed to study sources and cycling of inorganic and organic molecular compounds in natural ecosystems (*10*−*12*). The successes in applying stable isotope analysis to understand carbon cycling in natural environments offers hope that intrinsic variation in hydrocarbon isotopic compositions might provide another means of apportioning sources. Due to advances in instrumentation coupling separation methods (i.e., GC) with isotope ratio measurements, compound-specific isotope analysis (CSIA) has been applied to understand a number of biogeochemical cycles. Using this technique, individual components in a

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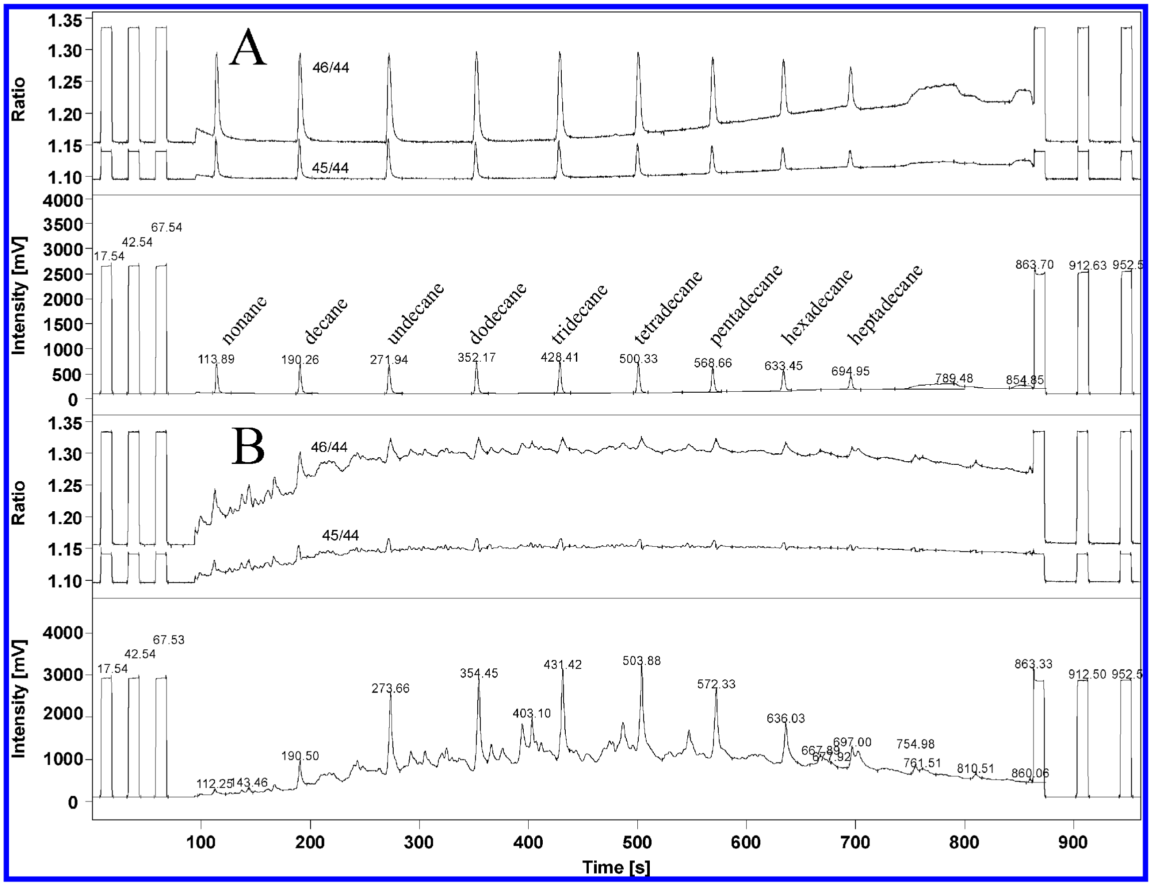

FIGURE 1. Sample chromatograms for *n*-Alkanes C_9 to C_{17} $\delta^{13}C$ analysis. A. Authenticated standards, B. Field sample run, *Mobil Facility* fuel (see text for explanation).

mixture are separated, combusted on-line and transferred to an isotope ratio mass spectrometer (IRMS), in some cases even with conformational data supplied by split flow to an ion trap mass spectrometer (ITMS). With CSIA, the isotope ratio of each component within a mixture can be measured and it can be accurately inferred in a concentration-independent fashion, if two hydrocarbon samples are from different sources.

The isotope ratios of individual components of a hydrocarbon mixture are set during plant photosynthesis (forming the organic matter), subsequent petrogenesis and refinery processes (*13*). As a result, a discreet source of oil will have a unique compound-specific isotope signature (*14*, *15*). Therefore, even though concentrations of individual components (or ratios of components) within the oil or fuel mixture may change over time—due to differential dilution, volatilization, or biodegradation—the isotope ratios of the individual components may remain the same. This may not be the case for some types of hydrocarbons, particularly volatiles (for instance benzene, toluene, ethylbenzene, and xylenes) and chlorinated hydrocarbons (i.e., chloroethylenes). Much of the CSIA literature revolves around using biodegradative fractionation factors for these constituents to confirm on-site bioremediation (*13*, *16*). In most aerobic environments, nonvolatile and semi-volatile hydrocarbons (aliphatic and polynuclear aromatics) appear to have little isotopic fractionation during biodegradation, vaporization, or photolytic conversion (*17*–*19*). This means that a discreet source of hydrocarbon (i.e., a singular spill) should provide a defined CSI signature that remains with the spill hydrocarbons irrespective of changes in concentration or concentration ratios, in any location the spill migrates.

The use of CSIA has just begun to be applied for source apportionment. In several studies, different sources of hydrocarbons were determined by comparing ratios of individual components of the hydrocarbon mixture (*20*, *21*). This approach has been fruitful as subtle variations in natural carbon isotope ratios allow for direct comparison of individual components. In addition to individual isotope comparisons, two-dimension comparisons are now more common coupling both $\delta^{13}C$ and $\delta^{15}N$ or δD analysis (*22*, *23*). What has been lacking is a comprehensive chemometric approach to isotope analysis providing a robust statistical framework for source-apportioning hydrocarbon mixtures.

In this manuscript, we report on a method for analyzing CSI values by a group of multivariate statistical tests which provides statistical probability that any two samples (or common mixtures of hydrocarbons) are the same; through principal components analysis (PCA) on the entire sample set and a reduced set based on match probabilities, a graphical representation of possible matches between unknowns and putative sources; and, finally, using hierarchical clustering, a potential relation between unknowns and potential source hydrocarbons.

Methodology

Standard *n*-Alkane Source. *n*-Alkane standards analyzed on the GC/ITMS/IRMS were purchased individually from Sigma-Aldrich, mixed in equal volume proportions, and diluted at a 1:50 ratio in methylene chloride. Chromatographic resolution was sufficient for resolving nonane through heptadecane (Figure 1A). Standard samples were run at least once with each batch of experimental samples (usually at the beginning and end of each batch). The average value for each standard (11 replicates) was used to calibrate values for each experimental batch.

Monte Carlo Simulation. To understand the operating parameters of the statistical method, a series of simulated

TABLE 1. Results of MANOVA Using the First Three Principal Component Scores (PCS) with All Ten Replicates and PCA−MANOVA Using Random-selected Triplicates from Simulated 10 Component, 10 Replicate δ^{13}C Data

variation	difference	P	*P* (1st 3 PCS)	PCA-Manova *P*(±capture)
2‰ variation	no difference	0.79	0.49	0.28 ± 0.036
2‰ variation	each component different by ≥ 2 ‰	$<10^{-5}$	$<10^{-5}$	0.003 ± 0.00001
2‰ variation	2 varied by 0.5 ‰	0.78	0.25	0.76 ± 0.003
2‰ variation	2 varied by 2 ‰	0.022	0.00070	0.064 ± 0.0008
2‰ variation	4 varied by 1 ‰	0.0077	0.00027	0.099 ± 0.004
1‰ variation	No difference	0.23	0.49	0.48 ± 0.06
1‰ variation	2 varied by 0.5 ‰	$<10^{-4}$	$<10^{-5}$	0.015 ± 0.0004
1‰ variation	4 varied by 1 ‰	$<10^{-5}$	$<10^{-5}$	0.0071 ± 0.0005
1‰ variation	2 varied by 1 ‰, 2 by 2 ‰	$<10^{-5}$	$<10^{-5}$	0.0001 ± $<10^{-5}$

[a] Variation denotes the variation specified for the random number generator (either 1 or 2‰). Difference indicates the manipulation between two simulated 10 replicate, 10 component data sets.

δ^{13}C data sets were created for comparison. These data sets consisted of 10 hydrocarbon components. The RAND function of Microsoft Excel was used to generate uniformly random δ^{13}C values with either a 1 or 2‰ range for each component. The overall range of values between variables was from −18 to −31‰. After generating a data set with 10 replicates of the same hypothetical sample, a second set was generated that simulated replicates of different samples by varying two through four components by a known offset, or in some instances generating a second random set with the same parameters (Table 1 and supplemental data). These two 10 × 10 matrixes were then exported to Matlab and analyzed by standard multivariate analysis of variance (MANOVA). The *P* value was recorded. Next, the two 10 × 10 matrixes were reduced by PCA analysis. The first three columns of PCA scores were then analyzed by MANOVA (to determine the applicability of the PCA−MANOVA described below with no restriction on replicates). The *P* value was recorded. Finally, the PCA−MANOVA method described below was applied to three replicates from each simulated 10 × 10 data set (giving a 3 × 10 test). The generated *P* value was also recorded for comparison (see Results and Discussion and Table 1). The simulated data is included as Supporting Information.

Fuel Tank Sampling. To determine if source differences could be detected, diesel fuel tanks at the Norfolk Naval Ship Yard (Craney Island) in Norfolk, VA (U.S.A.) were sampled on 21 February and 17 April 2001. Sampling was conducted through the top tank port with 100-ml sample containers attached to a lowering line. These tanks may hold “fresh” fuel. However, tanks are often back-fed from ships as well as inter-mixed, depending on local needs. Fuel samples were diluted 1:50 with methylene chloride before injection in the GC/ITMS−IRMS instrument.

Fuel Spill Samples. The Marine Environmental Response Branch, Marine Safety Office Hampton Roads, USCG provided samples consisting of neat floating hydrocarbons from a diesel spill discovered on the water column surface near the Berkley Bridge on the East Branch of the Elizabeth River in Norfolk, VA (36.84N × −76.28W). The spill (USCG case number 01000232) was discovered on 04 January 2001 and consisted of approximately 1500 gallons of diesel fuel. Three distinct spill samples were collected on the north side of the Elizabeth River (waterside), south side (Metro Machine) and directly under the Berkeley Bridge (Berkeley Bridge) which spans the river. Shipboard samples were collected by directly sampling tanks and bilges of the following local tugs and fuel depots: *Mobil Facility*, *Tarmac I*, *Tidewater Star*, *Bay King*, *Bay Eagle*, *Bay Hawk* (day tanks), *Bay Hawk* (port and starboard tanks), *Baytide Storage*, *Baytide* (day tanks), *Bay Hawk*, *Portsmouth Star*, *Arapano*, *Seletar Hope*, *Alert* (bilge), *Alert* (day tanks), and the *Sea Star*. Samples were sent directly to the Coast Guard analytical lab. Splits from these samples were then sent to NRL for GC−IRMS (CSIA) analysis. A representative chromatograph is shown (Figure 1B).

Chromatography. Methylene chloride-diluted (1:50) neat *n*-alkanes were injected (2 μL) into a Varian model 3400 gas chromatograph (GC) for separation on a 60 m, 250 μm diameter Supleco SPB-05 capillary column with 250 μm film thickness. The injector was operated in splitless mode in order to minimize potential isotope fractionation in the inlet (*24*). Column operating conditions were as follows: 40 °C for 1.00 min, ramp to 220 °C at 11.2 °C min^{-1} for 16.07 min, ramp to 290 °C at 35 °C min^{-1} for 2.00 min. The gas stream from the column was split with 80% of the flow directed through a Finnigan MAT combustion interface to a Finnigan MAT Delta S isotope ratio mass spectrometer (IRMS). The remaining flow was directed to a Finnigan MAT Magnum Ion Trap MS (ITMS) for conformational analysis. Known reference gas samples ($\delta^{13}C_{VPDB} = -45.01$) were injected into the source area of the IRMS in triplicate before and after sample introduction. Retention times were calculated based on known *n*-alkane standards. Field *n*-alkane samples were compared to the standard runs and verified using the National Institute of Standards and Technology (NIST) library using the ITMS and AMDIS (www.nist.gov) software. δ^{13}C values were calculated as

$$\delta^{13}\mathrm{C} = \left[\frac{R_s}{R_{std}} - 1\right]1000(‰) \tag{1}$$

where δ^{13}C is the stable carbon isotope ratio, *R* is the ^{13}C/^{12}C for stable carbon, s is the sample and std is the standard. Samples were referenced against CO_2 calibrated to Vienna PeeDee Belmenite (VPBD). All peak voltages were well within instrument linearity (1−6 V), or were discarded in subsequent analysis.

Statistical Analysis. Data from triplicate analyses were transferred to Matlab in which multivariate statistical tests were conducted using the statistical toolbox and custom-written function(s) described below. An initial data reduction was performed whereby individual hydrocarbon components not detected in all samples to be tested were removed from the set. This often led to the removal of nonane (C_9) and decane (C_{10}) from analysis. Data from CSIA lend themselves perfectly to multivariate analyses as each component of the mixture represents an independent variable impacted by initial enzymatic selection and diagenetic processing. As far as rejecting individual samples as a match for an unknown, the most powerful statistical test is an analysis of variance (ANOVA). With multivariate data, there is the option of performing one ANOVA test per variable and then pooling the results, or the option of performing a MANOVA. For this application, MANOVA is preferred, as error could be introduced by pooling the results of multiple variable-wise ANOVA tests. Specifically, while a Bonferroni correction can be applied to lower the significance level of each individual test to appropriately reduce the overall Type I error rate associated with pooling multiple ANOVA tests, this comes at the expense of increasing Type II errors (*25*). Unfortunately, there are conflicting forces at work in adapting data taken from typical CSIA to MANOVA analyses. The robustness of MANOVA is increased by having more variables (i.e., more isotope values individual components of the mixture), however, MANOVA requires that there be at least as many replicates as there are variables and is generally applied to data sets with large numbers of observations. The goal of this work was to provide a robust and workable solution for statistical postprocessing of CSIA data. It is impractical and expensive to perform 6−10 or more replicates using GC−IRMS instrumentation. Therefore, a method was needed to "adapt" data from CSIA to a form suitable for hypothesis testing. A number of attempts to model data variability from multiple injections of known standards proved unsatisfactory under varying conditions (i.e., closely versus nonclosely related sources).

This difficulty seems inherent to disciplines outside large population studies and has recently been addressed by Läuter et al. (*26*, *27*). Further adaptation of statistical methods for data with a small number of observations but a large number of variables was recently presented by Langsrud (*28*). These papers have demonstrated that one can re-project original data into decomposed variables and thus replace original data with new data capturing variability of the original data set, without affecting the results of subsequent multivariate tests, such as MANOVA (*26*). Because we assume that isotopic variation among components of a source hydrocarbon is not the result of any single factor, rather a combination of natural variation and refinery processing, a principal components analysis (PCA) is appropriate for describing the component variability and projecting that variability into a new data-space.

Although there is less need to normalize or otherwise scale δ^{13}C values (commonly done to reduce bias in PCA) as they are generally all the same order of magnitude, techniques such as mean-centering ensure direct comparison of the relative difference between samples rather than the distances of different samples from the origin of the measurement space. The function of PCA is to project data onto a lower-dimensional subspace composed of orthogonal axes that most efficiently describe the variation within the data. These axes are ordered according to how much of the variance of the original data they contain (*29*). Thus, we can initially assume that most of the data differences between multiple components in two mixtures will be captured in the first several principal components (PCs). If triplicate analyses are factored by PCA in a sample-wise fashion, i.e., triplicate vs triplicate, any significant sample to sample variability in the original data set will be represented in the first few principal components. This is because there is theoretically only one significant source of variation in such a data set, provided, of course, that the variance associated with each measurement in the original data is roughly the same.

In sample-wise comparisons we found an average of 98% ($n = 172$) of initial sample variability captured in the first three PCs. Therefore, our processing scheme involves first performing sample-wise PCA, then performing a MANOVA on the first three PCs (i.e., the number of original replicates) of the factored data set to test sample difference. Langsrud (*28*), suggests that variability captured in PCs not used in subsequent hypothesis testing should be presented along with probability values. In this work, we have propagated error in capturing only ~98% of the original sample variation by using the uncaptured variability (in PCs >3 for triplicate analysis) to create confidence intervals around probability values. For example, if 98% of the original variability was captured by sample-wise PCA and the subsequent MANOVA produced a probability of 0.10, a 2% confidence interval was calculated (i.e., ±0.002).

A Matlab program was created to cycle through all data and perform individual sample-wise PCA−MANOVA comparisons in the above manner. The number of individual tests (n) can be calculated as the sum of all integers less than the number of samples:

$$n = \sum_{Samples-1}^{j=1} j \tag{2}$$

The Matlab program calls a custom-written function producing a new data table specifying the test (i.e., sample 1 vs sample 2, and so on), the probability value (P), the capture percentage (CP) of the original variability, and the confidence interval. These data can then be copied to Microsoft Excel so that values can be sorted based on samples, P values, and so forth. The original data from sample tests having P values indicative of no statistical difference between samples ($P <$ 0.05) were then collated and analyzed by standard Matlab PCA and hierarchical clustering analyses (in the statistics toolbox). Samples having the largest P values (i.e., the greatest probability that the mean difference is not due to chance), the closest proximity to one another in PCA plots, and close hierarchical clustering were considered the greatest possible candidate matches.

The PCA−MANOVA process tests the probability of any two samples being statistically the same. In the PCA alone test, in which all samples having a P value greater than 0.05 in a given data set were included (not multiple pairwise analysis), variability in the original data set is used to screen for relation. The final multivariate test involved hierarchical clustering. This technique attempts to cluster relation among samples having multivariate data. Again, data from sample combinations having a P value greater than 0.05 were considered candidate matches. These data were averaged for each variable of each sample, then the means were processed using Matlab's pdist (p distribution) and linkage functions in the statistics toolbox. Output from this analysis was plotted in a dendrogram and putative matches (passing MANOVA and PCA critera) were verified by falling into hierarchical clusters. Clustering was used as an additional line of evidence apportioning unknown samples to a particular known source. The overall statistical processing routine (Figure 1) loosely follows the decision-making flowchart presented by Christensen et al., (*9*) whereby subsequent tests refine matches in order to arrive at the best possible statistically defensible apportionment.

Results and Discussion

Standards. The individual δ^{13}C for standard mix of *n*-alkanes consisting of decane (C_{10}), undecane (C_{11}), dodecane (C_{12}), tridecane (C_{13}), tetradecane (C_{14}), pentadecane (C_{15}), hexadecane (C_{16}), and heptadecane (C_{17}) ranged from −32.4 to −24.1‰ through 11 runs and resulted in a standard error (s.e.) from ±0.10 to ±0.14. The largest standard errors were observed in C_{12} and C_{13} where the peaks were most difficult to resolve. The average for the standard error of all eight *n*-alkanes was ±0.13‰. Previously reported values for BTEX, TNT, and PAHs have been measured in the range of ±0.3, ±0.1, and ±0.1‰, respectively (*21*, *22*, *30*). During the course of the running experimental batches (for all samples reported), the deviations from the standard averages ranged as high as 0.7‰.

TABLE II.[a] **Results of PCA−MANOVA Significance Tests for Sequential Sampling of Norfolk Fuel Storage Tanks**[a]

sample 1	sample 2	P	capture %	±
tank1 t1	tank1 t2	0.055	92.0	0.0044
tank2 t1	tank2 t2	0.343	92.5	0.026
tank3 t1	tank3 t2	0.029	94.1	0.0017
tank4 t1	tank4 t2	0.938	86.0	0.13
tank 1 t1	tank 2 t1	0.422	97.1	0.012
tank 1 t1	tank 3 t1	0.052	91.6	0.0044
tank 1 t1	tank 4 t1	0.857	89.9	0.087
tank 2 t1	tank 3 t1	0.328	96.4	0.012
tank 2 t1	tank 4 t1	0.933	84.2	0.15
tank 3 t1	tank 4 t1	0.683	92.5	0.051

[a] Results reflect C10 − C17 hydrocarbons.

Monte Carlo Simulation. By simulating δ^{13}C data with known levels of precision (i.e., 1 or 2‰), it was possible to determine under what parameters the described PCA−MANOVA tests are able to provide reliable estimates of the "true" *P* value. Using only three of the 10 replicates, at both 1 and 2‰ range for each component, PCA−MANOVA correctly showed no statistical difference in simulated replicate δ^{13}C values of the same sample (Table 1). At a 2‰ range for each component with 2 components varied by 0.5‰ between data sets, none of the statistical treatments showed a significant difference between the data sets. At 2‰ range per component and 2 components varied by 2‰ or 4 components varied by 1‰, both a MANOVA on the 10 × 10 data sets and a MANOVA on the first three principal components of the data sets (10 × 3 comparison) showed a statistical difference ($P < 0.05$). However, when three of the 10 replicates were chosen for the PCA−MANOVA method described in the manuscript, there was no statistical difference between the data sets ($P > 0.05$) (Table 1).

When the simulated data sets had a 1‰ range of precision for each component, the PCA−MANOVA method described here performed adequately for all tested scenarios. The PCA−MANOVA method was able to show a significant difference between triplicate samples taken from the 10 replicate data set with as small as a 0.5‰ shift in only two of the 10 components (Table 1). We conclude from this simulation that analytical precision should be ≤1‰ to adequately use the PCA−MANOVA method for determining statistical differences between multivariate CSIA data. Typical values for precision are less than 1‰, so this method should have broad applicability (cf., ref *31*). It should also be noted that the statistical processing routine presented here does not absolutely require standardization to known isotopic standards, now routine for accurate δ^{13}C reporting. Because normalization and PCA eventually create centered "new" variables, as long as the processes is systematic, one could reference analytical data to any standard (i.e., arbitrarily set the calibration standard to 0‰ and reference all experimental peaks to it).

Norfolk Tank Samples. Four fuel tanks (*1*−*4*) were sampled on 21 February 2001 and again on 17 April 2001. In addition, fuels from other tanks were sampled to determine the variability expected in Navy fuels. For security reasons, detailed information regarding the exact disposition of fuels is not presentable. Fuel within any given tank was a mixture of the original filling, additional fillings, and backfilling from ships. As most of these fuels were freshly refined, we were able to resolve C_{10}−C_{17} *n*-alkanes. Initial samples (February 01) showed no significant differences between any of the four tanks in pairwise tests (Table 2). In fact, several pairings (Tank 1 and Tank 4, and Tank 2 and Tank 4) had *P* values greater than 0.5 conceivably indicating identical sources.

Data were available for fuel transfers from Tanks 2−4. During the sampling period, Tank 2 had no refilling operations, and did not offer any significant difference in the sampled sources (Table 2). Fuel was offloaded by one ship to Tank 3 during the inter-sample period. Mixing in of this source likely resulted in a significant difference between the temporal samples (Table 2). Tanks 2 and 4 were the most interesting cases encountered in this study. Tank 2 was initially no different than Tank 4 ($P = 0.933$). It had no recorded refilling operations, yet had a lower *P* value (0.343) at t2. We attribute this unusual observation to the lower precision in the Tank 2 and Tank 4 data relative to the other tanks included in the data set (i.e., the average standard deviations). From the Monte Carlo simulation, we observed that lower precision data gave a higher deviation for the true *P* value (i.e., with many replicates). Over the course of 3 days in March 2001, Tank 4 received the offload of three separate ships (roughly 400 000 Gallons). Although we have been unable to verify the source of fuel on these ships, it is typical for fuel offloading to occur after joint deployments. These ships drew fuel from the facility sampled for this study. Records are not available for which tank fueled which ships (only receipts are tracked). However, if these ships drew from a common source (i.e., Tank 4), the source ratios would remain unchanged. Although a controlled study would be more useful in a forensics application, i.e., tracking both fueling and receipts from one tank over time, the purpose of this study was primarily to determine if statistical differences could be detected from tank to tank and over time.

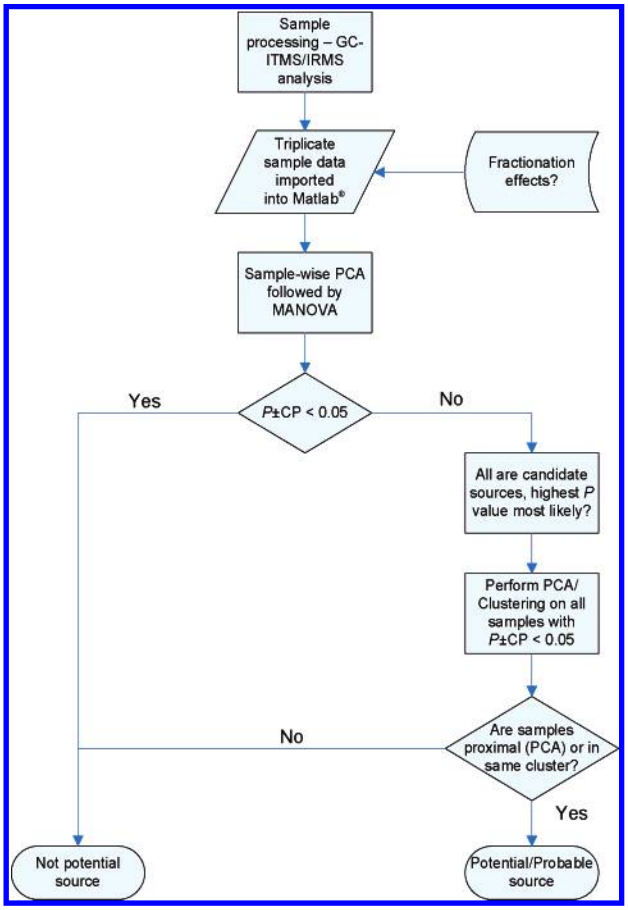

FIGURE 2. Statistical processing flowchart. Fractionation effects are addressed in the discussion section.

Fuel Spill in Elizabeth River. In developing this analysis, the U.S. Coast Guard expressed interest in coupling the capabilities of CSIA along with their current approach to identify potential spill sources after in-water releases. The Coast Guard findings for this spill were inconclusive. Several

TABLE 3. Results of PCA−MANOVA Significance Tests for Norfolk Spill Samples

sample 1	sample 2	P	capture %	±
Arapano	spill Metro Machine	0.114	98.2	0.00204
Bay Eagle	spill Metro Machine	0.178	98.7	0.00233
Bay Hawk D/T	spill Metro Machine	0.200	98.7	0.00262
Bay Hawk P&S tanks	spill Metro Machine	0.221	95.3	0.01039
Bay King	spill Metro Machine	0.929	96.1	0.03645
Mobil Facility	spill Metro Machine	0.130	96.4	0.00472
Seletar Hope	spill Metro Machine	0.134	90.5	0.01278
Arapano	spill at Waterside	0.082	99.1	0.00075
Bay Eagle	spill at Waterside	0.330	99.3	0.00234
Bay Hawk D/T	spill at Waterside	0.330	99.3	0.00219
Bay Hawk P&S tanks	spill at Waterside	0.197	99.0	0.00203
Bay King	spill at Waterside	0.165	99.0	0.00160
Mobil Facility	spill at Waterside	0.280	94.4	0.01569
Seletar Hope	spill at Waterside	0.162	97.5	0.00407
Arapano	sheen at Berkeley Bridge	0.015	99.1	0.00014
Bay Eagle	sheen at Berkeley Bridge	0.076	96.7	0.00251
Bay Hawk D/T	sheen at Berkeley Bridge	0.111	97.0	0.00333
Bay Hawk P&S Tanks	sheen at Berkeley Bridge	0.006	98.5	0.00009
Bay King	sheen at Berkeley Bridge	0.001	98.4	0.00002
Mobil Facility	sheen at Berkeley Bridge	0.022	99.2	0.00017
Seletar Hope	sheen at Berkeley Bridge	0.008	99.2	0.00006

[a] Results reflect those samples having *P* values above 0.05 for at least two of the three spill samples. C11 − C17 hydrocarbons were used for this analysis.

tug fuel samples were discounted by virtue of being a different fuel type (*Alert Bilge* and *Sea Star*) while most other samples were found to be light fuel oils with characteristics similar to the spill samples (*32*, *33*). Our statistical analysis started with performing pairwise PCA−MANOVAs for all samples. This amounted to 172 individual tests. *P* values ranged from 0.00094 to 0.93 while the percent capture of variability ranged from 91 to 100%. The resulting probabilities were screened for possible matches using the *P* values indicative of no significant difference (see Figure 2). By sorting the higher *P* values for each spill source, the original 16 suspect sources were reduced to 7, all having at least two out of three comparisons to the three spill sources with no significant difference (i.e., $P < 0.05$) (Table 3). Interestingly, the *Sheen* at Berkeley Bridge was found to be statistically different to both the spill at waterside and the spill at Metro Machine (which were, in turn, found to be statistically the same). The Coast Guard analysis classified all spill samples as slightly weathered. Although there is little evidence to suggest that hydrocarbon in the range used for this study fractionate isotopically during weathering (*19*, *34*), perhaps if the spills were all derived from a common source, some might have occurred. Another explanation is that the *Sheen* at Berkeley Bridge was the result of a separate release. Even though the isotope values for this sample were significantly different than those for the other spill samples, for purposes of this statistical comparison, the *Sheen* at Berkeley Bridge was treated as a source of the original spill.

The putative matches from the PCA−MANOVA analysis were then included in standard PCA (Figure 3A), and hierarchical clustering analyses to assess relatedness (Figure 3B). PCA analysis yielded 71% of variance captured in the first principal component. Twelve percent of initial sample variance was captured in the second principal component and almost 9% in the third component. The spill at Metro Machine and the spill at waterside were both positively loaded with respect to component one, and the *Sheen* at Berkeley Bridge was negatively loaded. The spill at *Metro Machine* was positively loaded with respect to component two while the other spill sources were negatively loaded. *Arapano*, *Bay Hawk* P&S (port and starboard) tanks, and the *Seletar Hope* showed the closest proximity to the spill at Metro Machine and spill at waterside sources in terms of both components, while *Bay Hawk* D/T (day tanks) was centrally loaded between all spill sources (Figure 3A). Based on the PCA analysis, it appears that the *Arapano*, *Bay Hawk* P&S Tanks, the *Seletar Hope,* and *Bay Hawk* D/T are the closest matches. The variability captured in the first two principal components placed *Mobile Facility*, the *Bay King* ,and the *Bay* Eagle a bit further from the sources. While the *Arapano*, the *Bay Hawk* P&S Tanks, and the *Seletar Hope* showed the greatest degree of similarity in PCA loadings to at least two of the spill sources, the PCA−MANOVA analysis indicated that these tugs were significantly different from the *Sheen* at Berkely Bridge (Table 3). The *Bay Eagle* and *Bay Hawk* D/T were the only samples showing no statistical difference for all three spill sources.

For the clustering analysis, the *Bay King* clustered with both the spill at Metro Machine and the spill at waterside. This cluster was in turn clustered with the *Bay Eagle*, *Bay Hawk* D/T, *Bay Hawk* P&S Tanks, and the *Arapano*. The distance of relatedness between the spill at Metro Machine and the spill at waterside and the tugs closest to the *y*-axis is remarkably similar as the horizontal line connecting the spill samples to the tug clusters is only about 0.1 units on the *y*-axis (Figure 3B). This indicates that while these tugs have various relations with each other, they all have virtually the same relation to the spill at waterside. *Mobile Facility* and the *Setelar Hope* are clustered together and only have about 0.1 unit of distance in relation to between the spill at Metro Machine and the spill at waterside. The *Sheen* at Berkely Bridge is the most distinct and shows very distant relation to both the other spills and the tugs used in this analysis.

The multivariate statistical approaches used for this forensic approach (Figure 2) include the ability to assess statistical differences in the collective means of the variables (PCA−MANOVA), the variability in the data set (PCA), and the statistical relation of samples (hierarchical clustering). Piecing together the results of all three multivariate analyses, one could make a strong case for the *Bay Hawk* D/T or *Bay Eagle* based on the fact that these were the only samples that showed no significant difference to all three of the spill sources. These samples also showed a great deal of related-

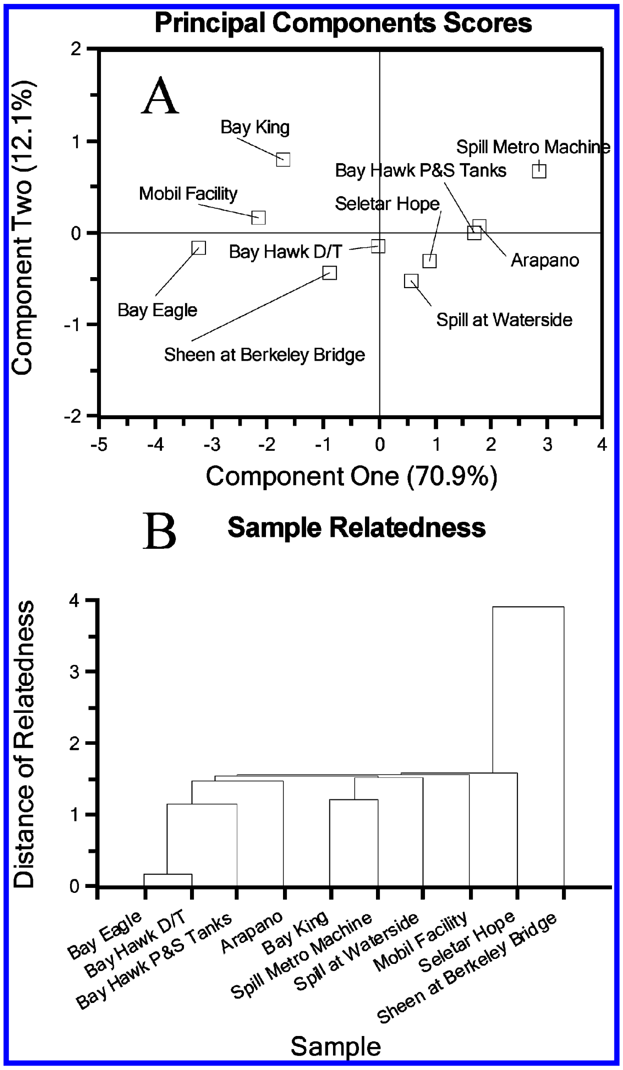

FIGURE 3. PCA (A) and hierarchical cluster (B) analysis of selected Norfolk fuel spill samples.

ness to one another based on the clustering analysis (Figure 3B). The *Bay King* was clustered most closely to the spill at Metro Machine and the spill at waterside (Figure 3B), but was negatively loaded with respect to component one in the PCA analysis, whereas each of these two spill sources was positively loaded (Figure 3B). The *Bay King* also showed the highest P value (0.93) for relation to the spill at Metro Machine, indicating that the samples were virtually the same. However, the *Bay King* was significantly different from the *Sheen* at Berkeley Bridge ($P = 0.001$); if all of the spill sources are considered to be the same, the *Bay King* fails to remain a candidate. If we make the assumption that all three spill samples represent the same source, we would conclude that the best possible match for the leaking tug is either the *Bay Hawk* D/T or the *Bay Eagle*. If we consider weathering a possibility (in terms of changing the isotope ratios), we would include the *Bay King* a strong candidate for the source of the spill.

Fractionation Analysis. Isotopic fractionation due to biodegradation, volatilization and weathering is a concern when attempting to use CSIA to source-apportion hydrocarbons. Many compounds undergo isotopic shifts during these processes and the observation of a predictable fractionation is often used as evidence for biodegradation or other remediation process (see refs *13*, *16*, *35* for recent reviews). In order to use a particular hydrocarbon mixture for source-apportionment, it is critical to understand what processes may introduce fractionation and the degree to which fractionation (if any) occurs. A controlled experiment was conducted by Mansuy et al. (*36*) in which fuel oil hydrocarbons were artificially weathered by evaporation (4 yr), water washing (2 months), and biodegradation (4 months of mixture with sewage sludge). These studies showed that *n*-alkane fractionation due to weathering processes was generally on the order of analytical precision for CSIA and should provide minimal interference in source-apportioning *n*-alkane hydrocarbons using the described method. In another biodegradation assay, no detectable fractionation was observed for *n*-alkanes (*18*). Therefore, we would expect there to be very little fractionation in the samples analyzed for this study. However, if fractionation factor(s) were known for components of a hydrocarbon mixture, measured isotope ratios could be altered mathematically to the pre-fractionated value(s). For instance, if anaerobic benzene biodegradation was known to cause a −3‰ shift in δ^{13}C value in the remaining source pool (cf., ref *35*), one could correct for this and alter the measured source value. Under this scenario, a measured benzene δ^{13}C of −32.0‰ would be corrected to −29.0‰ when apportioning source(s).

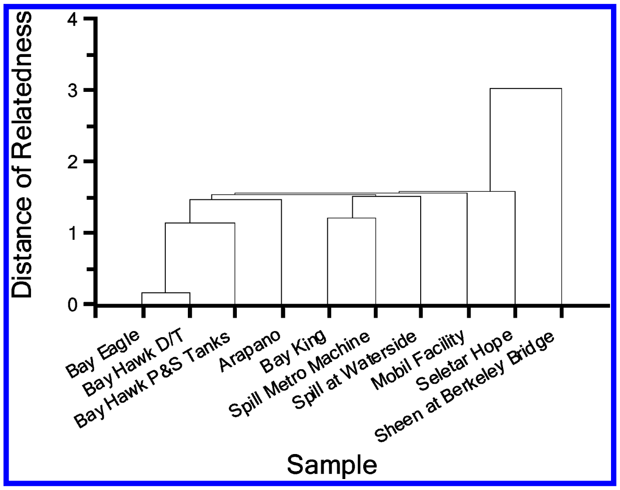

FIGURE 4. Cluster analysis of corrected Norfolk fuel spill samples.

In the present study with fuel oil spill samples, we determined that the *Sheen* at Berkeley Bridge sample was significantly different from the other two spill samples, spill at Metro Machine and spill at waterside. The U.S. Coast Guard analysis suggested that all of these potential source samples were from the same type of fuel oil, but that some weathering did occur before sample collection. Because the *Sheen* at Berkeley Bridge represented the most diffuse and most weathered source in this study, an attempt was made to mathematically correct the measured values with small shifts in isotope values. A figure in Mansuy et al. (*36*) measured the carbon isotope values of unweathered and weathered fuel oils. From their figure (Figure 4), we averaged the small shifts in isotope values from two sources of weathered and unweathered oils using the scale presented. Although our estimates are based soley on the figure, we used the following shifts to "correct" putative weathering in the *Sheen* at Berkeley Bridge sample: C_{12}−0, C_{13}−+0.625, C_{14}−+0.575, C_{15}−+0.325, C_{16}−+0.3, C_{17}−+0.5. While these correction factors are very close to the analytical precision of the IRMS (±0.3‰), they were used to demonstrate the proof of concept.

Once the values were corrected, statistical tests were rerun to determine if new significant differences were obtained for PCA−MANOVA tests, or different relationships evolved from hierarchical clustering analyses. Because PCA assesses variability, there should be no differences when correcting by the addition of constants. Using the putative correction values, a re-run of the PCA−MANOVA test showed the *Alert*

TABLE IV.[a] Results of PCA−MANOVA Significance Tests for Weathering-Corrected Norfolk Spill Samples[a]

sample 1	sample 2	P	capture %	±
"uncorrected values"				
spill Metro Machine	Sheen at Berkeley Bridge	0.004	99.2	0.00003
spill at waterside	Sheen at Berkeley Bridge	0.006	99.6	0.00002
Alert D/T	Sheen at Berkeley Bridge	0.047	95.5	0.00210
Bay Eagle	Sheen at Berkeley Bridge	0.076	96.7	0.00251
Bay Hawk D/T	Sheen at Berkeley Bridge	0.111	97.0	0.00333
	"corrected values"			
spill Metro Machine	Sheen at Berkeley Bridge	0.006	98.8	0.00007
spill at Waterside	Sheen at Berkeley Bridge	0.009	99.5	0.00005
Alert D/T	Sheen at Berkeley Bridge	0.083	95.9	0.00339
Bay Eagle	Sheen at Berkeley Bridge	0.111	95.8	0.00463
Bay Hawk D/T	Sheen at Berkeley Bridge	0.160	96.2	0.00609

[a] Results reflect C11 − C17 hydrocarbons.

D/T no longer as a significantly different source than the *Sheen* at Berkeley Bridge ($P = 0.047$ uncorrected; $P = 0.083$ corrected) (Table 4). Although the *Sheen* at Berkeley Bridge was still significantly different from the other spill sources, the corrected P value for comparison to the spill at waterside approached the 1% significance level ($P = 0.009$). When the putative sources identified in the previous section were analyzed by hierarchical clustering with the weathering-corrected *Sheen* at Berkeley Bridge values, the distance of relatedness between the *Sheen* at Berkeley Bridge and other sample clusters is decreased by roughly one arbitrary unit of relatedness (Figure 3B and Figure 4). It is unclear whether the spill at Berkeley Bridge is from the same source spill; however, with modest isotopic corrections for weathering it becomes more related to the other two spill sources and to tank samples from local tugs. Although fractionation of *n*-alkanes due to weathering has been shown to be minimal and essentially within analytical precision of IRMS instrumentation, if fractionation factors are known, correction could be applied before evaluating putative sources with unknown source samples (i.e., spills). This could be done in the initial stages of the proposed source comparison protocol (Figure 2).

Refinements and Applicability. In this work, we utilized existing data sets of stable isotopes collected from a single type of fuel hydrocarbon (diesel). In the years since these data were generated, the science of GC−combustion−IRMS has progressed significantly. With increasing numbers of "certified" standards available from NIST and the Internation Atomic Energy Agency, it is now possible to accurately calibrate each run by co-injecting a certified standard, which is then subject to the same chromatographic and combustion processes as the experimental sample. This allows more accurate reporting of isotope ratios than direct injection of calibration gas standards into the IRMS source (cf., ref *31*). In addition, new software from instrument manufacturers (in our case, Thermo-electron) allows for more sophisticated algorithms for peak detection and background determination.

One factor which may lead to variability in individual component isotope measurements is the presence of an unresolved complex mixture (UCM) found in fuel samples. This high background signal can potentially cause difficulty when trying to separate desired compound isotope values from unresolved compounds. Commercial IRMS instrumentation software can account for a background signal by using the isotopic signal of the preceding nonpeak signal in a chromatogram. Newer software (for example, Thermo Finnigan IsoDAT NT) may provide additional flexibility in removing background signals, although there appears to be limited literature on the application of the different background subtraction algorithms. If the individual compound signal is large, relative to the UCM signal, initial sample cleanup has been shown to be not necessary (*36*). In highly weathered samples (microbial degradation in particular), pretreatment of the sample to minimize the relative contribution of the UCM may be necessary (*36*, *37*). The spill samples used in this work were likely only lightly weathered so no pretreatment was performed. However, the influence of the UCM appears to not have been adequately addressed in the literature. A reasonable follow-on study would be to investigate the ability of IRMS software to accurately account for the UCM by mixing highly weathered UCM with varying concentrations of isotopically known alkane standards. Because all of the sample carbon is combusted to CO_2 before IRMS analysis, per-carbon influences of each fraction could be accounted for by determining the relative peak area of UCM and standard, then calculating the true integrated value using previously reported methods (cf., ref *38*).

Current work in our laboratory is devoted to applying refined analytical techniques to other analytes such as BTEX, fatty acids, and humic material oxidation products. No matter how precise and accurate a data set is, no rigorous and robust statistical method previously existed for evaluating similarity among samples. Because the emphasis of this paper is to define such a statistical technique rather than to concentrate on analytical methodology or site-specific factors, we chose archived data sets processed with relatively antiquated software (by today's standards). In order for the processing routine to work best, the data must conform to the following general guidelines: (1) the data should be systematically comparable, i.e., referenced to the same standard for all replicate measurements. The data need not be referenced to a known calibration standard, only all referenced to the same calibration standard; (2) the data should be as precise as possible, with less than or equal to 1‰ variation between replicate measurements; and (3) at least triplicate measurements should be made as the first three PCs scores from pairwise PCA analysis generally contain greater than 90% ($n = 172$) of the original data variability. This allows for a robust application of MANOVA, giving the true probably of sample relation.

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Supporting Information Available

The first Microsoft Excel spreadsheet (Simulated Data tab) contains the artificial data used to test the statistical processing routine. Two sets of random $\delta^{13}C$ values were produced as described in the green-blocked text (random number generator). The second tab (Tanks) contains the compiled data from the Norfolk tank samples. Each sample has three replicate analyses (Rep in column heading). Values from $C_{10}-C_{17}$ alkanes are presented. The third tab (Norfolk Tugs) contains data from the spill and tug samples from Norfolk, VA. The format is similar to the Tank tab. These data are provided so readers may reproduce our simulation results. A plain text file is available for the Matlab m-file (function) which performs the PCA-MANOVA routine described in the paper. It was uploaded as text file due to selection limitations for uploading. It can be renamed to custom_manova_pca_rep.m and used within Matlab to run the statistical test(s) described herein. This material is available free of charge via the Internet at http://pubs.acs.org.

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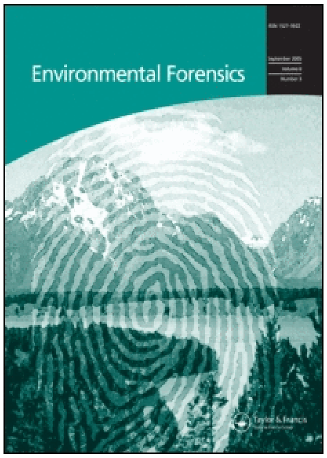

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Radiocarbon and Stable Carbon Isotope Analysis to Confirm Petroleum Natural Attenuation in the Vadose Zone

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Radiocarbon and Stable Carbon Isotope Analysis to Confirm Petroleum Natural Attenuation in the Vadose Zone

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CO_2 and CH_4 radiocarbon and stable carbon isotope ratios were used to assess natural attenuation at a fuel-contaminated soil site at the Norfolk Navy Base, Norfolk, VA (USA). Soil gas samples were collected spatially over a monitoring network in October 2002 and in March 2003. CO_2 and CH_4 from regions with high petroleum concentrations were ^{14}C-depleted relative to uncontaminated areas. ^{14}C-depleted methane suggested methanogenic hydrocarbon degradation. The difference in CO_2 age between background and plume-influenced areas indicated that approximately 90% of the CO_2 at the latter was petroleum derived, making contaminant the primary source of carbon for the microbial assemblage.

Keywords: natural attenuation, vadose zone, groundwater, carbon isotopes, carbon dioxide, methane, radiocarbon, stable carbon isotope analysis, soil gas, petroleum, methanogenesis

Introduction

Evaluating in situ biodegradation of organic contaminants requires an efficient, precise, and cost-effective monitoring strategy. Predicting environmental remediation timescales requires understanding contaminant turnover with respect to additional source introduction, contaminant transport and (bio)degradation. Multiple sources and their mixing may complicate remedial actions at many subsurface fuel-contaminated sites (Atmadja and Bagtzoglou, 2001; Macdonald and Kavanaugh, 1994; Marryott et al., 2002; Renner, 1998). Anthropogenic organic compound turnover depends on organic contaminant availability and inherent lability, nutrient availability, natural organic carbon concentrations, and seasonal physicochemical variability. Thus, defining the parameters necessary to substantiate natural attenuation is difficult and not without uncertainty (cf. Renner, 2000).

Stable carbon and radiocarbon isotope analysis ($\delta^{13}C$ and $\Delta^{14}C$, respectively) have been extensively used to assess basic biogeochemical roles in natural carbon cycling (Cherrier et al., 1999; Coffin et al., 1990; Druffel et al., 1992; Hullar et al., 1996; Peterson and Fry, 1987). More recently, stable carbon isotope analysis has been applied to identify contaminant carbon (Aggarwal and Hinchee, 1991) as well as biodegradation byproducts and residual contamination (Dempster et al., 1997; Lollar et al., 1999; Slater et al., 2001). Stable isotope techniques have also been applied to field settings by analyzing fractionation factors (Elsner et al., 2005; Lollar et al., 2001), addition of stable isotope labeled tracers (Fischer et al., 2006), and evaluating efficacy of active bioremediation strategies through monitoring production of contaminant-derived CO_2 (Mueller et al., 1995). In addition, as a biomarker approach, bacterial nucleic acid stable carbon isotopes were analyzed from beaches contaminated with oil from the Valdez oil spill to confirm bacterial hydrocarbon biodegradation in Prince William Sound, Alaska (Coffin et al., 1997). Aside from measuring fractionation factor(s) of residual contaminant pools, monitoring hydrocarbon biodegradation respiration products (e.g., CH_4 and CO_2) to confirm biodegradation may require the least analytical effort. This strategy has been applied to groundwater and vadose zone gases (Conrad et al., 1999).

However, definitive biodegradation confirmation may be hindered when contaminant and natural organic carbon stable isotope ratios overlap; for example, under aerobic conditions, when the CO_2 produced from biodegradation has "the same" isotopic ratios as both the contaminant and the background organic matter. In a system driven anaerobic by contaminant input, methane production yields ^{13}C-depleted CH_4 and ^{13}C-enriched CO_2 (Lollar et al., 2001). Without ancillary analyses, the similarity of ^{13}C content in atmospheric CO_2 ($\delta^{13}C$ approximately -7%) and CO_2 produced from organic matter degradation under methanogenic

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conditions may interfere with the ability of stable carbon isotope analysis to aid in confirming natural attenuation, particularly if one is trying to link the δ^{13}C value of CO_2 to the contaminant δ^{13}C value or cannot accurately estimate the atmospheric contribution to CO_2 in the vadose zone. Recent reviews provide considerable information on the application of stable isotope methods for observing and confirming contaminant biodegradation (Elsner et al., 2005; Meckenstock et al., 2004).

Clearly, when identification of hydrocarbon source(s) and fate(s) using stable isotope analyses is problematic due to an inability to distinguish between contaminant and natural organic carbon of overlapping isotope ranges, another strategy to confirm biodegradation must be undertaken. Natural abundance radiocarbon analysis is a complementary approach for tracing carbon assimilation and respiration through the microbial assemblage. ^{14}C-content can be used to distinguish between carbon sources of different age (Bauer et al., 1992, 1995, 1990; Cherrier et al., 1999). With a half-life of approximately 5700 years, petroleum sources are radiocarbon-free and thus provide a definitive end-member when analyzed against photosynthetic-based carbon sources, which contain modern CO_2 from the atmosphere. The percentage modern carbon (pMC) of living photosynthetic biomass is approxiamtely 110% because of nuclear bomb tests, which peaked before 1965 (Aelion et al., 1997). In contrast, petroleum carbon and CO_2 derived from its biodegradation have no ^{14}C and are thus 0 pMC. Given the two extreme end-members, one can readily differentiate the relative contributions of petroleum and plant biomass degradation in the total respired CO_2 pool.

Radiocarbon analysis of soil gas carbon dioxide was applied to study contaminant degradation and migration in the vadose zone of an aquifer contaminated with organic solvents (Suchomel et al., 1990). The results demonstrated distinct CO_2 radiocarbon signatures from control and contaminated sites. In a subsequent study, radiocarbon was used to investigate petroleum degradation in groundwater and soil gas (Conrad et al., 1997). In this study, definitive demonstration of petroleum biodegradation by stable carbon isotope analysis was hindered by CO_2 generated from methanogenesis. Radiocarbon analysis was able to confirm petroleum biodegradation. Radiocarbon was also used to survey groundwater contaminant degradation at a gasoline-contaminated site undergoing remediation with an air sparging/ soil vapor extraction system (Aelion et al., 1997). The CO_2 radiocarbon signature in the soil gas and groundwater-dissolved inorganic carbon suggested aerobic petroleum biodegradation produced 59–87% of the total CO_2. The approach was then applied to a chlorinated solvent plume wherein biodegradation was confirmed through the observation of the fossil end-member in soil gas CO_2 (Kirtland et al., 2003, 2005). These studies provide strong support for applying and coupling stable carbon and radiocarbon analyses to confirm and quantify natural attenuation.

This study focuses on the isotopic (^{13}C and ^{14}C) composition and concentration of CO_2 and CH_4 from the vadose zone of a site contaminated by fuel leakage at the Navy Ship Yard in Norfolk, VA, to evaluate petroleum natural attenuation in the soil and groundwater. The vadose zone is capped by an asphalt parking lot where 11 shallow and deep soil gas monitoring points (MPs) were installed. Recent evaluation of the petroleum plume was conducted by the Norfolk Navy Base to determine concentrations and transport rates. Data from late summer/early fall and late winter/ early spring sampling events are compared here. Our results support the application of CO_2 radiocarbon analysis to assess natural attenuation of contaminated soil and groundwater.

Methods and Materials

Site Description

The Norfolk Naval Station at Sewell's Point in Hampton Roads, VA, is the world's largest naval station and home port to 78 ships and 133 aircraft. There are 14 piers along a 4-mile waterfront, 15 aircraft hangars, and other military support facilities on the 3,400-acre area. Extensive refueling operations have and continue to release hydrocarbons into the subsurface environment at several locations. Groundwater flow adjacent to fuel plumes may leach soluble components and transport contaminants down gradient; in this case, towards the Elizabeth River, a tributary of the Chesapeake Bay. Fueling operations around the northwestern end of the base have released Bunker C and other fuels into the subsurface. A network of soil gas MPs was installed for monitoring petroleum concentrations in the soil and groundwater (Figure 1). Soil gas MPs 2, 3, and 11 are outside of the contaminated soil region and, therefore, were selected as control sites (Figure 1).

Vadose Zone Gas Sampling

The soil gas MPs are adjacent to those previously used by the base to survey the contaminated groundwater plume. Each MP had sampling ports at 72.6 (28.6 in) and 135 cm (53.2 in) below the soil surface (Figure 2). Vadose gas was collected using a 1-L gas-tight syringe after a 1-L purge and were transferred into Cali-5 1-L gas-tight bags (Calibrated Instruments, Inc., Hawthorne, NY, USA). Sampling was conducted in October 2002 and March 2003.

CO_2 Concentration

Carbon dioxide concentrations in the vadose gas samples were determined with a UIC Model 5011 coulometer (UIC, Inc., Joliet, IL, USA). The gas samples were injected into a UHP He stream (80 mL min^{-1}) at a rate 1 mL/ min^{-1} to avoid saturating the cathode solution of the coulometric cell. The percentage of carbon dioxide in the injected volume was calculated by multiplying the coulometer counts by a calibration factor determined with a 100% CO_2 standard (MG Industries, Malvern, PA, USA). Analytical precision of the standards and unknowns was $<1.5\%$ and $<3.0\%$, respectively, based on triplicate analysis.

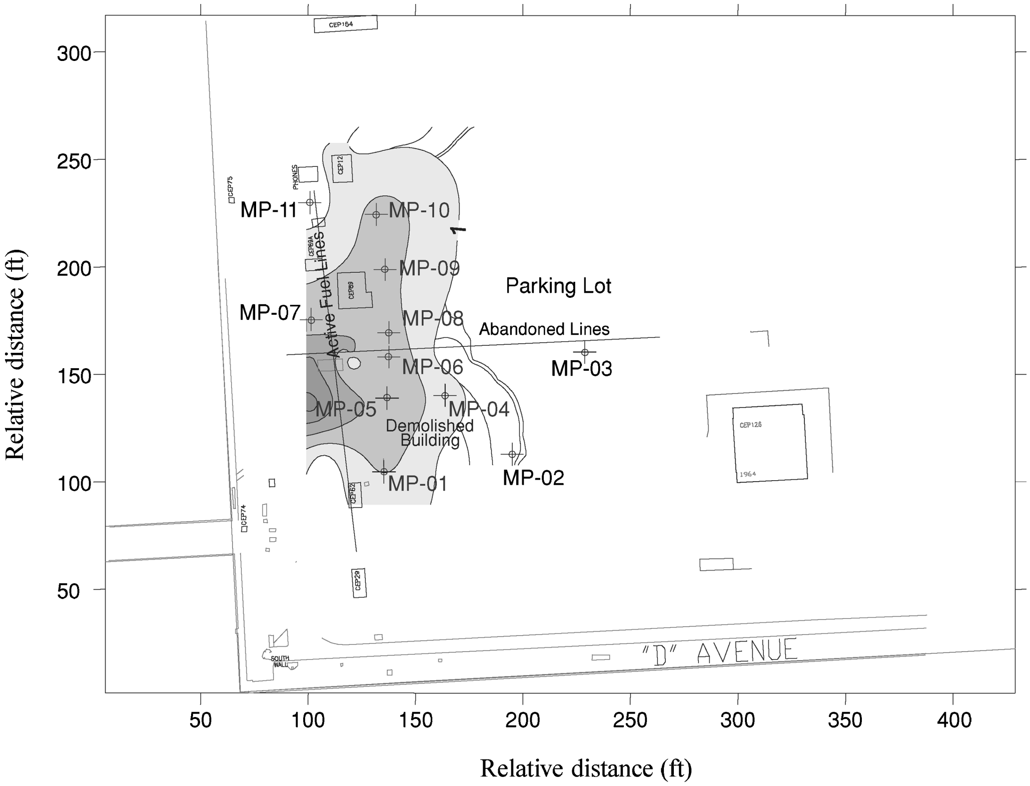

Figure 1. Soil gas monitoring point (MP) distribution. Shading represents petroleum contaminated soil regions. Points 2, 3, and 11 were used as control stations. Generalized groundwater flow is from right to left (east to west).

CH_4 Concentration

Methane concentrations were determined using a Shimadzu GC-14A gas chromatograph (GC) equipped with a flame ionization detector (FID). The gas was introduced through a manual gas-sampling valve and GC separation was achieved with a Poropak-Q column (8′ × 1/8″; Alltech Associates, Inc., Deerfield, IL, USA) at isothermal conditions (50°C). Methane concentrations were determined against certified gas standards (Scott Gas, Plumsteadville, PA, USA). Injection volumes ranged

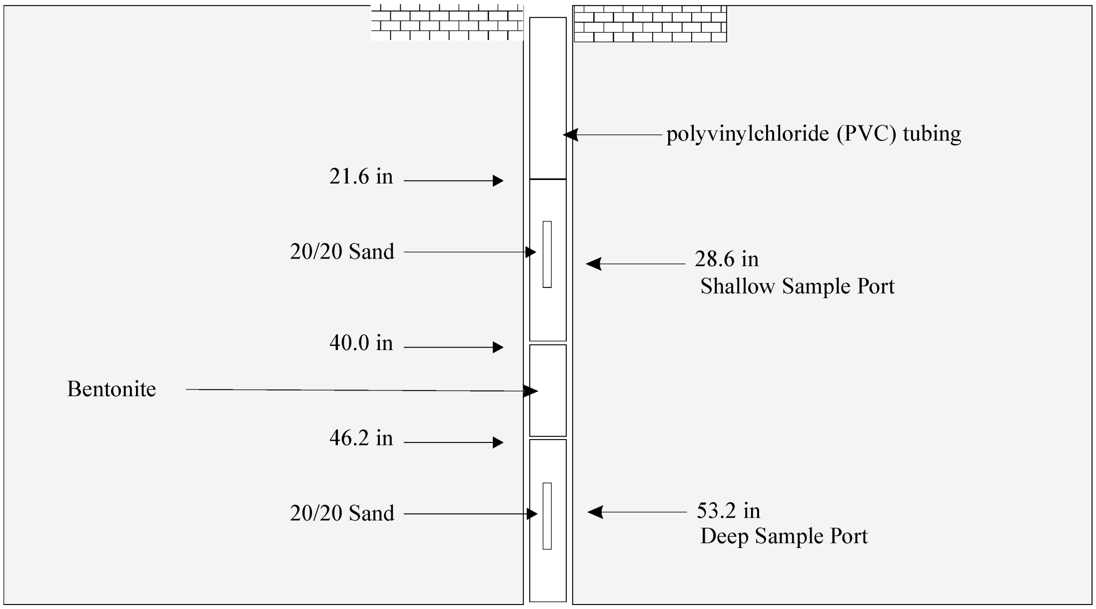

Figure 2. Soil gas monitoring point (MP) and sample port design.

from 2 to 10 mL of gas. Analytical precision from 3-mL standard injections was 0.2%.

Stable Carbon Isotope Analysis

Stable carbon isotope analysis was conducted on methane and CO_2 from the vadose zone. Sample volumes ranging between 0.5–10 μL were injected into a Trace GC Ultra (Thermo Electron, Waltham, MA, USA) containing a PoraPLOT Q 25 m × 0.32 μm capillary column (Varian, Inc., Palo Alto, CA, USA). Runs were isothermal at 50°C. Separated analytes (CH_4 and CO_2) were run through a Thermo-Finnigan GC Combustion III (Thermo-Fisher Scientific, Waltham, MA) interface and transferred to a Finnigan Delta Plus XP (Thermo-Fisher Scientific, Waltham, MA) isotope ratio mass spectrometer. Calibration was performed by co-injection of a laboratory standard CO_2, which in turn was calibrated against NBS-22 (National Institute of Standards and Technology [NIST, Gaithersburg, MD]). Stable carbon isotope ratios were expressed as:

$$\delta^{13}\mathrm{C} = \left[\frac{\mathrm{Rs}}{\mathrm{Rstd}} - 1\right] \times 1000\ (‰) \tag{1}$$

where δ^{13}C is the carbon isotope ratio, R_s is the $^{13}C/^{12}C$ for the unknown, and R_{std} is the $^{13}C/^{12}C$ for standard Pee Dee Belemnite (PDB). The detection limit was approximately 1 mg C, and the precision was ±0.3‰, based on triplicate measurements.

Radiocarbon Analysis

Gas samples for radiocarbon analysis of CO_2 and CH_4 were injected into an oxygen-enriched He carrier stream and cryogenically separated with a series of stainless steel loops immersed in dry ice/ethanol and liquid nitrogen. Methane was oxidized on-line to CO_2 over alumina pellets at 650°C and purified by cryogenic distillation. The CO_2 from each trap was cryogenically transferred to reactors and converted to graphite by iron catalyzed reduction with hydrogen (Pohlman et al., 2000; Vogel et al., 1987). The graphite was pressed into aluminum targets and analyzed with a 3-MV Pelletron tandem accelerator mass spectrometer with an multi-cathode source of negative ions by cesium sputtering MC-SNICS ion source (Grabowski et al., 2000). Radiocarbon values are reported as pMC and as the $D^{14}C$ notation relative to the NIST oxalic acid II standard (Stuiver and Polach, 1977).

Results

CO_2 and CH_4 Concentrations

CO_2 and CH_4 concentrations from the deep and surface soil gas MPs were generally not significantly different ($P < 0.05$), although some exceptions were observed (Table 1). The similarity in the vertical gas concentrations was likely a result of the asphalt cover over the contaminated zone, restricting exchange with atmospheric CO_2. In October 2002, the percentage CO_2 in vadose zone samples ranged from 4.6 to 23.2% (Table 1). The lowest values were found at control MPs 2 and 3 located at the upstream edge of the groundwater sampling region and control MP 11 at the down stream contaminant plume boundary (Figure 1). Methane was a substantially higher percentage of the vadose zone gas, with a range from non-detect to 60% (Table 1). In March 2003, CO_2 concentrations were substantially lower (0.1–17.6%). CH_4 concentrations were lower at MPs 1 and 10 and higher at MP 6, but otherwise similar to October (0.0–60%). In March, samples from MPs 2, 3, 10, and 11 had the lowest concentrations of both gases. Seasonal variation was observed in the vadose zone for MPs outside the contaminant plume, with CO_2 concentrations lower in March 2003. During both samplings the percentage CO_2 relative to percentage CH_4 was more evenly distributed than seasonal concentrations.

MPs 2, 3, and 11 were selected as control sites for this study based on previous groundwater contaminant distribution surveys conducted by the Navy base (Figure 1) and CO_2 and CH_4 concentrations determined during this study. CO_2 and CH_4 concentrations in control MPs were lower than the contaminated wells during both samplings. CH_4 in the control points was below detection (Table 1).

$\delta^{13}C$ of CO_2 and CH_4

$\delta^{13}CO_2$ ranged from −30.9 to 22.7‰ in October 2002 and from −27.3 to 9.99‰ in March 2003. Methane δ^{13}C ranged from–42.09 to −37.43‰ in October and −44.19 to −25.52‰ in March (Table 1). For soil gas stations over the plume, where there was detectable methane, CO_2 was generally enriched in ^{13}C. CO_2 was also ^{13}C-enriched in October relative to March (Table 1).

Percent Modern Carbon of CO_2 and CH_4

In October 2002, CO_2 pMC ranged from 0 to 24.9 (Table 1). A similar range was observed for March 2003 with values from 1.5 to 27.8 pMC. There was variation in values observed for each individual MP between the two samplings. For both sampling events, lower CO_2 concentrations coincided with a higher pMC (Table 1). CO_2 radiocarbon values at the control sites from both samplings were enriched in ^{14}C relative to plume values; however, control soil gas CO_2 was only 30 pMC or approximately 10,000 years old. Methane pMC for March was in a similar range of the values as vadose zone CO_2 at contaminated MPs, although it was never greater than 1.7 pMC. The CH_4 concentrations at the control sites were not high enough to measure radiocarbon.

Discussion

Gas Concentrations

The percentage CO_2 and CH_4 in October 2002 and March 2003 provides petroleum biodegradation evidence in the contaminated regions of the study site because concentrations were elevated relative to background values. CO_2 concentration in the vadose zone is a function of microbial respiration, plant root

Table 1. *Vadose zone data collected at the Norfolk Navy Base, Norfolk VA during October 2002 and March 2003. The standard deviations for percent carbon dioxide and methane and stable carbon isotope ratios of carbon dioxide and methane are calculated from 3 replicate runs. The standard deviation for pMC for CO_2 and CH_4 averaged 0.22 and 0.42, respectively. Not determined (ND) represents either no analysis was made (i.e. October 2002 methane) or gas concentrations below ~5% of soil gas which provided too little sample to obtain stable carbon or radiocarbon isotopes.*

	% CO_2	% CH_4	$\delta^{13}C$ CO_2	$\delta^{13}C$ CH_4	pMC CO_2	pMC CH_4
October 2002 Wells						
1S	19.6 ± 0.05	44.6 ± 0.88	8.36 ± 0.56	−37.4 ± 0.29	1.25	ND
1D	22.3 ± 0.12	52.9 ± 0.49	16.7 ± 0.24	−39.1 ± 0.71	−0.13	ND
2S	13.0 ± 0.17	0.05 ± 1.27	−30.9 ± 0.23	ND	ND	ND
2D	12.5 ± 0.13	0.00 ± 0.00	−30.3 ± 0.10	ND	ND	ND
3S	4.60 ± 0.25	0.00 ± 0.00	−22.3 ± 0.20	ND	13.8	ND
5S	22.6 ± 0.31	56.5 ± 5.39	22.7 ± 0.20	−40.7 ± 0.37	−0.20	ND
5D	23.2 ± 0.53	59.9 ± 0.87	22.7 ± 0.46	−40.3 ± 0.14	0.30	ND
6S	18.1 ± 0.39	18.4 ± 5.13	13.6 ± 1.90	−40.4 ± 0.16	1.56	ND
7S	19.5 ± 0.06	43.5 ± 5.04	6.84 ± 0.33	−41.8 ± 0.06	1.92	ND
8S	22.1 ± 0.25	56.2 ± 3.86	18.4 ± 0.35	−41.7 ± 0.58	0.90	ND
8D	22.5 ± 0.54	58.0 ± 4.05	19.7 ± 0.60	−42.5 ± 0.42	0.60	ND
10S	17.2 ± 0.31	50.6 ± 1.46	7.37 ± 0.19	−42.1 ± 0.44	2.67	ND
10D	18.1 ± 0.19	60.5 ± 1.89	20.6 ± 0.60	−42.1 ± 1.45	2.08	ND
11S	6.91 ± 0.34	0.08 ± 0.00	−28.7 ± 0.80	ND	22.7	ND
11D	7.04 ± 0.29	0.00 ± 0.00	−29.6 ± 0.39	ND	24.9	ND
March 2003 Wells						
1S	8.98 ± 0.02	21.30 ± 0.04	−20.7 ± 0.08	−25.5 ± 0.12	5.53	0.22
1D	14.50 ± 0.05	39.50 ± 0.08	−25.6 ± 0.30	−31.4 ± 0.13	1.90	1.52
2D	7.95 ± 0.06	0.00 ± 0.00	ND	ND	8.61	ND
3S	2.12 ± 0.03	0.01 ± 0.00	−13.2 ± 0.34	ND	27.8	ND
4D	11.70 ± 0.07	3.11 ± 0.00	ND	ND	4.09	1.62
5S	17.6 ± 0.05	59.2 ± 0.12	6.00 ± 0.43	−34.9 ± 0.11	ND	1.58
5D	17.40 ± 0.22	58.2 ± 0.12	−25.4 ± 0.32	−34.9 ±0.14	1.48	0.66
6S	16.30 ± 0.04	51.1 ± 0.10	−3.86 ± 0.23	−36.9 ± 0.18	ND	0.68
7S	11.70 ± 0.12	36.9 ± 0.07	−4.48 ± 0.29	−33.7 ± 0.33	3.48	0.41
7D	14.3 ± 0.10	44.1 ± 0.09	ND	ND	2.56	0.85
8S	16.00 ± 0.01	54.1 ± 0.11	2.19 ± 0.08	−38.8 ± 0.22	1.73	0.98
8D	16.70 ± 0.14	53.6 ± 0.11	2.57 ± 0.22	−39.1 ± 0.26	1.69	1.48
9S	11.60 ± 0.02	22.4 ± 0.04	−6.67 ± 0.45	−39.5 ± 0.04	3.63	1.24
9D	11.80 ± 7.72	21.8 ± 0.04	−5.61 ± 0.83	39.9 ± 0.04	2.77	0.98
10S	7.72 ± 0.07	6.65 ± 0.01	9.99 ± 0.32	−44.2 ± 0.11	2.68	1.43
10D	0.11 ± 0.00	0.00 ± 0.00	ND	ND	ND	ND
11S	1.26 ± 0.02	0.06 ± 0.00	ND	ND	ND	ND
11D	2.05 ± 0.01	0.00 ± 0.00	−27.3 ± 0.23	ND	14.52	ND

respiration, horizontal gas transport, and atmospheric CO_2 exchange. Because the study area was paved over, concentration equilibration with the atmosphere and CO_2 root respiration were likely minimal relative to other processes. The range in percentage CO_2 for the October and March samples (0.1–23.2%) was greater than another study where values ranged from 1–16% (Aelion et al., 1997). The higher percentage CO_2 measured at the Norfolk site may be a result of vadose zone gas trapping under the pavement or rapid petroleum biodegradation rates. There was a difference in the percentage CO_2 between October and March samplings (Table 1) with higher values observed in October. This difference was assumed to be seasonal. Microbial production and respiration rates were higher under warmer conditions before and at the time of the October sampling. CH_4 concentration in the soil gas was routinely in excess of 40% in contaminated region MPs during both samplings (Table 1). CH_4 was not detected in samples from the control MPs. There was a significant linear correlation ($P < 0.01$) between the percentage CO_2 and percentage CH_4 in the vadose zone with goodness of fit statistic (r^2) values of 0.93 for March (Figure 3). Although a significant correlation was not evident for October, the highest percentage CO_2 and CH_4 values plotted in the same region of the graph (Figure 4).

Although CH_4 to CO_2 ratios were never as high as the literature methanogenic value of 4:1 (Anderson and Lovley, 2000), the ratios ranged from 2 to 3, which is indicative of hydrocarbon conversion to CH_4 and CO_2 under field conditions.

Isotope Analyses

$\delta^{13}C$ for CO_2 and CH_4 fit well with the trends observed in percentage CO_2 and percentage CH_4. At MPs with high percent CO_2 and ^{13}C-enriched CO_2 signatures, CH_4 was consistently depleted in ^{13}C (Figures 5 and 6). In October 2002, CO_2 was more ^{13}C-enriched in contaminated areas than in March (Figures 5 and 6). March 2003 had more ^{13}C-depleted values for both CO_2 and CH_4. These ^{13}C-depleted signatures correspond to lower percentage CO_2 and percentage CH_4 and suggested

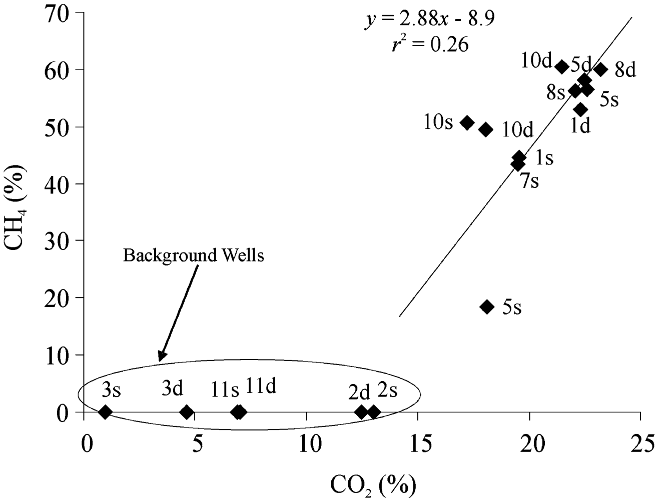

Figure 3. Vadose zone gas comparison with percentage CH_4 plotted relative to percentage CO_2 for the March 2003 sampling.

a lower microbial petroleum cycling rate (most likely due to lower temperature). Seasonal shifts in microbial metabolism rates in temperate aquatic environments are well documented (cf. Lomas et al., 2002). In MPs 2, 3, and 11, CH_4 concentrations were below the $\delta^{13}C$ limits of detection At these control sites the CO_2 was ^{13}C-depleted relative to the other sample MPs (Table 1). This depletion is consistent with aerobic hydrocarbon or soil organic matter degradation as the primary source(s) of respired soil CO_2, particularly so given the lack of significant root respiration or atmospheric CO_2 input. In other studies, vadose zone CO_2 was substantially depleted in ^{13}C relative to the data described here. For contaminated groundwater at a gas station in Columbia, South Carolina, the range for $\delta^{13}CO_2$ was -22.0 to -35.9‰ (Aelion et al., 1997). Similar results are observed for a PCB-contaminated Savannah River site, where the range for vadose zone CO_2 over the contaminated region was -26.8 to -21.1‰ (Kirtland et al., 2003). At these sites, the stable carbon isotope signature was determined to be a function of plant root respiration, atmospheric input, and microbial respiration.

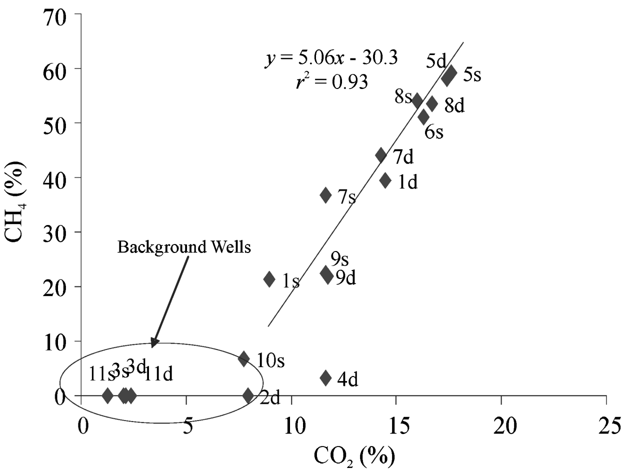

Figure 4. Vadose zone gas comparison with percentage CH_4 plotted relative to percentage CO_2 for the October 2002 sampling.

^{14}C isotope analysis can be used to differentiate between contemporary carbon respiration relative to the petroleum-derived carbon respiration. The technique is very sensitive because there is considerable analytical resolution between end-members (approximately 1200‰); for instance, carbon in atmospheric CO_2 is modern with pMC values up to 120 ($\Delta^{14}C = +200$‰) or even greater due to the influence of bomb-released ^{14}C, whereas carbon in fossil fuels is "radiocarbon dead" with a pMC of 0 ($\Delta^{14}C = -1000$‰). ^{14}C-depleted CO_2 and CH_4 occurred where CO_2 and CH_4 concentrations were elevated (Figure 7). For October 2002 and March 2003, CO_2 from the control MPs ranged from 8.6 to 27.8 pMC, which suggests isolation from atmospheric exchange and microbial degradation of aged plant material if one assumes there was no residual or lateral contributions from petroleum degradation.

Samples taken in March 2003 from MPs above the petroleum plume were more modern and variable with values ranging from 1.48 to 4.09 pMC compared with October 2002 when values were lower with pMC ranging from 0 to 2.06. This change in CO_2 pMC between the sampling events coincides with the variation in the vadose zone percentage CO_2. While the range in variation between the two sample dates was small, the differences were taken as evidence of slower microbial petroleum degradation in March.

Comparing CO_2 pMC and $\delta^{13}C$ reveals that ^{13}C-enriched values were coincident with depleted ^{14}C (Figure 8). In the control MPs (3 and 11), we observed lower $\delta^{13}C$ corresponding to greater pMC. Under aerobic biodegradation, one might expect incorporated carbon and $\delta^{13}CO_2$ to be similar to that of the hydrocarbon contaminant (cf. Pelz et al., 1998). In March 2003, MPs 1D and 5D had $\delta^{13}CO_2$ values in a range typical of fuel hydrocarbons (*cf.* Boyd et al., 2006; Mansuy et al., 1997), perhaps indicating aerobic hydrocarbon biodegradation (Figure 8). Fossil CO_2 with ^{13}C-enrichment indicates anaerobic hydrocarbon degradation under methanogenic conditions (see circled area in Figure 8) as preferential utilization of lighter CO_2 to produce CH_4 leaves a heavier residual CO_2 pool (*cf.* Anderson and Lovley, 2000; Kaplan et al., 1997).

CH_4 was consistently depleted in ^{14}C in the March 2003 sampling (Table 1). These data suggest that the CO_2 respired during petroleum degradation served as a terminal electron acceptor for methanogenesis. There were no clear trends (i.e., a significant regression model) when comparing radiocarbon content and stable carbon isotope values for CH_4 (Figure 9). The lightest $\delta^{13}CH_4$ value was greater than -45‰, whereas most values were between -40 and -30‰ (Table 1). These values were heavier than expected for biogenic methane production (*e.g.*, Kaplan et al., 1997) unless the CO_2 source signature was ^{13}C-enriched (Grossman et al., 2002). Because the site was covered by an asphalt parking lot and background CO_2 was low in radiocarbon (approximately 25 pMC), atmospheric exchange and root respiration are not likely to impact CO_2 age in the vadose zone. A greater likelihood is that the CO_2 source available

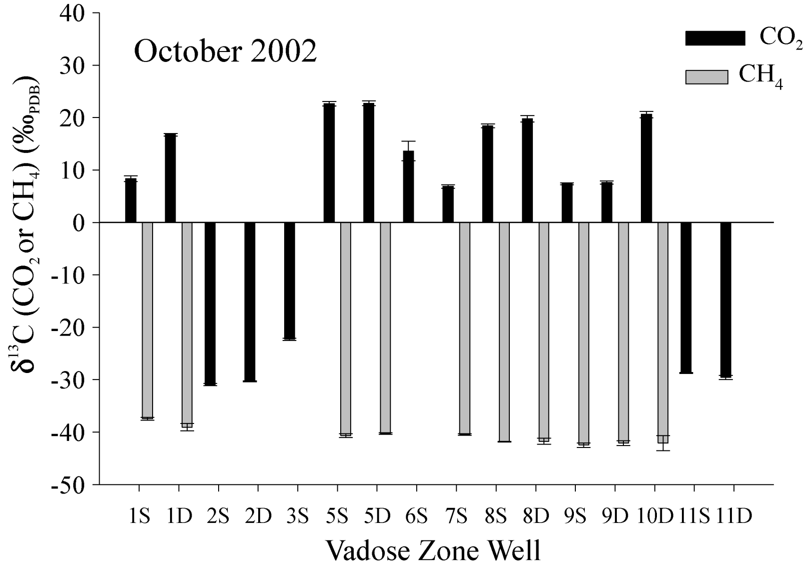

Figure 5. δ^{13}C of vadose zone CO_2 and CH_4 in October 2002.

on-site for methanogenesis is highly "recycled." Much of the CO_2 in the soil gas was ^{13}C-enriched (Table 1). As this CO_2 is converted to CH_4, the resultant $\delta^{13}CH_4$ values should be heavier than expected (e.g., Kaplan et al., 1997). This is further substantiated by the contrast of Norfolk vadose zone samples with those of other recently reported isotopic field studies. In the vadose zone of a petroleum-contaminated site in Columbia, SC, $\delta^{13}CO_2$ ranged from −35.9 to −22‰ (Aelion et al., 1997). At a TCE-contaminated site in Georgia $\delta^{13}CO_2$ in the vadose zone ranged from −26.8 to −21.1‰ (Kirtland et al., 2003). The ^{13}C-enriched CO_2 and CH_4 from this study likely indicated considerable carbon recycling in this "atmospherically closed" environment and simplified the interpretation of natural attenuation. Probably also due to the "closed" nature of the presently studied system, the range of pMC values (0.3 to 24.9) was ^{14}C-depleted relative to other studies (Aelion et al., 1997; Conrad et al., 1997; Kirtland et al., 2003, 2005). Others have reported pMC values for contaminated and uncontaminated vadose zones ranging from 14 to 128 pMC reflecting CO_2 input from the atmosphere and plant root respiration.

Biodegradation Quantification

A two-component radiocarbon mass balance approach was employed in an attempt to constrain the relative contaminant and natural organic matter contributions:

$$\Delta^{14}CO_2 = (\Delta^{14}C_{pet} \times f_{pet}) + [\Delta^{14}C_{NOM} \times (1 - f_{pet})] \quad (2)$$

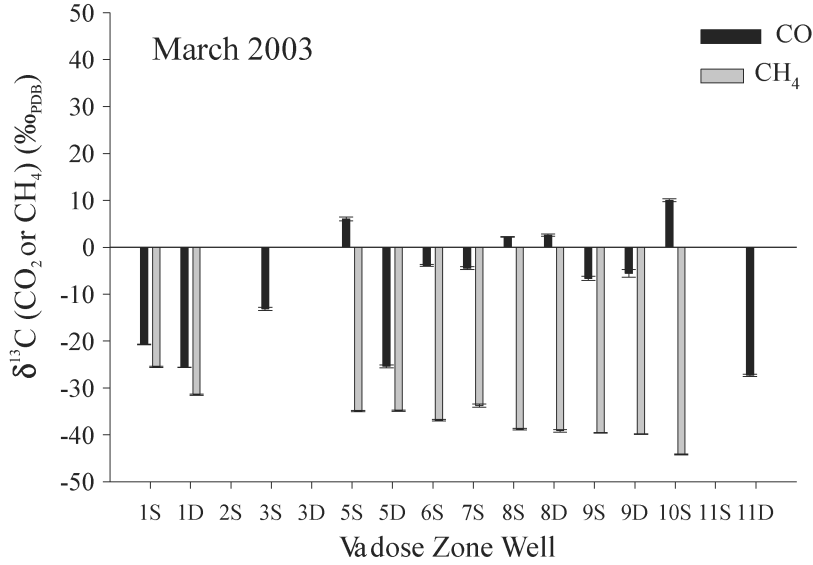

Figure 6. δ^{13}C of vadose zone CO_2 and CH_4 in March 2003.

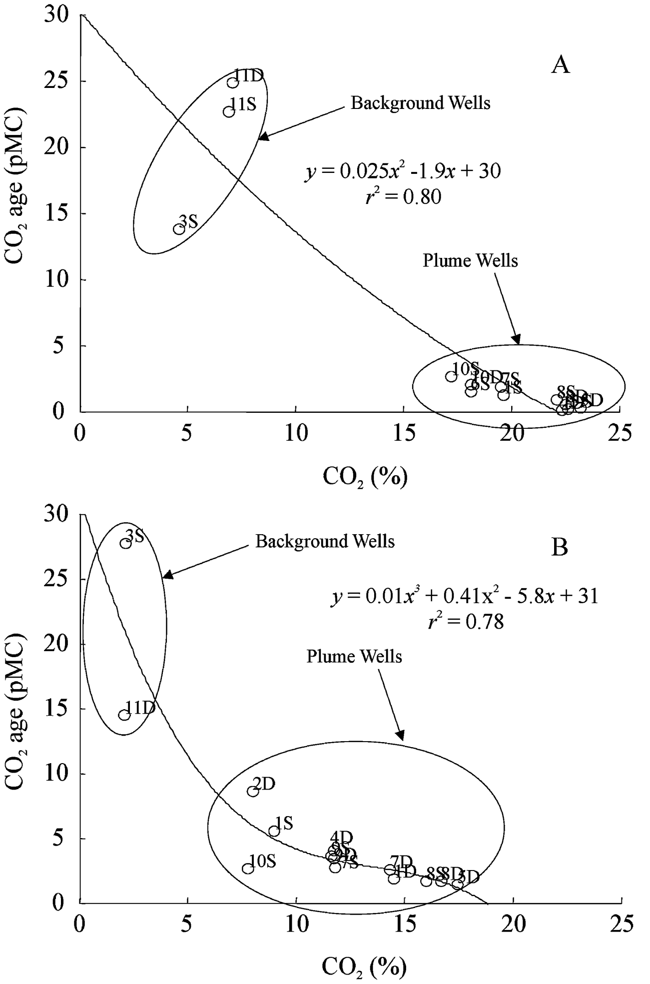

Figure 7. Percentage modern carbon (pMC) soil gas CO_2 relative percentage to CO_2 for A) October 2002 and B) March 2003.

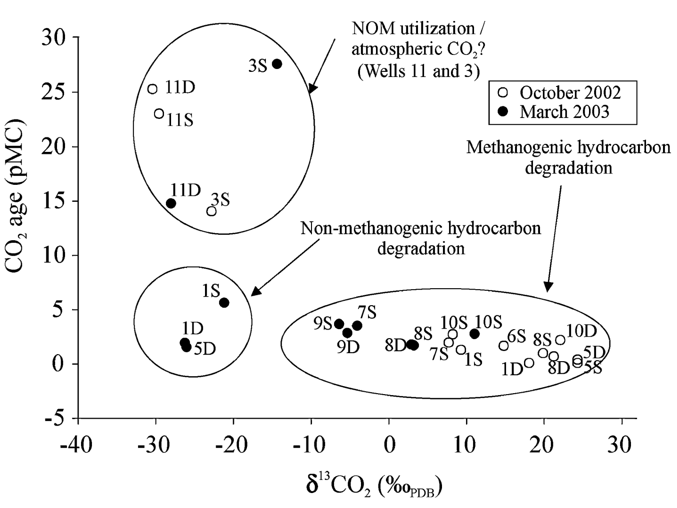

Figure 8. Percentage modern carbon (pMC) variation relative to the $\delta^{13}C$ in the vadose zone CO_2.

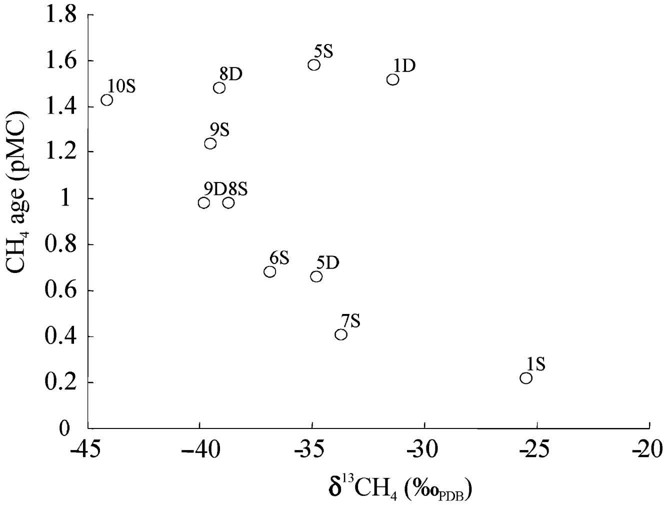

Figure 9. Comparison of radio and stable carbon isotope analysis of methane sampled from soil gas monitoring points (MPs). pMC, percentage modern carbon.

where $\Delta^{14}CO_2$, $\Delta^{14}C_{pet}$, $\Delta^{14}C_{NOM}$ are the soil gas CO_2, petroleum, and natural organic matter $\Delta^{14}C$ signatures, respectively, and f_{pet} is the fraction of CO_2 derived from petroleum. The $\Delta^{14}CO_2$ was measured directly (Table 1), the $\Delta^{14}C_{pet}$ was assumed to be −1000‰, and the $\Delta^{14}C_{NOM}$ was assumed to be represented by the average CO_2 $\Delta^{14}C$ from the control sites. The assumption that the CO_2 from the control sites accurately represents natural organic matter (NOM) $\Delta^{14}C$ no contaminant-derived CO_2 was present at those sites. If the average background radiocarbon CO_2 age (20.7 pMC) and the average plume CO_2 radiocarbon age (2.3 pMC) are converted to $\Delta^{14}C$ notation ($\Delta^{14}C = [pMC - 100] * 10$) and placed in the mass balance model, f_{pet} can be calculated as approximately 0.90, indicating that approximately 90% of the CO_2 found in vadose zone gas above the plume is derived from petroleum sources. Using the lowest background pMC value (8.6), the most conservative estimate of f_{pet} is 73%, still indicating that the majority of onsite soil gas CO_2 is derived from the petroleum end-member. This estimate is in line with the site in Columbia, SC, where a range from 58 to 86% was reported (Aelion et al., 1997). Although the overall CO_2 production rate is not currently known, its measurement would give a degradation rate for the hydrocarbon pool in the soil.

Conclusions

This study indicates that fuel hydrocarbons are a significant carbon source to in situ bacteria and that fuel hydrocarbon respiration resulted in the production of ^{14}C-depleted CO_2 (and ultimately CH_4) with stable carbon isotope ratios indicative of microbial methanogenesis. However, the most weathered samples indicated there is considerable recycling of carbon because $d^{13}C$ values for methane were uncharacteristically heavy (−45 to −26‰). The radiocarbon ages of CO_2 and CH_4 were definitive in showing that petroleum carbon makes up approximately 90%

of the carbon utilized by the in situ microbial assemblage. The radiocarbon measurement alone inconclusively demonstrates biodegradation is occurring on-site. Stable isotope ratios alone would likely fail to convey the importance of petroleum carbon to the CO_2 and CH_4 pools as considerable recycling led to relatively heavier than expected values (20–40‰). Coupling of radiocarbon and stable carbon isotope measurements allows a better understanding of onsite biogeochemical conditions (cf. Figure 8). These data provide strong support for the use of carbon isotope analyses to monitor natural attenuation and warrants more extensive seasonal and spatial sampling to better understand the dynamics of petroleum degradation under anoxic conditions. While sample analysis costs for radiocarbon are still relatively high (approximately $600 per sample), the installation of relatively inexpensive (approximately $150 each) soil gas MPs coupled with the results of stable isotope analyses may provide not only a definitive answer as to whether biodegradation is occurring but may provide considerable in situ biogeochemical information. Commercial laboratories are able to cryogenically separate CO_2 from "raw" soil gas samples (i.e., in Cali-5 bags). Methane analysis would likely add approximately $125 to the $600 per sample radiocarbon analysis. Again, though this seems high, several plume and background MPs could be installed, sampled, and analyzed for less than $5K, a fairly low cost to so definitely confirm onsite hydrocarbon biodegradation.

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