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AASERT-92 RESEARCH TRAINING FOR UNDERSTANDING THE FATE OF ENVIRONMENTAL POLLUTANTS

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6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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13. ABSTRACT *(Maximum 200 words)*

This award supported the training of two graduate students at Cornell University in their efforts to apply geochemical, physiological, and genetic approaches to understanding the fate of naphthalene in groundwater and sediments at a coal-tar contaminated field site. Three major scientific goals were accomplished. First, we extended methods and criterial for proving that environmental contaminants are actively being biodegraded in real time by using gas chromatography/mass spectrometry to detect a transient intermediate metabolite (1,2-dihydroxy-1,2-dihydronaphthalene) in site waters. Second, we applied molecular procedures to extend understanding of genetic processes that affect environmental contaminants by extracting DNA from site sediments, using PCR to amplify the naphthalene catabolic gene, and contrasting restriction digests of the site-and pure culture-derived genes. Third, using a combination of field and laboratory procedures, we tested several hypotheses for explaining the persistence of naphthalene in the field site. We concluded that in situ oxygen limitation and reduced bioavailability were the causes.

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FINAL REPORT

SUBMITTED TO:

U.S. AIR FORCE OFFICE OF SCIENTIFIC RESEARCH

DIRECTORATE OF CHEMISTRY AND LIFE SCIENCES

110 DUNCAN AVE., SUITE B-115

BOLLING AFB, DC 20322-6448

TO THE ATTENTION OF: DR. WALTER J. KOZUMBO

PROJECT OFFICER AT AFOSR

FOR AASERT GRANT #93-NO-073

ENTITLED

RESEARCH TRAINING FOR UNDERSTANDING THE FATE OF ENVIRONMENTAL POLLUTANTS

submitted by:

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Entire Grant period

July 1, 1993 - June 30, 1996

2.0 OBJECTIVES:

The objective was to support US citizens in acquiring expertise for understanding microbiological mechanisms that determine the fate of environmental pollutants. This was an AASERT augmentation grant tied to Dr. E. L. Madsen's AFOSR Grant #AFOSR-91-0436 "Geochemical, Genetic, and Physiological Control of Pollutant Biodegradation" (project period 30 March 1992 - 29 March 1995). The individuals supported by the AASERT grant were guided in their graduate curricula to gain knowledge in biochemistry, genetics, toxicology, microbiology, and environmental engineering by E. L. Madsen, a member of Cornell University's Graduate Fields of Microbiology and Environmental Toxicology.

3.0 STATUS OF EFFORT

The effort has been completed. Both this AASERT grant and its accompanying project have expired. By providing educational and research opportunities for American citizens (see below) at Cornell University, the goals were achieved. The list of peer reviewed publications (see below) attests to successful accomplishment of technical goals. We have applied molecular, geochemical, and microbiological methodologies to gain an understanding of processes that govern the environmental fate of pollutant compounds in contaminated sites.

4.0 ACCOMPLISHMENTS/NEW FINDINGS

Criteria for documenting *in situ* microbial metabolism of environmental pollutants have been established, tested, and extended. These criteria and the supporting methodologies are important for efforts by the AF and society at large in eliminating environmental pollutants. Of key importance is the fact that the studies carried out and reported here address intrinsic bioremediation, which may be the most widespread and economically feasible microbiological clean-up technology for contaminated sites.

5.0 PERSONNEL SUPPORTED

Associate Professor	Eugene L. Madsen	
Graduate Students	James B. Herrick	(Ph.D. January 1995, Microbiology; presently employed at Los Alamos National Laboratory)
	Robert L. Sandoli	(MS June 1994, Microbiology; Presently employed at Earth Tech, Inc.)
	Karen G. Stuart-Keil	(Ph.D. ≈ January 1998, Environmental Toxicology)
	Mark S. Wilson	(Ph.D. ≈ June 1997, Microbiology)
Undergraduate Assistant	Cynthia Mann	(presently enrolled in the toxicology graduate program at the University of North Carolina)

6.0 PUBLICATIONS

Madsen, E. L. 1996. Method for determining biodegradability. In: C.J. Hurst, *et al.* (ed.). Manual of Environmental Microbiology. American Society of Microbiology, Washington, DC. (In Press).

Madsen, E. L., C. L. Mann, and S. E. Bilotta. 1996. Oxygen limitations and aging as explanation for the persistence of naphthalene in coal-tar contaminated surface sediments. Environ. Toxicol. Chem. (**15**: In Press).

Madsen, E. L., C. T. Thomas, M. S. Wilson, R. L. Sandoli, and S. E. Bilotta. 1996. *In situ* dynamics of aromatic hydrocarbons (AHs) and bacteria capable of AH metabolism in a coal tar waste-contaminated field site. Environ. Sci. Technol. **30**:2412-2416.

Sandoli, R. S., W. C. Ghiorse, and E. L. Madsen. 1996. Regulation of microbial phenanthrene mineralization in sediments by sorbent-sorbate contact time, inoculum, and gamma irradiation-induced sterilization artifacts. Environ. Toxicol. Chem. (**15**:In Press).

Wilson, M. S., and E. L. Madsen. 1996. Field extraction of a unique intermediary metabolite indicative of real time *In situ* pollutant biodegradation. Environ. Sci. Technol. **30**:2099-2103.

Madsen, E. L. 1995. Impacts of agricultural practices on subsurface microbial ecology. In: D. Sparks (ed.). Advances in Agronomy Vol. 54, pp. 1-67.

Ghiorse, W. C., J. B. Herrick, R. L. Sandoli, and E. L. Madsen. 1995. Natural selection of PAH-degrading bacterial guilds at coal tar disposal sites. Environmental Health Perspectives **103** (Suppl. 5):107-111.

Moré, M. I., J. B. Herrick, M. C. Silva, W. C. Ghiorse, and E. L. Madsen. 1994. Quantitative cell lysis of indigenous microorganisms and rapid extraction of microbial DNA from sediment. Appl. Environ. Microbiol. **60**:1572-1580.

Herrick, J. B., E. L. Madsen, C. A. Batt, and W. C. Ghiorse. 1993. Polymerase chain reaction amplification of naphthalene catabolic and 16S rRNA gene sequences from indigenous sediment bacteria. Appl. Environ. Microbiol. **59**:687-694.

Madsen, E. L. and W. C. Ghiorse. 1993. Ground Water Microbiology: Subsurface ecosystem processes. pp. 167-213. In: Ford, T. (ed.) Aquatic Microbiology: An Ecological Approach. Blackwell Scientific Publications, Cambridge, MA.

National Research Council. 1993. *In situ* Bioremediation: When Does It Work? B. E. Rittmann, J. M. Tiedje, R. Brown (Executive Committee); E. L. Madsen (Rapporteur). National Academy Press, Washington, DC.

7.0 INTERACTIVE/TRANSITIONS

A. Participation at Meetings:

Wilson, M. S., and E. L. Madsen. 1995. Recovery of 1,2-dihydroxy-1,2-dihydronaphthalene from a contaminated field site indicates *in situ* naphthalene biodegradation. Abstract, Amer. Soc. Microbiol. Annu. Meet., Washington, DC. Q126, p. 421.

Wilson, M. W., J. B. Herrick, and E. L. Madsen. 1995. Analysis of intermediary metabolites from diverse bacteria indicate a highly conserved pathway for naphthalene catabolism. Abstract, Amer. Soc. Microbiol. Annu. Meet., Washington, DC. Q-411, p. 472.

Madsen, E. L., J. B. Herrick, M. S. Wilson, K. Stuart, R. S. Sandoli, and W. C. Ghiorse. 1995. Molecular and biochemical microbial ecology of a coal tar-contaminated waste site undergoing intrinsic bioremediation. Abstract, the Third International Bioreclamation Symposium, San Diego, CA.

Herrick, J. B., D. E. Hinman, K. G. Stuart, E. L. Madsen, and W. C. Ghiorse. 1994. Genetic and taxonomic variation in naphthalene catabolic bacterial populations native to coal tar waste-contaminated site. Abstract, Amer. Soc. Microbiol. Annu. Meet., Anaheim CA, LasVegas, NV. N-183, p. 348.

Madsen, E. L., C. T. Thomas, M. S. Wilson, and R. L. Sandoli. 1994. Mobility of polycyclic aromatic hydrocarbon (PAHs) and bacteria capable of PAH metabolism in a coal-tar contaminated field site. Abstract, Amer. Soc. Microbiol. Annu. Meet., Anaheim CA, LasVegas, NV. Q-135, p. 411.

Madsen, E. L., S. Bilotta-Best, R.L. Sandoli, L.W. Lion, and W.C. Ghiorse. 1993. Final Electron Acceptor Limitations and Sorption May Explain the Persistence of Polycyclic Aromatic Hydrocarbons in Coal-Tar Contaminated Surface Sediments. Abstract, Amer. Soc. Microbiol. Annu. Meet., Atlanta, GA.

Sandoli, R. L., E. L. Madsen, and W. C. Ghiorse. 1993. Macro- and micro-scale methodologies for understanding relationships between biodegradation and sorption reactions. NIEHS Biodegradation Conference, RTP, NC.

Herrick, J. B., M. Silva, E. L. Madsen, C. A. Batt and W. C. Ghiorse. 1993. Approaches to studying the genetics of indigenous biodegradative bacterial populations: The distribution and variation of *nah* genes at a coal-tar waste site. NIEHS Biodegradation Conference, RTP, NC.

B. Consultation:

U.S. Dept. Defense Tri-Service Workshop on Bioavailability of Organic Contaminants in Soils and Sediments, Invited speaker (April 9-12, 1995). Contact: W. Kozumbo, AFOSR.

U.S. Department of Energy, Contaminant Plumes Focus Area, Review Panel Member (June 6-7, 1995). Contact: Tom Brouns, Batelle Pacific Northwest Laboratories.

C. Transitions

As rapporteur for the National Academy of Sciences committee on *In situ* Bioremediation, Madsen wrote the first 2 drafts of the National Research Council Book: "*In situ* Bioremediation: When Does It Work?". This has had broad national influence and impacts — especially upon environmental remediation efforts by EPA and DOD

Merck Co., Inc. 1 March 1994 – 31 December 1995. Madsen collaborated with Merck personnel in documenting intrinsic bioremediation of solvents contaminating an alluvial aquifer. Contact: Richard Williams, phone: 703-298-4803.

8.0 NEW DISCOVERIES

None

9.0 HONORS/AWARDS

E. L. Madsen
Member of Editorial Board for Applied and Environmental Microbiology (recently appointed to a fourth 3-year term)
1992 National Academy of Sciences Committee on *In situ* Bioremediation

Karen G. Stuart
1996 Travel Grant from American Society of Microbiology