Annual Progress Summary for the IPEC project titled,

"Using Plants to Remediate Petroleum-Contaminated Soil "
EPA Grant Number: R827015-01-0
Principal Investigators:

Greg Thoma, Dept. of Chemical Engineering, 3202 Bell Engineering, University of Arkansas, Fayetteville, AR 72701, (479) 575-7374, gthoma@uark.edu
Susan Ziegler, Dept. of Biological Sciences, 416 Science Building, University of Arkansas, Fayetteville, AR 72701, (479) 575-6342, susanz@uark.edu
Duane Wolf, Dept. of Crop, Soil, and Environmental Sci., 115 Plant Sciences, University of Arkansas, Fayetteville, AR 72701, (479) 575-5739, dwolf@uark.edu
Industrial Collaborator:
Robert Reynolds, Shuler Drilling, 3514 West Hillsboro, El Dorado, AR, (870) 863-7234
Institution: University of Arkansas
EPA Project Officer: Bala Krishnan
Project Period: September 1, 2003 to August 31, 2004
Project Amount: $157,212
Research Category: Phytoremediation

This report covers the June 1, 2004 to August 31, 2004 period and summarizes our current IPEC phytoremediation studies that consist of an on-site field project in southern Arkansas, a laboratory study on microbial ecology, and a mathematical modeling project.

Progress Summary/Accomplishments:
Field Study

Materials and Methods

The field site in El Dorado, AR is located in a bermed crude oil storage/separation facility that was the site of an intentional spill in 1997 by vandals. The experimental plots consist of four replicates of the following treatments: (1) nonvegetated-nonfertilized control, (2) ryegrass (*Lolium multiflorum L.*) - fescue (*Festuca arundinacea Schreb.*) + fertilizer, and (3) bermudagrass (*Cynodon dactylon (L.) Pers.*) - fescue + fertilizer. Each field plot has 12 microplots (>soil socks=) that contain homogenized soil that allow monitoring of the field treatments, on a smaller scale, with less effect of field variability of the contaminant levels.

Sampling of the field site at El Dorado, AR occurred at 57 months after plot establishment and data for soil nutrient levels and soil TPH levels are being processed. Analyses for microbial parameters and shoot biomass are complete and statistical evaluation is underway.

Results and Discussion

For soil samples collected at 57 months, the microbial numbers show that bacterial and fungal numbers were greater in the vegetated-fertilized plots compared to the control plots (Fig. 1). There was no apparent difference between the fescue and bermudagrass treatments for bacterial or fungal numbers and numbers were within ranges expected for petroleum-contaminated soils. The number of petroleum-, PAH-, and alkane-degrader microorganisms suggested that levels were not different among the three treatments at the 57-month sampling (Fig. 2). Numbers were consistent with previous observations for the plots. Shoot biomass was similar for bermudagrass and fescue

vegetation and indicated substantial plant growth had occurred (Fig. 3).

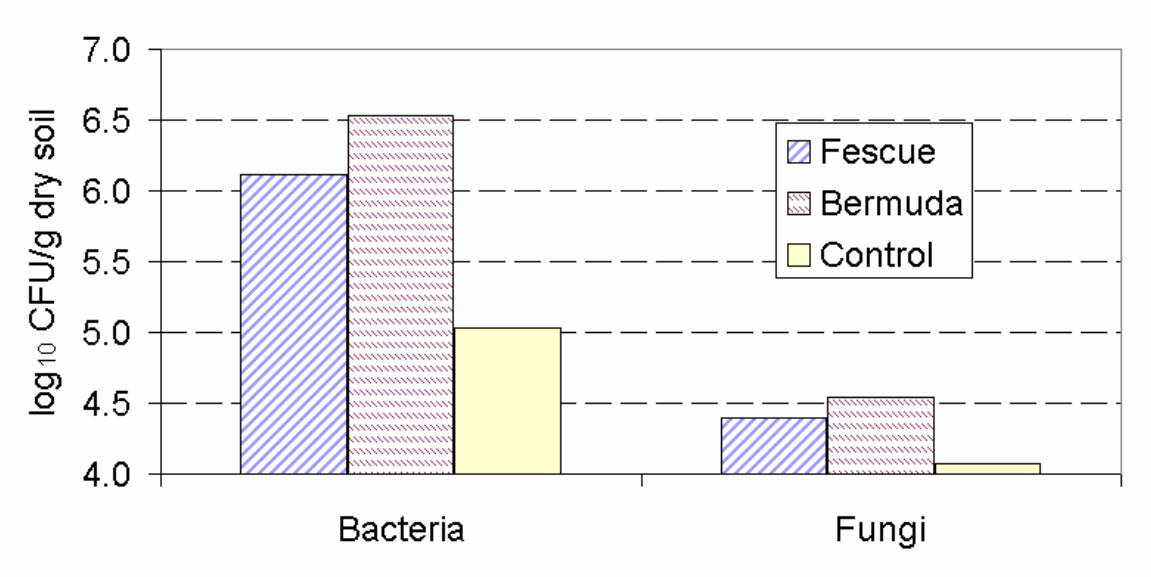

Fig. 1. Bacterial and fungal numbers for soil samples collected 57 months after plot establishment at the El Dorado field site. The control treatment was not fertilized or vegetated. The fescue and bermudagrass plots received fertilizer and lime to facilitate plant growth.

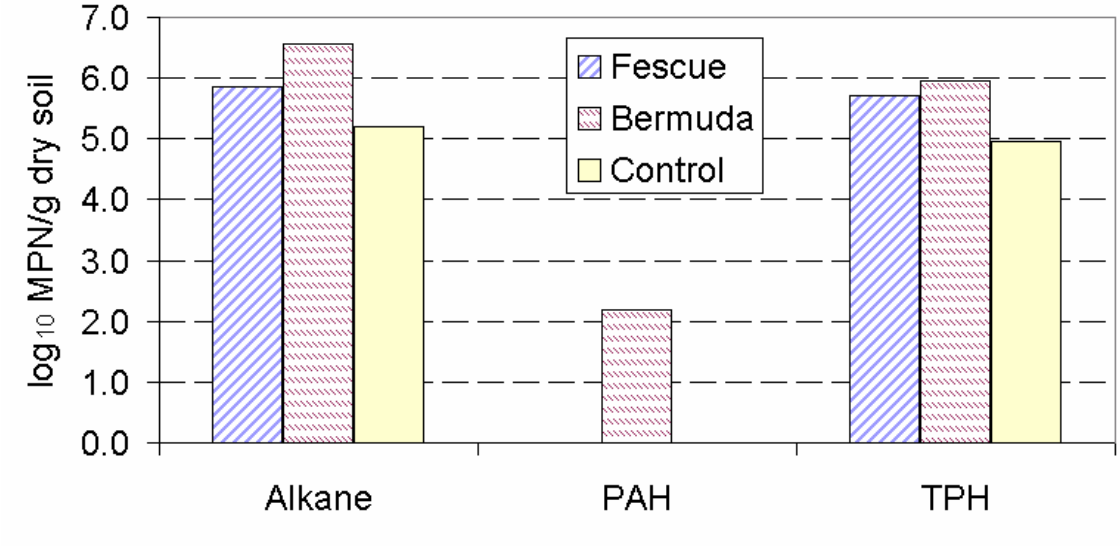

Fig. 2. Petroleum-, PAH-, and alkane-degrader microbial numbers for the three treatments at the El Dorado field site for samples collected 57 months after plot establishment.

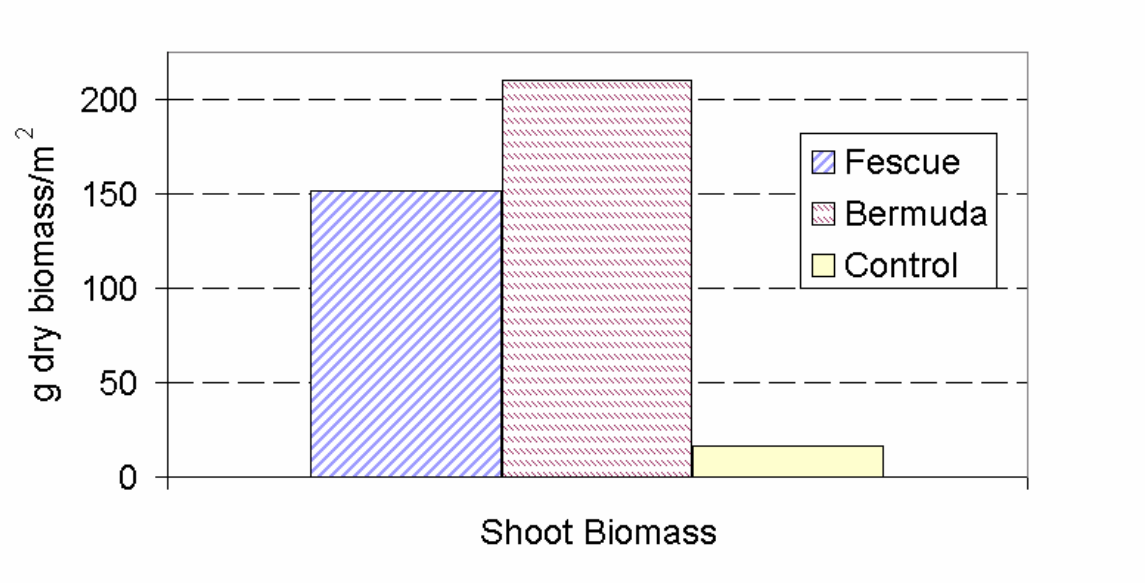

Figure 3. Shoot biomass production for the three treatments at the El Dorado field site for samples collected 57 months after plot establishment.

Mathematical Model

We have been investigating the behavior of a supporting model for calculation of the root and rhizosphere volume of fractal root systems. This supporting model will be used to help define the relationship between the fractional root volume and the rhizosphere. As the root density increases, there is greater overlap and interaction between the rhizosphere associated with adjacent roots. The current overall model does not completely account for this behavior. We have been testing the code on single segments to determine if the error in the calculation is dependent on the root segment orientation. As shown in Figure 4, over the range of angles allowed for the 'slicing plane angle' there is very little correlation to the angle, except for angles approaching 90^0. The average error for a computation with an ideal packing of volume elements of 5 per unit root radius is 0.001% with a range of -2.7% to 3.4%. The root mean square error is 0.33% (square root of the sum of squared errors). These simulations were performed for a single root segment with length 5 mm and radius of 1mm. The starting end point was chosen from a uniform random distribution of coordinates which fell inside the volume element centered on the origin. The direction vector was chosen by picking uniform random variates for the x,y, and z directions for the segment. Ten thousand segments were analyzed and the error reported is the 'voxel packing volume' compared to the analytically calculated volume for the segment. The minimum allowable angle is 37.5^0.

Microbial

Figure 5 presents results of PLFA analysis from January 2003. There is little variation detected in bulk microbial community among the treatments. Overall the soil community appears to be dominated by gram negative with some gram positive components including actinomycetes. The planned 13C-tracer studies will allow us to decipher which components of this overall community are involved in the degradation of certain classes of hydrocarbons. We are continuing collection and analysis of the PLFA data.

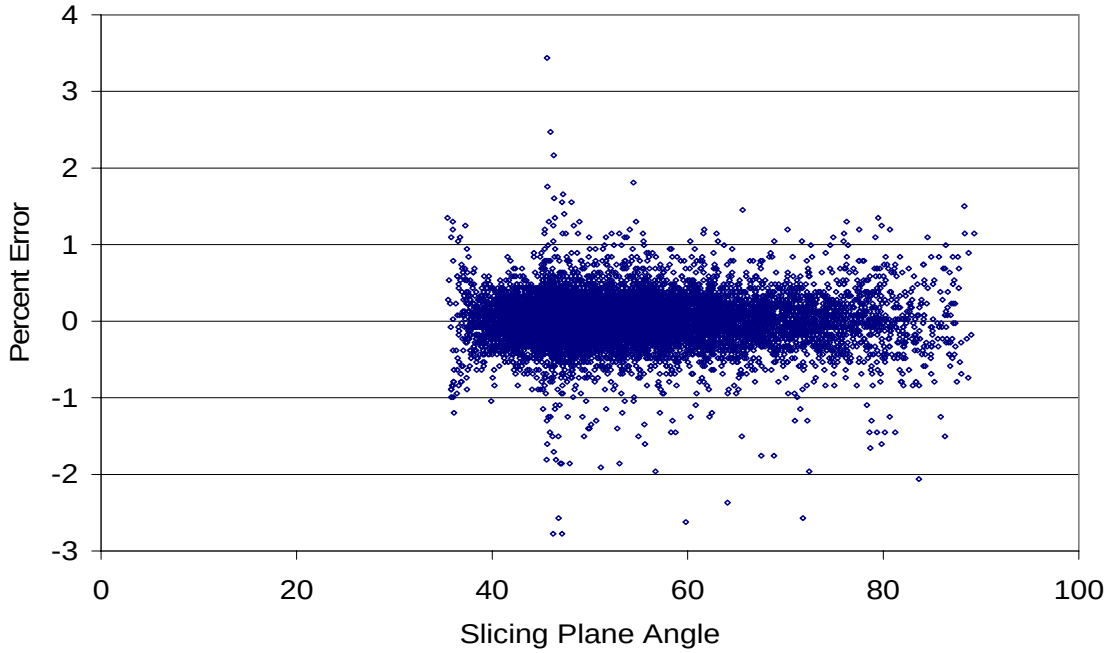

Figure 4. Calculated absolute error for a cylinder with a L/R ratio of 5. These calculations are for a volume element density of 5 per unit radius.

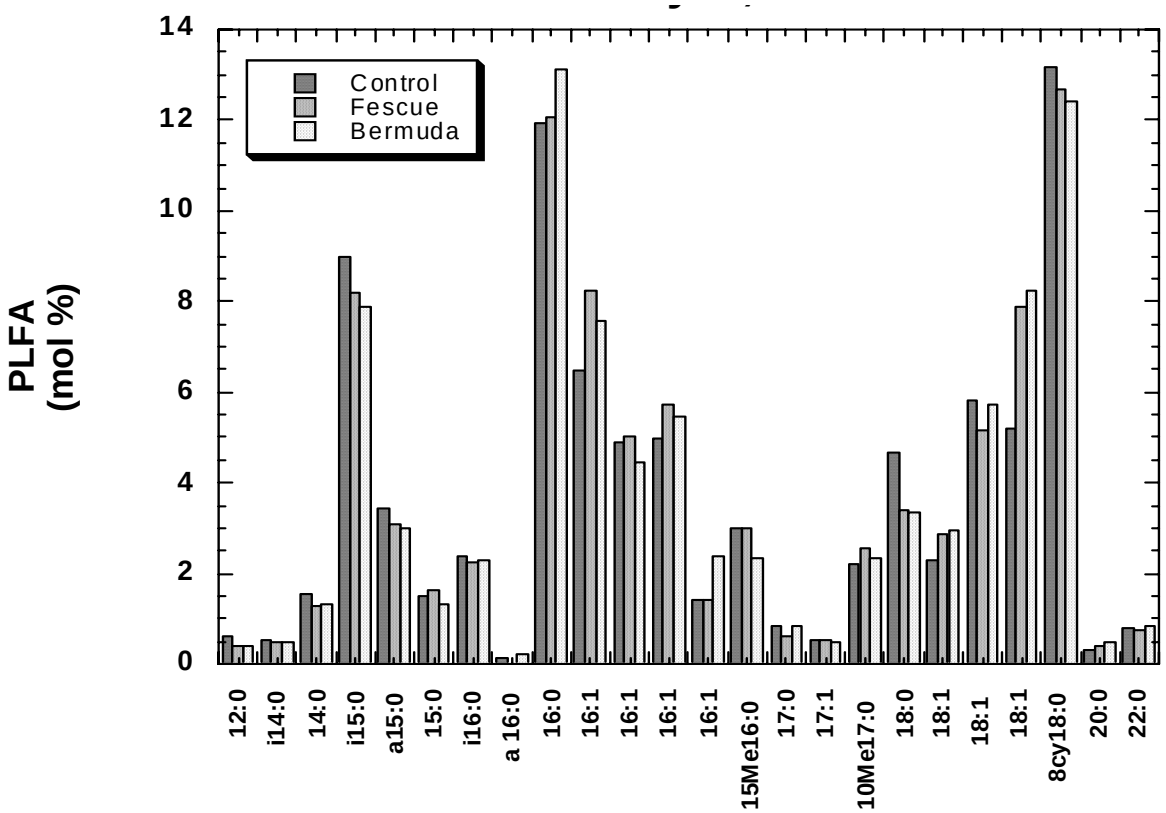

Figure 5. PLFA profile comparison across treatments.

Publications/Presentations:

Abstracts and titles of poster or oral presentations:

None in this period.

Abstracts and titles that have been submitted for presentation as posters or presentations in the future include:

Greer, K.M., S.E. Ziegler, G.J. Thoma, K.J. Davis, and D.C. Wolf. 2004. Influence of abiotic factors on hexadecane biodegradation in a Captina silt loam. *In* 11th Annual International Petroleum Environmental Conference. 12-15 October 2004. Albuquerque, NM. Integrated Petroleum Environmental Consortium, Tulsa, OK.

Lam, T.B., G. Thoma, D. Wolf, and S. Ziegler. . 2004. Novel approaches to measurement of rhizosphere effects in phytoremediation of oil-contaminated soils. *In* 11th Annual International Petroleum Environmental Conference. 12-15 October 2004. Albuquerque, NM. Integrated Petroleum Environmental Consortium, Tulsa, OK.

Greer, K.M., S.E. Ziegler, G.J. Thoma, K.J. Davis, and D.C. Wolf. 2004. Microbial degradation of hexadecane in soil. *In* Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, SSSA, Madison, WI.

Savin, M.C., S.E. Ziegler, G.J. Thoma, K.J. Davis, P.J. Tomlinson, and D.C. Wolf. 2004. Nematodes as ecological indicators during phytoremediation of a crude oil-contaminated soil. *In* Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, SSSA, Madison, WI.

Manuscripts submitted:

Ziegler, S.E., P.M. White, Jr., D.C. Wolf, and G.J. Thoma. 2004. Tracking the fate and recycling of ^{13}C-labeled glucose in soil: Lessons for stable isotope-labeling and biomarker studies. Soil Biology and Biochemistry.

Future Activities:

Our initial findings suggest that phytoremediation does reduce contaminant levels through the action of microbial communities associated with the rhizosphere. It is therefore important to develop successful agronomic management strategies that exploit this understanding. However, our detailed knowledge of the microbial ecology of the rhizosphere is lacking. We plan to use carbon-13 isotopic labeling of specific contaminants coupled with phospholipid fatty acid (PLFA) analysis to identify specifically which group of microbes are responsible for the degradation. We will continue to investigate the modes of action of a phytoremediation system; while keeping in mind that the ultimate goal remains site cleanup.

Supplemental Keywords:

Rhizosphere; rhizodegradation; species selection; Arkansas; South Central United States

Relevant Web Sites:

Remediation Technologies Development Forum: **www.rtdf.org**; IPEC: **ipec.utulsa.edu**