Third Quarter Progress Summary for the IPEC project titled,

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

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Project Period: September 1, 2003 to August 31, 2004
Project Amount: $157,212
Research Category:

This report covers the March 1, 2004 to May 30, 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.

Spring sampling of the field site at El Dorado, AR collected 18 May 2004 at 53 months after plot establishment and data for soil nutrient levels and soil TPH levels are being processed. Analyses for microbial parameters, shoot biomass, root biomass, root length, and root surface area are complete and statistical evaluation is underway.

Results and Discussion

Analysis of the soil samples collected 46 months after plot establishment shows the increase in nutrient levels and pH resulting from addition of fertilizer and lime (Table 1). The fertilized plots contained nutrient levels sufficient for plant growth.

For soil samples collected at 53 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 53-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).

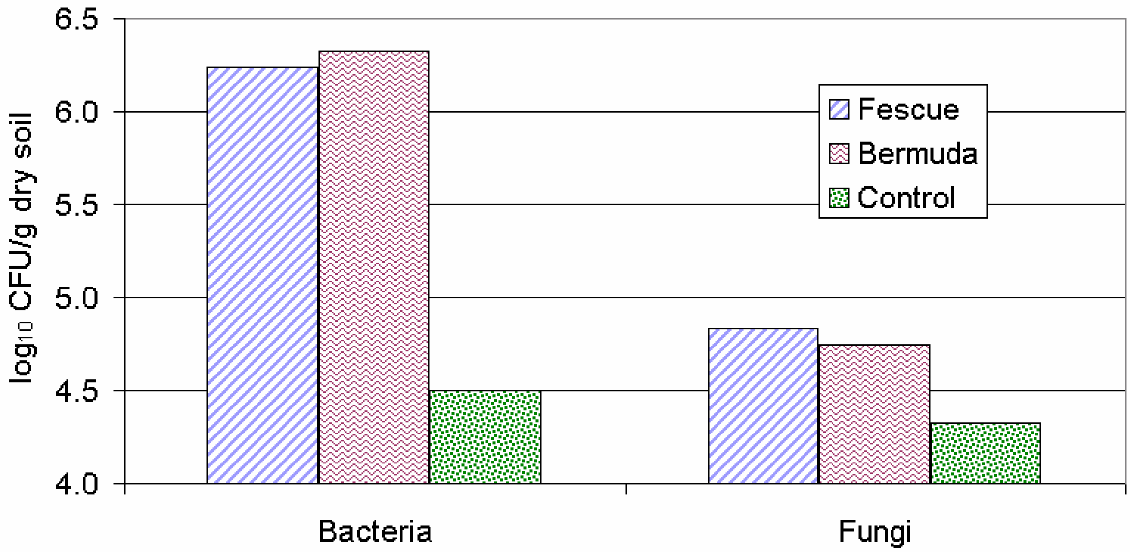

Fig. 1. Bacterial and fungal numbers for soil samples collected 53 months after plot establishment at the El Dorado field site. The control treatment was not fertilized or vegetated. The fescue and bermudagrass plots received fertilizer and lime to facilitate plant growth.

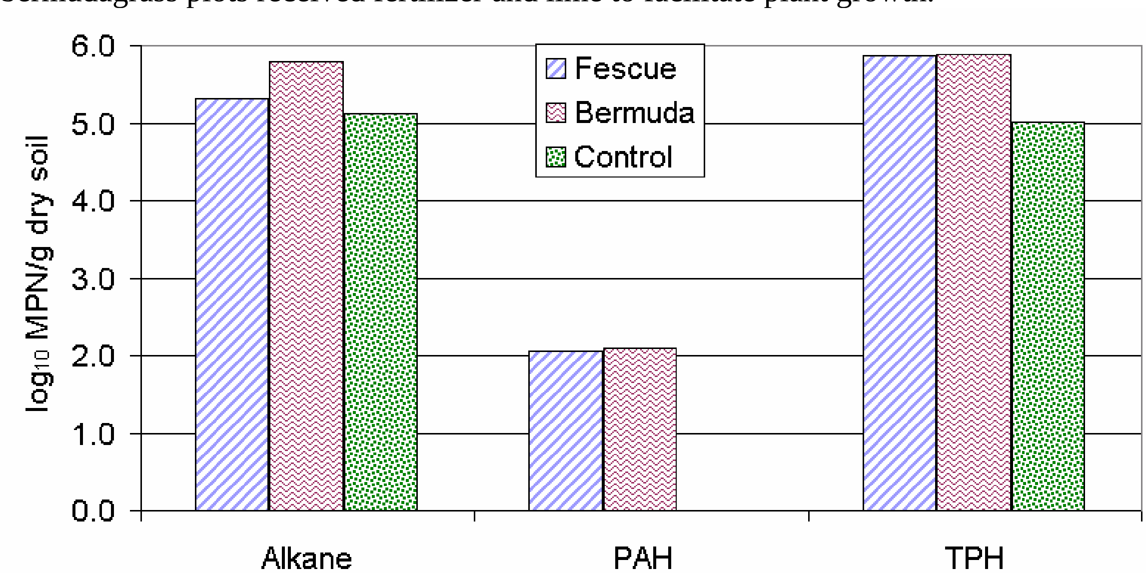

Fig. 2. Petroleum-, PAH-, and alkane-degrader microbial numbers for the three treatments at the El Dorado field site for samples collected 53 months after plot establishment.

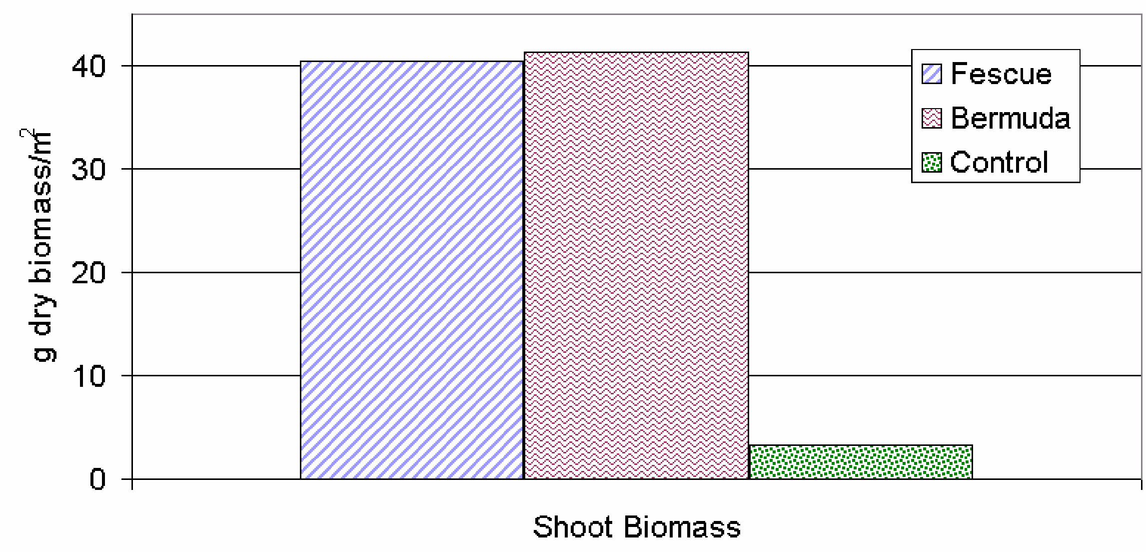

Figure 3. Shoot biomass production for the three treatments at the El Dorado field site for samples collected 53 months after plot establishment.

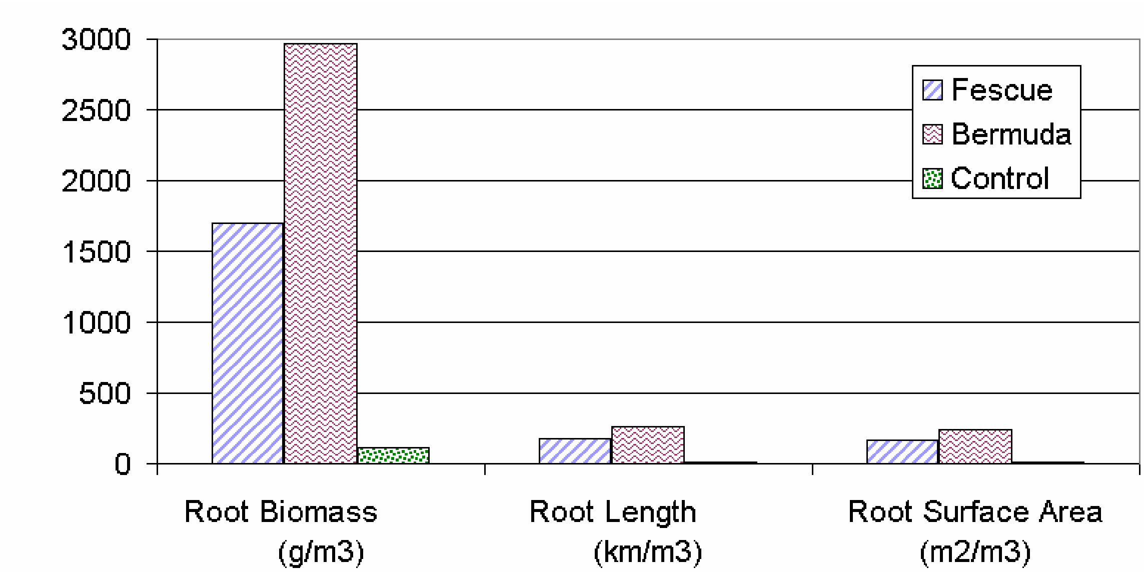

Figure 4. Root biomass, length, and surface area production for the three treatments at the El Dorado field site for samples collected 53 months after plot establishment.

Mathematical Model

Our supporting model for computation of the rhizosphere volume as a function of root morphology using a cubic voxel grid was tested to determine the source of the errors that were reported in the previous quarterly report. Analytical calculations of the number of cubic voxels that can fit in a cylinder of known size were compared with the computations of resulting from the computer program. The dependence of the computed volume on the ratio of voxel dimension to root radius was shown to be exactly as expected for a single, vertically oriented root segment. Analytical computations for tilted segments were not performed due to the relatively complex nature of this geometric configuration. However, because the testing showed that the computer code correctly computed the root volume, we can consider that the code has been validated and verified. During the testing, it was noted that the angular orientation of the segment to the principal planes of the voxel grid has an effect on the accuracy of the volume computation (compared to the known true volume of the cylindrical segment). this observation has brought into question one of the computational assumptions in the computer program: stepping of the voxel counting plane along the principal axis that is most nearly parallel to the root axis yields the most accurate volume estimate. We are performing sensitivity analysis to determine the most accurate approach for selecting the stepping plane. The next phase of this part of the modeling will be to incorporate a 'coring' subroutine. This is necessary due to memory limitations of the computers available for this work. The coring routine will take as input a sub volume of the soil containing the root and analyze only those segments that lie within the ' virtual core'. When this addition has been tested sensitivity analysis of the rhizosphere volume as a function of root volume will be performed and ultimately incorporated into the field scale model for phytoremediation effectiveness.

Microbial

The phospholipid fatty acid profiles have been determined for samples collected from the three treatment plots. Initial analysis of these data suggest some difference in the microbial community structure between planted and unplanted sites corresponding to elevated degradation rates and the degrader number data. Our next step is to subject these data to principle component analysis to generate aggregates from the multicomponent data sets for each sampling period and each treatment to statistically test for differences in microbial community structure among the different treatments over time.

We have complete a test run using ^{13}C-glucose to assess the feasibility of using ^{13}C-labeled substrates to determine the microbial groups responsible for hydrocarbon contaminant degradation in soil. A second experiment has been completed using ^{13}C-hexadecane. Samples from this experiment still need to be analyzed.

Publications/Presentations:

Abstracts and titles of poster or oral presentations given during this quarter include:

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.

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.

The field scale mathematical model will be extended to include climatic and field conditions that affect temperature and moisture level in the soil. In particular we are interested in modeling the differences that may arise associated with soil temperature as a function of vegetative cover.

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**

Table 1. Chemical properties of the crude oil contaminated soil samples collected 4 October 2003 at T = 46 months of the field study in El Dorado, AR.

		Mehlich 3 Extractable					Total	
Treatment	pH	P	K	Ca	Mg	Na	N	C
	(2:1)	mg/kg					%	
Control No Fertilizer No Vegetation	5.2	4	31	259	36	35	0.07	3.7
Fescue/Rye + Fertilizer	5.3	21	68	345	52	43	0.09	3.4
Bermudagrass + Fertilizer	5.4	33	87	424	65	70	0.09	3.4