

Second 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 December 1, 2003 to February 29, 2004 period and summarizes our current IPEC phytoremediation studies that consist of an on-site field project in southern Arkansas 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. The spring sampling is scheduled for May 2004 (t=53 mo).

Results and Discussion

As part of the Quality Assurance Project Plan, we have summarized data for the oil degraders, root parameters, and soil nutrient levels for samples collected during the Fall 2001 to Fall 2003. A previous report summarized data prior to the Fall 2001 sampling. Precision was calculated as the Relative Percent Difference (RPD)=[(n1-n2)/mean]100. Range was calculated as (n1-n2) for a duplicate analysis of a given sample. Completeness was determined as the percent of the required samples actually collected and analyzed.

OIL DEGRADER NUMBERS

- A. Precision: A total of 10 samples were analyzed in duplicate for Alkane, Petroleum, and PAH degrader numbers and the precision estimates for Alkane, Petroleum, and PAH degrader numbers exceeded the corrective action limit of 0.3 log units for 6, 4, and 5 samples, respectively (Fig. 1). The 0.3 log unit limit appeared to be too restrictive for the parameters analyzed. When the data were calculated as RPD, all alkane and petroleum degrader values were less than 20% (Fig. 2). The PAH degrader RPD did exceed 20% RPD in 4 of the 10 samples due to the extremely low values. We have modified the Quality Assurance Project Plan to reflect this change in our method of estimating precision for this parameter.
- B. Bias: Not applicable for the analyses.
- C. Completeness: Experimental protocol required collection of 12 samples for each of the five sample times and a total of 60 samples were collected and analyzed (Table 1).

ROOT PARAMETERS

- A. Precision: Plant root length, surface area, diameter, and volume were measured in duplicate for 15 samples over the five sample times and all values were below the corrective action limit of 20% (Fig. 3).
- B. Bias: Not applicable for the analyses.
- D. Completeness: Experimental protocol required collection of 12 samples for each of the five sample times and a total of 60 samples were collected and analyzed (Table 1).

SOIL NUTRIENT LEVELS

- A. Precision: Soil chemical parameters were measured in duplicate for ten samples over the five sample times. The calculated RPD for pH, electrical conductivity (EC), P, and K has a QC corrective action limit of 10%. For pH, all RPD values were below the 10% limit (Fig. 4). Six of the ten EC values were below the 10% limit. Due to the low extractable P and K values in the soil, 8 of the 10 P values and 4 of the 10 K values exceeded the 10% level. The QC corrective action limit for Mehlich 3 extractable Ca, Mg, Na, Zn, and Cu is 20%. Only one of the Ca and Mg values exceeded the limit. The 20% limit was more problematic for Na, Cu, and Zn because of the very low levels in the soil. Especially problematic were plots A1 and B2 that are unfertilized treatments where the inherently low soil nutrient levels resulted in increased RPD values.
- B. Bias: Not applicable for the analyses.
- C. Completeness: Experimental protocol required collection of 12 samples for each of the five sample times and a total of 60 samples were collected and analyzed (Table 1).

MATHEMATICAL MODEL

During the validation and verification for the supporting model used to compute the rhizosphere volume as a function of root length density and rhizosphere thickness, it was determined that for single root segments the computed root volume depended strongly on the exact location of the root segment endpoint with respect to the grid superimposed on the system that is used as a basis for defining the volumes; there is also a dependence of the computed volume on the orientation of the root segment with respect to the principal axes of the voxel grid. An element of the grid is called a

voxel, or volume element, and the data in Fig. 6 show the dependence of root position on the computed volume error. We are investigating the source of this computational error during the next quarter.

MICROBIAL COMMUNITY ASSESSMENT

Laboratory analyses of the phospholipid fatty acid (PLFA) profiles of plots from the field site for selected sample times have been completed and are being evaluated.

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:

Ziegler, S.E., J.M. Hamilton, G.J. Thoma, K.J. Davis, and D.C. Wolf. 2004. The influence of phytoremediation on microbial phospholipid fatty acid composition within hydrocarbon-contaminated soil. Submitted to 2004 ASA-CSSA-SSSA International Annual Meetings. 31 Oct. to 4 Nov. 2004 Seattle, WA.

Greer, K.M. Ziegler, S.E., G.J. Thoma, K.J. Davis, and D.C. Wolf. 2004. Microbial degradation of hexadecane in soil. Submitted to 2004 ASA-CSSA-SSSA International Annual Meetings. 31 Oct. to 4 Nov. 2004 Seattle, WA.

Manuscripts submitted:

Manuscripts published:

White, Jr., P.M., D.C. Wolf, G.J. Thoma, and C.M. Reynolds. 2003. Influence of Organic and inorganic soil amendments on plant growth in crude oil-contaminated soil. *Int. J. Phytoremed.* 5:381-397.

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.

Validation and verification of the mathematical model for root and rhizosphere volume will continue.

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. Number of samples collected and completeness of sample analyses for critical measurements.

	Sample Time (months)					Total
	21	30	36	42	46	
----- Field Study -----						
----- Samples Collected -----						
Oil Degradar Numbers	12	12	12	12	12	60
Root Parameters	12	12	12	12	12	60
Soil Nutrient Levels	12	12	12	12	12	60
----- Samples Analyzed -----						
Oil Degradar Numbers	12	12	12	12	12	60
Root Parameters	12	12	12	12	12	60
Soil Nutrient Levels	12	12	12	12	12	60
----- Completeness (%) -----						
Oil Degradar Numbers	100	100	100	100	100	100
Root Parameters	100	100	100	100	100	100
Soil Nutrient Levels	100	100	100	100	100	100

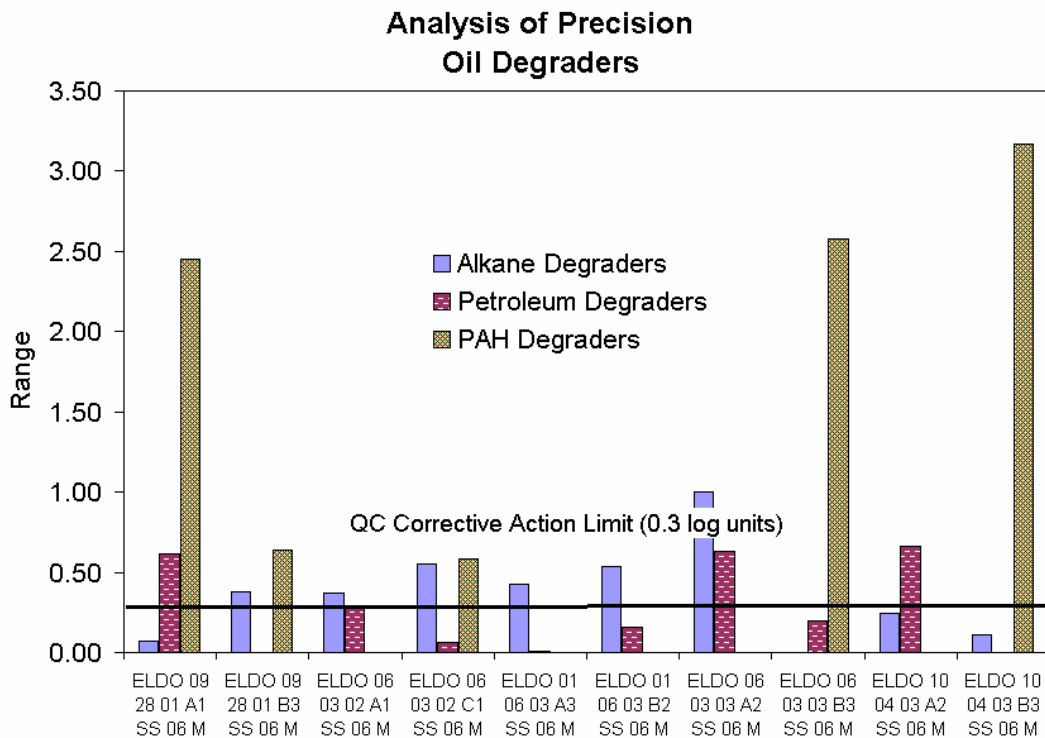


Fig. 1. Oil degrader precision results for ten soil samples from the IPEC study at El Dorado, AR.

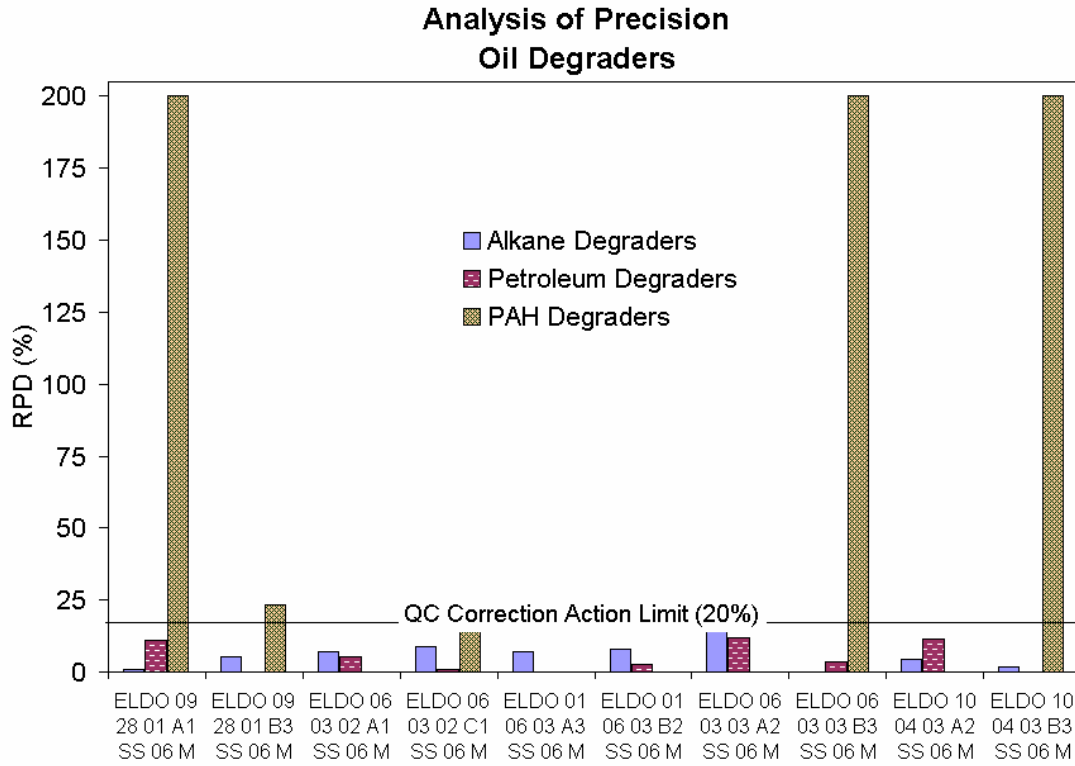


Fig. 2. Oil degrader precision results for ten soil samples from the IPEC study at El Dorado, AR.

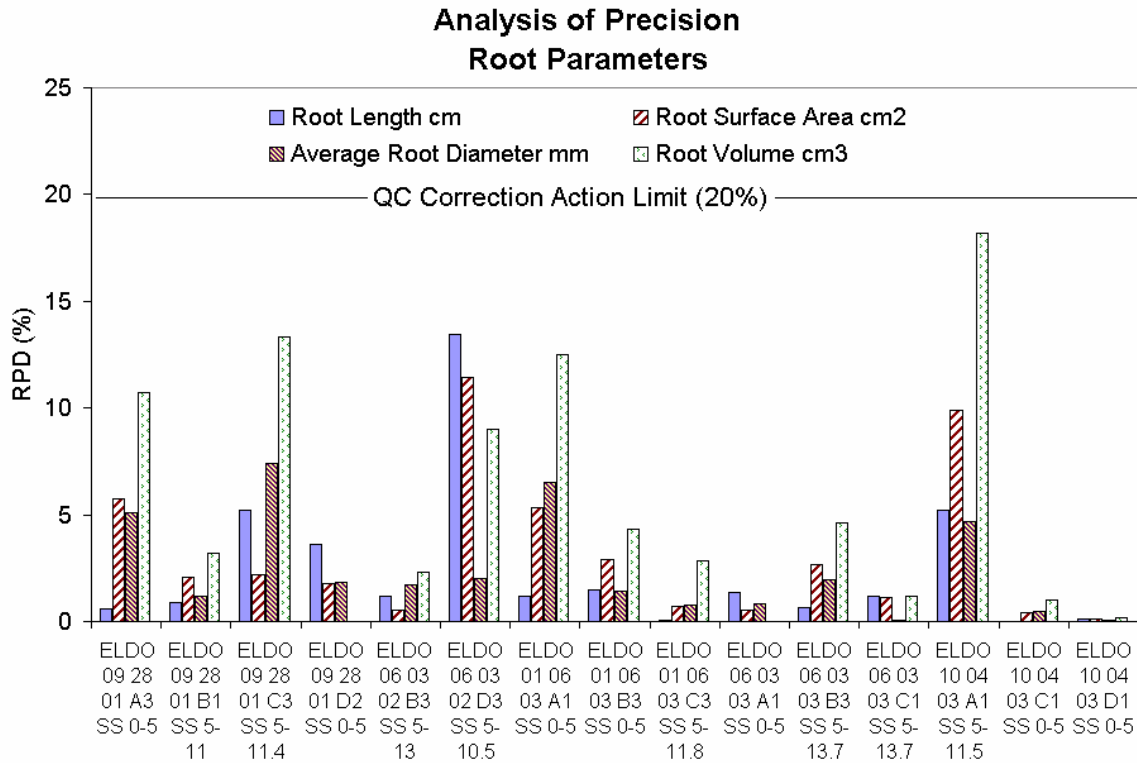


Fig. 3 The root parameter precision results for 15 samples from the IPEC study at El Dorado, AR.

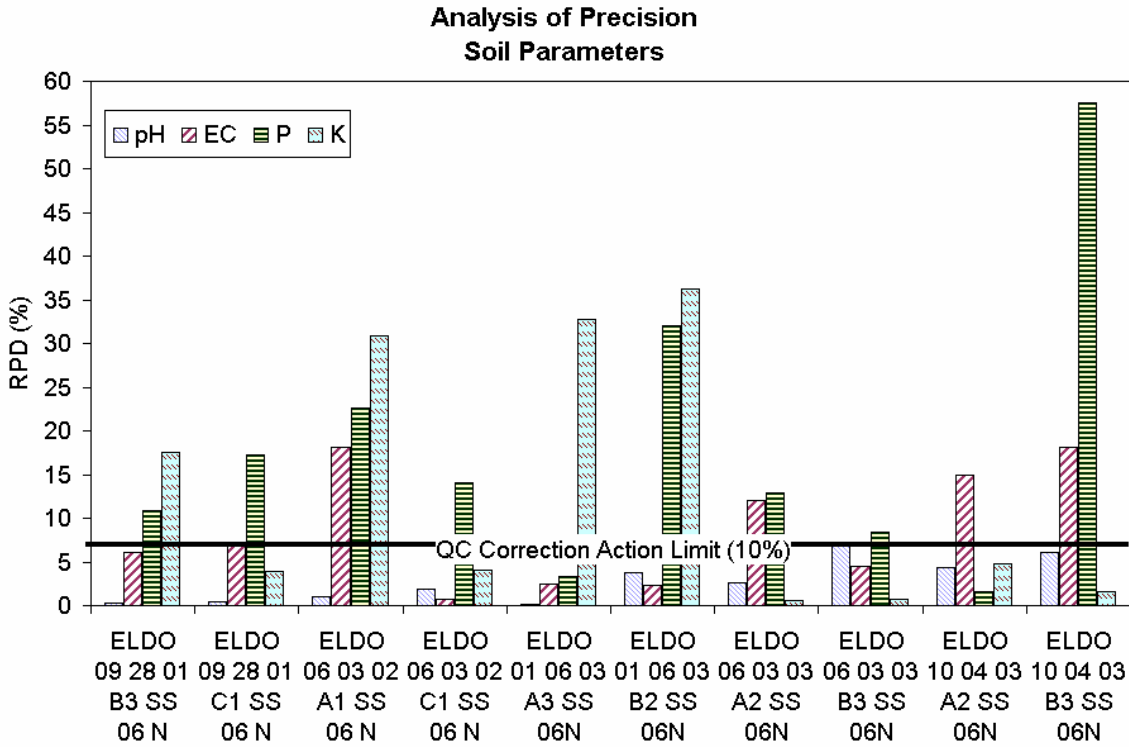


Fig. 4. Soil pH, electrical conductivity (EC), and Mehlich 3 extractable P and K precision results for ten samples over five sample times from the IPEC study at El Dorado, AR.

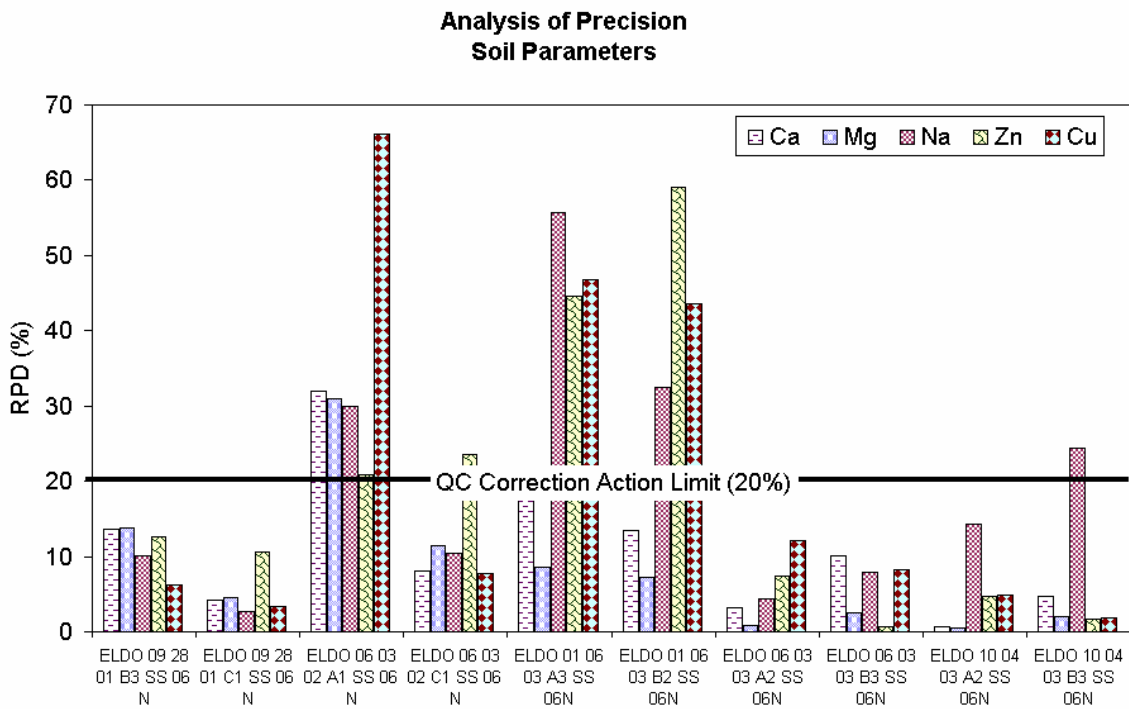


Fig. 5. Mehlich 3 extractable Ca, Mg, Na, Zn, and Cu levels in soil for ten samples over five sample times from the IPEC study at El Dorado, AR.

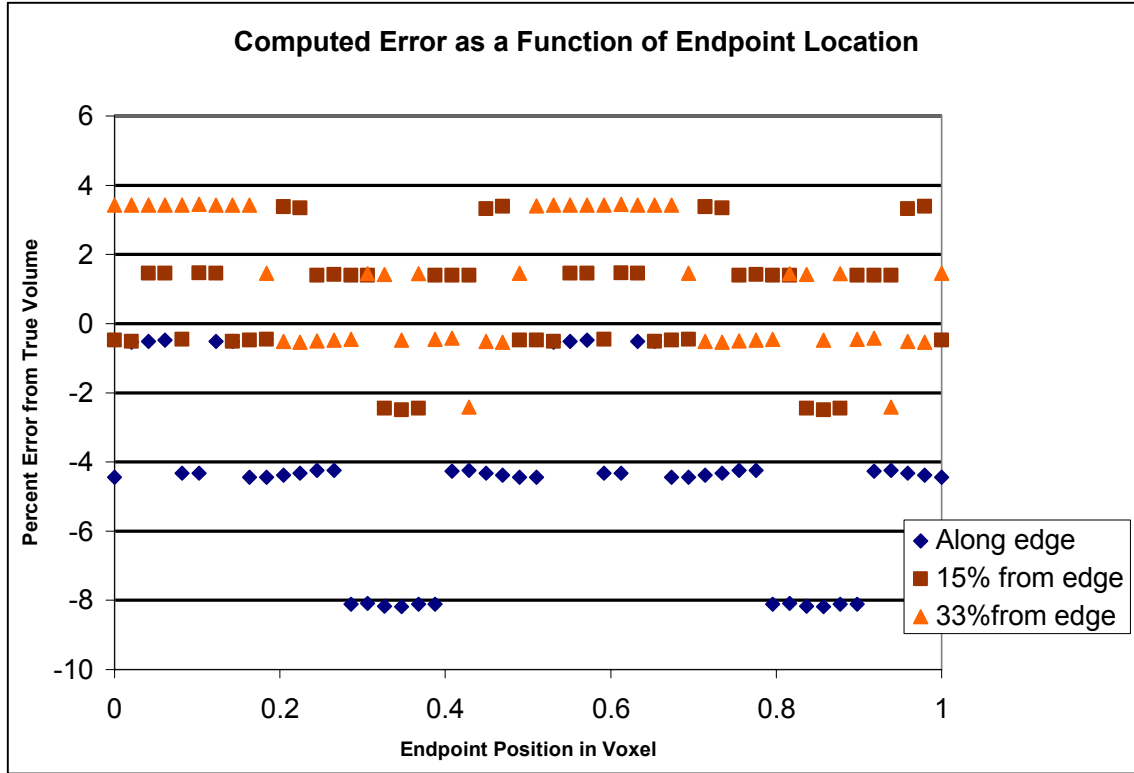


Fig 6. Validation results for voxel based calculation of root volume. We are investigating the cause of this behavior as part of the code validation. It is not clear at present if this is an artifact of the superposition of the square grid on the curved (cylindrical) root surface.