

# Using Plants to Remediate Petroleum-Contaminated Soil - Project Continuation – Quarterly Report

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**Title:** Using Plants to Remediate Petroleum-Contaminated Soil - Project Continuation

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**Institution:** University of Arkansas

**EPA Project Officer:** Bala Krishnan

**Project Period:** July 1, 2001 to June 30, 2002 ( N/C extension till May 15, 2003)

**Project Amount:** \$145,298

**Research Category:** Phytoremediation

This report covers the July 1, 2002 to October 1, 2002 period and summarizes our current IPEC phytoremediation studies that consist of an on-site field project in southern Arkansas and a mathematical modeling project.

## Field Study

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.

Soil samples collected 28 September 2001 at 21 months after plot establishment. As part of the Quality Assurance Project Plan, we have summarized data for the Total Petroleum Hydrocarbon (TPH) by GC/FID, TPH Criteria Working Group (TPHCWG) Total, Aliphatic, and Aromatic, PAH, biomarker (hopane), oil degraders, and root parameters. Precision was calculated as the Relative Percent Difference (RPD)=[(n1-n2)/mean]100. Bias was evaluated as percent of spiked surrogate recovery. Completeness was determined as the percent of the required samples actually collected and analyzed.

## TPH (GC/FID)

- A. Precision: One sample was analyzed in duplicate and had a RPD of less than the corrective action limit of 25% (Fig. 1).
- B. Bias: Recovery of the surrogates ortho-terphenyl, 5A-androstane, and d50-tetracosane, from 12 soil samples analyzed showed mean recovery values of 70, 68, and 64, respectively. The values approached the lower limit for our % surrogate recovery of 70% and no corrective action was initiated (Fig. 2).
- C. Completeness: Experimental protocol required collection of 12 samples for the sample time and all samples were collected and analyzed (Table 1).

## **TPHCWG**

- A. Precision: One sample was analyzed in duplicate and showed RPD of less than the corrective action limit of 25% (Fig. 1).
- B. Bias: Recovery of the surrogates ortho-terphenyl, 5A-androstane, and d50-tetracosane, from 12 soil samples analyzed showed that all values were between the 70 to 130% corrective action limits (Fig. 3).
- C. Completeness: Experimental protocol required collection of 12 samples for the sample time and all samples were collected and analyzed (Table 1).

## **PAH**

- A. Precision: One sample was analyzed in duplicate for 17 PAHs. None of the values were outside of the corrective action limit of 25%. Typical values are presented in Figure 4.
- B. Bias: Recovery of the four surrogates d8-naphthalene, d10-acenaphthene, d10-phenanthrene, and d12-benzo[a]pyrene from 12 samples analyzed showed 2 of the 48 samples were below the 70% corrective action limit. No corrective action was initiated (Fig. 5).
- C. Completeness: Experimental protocol required collection of 12 samples for the sample time and all samples were collected and analyzed (Table 1).

## **BIOMARKER (C30 17a(H)-hopane)**

- A. Precision: One sample was analyzed in duplicate and showed RPD of 6% and was less than the corrective action limit of 25%.
- B. Bias: Recovery of the surrogate D66-dotriacontane from 12 soil samples analyzed showed that all values were between the 70 to 130% corrective action limits (Fig. 6). The hopane concentrations for the 40 samples were consistent throughout the sample collection period (Fig. 7). This suggests the hopane biomarker can be used as a valid parameter to normalize TPH and PAH levels.
- C. Completeness: Experimental protocol required collection of 12 samples for the sample time and all samples were collected and analyzed (Table 1).

## **OIL DEGRADER NUMBERS**

- A. Precision: Two samples were analyzed in duplicate for Alkane, Petroleum, and PAH degrader numbers (Fig. 8). The PAH degrader number for one sample was 200% and was due to the very low value found in the sample. As the absolute value of an observation decreases, the percentage RPD typically increases. Five of the six observations had percentage RPD less than 20%.
- B. Bias: Not applicable for the analyses.
- C. Completeness: Experimental protocol required collection of 12 samples for the sample time and 12 samples were collected and analyzed (Table 1).

## **ROOT PARAMETERS**

- A. Precision: Plant root length, surface area, diameter, and volume were measured in duplicate for 4 samples and all values were below the corrective action limit of 20% (Fig. 9).
- B. Bias: Not applicable for the analyses.
- C. Completeness: Experimental protocol required collection of 12 samples for the sample time

and all samples were collected and analyzed (Table 1).

## **MICROBIAL ANALYSES**

Laboratory studies have been conducted assessing the  $^{13}\text{C}$  labeling of microbial phospholipid fatty acids (PLFA) in soil using  $^{13}\text{C}$  labeled glucose. Results suggest that the glucose was used to different extents by different microbial groups in the soil. Additionally, a fraction of the glucose was used solely as an energy source and lost as  $\text{CO}_2$ . We are currently developing the means to isolate, purify and analyze the  $\text{CO}_2$  evolved in our experiments for  $\delta^{13}\text{C}$  composition. This will enable us to partition the proportion of hydrocarbon contaminant lost to  $\text{CO}_2$  and incorporated into microbial biomass. Our results indicate that this approach may be used to identify those groups of microbes responsible for the degradation and incorporation of labeled hydrocarbons in soil. Additionally, we have successfully analyzed the microbial PLFA composition of hydrocarbon contaminated soil from our study site, enabling us to begin studies of contaminated soil both at the study site and in controlled experiments using labeled hydrocarbon contaminants. The soil from a single contaminated plot from our study site was dominated by bacterial biomarkers which were found to be as abundant as general PLFA markers such as hexadecanoic acid. The bacterial PLFA detected were primarily branched fatty acids indicating a dominance of gram positive bacteria over fungal biomass. Future studies are planned to (1) assess soil type and conditions on the incorporation and degradation of labeled hydrocarbons, (2) identify those microbial groups responsible for this degradation, and (3) to assess the microbial community composition of the established field plots where hydrocarbon degradation occurs.

### **Mathematical Model**

The mathematical model has been extended to include the effect of local climate and soil texture on the efficacy of phytoremediation. The climatic effect includes both the effect of temperature and soil moisture content on the degradation rate constants in the soil. The model can take as input daily temperature and precipitation observations, or daily, monthly, or annual averages. The temperature effect is based on an Arrhenius model with a basal rate at 298K. The soil water potential is used with a published correlation<sup>1</sup> to provide a correction factor for moisture stress. The two correction factors are multiplied together to give an overall correction to the degradation rate constants. Thus when weather patterns result in hot, dry conditions the degradation rate is decreased due to moisture stress, and it is maximized during warm moist conditions. Figure 10 presents the results of simulations matched to the El Dorado site. As shown in the figure, the choice of dataset does not significantly affect the model prediction; the implication is that short term and seasonal variations from the mean conditions do not play a significant role in the efficacy of the system.

### **Technology Transfer**

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

Thoma, G., D. Wolf, L. Thanh, and S. Ziegler. Mathematical modeling of phytoremediation of

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1 Gilmour, J.T. 1998. Carbon and nitrogen mineralization during co-utilization of biosolids and composts. p. 89-112. In Brown, S. Angle, J.S. and Jacobs, L. (eds.) Beneficial co-utilization of agricultural and industrial by-products. Kluwer Academic Publishers.

petroleum contaminated soils. *In* 9<sup>th</sup> Annual International Petroleum Environmental Conference. 22-25 October 2002. Albuquerque, NM.

White, Jr., P.M., G.J. Thoma, D.C. Wolf, and E.E. Gbur. 2002. Field scale phytoremediation of petroleum-contaminated soil. *In* 9<sup>th</sup> Annual International Petroleum Environmental Conference. 22-25 October 2002. Albuquerque, NM.

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

Thoma, G., D.C. Wolf, T.B. Lam, and S. Ziegler. 2002. Mathematical modeling of phytoremediation of petroleum contaminated soils. *In* Annual Meetings Am. Inst. Chemical Engineers. 3-8 Nov. 2002. Indianapolis, IN.

White, Jr., P.M., D.C. Wolf, G.J. Thoma, C.M. Reynolds, and E.E. Gbur. 2002. Effects of plants and fertilizer on the remediation of crude oil-contaminated soil. *In* Annual Meetings Abstracts [CD-ROM]. ASA, CSSA, SSSA, Madison, WI. (10-14 Nov. 2002. Indianapolis, IN).

Manuscripts submitted:

Thoma, G.J., T.B. Lam, and D.C. Wolf. 2002. Modeling phytoremediation for petroleum contaminated soil: Model development. *Accepted Int. J. Phytoremed.*

Thoma, G.J., T.B. Lam, and D.C. Wolf. 2002. A mathematical model of phytoremediation for petroleum contaminated soil: Sensitivity analysis. *Accepted Int. J. Phytoremed.*

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. Submitted to *Int. J. Phyto.*

Thesis completed:

White, Jr., P.M. 2002. Phytoremediation of crude oil-contaminated soil. M.S. thesis. University of Arkansas, Fayetteville.

Table 1. Number of samples collected and completeness of sample analyses for critical measurements.

	Sample Time (months)				Total
	0	6	17	21	
----- Field Study -----					
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<i>Samples Collected</i> -----					
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TPH GC/FID	4	12	12	12	40
TPHCWG (Total, Aliphatic, & Aromatic)	4	12	12	12	40
PAHs	4	12	12	12	40
Biomarker (Hopane)	4	12	12	12	40
Oil Degradation Numbers	0	12	12	12	36
Root Parameters	4	12	12	12	40
<i>Samples Analyzed</i> -----					
--					
TPH GC/FID	4	12	12	12	40
TPHCWG (Total, Aliphatic, & Aromatic)	4	12	12	12	40
PAHs	4	12	12	12	40
Biomarker (Hopane)	4	12	12	12	40
Oil Degradation Numbers	0	12	12	12	36
Root Parameters	4	12	12	12	40
<i>Completeness (%)</i> -----					
--					
TPH GC/FID	100	100	100	100	100
TPHCWG (Total, Aliphatic, & Aromatic)	100	100	100	100	100
PAHs	100	100	100	100	100
Biomarker (Hopane)	100	100	100	100	100
Oil Degradation Numbers	0	100	100	100	90
Root Parameters	100	100	100	100	100

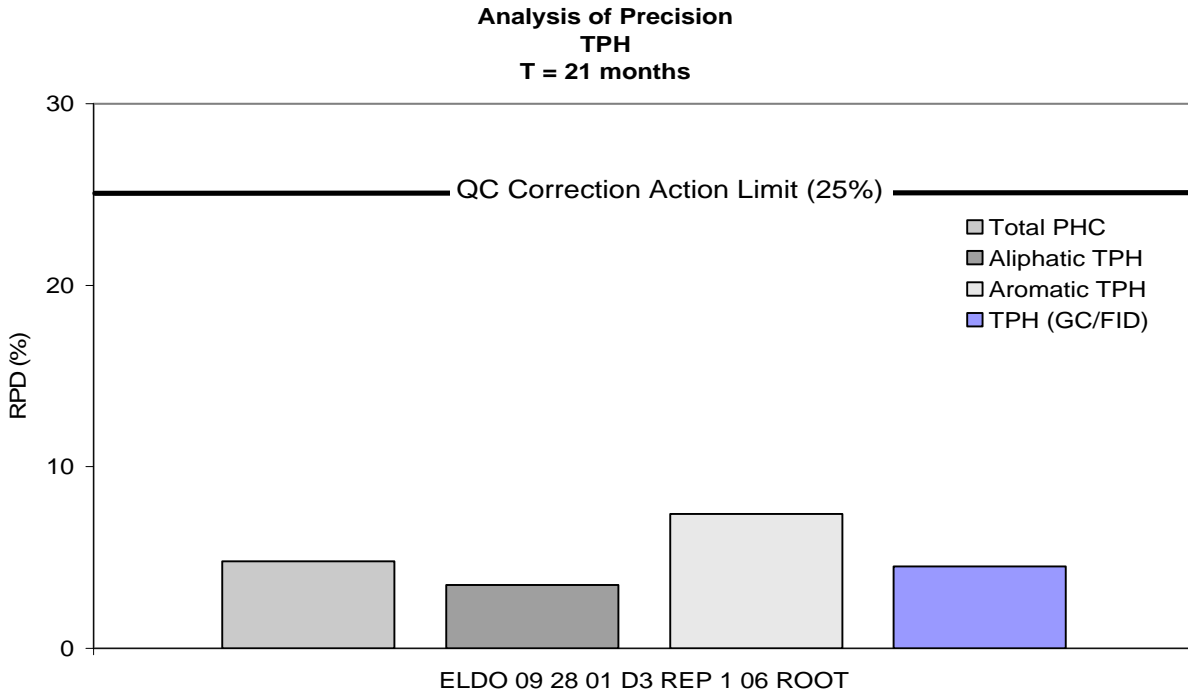


Figure 1. The TPH(GC/FID), CWG TPH Total, aliphatic, and aromatic precision results for one sample collected 9/28/01.

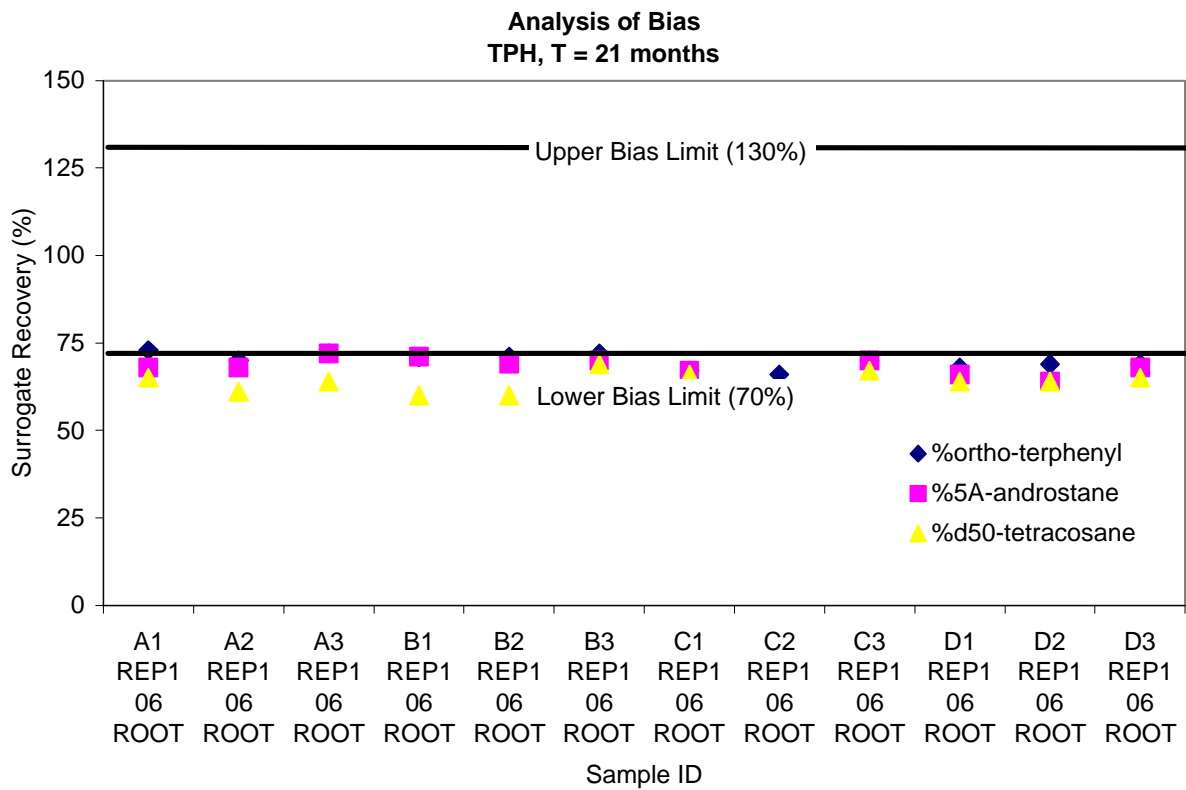


Figure 2. Percentage recovery of three surrogates from 12 samples from the IPEC study site.

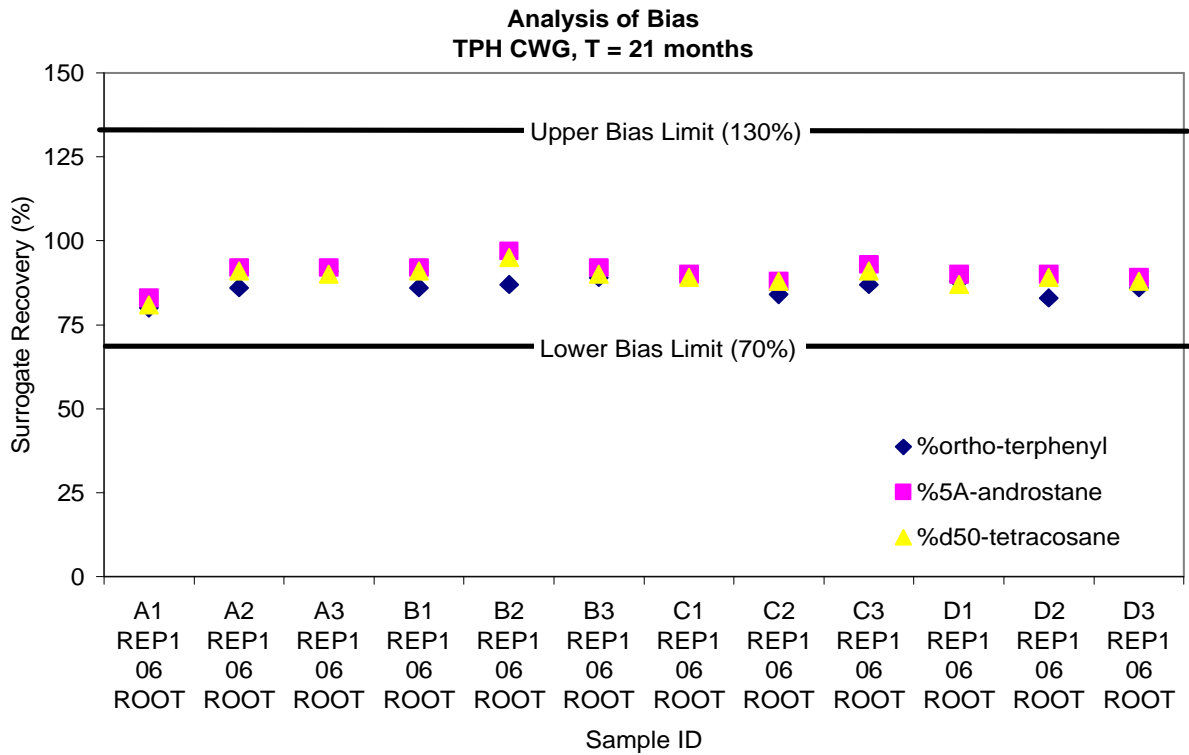


Figure 3. Percentage recovery of three surrogates from 12 samples from the IPEC study site.

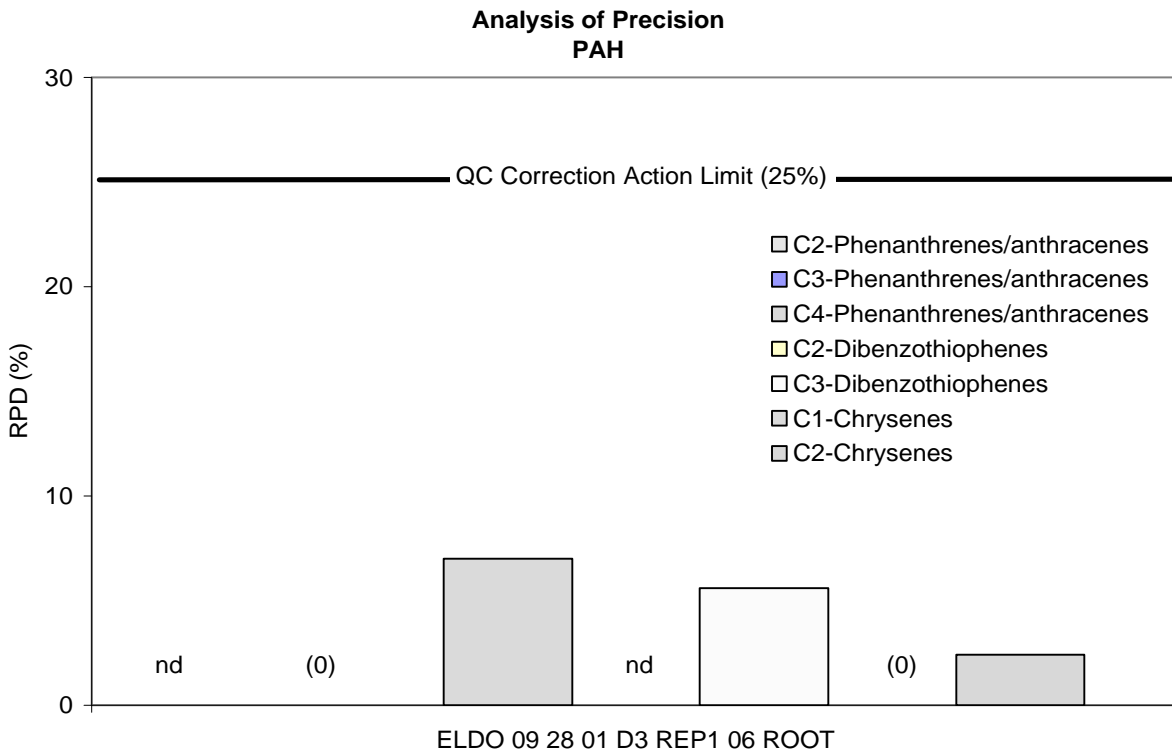


Figure 4. The PAH precision from one sample collected from the IPEC study site.

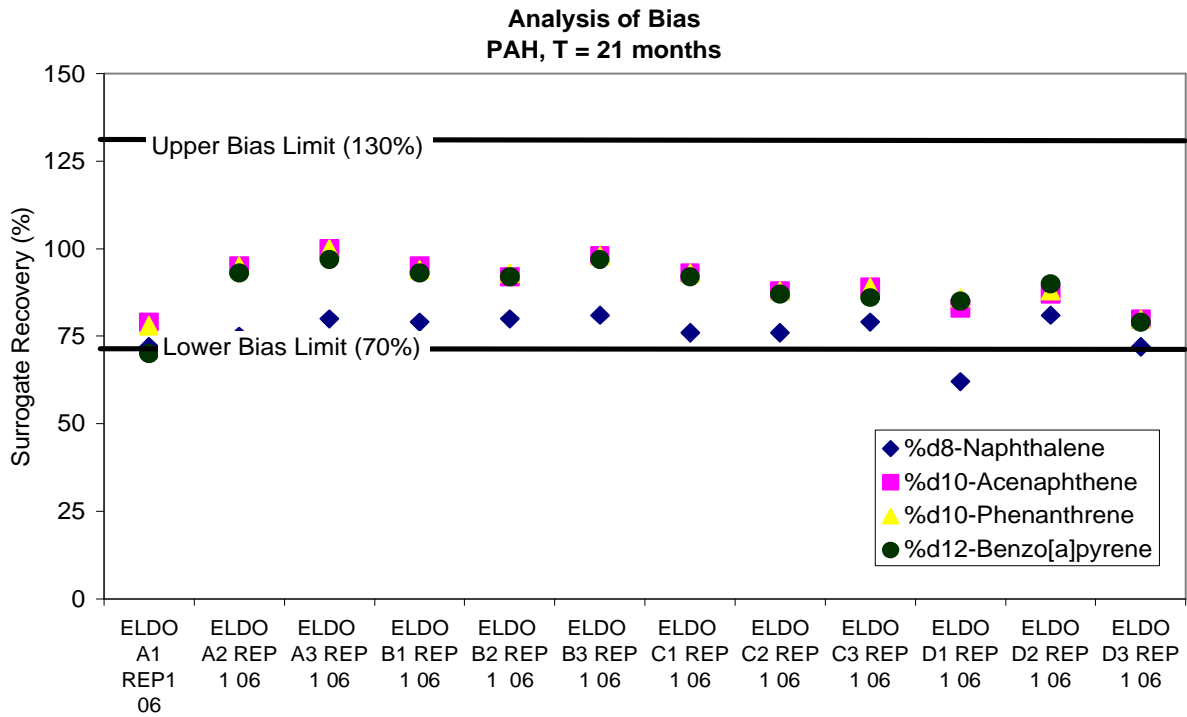


Figure 5. Percentage recovery of four PAH surrogates from the 12 samples from the IPEC site.

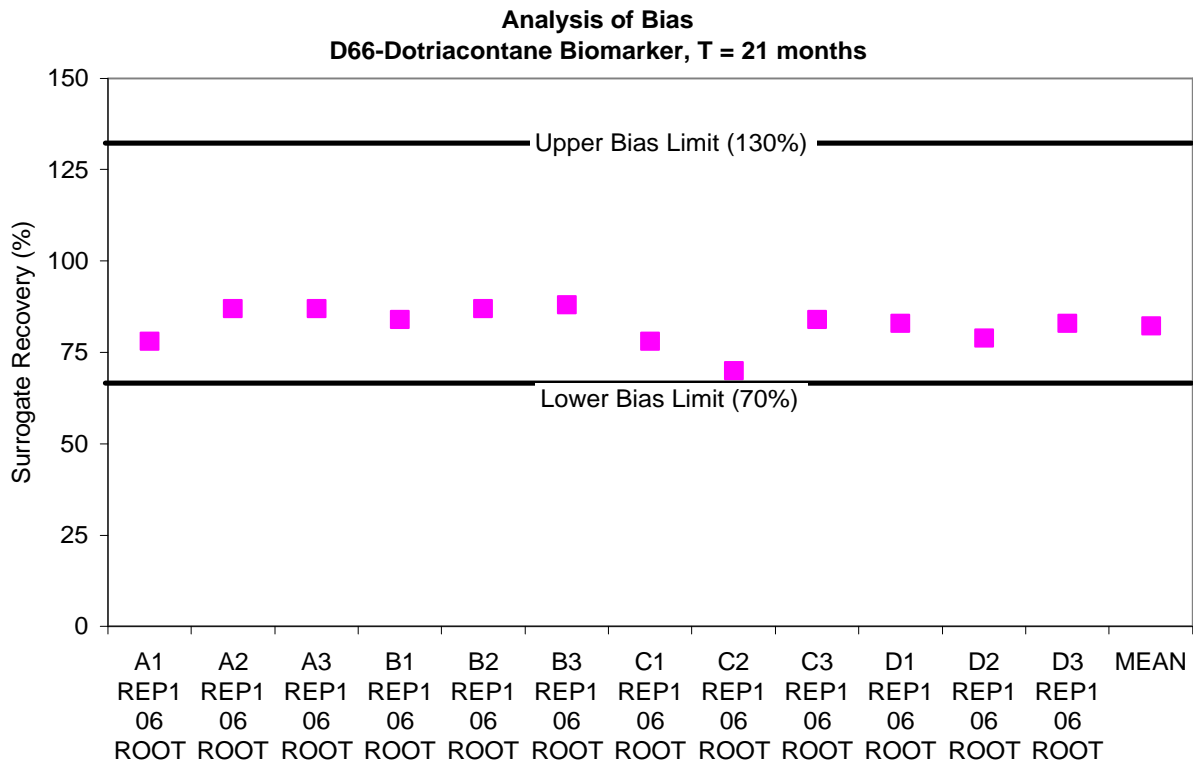


Figure 6. Percentage recovery of D66-Dotriacontane Biomarker from 12 IPEC samples.

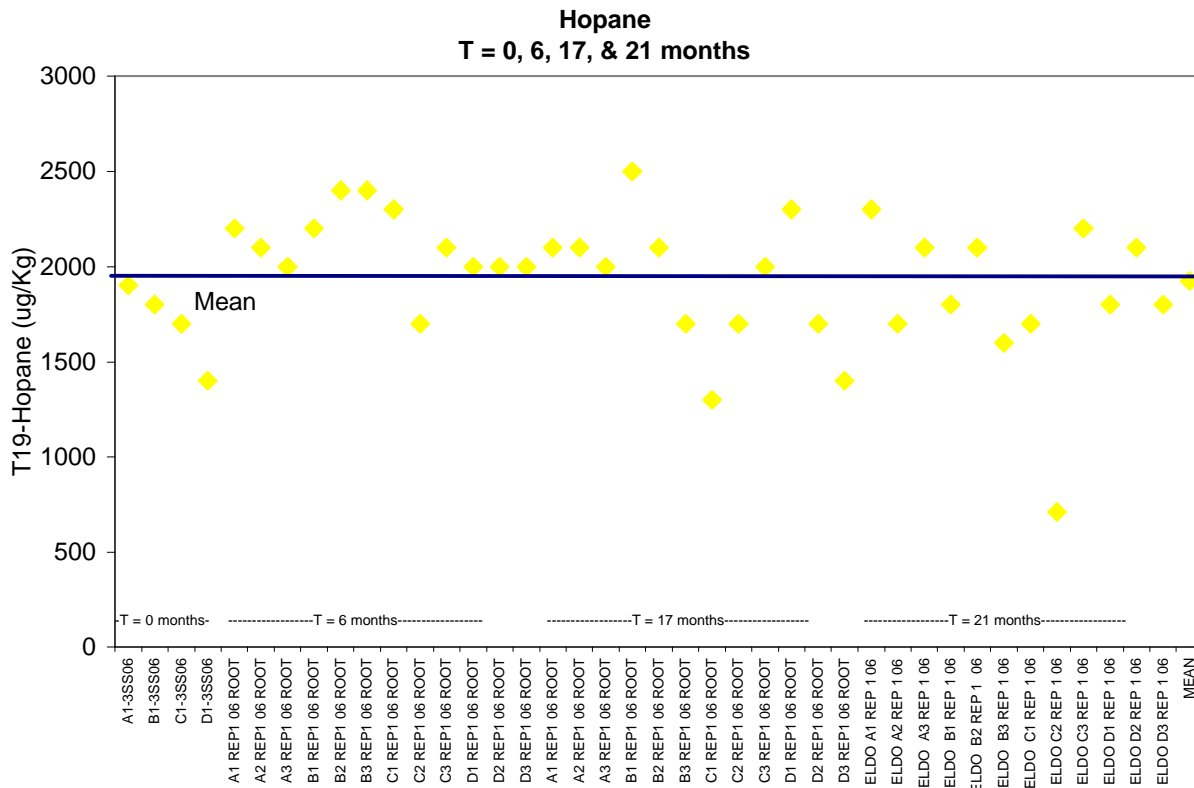


Figure 7. Hopane recovery from 40 soil samples collected from the IPEC study site.

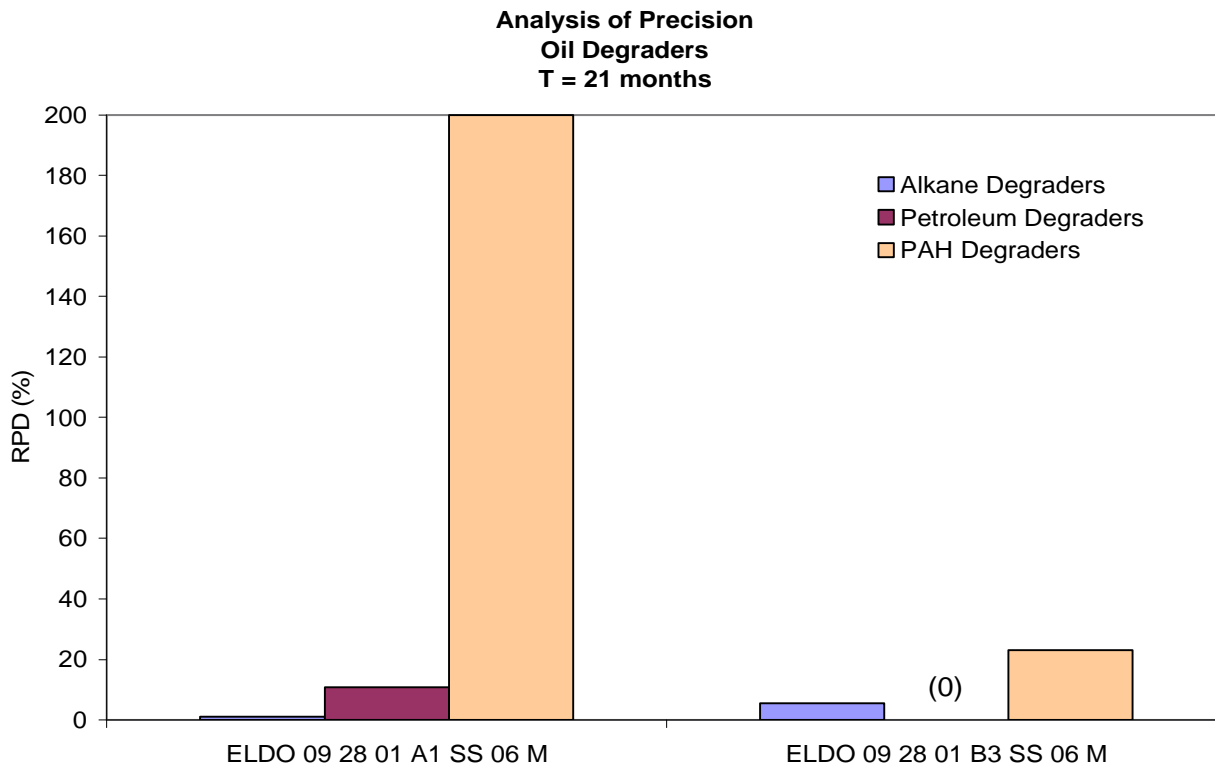


Figure 8. Oil degraders precision results for two soil samples from the IPEC study site.

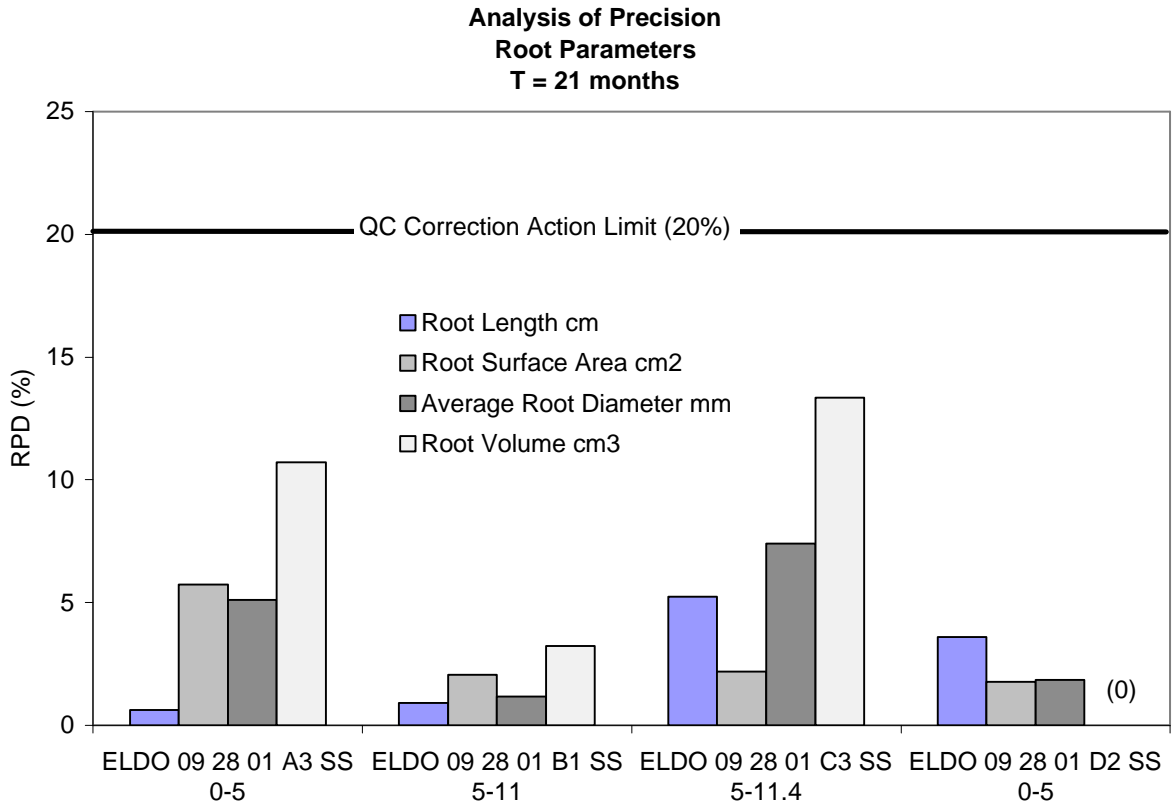


Figure 9. Root parameter precision results for four soil samples from the IPEC study site

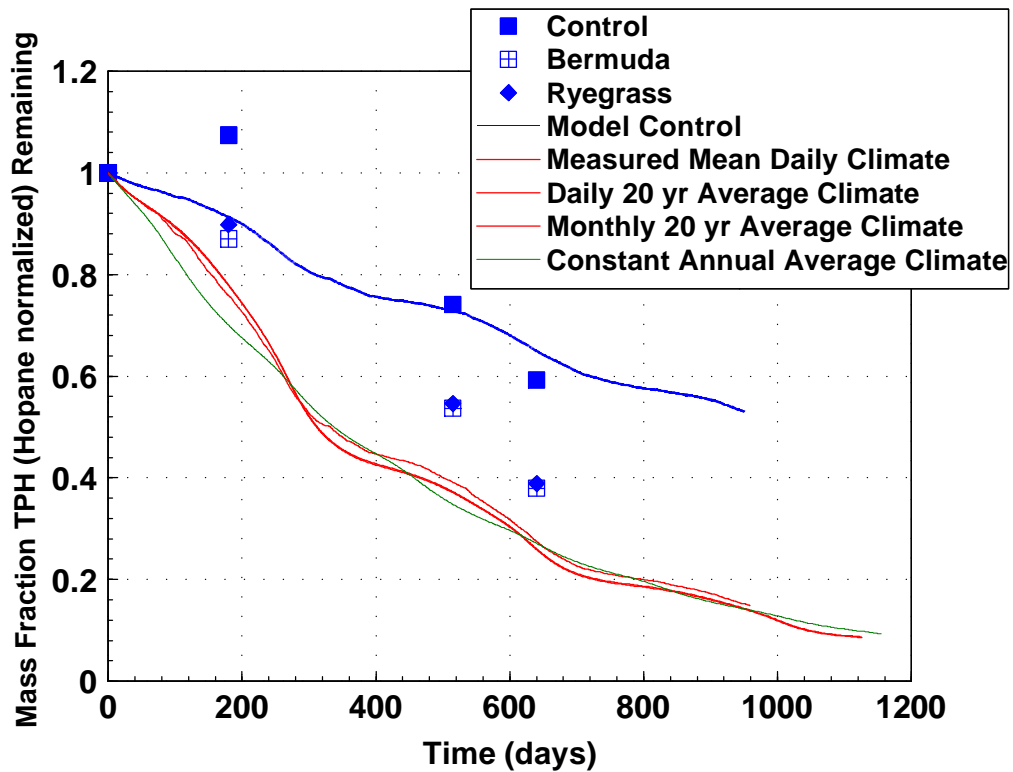


Figure 10. Effect of climate data set on model prediction.