

Molecular Characterization of Microbial 4 Fuel Contaminated Soil

in a JP-

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2 **MOLECULAR CHARACTERIZATION OF MICROBIAL COMMUNITIES**
3 **IN A J-P-4 FUEL CONTAMINATED SOIL**

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13 **ABSTRACT:** In this study, lipid biomarker characterization of the bacterial and eukaryotic
14 communities was combined with PCR-DGGE analysis of the eubacterial community to evaluate
15 correlation between JP-4 **fuel** concentration and community structure shifts. Vadose, capillary
16 **fringe** and saturated- soils were taken **from** cores within, up- and down-gradient of the
17 contaminant plume. Significant differences in biomass and proportion of Gram negative bacteria
18 were found inside and outside the plume. Sequence analysis of DGGE bands from within the
19 spill site suggested dominance by a limited number of phylogenetically diverse bacteria. Used in
20 tandem with pollutant quantification, these molecular techniques should facilitate significant
21 improvements over current assessment procedures for determination of remediation end points.
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23 **INTRODUCTION**

24 **Shifts** in microbial community structure provide a sensitive target for assay of the
25 progress of bioremediation. The dominant organisms of contaminated sites are likely to be active
26 in remediation of the contaminant. By combining PLFA analysis with PCR-DGGE analysis of
27 the bacterial community we document herein shifts in a field population structure resulting
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1 **Volatile Organic Compounds.** Samples were analyzed for **VOCs** on a HP-5890 series II Gas
2 Chromatograph (GC) with an HP 5972 mass selective (MS) detector as described by Fang *et al.*,
3 (1997). Separation was accomplished using an HP-624 GC column: 60 m x 0.25 mm i.d. (film
4 thickness $d_f = 1.8 \mu\text{m}$; Hewlett-Packard). For all 43 compounds detected, calibration curves were
5 linear between 1.0 $\mu\text{g/L}$ to 200 $\mu\text{g/L}$. Compounds were identified based on relative retention
6 time and verified by mass spectra. Concentrations of **VOCs** were calculated using the internal
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RESULTS AND DISCUSSION

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1 (DMA/PLFA) followed the order, capillary fringe> saturated>vadose zone. The relative DMA
2 concentration followed no discernable order in the remaining bore-holes.

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4 **DGGE analysis of microbial diversity.** DGGE analysis of triplicate sub-samples taken within
5 the heavily JP-4 impacted zone showed strong and reproducible banding and stratification at all
6 depths (Figure 2). Two sequences were recovered from all three depths and represented unknown
7 organisms from the P-subgroup proteobacteria. Four bands were absent from the saturated zone,
8 representing an α - and 3 β -proteobacterial sequences. The capillary fringe displayed 3 unique
9 bands, representing an uncultured bacterium associated with the Flexibacter-Cytophaga-
10 Bacteroides phylum, an α and a β -proteobacterium. A member of the Cytophaga-subgroup was
11 found in both capillary and saturated soil. Five sequences were recovered from only the saturated
12 zone, and represented members of the α , p- and e-subgroup proteobacteria, Flexibacter-
13 Cytophaga-Bacteroides- phylum and the Cram-positive phylum. None of these bands were
14 visible outside the plume, suggesting that these organisms were active in remediation of the JP-4.

15 CONCLUSION

16 Shifts in biomass content and community structure throughout the JP-4 contaminated soil
17 samples were detected related to the increased VOC concentration. Measured as PLFA, the
18 highest viable biomass levels were detected in the most highly contaminated site.

19 ACKNOWLEDGEMENTS

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21 Development Program (SERDP contract number 1 XSY887Y).

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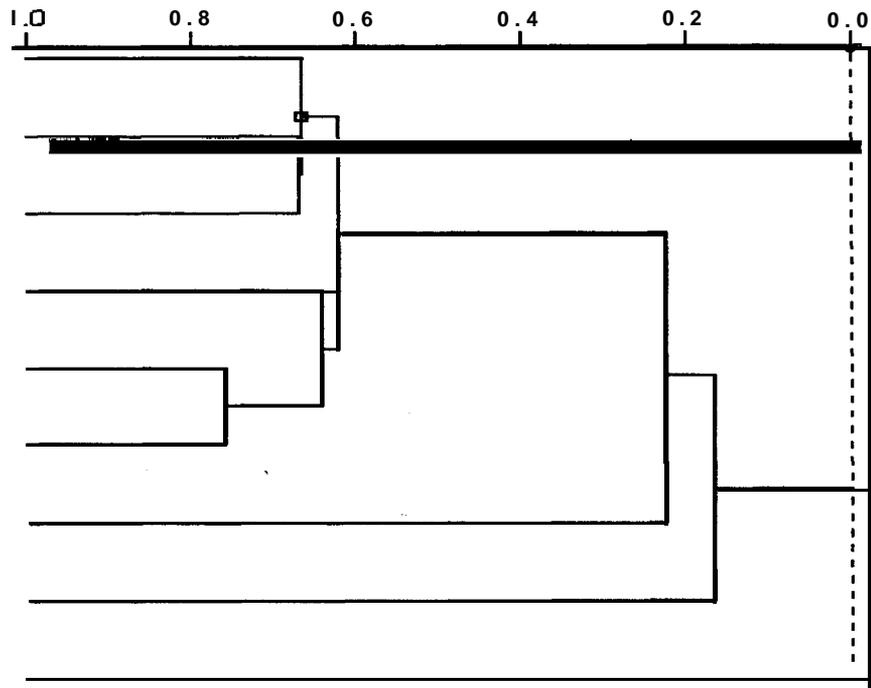
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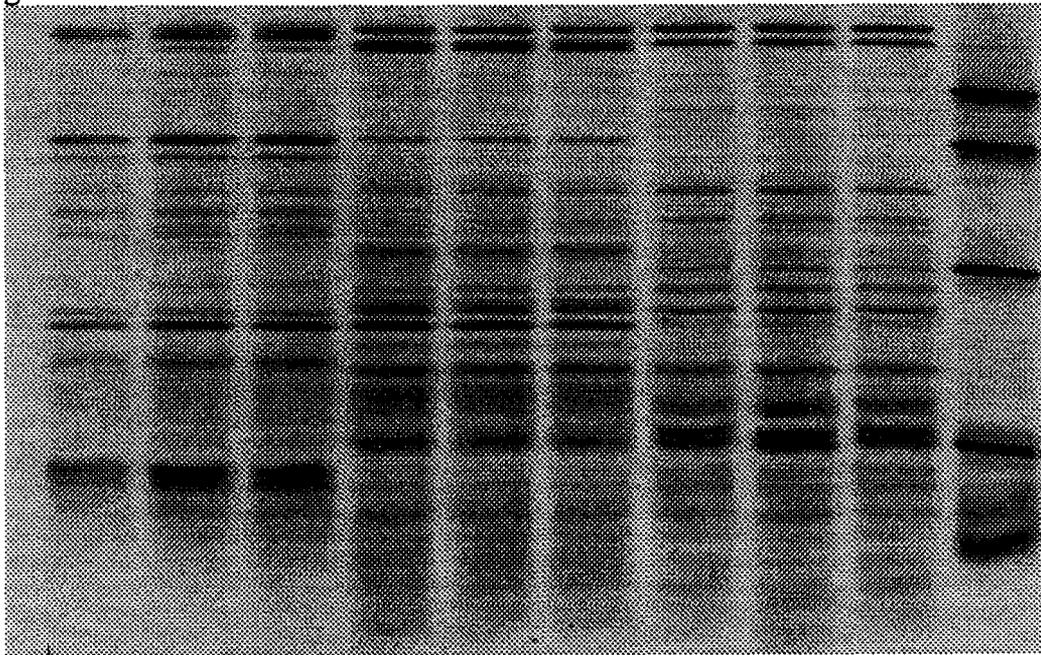
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3 Figure 1: A dendrogram representation of a hierarchical cluster analysis (single linkage based on
4 euclidean distance) for the bacterial PLFA profiles. DG, down-gradient; CS, crash site; UG, up-
5 gradient.



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7 Figure 2. DGGE-eubacterial community profile of soil from within the crash-site. The portion of
8 the gel shown represents the range of profiles that were found.
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