



# Microbial Community Structure in a Shallow Hydrocarbon-Contaminated Aquifer Associated With High Electrical Conductivity

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## ABSTRACT

Little is known about the complex interactions between microbial communities and electrical properties in contaminated aquifers. In order to investigate possible connections between these parameters a study was undertaken to investigate the hypothesis that the degradation of hydrocarbons by resident microbial communities causes a local increase in organic acid concentrations, which in turn causes an increase in native mineral weathering and a concurrent increase in the bulk electrical conductivity of soil. Microbial community structure was analyzed using a 96-well most probable number (MPN) method and rDNA intergenic spacer region analysis (RISA). Microbial community structure was found to change in the presence of hydrocarbon contaminants and these changes were consistently observed in regions of high electrical conductivity. We infer from this relationship that geophysical methods for monitoring the subsurface are a promising new technology for monitoring changes in microbial community structure and simultaneous changes in geochemistry that are associated with hydrocarbon degradation.

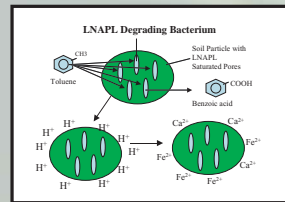
**Notice:** The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), approved this abstract as a basis for an oral presentation. The actual presentation has not been peer reviewed by EPA.

## HYPOTHESIS

- Microbial production of organic acids causes an increase in the dissolution of native soil particles, decreasing soil resistivity.

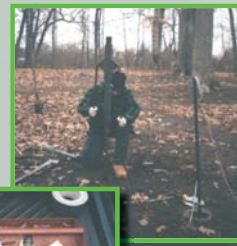
## RESEARCH QUESTIONS

- Are there changes in the composition of microbial communities at depths with anomalous resistivity readings?
- Is there evidence of increased weathering of soil particles in a contaminated vs. non-contaminated aquifer?



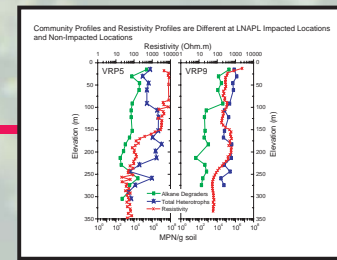
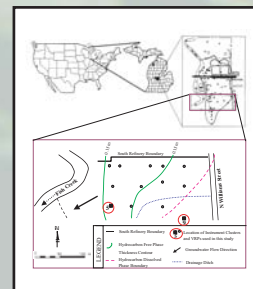
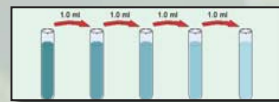
## MICROBIAL COMMUNITY ANALYSIS: CULTURE BASED APPROACH

- Soil Cores
  - Aseptic sampling
  - Most Probable Number (MPN)
    - 15 cm (6 in)
    - Heterotrophic microbes
    - Alkane degrading microbes



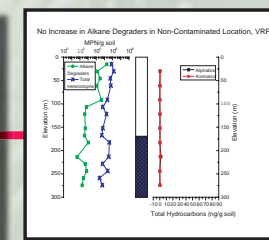
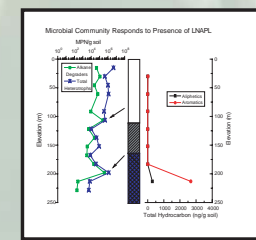
## Most Probable Number Analysis

- Extract bacterial cells from sand
- 10-fold serial dilution series
  - 5 Replicates
- Statistical Program computes MPN based on terminal dilution
- Make depth-wise profiles of MPN values
  - Heterotrophs
  - Alkane Degraders
- Calculate
  - What percentage of the heterotrophic community can degrade alkanes?



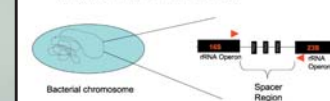
## MPN APPROACH RESULTS

- Zones of increased percentages of alkane degrading bacteria at contaminated sites
  - Concurrent with areas of contamination
  - Increased percentages of alkane degraders are not observed at non-contaminated control site
- Increased percentage of alkane degraders are concurrent with regions of anomalously low apparent resistivity

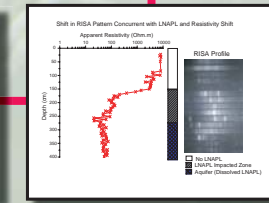
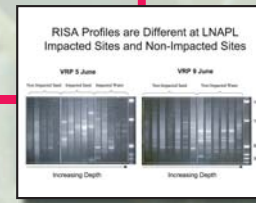


## Microbial Community Analysis: Molecular Approach

- Extractions of total chromosomal DNA from field soil samples
  - Community fingerprint analysis
  - RISA (DNA intergenic spacer region analysis)



- Isolate chromosomal DNA from soil organisms
- Amplify spacer region using PCR and primers specific for ends of 16S and 23S rDNA
- Separate bands on 2% agarose gel

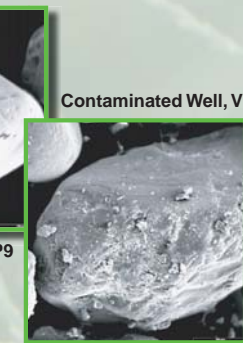


## SEM Images: Soil Grains



Control Well, VRP9

Bar = 100 μm



Contaminated Well, VRP5

## MOLECULAR APPROACH (RISA) RESULTS

- RISA pattern shifts are coincident with region of decreased resistivity
- Community profiles from contaminated sites have fewer, but more dominant members than non-contaminated sites
- RISA profiles from contaminated sites show increased depth-wise variability when compared to non-contaminated controls

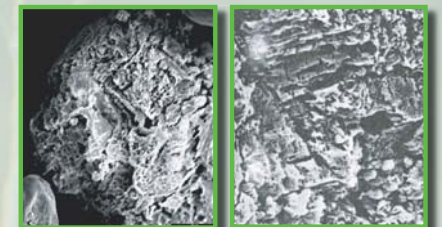
## CONCLUSIONS

- Microbial community structure measured by culture based and molecular methods responds to hydrocarbon contamination
- Alterations in both the cultural and molecular community structure are coincident with geophysical changes
- Increased mineral etching occurs in zones of high percentages of alkane degraders and in depths of low resistivity levels

## REFERENCES

- Heibert, F. K. and Bennett, P. C., Microbial control of silicate weathering in organic-rich ground water, *Science*, 258, 278-281, 1992.
- Berner, R.A. and Holdren, G. R., Jr. 1979. Mechanism of feldspar weathering-II. Observations of feldspars from soils. *Geochim. Cosmochim. Acta* 43:1173-1186.

## Extreme Etching: Aluminosilicate Deposits



This Study

Berner and Holdren, 1979