

# Soil Treatability Study

Energy Technology Engineering Center • U.S. Department of Energy

## *Bioremediation Study*

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U.S. DEPARTMENT OF  
**ENERGY**



# Bioremediation Study Overview

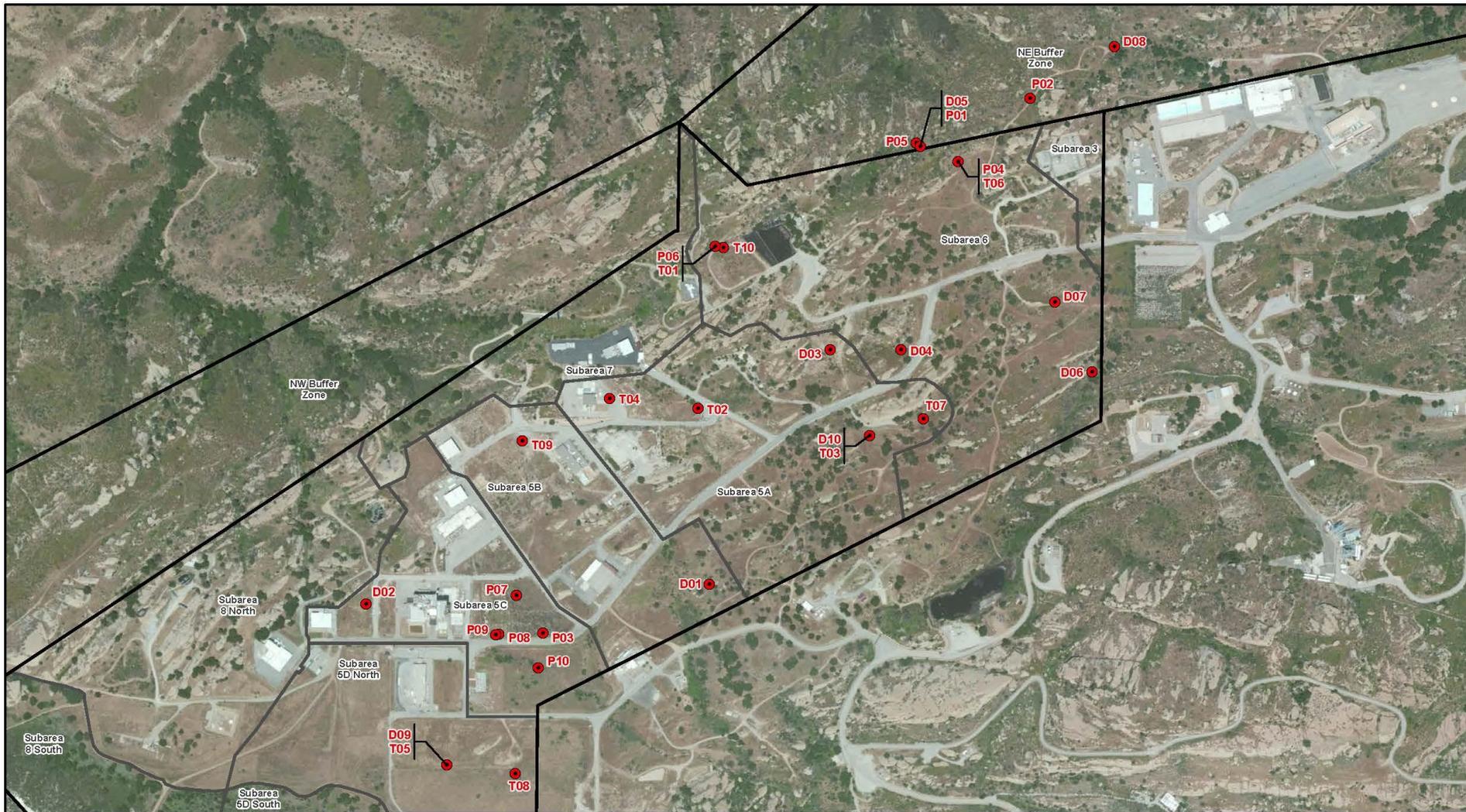
- Phase 1: Analysis of microbial communities in the field
  - Identify potential chemical-degrading microorganisms in Area IV soils
    - Terminal Restriction Fragment Analysis (TRFLP)
    - Culturing of microorganisms from Area IV soils & sequencing
    - Metagenomics for complete microbial characterization
- Phase 2: Laboratory microcosms using soil from Area IV
  - Incubate in the laboratory under controlled conditions
  - Measure biodegradation rates under natural attenuation conditions
  - Estimate efficacy of biostimulation and bioaugmentation



# Bioremediation Study: Phase 1 Status

- Study Plan in review
- Soil sampling locations selected
- Model microorganisms cultivated
- Arrangements made for metagenomic assays

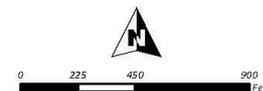
# Soil Sampling Locations for Phase 1 Field Study



- Legend**
- Potential Bioremediation Phase 1 Sample Location
  - ▭ Area IV Subarea
  - ▭ Area IV & Northern Buffer Zone

Service Layer Credit: Source: Esri, DigitalGlobe, GeoEye, i-cubed, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community.

**Proposed Bioremediation Treatability  
Study Phase 1 Sampling Locations**

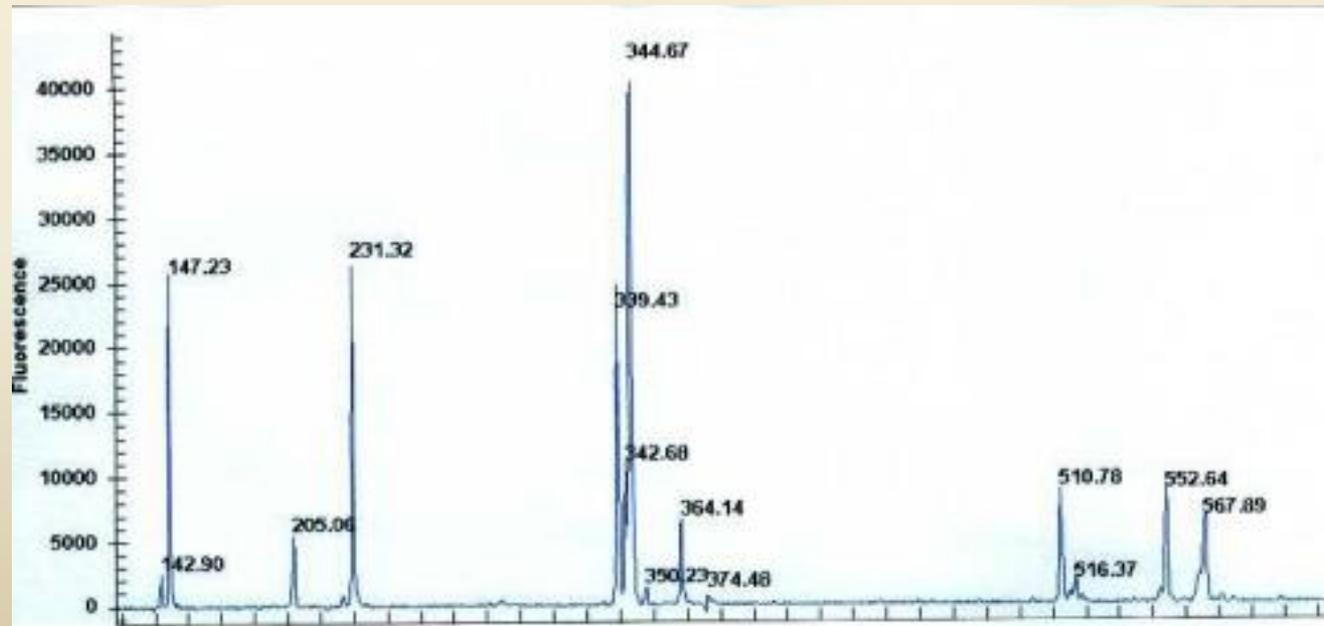


Santa Susana Field Laboratory  
Ventura County, California  
Figure 4-1



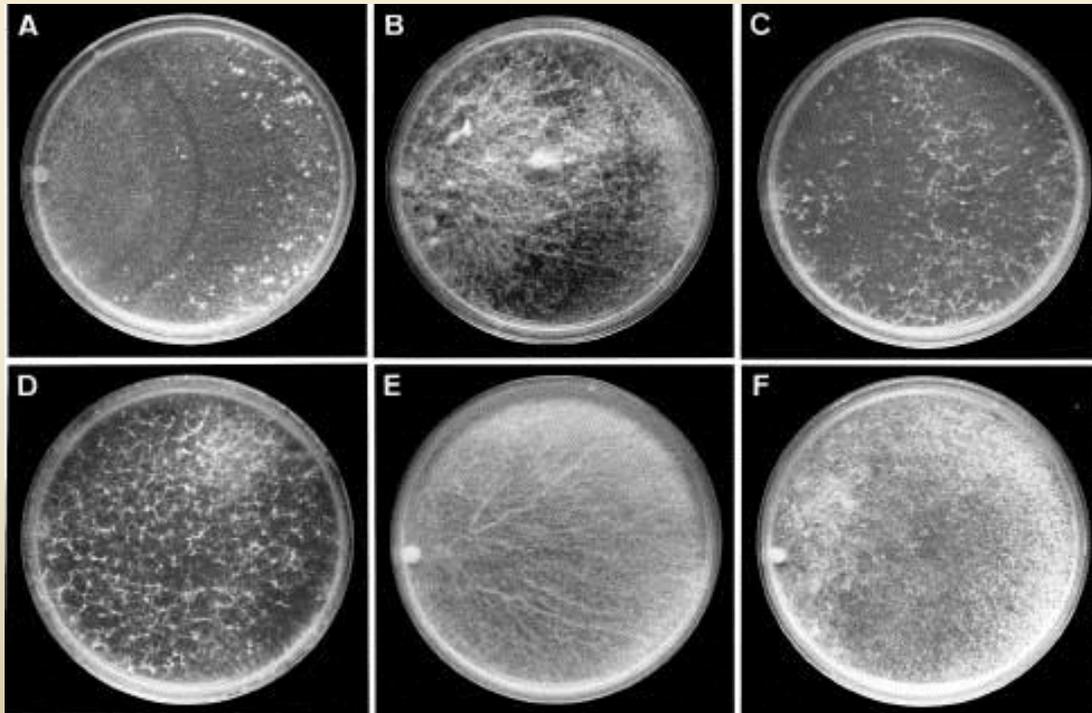
# Identification of soil microorganisms: Terminal Restriction Fragment (TRFLP) Analysis

- Provides the relative abundance of specific genetic sequences
- These genetic sequences can be correlated to microbial degraders using data from other analyses
- Gives a good indication of the microbial population diversity in a sample



# Isolating pure-cultures of soil bacteria and fungi from Area IV soils

- Grow on model compounds with no other carbon source
- Sequence 16S DNA
- Compare to known degraders of Area IV chemicals



e.g. *Phanerochaete chrysosporium*, White-Rot Fungi – PCB degrader

# Metagenomics



- Use of modern genomics techniques to characterize microbial communities directly in natural environments, without laboratory cultivation of individual species
- Provides genus and species level identification
- Provides relative populations of different classes of microorganisms
- Available through Sandia National Laboratory (at Livermore)

# Bioremediation Study: Phase 2 Status

- Soil collected and processed for use in microcosms



# Bioremediation Study: Deep Soil Collection



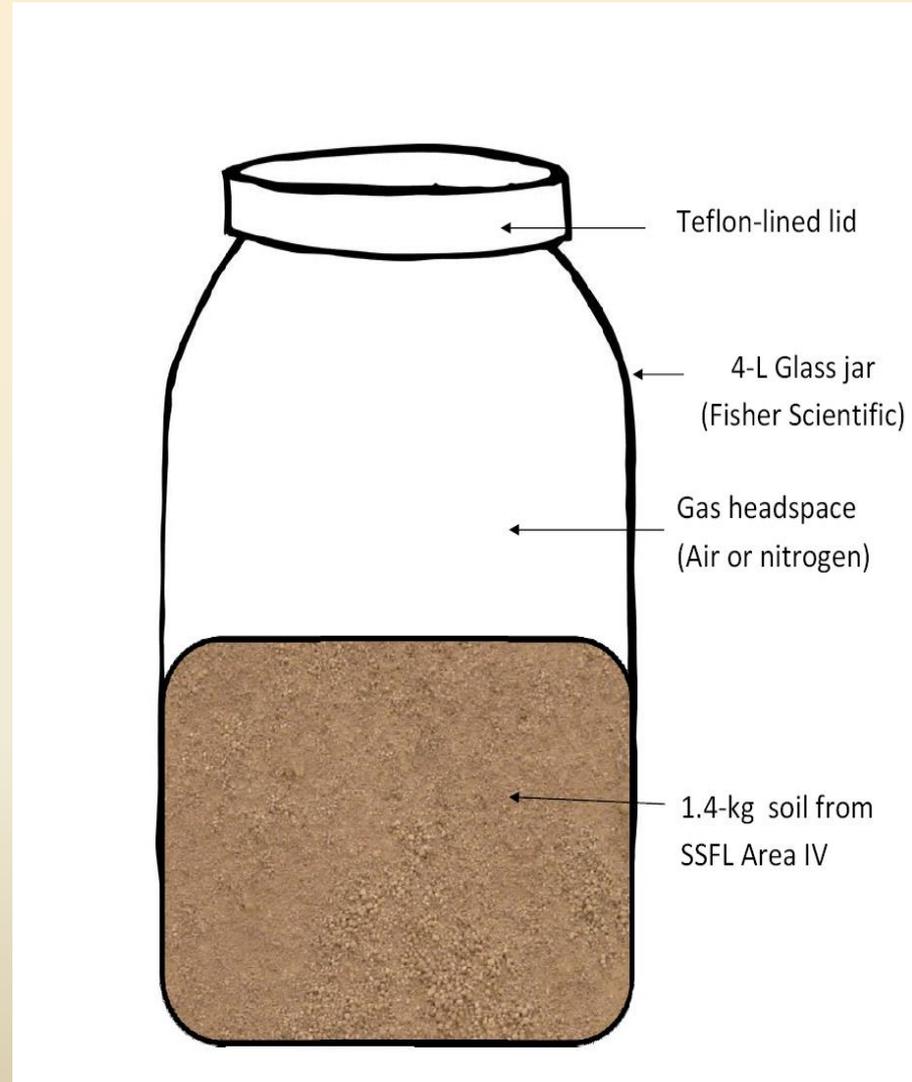
# Bioremediation Study: Soil Collection

Soil transported to Cal Poly  
in labeled Teflon-lined 5-gal buckets



# Bioremediation Study: Microcosm set up

- Incubate in the dark at 14°C to simulate soil conditions at SSFL
- Measure chemical concentrations in soil after 0, 4 and 9 months





# Bioremediation Study: Microcosm Amendments

	Microcosm Type
<b>Un-amended</b>	Sterilized
	Unsterilized
<b>Amended</b>	Fertilizer
	Rice hulls
	Rice hulls + fertilizer + <i>P. chrysosporium</i> fungi
	Soya lecithin
	Combination

# *Questions?*

