

**FINAL REPORT**  
**U.S. Department of Energy**

**Control of Biologically Active Degradation Zones By Vertical Heterogeneity: Applications In Fractured Media**

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### ***3. Executive Summary:***

The purpose of this research was to determine the relationship between of biologically active contaminant degradation zones in a fractured, subsurface medium and vertical geological heterogeneities. The research was performed on samples collected from three subsurface locations at the Test Area North (TAN) site at the Idaho National Engineering and Environmental Laboratory (INEEL) where a dissolved trichloroethylene (TCE) plume is migrating in the basalts and interbed sediments of the Snake River Plain (SRP) aquifer. The microbiological properties of paired cores and water samples from boreholes located in differing geochemical and flow environments within the plume were studied. A multi-level sampler was used to provide fine-scale vertical resolution of how geochemical and hydraulic properties effect important microbial processes.

Key among our findings was evidence of a large, multidimensional variability in the microbial distribution in the fractured basalts at TAN. We observed that the strongest correlate to microbial properties (i.e., biomass, key physiological groups) was the concentration of TCE in the groundwater and therefore the distance of the sampling site from the original waste injection well. In contrast with our hypothesis, in this fracture-flow environment we could not detect a significant relationship between any of the measured microbial parameters and the physical heterogeneities that are associated with multiple layers of basalt flows and which determine the primary flow paths of water and contaminants. In other words, in a given borehole microorganisms did not seem to prefer any specific strata in the vertically, and physically distinct, sampled sequences; however, higher biomass and physiological diversity seemed to be controlled mainly by the location of the borehole with respect to the original source of contamination.

The relevance, impact, and transfer of technologies associated with this research include: 1) subsurface sampling technologies provided to Idaho Water Resources Research Institute researchers and EM-40 scientists conducting tests for enhanced in situ bioremediation at TAN, 2) determination of the broad distribution of naturally-occurring TCE-degrading microorganisms in the TAN TCE plume, and 3) detection of methane in the aquifer at TAN which can be used by methanotrophs during TCE cometabolism. An improved understanding of the constraints on the activities and distribution of TCE-degrading organisms by the geochemical and hydrological environment allows

better decisions to be made regarding the use of remedial technologies such as natural attenuation and in situ bioremediation at geologically complex waste sites.

This research was multi-disciplinary with scientists from different national labs and universities involved in the characterization of microbial communities and the chemical characteristics of the aquifer. Portions of two Ph.D. dissertations were supported through this work (R.M. Lehman and S.P. O'Connell, both from Idaho State University). Briefly, their relevant results suggest: 1) partitioning of microorganisms between the attached and free-living phases in fracture-flow subsurface environments may be different than in porous-flow environments, 2) the use of dialysis cells or other colonization substrata incubated in a well do not accurately reflect the microbiota that are present in the native geologic (before the borehole existed) or present in the ambient groundwater, 3) indigenous microorganisms collected from wells that are different by virtue of TCE concentrations demonstrate physiological differences that can be used for ecological risk assessment and determination of relevant remediation endpoints.

#### **4. Research Objectives**

The key objective of this research was to determine the distribution of biologically active contaminant degradation zones in a fractured, subsurface medium with respect to vertical heterogeneities. Our expectation was that hydrogeological properties would determine the size, diversity, and activities of microbial communities in fractured basalt by controlling the fluxes and concentrations of aqueous constituents upon which these communities depend. We expected that microorganisms would be more abundant, of greater diversity, and of relatively higher metabolic activity within zones of high permeability that contain favorable concentrations of electron donors and acceptors; the composition and flux of these solutes will reflect the spatial continuity of interflow fracture and rubble zones. We further expected that the composition and dynamics of microbial communities associated with rock surfaces in fractured basalt aquifers could be predicted by the incubation of a native rock substratum placed at discreet, isolated intervals within a borehole.

#### **5. Methods and Results**

##### **Methods**

##### **Site description, lithology, aquifer characteristics, and hydrochemistry**

The Idaho National Engineering and Environmental Laboratory (INEEL) and Test Area North (TAN) are located on the Eastern Snake River Plain, a semi-arid high desert in southeastern Idaho, USA. The subsurface stratigraphy at the INEEL consists of multiple basalt flows that are interbedded with thin sedimentary zones consisting of silts and clays with sporadic presence of sand and gravel layers (1, 6). The basalt flows are the dominant lithologic feature and within a given flow, several well-recognized horizontally and vertically distributed facies may be present. The vertical facies (from flow bottom to top) are most important and include a basal rubble zone, a lower vesicular zone, a massive central columnar jointed zone, an upper vesicular zone, and a zone of platy jointed crust capping the flow. The porosity and permeability of the aquifer system is primarily attributed to the rubble zones, cooling fractures, and vesicular zones within the basalt sequences. The median thickness of basalt flow units in the TAN vicinity is approximately 4.5 m (11).

The active thickness of the Snake River Plain (SRP) aquifer beneath TAN is estimated to be between 64 and more than 274 m (11). At TAN the water table is notably flat and the direction of groundwater movement is generally from north to south. However, because of the flat water table, the local flow direction varies seasonally and is affected by the pumping of TAN production wells. Estimated rates of groundwater movement at TAN range between a low of 0.0006 m/d and a high of 1.3 m/d with a median value of 0.06 m/d (11). Aquifer transmissivity estimates for the TAN vicinity have a median value of 3,600 m<sup>2</sup>/d (range: 40 to 74,000 m<sup>2</sup>/d) (11) which is low compared to the regional aquifer transmissivity of 25,000 to 37,000 m<sup>2</sup>/d (19).

Groundwater contamination at TAN resulted from previous injection well disposal of liquid wastes to the SRP aquifer. From 1953 to 1970, organic, inorganic, and low-level radioactive wastewaters, and sanitary wastewaters were injected into a well known as TSF-05. Depth to groundwater in TSF-05 is approximately 63 meters below land surface (mbls). The TSF-05 injection well is the source of a trichloroethylene (TCE) plume that presently extends 3,100 m downgradient with concentrations ranging from 32,000 µg/L at the injection well to less than 5 µg/L at the distal end of the plume (21, 22). Although TCE is the primary contaminant of concern, additional groundwater contaminants include other volatile organic compounds tetrachloroethene, cis- and trans-1,2-dichloroethene, as well as radionuclides strontium-90, tritium, cesium-137, and uranium-234. The TCE plume resides between the top of the aquifer at ca. 61 mbls and the Q-R sedimentary interbed at 134 mbls (11). Generally, dissolved TCE is thought to migrate in the highly permeable zones in the basalts and become retarded in or diverted by the fine-grained, sedimentary interbeds. Distribution of the contaminant plume is subject to

fractures and rubble zones in the basalts that represent preferred flow paths for contaminants and other groundwater constituents.

Groundwater chemistries for wells at TAN are presented in Table 1. The wells in Table 1 are arranged from northwest to southeast (local flow direction) along the axis of the TCE plume. Contaminated wells are characterized by higher sodium and chloride values and these wells have lower dissolved oxygen values. The lower dissolved oxygen values reflect the disposal of organic sludge and may be due to either biotic or abiotic processes. Elevated bicarbonate concentration may reflect higher concentration in the injected waste, in situ mineralization of injected organic matter, or both.

### **Core Collection and Handling**

Subsurface cores were collected for microbiological and chemical analyses from the saturated zone from three boreholes located within the TCE plume at TAN (Figures 1 and 2). Cores were acquired from ca. 67 to 134 mbls. One borehole (TAN-37) was located proximal (35 m) to the injection well in a zone that is heavily influenced by the waste (2000-3000 ppb dissolved TCE). A second hole, TAN-33, was located 425 m from the injection well (ca. 900 ppb dissolved TCE). The third borehole, TAN-48, was 600 m from the injection well (ca. 100 ppb dissolved TCE). The cored intervals in TAN-37 and TAN-33 were bounded on the upper and lower ends by the PQ and QR sedimentary interbeds. In TAN-48, the cored intervals were only at the uppermost part of the aquifer near the PQ interbed. Drilling and coring were accomplished using reverse circulation with an air-water drilling fluid that had been previously disinfected by adding household bleach (final concentration: 10 ppm sodium hypochlorite). Cores were 8.1 cm diameter, 1.5 m long (maximum length) and 23, 19, and 5 cores for microbiological investigations were acquired from TAN-37, TAN-33, and TAN-48, respectively (Figure 2).

During coring, fluorescent, bacterial-sized, carboxylated microspheres (Polysciences Inc., Warrington, PA) were added to the cores in order to distinguish between core material that had contacted the drilling fluids and that which was relatively pristine (6). These microspheres were surrogates for microbial contamination that can occur during coring and were enumerated using epifluorescent microscopy of the core materials. Core quality was also assessed using perfluoromethylcyclohexane or PFMCH (Sigma, Inc.) as a chemical tracer of the drilling fluid. PFMCH was introduced in the drilling fluid using an HPLC pump (rate: 0.5-1 ml/min) and subsequently analyzed in the core materials using gas chromatography with an electron capture detector (6, 17). The indigenous microbial community was used as a tracer by comparing the community-level physiological profile (CLPP) response to multiple carbon sources in the drilling fluids to that in the cores (14). Samples of the drilling fluid were acquired by bailing water from the borehole at the start and end of each day of coring and the CLPP responses of these samples were compared to CLPP responses from cores acquired during the course of that day.

Within 0.5 h of arrival at land surface cores were transferred to an anaerobic glove bag for sample processing. Sample processing involved removal of the outside of the cores (core parings) to obtain the internal subcore which was used for sample analyses (6). On the day that cores were collected in the field, subcore materials were shipped by overnight courier to participating microbiologists at distant laboratories. Samples for molecular studies (e.g., PLFA or DNA extraction) were frozen in the field lab and shipped with dry ice while samples for culture-based investigations were refrigerated at 4 °C. Along with authentic core materials, investigators were periodically sent blind controls that included basalt combusted at 500 °C on two successive days and basalt spiked with high numbers of an active microbial culture. For each core interval, samples of the cores to be used for microsphere, perfluorocarbon, and indigenous microbial tracer analyses were obtained at the same time as samples for microbiological study.

Following coring each of the boreholes remained as open holes (without casing) to allow acoustic borehole televiewer study (12). Acoustic data were collected by Colog, Inc. (Golden CO) in each of the boreholes allowing macroscopic fracture analysis, determination of fracture distribution (GeoMechanics International, Palo Alto, CA), and the presence of rubble zones that form the interfaces between adjacent basalt flows and that serve as the

preferred flow paths for groundwater in the basalt aquifer. Conclusions regarding the distribution of rubble zones drawn from televiewer data were corroborated by direct observation of cores.

### **Microbiological Analyses**

Microbiological analyses conducted on pared core samples included biomass determinations using phospholipid fatty acid (PLFA) analyses (25). For each core that was collected, portions were aseptically inoculated into most probable number (MPN) series using several types of growth media that were selective for the enrichment of microorganisms known to degrade TCE. These culture-based enumerations of various physiologies included conditions specific for methanotrophs (26), propanotrophs (2), phenol-oxidizers (5), ammonia oxidizers (3), and iron reducers (16). Oligotrophic heterotrophs were enumerated by plate count on R2A medium (23). All cultural enumerations were incubated at room temperature for at least 90 days before counting.

DNA extraction, PCR amplification of 16S rDNA genes, clonal analysis, and denaturing gradient gel electrophoresis (DGGE) were performed on selected rock and water samples (see (15) for methods). Acetate mineralization was conducted on core samples as previously described (9) except that both anaerobic and aerobic incubations were conducted on splits of the samples.

### **Chemical Analyses**

TCE in groundwater and on basalt samples was determined using gas chromatography (Hewlett-Packard model 5890A) with a photoionization detector and a Hall detector. The detection limit for these analyses was 0.25 µg/kg.

## **Results**

### **Sample Quality and TCE Concentrations**

Data from intentionally introduced microsphere tracers during the coring of TAN-33 indicated that in all cases the subcores had fewer microspheres than the parings from the outside of the cores (Figure 3). In most cases, the numbers of microspheres between the outside and the inside of the cores exhibited a decrease of several orders of magnitude suggesting a comparable level of decrease in potential microbial contaminants that might have come from the drilling and sample handling process. With one exception, data from TAN-37 also indicate lower numbers of microspheres in the interior of the cores (data not shown); however, in many cases the decrease was not as pronounced as observed between core parings and subcores from TAN-33. During the coring of TAN-37 we evaluated the process by which the glove bag and the core processing tools were sterilized. These experiments suggested that our disinfection methods successfully reduced the numbers of cells that could be cultured from the glove bag and tools (data not shown); however, this cleaning of the sample processing equipment did not eliminate microspheres indicating that successive cores containing microspheres may contribute to an increasing level of this tracer on the sampling and sample handling equipment.

Community-level response to multiple carbon sources of indigenous microbial communities in the drilling fluids and communities in the cores allowed limited interpretation due to an absence of response in most of these samples. This suggests that the drilling fluid was a minimal source of microbial contamination during coring. This contrasts with past subsurface coring efforts in which the biomass of the microbial communities in the drilling fluids was several orders of magnitude higher than the biomass in the cores (7, 14). Together with the microsphere tracer data, the indigenous microbial tracers suggest that the cores were not microbially altered to any great extent as a result of core acquisition and handling.

Measurable levels of PFMCH were found in many of the TAN-33 samples and in all of the TAN-37 samples. For TAN-33, there was a generalized, ten-fold decrease in PFMCH concentrations between the core parings and the subcore (Figure 3); however, for many of the samples the concentrations were low (< 10 µg/kg core) and the parings and subcores from a common depth were indistinguishable. For TAN-37, quantitatively higher PFMCH additions overall were achieved during coring but as for TAN-33 in many cases PFMCH concentrations in the

parings were indistinguishable from that of the subcores (data not shown). PFMCH traces the movement of gases in the samples as a result of core acquisition and handling. Previous use of perfluorocarbon tracers while coring fractured basalt in the vadose zone indicates that the chemical can be forced at least 7 m into the formation ahead of the drill bit by the drilling fluid (6). Although we did not measure it, the extent of this gaseous alteration is probably significantly attenuated during coring TAN because our samples came from saturated basalts as opposed to the unsaturated basalts cored by Colwell et al. (6). Thus, the measurement of low quantities of PFMCH in our samples from TAN suggest that there may have been some perfusion of the drilling fluid gases into the samples during coring.

TCE concentrations determined by extracting the chlorinated compound from solid core samples from TAN-37, TAN-33, and TAN-48 were consistently low ( $\leq 3$ ,  $\leq 6$ , and  $\leq 6$   $\mu\text{g/L}$ , respectively) even though the water at those locations contained approximately 5000, 900 and 200  $\mu\text{g/L}$ , respectively. That the values for TCE adsorbed to the basalt were so low relative to the concentrations of TCE evident in groundwater at these locations is corroborated by evidence of low adsorption of TCE on basalt (10). Also, there is no apparent relationship between the concentration of TCE obtained from the solid core samples and the concentration in the surrounding groundwater. None of the core samples obtained from all three wells had TCE values that exceeded 6  $\mu\text{g/L}$  and in many of the samples TCE could not be detected, even though water samples from TAN-37 had > 40-fold higher TCE concentrations than water from TAN-48. In TAN-48, TCE concentrations were highest at 103 mbls, just above a series of fractures and an interflow zone, an area not sampled for microbial properties. The TCE concentrations in TAN-33 cores also peaked at 103 mbls, just above a zone of pervasive fractures that correspond to a perceived interflow zone as determined by the acoustic borehole televiewer data (Figure 2). In TAN-37, the highest TCE values in the cores occurred at depths between 68 and 79 mbls, near the top of the aquifer at this location. These data are consistent with acoustic borehole televiewer data that suggest the presence of proximal interflow zones (Figure 2). Cross-hole seismic tomography conducted in TAN-37 and neighboring wells also indicate that the primary flowpath for contaminant movement near the sources appears to exist at ca. 74 mbls in TAN-37 (8).

### **Microbiology**

Taken collectively for all samples in a given borehole, microbial biomass (cultured and non-cultured) in the basalt from the distal two boreholes (TAN-33 and -48) was at the limit of detection for many of the assays that were used to assess the microbial community. Rates of aerobic and anaerobic acetate mineralization were minimal for selected samples from these two boreholes. On the other hand, basalt from near the injection well (TAN-37) showed low but measurable biomass by PLFA (ca. 3 pmol/g), much higher levels of acetate mineralization, and positive enrichments for nearly all physiological types of microorganisms in many of the samples. When the microbial data from an individual borehole is averaged for all of the depths that were sampled within that borehole and then these data are compared among boreholes there is a strong positive relationship between these microbial measures and the proximity to the injection well (Figure 4). Increasing distance from the injection well (along the groundwater flow path) and diminishing concentrations of TCE are strong predictors of lower levels of microbial biomass, numbers of different physiological groups, and general microbial activity. Strong positive correlations of microbial properties to TCE concentration in the water from the different wells was evident (Table 2), although marked variability in the data from a given borehole was also apparent. When the microbial data from a single borehole (i.e., TAN-37) is considered with respect to the presence of rubble zones that have been verified as active flow zones there are no significant correlations (Table 3).

Key among our findings was evidence of a large, multidimensional variability in the microbial distribution in the fractured basalts at TAN. We observed that the strongest correlate to microbial properties (i.e., biomass, key physiological groups) was the concentration of TCE in the groundwater and therefore the distance of the sampling site from the original waste injection well. In contrast with our hypothesis, in this fracture-flow environment we could not detect a significant relationship between any of the measured microbial parameters and the physical heterogeneities that are associated with multiple layers of basalt flows and which determine the primary flow paths of water and contaminants. In other words, in a given borehole microorganisms did not seem to prefer any

specific strata in the vertically, and physically distinct, sampled sequences; however, higher biomass and physiological diversity seemed to be controlled mainly by the location of the borehole with respect to the original source of contamination.

While statistically significant correlations between microbial properties and the presence of hydraulically important flow zones were not apparent, cores from TAN-37 exhibited some evidence that microbial communities may be structured by the contrasting hydraulic conditions of the rubble zones relative to the massive basalt layers. Evidence of this comes from somewhat higher enumerations for some microbial physiologies near some of the rubble zones in TAN-37 (data not shown). TAN-37 lies within an area of active pumping of the aquifer and this creates a forced hydrologic gradient wherein some strata are continuously swept by water while others are outside the influence of the pumping. TAN-33 is away from the zone of influence of the active groundwater pumping and there is no evidence of a groundwater gradient in this location. Differences in microbial communities in vertically distinct zones in TAN-33 are inapparent and may be below the limits of detection by the methods that we have used in this study. Thus, geological heterogeneities may play an important role in structuring microbial communities if a significant aquifer gradient exists at a given location.

In TAN-37 cores, the following order of prevalence was discernible from MPN enumerations of microorganisms known to be capable of TCE co-metabolism: phenol oxidizers ( $< 10^5$  cells/g) > propanotrophs ( $< 10^4$  cells/g) >> methanotrophs ( $< 10^2$  cells/g) >> nitrifiers ( $< 2$  cells/g). Relative to TAN-37 cores, phenol-oxidizers and methanotrophs in TAN-33 were present at much lower concentrations:  $< 10^2$  and  $< 20$  cells/g, respectively. Dissimilatory metal reducing bacteria were present in about 75% and 25% of the TAN-37 and TAN-33 core samples, respectively, while these bacteria were not detected in the TAN-48 cores.

Microbial DNA was extracted from selected TAN-33 and TAN-37 samples and amplified using PCR with bacterial and archaeal primers. TAN-33 samples did not yield visible PCR bands but when spiked with as few as  $10^4$  cells/g samples PCR bands were apparent. Invisible PCR bands from two of the TAN-33 samples along with a full procedural blank (processed without basalt) were cloned and a subset of the clones was submitted to Amplified Ribosomal DNA Restriction Analysis (ARDRA). Six ARDRA types (all bacterial) were identified from the 113.3 m sample and three ARDRA types (all bacterial) were identified from the 125.4 m sample. Basic Local Alignment Search Tool (BLAST) was used to compare the sequences of these bacterial clones to those of known microorganisms. Most of the sequences that resulted from these clones were approximately 90% similar to known bacterial sequences. Extractions from selected TAN-37 samples indicate much higher levels of DNA than in TAN-33 samples. Bacterial PCR bands were recovered from all of the samples that were amplified except the combusted basalt control sample. Only one of the ten samples yielded an archaeal PCR band. Cloning and sequencing of these PCR bands indicate a diversity of microorganisms in the samples from the top of the aquifer in TAN-37 including types that are common in soil environments (e.g., Acinetobacter, Pseudomonas, and actinomycetes). These molecular analyses of the microbial communities in TAN-33 and 37 correspond to the findings of culture-dependent assays of microbial presence, both indicating a higher level of microbial biomass and diversity in the water closest to the injection well. In a complementary study, cells were filtered from water collected in TAN wells, the DNA was extracted from these cells and then amplified (with bacterial and archaeal primers), cloned, and separated using DGGE (18). DGGE patterns show evidence of diverse archaeal (five to nine species detected) and bacterial (11-28 species detected) communities in the groundwater.

Preliminary determination of microbial diversity in TAN-37 core samples from 63.5 m (basalt; top of the aquifer) and 125.7 m (sediment interbed; below the contaminated strata) were obtained using DGGE. The diversity profiles were different for the two samples, although they did share three common banding patterns. Both samples had about eight different bands, representative of eight putative different 16S rDNA fragments. Initial recovery and sequencing of one of the dominant bands common to both samples revealed that this product was most closely related to members of Acinetobacter. This genus is consistent with the results obtained from the clone library

developed for TAN-37 samples that came from 63.5 to 78.8 mbls. Most Acinetobacter spp. are nutritionally diverse and common inhabitants of soils.

In order to understand the subsurface distribution of a key TCE-degrading microbial assemblage, we conducted experiments examining the concentration of methanotrophs in several wells from the Snake River Plain aquifer (27). In addition to the culture-based assays for methanotrophs described above, water from TAN-37, TAN-33, TAN-48, and USGS-103 (an uncontaminated control well) was filtered to obtain free-living microbial cells. DNA extracts were obtained and subsequently diluted to extinction for use in a Most Probable Number-Polymerase Chain Reaction (MPN-PCR) assay to determine the number of methanotrophs irrespective of whether they can be grown in culture. This MPN-PCR research suggested that the numbers of methanotrophs in wells TAN-37, TAN-48, and USGS-103 as determined by molecular methods are comparable to the number of these cells determined by culture-based methods. Methanotrophs were not detected in TAN-33 samples using the MPN-PCR approach. By both methods of enumeration methanotrophs accounted for no more than 0.01% of the total number of cells enumerated in the water samples. Both methods yielded similar numbers of methanotrophs in the water (0-100 methanotrophs per ml). This is consistent with our original estimates of methanotrophs in TAN cores; that only low numbers of methanotrophs are present in the aquifer at TAN and the SRP aquifer at large. Besides the fact that relatively few studies compare culture-based methods to nonculture-based (molecular) methods as a means of enumerating cells in the environment, this work is significant in providing estimates of the distribution and numbers of a prominent TCE-degrading group of microbes.

One aspect of this research documented the possibility that modern calcite cementation has occurred in the aquifer at TAN (24). Evaluation of the latest generation of calcite cement, estimated at less than 50 years old, using catholuminescence indicated elevated Mn concentrations (up to 6400 ppm) when compared to other calcite in the basalts at TAN. This incorporation of higher levels of Mn in the recently precipitated calcite is a proxy for localized oxygen consumption near the TAN injection well. This depletion of oxygen is still evident in the plume close to the source of contamination. The geochemical signature within the calcite indicates a distinctive redox condition as a result of the contaminant plume and may be useful in characterizing historical occurrences in other contaminated groundwater systems that have a tendency to precipitate calcite.

## ***6. Relevance, Impact, and Technology Transfer***

This EMSP research has helped to address EM-40 needs in the cleanup of the waste plume in groundwater by: 1) determining the specific vertical location of contaminants in a model aquifer (the Snake River Plain aquifer) and 2) establishing the presence and distribution of naturally occurring microbial communities that are capable of contaminant degradation. As a result of this research studies can commence which will focus on estimates of the natural rates of TCE remediation in the aquifer at TAN. This EMSP research has assisted EM-40 and regulatory agencies that have responsibility for the cleanup activities, in determining where aggressive remediation must be conducted and where it is likely that natural attenuation of the contaminants will occur.

This project initiated the transition from basic subsurface microbiology and geochemistry field research fostered by the Office of Science, Office of Biological and Energy Research, Subsurface Science Program (SSP) to applied microbiology and geochemistry aimed at addressing EM needs in the field. This effort was formally initiated by convening a workshop at INEEL (March 1996) to bring DOE Environmental Restoration personnel and SSP scientists together to seek solutions to subsurface issues associated with the contamination of the fractured basalts at TAN. Technologies developed and deployed for sampling the subsurface at TAN through this EMSP research were transferred to Idaho Water Resources Research Institute scientists for their parallel research. Through a unique interaction between Operations and Research at the INEEL this EMSP project was responsible for the "aseptic" sampling of the subsurface at TAN as required by cleanup activities for EM-40. These samples were required by EM-40 researchers to conduct the laboratory tests to establish protocols for the enhanced in situ bioremediation (currently reductive dechlorination) at the TAN injection well. Other research determined that natural attenuation is occurring in the dissolved TCE plume at TAN (22) and the research described in this EMSP

final report determined the broad distribution in the dissolved TCE plume of naturally-occurring TCE-degrading microorganisms. These microorganisms may be responsible for this natural attenuation at low TCE concentrations and may be an essential part of the long-term solution to plume remediation at TAN. Our work also verified the presence of dissolved methane in the SRP aquifer and at TAN (27). Dissolved methane is the potential primary energy source for methanotrophs, microorganisms capable of aerobic chlorination (through cometabolism) of TCE.

The conclusion that natural attenuation is occurring in the dissolved TCE plume at TAN (22) and the results of our own EMSP research suggest that future research needs to focus on the specific biological processes responsible for the TCE attenuation and the rates at which these processes are occurring. Quantitative values for the rate of TCE disappearance and an estimate of the volumetric productivity of the aquifer microorganisms in this regard will be required before the EPA and Idaho DEQ accept natural attenuation as a viable option for remediation of TAN's dissolved TCE plume. However, acceptance of this treatment approach, which we hypothesize is occurring by indigenous microorganisms and a natural source of methane in the aquifer has the potential to spare considerable amounts of federal cleanup funds when compared to expensive engineered cleanup options.

This research has bridged an important gap between INEEL Operations and INEEL Research and Development. It is one of the key examples within the DOE Complex of how these two distinct activities at national laboratories can work towards a common goal. The results have directed current research activities towards a better understanding of the methanotrophs that may be responsible for measured TCE attenuation in the plume. Our data support the work performed by L Peterson and K Sorenson, INEEL Environmental Restoration scientists who have verified that enhanced TCE degradation is occurring as a result of lactate introduction (21). That group has completed an amendment to the Record of Decision (ROD) at TAN which promotes enhanced bioremediation near the injection well and natural attenuation in the distal plume. The amendment to the ROD still must be accepted by both the U.S. Environmental Protection Agency (EPA) and Idaho Department of Environmental Quality (DEQ). It is significant that while this EMSP project is distinct from the work that led to the amendment to the ROD, our basic research tasks have directly assisted their microbiological investigations that demonstrated enhanced bioremediation and then generated the ROD amendment.

The research activity has prompted new collaborations between Operations and R&D at the INEEL. A larger scale effort at this site is not needed; however, as noted above, further research into the specific microorganisms responsible for the TCE degradation and the rates at which such degradation can be expected remain unaddressed but are key issues related to the long-term stewardship of this site. These rates of microbially-mediated natural attenuation represent a fundamental scientific hurdle that must be considered to fully extend this work to support DOE Environmental Management problems and to confront the concerns of the EPA and the Idaho DEQ.

## ***7. Project Productivity***

The project was not as efficient as expected at the outset and it was significantly delayed over its lifetime. This is because of the difficulties associated with conducting research at a CERCLA site such as TAN. Although some of these constraints were anticipated when the proposal was written and handled accordingly, we found that research which is carried out in the context of remediation at a DOE site is subject to the whims of the regulatory agencies that oversee the remediation, the contract group that conducts the remediation, and the internal rules of the federal facility (in this case, INEEL). While EMSP has established the goal of funding research that is relevant to DOE's cleanup needs, it seems that individuals or organizations that are implementing the cleanup are not encouraged to accommodate scientists. For EMSP projects to successfully integrate research in to Operations at DOE sites, it is imperative that some inclusion of such research is fostered for selected remediation problems.

## **8. Personnel Supported**

**Research Fellowships** : Matt Downing (high school teacher, Shawnee HS, Marlton, NJ); Abe Suazo (high school teacher, Manitou HS, Manitou Springs, CO); Dustin Eaton (Shelley HS, Science Action Team), Sara Gilk (undergraduate fellowship, Whitman College)

**Graduate Students**: R. Michael Lehman (Idaho State Univ.), Sean O'Connell (Idaho State Univ.), Costantino Vetriani (Rutgers Univ.)

**Postdoctoral fellows** : Mark Wilson (INEEL), Ken Tobin (Princeton Univ.)

**Faculty**: Ken Tobin (Univ. Illinois - Chicago)

## **9. Publications**

- O'Connell, S.P., R.M. Lehman, F. S. Colwell and M.E. Watwood. 1997. Microbiological monitoring of contaminants in a fractured basalt aquifer, pp. 111-116. In, *In Situ and On-site Bioremediation: Volume 4. The Fourth International Symposium*. Battelle Press. Columbus, Ohio.
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- Colwell, F. 2001. Constraints on the distribution of microorganisms in subsurface environments, pp. 71-95. In (J. Fredrickson and M. Fletcher, eds.), *Subsurface Microbiology and Biogeochemistry*. John Wiley and Sons. New York, NY.
- Lehman, R.M., F.S. Colwell, and G.A. Bala. 2001. Attached and unattached microbial communities in a simulated basalt aquifer under fracture- and porous-flow conditions. *Appl. Environ. Microbiol.* 67: 2799-2809.
- Wilson, M.S., R.M. Lehman, F. Colwell, and S. Gilk. Enumeration of methanotrophic bacteria in a deep basalt aquifer using molecular and culture-based approaches. *FEMS Microbiol. Ecol.* In preparation.
- Lehman, R.M., S.P. O'Connell, A. Banta, J.K. Fredrickson, F. Brockman, A.-L. Reysenbach, F.S. Colwell. Obtaining a representative sample of subsurface microflora: Attached and unattached bacteria in a fractured basalt aquifer. In preparation.
- Colwell et al. Vertical distribution of microbial communities related to geochemical and hydrological variations in a fractured basalt aquifer. In preparation.
- McKinley et al. Fine-scale aquifer chemistry determined by fracture distribution and proximity to a waste plume. In preparation.
- Lehman et al. Oligotrophic microbial communities in a basalt aquifer. In preparation.
- O'Connell, S.P. Ph.D. Dissertation. Idaho State University. In preparation.

## **10. Interactions**

Principal investigators funded through this research participated in the following interactive activities:

- Invited speaker, 1996 American Geophysical Union Annual Meeting (12/96, San Francisco, CA)
- Two papers presented at the 1998 Annual Meeting for the American Society for Microbiology (5/97, Atlanta, GA)
- Poster presentation at the American Geophysical Union Annual Meeting (12/97, San Francisco, CA)

- Poster presentation at the EMSP National Workshop (7/98, Chicago, IL)
- Invited speaker, Lawrence Berkeley National Laboratory, Center for Environmental Biotechnology, “Geological Constraints on the Distribution of Microorganisms in Subsurface Environments” (2/99, Berkeley, CA)
- Three papers presented at the International Symposium on Subsurface Microbiology. At this meeting the PI coordinated and convened a session on Contaminated Fractured Rock Environments (8/99, Vail, CO)
- Invited speaker, Humboldt State University, “Geological Constraints on the Distribution of Microorganisms in Subsurface Environments” (11/99, Arcata, CA)
- Invited speaker in Success Stories session at EMSP National Workshop; poster presentation at same meeting (4/00, Atlanta, GA)
- Invited speaker at Department of Energy-HQ, Environmental Management Seminar Series (5/00, Washington, DC)
- Poster presentation at the EPA/DOE Fractured Rock Workshop (12/00, Providence, RI)

### ***11. Transitions***

Much of the early research on this project was transitioned to the Idaho Water Resources Research Institute (contact: Ron Crawford, Univ. of Idaho) and to INEEL scientists involved in the Environmental Restoration at the TAN site (contacts: Lance Peterson and Kent Sorenson, INEEL).

### ***12. Patents***

None to report.

### ***13. Future Work***

Remaining work on this project is the completion of manuscripts that describe the results of our research at TAN. These data should be published within the next year. We intend to continue the research by focusing on the microbial communities that are responsible for the degradation of the TCE in the dissolved plume at TAN. The responsible microorganisms are believed to be either methanotrophs, phenol-oxidizers, or both groups working together. Although these proposed follow-on studies were submitted as a new EMSP proposal, the work was not accepted for funding. Our intent is to address the reviewer’s concerns and resubmit when an appropriate call for proposals is identified. The key objectives of the future work would involve determining the communities responsible for natural attenuation of the TCE plume and use bioreactors (e.g., retentostats (13)) in order to obtain realistic in situ rates of TCE dechlorination. The eventual product of the research would be a volumetric productivity for the subsurface at TAN with respect to TCE destruction. This value, calculated for different locations within the plume, would allow the EPA and Idaho DEQ to evaluate natural attenuation of the plume as a remediation solution.

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### ***15. Feedback***

None to offer.

## 16. Tables and Figures

Table 1. Water chemistry values for TAN-33 and TAN-37. Values are mg/L, except where noted. Data from (4, 20).

Parameter	TAN-33	TAN-37
Distance from injection well (m)	425	35
dissolved oxygen	6-7	3-4
pH	8	8
temperature ( °C)	12-13	12-13
total organic carbon	< 0.5	1
dissolved inorganic carbon	208-220	ND
total chlorinated alkenes	< 1	1-5
conductivity (µS/cm)	600	700
<sup>3</sup> H (µCi/L)	4	4
<sup>90</sup> Sr (pCi/L)	< 0.2	150
o-phosphate	< 1.0	< 1.0
nitrate	9	10
ammonium	< 0.1	0.5
sulfate	30	40
chloride	80	90
calcium	72000	68000
magnesium	18000	21000
potassium	3300	4800
sodium	25000	40000

ND = Not Determined

Table 2. Large scale horizontal variations in microbial properties and correlations to TCE concentration in the water from the respective wells. Data used to generate these relationships included all microbial parameters measured in wells TAN-37, 33, and 48.

Microbiological parameter	Coefficient of variation (%)	Correlation with aqueous TCE concentration
methanotrophs	173	0.998
propanotrophs	173	0.998
nitrifiers	173	0.998
phenol-oxidizers	169	0.999
CLPP	169	0.998
PLFA biomass	62	0.998
aerobic acetate mineralization	134	0.999
anaerobic acetate mineralization	164	0.999
dissimilatory metal reducers	115	0.965

Table 3. Large scale vertical variations in microbial properties and correlations of microbial properties and sample depth to strata that were determined to be active flow zones or primary flow paths for contaminants. All data from TAN-37.

Microbiological parameter	Coefficient of variation (%)	Correlation with hydraulically active flow zones
sample depth		-0.383
methanotrophs	247	0.263
propanotrophs	206	0.445
nitrifiers	480	-0.187
phenol-oxidizers	251	0.181
CLPP	32	0.410
PLFA biomass	55	-0.009
aerobic acetate mineralization	19	0.045
anaerobic acetate mineralization	49	0.438
colony forming units	280	-0.169

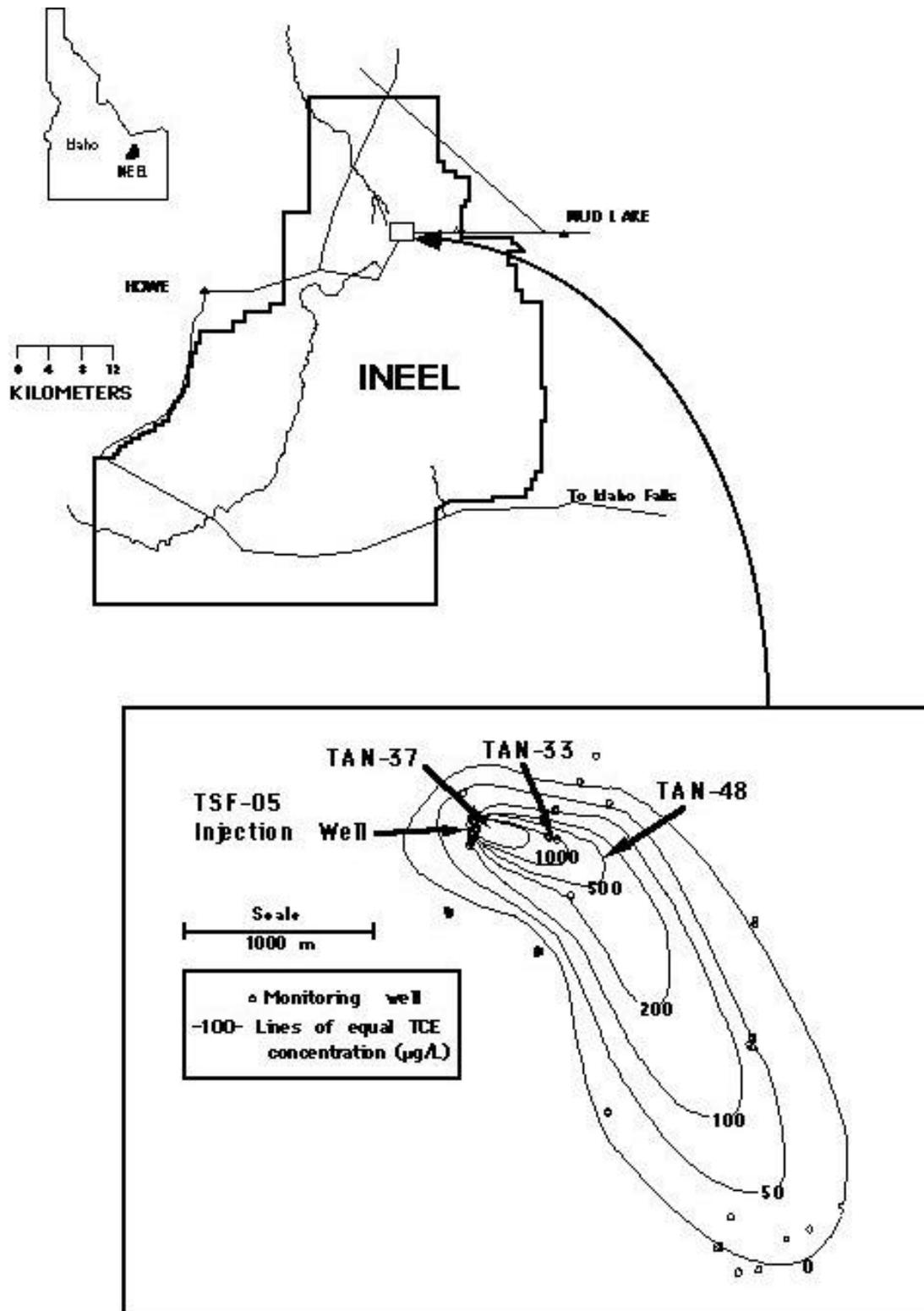


Figure 1. Location of the Idaho National Engineering and Environmental Laboratory (INEEL) and Test Area North (TAN) site. Inset shows a plan view of the TCE plume in the aquifer at TAN, the wells (TAN-37, 33, and 48) that were sampled in this study, and the isopleths for TCE contamination at the site.

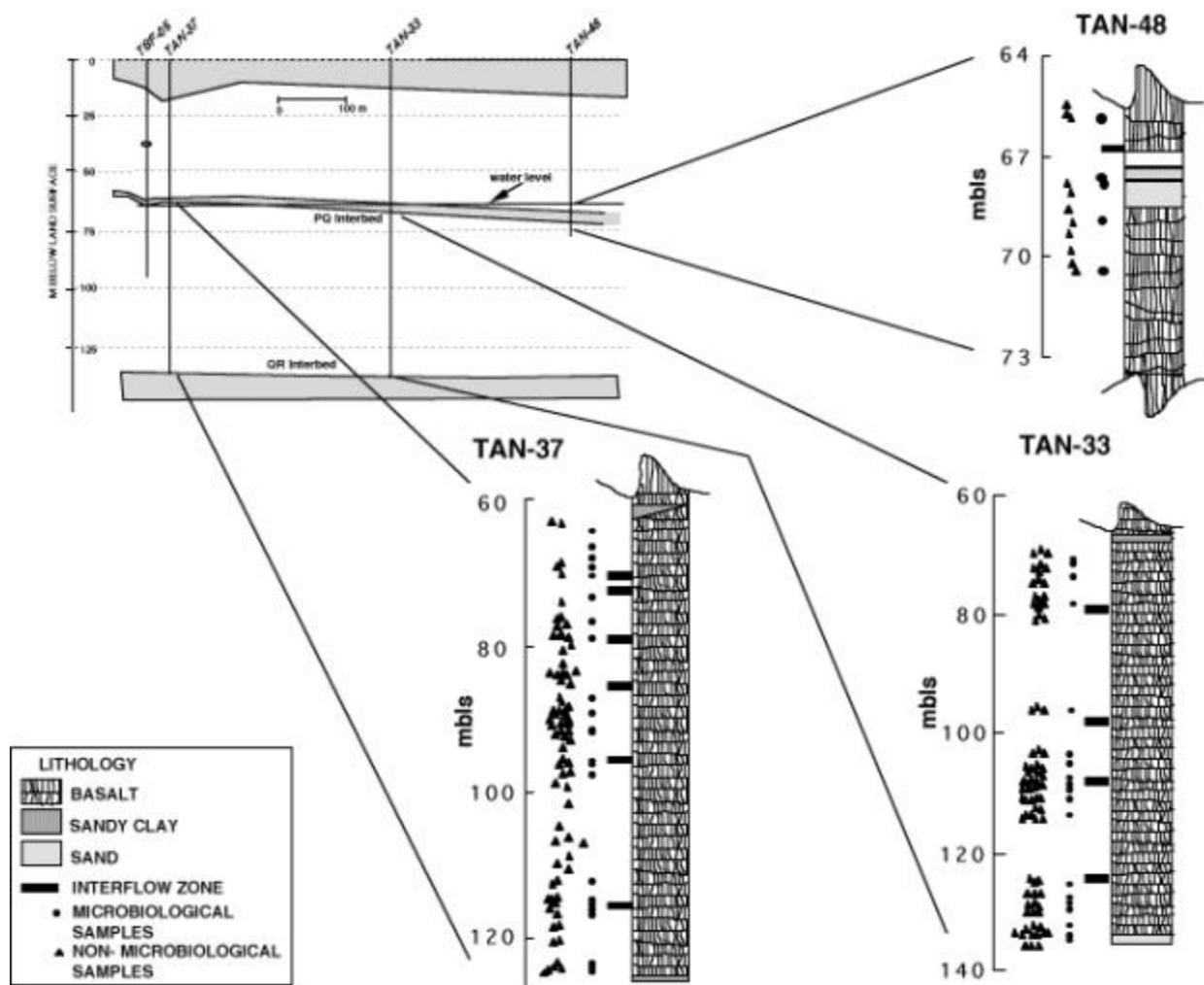


Figure 2. Cross-sections of the boreholes sampled during this study and their location relative to the TAN injection well (TSF-05). Generalized cross-section at the top showing all three boreholes is vertically exaggerated. Specific borehole cross-sections identify microbiological and non-microbiological sample locations as well as the inferred interflow zones that represent the contact between adjoining basalt flows where preferred fluid flow is likely to occur.

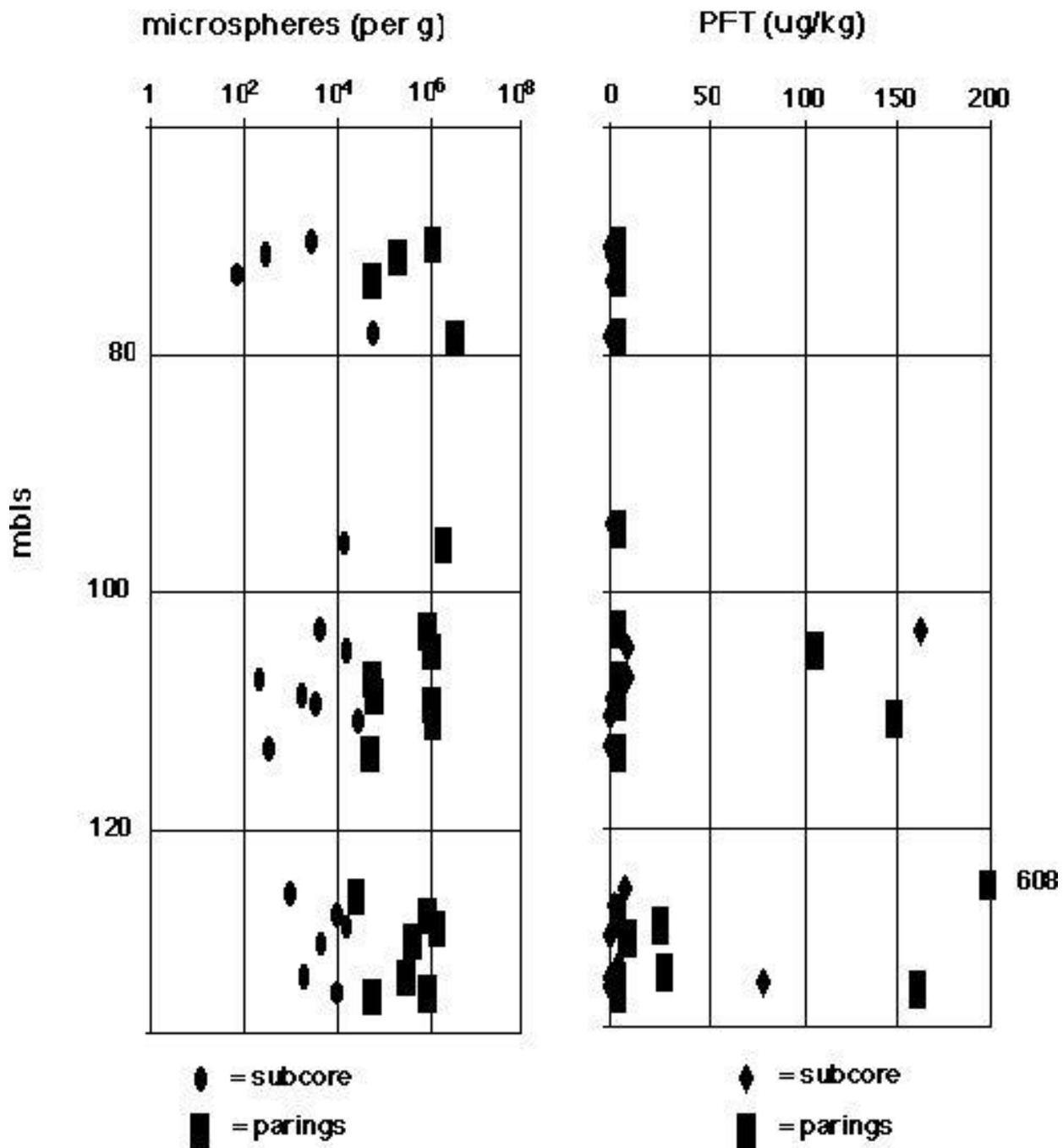


Figure 3. Numbers of fluorescent microspheres (per g of core) and of perfluorocarbon tracer (per kg of core) determined in subcore and parings from TAN-33.

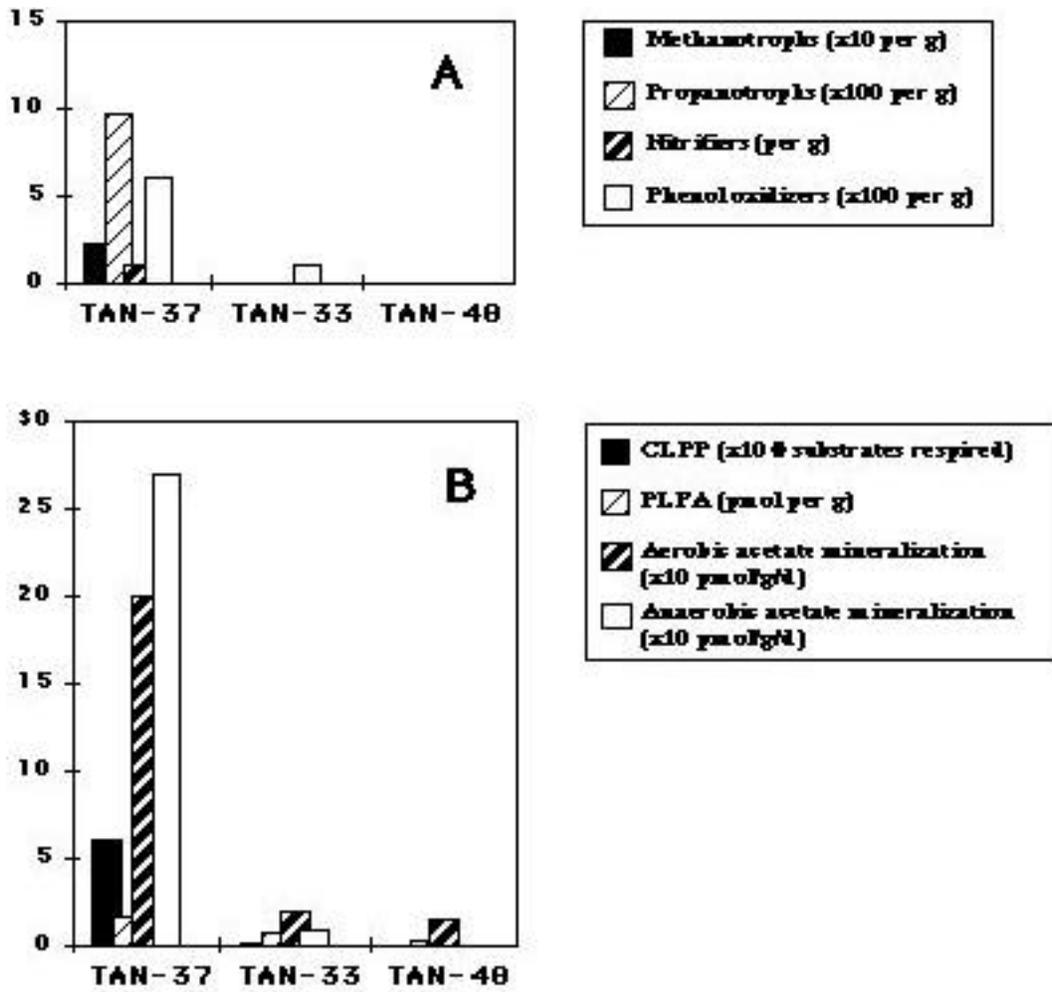


Figure 4. Mean values for enumerated microbial physiologies relevant to TCE co-metabolism (A) and for microbial biomass and activity determinations (B) measured in core materials collected along the horizontal axis of the TAN TCE plume. Concentrations of dissolved TCE decrease from left to right in the boreholes shown in the figures. Y-axes are scaled according to the designations for each of the microbial parameters.