Comparison of Bacteria from Deep Subsurface Sediment and Adjacent Groundwater

Terry C. Hazen, Luis Jiménez,[1] Carl B. Fliermans,[1] and Geralyne López de Victoria,[2]
[1]Environmental Sciences Section, Savannah River Laboratory,
Westinghouse Savannah River Company, Aiken, SC
[2]Center for Environmental Biotechnology, University of Tennessee, Knoxville, Tennessee.

Abstract

Samples of groundwater and the enclosing sediments were compared for densities of bacteria using direct (acridine orange direct staining) and viable (growth on 1% PTYG medium) count methods. Sediments to a depth of 550 m were collected from boreholes at three sites on the Savannah River Site near Aiken, SC using techniques to insure a minimum of surface infiltration. Clusters of wells screened at discrete intervals were established at each site. Bacteria densities in sediment were higher by both direct and viable count than they were in groundwater samples. Differences between direct and viable counts were much greater for groundwater samples than sediment samples. Densities of bacteria in sediment ranged from less than 1.00×10^6 bacteria/g dry weight (gdw) up to 5.01×10^8 bacteria/gdw for direct counts, while viable counts were less than 1.00×10^3 CFU/gdw to 4.07×10^7 CFU/gdw. Bacteria densities in groundwater were 1.00×10^3 - 6.31×10^4 bacteria/ml and 5.75 - 4.57×10^2 CFU/ml for direct and viable counts, respectively. Isolates from sediment were also found to assimilate a wider variety of carbon compounds than groundwater bacteria. The data suggests that oligotrophic aquifers have unique and dense bacterial communities that are attached and not reflected in groundwater that is found in the strata. Effective *in situ* bioremediation of contamination in these aquifers may require sediment sampling.

Introduction

Organic xenobiotic chemical contamination of groundwater has become the most important pollution problem of industrialized nations of the world. More than 15% of community drinking water supplies in the United States have been found to be contaminated with carcinogenic, chlorinated hydrocarbons.[7] Identification of previously unknown waste disposal sites that are impacting groundwater occurs almost daily, and thus, the extent of the problem is undoubtedly greater than what any of the current data suggest. Indeed, our reliance on groundwater in the United States has steadily increased over the past 30 years, not only for drinking water but also for industrial processes, agricultural irrigation, etc.[7] As sources of clean surface water steadily decline, our reliance on groundwater will undoubtedly continue to increase far into the next century. Thus, with increasing urgency we have been seeking ways to clean up (i.e., remediate, contaminated groundwater). Since many aquifers are quite deep and contain tremendous volumes of water with slow turnover times, *in situ* bioremediation is very attractive and in some cases may be the only recourse.

In situ bioremediation has been practiced for more than 30 years by petroleum industries, (e.g. petroleum land farming).[4] The initial process of inorganic nutrient infiltration of groundwater to stimulate biodegradation by indigenous bacteria in groundwater contaminated with petroleum was patented by R. L. Raymond in 1974 (U. S. Patent 3,846,290). However, widespread application of this technology has not occurred due to limited successes. This was due in part to the paucity of knowledge concerning the microbial ecology of subsurface sediments.[13] During the 1980s, several laboratories, including ours, began studying the biogeochemistry of subsurface sediments in order to understand and control biodegradation processes in groundwater.

The United States Department of Energy's Office of Health and Energy Research began a comprehensive program to study subsurface microbiology in 1985.[9] During this program, four sites were chosen at the DOE's Savannah River Site near Aiken, SC. Sediments were sampled using special recovery techniques from the surface to bedrock, 550 m in the deepest borehole.[23] (For a comprehensive description of these studies see Fliermans and Balkwill,[9] and volume seven of the *Geomicrobiology Journal.*) At three of the sites, a series of well clusters (6-12) were established so that groundwater would be recovered from discrete strata. The purpose of the present study was to compare the microbiology of the sediments with the adjacent groundwater. These studies were undertaken in order to determine the efficacy of using groundwater to monitor the microbiology of aquifers, especially as it may apply to *in situ* bioremediation.

Materials and Methods

Study sites. Sediment samples were taken by aseptic coring from three sites (P24, P28, P29) at the Savannah River Site, near Aiken, SC. (For a thorough description of the geology and hydrology of these sites and this area of South Carolina, see Sargent and Fliermans.[26] For a general description of the U. S. Department of Energy's Deep Probe Project findings, see Fliermans and Balkwill[9] and volume 7, numbers 1 and 2 of the *Geomicrobiology Journal.*) Water samples

were taken from a cluster of wells at the same site. All of these wells were established within 20 m of the borehole used for sediment sampling. The wells were built using carbon steel casing, stainless steel screens, gravel packs, and dedicated, 9 gpm pumps. Wells in each cluster were single screened at specific geological formations over a 3-m interval to provide water from specific aquifers or segments of aquifers. Table 1 provides a listing of wells, their physical description, and representative physical-chemical data.

Sediment analysis. Bacteriological data from sediment analysis were taken from Balkwill[1] and Sinclair and Ghiorse.[27] For a detailed description of sediment sampling techniques, see Phelps et al.[23]

Water analysis. Wells were flushed by pumping until at least three well volumes of water had been evacuated and the pH and conductivity were stable, (i.e., less than 1% change in one hour; sterile, one liter bottles were filled with water for bacteria counts), and all samples were placed on ice and transported to the laboratory for analysis within 1-3 hours. Viable counts were determined by filtering 1, 10, and 100 ml of water through 0.45 µm pore size, 47 mm, HA type, membrane filters (Millipore Corp., Bedford, MA). Filters were placed on 1% PTYG medium[1] and incubated at 23°C for two weeks. Bacterial colonies were counted with the aid of a stereo microscope. Total cell counts were determined by direct count (AODC) methods using acridine orange.[17,22] Activity was estimated by dividing the log density of AODC's by the log density of viable counts on 1% PTYG.

Bacteria Isolation and Assimilation. Deep subsurface bacterial isolates from sediments used in the physiological studies were provided from the Subsurface Microbiology Culture Collection (SMCC) by Dr. David Balkwill of Florida State University, Tallahassee, FL. (For further details on the isolation of deep subsurface bacteria from sediment and media used, see Balkwill et al.[3] and Balkwill and Ghiorse.[2] Water isolates were obtained from random colony selections from 1% PTYG medium. Both water and sediment isolates were analyzed for physiological capabilities using the API-NFT Rapid tests as described by Balkwill et al.[3] and Bone and Balkwill.[5]

Data analysis. The data were analyzed by using prepared programs for Macintosh computers. Factorial analyses of variance were used to test for differences between sites, samples and methods. Data were subjected to the appropriate transformation before statistical analysis by the method of Zar.[30] Any probability less than or equal to 0.05 was considered significant.

Results

Direct enumeration of bacteria in sediments using AODC revealed from less than 1.00×10^6 bacteria/gdw up to 5.01×10^8 bacteria/gdw (Table 2). Viable counts using 1% PTYG medium varied from no detectable growth (i.e., less than 1.00×10^3 CFU/gdw) up to 4.07×10^7 CFU/gdw. Due to variations between samples within sites and within geological formations, a factorial analysis of variance demonstrated no significant differences between sites or between geological formations. However, differences between viable counts and direct counts were significant ($F = 66$;

DF = 1, 85; P < 0.0001). Direct counts were usually the same, but for some samples they were two orders of magnitude higher than viable counts (Table 2). (For further analysis of sediment enumeration techniques and geochemistry see Balkwill[1] and Sinclair and Ghiorse.[27]

Groundwater AODC measurements ranged from 1.00 x 10^3 to 6.31 x 10^4 bacteria/ml (Table 3), while bacteria densities measured by viable counts on 1% PTYG ranged from 5.75 to 4.57 x 10^2 CFU/ml. As with the sediment enumeration, a factorial analysis of variance revealed that variability within sites and within geological formations prevented density differences between sites and between geological formations from being significant. Differences between direct counts and viable counts were very significant (F = 155; DF = 1, 36; P < 0.00001). The average difference between viable and direct counts for sediment samples ranged from two to three orders of magnitude.

Comparison of viable counts from the sediment and water demonstrated very significant differences (Table 4; F = 85; DF = 1, 36; P < 0.0001). Differences ranged from two to five orders of magnitude, with an average of three. Comparison of direct counts from the sediment and water also revealed even greater differences (Table 5; F = 232; DF = 1, 36; P < 0.00001). Sediment direct counts were from three to five orders of magnitude (average = 4) higher than adjacent groundwater densities.

As an index of activity, the ratio of direct to viable counts (D/V) was calculated for both sediment and water and compared (Table 6). The D/V ratio was significantly higher for water (F = 14.5; DF = 1, 36; P < 0.001). Indeed, all but three of the samples had a higher water D/V than the sediments and only one of these was significant. The average D/V was 75% larger for water samples. D/V was not significantly different by site or geological formation as analyzed by factorial analysis of variance. Isolates from both water and sediment collected at the same sampling interval were compared in terms of their physiological abilities using the API-NFT test (Table 7). The first nine metabolic tests were not significantly different between water and sediment isolates. However, significantly higher proportions of sediment isolates were able to utilize the carbon compounds that were assayed (F= 3.32; DF = 1, 22; P < 0.01). Eleven of 13 compounds were assimilated at a higher frequency by the sediment isolates.

Discussion

The detailed studies done by several investigators on the sediment samples described in this study have defined many microbiological parameters.[8,9] Great care was taken to collect samples with a minimum of surface and drilling mud contamination.[23] Sixteen separate lines of evidence using a variety of tracers and microbiological assays suggests that fewer than 10% of the sediment samples were compromised.[8] The sediment samples demonstrated that overall bacteria were present in high densities (10^7 AODC or CFU/gdw) and were able to rapidly utilize a wide array of carbon compounds. This was indicated by the close agreement of viable and direct counts (1-2 orders of magnitude) and the greater frequency of isolates capable of assimilating carbon compounds found in the API-NFT assay.

Related studies of these same isolates have also demonstrated their ability to degrade a variety of toxic compounds, such as trichloroethylene, quinoline, phenol, and 4-methoxybenzoic acid.[6,10,16]

Poindexter[24] suggested that bacteria adapted to oligotrophic conditions might be expected to have broader uptake systems, having adapted to utilization of a broader range of substrates. Similar findings have been reported for oligotrophic bacteria in Antarctica.[29] The sediments and water in the present study were oligotrophic based on the low concentrations of phosphorous and nitrogen and recalcitrant organic matter since dissolved organic carbon was between 3-9 ppm.[11] Thus, even though the sediment bacteria in our study may be living in an oligotrophic, recalcitrant environment, they were able to maintain a high standing biomass in a quiescent state and could almost immediately respond to small increases in nutrients like those found in the low nutrient, 1% PTYG medium. This is quite different from oligotrophic aquatic systems where differences between direct and viable counts for the same samples are typically 4-5 orders of magnitude.[18,25] This was further supported by the data of Tunlid et al.,[28] who showed that the phospholipid fatty acid profiles of bacteria in these sediments were under nutrient stress. Balkwill[1] and others[2,5] showed that bacteria from these deeper sediments had greater metabolic flexibility than surface sediment bacteria, growing rapidly on both low nutrient and high nutrient media. Other studies on the same isolates[12] showed an increasing frequency of plasmids among isolates with increasing depth at these sites. This suggested that as the environment becomes more recalcitrant it becomes more metabolically economical to increase the plasmid burden in order to increase the number of degradable compounds.

In contrast to sediments, groundwater adjacent to the sediments had direct counts that were 2-3 orders of magnitude lower and viable counts that were 3-5 orders of magnitude lower than sediment densities. The ratios of direct to viable counts were much greater for groundwater samples. Thus groundwater bacteria were not only much lower in density, but they were under greater stress because they were much less capable of responding to nutrient input (i.e., growth on low nutrient medium: 1% PTYG). This was further supported by the lower frequency of isolates that could assimilate API-NFT compounds.

Studies in Germany[20,21] comparing sediment and groundwater bacterial communities also found that densities of bacteria in the sediment by viable counts were 2-3 orders of magnitude higher than nearby groundwater. In their studies, viable counts for water were quite similar to the results in this study; however, sediment densities were significantly lower. Combined with the present study, these findings suggest that these aquifers are comprised of epilithic communities. In other words, the microbial communities of these aquifers are nearly all attached. It appears that the microorganisms that are free in the groundwater are either dead or stressed attached bacteria or organisms that are being transported through the aquifer having their origins in the recharge zone of the aquifer. It is unlikely that these planktonic bacteria exist as a free living community due to their poor culturability and the extremely low nutrient levels of the water.[25]

Support for a recharge zone or sediment independent origin of the groundwater bacteria is further evidenced by our laboratories finding that three DNA probes from sediment bacteria, one from each site,[19] did not show significant homology, < 26%, with any of 23 groundwater isolates. API-NFT assays could not be used for identification of sediment bacteria because isolates with identical phenotypes by these assays did not have similar DNA homologies or even similar mol% G+C composition of the DNA.[19] Identification comparison of sediment and water isolates will therefore not be possible until sediment phenotypes can be differentiated and further characterized.

Conclusion

The present work has demonstrated that deep oligotrophic aquifers have large attached communities of potentially very active indigenous bacteria that are not reflected in the groundwater from that aquifer. This has serious implications for the *in situ* bioremediation of contaminated aquifers, since monitoring of groundwater is the principal method used to characterize and control biodegradation by indigenous bacteria stimulated by nutrient infiltration. Groundwater monitoring will not indicate community or population numbers, or physiological activity of the sediment attached microorganisms, which are the principal biologically active component of these aquifers. Harvey et al.[15] and Harvey and George[14] have shown that shallow, eutrophic, rapidly moving aquifers, behave quite differently, in that there are no significant differences between groundwater and attached sediment communities. This is reasonable because attachment in such an environment would have no significant advantage, unlike the oligotrophic deep aquifers.

Thorough characterization and control of deep aquifer communities, necessary for any bioremediation effort that utilizes stimulation of indigenous bacteria, may require deep sediment sampling. Deep sediment sampling and recovery of uncontaminated samples for microbial analysis may make this type of *in situ* bioremediation cost prohibitive. A better understanding of biogeochemistry, microbial composition and synecology of these deep aquifers would seem warranted before any severe manipulation by man is tried.

Acknowledgements

We are grateful for the technical assistance of W. Fulmer, J. J. Foreman, and C. Betivas. The information contained in this article was developed during the course of work under contract DE-AC09-76SR00001 between the U.S. Department of Energy and Westinghouse Savannah River Company and in part by contract DE-AC05-760R000033 between the U.S. Department of Energy and Oak Ridge Associated Universities. The U.S. Department of Energy, Office of Health and Environmental Research supported several investigators that provided ancillary information through their Subsurface Science Program, under the direction of F. Wobber.

References

1. Balkwill, D. L., 1989. "Numbers, Diversity, and Morphological Characteristics of Aerobic, Chemoheterotrophic Bacteria in Deep Subsurface Sediments from a Site in South Carolina". *Geomicrobiol. J.* **7**:33-5.

2. Balkwill, D. L. and W. C. Ghiorse, 1985. "Characterization of Subsurface Bacteria Associated with Two Shallow Aquifers in Oklahoma". *Appl. Environ. Microbiol.* **50**:580-588.

3. Balkwill, D. L., J. K. Fredrickson, and J. M. Thomas, 1989. "Vertical and Horizontal Variations in the Physiological Diversity of the Aerobic Chemoheterotrophic Bacterial Microflora in Deep Southeast Coastal Plain Subsurface Sediments". *Appl. Environ. Microbiol.* **55**:1058-1065.

4. Bartha, R. and I. Bossert. "The Treatment and Disposal of Petroleum Wastes." R. Atlas (ed.), *Petroleum Microbiology*. Macmillan Publishing Co., NY, 1984.

5. Bone, T. L. and D. L. Balkwill, 1988. "Morphological and Cultural Comparison of Microorganisms in Surface Soil and Subsurface Sediments at a Pristine Study Site in Oklahoma". *Microb. Ecol.* **16**:*49-64*.

6. Brockman, F. J., B. A. Donovan, R. J. Hicks, and J. K. Fredrickson, 1989. "Isolation and Characterization of Quinoline-Degrading Bacteria from Subsurface Sediments". *Appl. Environ. Microbiol.* **55**:1029-1032.

7. Craun, G. F. "*Waterborne Diseases in the United States*". CRC Press, Boca Raton, FL, 1986.

8. Fliermans, C. B., 1989. "Microbial Life in the Terrestrial Subsurface of Southeastern Coastal Plain Sediments". *Haz. Was. Haz. Mat.* **6**:155-171.

9. Fliermans, C. B. and D. L. Balkwill, 1989. "Microbial Life in Deep Terrestrial Subsurfaces". *Bio Science*. **39**:370-377.

10. Fliermans, C. B., T. J. Phelps, D. Ringelberg, A. T. Mikell, and D. C. White, 1988. "Mineralization of Trichloroethylene by Heterotrophic Enrichment Cultures". *Appl. Environ. Microbiol.* **54**:1709-1714.

11. Fredrickson, J. K., T. R. Garland, R. J. Hicks, J. M. Thomas, S. W. Li, and K. M. McFadden, 1989. "Lithotrophic and Heterotrophic Bacteria in Deep Subsurface Sediments and Their Relation to Sediment Properties". *Geomicrobiol. J.* **7**:53-66.

12. Fredrickson, J. K., R. J. Hicks, S. W. Li, and F. J. Brockman, 1988. "Plasmid Incidence in Bacteria from Deep Subsurface Sediments". *Appl. Environ. Microbiol.* **54**:2916-2923.

13. Ghiorse, W. C. and J. T. Wilson, 1988. "Microbial Ecology of the Terrestrial Subsurface". *Adv. Appl. Microbiol.* **33**:107-172.

14. Harvey, R. W. and L. H. George, 1987. "Growth Determination for Unattached Bacteria in a Contaminated Aquifer". *Appl. Environ. Microbiol.* **53**:2992-2996.

15. Harvey, R. W., R. L. Smith, and L. H. George, 1984. "Effect of Organic Contamination Upon Microbial Distributions and Deterotrophic Uptake in a Cape Cod, Mass. Aquifer". *Appl. Environ. Microbiol.* **48:**1197-1202.

16. Hicks, R. J. and J. M. Fredrickson, 1989 "Aerobic Metabolic Potential of Microbial Populations Indigenous to Deep Subsurface Environments". *Geomicrobiol. J.* **7**:67-77.

17. Hobbie, J. E., R. J. Daley, and S. Jasper, 1977. "Use of Nuclepore Filters for Counting Bacteria by Fluorescence Microscopy". *Appl. Environ. Microbiol.* **33**:1225-1228.

18. Jannasch, H. W. and G. E. Jones, 1959. "Bacterial Populations in Sea Water as Determined by Different Methods of Renumeration". *Limnol. Oceanogr.* **4**: 128-139.

19. Jiménez, L., 1990. "DNA Comparison of Deep Terrestrial Subsurface Bacteria Isolated from Different Geological and Hydrological Strata". *Appl. Environ. Microbiol.* **56**:2108-2113.

20. Kölbel-Boelke, J., B. Tienken, and A. Nehrkorn, 1988. "Microbial Communities in the Saturated Groundwater Environment. I. Methods of Isolation and Characterization of Heterotrophic Bacteria". *Microb. Ecol.* **16**:17-29.

21. Kölbel-Boelke, J., E. Anders, and A. Nehrkorn, 1988. "Microbial Communities in the Saturated Groundwater Environment, I. Diversity of Bacterial Communities in a Pleistocene Sand Aquifer and Their In Vitro Activities". *Microb. Ecol.* **16**:31-48.

22. López-Torres, A. J., L. Prieto, and T. C. Hazen, 1988. "Comparison of the *In Situ* Survival and Activity of Klebsiella pneumoniae and Escherichia Coli in Tropical Marine Environments". *Microb. Ecol.* **15**:41-57.

23. Phelps, T. J., C. B. Fliermans, T. R. Garland, S. M. Phiffner, and D. C. White, 1989. "Methods for Recovery of Deep Terrestrial Subsurface Sediments for Microbiological Studies". *J. Microbiol. Meth.* **9**:267-280.

24. Poindexter, J., 1981. "Oligotrophy: Fast and Famine Existence". *Adv. Micro. Ecol.* **5**:63-90 .

25. Roszak, D. B. and R. R. Colwell, 1987. "Survival Strategies of Bacteria in the Natural Environment". *Microbiol. Rev.* **51**:365-379.

26. Sargent, K. A. and C. B. Fliermans, 1989. "Geology and Hydrology of the Deep Subsurface Microbiology Sampling Sites at the Savannah River Plant, South Carolina", *Geomicrobiol. J.* **7**:3-14.

27. Sinclair, J. L. and W. C. Ghiorse, 1989. "Distribution of Aerobic Bacteria, Protozoa, Algae, and Fungi in Deep Subsurface Sediments". *Geomicrobiol. J.* **7**:15-32.

28. Tunild, A., D. Ringelberg, T. J. Phelps, C. Low, and D. C. White, 1989. "Measurement of Phospholipid Fatty Acids at Picomolar Concentrations in Biofilms and Deep Subsurface Sediments Using Gas Chromatography and Chemical Inonization Mass Spectrometry". *J. Microbiol. Meth.* **10**:139-153.

29. Upton, A. C. and D. B. Nedwell, 1989. "Nutritional Flexibility of Oligotrophic and Copiotrophic Antarctica Bacteria with Respect to Organic Substrates". *FEMS Microb. Ecol.* **62**:1-6.

30. Zar, J. H. *Biostatistical Analysis.* (2nd ed). Prentice-Hall, Englewood Cliffs, NJ, 1984.

Tables

Table 1. Physical and Chemical Descriptions of Cluster Wells.

Well units	Screen (ft)	Geological Formation	Temp (°C)	pH	Cond (µmohs)	Redox (mv)	D O (mg/l)
P28A	330-341	Pee Dee	20.6	6.0	44	0.102	3.8
P28TE	404-415	Black Creek	20.1	6.3	42	0.122	8.1
P28TD	494-505	Black Creek	24.9	7.1	118	0.127	0.3
P28TC	553-564	Middendorf	21.7	9.9	281	-0.023	0.5
P28TB	618-640	Middendorf	22.0	5.5	30	0.125	3.2
P28TA	747-780	Middendorf	21.9	5.7	50	0.161	4.2
P24D	45-65	Tobacco Road	21.0	6.8	55	0.145	9.1
P24C	130-150	Dry Branch	26.6	6.7	45	0.327	8.3
P24B	219-230	McBean	21.1	9.5	149	0.198	8.3
P24A	304-315	Congaree	23.1	10.7	187	ND	6.0
P24TD	489-500	Pee Dee	21.6	6.8	55	-0.043	1.0
P24TC	584-595	Pee Dee	21.8	5.9	97	-0.008	0.1
P24TB	795-805	Middendorf	22.2	6.2	60	0.120	3.2
P24TA	950-972	Middendorf	21.7	10.9	98	ND	2.8
P29D	93-113	Dry Branch	19.1	5.8	40	0.183	7.4
P29C	130-140	Congaree	20.0	5.6	103	0.102	2.5
P29B	180-190	Ellenton	20.8	6.4	39	0.141	8.3
P29A	304-315	Pee Dee	22.3	6.1	76	0.112	0.2
P29TD	408-430	Black Creek	21.3	5.3	60	0.057	5.7
P29TC	488-510	Middendorf	21.7	6.6	86	0.067	5.2
P29TA	669-690	Middendorf	21.7	5.3	29	0.143	6.7

* Screen = Screened interval of well below surface, Temp = Temperature, Cond = Conductivity, DO= Dissolved Oxygen, ND = Not Done.

Table 2. Sediment Direct vs. Viable Counts for Selected Sediment Samples.

	P28		P24		P29	
Formation	AODC[1]	PTYG[2]	AODC[1]	PTYG[2]	AODC[1]	PTYG[2]
Surface Soil	8.4	6.38	8.6	6.49	8.7	6.59
Tobacco Road	6.6	2.88	6.9	4.09	7.2	5.67
Dry Branch	7.6	5.58	6.4	6.37	6.7	6.20
McBean	-	-	6.6	4.81	-	-
Congaree	7.7	7.01	7.2	6.48	7.6	6.72
Williamsburg	7.3	3.84				
Ellenton	-	-	7.1	5.76	6.5	N G
	-	-	7.1	3.22	-	-
Pee Dee	7.8	7.29	6.0	3.24	7.0	4.74
	6.6	N G	7.2	6.65	-	-
Black Creek	7.6	5.16	7.2	4.97	6.6	N G
	7.3	3.27	7.0	4.98	7.2	5.54
	-	-	6.5	2.12	-	-
Middendorf	6.7	5.72	7.4	4.40	7.2	6.56
	<6.0	3.68	6.8	N G	7.2	6.28
	7.5	7.16	7.4	6.46	6.7	2.25
	7.7	7.61	7.4	2.48	7.4	5.90
	6.3	6.40	-	-	7.0	5.20
	<6.0	3.90	-	-	7.2	5.93
					7.8	6.89
Mean	7.14	5.259	7.05	4.720	7.20	5.365

All values are $\log_{10}$ densities, per gram dry weight soil.
[1]From Sinclair and Ghiorse (1989).
[2]From Balkwill (1989).
NG = No growth on lowest dilution (10^{-3}).

Table 3. Water Direct vs. Viable Counts for Selected Water Samples.

	P24		P28		P29	
Formation	AODC	PTYG	AODC	PTYG	AODC	PTYG
Surface Soil						
Tobacco Road	3.5	2.39	-	-	-	-
Dry Branch	3.7	2.66	-	-	4.8	2.02
McBean	3.7	1.78	-	-	-	-
Congaree	3.4	0.88	-	-	3.6	2.63
Williamsburg	-	-	-	-	-	-
Ellenton	-	-	-	-	4.4	2.05
Pee Dee	3.0	1.75	3.4	1.75	4.2	1.89
	3.1	2.26	-	-	-	-
Black Creek	-	-	3.8	1.40	4.2	2.33
	-	-	3.5	1.43	-	-
Middendorf	4.3	0.87	3.4	1.43	-	-
	-	-	3.3	1.48		
	3.4	0.98	-	-	3.5	0.76
	-	-	3.2	1.39		
	-	-	-	-	3.4	0.81
Mean	3.51	1.696	3.43	1.432	4.01	1.784

All values are $\log_{10}$ densities per ml water.

Table 4. Water vs. Sediment Samples Using Plate Counts on 1% PTYG.

	P24		P28		P29	
Formation	Water	Sediment*	Water	Sediment	Water	Sediment
Surface Soil	-	-	-	-	-	-
Tobacco Road	2.39	4.09	-	-	-	-
Dry Branch	2.66	6.37	-	-	2.02	6.20
McBean	1.78	4.81	-	-	-	-
Congaree	0.88	6.48	-	-	2.63	6.72
Williamsburg	-	-	-	-	-	-
Ellenton	-	-	-	-	2.05	NG
Pee Dee	1.75	3.24	1.46	7.29	1.89	4.74
	2.26	6.65	-	-	-	-
Black Creek	-	-	1.40	5.16	2.33	NG
			1.43	3.27	-	-
Middendorf	0.87	4.40	1.43	5.72	-	-
	-	-	1.48	7.16	-	-
	0.98	2.48	-	-	0.76	5.90
	-	-	1.39	3.90	-	-
	-	-	-	-	0.81	6.89
Mean	1.696	4.815	1.432	5.417	1.784	5.207

All values are $\log_{10}$ densities, per gram dry weight soil or per ml water.

*From Balkwill (1989).

NG = No Growth on lowest dilution (10^{-3}).

Table 5. Water vs. Sediment Sample Using Acridine Orange Direct Counts.

	P24		P28		P29	
Formation	Water	Sediment*	Water	Sediment	Water	Sediment
Surface Soil	-	-	-	-	-	-
Tobacco Road	3.5	6.9	-	-	-	-
Dry Branch	3.7	6.4	-	-	4.8	6.7
McBean	3.7	6.6	-	-	-	-
Congaree	3.4	7.2	-	-	3.6	7.6
Williamsburg	-	-	-	-	-	-
Ellenton	-	-	-	-	4.4	6.5
Pee Dee	3.0	6.0	3.4	7.8	4.2	7.0
	3.1	7.2	-	-	-	-
Black Creek	-	-	3.8	7.6	4.2	6.6
	-	-	3.5	7.3	-	-
Middendorf	4.3	7.4	3.4	6.7	3.5	7.4
	-	-	3.3	7.5	-	-
	3.4	7.3	-	-	-	-
	-	-	3.2	<6.0	-	-
	-	-	-	-	3.4	7.8
Mean	3.51	6.88	3.43	7.15	4.01	7.09

All values are $\log_{10}$ densities, per gram dry weight soil or per ml water.

*From Sinclair and Ghiorse (1989).

Table 6. Ratio of Direct to Viable Counts for Selected Water and Sediment Samples.

	P24		P28		P29	
Formation	Water	Sediment*	Water	Sediment	Water	Sediment
Surface Soil	-	-	-	-	-	-
Tobacco Road	1.46	1.69	-	-	-	-
Dry Branch	1.39	1.00	-	-	2.38	1.08
McBean	2.08	1.37	-	-	-	-
Congaree	3.86	1.11	-	-	1.37	1.13
Williamsburg	-	-	-	-	-	-
Ellenton	-	-	-	-	2.15	2.17
Pee Dee	1.71	1.85	2.33	1.07	2.22	1.48
	1.37	1.08	-	-	-	-
Black Creek	-	-	2.71	1.47	1.80	2.20
	-	-	2.45	2.23	-	-
Middendorf	4.94	1.68	2.38	1.17	4.61	1.25
	-	-	2.23	1.05	-	-
	3.47	2.94	-	-	-	-
	-	-	2.30	1.54	-	-
	-	-	-	-	4.20	1.13
Mean	2.537	1.592	2.400	1.422	2.674	1.492

All values are ratios (AODC/PTYG) of $\log_{10}$ densities per gram dry weight soil or per ml water,

*From Sinclair and Ghiorse (1989) and Balkwill (1989).

Table 7. Percent of Bacterial Isolates from Sediments and Groundwater that Tested Positive for API-NFT Compounds.

	Sediment	Water
Metabolic Tests		
Nitrate reductase	58	51
Tryptophanase	<1	0
Arginine Dihydrolase	5	15
Urease	42	16
Esculin Hydrolysis	54	ND
Gelatinase	32	37
ß-Galactosidase	56	44
OxidaseE	39	37
Glucose Fermentation	1	2
Aeroboic assimilation tests		
D-Glucose	86	64
L-Arabinose	51	36
D-Mannose	56	36
D-Mannitol	61	47
N-Acetyl-D-Glucosamine	35	45
Malosee	69	60
D-Glucose	70	48
Caprate	15	20
AdipateE	53	31
L-Malate	76	53
Citrate	44	42
Phenylacette	36	11
Total Number of Isolates	430	120

Values are a percentage of total isolates that tested positive, all three sites (P24, P28, P29) combined.

Figures

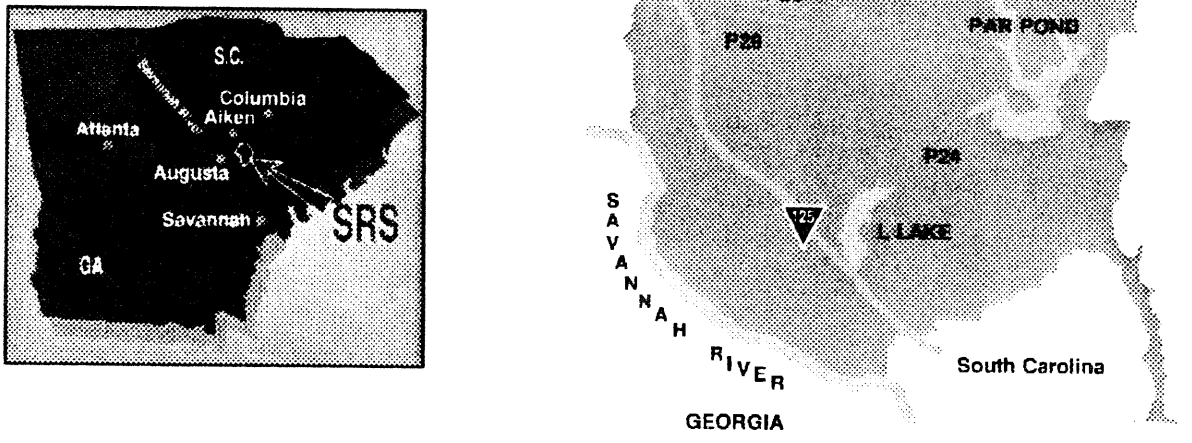
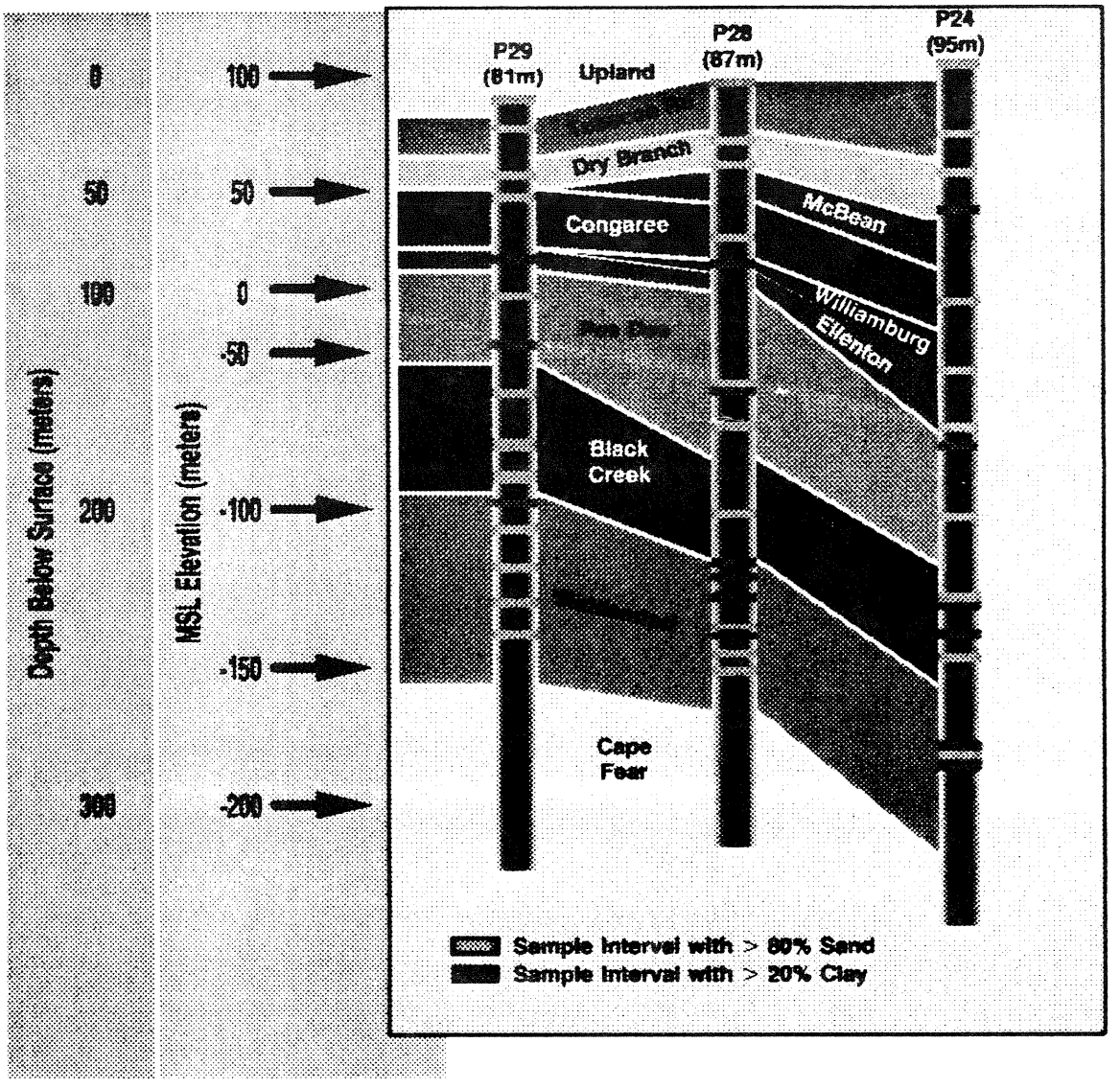


Figure 1. Map of Savannah River Site and Well Clusters P24, P28, and P29 (continued on following page).


Depth Below Surface (meters)
0
50
100
200
300
MSL Elevation (meters)
100
50
0
-50
-100
-150
-200
P29
(81m)
Upland
P28
(87m)
P24
(95m)
Dry Branch
Congaree
McBean
Williamburg
Ellenton
Black
Creek
Cape
Fear
Sample Interval with > 80% Sand
Sample Interval with > 20% Clay