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ENVIRONMENTAL DIAGNOSTIC ANALYSIS OF GROUND WATER BACTERIA AND THEIR INVOLVEMENT IN UTILIZATION OF AROMATIC COMPOUNDS (U)

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DISCLAIMER

Environmental Diagnostic Analysis of Ground Water Bacteria and Their Involvement in Utilization of Aromatic Compounds

by

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This dissertation is dedicated to:

my two children,

John and Elizabeth

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ABSTRACT

John Edmund Wear, Jr.

Environmental Diagnostic Analysis of Ground Water Bacteria
and their Involvement in Utilization of Aromatic Compounds

Dissertation under the direction of Terry C. Hazen, Ph.D., Adjunct Professor of Biology, Wake Forest University and Principal Scientist, Savannah River Technology Center.

The objective of this study was to examine the hypothesis that select functional groups of bacteria from pristine sites have an innate ability to degrade synthetic aromatics that often contaminate groundwater environments, due to exposure to naturally occurring recalcitrant aromatics in their environment.

Ground water was pumped at monthly intervals from twelve wells at four different sites. Two of these sites could be considered pristine. The other two sites were contaminated, one with trichloroethylene, the other with polyaromatic hydrocarbons and possible sulfur compounds. The bacteria in shallower wells tended to have both the greatest direct counts and heterotrophic viable counts. Acridine orange direct count differences between any of the wells were less than one order of magnitude. In contrast, viable counts had several orders of magnitude difference between wells. This illustrates that there are great variations in physiological states and metabolic needs of bacteria in these different aquifers. All but one of the wells studied demonstrated higher counts on low nutrient media than high nutrient media, suggesting the oligotrophic nature of these groundwater environments.

This study demonstrates that subsurface microbial communities are capable of utilizing lignin and humic acid breakdown products. Utilizers of these compounds were

found to be present in most all the wells tested. Even the deepest aquifer tested had utilizers present for all six of the aromatics tested. Highest counts for the aromatics tested were observed with the naturally occurring breakdown products of either lignin or humic acid. Carboxylic acids were found to be an important sole carbon source for groundwater bacteria possibly explained by the fact that they are produced by the oxidative cleavage of aromatic ring structures. The carbohydrate sole carbon sources that demonstrated the greatest densities were ones commonly associated with humics. This study indicates that utilization of naturally occurring aromatic compounds in the subsurface is an important nutritional source for groundwater bacteria. In addition, it suggests that adaptation to naturally occurring recalcitrant substrates is the origin of degradative pathways for xenobiotic compounds with analogous structure. This work has important implications for in situ bioremediation as a method of environmental cleanup.

INTRODUCTION

Ground water and ground water contamination. Potable ground water is vital to everyone. Ground water makes up over 95% of the world's freshwater reserves and is the main source of drinking water for a large percentage of the world's people. Nearly half of the population of the United States uses ground water from wells or springs as their primary source of drinking water (Bouwer, 1984). Thirty-six percent of the municipal public drinking water supply comes from ground water and seventy-five percent of major U. S. cities depend on ground water as their major water source (Pye and Patrick, 1983).

As a result of various human activities, particularly those involving disposal of chemical waste to the land, the quality of ground water is becoming increasingly threatened. During this century there has been a phenomenal expansion of the chemical industry. Due to this expansion there has been an increase in hazardous waste production. Total annual world production of synthetic organic chemicals is over 300 million tons. In addition, more than 1000 new compounds are marketed each year (Fewson, 1988). It was estimated that over 57 million metric tons of hazardous industrial waste were produced in the US during 1980 alone (Keswick, 1984). For these reasons, pollution of ground water due to hazardous waste is becoming a very serious problem. According to J. H. Lehr of the Association of Ground water Scientists and Engineers in Worthington, Ohio, more than 10 million Americans probably now use tap water with contaminant levels that exceed EPA standards (Knox, 1988). Each year our total withdrawals and our dependency on ground water increase, thus amplifying the impact that pollution has upon us. Industrial waste landfills are the most common source of serious ground water contamination because of their potential for leaching a wide variety of hazardous substances into underlying ground water (Keswick, 1984).

One of the best known cases of industrial waste landfill pollution is the Love Canal Landfill in Niagara Falls, New York. Hooker Chemical Co. disposed of more than 43,000 tons of waste from 1942 to 1952 containing many hazardous chemicals. The chemicals disposed of included dioxin, hexachlorobenzene, tetrachloroethylene, chloroform, dichloroethane, benzene, hexachloride, trichloroethylene, toluene, and phenol. These substances have migrated from the landfill to other areas, and have affected both ground and surface waters. The cost to clean up this site was estimated at 45 million dollars (Keswick, 1984). The Superfund bill was passed by Congress in 1980 to assist in the cleanup of sites like this one, the greater portion of the money coming from industry. Originally the Superfund was to raise four billion dollars to be used in the clean up of hazardous waste. This amount has been increased substantially since. A 1983 report by the Office of Technology Assessment (OTA) estimated that 10,000 sites or more may require cleanup by Superfund (Goldsmith and Hildyard, 1988).

Conventional methods for cleaning up toxic waste polluted sites include air stripping, charcoal filtration and incineration. These methods tend to be expensive and essentially move the pollutant from one part of the environment to another. Air stripping volatilizes toxic waste, thus releasing them into the atmosphere. Charcoal filtration can efficiently remove toxic waste but only captures the toxic waste by adsorption and does not destroy it. These wastes can potentially leach out of the charcoal once the charcoal has been disposed of in the landfill. High temperature incineration can, if properly operated, have very good destruction efficiencies. But, if not operated correctly, incineration can result in the release of wastes that were not destroyed, or in the formation and release of new products of incomplete combustion (Goldsmith and Hildyard, 1988).

There is a tremendous potential for utilizing subsurface microorganisms to clean-up toxic waste contaminated sites. Many contaminants in solution in the ground water, as well as vapors in the vadose (unsaturated) zone, can be completely degraded and possibly

utilized or transformed into new compounds by naturally occurring indigenous microbial populations. In fact, the complete degradation of organic molecules in water and soils is almost always a consequence of microbial activity (Alexander, 1980). This biodegradation can often result in the transformation of hazardous organic chemicals into innocuous products.

A term used to describe the utilization of microorganisms to clean up contaminated sites by biodegradation of the contaminants is bioremediation. For bioremediation to be an effective method of cleaning up toxic waste sites, some assistance must be given the microorganisms so that they can carry out biodegradation more efficiently. According to John T. Wilson of the EPA's Kerr Laboratories, without human intervention microorganisms typically degrade only about 1% of the hydrocarbon pollution flowing past them (Knox, 1988). There are a number of ways that the subsurface environment can be modified to increase the rates at which biodegradation will occur. Since oxygen is frequently one of the major limiting factors, dissolved oxygen can be increased by injecting hydrogen peroxide into the subsurface through injection wells. Other limiting nutrients like nitrogen and phosphorus can also be injected. The amounts of these nutrients are determined by the specific environmental conditions and the requirements of the microorganisms that have the potential to break down the pollutant.

Altering the subsurface environment in order to stimulate the indigenous microorganisms to degrade xenobiotic contaminants is called in situ bioremediation. In situ bioremediation of aquifers is a promising method for cleaning up contaminated sites in a cost effective manner. An important aspect of in situ bioremediation is that the physical removal of contaminated soils is not necessary, which significantly reduces cost and public health risks (Wilson et al., 1986). There are some problems associated with in situ bioremediation. Upon injection of nutrients, so much bacterial biomass may be

produced that the flow of water in that vicinity is restricted. Another potential problem is that compounds that are even more toxic than the contaminant itself may be produced. For example, under anaerobic conditions trichloroethylene may be transformed into vinyl chloride a much more toxic compound (Wilson and Wilson, 1985). A fundamental problem with in situ bioremediation is the inability of microorganisms to break down specific contaminants due to the structure of the contaminant. Recalcitrance, or the ability of a substance to remain in a particular environment in an unchanged form, can be increased as a result of polymerization and branching, and the presence of chlorine atoms on the molecule. The greater the number of attached chlorine atoms per molecule relative to the total number of carbon atoms, the more difficult it is for a compound to be broken down (Alexander, 1965; Fewson, 1988).

Cleavage of the carbon-halogen bond is endergonic and thus requires a substantial energy input. The energetics of the degradation of extensively halogenated carbon compounds is such that there is little prospect for microorganisms to adapt to many of these compounds as growth substrates (Atlas and Bartha, 1987). Other factors may also affect the biodegradation of specific pollutants. The pollutants may be present in mixtures that are incompatible for effective degradation or they may be in too low or too high a concentration. Genetic factors may also be involved, for instance, the inducer for a pathway may not be present, or the pathway may be blocked by inhibitors (Lindow et al., 1989).

In addition to the recalcitrance of certain contaminants, other factors can affect in situ bioremediation. Several environmental factors are known to influence the capacity of the indigenous microbial population to degrade contaminants. These factors include dissolved oxygen levels, pH, temperature, oxidation-reduction potential, availability of mineral nutrients, salinity, concentration of specific pollutants, and the nutritional quality of dissolved organic carbon in the ground water (Wilson et al., 1986). In some situations

the pollutant is broken down or modified even though it is not utilized for growth by the microorganism. This process, called co-metabolism, occurs while the microorganism is metabolizing another substrate (Horvath, 1972). Co-metabolism may prove useful as a means of utilizing bacteria to degrade recalcitrant halogenated organics, as, for example, the breakdown of trichloroethylene (TCE) by Pseudomonas putida harboring the TOL plasmid (Nelson et al., 1988). While these strains do not utilize TCE, it is broken down by enzymes that are produced by these microorganisms for the metabolism of another substrate.

Probably the best way to study the potential for in situ bioremediation is by examining the sediments, but our technology at the present time does not enable us to do this in a cost effective way. Also, since samples of subsurface sediments are taken at only one point and the sampling process is destructive, the precise point at which the sample was taken cannot be sampled again, although nearby sites could be sampled.

An alternative to sampling sediments is to study the ground water. Water sampling has several advantages over sediment sampling. Ground water can be sampled at different intervals, therefore, subsurface microorganisms can be studied as the parameters of interest change with time. These variations may be due, for instance, to precipitation and seasonal changes affecting the rate of recharge of the aquifer and causing the levels of other parameters such as pollutant concentration to fluctuate. A major disadvantage of ground water sampling is that under many conditions the ground water may not be indicative of the microbial communities actually associated with the surrounding sediments (Hazen et al., 1991; Kolbel-Boelke et al., 1988). It has been clearly shown that the microbial flora of sediment and ground water environments decrease in similarity with increasing depth and as conditions go from eutrophic to oligotrophic (Hazen et al., 1991). Thus, the value of using ground water methods is greatest in near-surface environments which are usually where contaminants are found.

The importance of subsurface microorganisms in controlling the quality of ground water has recently become apparent (Wilson and McNabb, 1983). Yet, relatively little is known about the microbiology of ground water (McNabb and Mallard, 1984). Early microbiological studies concluded that the sediments below the root zone were essentially devoid of life (Waksman, 1916). However, these early studies depended solely on the culturability of the microorganisms, and the high nutrient culture methods employed were subsequently demonstrated to be of limited value in determining the bacterial densities in the subsurface environment. These erroneous conclusions delayed progress in the study of the microorganisms in subsurface habitats for many years later. For example, a comprehensive search of the literature by United States Environmental Protection Agency scientists in the early 1970's revealed virtually no information on the indigenous microflora of common drinking-water aquifers between a few meters to several hundred meters deep (Ghiorse and Wilson, 1988).

Not only is it now clear that microbial communities exist in deep subsurface environments but it has also been demonstrated that these microorganisms are abundant. For example, Wilson et al. (1983b) found that the numbers of microorganisms in a shallow water-table aquifer were surprisingly high and did not appear to decrease dramatically with increasing depth. Indeed, researchers associated with the Department of Energy's Deep Probe project demonstrated that microbial life was abundant at far greater depths (greater than 300 m) than ever before aseptically sampled (Fredrickson and Hicks, 1987).

The density of heterotrophic microorganisms is often limited by the concentration of metabolizable carbon (Poindexter, 1981; Atlas and Bartha, 1987). For microorganisms to be abundant in deep subsurface habitats, carbon sources must be available that would support growth. Subsurface areas not associated with petroleum, peat, or coal deposits usually have low organic carbon concentrations and, hence, can be considered

oligotrophic (Ghiorse and Balkwill, 1983; White et al., 1983). The populations of subsurface bacteria found in these areas most closely resemble populations seen in oligotrophic aquatic environments (Kuznetsov et al., 1979). Since primary production in the subsurface is low, bacterial cells must utilize carbon sources leached from the surface or deposited in the adjacent geological strata. Microbes in the subsurface environment are forced to exist "on the stuff that no one bothered to eat" (Wilson and McNabb, 1983).

Due to the low organic carbon, competition is likely to be extreme, with selection favoring microorganisms that can utilize carbon sources that might otherwise not be exploited (Jiménez, 1989). Selection, under these conditions, might result in microorganisms in the subsurface environment with unusual pathways for biotransformations that are very different from those of microbes found at the surface (Wilson and McNabb, 1983; McCarty et al., 1984).

A relatively new source of nutrients in many aquifers is organic contamination. Many microorganisms have adapted to the pollution that has entered their environment and, in fact, can utilize it as a source of energy and organic carbon. Because of the low levels of metabolizable carbon in these environments, the addition of hydrocarbon pollution enables many of these microorganisms to flourish (Harvey et al., 1984; Knox, 1988).

In some situations these organic contaminants are not readily utilized as carbon sources but instead remain in the environment for extremely long times. One reason is that many of these compounds are xenobiotics, these are compounds that are man-made and therefore have chemical structures to which these microorganisms have not been exposed (Fewson, 1988). Since the microorganisms have not been exposed to these chemicals there has been no selection for organisms able to utilize these potential organic carbon sources. Therefore these compounds are often resistant to biodegradation.

Mechanisms of genetic adaptation.

A number of mechanisms have been suggested that might account for genetic adaptation of microorganisms to xenobiotic compounds. One possible way a catalytic pathway may arise is for a mutation to occur that enables a bacterium to utilize a substrate that it could not otherwise utilize or would not utilize efficiently. A mechanism that would allow this to occur (without loss of the ability to utilize the original substrate) would be gene duplication followed by sequence divergence. The original gene might remain functional while the duplicated gene is free to undergo mutational events that might increase its catabolic specificity for other substrates (Clarke, 1984). For example, chemostat cultures of Escherichia coli that were maintained on low concentrations of lactose, have been shown to undergo duplication of the lac genes and as a result experience more rapid growth and a greater capability to store food reserves than those bacteria with single copies (Horiuchi et al., 1962). There have been similar observations with other species (Inderlied and Mortlock, 1977; Rigby et al., 1974).

A complication with genetic adaptation is that an accompanying regulatory mutation may need to occur. For example, an existing catalytic pathway might enable the bacteria to break down the contaminant but a regulatory mutation needs to occur in order that the pathway be induced (Mortlock, 1981). Another possibility is that a mutation may alter the specificity of the first enzyme in a pathway that has already undergone a constitutive mutation, followed later by a regulatory mutation that induces the pathway only when the new substrate is present (Hall, 1984). Both of these events might occur following gene duplication.

Transposons may play an important role in adaptation of bacteria to xenobiotics. Transposons carry genes that allow the transposon to move from one DNA replicon to another, for example, from chromosome to conjugative plasmid (Beringer and Hirsch, 1984). Some transposons are known to contain active catabolic genes (Cornelis et al.,

1978), and there is evidence that transposons are transferred widely. Transposon TN1, which contains a gene for ampicillin resistance, has been observed all over the world in a number of different genera (Heffron et al., 1975).

Once a pathway exists for the utilization of a novel substrate, mechanisms such as transformation, transduction and conjugation could allow the rapid dissemination of those genes throughout bacterial populations. Horizontal transfer by conjugative plasmids may play an important part in the formation of novel pathways. Studies have shown a higher frequency of plasmids in bacterial isolates from environments where nutritional or pollution stress exists (Wickham and Atlas, 1988; Fredrickson et al., 1988). A number of plasmids have been found that enable their host to degrade compounds that would otherwise remain in the environment as pollutants. Many bacterial genes for xenobiotic degradation have originated from strains isolated from contaminated waste sites and these genes are often found on plasmids (Lindow et al., 1989).

Plasmids are autonomously replicating covalently closed, circular extrachromosomal DNA. They can be inherited without being linked to the chromosome (Crosa and Falkow, 1981; Hardy, 1986). They are not essential for survival but they may encode genetic determinants that permit the host to survive better in an adverse environment or to compete better with others occupying the same ecological niche (Crosa and Falkow, 1981). Plasmids could assist genetic adaptation to xenobiotics because there is not only a greater potential for plasmids to transfer to other microorganisms but also for plasmids to recombine with each other to produce new arrangements of genes (Beringer and Hirsch, 1984). Studies have shown that the evolution of an interacting microbial community may be more important in the evolution of a novel pathway than a series of evolutionary events occurring within one organism (Slater, 1984). Several studies using chemostats have indicated that it is possible to observe gene transfer and the formation of novel pathways using mixed cultures of bacteria and mobilizable plasmids

(Kellogg et al., 1981; Knackmuss, 1981, Slater et al., 1984). Evidence exists that relicons such as plasmids can be disseminated widely, even to other genera. Plasmid RP1 has been identified as appearing unchanged in 17 different bacterial genera (Slater, 1984).

Almost all of the plasmids characterized to date that have genes for xenobiotic catabolism have been from gram negative bacteria, predominantly Pseudomonas (Lindow et al., 1989). The most studied and best understood catabolic plasmid is the TOL plasmid. The archetype TOL plasmid was first described in 1974 (Williams and Murray, 1974). This plasmid codes for enzyme pathways that enable the microorganism harboring it to degrade toluene, m-xylene, p-xylene and other similar organic substances (Figure 1). TOL plasmids are usually found in Pseudomonas species (Burlage et al., 1989). The toluene utilizing genes of the TOL plasmid may not be confined to plasmids, but may also be located on chromosomes. Genes that are almost identical to those on the TOL plasmid have been detected in the chromosome of an isolate of Pseudomonas putida that was able to live on toluene as a sole carbon source (Sinclair et al., 1987). The host range of the TOL plasmid possibly includes other genera. For example, a TOL-like plasmid has been described in Alcaligenes eutrophus (Hughes et al., 1984).

The ubiquity of the TOL plasmid has been examined using standard methods of enrichment and isolation. Williams and Worsey (1976) isolated thirteen bacteria from soil samples using selective enrichment on m-toluate minimal medium. All thirteen carried plasmids that were similar to the archetype TOL plasmid although these plasmids did show variations in their properties.

The TOL plasmid can dissociate into two or more separate plasmids that are capable of independent replication. Dissociation gives rise to a non-conjugative plasmid, which specifies the degradative pathway, and to a separate conjugative plasmid. The fragment of TOL which can be inserted into unrelated DNA molecules has a molecular weight of about 40 X 10^6 daltons. TOL plasmids can also dissociate to lose a fragment

that has a molecular weight of about 30 X 10^6 daltons. This appears to involve recombination between directly repeated 1400 base pair sequences (Hardy, 1986).

Catabolic plasmids which appear to be related to the TOL plasmid include the NAH plasmid (Dunn and Gunsalus, 1973) which codes for naphthalene degradation, the OCT plasmid (Chakrabarty et al., 1973) which codes for n-octane degradation, the SAL plasmid which codes for salicylate degradation (Chakrabarty, 1972) and the CAM plasmid which codes for camphor degradation (Rheinwald et al., 1973). All of these plasmids were originally found in different strains of Pseudomonas putida.

Carbon sources in ground water environments.

Organic compounds in ground water consist mainly of recalcitrant humic and fulvic acids, tannins and lignins (Wallis et al., 1981). These recalcitrant substances and their breakdown products may be a major carbon and energy source for bacteria in sediments and ground water. Humic substances (Figure 2) are a major reservoir of organic carbon in aquifers (Aiken et al., 1985), and can consist of 21% to 66% of the total dissolved organic carbon (Thurman, 1985). Humic acid resists degradation by microorganisms as evidenced by the long residence times of this compound (Campbell et al., 1967). Radiocarbon dating has demonstrated humic compounds thousands of years old in some soil (Jenkinson and Rayner, 1977). The recalcitrance of humic molecules is due to their molecular weight and complex structure, both resulting from disorderly condensation and extensive co-polymerization and crosslinking (Stout et al., 1981).

There are at least two hypotheses to account for the origin of humic substances in ground water, one, that humic material originates in overlying soils and is leached from organic matter moving through the vadose zone and into recharge waters, and two, that humic substances are leached from kerogen in the sediment of the aquifer (Thurman,

1985). In fact, kerogen has been described as the most common organic material on earth (Brownlow, 1979).

Lignin (Figure 3) is second only to cellulose as a reservoir of metabolically derived carbon in the biosphere (Zelkus, 1981). One function of lignin in plants is to impede microbial destruction of plant tissues, which might explain in part why it is the most recalcitrant natural product (Zelkus, 1981). In certain environments the recalcitrance of lignin can result in the formation of lignites and coals, the major forms of fossilized organic matter on earth (Zelkus, 1981). Among the reasons for the recalcitrance of lignin are that it is slowly metabolized because it is an insufficient energy source for microbial growth, its insolubility in water, and the inaccessibility to cleavage of its intermolecular bonds. It also appears that lignolytic microbes require oxygen, since lignin does not degrade in anaerobic environments (Zelkus, 1981). The breakdown of lignin is important in humic acid formation (Brownlow, 1979). Although lignin is resistant to degradation, there are known lignin-degrading fungi, actinomycetes, and bacteria (Crawford, 1981; Crawford and Crawford, 1984).

Because the concentration of organic carbon in ground water is commonly less than 1 mg carbon per liter (Leenheer et al., 1974), it is difficult to isolate and characterize the breakdown products of these compounds, and for this reason there are relatively few studies of these substances in ground water (Thurman, 1985). Therefore, data are not available on naturally occurring aromatic breakdown products of lignin and humic acid in ground water.

The oxidation of both lignin and humic acid (Figure 4) results in a mixture of phenolic aldehydes and acids (Atlas and Bartha, 1987). Laboratory investigations indicate what some of these compounds might be. Lignin and humic acid must be broken down extracellularly before the chemical subunits enter the cell. For example, the white-rot fungi must break down lignin extracellularly to fragments small enough to diffuse to

and enter the hyphae. Chen et al. (1982) identified nine aromatic acids produced during the degradation of lignin by the white-rot fungus Phanerochaete chrysosporium. These compounds included p-hydroxybenzoic acid, vanillic acid, isovanillic acid, veratric acid, dehydrovanillic acid, and 4-hydroxy-5-methoxypthalic acid. Crawford (1981) used gas chromatography and G.C.-mass spectrometry to identify several simple lignin derivatives, including p-hydroxybenzoic acid, vanillic acid, protocatechuic acid, p-coumaric acid, syringic acid, and ferulic acid, from cultures of the aerobic lignolytic actinomycete Streptomyces viridosporus. A study by Donnelly and Crawford (1988) demonstrated that p-coumarate was the dominant compound in culture supernatants of a species of Streptomyces.

Therefore, from these studies, it would appear that aromatic monomers should occur in ground water wherever lignin or humic acid is being broken down. If this is true then these aromatic compounds should be a source of metabolizable carbon for bacteria in subsurface environments. In addition, aromatic compounds with similar structures might be degraded as well. Prior evidence has demonstrated this possibility.

A study of microbes in shallow subsurface soil demonstrated that these microbes could degrade toluene (Wilson et al., 1983a). A study by Ventullo and Larson (1985) found that benzoic acid was rapidly mineralized in ground water. Ward (1985) demonstrated that benzoic acid was mineralized in both anaerobic and aerobic soils. Suflita and Miller (1985), using ground water aquifer microcosms, demonstrated that the microflora of ground water aquifers have the potential for degrading phenolic substrates. Phenol was metabolized in all the tested ground water habitats. Dobbins et al. (1987) demonstrated that there was great variation in mineralization potential of phenol between differing soil types and subsurface horizons. Federle (1988) demonstrated that phenol, benzoic acid and benzylamine (all monosubstituted aromatic compounds) were mineralized rapidly in two shallow (20 m) soil profiles. In addition, the variability in

mineralization activity within a soil profile could exceed the mineralization activity between two quite different profiles. Hicks and by Fredrickson (1989) studied mineralization of acetate, phenol and 4-methoxybenzoic acid in deep subsurface sediments at SRS. They found that biodegradation of the labeled substrates did not appear to be related to depth and was often as high or higher in the lower strata as it was in the upper strata or in the surface soil. A number of other studies have isolated bacteria capable of utilizing the breakdown products of lignin and humic acid (Gonzalez et al., 1986; Ball et al., 1989).

Therefore, it would appear that utilization of aromatic compounds found naturally that are breakdown monomers of lignin and humic acid or other complex aromatic substances may allow microbes in the subsurface to genetically adapt to the utilization of xenobiotic aromatic compounds introduced into the environment.

Objectives. My hypothesis is that, due to exposure to naturally occurring recalcitrant aromatics in their environment, select functional groups of bacteria from pristine sites have an innate ability to degrade synthetic aromatics that often contaminate ground water environments. This study differs from previous studies that have examined this area in that naturally occurring breakdown monomers of lignin and humic acid were used and the indigenous bacteria capable of using these compounds were quantified. This hypothesis was tested in the following ways: First, by determining the density of select functional groups of bacteria responsible for the degradation of certain naturally occurring and synthetic aromatics in pristine and contaminated ground water environments. Second, by determining the innate ability of microbial communities from pristine and contaminated environments to degrade synthetic aromatic compounds. Third, by examining the ways in which abiotic factors might affect the density and catabolic potential of these microbes to degrade aromatics.

MATERIALS AND METHODS

Sample sites. This research was conducted at the Department of Energy's Savannah River Site, a 768 km^2 nuclear production facility, producing nuclear materials for national defense, medical applications and the space program. Located in the Upper Atlantic Coastal Plain, near Aiken, South Carolina (Figure 5), the site is operated by Westinghouse Savannah River Company for the United States Department of Energy. It was built in the early 1950's and has five different nuclear reactors, one of which is still functional. Therefore, it has all the processing wastes associated with these types of operations, including solvents, petroleum and coal pile leachates. Geologically, the surface at the site is composed of approximately 400 m of unconsolidated sands, clayey sands and sandy clays that overlie a basement of dense metamorphic rock, igneous intrusives, or consolidated sedimentary rock. For a complete geological description see Sargent and Fliermans (1989). Ground water was pumped at monthly intervals from twelve wells at four different sites: P28, P29, DCB, and MSB (Figure 6). The screened interval (well casing water intake holes) for these wells ranges from depths of 33.2 m to 210.3 m (Table 1). Two of the most common ground water contaminants at the Savannah River Site have been trichloroethylene and polyaromatic hydrocarbons. Trichloroethylene was used in the past for used for degreasing target elements. The MSB site is associated with a leaky process sewer line that has released trichloroethylene into the surrounding soil and subsequently into the underlying ground water. This site has the highest trichloroethylene contamination of all four sites with a mean concentration of 41,100 ppb. Although the Savannah River Site has had a number of different nuclear reactors, energy is produced using coal. Therefore coal piles are present and the accompanying contamination by polyaromatic hydrocarbons. The DCB site is associated

with coal piles and is contaminated with a heterogeneous mixture of trichloroethylene, polyaromatic hydrocarbons and possible sulfur compounds. The DCB site has a mean TCE concentration of 60 ppb. The P28 (Figure 7) and P29 (Figure 8) sites could be considered pristine. The direction of vertical flow in these wells is downward for the Congaree but upward for the formations sampled below the Congaree which in this study were the Pee Dee and Middendorf formations (Sargent and Fliermans, 1989). Two wells, P29TA and P28TA are wells bored and sampled for the Department of Energy's Deep Subsurface Microbiology Program (Fredrickson et al., 1987; Fredrickson et al., 1989; Fliermans and Balkwill, 1989). For a complete discussion of the Deep Subsurface Microbiology Program results see volume 7 of the Geomicrobiology Journal.

Sampling ground water. A portable generator supplied electrical power to the submersible pumps already in place at each well site. A Hydrolab surveyor model SVR2-SU sonde unit (Hydrolab Inc., Austin, TX) was connected to a valve at the surface. Since water in the well casing exchanges gases with the atmosphere and interacts with the well casing, it is not representative of the water in the aquifer. Therefore, the water was pumped from each well until the conductivity and pH of the water being purged stabilized (Lee and Jones, 1983; US EPA, 1987, Handbook 625/6-87/016), at which point a sterile 4 liter and 250 ml Nalgene bottle was filled aseptically for bacterial analysis. A 30 ml amber glass bottle with teflon septa also was filled for chemical determination using gas chromatography.

Aromatic compounds used in this study. The ability of the indigenous bacteria to utilize each of six aromatic compounds was quantified in this study (Figure 9). Ferulic acid, p-coumaric acid and isovanillic acid represent lignin and humic acid breakdown products (Chen et al., 1982; Crawford, 1981; Donnelly and Crawford, 1988). Salicylic acid is produced by plants and is an intermediate in the degradation of some polyaromatic

compounds (Cerniglia, 1984). Salicylate (also known as 2-hydroxybenzoate) has been identified as an intermediate in the naphthalene degradation pathway and as an inducer of the nah operon carried on the NAH7 plasmid for the degradation of naphthalene (Schell, 1985). Phenol represents a class of ground water contaminants (MacRae, 1989). M-Toluic acid is a substrate utilized by Pseudomonas putida MT-2 which harbors the TOL plasmid and has been used as an enrichment medium for the isolation of TOL plasmids in a number of studies (Williams and Worsey, 1976).

Aromatic utilization. In order to quantify the number of bacteria capable of utilizing aromatic compounds, a most probable number method was used. The bacterial cells in ground water were concentrated by filtration. One hundred ml, 10 ml and 1 ml aliquots of ground water were taken from the 250 ml sample flask and were filtered through 0.22 μm pore size Nuclepore polycarbonate filters (Costar Corp., Cambridge, MA). Filters were then placed in tubes containing 7 ml of modified Stanier's minimal media (Stanier et al., 1966) and 100 ppm of the aromatic to be tested. Smaller volumes, 0.1 ml and 0.01 ml, were dispensed directly into the tube without filtration. Five replicates were made for each volume. After three weeks of incubation at 25°C a 600 ml aliquot was removed and filtered using a 0.45 μm Durapore filter (Millipore Corp., Bedford, MA) and examined for the disappearance of the aromatic substrate using a Gilford Response UV spectrophotometer (Ciba Corning Diagnostics Corp., Norwood, MA). (Note: Stanier's minimal media has been used in sole carbon source studies of aerobic Pseudomonads; Stanier et al., 1966). Preliminary experiments indicated that the greatest number of bacteria were observed when using the medium as specified by the author and with the addition of 100 ppm of the aromatic carbon source.

Toluene enrichment. Ground water (100 ml) was filtered through 0.22 μm pore size Nuclepore polycarbonate filters (Costar Corp., Cambridge, MA) and the filters were placed in cotton stoppered tubes of Stanier's minimal media with no carbon source. The

tubes were then placed in jars with an atmosphere saturated with toluene. Additional tubes containing Stanier's minimal media were inoculated with 1000 ppm or 100 ppm toluene and capped with a teflon septum.

Lysis of bacterial cells for PCR amplification. A 200 µl volume of the Stanier's minimal medium enriched with one of the aromatics was placed in a sterile 0.5 ml microfuge tube, centrifuged and washed once with sterile ultra-filtered water. Tubes were then placed in dry ice for 10 minutes, removed then heated to 50°C for 1 minute in a thermal cycler (DNA Thermal Cycler, Perkin-Elmer Corporation, Norwalk, CT). The freeze-thaw procedure was repeated six times. After every second freeze-thaw cycle, the tubes were vortexed for 15 seconds then centrifuged briefly to bring fluid to the bottom of the tube. After all six freeze thaw cycles were completed, the tubes were placed in the thermal cycler at 85°C for 5 minutes to inactivate nucleases. Tubes were then ready for the addition of the PCR mix (Bej et al., 1991).

PCR amplification of the catechol dioxygenase gene. The nucleotide sequences for the catechol dioxygenase gene from both the NAH and TOL plasmids have been reported (Harayama et al., 1987). By comparing both segments it was possible to determine highly conserved regions that may be suitable to use as oligonucleotide primers. Three positive strand primers and three negative strand primers were chosen and combinations of all possible pairs of these primers were tested. The pair of primers selected in this study were the pair that produced the greatest amount of amplicon using the model organism for this study (Pseudomonas putida mt-2 which harbors the TOL plasmid). These were 20-base oligomers specific for sites flanking the target sequence on the catechol dioxygenase gene. To determine that the amplicon produced was the correct sequence, a restriction digest was performed using several different restriction enzymes and the sizes of the fragments compared with known size standards (Sambrook et al., 1989).

PCR amplification was performed using AmpliTaq DNA polymerase and a GeneAmp kit (Cetus Corp., Emeryville, CA) as described by Saiki et al. (1988). In order to determine suitable primers for amplification of the catechol 2,3 dioxygenase gene, regions in the gene were first chosen that were identical in both the NAH and TOL plasmids. These regions would be less likely to undergo mutation and therefore the chances of finding this sequence in the environment using PCR would be improved. A number of positive and negative strand primers were synthesized with sequences that were from these conserved sequences. Pairs of primers were then tested using the model bacterium, Pseudomonas putida mt-2 (which harbors the TOL plasmid). PCR primers were chosen using the following guidelines: a length of 18 to 28 bases, 50% to 60% G+C content, the calculated thermal melting points for the bases should be balanced, complementarity at the 3' ends should be avoided, and there should be no (or little) internal homology capable of forming hairpin structures (Innes and Gelfand, 1990). The primer pair 5' GGG CCG TGT CTA TCT GAA GGC 3' and 5' GGT TAC CGG ACG GGT CGA AG 3' was chosen because it gave the highest yield of amplicon when PCR was performed on Pseudomonas putida mt-2. The calculated T_m for these two primers was 68°C and 66°C, respectively. These primers were synthesized to these specifications and purified using polyacrylamide gel electrophoresis by Synthetic Genetics, San Diego, California. The protocol for PCR was 10 min at 95°C followed by 30 cycles of 1 min at 95°C, 1 min at 60°C, and 1 min at 72°C then finally 3 min at 72°C.

Testing ground water samples for chlorinated organics. Samples were taken in amber (30 ml) glass bottles and sealed with teflon septa. Upon reaching the laboratory 10 ml aliquots of ground water were dispensed into 20 ml serum vials with teflon-sealed crimp tops and refrigerated until ready to test using gas chromatography. Sealed samples were tested the day the sample was taken using a headspace sampling method. When volatile organics in water are allowed to come to equilibrium with the vapor head space,

the concentration in the head space is proportional to the concentration in the water (Micure, 1981). Samples were processed using an automated head space gas sampling device (Model 19395A, Hewlett Packard, Avondale, PA) with a bath temperature of 75°C. Trichloroethylene was analyzed using a Hewlett Packard 5890 gas chromatograph (Hewlett Packard, Avondale, PA) equipped with a 60m X 0.75 mm I. D. VOCOL capillary column (Supelco, Bellefonte, PA) and an electron-capture detector (ECD). The following conditions were used: oven temperature, 32°C for 8 min then increased 4°C/min until reaching 80°C, then held at 80°C for 1 minute; injector temperature, 175°C; and detector temperature 325°C. The split ratio was set at 1:1. The carrier gas was He and the make-up gas was N_2. Quantitative analyses were made for TCE by relating peak heights of samples to those of prepared standard curves.

Direct counts of ground water bacteria. Acridine orange direct counts were used to enumerate ground water bacteria. This is a method that is widely used and has been demonstrated to be one of the best methods available today for enumeration of bacteria in environmental samples (Daley, 1979). It is useful when enumerating the entire population of microorganisms but it does not distinguish between specific organisms or groups. Samples (4.4 liters) were concentrated to 10 ml using continuous flow centrifugation. Ten µl aliquots were applied to the eight wells of a toxoplasmosis slide (Cel-Line Associates Inc., N. J.) and heat fixed by placing the slide on a slide warmer at 50°C until dry. Slides were stained with acridine orange (Difco SpotTest #3561-26-5) for 2 minutes, rinsed with filtered distilled water and bacterial numbers counted using a Zeiss epifluorescence microscope (Lopez de Victoria, 1989).

Plate counts. Two media were used for heterotrophic viable plate counts, PTYG and 1% PTYG. PTYG agar contains the following ingredients per liter of distilled water: glucose, 10 g; yeast extract, 10 g; peptone, 5 g; Trypticase, (Becton Dickinson Microbiology Systems, Cockeysville, MD), 5 g; $MgSO_4 \cdot 7H_2O$, 0.6 g; $CaCl_2.H_2O$, 0.07

g; agar, 15.0 g (Balkwill and Ghiorse, 1985). The 1% PTYG agar is a 1:100 dilution of PTYG, except that the concentrations of $MgSO_4 \cdot 7H_2O$, $CaCl_2.H_2O$ and agar are the same as with PTYG. Previous studies have demonstrated that different populations are generally isolated on the two types of media. Therefore, both media were used to get a better approximation of heterotrophic bacterial numbers (Balkwill et al., 1989).

One, 10 and 100 ml samples of well water were filtered through 0.45 μm pore size Millipore filters (47 μm diameter, type HA). Filters were placed on duplicate plates of PTYG and 1% PTYG, incubated at 27°C and read using a binocular microscope (Wild Heerbrug model M5, Switzerland) after 5 days.

Percentage of active cells. The percentage of active cells as measured by acridine orange is believed to be a measure of the percentage of actively growing and synthesizing bacterial cells in the microbial community (Daley, 1979; Lopez-Torres et al., 1988). When acridine orange attaches as a monomer to double-stranded DNA it will fluoresce green, and when attached as a dimer to single-stranded RNA or denatured DNA it will fluoresce orange-red. Therefore, inactive cells containing mostly double-stranded DNA fluoresce green and actively growing cells with large amounts of RNA should fluoresce orange-red (Daley, 1979). One drawback of this method is that dead cells with denatured DNA would also fluoresce orange-red. For this reason it is important to count discrete cells and not cells that appear to have disrupted membranes. Using this method the percentage of active cells is the percentage of red fluorescing cells in the total community of red and green cells.

Biolog-MPN quantification of sole carbon source utilization. The Biolog technology is based on tetrazolium dye reduction as an indicator of sole-carbon source utilization (Bochner, 1989a; Bochner, 1989b). The Biolog GN microplate (Biolog Inc., Hayward, CA) classifies Gram negative bacteria by testing for 95 different sole carbon sources (Table 2). A study by Garland and Mills (1991) examined bacterial communities

by the direct addition of water samples to plates but no quantification was made of the densities of bacteria capable of using each carbon source. To quantify the densities of bacteria capable of using each carbon source, the present study combined a most probable number assay and the direct addition of ground water samples to Biolog GN microplates. Ten plates were inoculated directly with ground water. Additional Biolog GN plates were inoculated with ten-fold dilutions of ground water. Plates were placed in whirl-pak bags and incubated at 25°C for two weeks. At the end of two weeks the plates were read using a Biolog Microstation System and MicroLog Software release 2.01 (Biolog, Inc). The most probable number of bacteria per milliliter was calculated using a Basic computer program written by Hurley and Roscoe (1983).

Data analysis. Two different analysis of variance tests using Systat (Systat: Version 5.2 Edition, Evanston, IL) and JMP, version 2 (SAS Institute Inc., Cary, NC), were conducted for density, physical and chemical data. The first was a standard single-factor ANOVA, where variances are assumed to be homogeneous (Sokal and Rohlf, 1981). However, the Hartley F-max test (Sokal and Rohlf, 1981) revealed significant deviations from homoscedasticity for many of the data examined as so indicated in the appendix. The data for any single factor ANOVA given passed the Hartley Fmax test. Skew and kurtosis were reduced by transformation for many of the physical and chemical parameters. Log(Y+1) transformation allowed the distributions of density data to assume homogeneous variances in most cases. Dissolved oxygen, oxidation-reduction potential, conductivity, and trichloroethylene data were transformed using square root, $(Y+2)^3$, arc sine square root, and log transformations, respectively. Of these parameters, transformation allowed the distribution of only one, dissolved oxygen, to assume homogeneous variances. When variances were homoscedastic, pairwise comparisons were made using the Tukey method because it provides narrower confidence limits than methods used for general contrasts. In order to analyze heteroscedastic data a second

ANOVA test, the Welch ANOVA (Welch, 1951; Brown and Forsythe 1974), was conducted. This test does not require that variances be homogeneous. Data not passing the Hartley Fmax Test was analyzed using the Welch ANOVA. The group means multiple-range-tests of all heteroscedastic data were conducted according to the Games and Howell procedure (Games and Howell, 1976; Sokal and Rohlf, 1981). This method does not assume equal variances or equal sample sizes. When variances are not equal, counter-intuitive results may occur, with closer groups sometimes appearing significantly different while more distant groups with larger variances may not. For all tests a probability level of 0.05 was assumed to be the critical level of significance.

RESULTS

Density of aromatic utilizers. The first objective of this study was to determine the density of select functional groups of bacteria responsible for the degradation of certain naturally occurring and synthetic aromatics in pristine and contaminated ground water environments. The densities of bacteria capable of utilizing the aromatics tested ranged from higher than 800,000 cells per liter (beyond the range of the assay) to less than one cell per liter (below detectable limits) depending on the aromatic compound and the well tested (Table 3).

One area of interest was whether naturally occurring aromatic compounds were utilized by the indigenous species of pristine ground water aquifers. A comparison of the pristine sites, P28 and P29, (Figure 10) demonstrated that the naturally occurring aromatic monomers p-coumarate, ferulate, isovanillate and salicylate were utilized in all six wells. The compound representing a class of contaminants, phenol, was utilized in all the P28 wells but only in the Congaree formation in the P29 well cluster. M-toluate was utilized in the Pee Dee and Middendorf formations of the P28 cluster and only the Congaree in the P29 cluster. Utilizers for all six aromatic compounds were found in well P28TA, a pristine well screened in the Middendorf formation and the deepest well in this study.

Carbon sources utilized by ground water bacteria. In order to study the carbon sources utilized by ground water bacteria in more detail, a method was developed using Biolog-GN microtiter plates in combination with a most probable number enumeration method. Using this method it was possible to quantify the number of bacteria capable of utilizing a variety of additional sole carbon sources. The Biolog sole carbon source α -D-glucose was found to be highly correlated with heterotrophic plate counts on 1% PTYG

($r=0.843$, $df=7$, $p<0.01$) and PTYG ($r=0.804$, $df=7$, $p<0.01$). This is important since glucose is a major component of PTYG and 1% PTYG therefore validating the usage of the Biolog MPN method used in this study.

Densities were found to be significantly higher for some classes of carbon compounds. A Welch ANOVA indicated that there were significant differences ($F=13.5$, $df=2$ and 356.2, $p<0.001$) between the densities of utilizers of carboxylic acid, amino acid and carbohydrate utilizers. A Games and Howell multiple-range-comparison test demonstrated that the density of carboxylic acid utilizers was significantly higher as compared to amino acid or carbohydrate sole carbon source densities. Large numbers of different carboxylic acids (Table 4) gave high densities, in contrast to different carbohydrates (Table 5) and amino acids (Table 6). In addition, carbohydrates that gave the highest densities of the carbohydrates tested were those commonly associated with humic acids.

Direct counts and heterotrophic viable counts. The total numbers of bacteria in an aquifer could be an important determinant of the total numbers of aromatic utilizers. Two methods were used to determine bacterial density; acridine orange direct counts and heterotrophic plate counts. Acridine orange direct counts give an indication of the total numbers of all types of bacteria. Heterotrophic plate counts provide an indication of the total number of bacteria that could grow on a particular media under the incubation conditions used. In general, utilizers for each aromatic made up less than 2% of the bacteria when compared to heterotrophic viable counts on 1% PTYG agar and a much smaller percentage as compared to direct counts.

Bacterial numbers decreased with increasing depth below the surface. This was true for both methods of bacterial density determination; acridine orange direct counts and heterotrophic plate counts. In addition, the densities for aromatic utilizers were highest in the shallowest wells (depths less than 63 meters) with the exception of the

DCB7 well. A significant negative correlation between depth and acridine orange direct counts for all wells was observed ($r=-0.224$, $df=227$, $p<0.01$). A Welch ANOVA indicated significant differences in bacterial densities for the different wells as determined by acridine orange direct counts ($F= 8.7$, $df=10$ and 83.2, $P<0.001$). Only one site, the P29 site, did not have higher direct counts in the shallower wells (Figure 11). The shallowest well (DCB7) had the highest direct counts which were significantly greater than three of the eleven wells in this study.

As with direct counts there was a significant negative correlation between depth and viable counts on PTYG ($r=-0.514$, $df=168$, $p<0.01$) and 1% PTYG ($r=-0.363$, $df=242$, $p<0.01$). Single factor ANOVA indicated a significant difference in viable counts between sites on PTYG ($F= 20.6$, $df=2$ and 87, $p< 0.0001$) and 1% PTYG ($F=33.5$, $df=2$ and 177, $p< 0.0001$). Densities were significantly higher for ground water samples at the MSB site than either the P28 or P29 sites for PTYG and for 1% PTYG as determined by Tukey multiple comparison tests. Significant differences were observed between the P29 and P28 sites for densities on either PTYG or 1% PTYG. There was a significant difference between some well viable counts on PTYG agar ($F=16.3$, $df=10$ and 30.7, $p<0.0001$) and 1% PTYG agar ($F=17.9$, $df=10$ and 83, $p<0.0001$) as determined by Welch ANOVA. Viable counts on 1% PTYG (Figure 12) were significantly lower for well DCB7 than for the other five shallowest wells, but were not significantly different from the four deep well densities on 1% PTYG. DCB7 counts were also much lower on PTYG agar but a significant difference was not detected due to the high variance in PTYG counts associated with this well (Figure 13). The four deepest wells each had counts on both media that were significantly lower than all the wells that were shallower except well DCB7. Therefore depth appears to be an important factor influencing both the total numbers of bacteria and species capable of utilizing aromatic compounds.

Percentage of active cells. A single factor ANOVA demonstrated a significant difference in mean activity between the different wells ($F=8.94$, $df=10$ and 209, $P<0.0001$). The wells with the highest densities of aromatic utilizers (MSB11B, MSB11C, MSB11D) also had the highest percentage of active cells as measured by acridine orange. The mean activities of wells MSB11B (77%) and MSB11D (75%) were significantly higher than well P29TA (53%) but not P28TA (64%). The latter two wells are screened in the Middendorf formation. Well DCB7, which had the lowest densities of aromatic utilizers, had the lowest mean activity (28%) which was significantly lower than all the other wells tested (Figure 14). This lower activity may be a result of the extremely low pH of this well.

Detection of the XylE gene. The second objective of this study was to determine the innate ability of microbial communities from pristine and contaminated environments to degrade synthetic aromatic compounds. Amplification by the polymerase chain reaction was performed as a means of detection of the catechol 2,3-dioxygenase (XylE) gene. This gene was chosen because it is the ring cleavage gene in the degradation of aromatics by the TOL (toluene degradation) and NAH (naphthalene degradation) plasmids. Enrichments containing minimal media and one of the aromatics (either toluene, m-toluate, salicylate, ferulate, p-coumarate, isovanillate or phenol) were made for all wells. After a two week incubation the enrichments were tested for the presence of the XylE gene sequence. In addition a combined total of over 250 isolates (from the six different aromatic compounds and nine wells) were tested. The positive control, Pseudomonas putida mt-2, gave positive signals (a visible amplicon as detected with gel electrophoresis). No positive signals were observed with either the enrichments or the isolates.

Physical and chemical parameters. The third objective of this study was to examine the ways in which abiotic factors might affect the density and catabolic potential

of these microbes to degrade aromatics. The temperature, dissolved oxygen, conductivity, redox and salinity were measured simultaneously as samples were taken (Table 7). A two factor ANOVA indicated significant interaction between well and time for temperature, pH, and conductivity. This indicates that over time, these parameters are not changing consistently for the different wells tested. This would be expected since the shallower wells, specifically the wells screened in or above the Congaree formation, would be influenced by changes in precipitation.

Dissolved oxygen. As mentioned previously, depth below the surface appears to be an important factor influencing the density of both aromatic degraders and total numbers of bacteria. One parameter that might be affected by depth is the dissolved oxygen level. Shallower wells are recharged not only from the recharge zone but also from percolation of the water down from the surface therefore higher dissolved oxygen levels in these wells would be expected. A Welch ANOVA indicated significant differences in dissolved oxygen by site ($F= 62.9$, $df=2$ and 184.5, $P<0.0001$) and factorial analysis of variance indicated significant differences in dissolved oxygen by well ($F=11.7$, $df=10$ and 165, $P<0.0001$). The MSB wells had mean dissolved oxygen levels that ranged from a mean of 6.9 to 7.0 ppm. The MSB site (with shallower wells than either the P28 or P29 site) had significantly higher dissolved oxygen than either of these sites. There was not a significant difference between the P29 and P28 dissolved oxygen levels. The lowest mean dissolved oxygen levels were observed in the two deepest wells of the P29 site (Figure 15). Well P29TA and well P29TC had mean dissolved oxygen levels of 2.7 ppm and 2.0 ppm respectively. Well DCB7 (4.3 ppm) had significantly higher dissolved oxygen than the P29 and P28 wells but not the MSB wells. Dissolved oxygen correlated positively with viable counts on both 1% PTYG ($r=0.176$, $df=201$, $p<0.05$) and PTYG ($r=0.369$, $df=135$, $p<0.01$) agar. Therefore oxygen levels may be one

of the more important factors influencing density of both total numbers of bacteria and of aromatic utilizers.

Oxidation-reduction potential. The ability of a bacterium to carry out oxidation-reduction reactions depends on the oxidation-reduction state of the environment (Atlas and Bartha, 1987). Redox potentials are affected by the oxygen sink or the demand for oxygen itself which is determined by the presence of degradable organic carbon and bacterial activity (Bouwer, 1984). Earlier studies by Fredrickson et al. (1989) indicated that the SRS sediments were not highly reduced. Similar findings were observed in this study, with the oxidation-reduction potential ranging from +0.51 in the DCB7 well to +0.14 in the P29TC well. Depth appears to be a factor when considering the oxidation-reduction potentials of the various wells studied. A significant negative correlation was observed between well depth and oxidation-reduction potential ($r=-0.470$, $df=219$, $p<0.01$) and, as would be expected, there was a positive correlation between dissolved oxygen and oxidation-reduction potential ($r=0.363$, $df=193$, $p<0.01$). Graphical analysis of the oxidation-reduction potential of the wells (Figure 16) demonstrated a similar pattern as that observed with dissolved oxygen. Wells with depths less than 63 meters had mean oxidation-reduction potentials greater than 0.325 volts whereas wells with depths greater than 63 meters had mean oxidation-reduction potentials less than 0.287 volts. Density may also be influenced by oxidation-reduction potential. The oxidation-reduction potential correlated with direct counts ($r=0.152$, $df=211$, $p<0.01$) and with viable counts on 1% PTYG agar ($r=0.240$, $df=219$, $p<0.01$) and PTYG agar ($r=0.173$, $df=145$, $p<0.05$). Therefore as with the dissolved oxygen, the oxidation-reduction potential does appear to be more reducing in the deeper wells and may therefore be a factor determining numbers of bacteria specifically aromatic utilizers in these ground water environments.

Ground water pH. A factor that profoundly affects all microorganisms is pH. Welch ANOVA indicated significant differences in pH by site ($F=76.2$, $df=2$ and 159, $P<0.0001$) and well ($F=44.5$, $df=9$ and 68.5, $p<0.0001$). The P28 site (pH 7.4) had a significantly greater mean pH compared to the P29 site (pH 6.0) and MSB site (pH 4.9). Well P28TB (Figure 17) had the highest pH mean (7.27). Well DCB7 had the lowest pH mean (pH 2.57). Because of the presence of contaminant polyaromatic hydrocarbons and because direct counts demonstrated high numbers of bacteria present in these wells, the DCB7 well might be expected to have a high number of aromatic utilizers, instead, this well had utilizers for only coumarate and ferulate and densities were low for both compounds (Figure 18). In addition, low viable count densities were also observed. Therefore utilizers of aromatic compounds may be present but unable to acclimate to the higher pH of the media.

Temperature. The temperature of these ground water wells was extremely stable and did not differ more than one or two degrees over the entire year for any one well. The mean temperature ranged from 18.5 in the MSB11D well to 22.3 for the DCB7 well. A Welch ANOVA demonstrated a significant difference in mean temperature between some of these wells ($F=40.0$; $df=10$ and 65.5; $p<0.0001$), with well DCB7 having a significantly higher mean temperature than 8 of the 10 other wells (Figure 19) as demonstrated by a Games and Howell multiple comparison of means test. The deeper wells, P28TA, TB and A and P29TA, TC had significantly higher temperatures than the shallower wells, e.g., all MSB wells and the P29C well excluding well DCB7.

It is interesting that the shallowest and deepest wells had significantly higher temperatures than wells between these depths. The temperature of ground water responds to seasonal variations in the heat received at the Earth's surface from the Sun and by movement of heat from the Earth's interior. The movement of heat from the Earth's interior causes ground water temperatures to increase with depth. This increase is

referred to as the geothermal gradient and is approximately 1.8°C per 100 m in areas underlain by thick sections of sedimentary rocks (Heath, 1984). This would explain the significantly higher temperatures of the deeper wells. The increased temperature of the DCB7 could possibly be explained as a result of solar radiation, since it is much shallower than any of the other wells studied.

Temperature correlated with a number of other factors including heterotrophic viable count density and dissolved oxygen but because the variations in temperature between and within each well were so small it is very likely that these correlations were actually due to other factors. For example, the wells with the highest temperatures, DCB7 and the four Middendorf wells (P29TA, P29TC, P28TA and P28TB), had the lowest heterotrophic viable counts. The lower heterotrophic viable counts in the DCB7 well were probably related to the low pH of that well. The lower heterotrophic viable counts of the Middendorf wells may be a result of the lower dissolved oxygen levels of these wells or to some other depth related factor. Because the range of temperatures between wells and the variations in temperature within a well were so small it is very doubtful that temperature would affect utilization of aromatic compounds in these wells.

Conductivity. Conductivity is a numerical expression of the ability of an aqueous solution to carry an electric current. Factors that affect conductivity include the concentration of ions, and the temperature, among other factors (Clesceri et al., 1989). A Welch ANOVA demonstrated a significant difference between wells ($F=51.3$, $df=10$ and 73.9, $p<0.0001$). Conductivity was significantly higher in the DCB7 well (Figure 20) than all the other wells tested as determined by a Games and Howell multiple comparison of means test. The elevated conductivity of the DCB7 well is most likely due to metal ions associated with the contamination at this site.

Salinity. Salinity was at detectable levels at only the DCB site. The salinity measurements taken by the Hydrolab are based on conductivity. Therefore, the salinity

values at the DCB7 site are most likely due to the elevated levels of metal ions that are found at this site.

Trichloroethylene. The MSB site, which was highly contaminated with trichloroethylene, had the greatest numbers of aromatic utilizers and all three wells had utilizers for all six aromatics tested. Well MSB11C had the highest mean TCE concentration (147,719 ppb), and MSB11A had the lowest (22 ppb) of the four MSB wells (Figure 21). A decrease in trichloroethylene concentration occurred during this study in some of the wells at the MSB site. This decrease may have been due to the injection of air into wells nearby followed by increased biodegradation, to air stripping processes, or possibly the migration of the contaminant plume away from the MSB site sampled. Significant negative correlations were observed in trichloroethylene concentration over time in 3 of the 4 wells, well MSB11A ($r=-0.867$, $df=12$, $p<0.001$), MSB11C ($r=-0.7023$, $df=7$, $p<0.05$) and MSB11D ($r=-0.9476$, $df=12$, $p<0.001$). Contamination of ground water with elevated levels of trichloroethylene did not appear to decrease either the numbers of aromatic degrading bacteria or the total numbers of bacteria. In fact, the MSB wells (Figure 22) had both the highest numbers of aromatic utilizers and all three wells had utilizers for all six of the aromatics tested. Although higher densities were observed for both viable counts and for utilizers of aromatic compounds with bacteria from the MSB wells, it is difficult to determine whether TCE is an important factor determining bacterial numbers. Dissolved oxygen and depth may be a greater influence since the MSB wells are shallower and have higher dissolved oxygen levels than five of the six wells from the P28 and P29 sites.

DISCUSSION

Utilization of recalcitrant aromatic compounds in the subsurface. If lignin and humic acid breakdown products such as p-coumarate, ferulate and isovanillate are metabolizable carbon sources being utilized by subsurface bacteria then we might expect utilizers of these compounds to be present in most all the wells tested, and this is what was observed. Even the deepest aquifer tested (P28TA, Middendorf aquifer) had utilizers present for all six of the aromatics tested. The aquifers in both the Pee Dee and Middendorf formations could be considered pristine. The positive head pressures associated with the ground water in the formations below the Congaree causes water movement to be upward to overlying formations (Sargent and Fliermans, 1989). Therefore, contaminants that might exist at the surface would not tend to move downward into the deeper aquifers.

Of the six compounds tested, the highest counts were observed with p-coumarate, ferulate and isovanillate, all of which have been observed as breakdown products of either lignin or humic acid. In addition, an examination of P. putida mt-2 (harboring the TOL plasmid), demonstrated that it was capable of utilizing many of the aromatic substances tested in this study (Table 8). This does not necessarily mean that this gene would be involved in the utilization of these other naturally occurring aromatics. It would be an indication that the bacterium is capable of existing on these types of compounds and could have depended on similar compounds for it's existence in it's native environment. This might serve to explain to some degree the origin of these genes.

Other supporting evidence that subsurface bacteria are utilizing lignin and humic acid breakdown products is the differences in density of bacteria utilizing carboxylic acids, carbohydrates and amino acids. As can be observed in Table 4, a large number of

carboxylic acids were utilized by ground water bacteria. Similar results were found for sediment bacteria (Fredrickson et al., 1991). This may be an indication of what compounds these ground water bacteria are actually subsisting on and are best adapted to. Humic acid molecules have a large number of carboxyl groups associated with them. In addition, cleavage of aromatic ring structures by oxidative reactions results in the formation of carboxylic acids. The type of side group(s) attached to the aromatic ring would determine the type of carboxylic aromatic produced by oxidation of the aromatic ring. Therefore, we might expect to see greater numbers of utilizers of carboxylic acids by bacteria that are exposed to these compounds in the environment. For most of the wells studied the compounds with the greatest numbers of utilizers were carboxylic acids.

Carbohydrates and amino acids did not appear to be as important as sole carbon sources to ground water bacteria (Table 5 and 6). There is evidence that as humics descend through the soil and sediment column many of the readily utilizable carbon sources, for example carbohydrates, are removed. Humic substances in soil commonly vary from 5 to 10% carbohydrates (Stevenson, 1982). The carbohydrate content of humic substances from a number of different aquifers was tested and all were found to be below the detection limit of 0.1% (Thurman, 1985). Therefore we might not expect carbohydrate utilization to be as important in ground water environments. With Pseudomonas putida mt-2, sixteen of 24 carboxylic acids were utilized as sole carbon sources whereas only 3 of 28 carbohydrates were utilized (Table 9).

Some carbohydrates are associated with humic acid molecules. Table 10 lists carbohydrates commonly found associated with humic compounds and the relative proportion each makes up of the total carbohydrates (Uzaki and Ishwatari,1983). When these are compared with the carbohydrates giving the greatest densities in the pristine wells (Table 11) it can be seen that all the carbohydrates giving greatest densities are ones commonly associated with humics. In addition, all four of the Middendorf wells had the

highest carbohydrate densities with L-arabinose as the sole carbon source. Therefore, it appears that bacteria in ground water environments may be adapted to and utilize the carbohydrates associated with humic compounds when these carbohydrates are present.

Interactions between bacteria and contaminants. The highest densities of aromatic utilizers were at the MSB site. This is important because it demonstrates that contamination of ground water with elevated levels of chlorinated hydrocarbons did not decrease the numbers of aromatic degrading bacteria. A number of bacteria have been isolated that are capable of co-metabolizing trichloroethylene while metabolizing aromatic compounds (Harker and Kim, 1990; Nelson et al., 1986; Nelson et al., 1988; Wackett and Gibson, 1988). Prevalence of aromatic degraders at even the deepest aquifers demonstrates that the potential exists that similar bacteria may already be present in ground water that could co-metabolize TCE. Utilizing these indigenous species for bioremediation avoids the pitfalls of introducing genetically engineered species or species not indigenous to these waters. This makes a strong case for using bacteria that are already present in contaminated aquifers as tools for the clean up of the aquifer.

Physical factors such as pH appear to influence the distribution of aromatic utilizers. Even though we would expect aromatic utilizers to be present in polyaromatic hydrocarbon contaminated water, they were lower in density than the other wells tested and had the least number of different aromatics utilized. Therefore, from what has been observed in this study, the pH could affect the utilization of the aromatic in two ways. The most immediate affect would be to limit the types of aromatic utilizers able to survive low pH. Another possibility is that many of the bacteria that could utilize these aromatics were unable to acclimate to the higher pH of the media.

Types of bacteria in Savannah River Site Aquifers. Sinclair and Ghiorse (1989) found that dissolved organic carbon was not a good predictor of microbial abundance. Microbial numbers varied from layer to layer and correlated with changes in sediment,

pH and concentration of metallic cations. Of all the environmental factors, texture was the best predictor of abundance and activity in the deep subsurface sediments. Sandy samples from deeper formations demonstrated elevated diversity numbers. Low populations of culturable bacteria were found in the high clay sediments. This was possibly due to decreased hydraulic conductivity although the pH of these sediments was also low (Fredrickson et al., 1989)

The percentage of sand versus clay appears to determine the relative percentages of gram negative and gram positive bacteria. Sinclair and Ghiorse (1989) found a strong association of Gram positive isolates with samples containing more than 50% clay. These samples yielded 100% gram-positive and no gram-negative isolates whereas samples containing less than 30% clay yielded 21% gram positive and 79% gram-negative. In no cases were gram positives completely excluded from samples with very high sand content. Isolates from both media (PTYG and 1% PTYG) and from three of the MSB wells (Table 12) had between 84% and 96% gram negative bacteria. This agrees with other studies that have indicated that gram-negative bacteria are more abundant than gram-positive bacteria in sandy aquifer sediments and ground water (Hirsch and Rades-Rohkohl, 1988; Kolbel-Boelke et al., 1988). Gram negative bacteria may be at an adaptive advantage in the dilute aquatic environments of sandy aquifer sediments (Sinclair and Ghiorse, 1989). These advantages include the structure of the gram-negative cell envelope which is believed to provide greater survival advantages in dilute aquatic environments (Brock and Madigan, 1987) and the chemical properties of the outer membrane of gram negatives which allow for alterations of surface hydrophobicity during cycling between attached and detached states (Beveridge, 1981).

API-NFT on isolates taken from PTYG and 1% PTYG from three of the MSB wells indicated that a large number of these isolates were possibly Pseudomonads. Balkwill et al. (1989) had similar findings with sediment isolates from the Savannah

River Site. These included isolates from the P28 and P29 sites. He found that a large number appeared to be Pseudomonads. This would be of possible advantage for in situ bioremediation because this group has been shown to frequently harbor catabolic plasmids (Lindow et al., 1989; Sayler and Blackburn, 1989). Plasmid studies have been conducted at this site on isolates from sediment samples. Fredrickson et al. (1988) demonstrated an increasing frequency of plasmids among isolates with increasing depth at these sites. These plasmids would benefit bacteria in environments where carbon sources are not as readily available. As the environment becomes more recalcitrant it becomes more metabolically economical for the bacteria to increase their plasmid burden in order to increase the number of compounds available for degradation (Hazen et al., 1991).

Role of the XylE gene in utilization of aromatic compounds in ground water environments. One objective of this study was to determine what part a single gene such as the catechol-2,3-dioxygenase (XylE) gene might have on the utilization of aromatics in ground water. Several studies have been conducted that have examined the prevalence of the TOL plasmid in ground water and sediments. A study by Ogunseitan et al. (1987) found there was no homology of TOL DNA with DNA of bacteria from relatively shallow aquifer sediments. Fredrickson et al. (1988) using a whole TOL plasmid probe found homology with plasmids from four bacteria isolated from sediments at the Savannah River Site. In addition, two isolates showed homology with chromosomal regions. Because plasmids which hybridized with whole TOL plasmid probes were found in previous studies in sediments from some of these same formations at the Savannah River Site used in this study it was believed that the XylE sequence should be detectable. Identification of isolates harboring this gene might indicate the prevalence of the sequence and provide some indication of it's involvement in genetic adaptation. Two methods were used in this study to test for the presence of the XylE gene sequence. The

first method was to examine enrichments. Enrichments for all aromatics including toluene were tested for the presence of the XylE gene. In addition, approximately 250 aromatic utilizers from these ground water samples were examined. These included utilizers of m-toluate, p-coumarate, ferulate, isovanillate, salicylate and phenol. Under stringent conditions (a 60°C reannealing step, which was the Tm-6°C) none of the isolates demonstrated an amplicon although an amplicon was observed with the model organism (Pseudomonas putida mt-2, which harbors the TOL plasmid).

Therefore, the catechol 2,3 dioxygenase gene found on the TOL and NAH plasmids does not appear to play a role in the degradation of aromatic compounds in these ground water environments. Degradation of aromatic compounds may have been due to diverged genes or different genes altogether. This implies that the homology detected with whole TOL plasmid probes (Fredrickson et al., 1988) may have been to sequences not related to the catabolic pathway that are commonly found on many plasmids (such as sequences for an origin of replication) or to sequences that are related to the pathway but have undergone a high degree of divergence. More specific TOL plasmid probes may better define this homology and aid in the development of new strategies for primer design.

Ground water versus sediment sampling. The nutritional studies performed indicate that under certain conditions ground water sampling may be indicative of what is occurring in the sediments. Comparisons between this study using ground water samples and other studies conducted on sediment samples from this site have been surprisingly similar. Tween 40 and Tween 80, polymers of fatty acids, were found to be utilized by large numbers of ground water bacteria in this study. This agrees with results from two other subsurface studies. Kolbel-Boelke et al. (1988) found that Tween 40 was commonly metabolized by bacterial isolates from sediment (71%) and ground water (52%). The substrate most commonly metabolized by Middendorf and Cape Fear

sediment isolates from the Savannah River Site was Tween 40 which was metabolized by 85% of the isolates. Tween 80 was metabolized by more than 50% of the isolates (Fredrickson et al., 1991). There are at least two possible explanations for the high densities observed with this compound. The compound may be similar in structure to compounds that are commonly used as a carbon sources in the subsurface. Another possibility is that since the compounds are polymers, and do not readily diffuse into the cell, there is less toxicity to those species that might be inhibited by similar but smaller and more readily diffusible compounds.

Another substrate that was utilized by large numbers of ground water bacteria was b-hydroxybutyric acid. Similar results were observed by Fredrickson et al. (1991). Middendorf and Cape Fear sediment isolates commonly metabolized organic acids and b-hydroxybutyric acid. b-hydroxybutyric acid can accumulate intracellularly as the polymer poly-b-hydroxybutyric acid (PHB) in some bacteria and can be an important survival mechanism for bacteria in oligotrophic subsurface environments (Fredrickson et al., 1991). These large granules of reserve material are present in amounts that vary with nutritional conditions and allow storage of carbon in a form that is osmotically inert (Stanier et al., 1986). PHB accumulates during times when carbon sources are abundant and is catabolized during times when nutrients are limited, thereby allowing the cells to maintain viability. Accumulation of PHB occurs under conditions of unbalanced growth, e.g., when an inorganic nutrient such as nitrogen is limiting and utilizable reduced carbon is in excess (Karl, 1986). A study by Sinclair and Ghiorse (1989) demonstrated that 74% of the bacteria isolated from the deep sandy subsurface at SRS had deposits of a lipophilic storage material assumed to be poly-b-hydroxybutyric acid (Fredrickson et al., 1991). The MSB11C b-Hydroxybutyric acid utilizers had the highest density of any compound tested.

Results with ground water isolates using API-NFT tests were very similar to studies with sediment isolates. Using API-NFT tests, Balkwill et al. (1989) found that physiological traits of aerobic chemoheterotrophs isolated from Savannah River Site surface soils on both PTYG and 1% PTYG plating media differed markedly from subsurface isolates. The surface soil bacteria more readily utilized most of the carbon sources offered in the assimilation tests; 8 of the 12 sources were used by at least 75% (often by more than 85%) of the surface soil isolates (compared with 3 of the 12 sources for the subsurface isolates). Similar results were observed in this study with ground water isolates from the MSB site wells. Of the 12 carbohydrates tested for using API-NFT, 75% of the ground water isolates used three or fewer carbohydrates. In Balkwill's study surface and subsurface bacteria responded similarly to the other API tests, except that a significantly larger proportion of the surface soil isolates hydrolyzed esculin and gelatin, whereas a significantly larger percentage of subsurface isolates reduced nitrate. Isolates from the same two isolation media but from ground water and not sediment samples gave similar results (Table 13). As the depth of the well increased from 50 m (MSB11B well) to 63 M (MSB11D well) the number of bacteria capable of gelatin hydrolysis decreased and the number capable of nitrate reduction increased. Balkwill found that most of the bacteria isolated on PTYG and 1% PTYG from sediments at SRS were oxidative rather than fermentative (82% assimilating glucose aerobically but only 4% fermenting it). Isolates taken from the MSB ground water gave similar results with 74% assimilating glucose aerobically and none of the isolates fermenting glucose. Similarity in results for assimilation of glucose may be a result of using the same isolation media (PTYG and 1% PTYG) since there is some selection for isolates that could use glucose aerobically with this media.

Bacterial densities and diversity in ground water. As can be observed in Figure 23, all but one of the wells studied demonstrated higher counts on 1% PTYG (the low

nutrient media) than PTYG (the high nutrient media). This demonstrates the oligotrophic nature of these ground water environments. The exception was well DCB7 which had higher PTYG counts. This might be expected since this well had high levels of organic carbon due to polyaromatic hydrocarbon contamination.

In studies by Balkwill et al. (1989) using the same two types of media (PTYG and 1% PTYG) but with sediment isolates, physiologically distinct types of bacteria were isolated on the two media, with only 11% overlap between the two groups. Similar differences between the two media were evident as observed from the results of the oxidase tests performed on MSB site isolates taken from the two different media. Oxidase reactions for isolates were very different, 45% of the PTYG isolates were oxidase positive whereas only 5% of the 1% PTYG isolates were oxidase positive (Table 9).

Earlier studies at this site demonstrated that ground water adjacent to 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 ground water samples (Hazen et al, 1991). The acridine orange direct count difference between any of the wells was less than one order of magnitude. This differed from viable counts in which there were several orders of magnitude difference between wells (Figure 24). DCB7, had the lowest viable counts and the highest direct counts. The bacteria in this well may not have been able to acclimate to the higher pH of the plate count media. This demonstrates that even though bacteria can be visualized and the densities in different aquifers are similar, there are great variations in physiological states and metabolic needs of bacteria in these different aquifers.

A number of sediment studies have been conducted that provide insight into bacterial diversity and numbers in aquifer sediments. Kolbel-Boelke et al. (1988) studied aquifer sediments (depths to 35 m) in the Federal Republic of Germany and concluded

that the microbial community at each depth was both very diverse and distinctly different from those at other depths. Balkwill et al. (1989) reported that different geological formations at the Savannah River Site contained distinct types of bacteria (strains with different API-NFT patterns). Sinclair and Ghiorse (1989) found similar results with samples from the same site. They observed no decline in diversity or numbers with increasing depth. Dramatic differences were observed in the utilization of sole carbon sources between sites and strata in this study. This demonstrates that different approaches might need to be developed that are suitable to the microflora of a particular stratum in order to stimulate the in situ biodegradation of a contaminant at particular site.

The percentage of active cells in ground water environments. Phelps et al. (1989) demonstrated that when comparing sediments from the various formations at the pristine sites the highest activities were in the water-bearing sands of the Congaree. Of the pristine wells, well P29C was the only well studied that lies in the Congaree formation. This well had significantly higher activity than the other two wells studied at the P29 site. There was significantly greater activity from samples from the MSB site as compared to the other sites, P29 and P28. These results were supported by the findings of Phelps et al. (1988). In their study, zones containing TCE concentrations greater than 500 mg per liter were devoid of microbial activity. At the onset of this study the well with the highest TCE concentration, MSB11C, had a mean concentration of TCE of 123,356 ppb, which was well below the level found to be devoid of life. As a whole the MSB site was significantly higher in densities on both PTYG and 1% PTYG than wells from the other sites. Phelps et al. (1988) found that above and below the TCE laden zones were areas that exhibited greater metabolic activity than near surface soils. As TCE concentrations decreased and water availability increased, microbial activities were greater than in near-surface sediments. Several other factors may contribute to the increased activity of the MSB wells. The MSB site had significantly higher dissolved oxygen levels as compared

to the other sites. As a whole, the depths of the wells of this site are shallower as compared to the wells of the P28 site and two of the wells of the P29 site. Well P29C, which was of similar depth with wells in the MSB site, did not differ significantly in activity. Therefore it appears that depth may also have been important in determining activity. In addition, there was a great deal of disturbance to the aquifer sediments by nearby drilling operations that may have increased nutrient levels and activity.

In conclusion, this study demonstrates the importance of aromatic compounds as nutritional sources for bacteria in the subsurface. A number of findings provide supporting evidence. Aromatic utilizers were found in all the geologic formations tested and in both contaminated and pristine aquifers. Carboxylic acids (which can be produced by the oxidative cleavage of aromatic ring structures) were found to be an important sole carbon source for ground water bacteria. In addition, carbohydrates giving greatest densities were found to be ones commonly associated with humics. These findings are important since aromatic utilizers in ground water environments may play an important part in the biodegradation of contaminants either metabolically or co-metabolically. The presence of these aromatic utilizers in even the deepest aquifers would indicate that the potential exists for genetic adaptation to xenobiotic compounds with analogous structure in even relatively pristine aquifers. These findings, therefore, have important implications for in situ bioremediation as a method of cleanup of contaminated aquifers.

CONCLUSIONS

1. Aromatic monomers that are breakdown products of lignin and humic acid were utilized by bacteria in all the aquifers tested, including the Middendorf, a very deep aquifer.
2. Highest counts for the aromatics tested were on the more common naturally occurring aromatic and humic acid breakdown products.
3. Utilizers of phenol, which represented a class of ground water contaminants, and salicylate, an intermediate in the breakdown of polyaromatic hydrocarbons, were present in all of the pristine aquifers tested.
4. Elevated levels of trichloroethylene, a compound co-metabolized by some aromatic degraders, did not decrease the numbers of bacteria capable of utilizing the aromatics tested.
5. Carboxylic acids appear to be an important source of carbon for bacteria in ground water environments.
6. Carbohydrates utilized by ground water bacteria appear to be those commonly present in humics.
7. Great variations existed in sole carbon source utilization between sites and formations.
8. Ground water may be characteristic of the associated sediments in some aquifers.
9. Shallower aquifers had both higher dissolved oxygen and higher direct counts and heterotrophic viable counts.
10. Several orders of magnitude difference were observed between viable counts from different wells. Direct counts on the same wells varied less than an order of magnitude. This implies that there is a high degree of variation in the physiological states and metabolic needs of the bacteria from the ground water from different formations.

11. Higher counts were observed on low nutrient media as compared to high nutrient media suggesting that ground waters are predominantly oligotrophic in nature.

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Table 1.

Screened interval for ground water wells sampled in this study.

Site	Well	Screened interval (meters)
P28	P28A	101 - 104
	P28TB	188 - 195
	P28TA	228 - 238
P29	P29C	40 - 43
	P29TC	149 - 155
	P29TA	204 - 210
MSB	MSB11A	40 - 43
	MSB11B	49 - 51
	MSB11C	54 - 55
	MSB11D	62 - 63
DCB	DCB7	33 - 39

Table 2.

Sole Carbon Sources in Biolog GN Microtitre Plates.

Carbon Sources		
Carbohydrates	Carboxylic acids	Amino Acids
N-Acetyl-D-galactosamine	Acetic acid	D-Alanine
N-Acetyl-D-glucosamine	cis-Aconitic acid	L-Alanine
Adonitol	Citric acid	L-Alanylglycine
L-Arabinose	Formic acid	L-Asparagine
D-Arabitol	D-Galactonic acid lactone	L-Aspartic acid
Cellobiose	D-Galacturonic acid	L-Glutamic acid
i-Erythritol	D-Gluconic acid	Glycyl-L-asparitic acid
D-Fructose	D-Glucosaminic acid	Glycyl-L-glutamic acid
L-Fucose	D-Glucuronic acid	L-Histidine
D-Galactose	α-Hydroxybutyric acid	Hydroxy-L-proline
Gentiobiose	β-Hydroxybutyric acid	L-Leucine
α-D-Glucose	γ-Hydroxybutyric acid	L-Ornithine
m-Inositol	p-Hydroxyphenylacetic acid	L-Phenylalanine
α-D-Lactose	Itaconic acid	L-Proline
Lactulose	α-Ketobutyric acid	L-Pyroglutamic acid
Maltose	α-Ketoglutaric acid	D-Serine
D-Mannitol	α-Ketovaleric acid	L-Serine
D-Mannose	D,L-Lactic acid	L-Threonine
D-Melibiose	Malonic acid	D,L-Carnitine
β-Methyl D-glucoside	Propionic acid	γ-Aminobutyric acid
D-Psicose	Quinic acid	
D-Raffinose	D-Saccharic acid	Aromatics
L-Rhamnose	Sebacic acid	
D-Sorbitol	Succinic acid	Urocanic acid
Sucrose		Inosine
D-Trehalose	Alcohols	Uridine
Turanose		Thymidine
Xylitol	2,3-Butanediol	
	Glycerol	Brominated Chemicals
Esters		
	Amides	Bromosuccinic acid
Methylpyruvate		
Mono-methylsuccinate	Succinamic acid	Amines
	Glucuronamide	
Polymers	Alaninamide	Phenylethylamine
		Putrescine
α-Cyclodextrin	Phosphoralated Compounds	2-Aminoethanol
Dextrin		
Glycogen	D,L-α-Glycerol phosphate	
Tween40	Glucose-1-phosphate	
Tween80	Glucose-6-phosphate	

Table 3.

Utilization of aromatics by ground water microorganisms.

WELL	P28A	P28TB	P28TA
p-Coumarate	17300 ± 11200[a]	177 ± 82	477 ± 350
Ferulate	>800000[b]	246 ± 120	477 ± 350
Isovanilate	101 ± 47	34 ± 16	354 ± 209
Phenol	129 ± 81	850 ± 854	3 ± 6
Salicylate	3960± 2330	20 ± 10	88 ± 45
Toluate	2 ± 2	BDL	1 ± 1

WELL	P29C	P29TC	P29TA
p-Coumarate	1300± 875	33 ± 15	305 ± 155
Ferulate	1300± 875	66 ± 35	85 ± 431
Isovanilate	195 ± 88	26 ± 11	305 ± 155
Phenol	61 ± 53	BDL	BDL
Salicylate	44 ± 23	6 ± 3	2 ± 3
Toluate	2400 ± 1570	BDL	BDL

WELL	MSB11B	MSB11C	MSB11D
p-Coumarate	2300± 1.840	>800000	>800000
Ferulate	4170± 3.680	>800000	>800000
Isovanilate	67± 36	22200± 14400	64 ± 34
Phenol	240± 220	>800000	34800 ± 25100
Salicylate	20± 823	>800000	697 ± 662
Toluate	240± 220	1050± 963	7 ± 4

WELL	DCB7
p-Coumarate	23 ± 21
Ferulate	23 ± 21
Isovanilate	BDL[c]
Phenol	BDL
Salicylate	BDL
Toluate	BDL

[a]All densities in most probable number of cells/liter ± one standard error

[b] above maximum detectable limit

[c]BDL = below detectable limits

Table 4

Relative proportion of isolates utilizing carboxylic acids.

Carboxylic acids	MSB11B	MSB11C	MSB11D	P28A	P28TA	P28TB	P29C	P29TA	P29TC
Acetic acid	18.52	0.47	52.93	32.91	1.48	10.59	10.09	43.29	19.24
alpha-Hydroxybutyric acid	4.01	0.49	7.18	0.00	0.00	10.00	4.01	19.08	2.96
alpha-Ketobutyric acid	2.12	0.36	8.74	6.28	0.44	0.00	10.09	0.00	0.00
alpha-Ketoglutaric acid	6.38	0.57	31.24	43.26	6.41	24.02	9.13	76.86	25.29
alpha-Ketovaleric acid	0.65	0.36	6.70	2.96	0.00	4.99	6.38	0.00	0.00
beta-Hydroxybutyric acid	18.52	100.00	100.00	100.00	39.74	42.65	18.52	58.48	13.43
cis-Aconitic acid	1.97	15.70	37.31	32.91	0.00	10.00	10.09	0.00	0.00
Citric acid	1.49	0.49	9.44	2.96	4.88	10.00	10.09	28.80	10.00
D,L-Lactic acid	40.26	0.57	22.13	25.29	10.43	32.45	6.38	40.81	25.29
D-Galactonic acid lactone	3.26	0.57	12.28	25.29	6.41	10.59	10.09	43.29	14.24
D-Galacturonic acid	6.38	0.49	9.44	14.24	0.44	10.59	4.01	8.99	14.24
D-Gluconic acid	3.26	45.74	24.73	25.29	39.74	32.45	18.52	43.29	19.24
D-Glucosaminic acid	0.29	0.57	11.21	14.24	0.00	0.00	18.52	0.00	0.00
D-Glucuronic acid	2.12	9.85	26.25	14.24	0.00	10.00	0.88	19.08	10.00
D-Saccharic acid	4.01	9.85	16.14	10.00	0.00	0.00	10.09	0.00	6.28
Formic acid	100.00	0.09	12.28	25.29	0.00	39.51	0.09	8.99	19.24
gamma-Hydroxybutyric acid	100.00	0.75	21.91	32.91	39.74	42.65	18.52	43.29	43.26
Itaconic acid	2.12	21.91	5.31	6.28	0.00	30.29	18.52	8.99	6.28
Malonic acid	0.21	0.35	12.97	25.29	0.00	10.59	18.52	0.00	6.28
p-Hydroxyphenylacetic acid	1.25	0.49	49.24	10.00	0.00	0.00	10.09	30.39	2.96
Propionic acid	1.49	0.21	25.81	25.29	0.93	10.00	6.38	100.00	10.00
Quinic acid	1.97	0.36	37.31	10.00	14.40	4.99	6.38	0.00	10.00
Sebacic acid	10.09	9.85	22.13	25.29	0.00	16.86	9.13	19.08	2.96
Succinic acid	18.52	0.35	26.25	25.29	12.16	100.00	10.09	0.00	59.30

Percentages for each groundwater sample were calculated by taking the density of sole carbon source utilization for that carbon source as a percentage of the acridine orange direct count density for that particular well. The highest percentage for each well was normalized by making it 100%. All other values for that well were then adjusted by the same factor.

Outlined percentages represent the ten highest densities for that sample (if there were several values at the density of the tenth highest density then all of those carbon sources were included).

Table 5.

Relative proportion of isolates utilizing carbohydrates.

Carbohydrate	MSB11B	MSB11C	MSB11D	P28A	P28TA	P28TB	P29C	P29TA	P29TC
Adonitol	0.21	9.85	0.00	2.96	0.00	4.99	0.00	0.00	0.00
alpha-D-Glucose	18.52	4.53	16.14	30.06	3.47	16.86	0.58	19.08	14.24
alpha-D-Lactose	0.00	4.53	9.44	0.00	0.00	16.86	0.00	8.99	0.00
beta-Methyl D-glucoside	2.12	0.36	16.14	25.29	0.00	10.59	0.00	0.00	0.00
Cellobiose	10.09	4.53	22.13	14.24	0.00	16.86	0.14	0.00	0.00
D-Arabitol	0.65	2.47	2.34	17.97	0.00	0.00	0.20	8.99	0.00
D-Fructose	0.65	9.85	14.38	25.29	2.50	32.45	6.18	8.99	13.43
D-Galactose	3.26	9.85	16.14	19.24	7.55	16.86	10.09	8.99	2.96
D-Mannitol	0.65	4.53	4.97	6.28	39.74	10.00	0.15	0.00	2.96
D-Mannose	10.09	4.53	6.70	19.24	0.00	16.86	1.74	30.39	14.24
D-Melibiose	4.01	0.57	7.18	0.00	0.00	4.99	0.20	19.08	5.93
D-Psicose	0.09	0.06	5.31	14.24	0.93	16.86	0.04	19.08	9.48
D-Raffinose	0.04	0.49	3.73	19.24	0.00	10.59	0.29	0.00	0.00
D-Sorbitol	0.49	7.85	7.18	6.28	0.00	22.65	0.04	8.99	0.00
D-Trehalose	0.86	7.85	22.13	30.06	0.44	0.00	0.09	18.02	0.00
Gentiobiose	2.12	0.49	12.28	13.43	0.00	0.00	0.15	8.99	0.00
i-Erythritol	0.14	0.21	2.21	0.00	8.71	24.02	0.00	30.39	10.00
L-Arabinose	40.26	9.85	7.18	23.43	61.98	46.47	10.09	91.34	83.72
L-Fucose	0.52	0.28	5.31	19.24	5.91	30.29	18.52	58.48	29.83
L-Rhamnose	2.12	0.43	11.21	6.28	1.40	24.02	0.49	8.99	9.48
Lactulose	0.38	4.06	3.73	0.00	0.00	4.99	0.00	8.99	0.00
m-inositol	0.45	0.31	2.34	0.00	0.00	0.00	0.14	0.00	0.00
Maltose	4.01	0.57	16.14	9.42	0.00	21.37	0.49	0.00	10.00
N-Acetyl-D-galactosamine	0.29	0.31	3.73	6.28	0.00	16.86	0.00	0.00	2.96
N-Acetyl-D-glucosamine	6.38	2.47	18.85	14.24	0.00	16.86	0.35	30.39	2.96
Sucrose	2.12	0.98	26.25	2.96	0.00	0.00	0.29	0.00	0.00
Turanose	0.20	1.51	8.74	10.00	0.00	0.00	0.00	19.08	9.48
Xylitol	0.38	2.47	0.00	2.96	0.00	0.00	0.00	18.02	0.00

Percentages for each groundwater sample were calculated by taking the density of sole carbon source utilization for that carbon source as a percentage of the acridine orange direct count density for that particular well. The highest percentage for each well was normalized by making it 100%. All other values for that well were then adjusted by the same factor.

Outlined percentages represent the ten highest densities for that sample (if there were several values at the density of the tenth highest density then all of those carbon sources were included).

Table 6.

Relative proportions of isolates utilizing amino acids.

Amino Acids	MSB11B	MSB11C	MSB11D	P28A	P28TA	P28TB	P29C	P29TA	P29TC
D,L-Carnitine	0.35	0.43	5.31	14.24	0.00	0.00	0.00	0.00	0.00
D-Alanine	0.89	0.57	14.38	2.96	0.00	0.00	10.09	0.00	0.00
D-Serine	0.04	0.09	0.00	0.00	0.00	4.99	0.15	0.00	0.00
gamma-Aminobutyric acid	1.50	0.31	14.38	2.96	0.00	4.99	6.38	19.08	6.28
Glycyl-L-asparitic acid	0.65	0.57	16.14	19.24	0.88	4.99	0.13	0.00	0.00
Glycyl-L-glutamic acid	4.01	15.70	52.93	19.24	1.87	10.59	6.38	0.00	2.96
Hydroxy-L-proline	0.76	0.43	3.73	10.00	0.00	4.99	0.21	0.00	2.96
L-Alanine	3.26	0.66	26.25	6.28	0.00	0.00	10.09	0.00	6.28
L-Alanyl-glycine	1.50	0.31	22.13	10.00	0.00	16.86	18.52	0.00	2.96
L-Asparagine	9.13	0.57	31.24	14.24	0.00	22.65	10.09	0.00	10.00
L-Aspartic acid	4.01	0.41	22.13	43.26	0.44	4.99	10.09	0.00	6.28
L-Glutamic acid	4.01	0.43	31.24	32.91	0.93	32.45	2.12	0.00	9.48
L-Histidine	2.12	0.23	16.14	0.00	0.00	10.59	18.52	0.00	0.00
L-Leucine	1.49	0.49	26.25	10.00	0.88	16.86	2.12	0.00	2.96
L-Ornithine	0.88	0.43	11.11	0.00	0.00	4.99	0.14	0.00	6.28
L-Phenylalanine	0.86	0.57	8.74	0.00	0.00	4.99	4.01	0.00	0.00
L-Proline	1.23	0.41	30.59	29.83	0.00	4.99	1.25	0.00	5.93
L-Pyroglutamic acid	1.49	1.56	22.13	25.29	21.03	10.59	10.09	0.00	6.28
L-Serine	0.60	0.26	7.18	2.96	0.00	4.99	10.09	0.00	2.96
L-Threonine	0.35	2.47	22.13	2.96	0.44	0.00	2.12	0.00	10.00

Percentages for each groundwater sample were calculated by taking the density of sole carbon source utilization for that carbon source as a percentage of the acridine orange direct count density for that particular well. The highest percentage for each well was normalized by making it 100%. All other values for that well were then adjusted by the same factor.

Outlined percentages represent the ten highest densities for that sample (if there were several values at the density of the tenth highest density then all of those carbon sources were included).

Table 7.

Physical and chemical parameters for ground water wells (mean ± standard deviation).

Well	Temperature (°C) n=16	Dissolved oxygen (mg/l) n=16	Redox (volts) n=16	Conductivity (ms/cm) n=18	pH n=18	Salinity (ppt) n=20
DCB7	22.3 ± 1.7	4.1 ± 1.8	0.511 ± 0.153	4.416 ± 2.297	2.6 ± 0.7	1.9 ± 1.3
MSB11A	18.5 ± 0.7	5.0 ± 1.9	0.394 ± 0.081	0.044 ± 0.019	6.1 ± 0.6	0.0 ± 0.0
MSB11B	18.6 ± 0.6	7.0 ± 1.9	0.437 ± 0.095	0.046 ± 0.017	5.7 ± 0.6	0.0 ± 0.0
MSB11C	18.6 ± 0.5	6.9 ± 1.9	0.474 ± 0.082	0.069 ± 0.017	4.5 ± 0.7	0.0 ± 0.0
MSB11D	18.5 ± 0.4	7.0 ± 1.9	0.503 ± 0.064	0.032 ± 0.007	4.4 ± 0.6	0.0 ± 0.0
P28A	19.8 ± 0.3	2.3 ± 2.4	0.113 ± 0.302	0.119 ± 0.075	7.1 ± 1.2	0.0 ± 0.0
P28TB	19.8 ± 0.4	3.0 ± 2.8	0.034 ± 0.270	0.213 ± 0.161	7.9 ± 1.9	0.0 ± 0.0
P28TA	20.0 ± 0.6	3.3 ± 2.1	0.032 ± 0.186	0.066 ± 0.067	7.3 ± 1.0	0.0 ± 0.0
P29C	19.4 ± 0.4	3.8 ± 2.1	0.355 ± 0.109	0.080 ± 0.011	5.5 ± 0.5	0.0 ± 0.0
P29TC	20.2 ± 0.2	2.0 ± 2.3	0.140 ± 0.246	0.077 ± 0.010	6.4 ± 0.7	0.0 ± 0.0
P29TA	20.1 ± 0.7	2.7 ± 3.1	0.244 ± 0.239	0.038 ± 0.016	6.0 ± 0.7	0.0 ± 0.0

Table 8.

Aromatic compounds utilized by Pseudomonas putida mt-2

p-Coumarate	+[a]
Ferulate	+
Isovanillate	-
Phenol	-
Salicylate	+
m-Toluate	+

[a] Positive values represent enrichment tubes in which dissapearance of the aromatic substrate occurred.

Table 9.

Biolog sole carbon source utilization for Pseudomonas putida mt-2 (pWW0).

	Carbohydrates		Carboxylic acids		Amino Acids		Esters
	N-Acetyl-D-galactosamine	+	**Acetic acid**	+	**D-Alanine**	+	**Methylpyruvate**
	N-Acetyl-D-glucosamine	+	**cis-Aconitic acid**	+	**L-Alanine**	+	**Mono-methylsuccinate**
	Adonitol	+	**Citric acid**	+	**L-Alanylglycine**		
	L-Arabinose	+	**Formic acid**	+	**L-Asparagine**		Polymers
	D-Arabitol		D-Galactonic acid lactone	+	**L-Aspartic acid**		
	Cellobiose	+	**D-Galacturonic acid**	+	**L-Glutamic acid**		α-Cyclodextrin
	i-Erythritol	+	**D-Gluconic acid**		Glycyl-L-asparitic acid		Dextrin
+	**D-Fructose**		D-Glucosaminic acid	+	**Glycyl-L-glutamic acid**		Glycogen
	L-Fucose	+	**D-Glucuronic acid**	+	**L-Histidine**	+	**Tween40**
	D-Galactose		α-Hydroxybutyric acid	+	**Hydroxy-L-proline**	+	**Tween80**
	Gentiobiose	+	**β-Hydroxybutyric acid**	+	**L-Leucine**		
+	**α-D-Glucose**	+	**γ-Hydroxybutyric acid**	+	**L-Ornithine**		Amides
	m-Inositol		p-Hydroxyphenylacetic acid	+	**L-Phenylalanine**		
	α-D-Lactose		Itaconic acid	+	**L-Proline**		Succinamic acid
	Lactulose		α-Ketobutyric acid	+	**L-Pyroglutamic acid**		Glucuronamide
	Maltose	+	**α-Ketoglutaric acid**		D-Serine	+	**Alaninamide**
	D-Mannitol	+	**α-Ketovaleric acid**	+	**L-Serine**		
+	**D-Mannose**	+	**D,L-Lactic acid**		L-Threonine		Phosphoralated Compounds
	D-Melibiose		Malonic acid	+	**D,L-Carnitine**		
	β-Methyl D-glucoside	+	**Propionic acid**	+	**γ-Aminobutyric acid**		D,L-α-Glycerol phosphate
	D-Psicose	+	**Quinic acid**				Glucose-1-phosphate
	D-Raffinose	+	**D-Saccharic acid**		Aromatics		Glucose-6-phosphate
	L-Rhamnose		Sebacic acid				
	D-Sorbitol	+	**Succinic acid**	+	**Urocanic acid**		Amines
	Sucrose			+	**Inosine**		
	D-Trehalose		Alcohols		Uridine	+	**Phenylethylamine**
	Turanose			+	**Thymidine**	+	**Putrescine**
	Xylitol	+	**2,3-Butanediol**			+	**2-Aminoethanol**
		+	**Glycerol**				
	Brominated Chemicals						
+	**Bromosuccinic acid**						

Table 10.

Carbohydrates commonly found associated with humic acid, humin and fulvic acid and the relative proportion each makes up of the total carbohydrates (Uzaki and Ishwatari, 1983).

Glucose	31.9 %
Galactose	16.7 %
Mannose	15.4 %
Xylose	9.5 %
Arabinose	7.8 %
Ribose	1.7 %
Fucose	6.1 %
Rhamnose	9.2 %

Table 11.

Carbohydrates giving greatest density in the pristine wells. These compounds are the carbohydrates giving the greatest density of 28 carbohydrates tested for each of the wells.

Well	Carbon Source
P28A	α-D-Glucose
P28TB	L-Arabinose
P28TA	L-Arabinose
P29C	L-Fucose
P29TC	L-Arabinose
P29TA	L-Arabinose

Table 12.

API-NFT results for isolates taken from three of the MSB cluster wells. Fifty isolates were taken at random 1% PTYG and 50 isolates were taken randomly from PTYG for each of the wells. These results represent the pooled results from PTYG and 1% PTYG for each well.

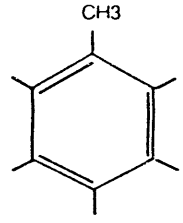
Well	MSB11B % Positive	MSB11C % Positive	MSB11D % Positive
Gram negative	96	84	89
Nitrate reduction	6	27	44
Tryptophanase	0	0	2
Glucose fermentation	0	0	0
Arginine dihydrolase	0	0	0
Urease	12	36	18
Esculin hydrolysis	52	49	47
Gelatinase	28	2	0
β -Galactosidase	74	33	36
Oxidase	14	31	29

Table 13.

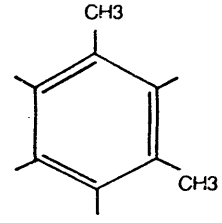
API-NFT results and Gram stain reaction for isolates taken from PTYG and 1% PTYG. Fifty isolates were taken at random from 1% PTYG agar plates and 50 isolates were taken randomly from PTYG agar plates from wells MSB11B, MSB11C, and MSB11D. These results represent the pooled results from all three wells.

Media	PTYG % Positive	1% PTYG % Positive
Gram negative	88	92
Nitrate reduction	15	36
Tryptophanase	1	0
Glucose fermentation	0	0
Arginine dihydrolase	0	0
Urease	20	22
Esculin hydrolysis	34	63
Gelatinase	0	19
β -galactosidase	41	54
Oxidase	45	5

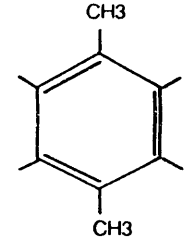
Figure 1. Known substrates for TOL plasmid coded enzymes.



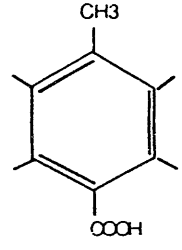
Toluene



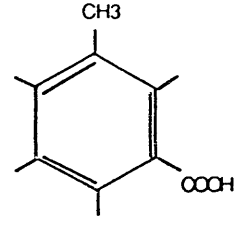
m-Xylene



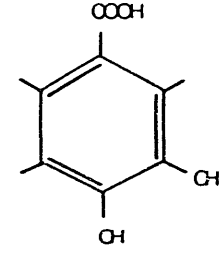
p-Xylene



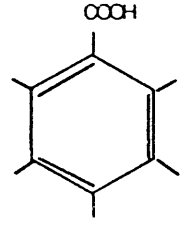
p-Toluic acid



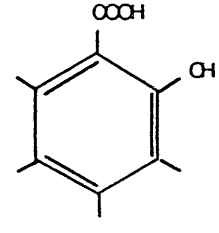
m-Toluic acid



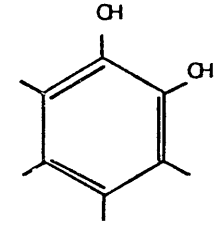
Protocatechuic acid



Benzoic acid



Salicylic acid



Catechol

Figure 2. Proposed type structure of humic acid.

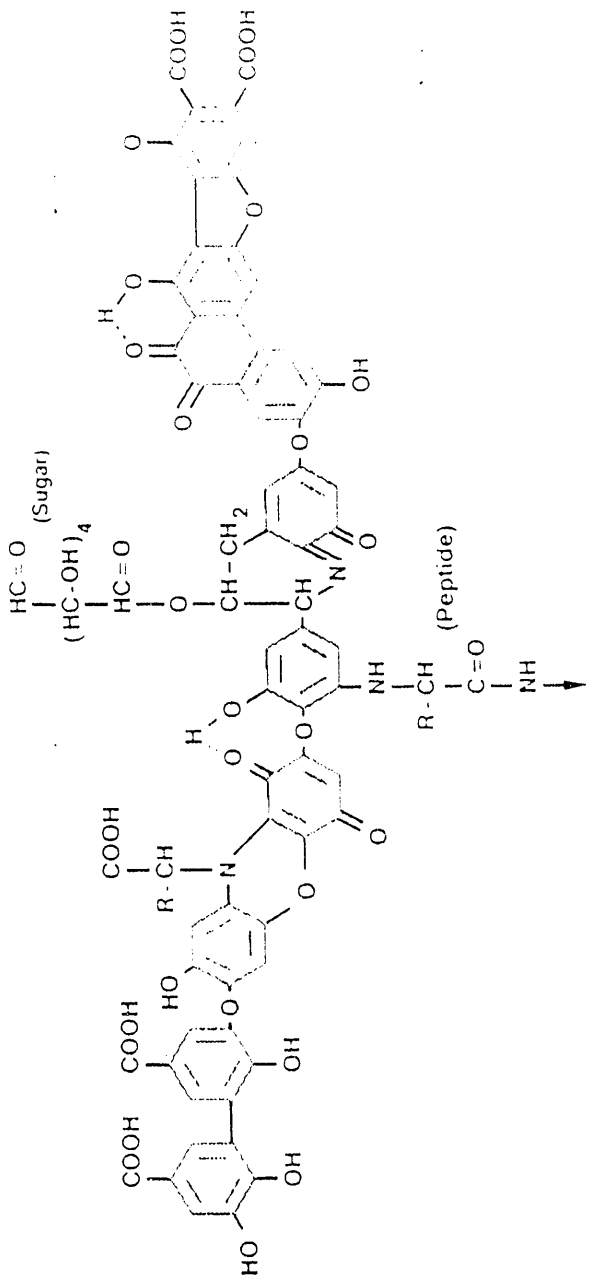

COOH
COOH
O
O
H
O
O
O
OH
O
(Sugar)
HC=O
(HC-OH)4
HC=O
O
CH-CH2
CH
N
O
O
(Peptide)
NH
R-CH
C=O
NH
H
O
O
O
O
COOH
R-CH
N
O
HO
O
COOH
OH
COOH
OH
HO

Figure 3. Proposed type structure of lignin.

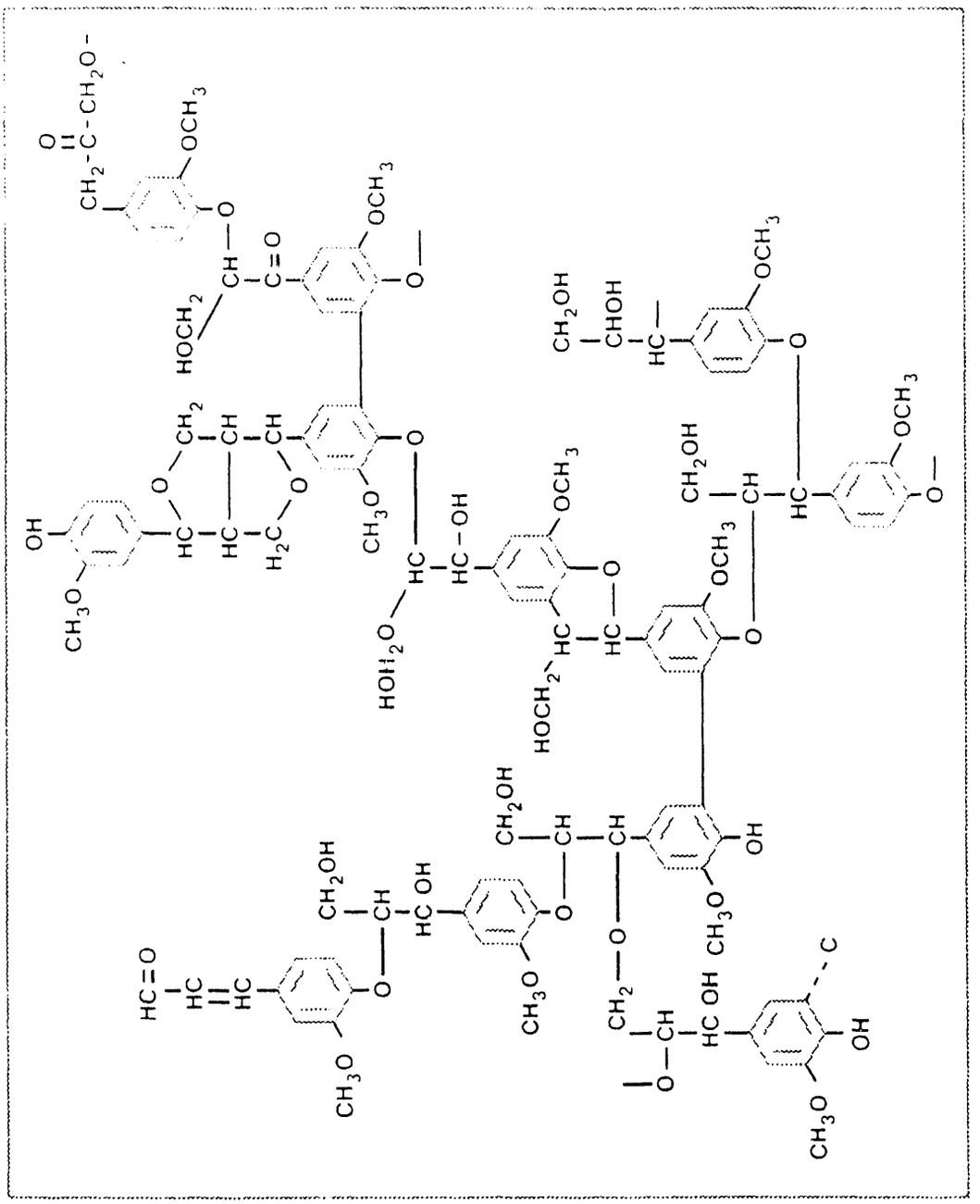
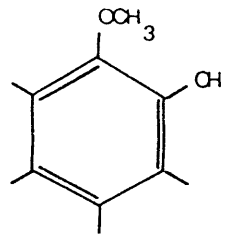
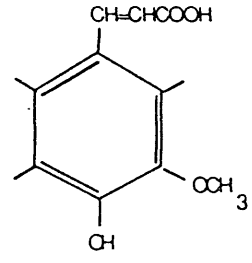


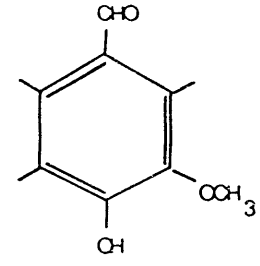
Figure 4. Selected breakdown products resulting from the oxidation of lignin and humic acid.



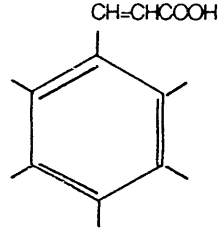
Guaiacol



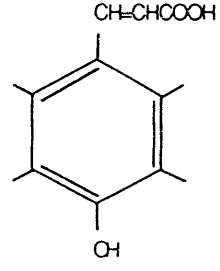
Ferulic Acid



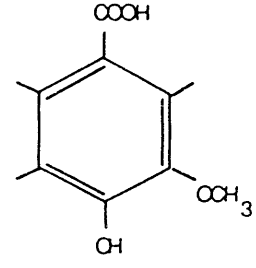
Vanillin



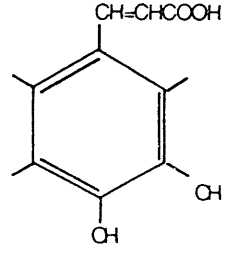
Cinnamic acid



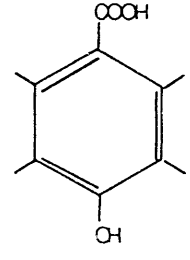
p-Coumaric acid



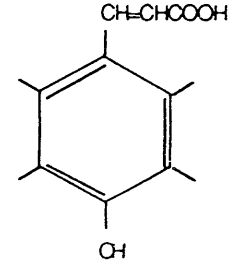
Vanillic acid



Caffeic acid



p-Hydroxybenzoic acid



p-Hydroxycinnamic acid

Figure 5. Map of South Carolina showing the location of the Savannah River Site.

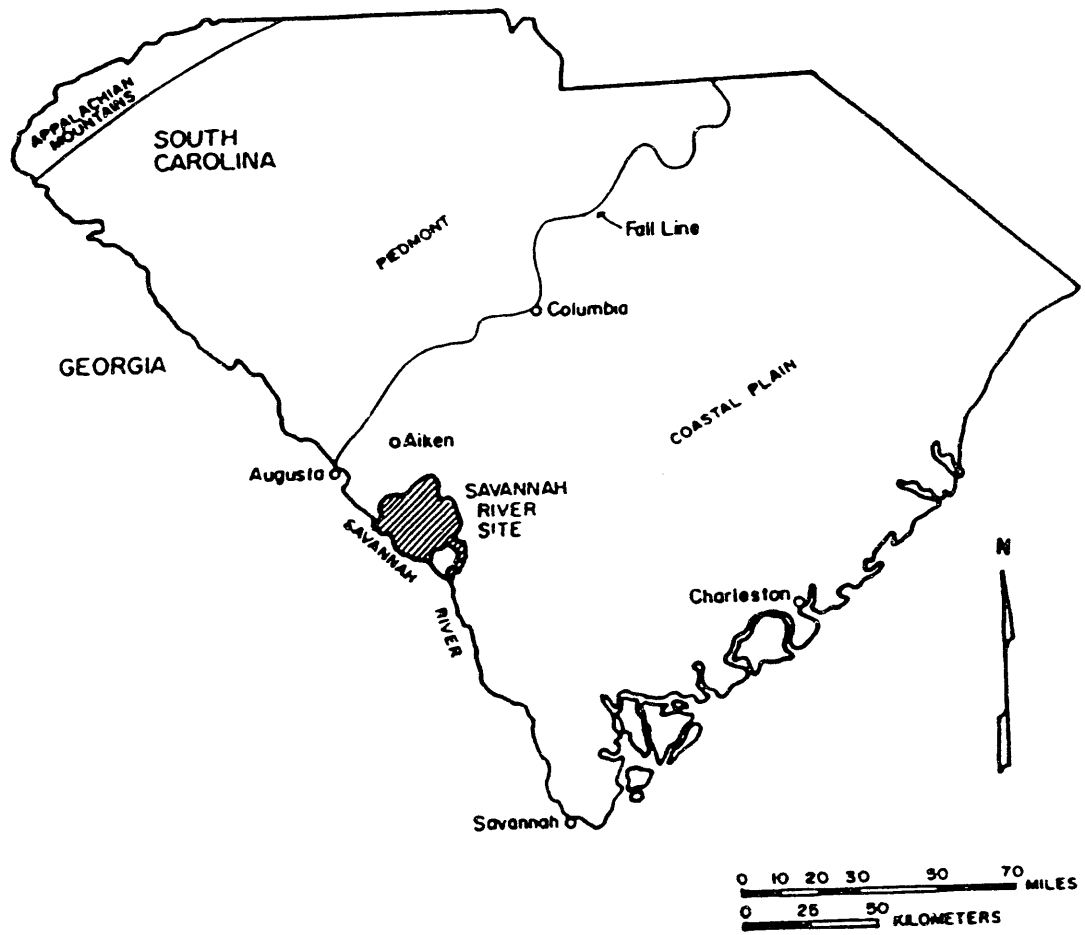

APPALACHIAN MOUNTAINS
SOUTH CAROLINA
PIEDMONT
Fall Line
Columbia
GEORGIA
COASTAL PLAIN
Aiken
Augusta
SAVANNAH RIVER SITE
SAVANNAH RIVER
Charleston
Savannah
N
0 10 20 30 50 70 MILES
0 25 50 KILOMETERS

Figure 6. Map of the Savannah River Site showing the location of the well sites and the microbial ecology laboratory.

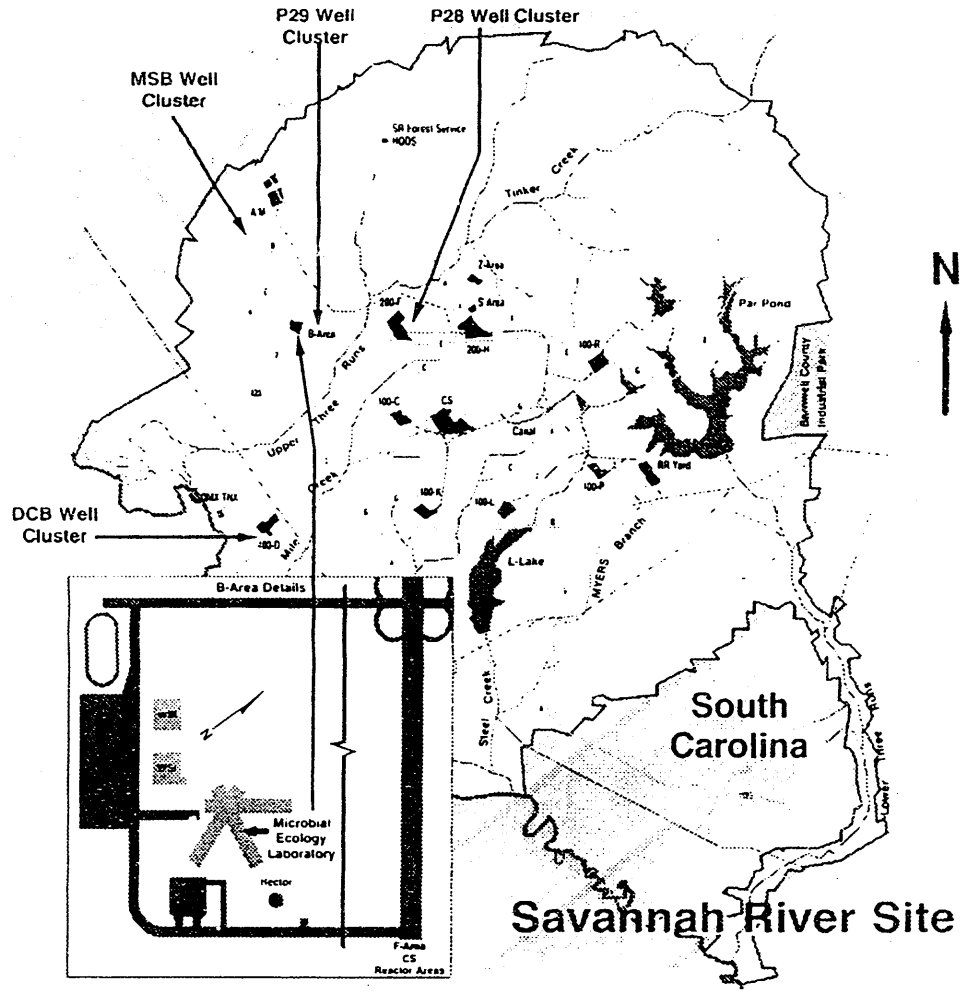

P29 Well Cluster
P28 Well Cluster
MSB Well Cluster
DCB Well Cluster
N
Par Pond
L-Lake
B-Area Details
Microbial Ecology Laboratory
South Carolina
Savannah River Site

Figure 7. Geological crossection of the P28 site showing the formations sampled.

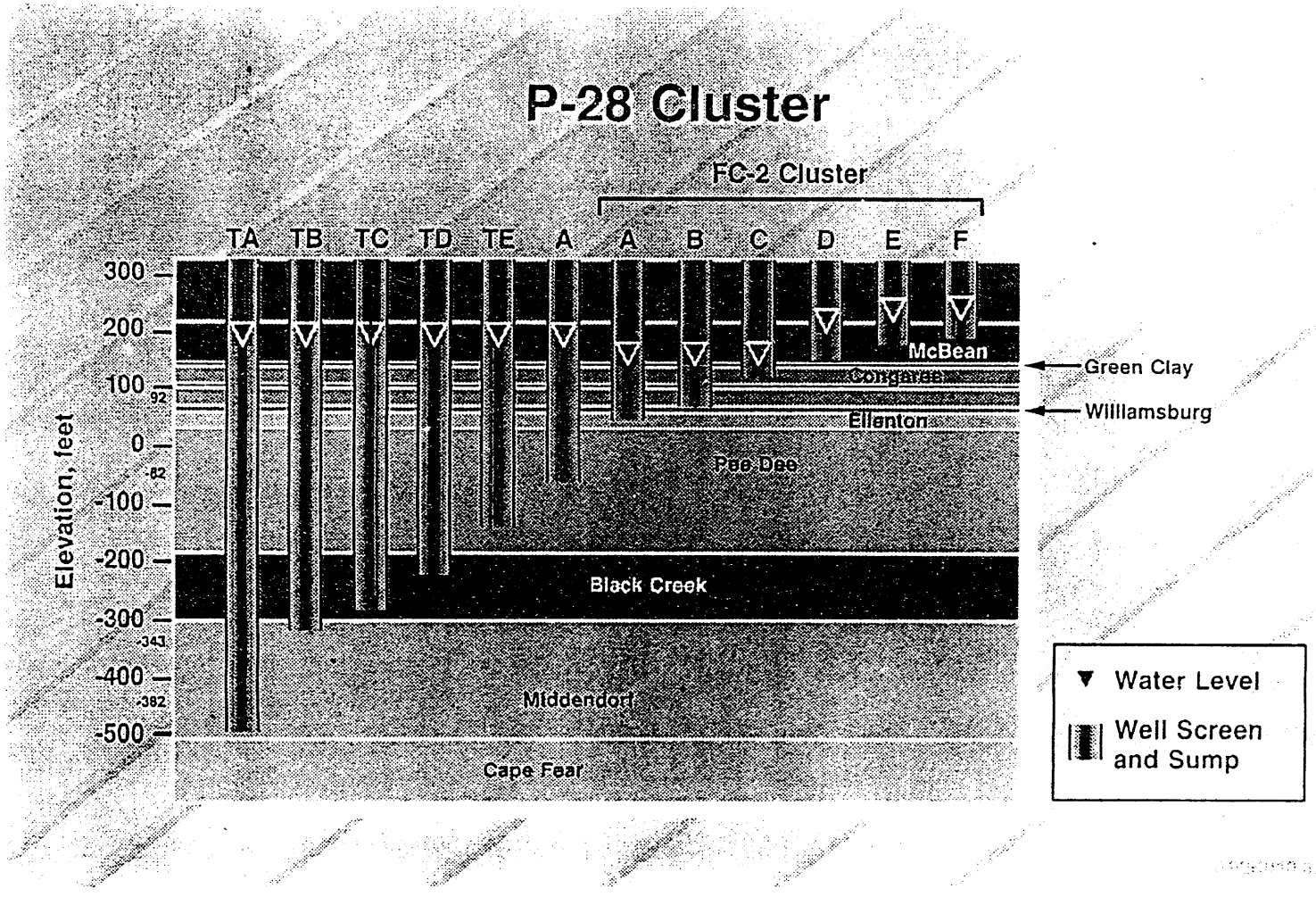
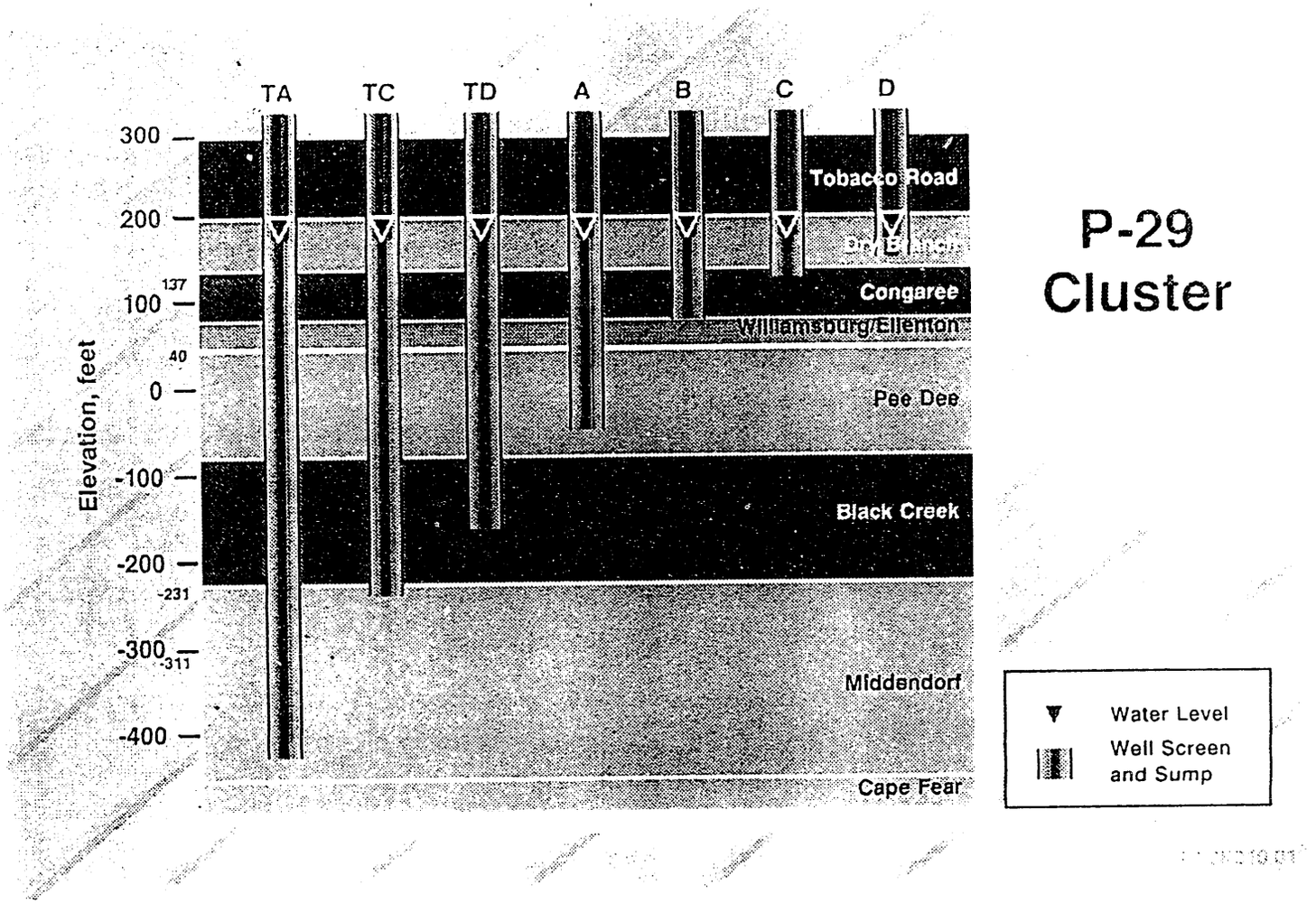
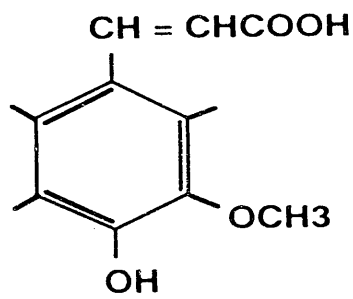

P-28 Cluster
FC-2 Cluster
TA
TB
TC
TD
TE
A
A
B
C
D
E
F
300
200
100
92
0
-82
-100
-200
-300
-341
-400
-382
-500
Elevation, feet
McBean
Congaree
Ellenton
Pee Dee
Black Creek
Middendorf
Cape Fear
Green Clay
Williamsburg
Water Level
Well Screen and Sump

Figure 8. Geological crossection of the P29 site showing the formations sampled.

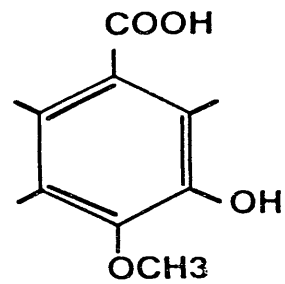

TA
TC
TD
A
B
C
D
300
200
100
137
40
0
-100
-200
-231
-300
-311
-400
Elevation, feet
Tobacco Road
Congaree
Williamsburg/Ellenton
Pee Dee
Black Creek
Middendorf
Cape Fear
Water Level
Well Screen and Sump

P-29 Cluster

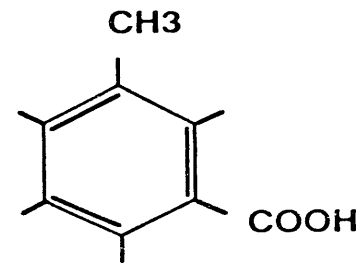
Figure 9. Aromatic compounds used in this study.



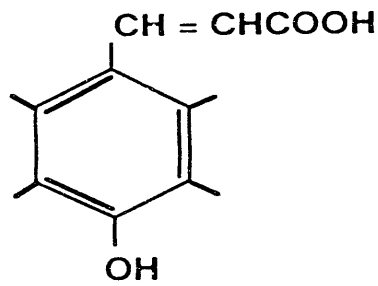
FERULIC ACID



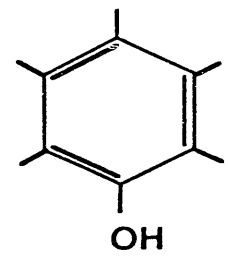
ISOVANILLIC ACID



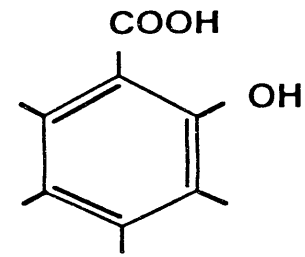
M-TOLUIC ACID



P-COUMARIC ACID



PHENOL



SALICYLIC ACID

Figure 10. Utilization of aromatic compounds by bacteria in the P28 and P29 wells. (Most probable number ± standard error of the mean).

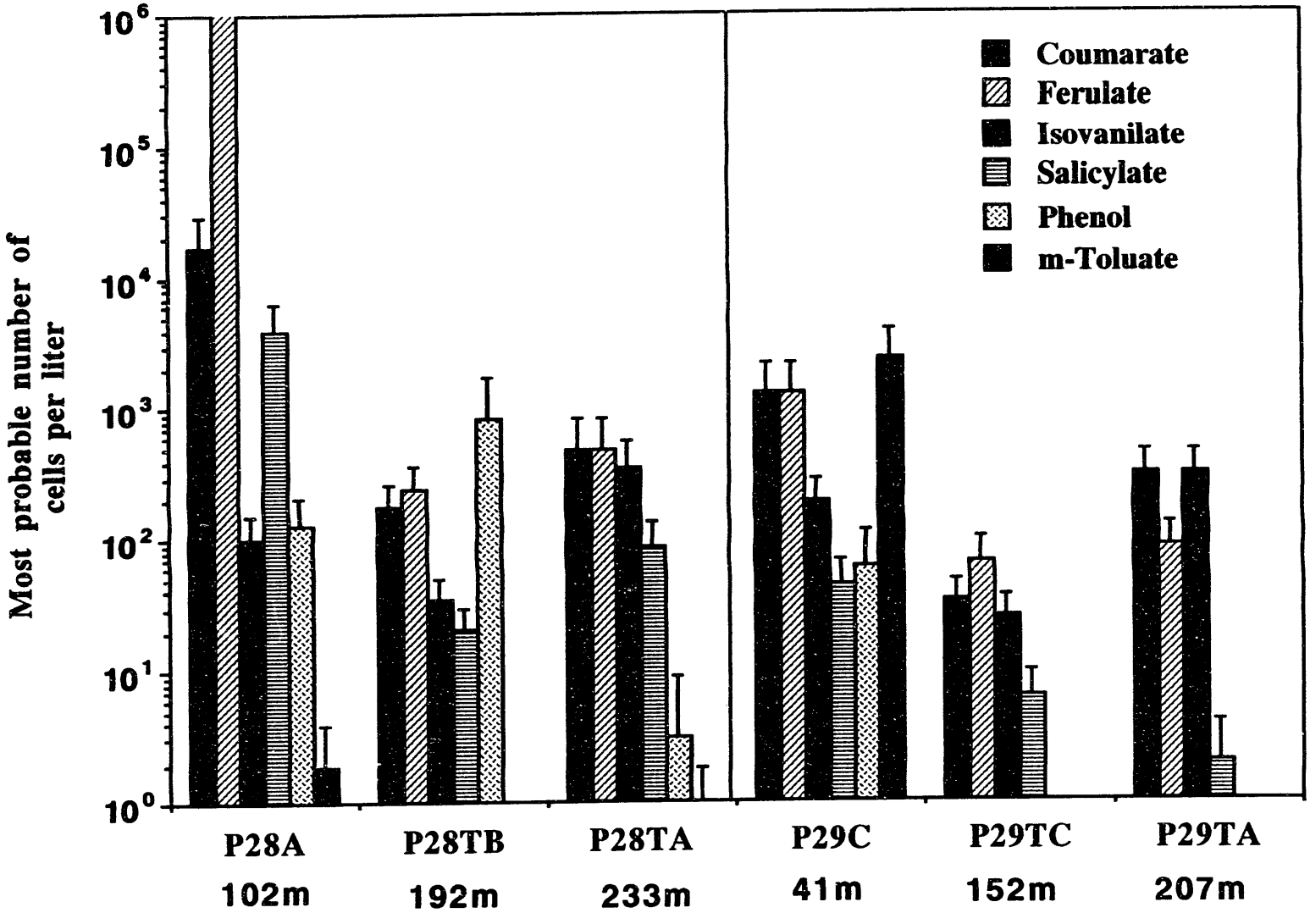

Most probable number of cells per liter
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Coumarate
Ferulate
Isovanilate
Salicylate
Phenol
m-Toluate
P28A
102m
P28TB
192m
P28TA
233m
P29C
41m
P29TC
152m
P29TA
207m

Figure 11. Acridine orange direct counts (cells/ml) by well. Samples were taken between 3/29/90 and 5/08/91. (Mean ± standard error of the mean; n=12).

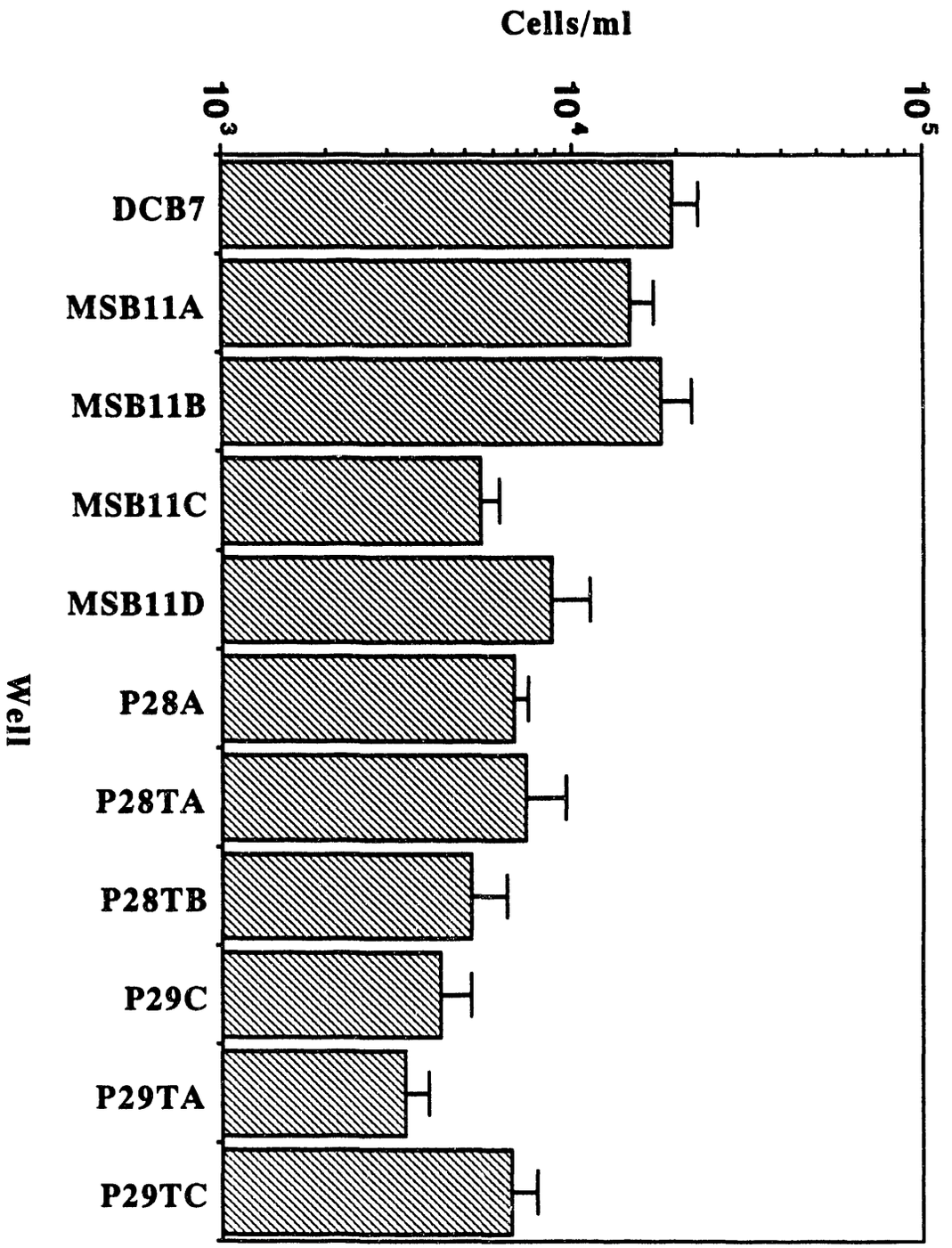

Cells/ml
10^3
10^4
10^5
Well
DCB7
MSB11A
MSB11B
MSB11C
MSB11D
P28A
P28TA
P28TB
P29C
P29TA
P29TC

Figure 12. Heterotrophic plate counts (CFU/ml) on 1% PTYG by well. Samples were taken between 2/28/90 and 5/24/91. (Mean ± standard error of the mean; n=12).

CFU/ml
10^0
10^1
10^2
10^3
10^4
WELL
DCB7
MSB11A
MSB11B
MSB11C
MSB11D
P28A
P28TA
P28TB
P29C
P29TA
P29TC

Figure 13. Heterotrophic plate counts (CFU/ml) on PTYG by well. Samples were taken between 2/28/90 and 3/15/91. (Mean ± standard error of the mean; n=8).

CFU/ml
10^0
10^1
10^2
10^3
WELL
DCB7
MSB11A
MSB11B
MSB11C
MSB11D
P28A
P28TA
P28TB
P29C
P29TA
P29TC

Figure 14. Percentage of active cells as measured by acridine orange. Samples were taken between 6/19/89 and 5/08/91. (Mean ± standard error of the mean; n=20).

Percentage of active cells
0
20
40
60
80
100
Well
DCB7
MSB11A
MSB11B
MSB11C
MSB11D
P28A
P28TA
P28TB
P29C
P29TA
P29TC

Figure 15. Dissolved oxygen (mg/l) by well. Samples were taken between 7/06/89 and 5/24/91. (Mean ± standard error of the mean; n=16).

Dissolved oxygen
(mg/l)
0
2
4
6
8
WELL
DCB7
MSB11A
MSB11B
MSB11C
MSB11D
P28A
P28TA
P28TB
P29C
P29TA
P29TC

Figure 16. Oxidation-reduction potential (mV) by well. Samples were taken between 7/17/90 and 2/22/91. (Mean ± standard error of the mean; n=16).

Oxidation-reduction potential (mV)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
WELL
DCB7
MSB11A
MSB11B
MSB11C
MSB11D
P28A
P28TA
P28TB
P29C
P29TA
P29TC

Figure 17. Mean pH by well. Samples were taken between 6/24/89 and 3/15/91. (Mean ± standard error of the mean; n=18).

pH
0
3
6
9
WELL
DCB7
MSB11A
MSB11B
MSB11C
MSB11D
P28A
P28TA
P28TB
P29C
P29TA
P29TC

Figure 18. Utilization of aromatic compounds by bacteria in the DCB7 well. (Most probable number ± standard error of the mean).

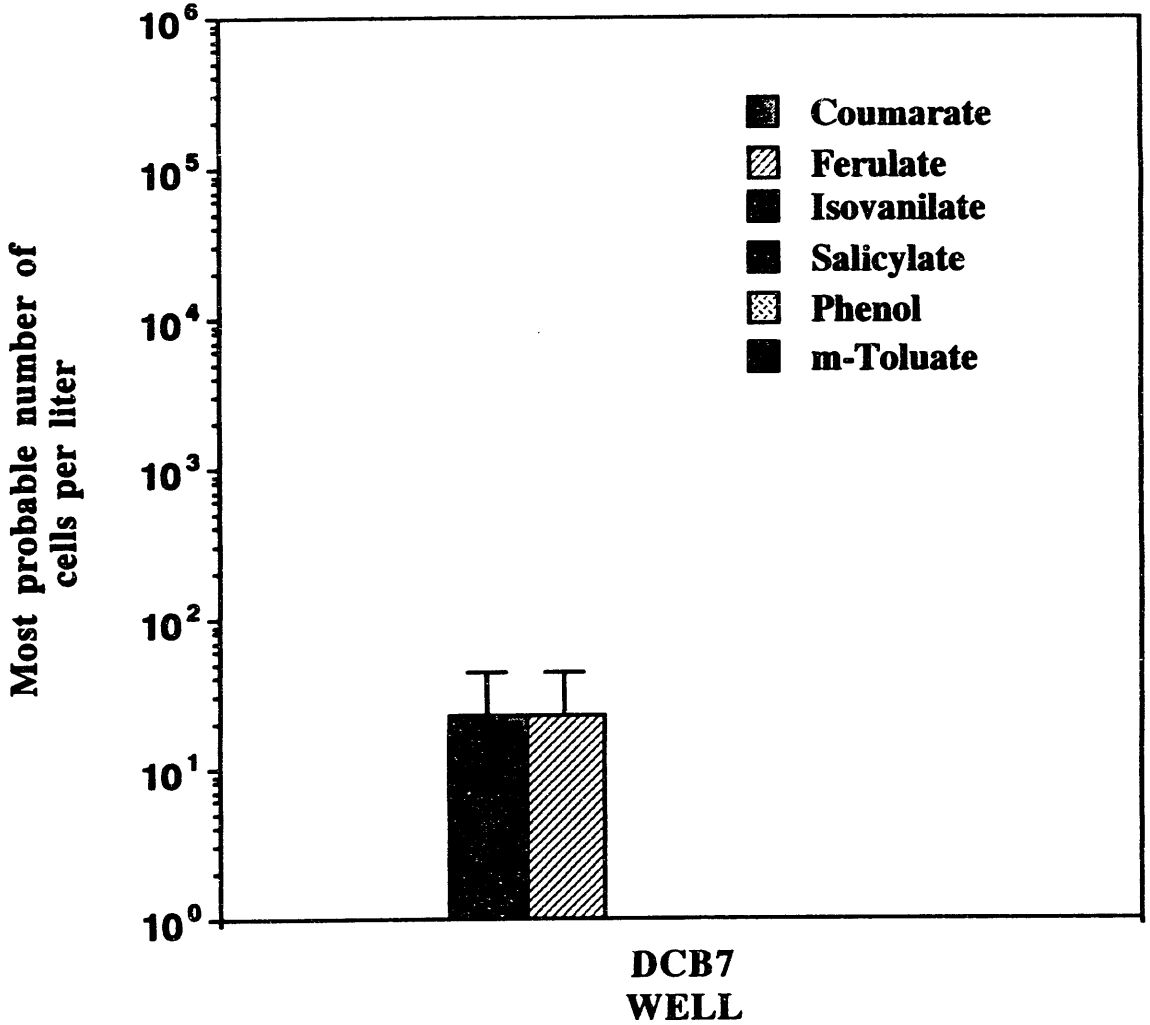

Most probable number of cells per liter
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Coumarate
Ferulate
Isovanilate
Salicylate
Phenol
m-Toluate
DCB7
WELL

Figure 19. Mean temperature (°C) by well. Samples were taken between 7/14/89 and 3/8/91. (Mean ± standard error of the mean; n=16).

Temperature
(degrees C)
17
18
19
20
21
22
23
Well
DCB7
MSB11A
MSB11B
MSB11C
MSB11D
P28A
P28TA
P28TB
P29C
P29TA
P29TC

Figure 20. Mean conductivity (ms/cm) by well. Samples were taken between 6/19/89 and 5/8/91. (Mean ± standard error of the mean; n=18).

Conductivity
(ms/cm)
0
1
2
3
4
5
DCB7
MSB11A
MSB11B
MSB11C
MSB11D
P28A
P28TA
P28TB
P29C
P29TA
P29TC
Well

Figure 21. Mean trichloroethylene (ppb) by well. Samples were taken between 10/18/90 and 2/22/91. (Mean ± standard error of the mean; n=3).

Trichloroethylene
(ppb)
log10(x+1)
0
1
2
3
4
5
6
MSB11A
MSB11B
MSB11C
MSB11D
WELL

Figure 22. Utilization of aromatic compounds by bacteria in the MSB well. (Most probable number ± standard error of the mean).

Most probable number of cells per liter
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Coumarate
Ferulate
Isovanilate
Salicylate
Phenol
m-Toluate
MSB11B
MSB11C
MSB11D
WELL

Figure 23. Mean heterotrophic plate counts on low nutrient (1% PTYG) and high nutrient (PTYG) media. Samples were taken between 2/28/90 and 5/24/91. (Mean ± standard error of the mean; n=12).

CFU/ml
10^0
10^1
10^2
10^3
10^4
WELL
DCB7
MSB11A
MSB11B
MSB11C
MSB11D
P28A
P28TB
P28TA
P29C
P29TC
P29TA
1% PTYG
PTYG

Figure 24. Mean bacterial densities as determined by acridine orange direct counts compared with heterotrophic plate counts on 1% PTYG media. Samples were taken between 2/28/90 and 5/24/91. (Mean ± standard error of the mean; n=12).

Cells/ml or
CFU/ml
10^0
10^1
10^2
10^3
10^4
10^5
DCB7
MSB11A
MSB11B
MSB11C
MSB11D
P28A
P28TB
P28TA
P29C
P29TC
P29TA
Well
AODC
1% PTYG

Table A-1.

Analysis of groundwater temperature by well and time. Two factor analysis of variance was performed with samples taken between 7/14/89 and 3/8/91 (n=16).

Summary of Fit

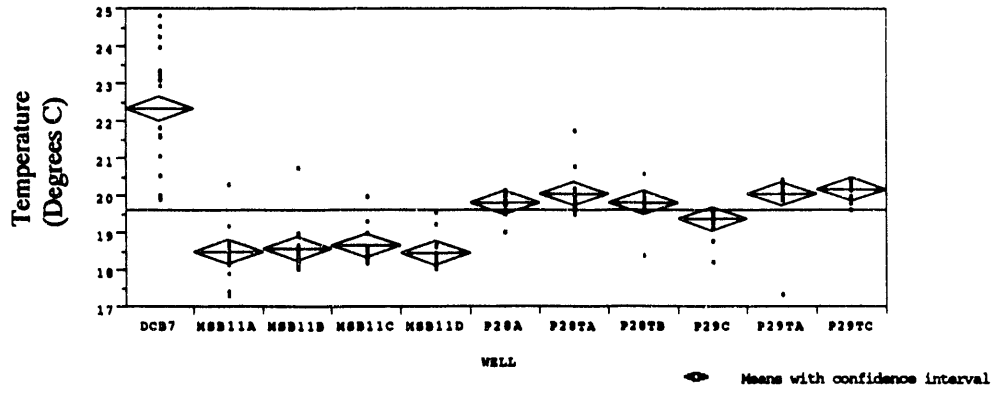
Rsquare	0.895952
Root Mean Square Error	0.582716
Mean of Response	19.62602
Observations (or Sum Wgts)	176

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
WELL	10	10	205.48727	60.5161	0.0000
TIME	7	7	6.21639	2.6153	0.0168
WELL*TIME	70	70	45.60066	1.9185	0.0019

Table A-2.

Analysis of ground water temperature by well. Samples were taken between 7/14/89 and 3/8/91 (n=16).



Well	Count	Mean	Std Dev
DCB7	16	22.34	1.70
MSB11A	16	18.49	0.68
MSB11B	16	18.60	0.63
MSB11C	16	18.65	0.47
MSB11D	16	18.47	0.42
P28A	16	19.84	0.26
P28TA	16	20.04	0.55
P28TB	16	19.83	0.44
P29C	16	19.38	0.37
P29TA	16	20.06	0.73
P29TC	16	20.20	0.25

Welch Anova			
F Ratio	DF Numerator	DF Denominator	Prob>F
39.9930	10	65.524	0.0000

Comparison of equality of means for oxidation-reduction potential of wells as determined by Games and Howell (1976) method (α=0.05). Positive values represent significant differences.

	MSB11D	MSB11A	MSB11B	MSB11C	P29C	P28TB	P28A	P28TA	P29TA	P29TC	DCB7
MSB11D	-	-	-	-	+	+	+	+	+	+	+
MSB11A	-	-	-	-	-	+	+	+	+	+	+
MSB11B	-	-	-	-	-	+	+	+	+	+	+
MSB11C	-	-	-	-	+	+	+	+	+	+	+
P29C	+	-	-	+	-	-	-	-	-	+	+
P28TB	+	+	+	+	-	-	-	-	-	-	+
P28A	+	+	+	+	-	-	-	-	-	-	+
P28TA	+	+	+	+	-	-	-	-	-	-	+
P29TA	+	+	+	+	-	-	-	-	-	-	-
P29TC	+	+	+	+	+	-	-	-	-	-	-
DCB7	+	+	+	+	+	+	+	+	-	-	-

Table A-3.

Analysis of groundwater dissolved oxygen by site and time. Data was transformed by taking the square root. Two factor analysis of variance of samples taken between 7/14/89 and 3/8/91 (n=48).

Summary of Fit

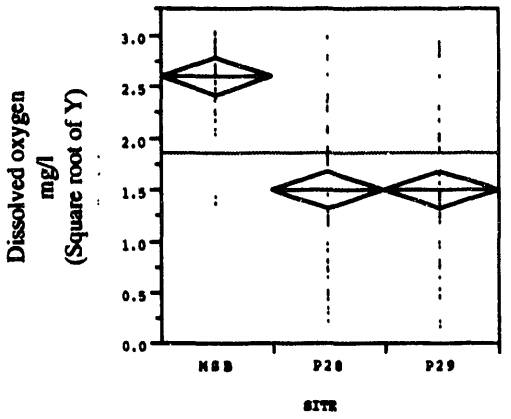
Rsquare	0.458168
Root Mean Square Error	0.683357
Mean of Response	1.870974
Observations (or Sum Wgts)	144

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
SITE	2	2	39.174711	41.9451	0.0000
TIME	7	7	1.927493	0.5897	0.7633
SITE*TIME	14	14	6.282307	0.9609	0.4971

Table A-4.

Analysis of groundwater dissolved oxygen by site. Data was transformed by taking the square root. Single factor analysis of variance was performed with samples taken between 7/14/89 and 3/8/91 (n=48).



Well	Count	Mean	Std Dev
P29	48	1.4999	0.7784
P28	48	1.5044	0.7740
MSB	48	2.6086	0.4024

Welch Anova F Ratio	DF Numerator	DF Denominator	Prob>F
62.9348	2	84.449	0.0000

Comparison of equality of means for oxidation-reduction potential of wells as determined by Games and Howell (1976) method (α=0.05). Positive values represent significant differences.

	P29	P28	MSB
P29	-	-	+
P28	-	-	+
MSB	+	+	-

Table A-5.

Analysis of groundwater dissolved oxygen by well and time. Data was transformed by taking the square root. Two factor analysis of variance of samples taken between 7/14/89 and 3/8/91 (n=16).

Summary of Fit

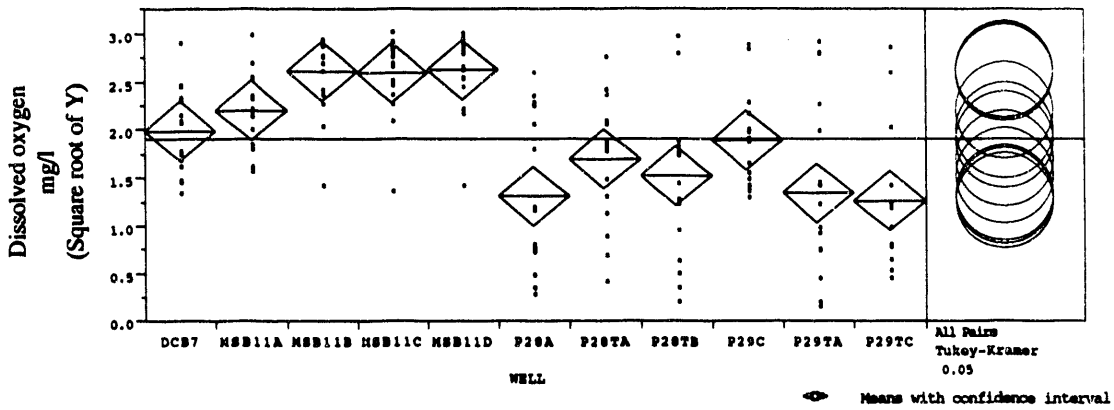
Rsquare	0.684666
Root Mean Square Error	0.63035
Mean of Response	1.912123
Observations (or Sum Wgts)	176

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
WELL	10	10	45.173109	11.5702	0.0000
TIME	7	7	2.912153	0.6875	0.6823
WELL*TIME	70	70	28.034651	1.0079	0.4827

Table A-6.

Analysis of groundwater dissolved oxygen by well. Data was transformed by taking the square root. Single factor analysis of variance was performed with samples taken between 7/14/89 and 3/8/91 (n=16). Variances were homogeneous as determined by the Hartley F max test.



Well	Count	Mean	Std Dev
DCB7	16	1.988	0.441
MSB11A	16	2.207	0.434
MSB11B	16	2.607	0.412
MSB11C	16	2.595	0.417
MSB11D	16	2.624	0.405
P28A	16	1.302	0.802
P28TA	16	1.692	0.646
P28TB	16	1.519	0.858
P29C	16	1.896	0.489
P29TA	16	1.347	0.958
P29TC	16	1.256	0.697

Summary of Fit

Rsquare	0.414598
Root Mean Square Error	0.627225
Mean of Response	1.912123
Observations (or Sum Wgts)	176

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	10	45.97311	4.59731	11.6858
Error	165	64.91287	0.39341	Prob>F
C Total	175	110.88598		0.0000

Comparisons for all pairs using Tukey HSD (α=.05)

q* 3.26445

Abs(Dif)-LSD	MSB11D	MSB11B	MSB11C	MSB11A	DCB7	P29C	P28TA	P28TB	P29TA	P28A	P29TC
MSB11D	-0.72392	-0.70711	-0.69455	-0.30647	-0.08798	0.003585	0.207604	0.380905	0.552787	0.598562	0.644007
MSB11B	-0.70711	-0.72392	-0.71136	-0.32328	-0.10479	-0.01322	0.190795	0.364096	0.535978	0.581753	0.627199
MSB11C	-0.69455	-0.71136	-0.72392	-0.33583	-0.11734	-0.02578	0.178239	0.351540	0.523422	0.569196	0.226560
MSB11A	-0.30647	-0.32328	-0.33583	-0.72392	-0.50543	-0.41386	-0.20984	-0.03654	0.135340	0.181114	0.008071
DCB7	-0.08798	-0.10479	-0.11734	-0.50543	-0.72392	-0.63235	-0.42833	-0.25503	-0.08315	-0.03738	-0.08349
P29C	0.003585	-0.01322	-0.02578	-0.41386	-0.63235	-0.72392	-0.5199	-0.3466	-0.17471	-0.12894	-0.28751
P28TA	0.207604	0.190795	0.178239	-0.20984	-0.42833	-0.5199	-0.72392	-0.55062	-0.37873	-0.33296	-0.46081
P28TB	0.380905	0.364096	0.351540	-0.03654	-0.25503	-0.3466	-0.55062	-0.72392	-0.55203	-0.50626	-0.6327
P29TA	0.552787	0.535978	0.523422	0.135340	-0.08315	-0.17471	-0.37873	-0.55203	-0.72392	-0.67814	
P28A	0.598562	0.581753	0.569196	0.181114	-0.03738	-0.12894	-0.33296	-0.50626	-0.67814	-0.72392	-0.67847
P29TC	0.644007	0.627199	0.614642	0.226560	0.008071	-0.08349	-0.28751	-0.46081	-0.6327	-0.67847	-0.72392

Positive values show pairs of means that are significantly different.

Table A-7.

Analysis of groundwater oxidation-reduction potential by site and time. Data was transformed by $(Y+2)^3$. Two factor analysis of variance was performed with samples taken between 6/19/89 and 5/08/91 (n=48).

Summary of Fit

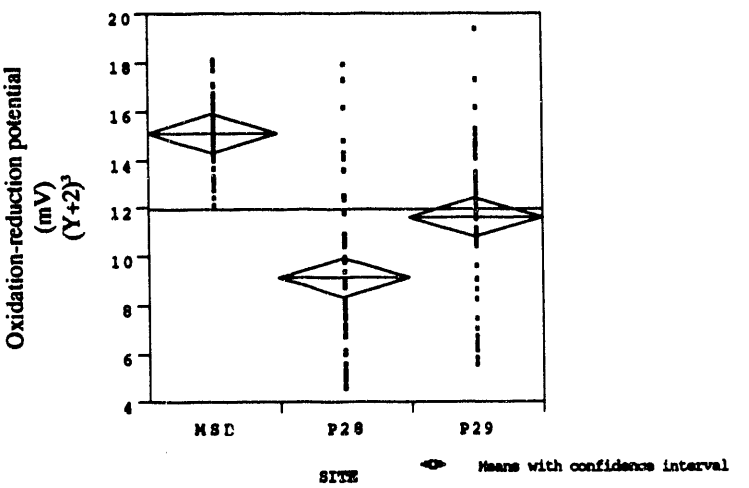
Rsquare	0.669288
Root Mean Square Error	2.373966
Mean of Response	11.9765
Observations (or Sum Wgts)	144

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
SITE	2	2	875.05537	77.6348	0.0000
TIME	7	7	317.93447	8.0592	0.0000
SITE*TIME	14	14	175.66609	2.2264	0.0103

Table A-8.

Analysis of groundwater oxidation-reduction potential by site. Data was transformed by $(Y+2)^3$. Samples were taken between 6/19/89 and 5/08/91 (n=48).



Well	Count	Mean	Std Dev
P28	48	9.134	3.469
P29	48	11.650	3.233
MSB	48	15.146	1.551

Welch Anova F Ratio	DF Numerator	DF Denominator	Prob>F
71.4642	2	81.684	0.0000

Comparison of equality of means for oxidation-reduction potential of wells as determined by Games and Howell (1976) method (α=0.05). Positive values represent significant differences.

	P28	P29	MSB
P28	-	+	+
P29	+	-	+
MSB	+	+	-

Table A-9.

Analysis of groundwater oxidation-reduction potential by well and time. Data was transformed by $(Y+2)^3$. Two factor analysis of variance was performed with samples taken between 6/19/89 and 5/08/91 (n=16).

Summary of Fit

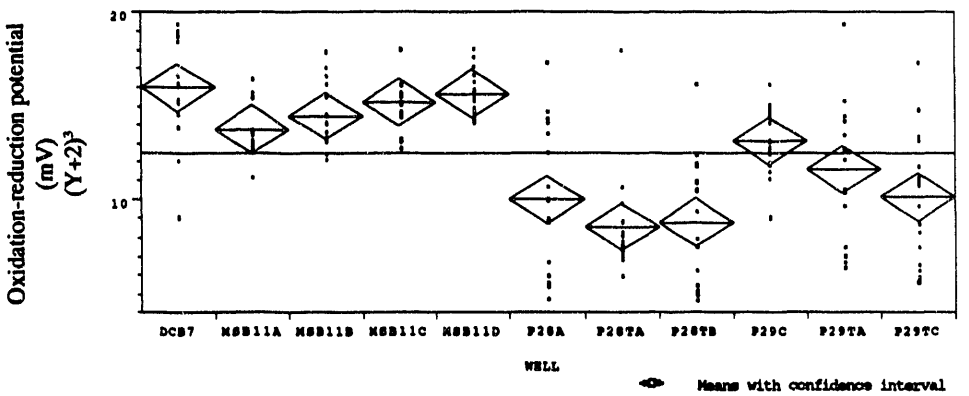
Rsquare	0.797712
Root Mean Square Error	2.370063
Mean of Response	12.50361
Observations (or Sum Wgts)	176

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
WELL	10	10	1233.8632	21.9658	0.0000
TIME	7	7	340.2842	8.6541	0.0000
WELL*TIME	70	70	375.1519	0.9541	0.5785

Table A-10.

Analysis of groundwater oxidation-reduction potential by well. Data was transformed by $(Y+2)^3$. Samples were taken between 6/19/89 and 5/08/91 (n=16).



Well	Count	Mean	Std Dev
DCB7	16	15.988	2.692
MSB11A	16	13.763	1.422
MSB11B	16	14.528	1.725
MSB11C	16	15.192	1.518
MSB11D	16	15.718	1.226
P28A	16	9.977	4.103
P28TA	16	8.595	2.794
P28TB	16	8.830	3.450
P29C	16	13.131	1.776
P29TA	16	11.653	3.594
P29TC	16	10.167	3.462

Welch ANOVA of Oxidation-Reduction Potential by well.

F Ratio	DF Numerator	DF Denominator	Prob>F
17.7070	10	65.612	0.0000

Comparison of equality of means for oxidation-reduction potential of wells as determined by Games and Howell (1976) method (α=0.05). Positive values represent significant differences.

	P28TA	P28TB	P28A	P29TC	P29TA	P29C	MSB11A	MSB11B	MSB11C	MSB11D	DCB7
P28TA	-	-	-	-	-	+	+	+	+	+	+
P28TB	-	-	-	-	-	-	+	+	+	+	-
P28A	-	-	-	-	-	-	-	-	-	+	+
P29TC	-	-	-	-	-	-	-	-	+	+	-
P29TA	-	-	-	-	-	-	-	-	-	-	-
P29C	+	-	-	-	-	-	-	-	-	-	-
MSB11A	+	+	-	-	-	-	-	-	-	-	-
MSB11B	+	+	-	-	-	-	-	-	-	-	-
MSB11C	-	+	-	+	-	-	-	-	-	-	-
MSB11D	+	+	+	+	-	-	-	-	-	-	-
DCB7	+	-	+	-	-	-	-	-	-	-	-

Table A-11.

Analysis of groundwater pH by site and time. Two factor analysis of variance was performed with samples taken between 6/19/89 and 5/08/91 (n=54).

Summary of Fit

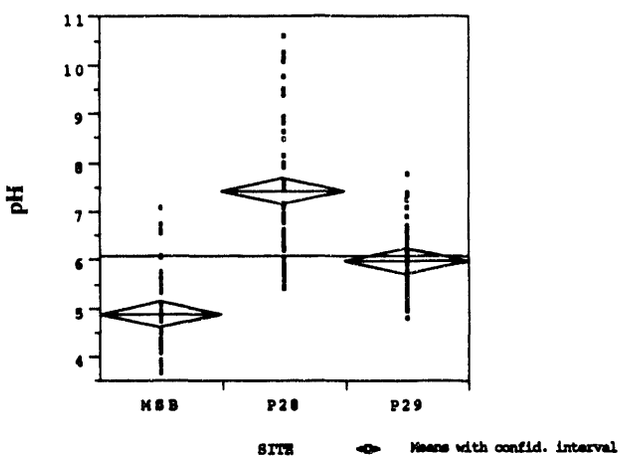
Rsquare	0.616886
Root Mean Square Error	0.997169
Mean of Response	6.094259
Observations (or Sum Wgts)	162

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
SITE	2	2	171.49258	86.2339	0.0000
TIME	8	8	12.07777	1.5183	0.1564
SITE*TIME	16	16	32.57623	2.0476	0.0142

Table A-12.

Analysis of groundwater pH by site. Samples were taken between 6/19/89 and 5/08/91 (n=54).



Well	Count	Mean	Std Dev
MSB	54	4.90	0.84
P28	54	7.41	1.46
P29	54	5.98	0.73

Welch Anova F Ratio	DF Numerator	DF Denominator	Prob>F
64.9599	2	100.61	0.0000

Comparison of equality of means for oxidation-reduction potential of wells as determined by Games and Howell (1976) method (α=0.05). Positive values represent significant differences.

	MSB	P29	P28
MSB	-	+	+
P29	+	-	+
P28	+	+	-

Table A-13.

Analysis of groundwater pH by well and time. Two factor analysis of variance was performed with samples taken between 6/19/89 and 5/08/91 (n=16).

Summary of Fit

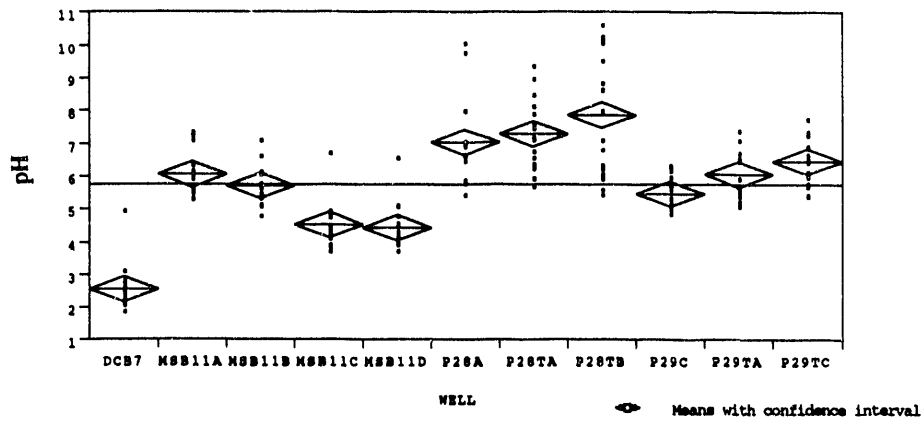
Rsquare	0.893843
Root Mean Square Error	0.780662
Mean of Response	5.770556
Observations (or Sum Wgts)	198

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
WELL	10	10	407.39149	66.8476	0.0000
TIME	8	8	11.10209	2.2771	0.0279
WELL*TIME	80	80	89.51781	1.8361	0.0021

Table A-14.

Analysis of groundwater pH by well. Samples were taken between 6/19/89 and 5/08/91 (n=18)



Well	Count	Mean	Std Dev
DCB7	18	2.57	0.68
MSB11A	18	6.06	0.64
MSB11B	18	5.70	0.57
MSB11C	18	4.54	0.66
MSB11D	18	4.44	0.63
P28A	18	7.06	1.25
P28TA	18	7.27	1.05
P28TB	18	7.90	1.89
P29C	18	5.48	0.48
P29TA	18	6.03	0.71
P29TC	18	6.43	0.68

Welch ANOVA of pH by well.

F Ratio	DF Numerator	DF Denominator	Prob>F
54.2627	10	74.593	0.0000

Comparison of equality of means for pH of wells as determined by Games and Howell (1976) method (α=0.05). Positive values represent significant differences.

	DCB7	MSB11D	MSB11C	P29C	MSB11B	P29TA	MSB11A	P29TC	P28A	P28TA	P28TB
DCB7	-	+	+	+	+	+	+	+	+	+	+
MSB11D	+	-	-	+	+	+	+	+	+	+	+
MSB11C	+	-	-	+	+	+	+	+	+	+	+
P29C	+	+	+	-	-	-	-	+	+	+	-
MSB11B	+	+	+	-	-	-	-	-	-	+	-
P29TA	+	+	+	-	-	-	-	-	-	-	-
MSB11A	+	+	+	-	-	-	-	-	-	-	-
P29TC	+	+	+	+	-	-	-	-	-	-	-
P28A	+	+	+	+	-	-	-	-	-	-	-
P28TA	+	+	+	+	+	-	-	-	-	-	-
P28TB	+	+	+	-	-	-	-	-	-	-	-

Table A-15.

Analysis of groundwater conductivity by well and time. Data was transformed using the arc sine square root. Two factor analysis of variance was performed with samples taken between 6/19/89 and 5/08/91 (n=18).

Summary of Fit

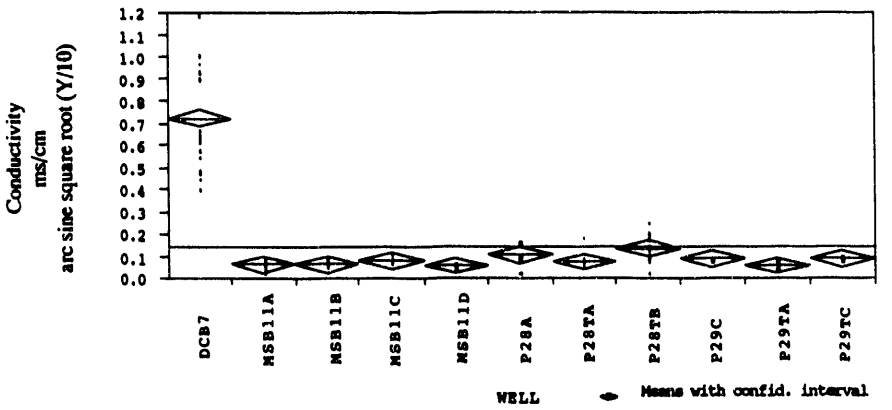
Rsquare	0.80437
Root Mean Square Error	0.071045
Mean of Response	0.265323
Observations (or Sum Wgts)	180

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
WELL	9	9	1.0705006	23.5656	0.0000
TIME	8	8	0.1384568	3.4289	0.0017
WELL*TIME	72	72	0.6588421	1.8129	0.0038

Table A-16.

Analysis of groundwater conductivity by well. Samples were transformed using arc sine square root. Samples were taken between 6/19/89 and 5/08/91 (n=18).



Well	Count	Mean	Std Dev
MSB11D	18	0.0563	0.0061
P29TA	18	0.0606	0.0124
MSB11A	18	0.0647	0.0163
MSB11B	18	0.0667	0.0115
P28TA	18	0.0769	0.0274
MSB11C	18	0.0824	0.0098
P29TC	18	0.0877	0.0060
P29C	18	0.0893	0.0061
P28A	18	0.1034	0.0364
P28TB	18	0.1357	0.0570
DCB7	18	0.7260	0.2475

Welch ANOVA of conductivity by well.

F Ratio	DF Numerator	DF Denominator	Prob>F
51.3898	10	73.958	0.0000

Comparison of equality of means for pH of wells as determined by Games and Howell (1976) method (α=0.05). Positive values represent significant differences.

	MSB11D	P29TA	MSB11A	MSB11B	P28TA	MSB11C	P29TC	P29C	P28A	P28TB	DCB7
MSB11D	-	-	-	-	-	+	+	+	+	+	+
P29TA	-	-	-	-	-	+	+	+	-	+	+
MSB11A	-	-	-	-	-	-	+	+	-	+	+
MSB11B	-	-	-	-	-	-	+	+	-	-	+
P28TA	-	-	-	-	-	-	-	-	-	-	+
MSB11C	+	+	-	-	-	-	-	-	-	-	+
P29TC	+	+	+	+	-	-	-	-	-	-	+
P29C	+	+	+	+	-	-	-	-	-	-	+
P28A	+	-	-	-	-	-	-	-	-	-	+
P28TB	+	+	+	-	-	-	-	-	-	-	+
DCB7	+	+	+	+	+	+	+	+	+	+	+

Table A-17.

Analysis of ground water acridine orange direct counts by site and time. Data was transformed using $Log_{10}(Y+1)$. Two factor analysis of variance was performed on samples taken between 6/19/89 and 5/08/91 (n=60).

Summary of Fit

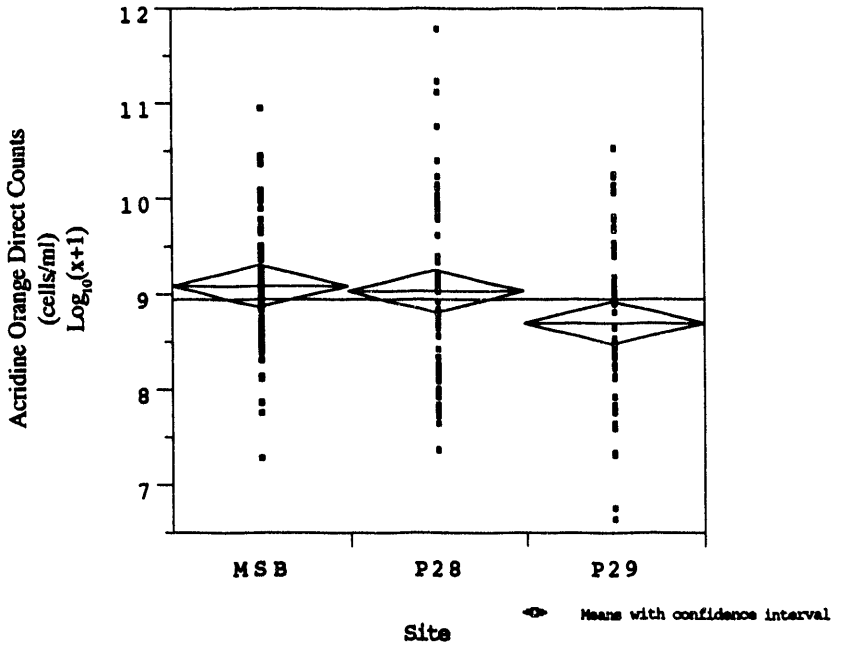
Rsquare	0.466545
Root Mean Square Error	0.719534
Mean of Response	8.945716
Observations (or Sum Wgts)	180

Effect Test

Source	Nparm	DF	Sum of Squares	Prob>F
Site	2	2	5.026115	0.0091
Time	9	9		0.0000
Site*Time	18	18		0.0005

Table A-18.

Analysis of ground water acridine orange direct counts by site. Data was transformed using $Log_{10}(Y+1)$. Samples were taken between 6/19/89 and 5/08/91 (n=60).



Well	Number	Mean	Std Dev
MSB	60	9.0899	0.6977
P28	60	9.0357	0.9909
P29	60	8.7115	0.9558

Welch Anova

F Ratio	DF Numerator	DF Denominator	Prob>F
3.1881	2	114.7	0.0449

Comparison of equality of means for pH of wells as determined by Games and Howell (1976) method (α=0.05). Positive values represent significant differences.

	P29	P28	MSB
P29	-	-	-
P28	-	-	-
MSB	-	-	-

Table A-19.

Analysis of ground water acridine orange direct counts by well and time. Data was transformed using $Log_{10}(Y+1)$. Two factor analysis of variance was performed on samples taken between 6/19/89 and 5/08/91 (n=20).

Summary of Fit

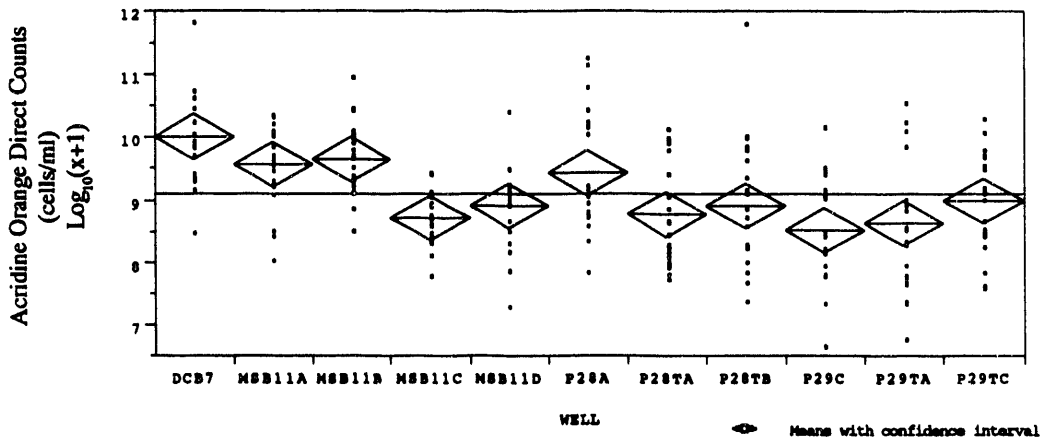
Rsquare	0.723187
Root Mean Square Error	0.687493
Mean of Response	9.097208
Observations (or Sum Wgts)	220

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
WELL	10	10	46.527172	9.8440	0.0000
Time	9	9	35.806424	8.4175	0.0000
WELL*Time	90	90	53.495601	1.2576	0.1256

Table A-20.

Analysis of ground water acridine orange direct counts by well. Data was transformed using $Log_{10}(Y+1)$. Samples were taken between 6/19/89 and 5/08/91 (n=20).



Well	Count	Mean	Std Dev
P29C	20	8.5116	0.3877
P29TA	20	8.6201	1.1377
MSB11C	20	8.7229	0.4217
P28TA	20	8.7729	0.8226
MSB11D	20	8.8983	0.6410
P28TB	20	8.9173	1.0857
P29TC	20	9.0027	0.7838
P28A	20	9.4171	0.9762
MSB11A	20	9.5625	0.6508
MSB11B	20	9.6487	0.6431
DCB7	20	9.9954	0.7108

Welch Anova

F Ratio	DF Numerator	DF Denominator	Prob>F
8.6616	10	83.249	0.0000

Comparison of equality of means for pH of wells as determined by Games and Howell (1976) method (α=0.05). Positive values represent significant differences.

	P29C	P29TA	MSB11C	P28TA	MSB11D	P28TB	P29TC	P28A	MSB11A	MSB11B	DCB7
P29C	-	-	-	-	-	-	-	-	-	-	-
P29TA	-	-	-	-	-	-	-	-	-	-	-
MSB11C	-	-	-	-	-	-	-	-	+	+	+
P28TA	-	-	-	-	-	-	-	-	-	-	+
MSB11D	-	-	-	-	-	-	-	-	-	-	+
P28TB	-	-	-	-	-	-	-	-	-	-	-
P29TC	-	-	-	-	-	-	-	-	-	-	-
P28A	-	-	-	-	-	-	-	-	-	-	-
MSB11A	-	-	+	-	-	-	-	-	-	-	-
MSB11B	-	-	+	-	-	-	-	-	-	-	-
DCB7	-	-	+	+	+	-	-	-	-	-	-

Table A-21.

Analysis of ground water heterotrophic viable counts on 1% PTYG (low nutrient) agar by site and time. Data was transformed using $Log_{10}(Y+1)$. Two factor analysis of variance was performed on samples taken between 6/19/89 and 5/08/91 (n=60).

Summary of Fit

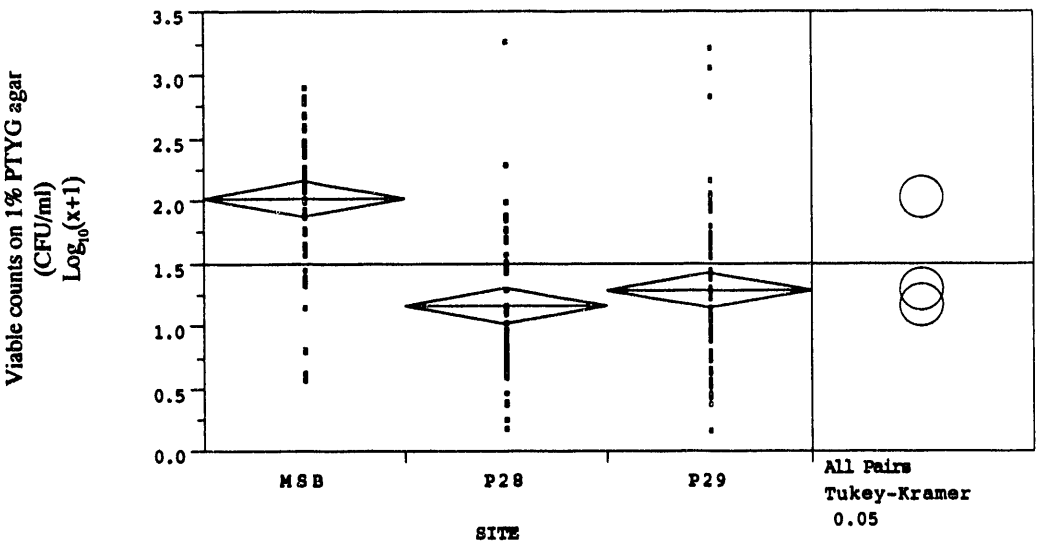
Rsquare	0.497514
Root Mean Square Error	0.558074
Mean of Response	1.49231
Observations (or Sum Wgts)	180

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
SITE	2	2	25.502942	40.9427	0.0000
TIME	9	9	15.992211	5.7053	0.0000
SITE*TIME	18	18	4.759549	0.8490	0.6403

Table A-22.

Analysis of ground water viable counts on 1% PTYG (low nutrient) agar by site. Data was transformed using $Log_{10}(Y+1)$. Samples were taken between 6/19/89 and 5/08/91 (n=60). Variances were homogeneous as determined by the Hartley F-max test.



Summary of Fit

Rsquare	0.274309
Root Mean Square Error	0.617397
Mean of Response	1.49231
Observations (or Sum Wgts)	180

Well	Count	Mean	Std Dev
MSB	60	2.0191	0.5691
P28	60	1.1627	0.6162
P29	60	1.2952	0.6633

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	25.502942	12.7515	33.4527
Error	177	67.468770	0.3812	Prob>F
C Total	179			0.0000

Comparisons for all pairs using Tukey HSD

q* 2.36365 Alpha= 0.05

Abs(Dif)-LSD	MSB	P29	P28
MSB	-0.26643	0.457528	0.590002
P29	0.457528	-0.26643	-0.13396
P28	0.590002	-0.13396	-0.26643

Positive values show pairs of means that are significantly different.

Table A-23.

Analysis of ground water heterotrophic viable counts on 1% PTYG (low nutrient) agar by well and time. Data was transformed using $Log_{10}(Y+1)$. Two factor analysis of variance was performed on samples taken between 6/19/89 and 5/08/91 (n=20).

Summary of Fit

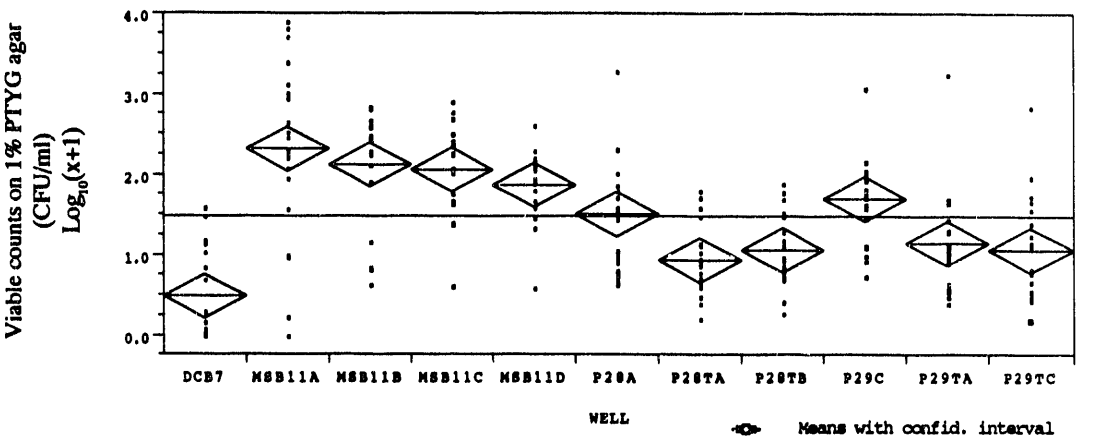
Rsquare	0.813479
Root Mean Square Error	0.513894
Mean of Response	1.474919
Observations (or Sum Wgts)	220

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
WELL	10	10	67.921638	25.7194	0.0000
TIME	9	9	22.973780	9.6659	0.0000
WELL*TIME	90	90	35.799534	1.5062	0.0204

Table A-24.

Analysis of ground water viable counts on 1% PTYG (low nutrient) agar by well. Data was transformed using $Log_{10}(Y+1)$. Samples were taken between 6/19/89 and 5/08/91 (n=20).



WELL	Number	Mean	Std Dev
DCB7	20	0.4879	0.5552
P28TA	20	0.9258	0.4382
P29TC	20	1.0477	0.6625
P28TB	20	1.0675	0.4380
P29TA	20	1.1428	0.6145
P28A	20	1.4948	0.7814
P29C	20	1.6949	0.5409
MSB11D	20	1.8720	0.4275
MSB11C	20	2.0598	0.5599
MSB11B	20	2.1255	0.6878
MSB11A	20	2.3054	1.1141

Welch Anova

F Ratio	DF Numerator	DF Denominator	Prob>F
17.8924	10	83.424	0.0000

Comparison of equality of means as determined by Games and Howell (1976) method (α=0.05). Positive values represent significant differences.

	DCB7	P28TA	P29TC	P28TB	P29TA	P28A	P29C	MSB11D	MSB11C	MSB11B	MSB11A
DCB7	-	-	-	-	-	-	+	+	+	+	+
P28TA	-	-	-	-	-	-	+	+	+	+	-
P29TC	-	-	-	-	-	-	-	-	+	+	-
P28TB	-	-	-	-	-	-	-	+	+	+	-
P29TA	-	-	-	-	-	-	-	-	+	+	-
P28A	-	-	-	-	-	-	-	-	-	-	-
P29C	+	+	-	-	-	-	-	-	-	-	-
MSB11D	+	+	-	+	-	-	-	-	-	-	-
MSB11C	+	+	+	+	+	-	-	-	-	-	-
MSB11B	+	+	+	+	+	-	-	-	-	-	-
MSB11A	+	-	-	-	-	-	-	-	-	-	-

Table A-25.

Analysis of ground water heterotrophic viable counts on PTYG agar by site and time. Two factor analysis of variance was performed on samples taken between 2/28/90 and 2/11/91 (n=30).

Summary of Fit

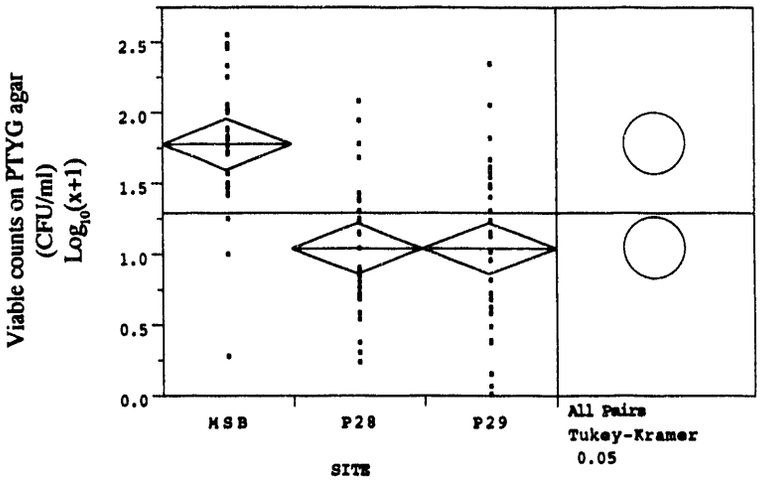
Rsquare	0.39096
Root Mean Square Error	0.524369
Mean of Response	1.294071
Observations (or Sum Wgts)	90

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
SITE	2	2	10.866060	19.7591	0.0000
TIME	4	4	0.958405	0.8714	0.4852
SITE*TIME	8	8	1.413539	0.6426	0.7395

Table A-26.

Analysis of ground water heterotrophic viable counts on PTYG (high nutrient) agar by site. Data was transformed using $Log_{10}(Y+1)$. Samples were taken between 2/28/90 and 3/15/91 (n=30).



Well	Number	Mean	Std Dev
MSB	30	1.7855	0.4610
P28	30	1.0502	0.4715
P29	30	1.0466	0.5984

Summary of Fit

Rsquare	0.320909
Root Mean Square Error	0.514102
Mean of Response	1.294071
Observations (or Sum Wgts)	90

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	10.866060	5.43303	20.5562
Error	87	22.994186	0.26430	Prob>F
C Total	89	33.860246		0.0000

Comparisons for all pairs using Tukey HSD

q* 2.38450

Abs(Dif)-LSD	MSB	P28	P29
MSB	-0.31652	0.418792	0.422338
P28	0.418792	-0.31652	-0.31297
P29	0.422338	-0.31297	-0.31652

Positive values show pairs of means that are significantly different.

Table A-27.

Analysis of ground water heterotrophic viable counts on PTYG agar by well and time. Two factor analysis of variance was performed on samples taken between 2/28/90 and 2/11/91.

Summary of Fit

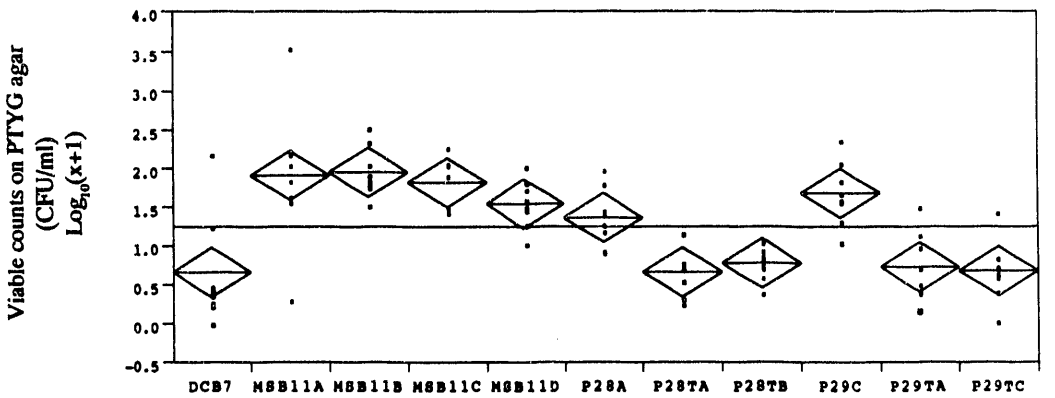
Rsquare	0.810768
Root Mean Square Error	0.424259
Mean of Response	1.253913
Observations (or Sum Wgts)	88

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
WELL	10	10	24.963289	13.8688	0.0000
TIME	3	3	0.547035	1.0131	0.3960
WELL*TIME	30	30	8.422419	1.5597	0.0880

Table A-28.

Analysis of ground water heterotrophic viable counts on PTYG (high nutrient) agar by well. Data was transformed using $Log_{10}(Y+1)$. Samples were taken between 2/28/90 and 2/11/91 (n=8). Distribution was normal as determined by the Shapiro-Wilk W test (Shapiro et al., 1968; Zar, 1984).



Well	Number	Mean	Variance
DCB7	8	0.655	0.509
MSB11A	8	1.918	0.805
MSB11B	8	1.961	0.107
MSB11C	8	1.830	0.099
MSB11D	8	1.551	0.100
P28A	8	1.362	0.144
P28TA	8	0.656	0.081
P28TB	8	0.768	0.042
P29C	8	1.687	0.171
P29TA	8	0.728	0.200
P29TC	8	0.678	0.154

Welch Anova

F Ratio	DF Numerator	DF Denominator	Prob>F
16.2779	10	30.655	0.0000

Comparison of equality of means as determined by Games and Howell (1976) method (α=0.05). Positive values represent significant differences.

	DCB7	P28TA	P29TC	P29TA	P28TB	P28A	MSB11D	P29C	MSB11C	MSB11A	MSB11B
DCB7	-	-	-	-	-	-	-	-	-	-	-
P28TA	-	-	-	-	-	-	+	+	+	-	+
P29TC	-	-	-	-	-	-	-	-	+	-	+
P29TA	-	-	-	-	-	-	-	-	+	-	+
P28TB	-	-	-	-	-	-	+	+	+	-	+
P28A	-	-	-	-	-	-	-	-	-	-	-
MSB11D	-	+	-	-	+	-	-	-	-	-	-
P29C	-	+	-	-	+	-	-	-	-	-	-
MSB11C	-	+	+	+	+	-	-	-	-	-	-
MSB11A	-	-	-	-	-	-	-	-	-	-	-
MSB11B	-	+	+	+	+	-	-	-	-	-	-

Table A-29.

Analysis of the percentage of active cells (as determined by acridine orange) by site and time. Data was transformed using arc sin square root. Two factor analysis of variance was performed on samples taken between 6/19/89 and 5/08/91 (n=60).

Summary of Fit

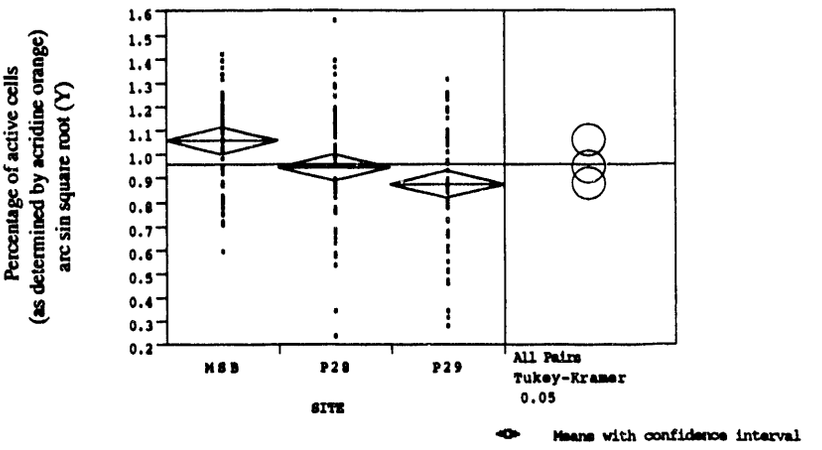
Rsquare	0.426259
Root Mean Square Error	0.204545
Mean of Response	0.960232
Observations (or Sum Wgts)	180

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
SITE	2	2	1.0966804	13.1060	0.0000
TIME	9	9	2.4470406	6.4986	0.0000
SITE*TIME	18	18	1.1188770	1.4857	0.1023

Table A-30.

Analysis of the percentage of active cells (as determined by acridine orange) by site. Data was transformed using arc sin square root. Samples were taken between 6/19/89 and 5/08/91 (n=60). Variances were homogeneous as determined by the Hartley F-max test.



Well	Count	Mean	Std Dev
MSB	60	1.062	0.192
P28	60	0.946	0.255
P29	60	0.873	0.255

Summary of Fit

Rsquare	0.10026
Root Mean Square Error	0.235803
Mean of Response	0.960232
Observations (or Sum Wgts)	180

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	1.096680	0.548340	9.8617
Error	177	9.841736	0.055603	Prob>F
C Total	179	10.938416		0.0001

Comparisons for all pairs using Tukey HSD

q*
2.36365

Abs(Dif)-LSD	MSB	P28	P29
MSB	-0.10176	0.014518	0.087821
P28	0.014518	-0.10176	-0.02845
P29	0.087821	-0.02845	-0.10176

Positive values show pairs of means that are significantly different.

Table A-31.

Analysis of the percentage of active cells (as determined by acridine orange) by well and time. Data was transformed using arc sin square root. Two factor analysis of variance was performed on samples taken between 6/19/89 and 5/08/91 (n=20).

Summary of Fit

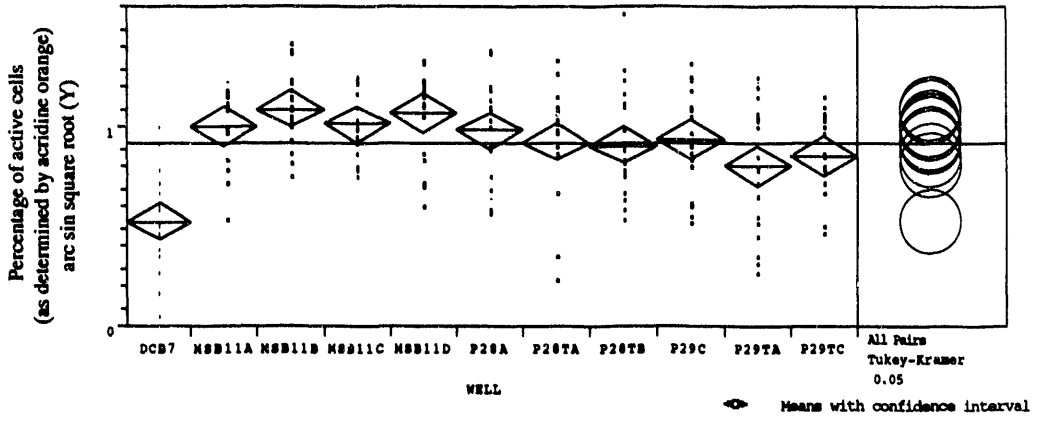
Rsquare	0.71156
Root Mean Square Error	0.206624
Mean of Response	0.925653
Observations (or Sum Wgts)	220

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
WELL	10	10	4.8772895	11.4240	0.0000
TIME	9	9	2.1694398	5.6460	0.0000
WELL*TIME	90	90	4.5386306	1.1812	0.2019

Table A-32.

Analysis of the percentage of active cells (as determined by acridine orange) by well. Data was transformed using arc sin square root. Samples were taken between 6/19/89 and 5/08/91 (n=20). Variances were homogeneous as determined by the Hartley F-max test.



Well	Count	Mean	Std Dev
DCB7	20	0.530	0.246
MSB11A	20	1.010	0.192
MSB11B	20	1.096	0.181
MSB11C	20	1.017	0.173
MSB11D	20	1.074	0.219
P28A	20	0.989	0.238
P28TA	20	0.934	0.267
P28TB	20	0.914	0.265
P29C	20	0.940	0.237
P29TA	20	0.815	0.318
P29TC	20	0.863	0.190

Summary of Fit

Rsquare	0.299558
Root Mean Square Error	0.233594
Mean of Response	0.925653
Observations (or Sum Wgts)	220

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	10	4.877290	0.487729	8.9383
Error	209	11.404344	0.054566	Prob>F
C Total	219	16.281634		0.0000

Comparisons for all pairs using Tukey HSD

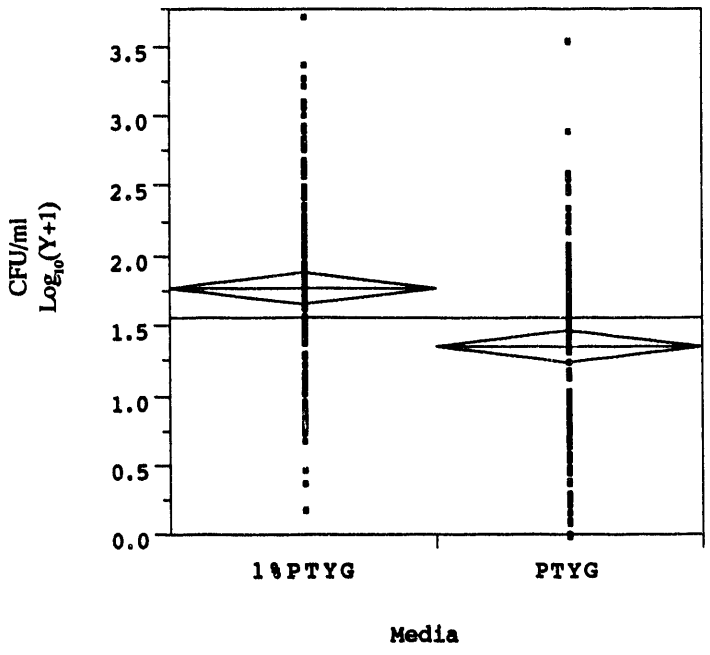
q* 3.25477

Abs(Dif)-LSD	MSB11B	MSB11D	MSB11C	MSB11A	P28A	P29C	P28TA	P28TB	P29TC	P29TA	DCB7
MSB11B	-0.24043	-0.21815	-0.16084	-0.15465	-0.13368	-0.08414	-0.07854	-0.05836	-0.00721	0.040674	0.325977
MSB11D	-0.21815	-0.24043	-0.18312	-0.17692	-0.15596	-0.10642	-0.10082	-0.08064	-0.02949	0.018399	0.303702
MSB11C	-0.16084	-0.18312	-0.24043	-0.23423	-0.21327	-0.16373	-0.15813	-0.13795	-0.08679	-0.03891	0.246393
MSB11A	-0.15465	-0.17692	-0.23423	-0.24043	-0.21946	-0.16992	-0.16432	-0.14414	-0.09299	-0.0451	0.240199
P28A	-0.13368	-0.15596	-0.21327	-0.21946	-0.24043	-0.19089	-0.18529	-0.16511	-0.11395	-0.06607	0.219234
P29C	-0.08414	-0.10642	-0.16373	-0.16992	-0.19089	-0.24043	-0.23483	-0.21465	-0.16349	-0.11561	0.169695
P28TA	-0.07854	-0.10082	-0.15813	-0.16432	-0.18529	-0.23483	-0.24043	-0.22025	-0.16909	-0.12121	0.164095
P28TB	-0.05836	-0.08064	-0.13795	-0.14414	-0.16511	-0.21465	-0.22025	-0.24043	-0.18927	-0.14139	0.143915
P29TC	-0.00721	-0.02949	-0.08679	-0.09299	-0.11395	-0.16349	-0.16909	-0.18927	-0.24043	-0.19254	0.092762
P29TA	0.040674	0.018399	-0.03891	-0.0451	-0.06607	-0.11561	-0.12121	-0.14139	-0.19254	-0.24043	0.044876
DCB7	0.325977	0.303702	0.246393	0.240199	0.219234	0.169695	0.164095	0.143915	0.092762	0.044876	-0.24043

Positive values show pairs of means that are significantly different.

Table A-33.

Comparison of densities for heterotrophic viable counts on PTYG (high nutrient) and 1% PTYG (low nutrient) media.



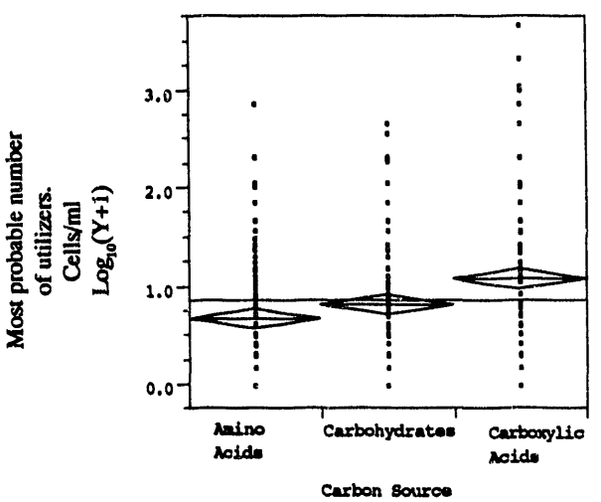
Media	Count	Mean	Std Dev
1%PTYG	132	1.7767	0.7179
PTYG	132	1.3529	0.7010

t-Test

t-Test	DF	Prob>|t|
4.85290024	262	0.0000

Table A-34.

Comparison of densities of carbohydrate, carboylic acid and amino acid utilizers. Data was transformed using Log_{10} (Y+1).



Carbon Source Groups	Count	Mean	Std Dev
Amino Acids	180	0.669	0.665
carbohydrate	180	0.818	0.712
Carboxylic acids	180	1.070	0.795

Welch ANOVA

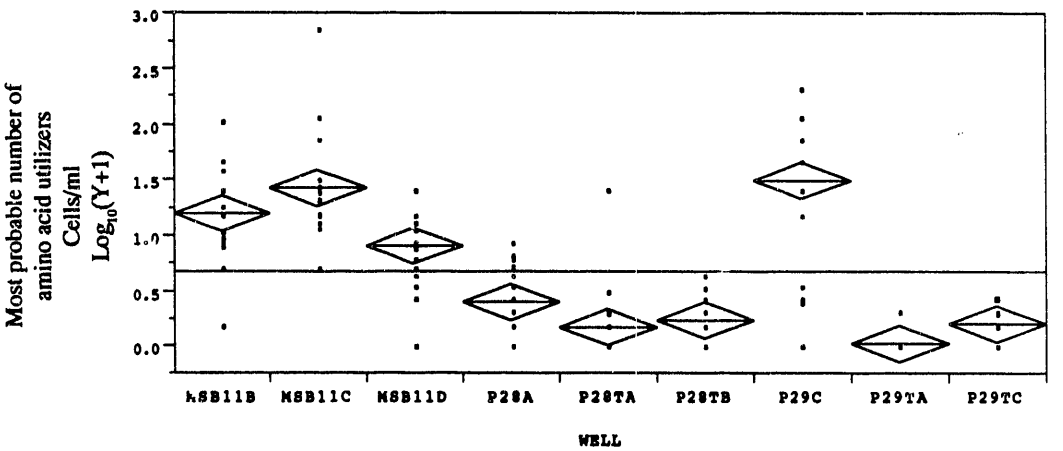
F Ratio	DF Numerator	DF Denominator	Prob>F
13.5082	2	356.18	0.0000

Comparison of equality of means for densities of amino acid utilizing bacteria as determined by Games and Howell (1976) method (a=.05).

	Amino Acids	Carbohydrate	Carboxylic acids
Amino Acids	-	-	+
Carbohydrate	-	-	+
Carboxylic Acids	+	+	-

Table A-35.

Comparison of densities of amino acid utilizers between wells. Data was transformed using Log_{10} (Y+1).



Well	Count	Mean	Std Dev
P29TA	20	0.016	0.071
P28TA	20	0.160	0.330
P29TC	20	0.204	0.159
P28TB	20	0.229	0.176
P28A	20	0.397	0.300
MSB11D	20	0.905	0.321
MSB11B	20	1.189	0.422
MSB11C	20	1.433	0.437
P29C	20	1.484	0.741

Welch ANOVA

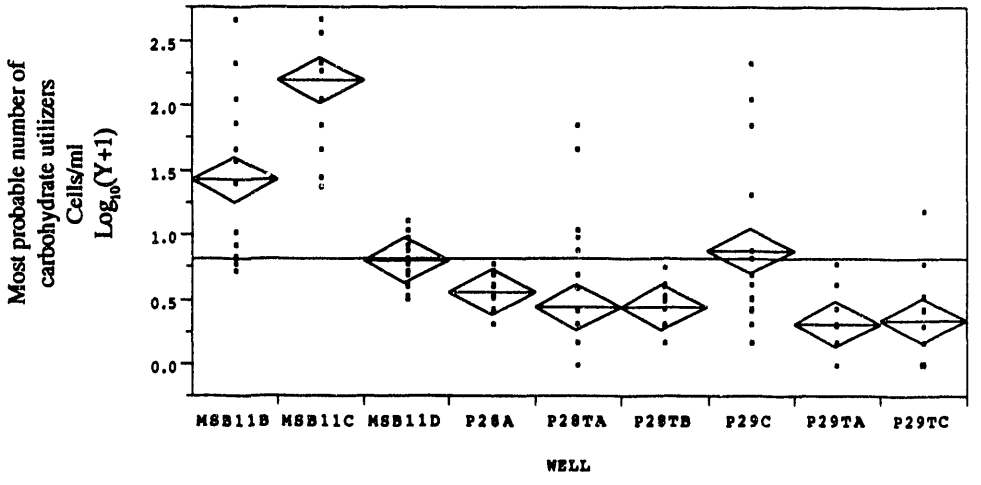
F Ratio	DF Numerator	DF Denominator	Prob>F
65.4361	8	68.176	0.0000

Comparison of equality of means for densities of amino acid utilizing bacteria as determined by Games and Howell (1976) method (a=.05).

	P29TA	P28TA	P29TC	P28TB	P28A	MSB11D	MSB11B	MSB11C	P29C
P29TA	-	-	+	+	+	+	+	+	+
P28TA	-	-	-	-	-	+	+	+	+
P29TC	+	-	-	-	-	+	+	+	+
P28TB	+	-	-	-	-	+	+	+	+
P28A	+	-	-	-	-	+	+	+	+
MSB11D	+	+	+	+	+	-	-	-	-
MSB11B	+	+	+	+	+	-	-	-	-
MSB11C	+	+	+	+	+	-	-	-	-
P29C	+	+	+	+	+	-	-	-	-

Table A-36.

Comparison of densities of carbohydrate utilizers between wells. Data was transformed using Log_{10} (Y+1).



Well	Count	Mean	Std Dev
P29TA	20	0.302	0.180
P29TC	20	0.336	0.303
P28TA	20	0.434	0.586
P28TB	20	0.444	0.142
P28A	20	0.557	0.159
MSB11D	20	0.797	0.189
P29C	20	0.878	0.674
MSB11B	20	1.428	0.563
MSB11C	20	2.188	0.428

Welch ANOVA

F Ratio	DF Numerator	DF Denominator	Prob>F
51.4053	8	70.378	0.0000

Comparison of equality of means for densities of carboxylic acid utilizing bacteria as determined by Games and Howell (1976) method (a=.05).

	P29TA	P29TC	P28TA	P28TB	P28A	MSB11D	P29C	MSB11B	MSB11C
P29TA	-	-	-	-	-	+	-	+	+
P29TC	-	-	-	-	-	+	-	+	+
P28TA	-	-	-	-	-	-	-	+	+
P28TB	-	-	-	-	-	+	-	+	+
P28A	+	-	-	-	-	-	-	+	+
MSB11D	+	+		+	-	-	-	-	+
P29C	-	-	-	-	-	-	-	-	+
MSB11B	+	+	+	+	+	-	-	-	+
MSB11C	+	+	+	+	+	+	+	+	-

Table B-1.

Correlation matrix of the DCB7, MSB11A, MSB11B, MSB11C, MSB11D, P29C, P29TC, P29TA, P28A, P28TB, and P28TA wells. Statistically significant correlations (P<0.05) are underlined.

	Depth	Temp	pH	DO	Cond	Salinity	Redox	TCE	AODC	Activity	1% PTYG	PTYG
Depth	1.000											
Temp	0.168[a]	1.000										
pH	0.411	0.016	1.000									
DO	0.424	-0.257	-0.264	1.000								
Cond	-0.260	0.494	-0.150	-0.015	1.000							
Salinity	-0.252	0.484	-0.119	-0.008	0.993	1.000						
Redox	-0.470	-0.970	-0.368	0.363	0.193	0.197	1.000					
TCE	-0.235	-0.198	-0.304	0.299	-0.041	-0.025	0.241	1.000				
AODC	-0.224	0.001	-0.053	0.044	0.273	0.260	0.152	-0.043	1.000			
Activity	-0.032	-0.319	0.146	0.115	-0.354	-0.317	-0.018	0.024	-0.108	1.000		
1% PTYG	-0.363	-0.298	-0.020	0.176	-0.311	-0.285	0.240	0.212	-0.111	0.364	1	
PTYG	-0.314	-0.425	-0.125	0.369	0.298	-0.282	0.173	0.182	0.123	0.317	0.669	1.000

Frequency Table

	Depth	Temp	pH	DO	Cond	Salinity	Redox	TCE	AODC	Activity	1% PTYG	PTYG
Depth	256											
Temp	234	234										
pH	234	234	234									
DO	203	203	203	203								
Cond	234	234	234	203	234							
Salinity	234	234	234	203	234	234						
Redox	221	221	221	195	221	221	221					
TCE	198	186	186	164	186	186	177	198				
AODC	229	226	226	198	226	226	213	185	229			
Activity	229	226	226	198	226	226	213	185	229	229		
1% PTYG	244	234	234	203	234	234	221	195	229	229	244	
PTYG	170	160	160	137	160	160	147	148	155	155	170	170

[a] Significant correlations are underlined.

df	alpha=0.05	alpha=0.01	df	alpha=0.05	alpha=0.01
135	0.168	0.219	170	0.150	0.196
140	0.165	0.215	180	0.145	0.190
145	0.162	0.212	190	0.142	0.185
150	0.159	0.208	200	0.138	0.181
160	0.154	0.202	250	0.124	0.162

Table B-2.

Correlation matrix of the P28 Well Site (P28A, P28TB, and P28TA). Statistically significant correlations (P<0.05) are underlined.

	Depth	Temp	pH	DO	Cond	Redox	AODC	Activity	1% PTYG	PTYG
Depth	1.000									
Temp	0.210	1.000								
pH	0.105	-0.011	1.000							
DO	0.144	0.164	-0.037	1.000						
Cond	-0.094	-0.010	0.655	-0.043	1.000					
Redox	-0.127	-0.183	-0.370	0.118	-0.249	1.000				
AODC	-0.251[a]	-0.212	0.019	-0.045	0.125	-0.477	1.000			
Activity	-0.100	-0.146	-0.061	0.081	-0.070	0.102	-0.052	1.000		
1% PTYG	-0.398	-0.156	-0.031	-0.213	0.026	0.263	-0.090	0.045	1.000	
PTYG	-0.648	-0.088	-0.163	0.014	-0.156	0.131	0.208	-0.002	0.648	1.000

Frequency Table

	Depth	Temp	pH	DO	Cond	Redox	AODC	Activity	1% PTYG	PTYG
Depth	72									
Temp	63	63								
pH	63	63	63							
DO	55	55	55	55						
Cond	63	63	63	55	63					
Redox	60	60	60	52	60	60				
AODC	66	63	63	55	63	60	66			
Activity	66	63	63	55	63	60	66	66		
1% PTYG	69	63	63	55	63	60	66	66	69	
PTYG	48	42	42	35	42	39	45	45	48	48

[a] Significant correlations are underlined.

df	alpha=0.05	alpha=0.01	df	alpha=0.05	alpha=0.01
32	0.339	0.442	52	0.268	0.348
36	0.320	0.413	58	0.254	0.330
40	0.304	0.393	60	0.250	0.325
43	0.294	0.380	64	0.242	0.315
46	0.285	0.368	66	0.239	0.310
50	0.273	0.354	70	0.232	0.302

Table B-3.

Correlation matrix of the P29 well site (P29C, P29TC, and P29TA). Statistically significant correlations (P<0.05) are underlined.

	Depth	Temp	pH	DO	Cond	Redox	AODC	Activity	1% PTYG	PTYG
Depth	1									
Temp	0.561[a]	1								
pH	0.383	0.424	1							
DO	-0.261	-0.303	-0.219	1						
Cond	-0.553	-0.074	-0.154	0.015	1					
Redox	-0.124	-0.146	-0.182	0.223	-0.049	1				
AODC	0.058	-0.145	-0.108	-0.179	0.119	-0.247	1			
Activity	-0.195	-0.051	0.057	-0.048	-0.029	-0.119	-0.154	1		
1% PTYG	-0.451	-0.154	-0.046	0.076	0.085	0.151	-0.24	0.312	1	
PTYG	-0.678	-0.706	-0.345	0.325	-0.007	0.065	0.057	0.263	0.543	1

Frequency Table

	Depth	Temp	pH	DO	Cond	Redox	AODC	Activity	1% PTYG	PTYG
Depth	69									
Temp	66	66								
pH	66	66	66							
DO	60	60	60	60						
Cond	66	66	66	60	66					
Redox	63	63	63	57	63	63				
AODC	63	63	63	57	63	60	63			
Activity	63	63	63	57	63	60	63	63		
1% PTYG	69	66	66	60	66	63	63	63	69	
PTYG	48	45	45	42	45	42	42	42	48	48

[a] Significant correlations are underlined.

df	alpha=0.05	alpha=0.01	df	alpha=0.05	alpha=0.01
40	0.304	0.393	58	0.254	0.330
43	0.294	0.380	60	0.250	0.325
46	0.285	0.368	64	0.242	0.315
54	0.263	0.341	66	0.239	0.310

Table B-4.

Correlation matrix of the MSB well site (MSB11A, MSB11B, MSB11C, and MSB11D). Statistically significant correlations (P<0.05) are underlined.

	Depth	Temp	pH	DO	Cond	Redox	TCE	AODC	Activity	1% PTYG	PTYG
Depth	1.000										
Temp	-0.012	1.000									
pH	-0.694[a]	-0.029	1.000								
DO	0.296	-0.152	-0.007	1.000							
Cond	-0.151	0.013	0.044	0.008	1.000						
Redox	0.472	-0.064	-0.292	0.146	-0.125	1.000					
TCE	0.225	-0.031	-0.433	0.190	0.415	0.300	1.000				
AODC	-0.401	0.016	0.503	-0.094	-0.188	-0.212	-0.307	1.000			
Activity	0.040	-0.227	0.134	-0.017	0.089	0.051	-0.218	-0.043	1.000		
1% PTYG	-0.221	0.170	0.180	-0.367	0.194	0.119	-0.039	0.006	0.141	1.000	
PTYG	-0.185	0.129	-0.005	0.125	0.078	-0.172	-0.021	-0.007	0.079	0.116	1.000

Frequency Table

	Depth	Temp	pH	DO	Cond	Redox	TCE	AODC	Activity	1% PTYG	PTYG
Depth	92										
Temp	83	83									
pH	83	83	83								
DO	68	68	68	68							
Cond	83	83	83	68	83						
Redox	76	76	76	66	76	76					
TCE	55	55	55	47	55	52	55				
AODC	80	80	80	67	80	73	54	80			
Activity	80	80	80	67	80	73	54	80	80		
1% PTYG	83	83	83	68	83	76	55	80	80	83	
PTYG	58	58	58	47	58	51	51	55	55	58	58

[a] Significant correlations are underlined.

df	alpha=0.05	alpha=0.01	df	alpha=0.05	alpha=0.01
45	0.288	0.372	70	0.232	0.302
49	0.276	0.358	74	0.226	0.294
50	0.273	0.354	78	0.220	0.286
52	0.268	0.348	80	0.217	0.283
56	0.259	0.336	82	0.215	0.280
64	0.242	0.315	90	0.205	0.267
66	0.239	0.310			

Table B-5.

Correlation matrix of aromatic sole carbon source utilization (n=9). Statistically significant correlations (P<0.05) are underlined.

	p-Coumarate	Ferulate	Isovanillate	Phenol	Salicylate	m-Toluate	Urocanic acid	Inosine	Uridine	Thymidine
p-Coumarate	1.000									
Ferulate	0.904	1.000								
Isovanillate	0.575	0.433	1.000							
Phenol	0.879	0.678	0.850	1.000						
Salicylate	0.681	0.628	0.962	0.865	1.000					
m-Toluate	0.145	0.038	0.420	0.272	0.351	1.000				
Urocanic acid	0.236	0.158	0.056	0.147	0.050	0.806	1.000			
Inosine	0.542	0.385	0.513	0.572	0.495	0.835	0.779	1.000		
Uridine	0.742	0.538	0.895	0.955	0.882	0.418	0.207	0.638	1.000	
Thymidine	0.583	0.393	0.705	0.738	0.682	0.395	0.259	0.742	0.769	1.000
TCE	0.623	0.479	0.993	0.884	0.970	0.404	0.070	0.547	0.928	0.765
Depth	-0.586	-0.571	-0.287	-0.450	-0.379	-0.614	-0.685	-0.888	-0.469	-0.670
Temperature	-0.740	-0.677	-0.344	-0.577	-0.425	-0.424	-0.496	-0.821	-0.536	-0.702
pH	-0.726	-0.505	-0.488	-0.681	-0.470	-0.478	-0.431	-0.799	-0.593	-0.736
DO	0.711	0.499	0.464	0.696	0.451	0.291	0.370	0.734	0.685	0.792
Conductivity	-0.317	-0.167	-0.132	-0.196	-0.064	-0.104	-0.139	-0.349	-0.031	-0.382
Redox	0.732	0.538	0.432	0.662	0.434	0.386	0.370	0.788	0.608	0.754
AODC	-0.016	0.230	-0.277	-0.216	-0.120	-0.416	-0.208	-0.120	-0.218	0.145
Activity	0.658	0.650	0.218	0.467	0.319	0.155	0.442	0.595	0.430	0.586
1% PTYG	0.671	0.608	0.425	0.579	0.485	0.487	0.500	0.855	0.594	0.764
PTYG	0.611	0.612	0.332	0.471	0.417	0.506	0.565	0.831	0.498	0.656

[a] Significant correlations are underlined.

df	alpha=0.05	alpha=0.01
7	0.666	0.798

Table B-6.

Correlation matrix of carbohydrate sole carbon source utilization (n=9). Statistically significant correlations (P<0.05) are underlined.

	L-Arabinose	D-Arabitol	Cellobiose	N-Acetyl-D-galactosamine	N-Acetyl-D-glucosamine	Adonitol	i-Erythritol	Maltose	D-Mannitol	D-Mannose
TCE	0.534	0.887[a]	0.692	0.826	0.684	0.982	0.565	0.513	0.773	0.674
Depth	-0.437	-0.652	-0.710	-0.517	-0.745	-0.395	0.385	-0.841	-0.172	-0.777
Temperature	-0.378	-0.689	-0.800	-0.594	-0.834	-0.445	0.240	-0.837	-0.244	-0.751
pH	-0.367	-0.617	-0.609	-0.457	-0.684	-0.475	0.018	-0.628	-0.297	-0.628
DO	0.540	0.670	0.891	0.722	0.880	0.565	0.227	0.863	0.536	0.729
Conductivity	-0.308	-0.220	-0.219	0.032	-0.286	-0.071	-0.083	-0.195	-0.209	-0.230
Redox	0.392	0.662	0.773	0.583	0.833	0.491	-0.039	0.776	0.291	0.720
AODC	0.060	0.091	0.360	0.270	0.287	-0.053	-0.236	0.381	-0.095	0.214
Activity	0.430	0.546	0.792	0.606	0.720	0.362	0.005	0.788	0.458	0.579
1% PTYG	0.535	0.778	0.891	0.680	0.917	0.550	-0.139	0.916	0.360	0.861
PTYG	0.515	0.731	0.839	0.605	0.857	0.465	-0.238	0.879	0.317	0.818

	D-Fructose	L-Fucose	D-Galactose	Gentiobiose	α-D-Glucose	m-Inositol	α-D-Lactose	Lactulose	D-Melibiose	Xylitol
TCE	0.809	0.132	0.699	0.598	0.662	0.790	0.948	0.954	0.575	0.941
Depth	-0.681	-0.443	-0.695	-0.796	-0.675	-0.723	-0.330	-0.452	-0.675	-0.407
Temperature	-0.587	-0.202	-0.643	-0.903	-0.727	-0.762	-0.436	-0.562	-0.754	-0.496
pH	-0.603	-0.279	-0.602	-0.715	-0.563	-0.718	-0.559	-0.606	-0.673	-0.523
DO	0.569	0.057	0.684	0.893	0.826	0.846	0.584	0.718	0.862	0.600
Conductivity	-0.087	-0.084	-0.238	-0.406	-0.319	-0.326	-0.064	-0.199	-0.394	-0.204
Redox	0.552	0.150	0.609	0.860	0.693	0.787	0.542	0.649	0.806	0.561
AODC	-0.26	-0.446	-0.138	0.381	0.379	0.072	-0.276	-0.037	0.283	0.018
Activity	0.434	-0.004	0.579	0.825	0.759	0.646	0.309	0.459	0.645	0.371
1% PTYG	0.664	0.254	0.745	0.958	0.843	0.859	0.475	0.647	0.846	0.601
PTYG	0.637	0.304	0.738	0.920	0.804	0.792	0.364	0.542	0.766	0.511

	β-Methyl-D-glucoside	D-Psicose	D-Raffinose	L-Rhamnose	D-Sorbitol	Sucrose	D-Trehalose	Turanose
TCE	0.514	0.519	0.824	0.572	0.947	0.648	0.881	0.912
Depth	-0.664	-0.145	-0.634	-0.747	-0.492	-0.814	-0.617	-0.491
Temperature	-0.797	-0.262	-0.588	-0.756	-0.581	-0.905	-0.741	-0.609
pH	-0.503	-0.188	-0.510	-0.609	-0.563	-0.807	-0.698	-0.682
DO	0.834	0.254	0.484	0.887	0.706	0.957	0.765	0.639
Conductivity	-0.188	0.141	0.077	-0.240	-0.078	-0.421	-0.320	-0.313
Redox	0.712	0.212	0.499	0.740	0.627	0.907	0.746	0.668
AODC	0.584	0.217	-0.142	0.253	-0.010	0.185	0.088	0.025
Activity	0.867	0.319	0.434	0.766	0.490	0.815	0.638	0.445
1% PTYG	0.850	0.212	0.633	0.863	0.666	0.950	0.784	0.630
PTYG	0.823	0.156	0.622	0.820	0.578	0.889	0.716	0.517

[a] Significant correlations are underlined.

df	alpha=0.05	alpha=0.01
7	0.666	0.798

Table B-7.

Correlation matrix of polymer sole carbon source utilization (n=9). Statistically significant correlations (P<0.05) are underlined.

	α-Cyclodextrin	Dextrin	Glycogen	Tween 40	Tween 80
TCE	0.455	0.589	0.825	0.066	0.669
Depth	-0.651	-0.285	-0.652	-0.454	-0.767
Temperature	-0.775[a]	-0.299	-0.744	-0.370	-0.700
pH	-0.570	-0.646	-0.662	-0.239	-0.670
DO	0.866	0.200	0.879	0.522	0.707
Conductivity	-0.388	-0.283	-0.220	-0.259	-0.342
Redox	0.753	0.415	0.773	0.290	0.670
AODC	0.571	-0.691	0.122	0.141	-0.017
Activity	0.818	-0.056	0.687	0.625	0.591
1% PTYG	0.843	0.206	0.835	0.462	0.796
PTYG	0.790	0.113	0.762	0.513	0.772

[a] Significant correlations are underlined.

df	alpha=0.05	alpha=0.01
7	0.666	0.798

Table B-8.

Pearson correlation matrix of carboxylic acid sole carbon source utilization (n=9). Statistically significant correlations (P<0.05) are underlined.

WELL	Acetic acid	α -Hydroxy-butyric acid	β -Hydroxy-butyric acid	γ -Hydroxy-butyric acid	cis-Aconitic acid	Citric acid	Formic acid	D-Galactonic acid lactone
p-Coumarate	0.292	0.247	0.590	-0.043	0.653	0.154	0.067	0.178
Ferulate	0.223	0.081	0.465	-0.111	0.554	-0.027	0.094	0.099
Isovanillate	0.113	0.360	0.749	0.070	0.680	0.345	-0.079	0.305
Phenol	0.191	0.339	0.697	-0.025	0.705	0.254	0.002	0.196
Salicylate	0.112	0.288	0.723	-0.004	0.707	0.232	-0.038	0.248
Toluate	0.637	0.783	0.682	0.540	0.783	0.930	-0.051	0.865
TCE	0.159	0.395	0.758	0.085	0.772	0.535	0.082	0.382
Depth	-0.878	-0.783	-0.675	-0.549	-0.857	-0.436	-0.502	-0.721
Temperature	-0.729	-0.727	-0.647	-0.473	-0.807	-0.511	-0.543	-0.607
pH	-0.855	-0.647	-0.837	-0.302	-0.726	-0.562	-0.201	-0.544
DO	0.702	0.705	0.758	0.546	0.721	0.510	0.584	0.523
Conductivity	-0.432	-0.286	-0.516	-0.324	-0.195	-0.341	-0.139	-0.374
Redox	0.743	0.721	0.662	0.401	0.743	0.516	0.454	0.536
AODC	0.247	0.058	-0.054	0.282	-0.083	-0.293	0.798	-0.067
Activity	0.684	0.514	0.665	0.584	0.616	0.362	0.857	0.489
1% PTYG	0.865	0.809	0.780	0.590	0.860	0.584	0.625	0.683
PTYG	0.867	0.788	0.761	0.630	0.844	0.588	0.627	0.718

	D-Galactaronic acid	D-Gluconic acid	D-Glucos-aminic acid	D-Glucuronic acid	p-Hydroxy-phenylacetic acid	D-Saccharic acid	Sebacic acid	Succinic acid
p-Coumarate	0.252	0.452	0.360	0.710	0.492	0.523	0.511	0.007
Ferulate	0.180	0.285	0.321	0.563	0.399	0.395	0.418	-0.064
Isovanillate	0.296	0.785	0.375	0.800	0.296	0.643	0.695	-0.003
Phenol	0.274	0.845	0.375	0.838	0.436	0.614	0.588	0.012
Salicylate	0.282	0.708	0.377	0.808	0.288	0.627	0.602	-0.038
Toluate	0.730	0.776	0.940	0.909	0.807	0.814	0.757	0.559
TCE	0.343	0.772	0.502	0.847	0.525	0.675	0.643	0.042
Depth	-0.868	-0.536	-0.797	-0.709	-0.878	-0.855	-0.861	-0.628
Temperature	-0.767	-0.494	-0.650	-0.805	-0.855	-0.788	-0.875	-0.465
pH	-0.593	-0.662	-0.621	-0.788	-0.815	-0.747	-0.702	-0.246
DO	0.671	0.595	0.442	0.807	0.655	0.722	0.761	0.519
Conductivity	-0.273	-0.261	-0.191	-0.287	-0.428	-0.301	-0.235	-0.077
Redox	0.671	0.510	0.558	0.814	0.799	0.750	0.772	0.339
AODC	0.224	-0.283	-0.282	0.022	-0.166	-0.046	0.096	0.287
Activity	0.624	0.444	0.388	0.611	0.529	0.584	0.630	0.613
1% PTYG	0.846	0.596	0.667	0.862	0.819	0.857	0.906	0.567
PTYG	0.854	0.567	0.698	0.788	0.815	0.828	0.886	0.617

	Itaconic acid	α -Keto-butyric acid	α -Keto-glutaric acid	α -Keto-valeric acid	D,L-Lactic acid	Malonic acid	Propionic acid	Quinic acid
p-Coumarate	0.408	0.288	0.239	0.335	0.082	0.322	0.282	0.333
Ferulate	0.265	0.197	0.163	0.212	0.023	0.286	0.207	0.192
Isovanillate	0.711	0.294	0.210	0.385	0.127	0.263	0.116	0.222
Phenol	0.607	0.280	0.192	0.379	0.085	0.287	0 173	0.281
Salicylate	0.666a	0.264	0.177	0.349	0.072	0.289	0.102	0.172
Toluate	0.870	0.875	0.778	0.933	0.538	0.915	0.792	0.691
TCE	0.730	0.513	0.729	0.803	0.158	0.777	0.130	0.729
Depth	-0.720	-0.833	-0.810	-0.814	-0.661	-0.774	-0.811	-0.647
Temperature	-0.617	-0.721	-0.729	-0.694	-0.614	-0.572	-0.747	-0.576
pH	-0.609	-0.617	-0.609	-0.629	-0.419	-0.522	-0.696	-0.542
DO	0.590	0.606	0.628	0.572	0.664	0.316	0.506	0.612
Conductivity	-0.090	-0.297	-0.421	-0.189	-0.350	-0.053	-0.425	-0.418
Redox	0.603	0.639	0.648	0.625	0.547	0.445	0.696	0.517
AODC	-0.201	-0.047	0.079	-0.198	0.335	-0.276	-0.116	-0.093
Activity	0.391	0.553	0.607	0.459	0.667	0.311	0.412	0.638
1% PTYG	0.715	0.780	0.787	0.739	0.725	0.575	0.736	0.613
PTYG	0.683	0.809	0.819	0.753	0.747	0.620	0.757	0.643

[a] Significant correlations are underlined.

df	alpha=0.05	alpha=0.01
7	0.666	0.798

Table B-9.

Correlation matrix of amino acid sole carbon source utilization (n=9). Statistically significant correlations (P<0.05) are underlined.

	L-Glutamic acid	Glycyl-L-Aspartic acid	Glycyl-L-Glutamic Acid	D- Alanine	L-Alanine	L-Alanyl-glycine	L-Asparagine	L-Aspartic acid	L-Histidine	L-Threonine
TCE	0.330[a]	0.723	0.728	0.413	0.362	0.224	0.273	0.250	0.198	0.748
Depth	-0.922	-0.730	-0.818	-0.846	-0.911	-0.849	-0.910	-0.929	-0.788	-0.775
Temperature	-0.851	-0.832	-0.803	-0.745	-0.820	-0.701	-0.805	-0.804	-0.668	-0.732
pH	-0.573	-0.663	-0.740	-0.712	-0.740	-0.585	-0.616	-0.599	-0.585	-0.822
DO	0.779	0.866	0.810	0.627	0.700	0.545	0.704	0.605	0.583	0.704
Conductivity	-0.146	-0.281	-0.284	-0.294	-0.361	-0.143	-0.204	-0.255	-0.208	-0.345
Redox	0.702	0.765	0.767	0.696	0.756	0.603	0.704	0.647	0.617	0.746
AODC	0.331	0.242	-0.045	-0.193	-0.001	-0.105	0.173	0.163	-0.129	-0.260
Activity	0.836	0.856	0.696	0.512	0.616	0.498	0.667	0.666	0.479	0.516
1% PTYG	0.909	0.875	0.869	0.780	0.854	0.731	0.860	0.842	0.713	0.756
PTYG	0.936	0.847	0.841	0.785	0.853	0.765	0.875	0.891	0.733	0.695

	Carnitine	γ-amino butyric acid	hydroxy-L-proline	L-Leucine	L-Ornithine	L-Phenyl-alanine	L-Proline	L-Pyro-glutamic acid	D-Serine	L-Serine
TCE	0.804	0.309	0.746	0.466	0.692	0.504	0.449	0.462	0.804	0.300
Depth	-0.640	-0.845	-0.807	-0.889	-0.746	-0.818	-0.984	-0.621	-0.546	-0.794
Temperature	-0.754	-0.748	-0.802	-0.842	-0.808	-0.735	-0.946	-0.528	-0.446	-0.629
pH	-0.611	-0.719	-0.638	-0.699	-0.769	-0.702	-0.756	-0.489	-0.474	-0.594
DO	0.763	0.624	0.787	0.820	0.919	0.693	0.774	0.554	0.489	0.483
Conductivity	-0.250	-0.337	-0.195	-0.235	-0.347	-0.245	-0.294	-0.272	0.056	-0.181
Redox	0.706	0.723	0.733	0.768	0.848	0.715	0.815	0.450	0.459	0.562
AODC	0.308	-0.126	0.267	0.038	0.161	-0.178	0.212	-0.174	-0.304	-0.226
Activity	0.724	0.468	0.713	0.760	0.718	0.525	0.798	0.577	0.263	0.387
1% PTYG	0.804	0.780	0.896	0.891	0.865	0.797	0.950	0.600	0.565	0.669
PTYG	0.752	0.772	0.861	0.892	0.773	0.782	0.955	0.636	0.526	0.690

[a] Significant correlations are underlined.

df	alpha=0.05	alpha=0.01
7	0.666	0.798

Table B-10.

Correlation matrix of phosphorylated compound sole carbon source utilization (n=9). Statistically significant correlations (P<0.05) are underlined.

	D,L-α-Glycerol phosphate	Glucose-1-phosphate	Glucose-6-phosphate
TCE	0.789[a]	0.898	0.953
Depth	-0.330	-0.500	-0.462
Temperature	-0.308	-0.557	-0.536
pH	-0.252	-0.483	-0.496
DO	0.171	0.636	0.651
Conductivity	0.064	-0.156	-0.041
Redox	0.203	0.553	0.556
AODC	0.099	0.191	0.049
Activity	0.229	0.510	0.484
1% PTYG	0.349	0.674	0.628
PTYG	0.330	0.611	0.550

[a] Significant correlations are underlined.

df	alpha=0.05	alpha=0.01
7	0.666	0.798

Table B-11.

Correlation matrix of ester sole carbon source utilization (n=9). Statistically significant correlations (P<0.05) are underlined.

	Methylpyruvate	Mono-methylsuccinate
TCE	-0.057	0.717
Depth	-0.507	-0.730
Temperature	-0.378	-0.732
pH	-0.342	-0.722
DO	0.399	0.737
Conductivity	-0.357	-0.321
Redox	0.316	0.739
AODC	-0.066	-0.184
Activity	0.448	0.549
1% PTYG	0.423	0.810
PTYG	0.476	0.781

[a] Significant correlations are underlined.

df	alpha=0.05	alpha=0.01
7	0.666	0.798

Table B-12.

Correlation matrix of brominated sole carbon source utilization (n=9). Statistically significant correlations (P<0.05) are underlined.

	Bromosuccinic acid	Succinic Acid
TCE	0.032	0.042
Depth	-0.786	-0.628
Temperature	-0.684	-0.465
pH	-0.435	-0.246
DO	0.651	0.519
Conductivity	-0.224	-0.077
Redox	0.561	0.339
AODC	0.381	0.287
Activity	0.718	0.613
1% PTYG	0.748	0.567
PTYG	0.785	0.617

[a] Significant correlations are underlined.

df	alpha=0.05	alpha=0.01
7	0.666	0.798

Table B-13.

Correlation matrix of amine sole carbon source utilization (n=9). Statistically significant correlations (P<0.05) are underlined.

	Phenylethylamine	Putrescine	2-Aminoethanol
TCE	0.952[a]	0.809	0.143
Depth	-0.424	-0.574	-0.698
Temperature	-0.529	-0.659	-0.463
pH	-0.659	-0.513	-0.491
DO	0.639	0.593	0.224
Conductivity	-0.211	-0.145	-0.107
Redox	0.617	0.571	0.374
AODC	-0.238	0.258	-0.334
Activity	0.400	0.617	0.182
1% PTYG	0.553	0.695	0.456
PTYG	0.445	0.664	0.489

[a] Significant correlations are underlined.

df	alpha=0.05	alpha=0.01
7	0.666	0.798

Table B-14.

Pearson correlation matrix of amide sole carbon source utilization (n=9). Statistically significant correlations (P<0.05) are underlined.

	Succinamic acid	Glucaronamide	Alaninamide
TCE	0.258	0.831	0.668
Depth	-0.802[a]	-0.750	-0.871
Temperature	-0.639	-0.747	-0.817
pH	-0.468	-0.665	-0.740
DO	0.567	0.793	0.761
Conductivity	-0.120	-0.187	-0.237
Redox	0.493	0.713	0.769
AODC	0.024	0.151	-0.040
Activity	0.638	0.686	0.631
1% PTYG	0.702	0.827	0.882
PTYG	0.759	0.767	0.858

[a] Significant correlations are underlined.

df	alpha=0.05	alpha=0.01
7	0.666	0.798

Table C-1

Relative proportion of isolates utilizing alcohols, amides, amines, and aromatics.

Carbon Source	MSB11B	MSB11C	MSB11D	P28A	P28TA	P28TB	P29C	P29TA	P29TC
Alcohols									
2,3-Butanediol	0.00	0.05	3.54	0.00	0.00	0.00	0.00	0.00	0.00
Glycerol	1.74	0.31	**37.31**	**43.26**	**100.00**	24.02	**40.26**	0.00	**35.00**
Amides									
Alaninamide	4.01	**9.85**	30.59	14.24	0.00	10.00	10.09	0.00	2.96
Glucuronamide	3.26	**9.85**	26.25	25.29	0.93	24.02	0.75	0.00	23.43
Succinamic acid	4.01	0.75	**37.31**	**43.26**	6.41	**46.76**	**18.52**	0.00	17.97
Amines									
2-Aminoethanol	0.15	0.11	7.18	6.28	0.00	4.99	10.09	0.00	14.24
Phenylethylamine	0.00	0.16	2.34	0.00	0.00	0.00	0.00	0.00	0.00
Putrescine	0.09	0.16	2.34	14.24	0.00	0.00	0.00	0.00	0.00
Aromatics									
Inosine	0.65	0.49	12.28	2.96	0.00	0.00	2.12	0.00	2.96
Thymidine	0.21	0.12	2.34	0.00	0.00	0.00	0.04	0.00	6.28
Uridine	0.04	0.35	3.73	0.00	0.00	10.59	0.04	0.00	0.00
Urocanic acid	1.74	0.46	**100.00**	**43.26**	10.95	**42.65**	**100.00**	0.00	**30.06**

Percentages for each groundwater sample were calculated by taking the density of sole carbon source utilization for that carbon source as a percentage of the acridine orange direct count density for that particular well. The highest percentage for each well was normalized by making it 100%. All other values for that well were then adjusted by the same factor.

Outlined percentages represent the ten highest densities for that sample (if there were several values at the density of the tenth highest density then all of those carbon sources were included).

Table C-2.

Relative proportion of isolates utilizing brominated chemicals, esters, phosphorylated compounds, and polymers.

Carbon Source	MSB11B	MSB11C	MSB11D	P28A	P28TA	P28TB	P29C	P29TA	P29TC
Brominated chemicals									
Bromosuccinic acid	**40.26**	0.27	**52.93**	25.29	2.85	**42.65**	10.09	0.00	**32.91**
Esters									
Methylpyruvate	10.09	0.49	**81.34**	**49.94**	**63.28**	**74.51**	**31.57**	**91.34**	**100.00**
Mono-methylsuccinate	4.01	**9.85**	**37.31**	29.83	5.15	30.29	10.09	**100.00**	6.28
Phosphoralated Compounds									
D,L-alpha Glycerol phosphate	0.00	0.16	0.00	19.24	0.00	0.00	0.00	0.00	2.96
Glucose-1-phosphate	0.15	0.16	0.00	2.96	0.00	0.00	0.00	0.00	0.00
Glucose-6-phosphate	0.09	0.24	1.10	2.96	0.00	4.99	0.00	0.00	0.00
Polymer									
alpha-Cyclodextrin	6.38	0.36	12.28	5.93	0.00	0.00	0.00	0.00	2.96
Dextrin	0.00	0.25	14.38	10.00	1.33	10.59	0.45	**58.48**	19.24
Glycogen	4.01	7.85	26.25	10.00	0.93	32.45	0.35	19.08	2.96
Tween40	**18.52**	0.57	**52.93**	29.83	**100.00**	**65.00**	12.00	8.99	**43.26**
Tween80	**31.57**	**45.74**	**52.93**	**59.30**	**14.74**	32.45	**40.26**	**58.48**	**70.35**

Percentages for each groundwater sample were calculated by taking the density of sole carbon source utilization for that carbon source as a percentage of the acridine orange direct count density for that particular well. The highest percentage for each well was normalized by making it 100%. All other values for that well were then adjusted by the same factor.

Outlined percentages represent the ten highest densities for that sample (if there were several values at the density of the tenth highest density then all of those carbon sources were included).

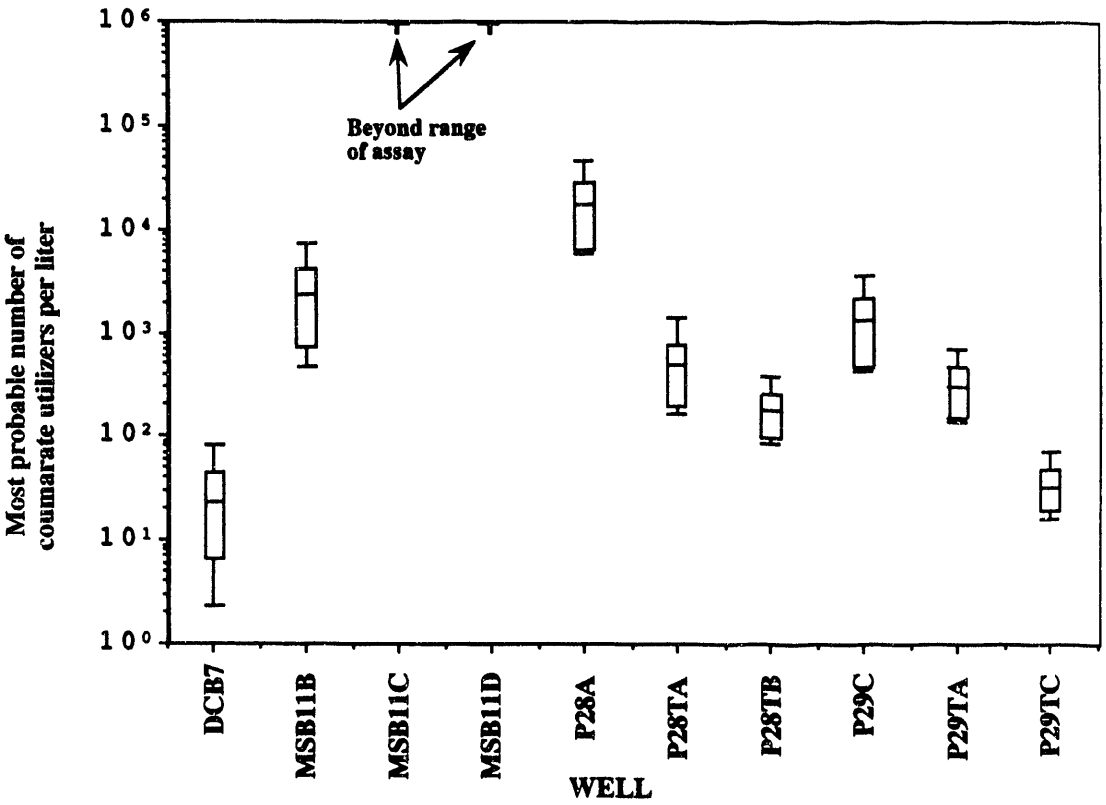
Table C-3.

Number of different compounds utilized in each well.

	Alcohols	Amides	Amines	Amino Acids	Aromatics	Brominated Chemicals	Carbo-hydrates	Carboxylic Acids	Esters	Phosphorylated Compounds	Polymers
MSB11B	1	3	2	20	4	1	27	24	2	2	4
MSB11C	2	3	3	20	4	1	28	24	2	3	5
MSB11D	2	3	3	19	4	1	26	24	2	1	5
P28A	1	3	2	16	2	1	23	23	2	3	5
P28TA	1	2	0	7	1	1	10	13	2	0	4
P28TB	1	3	1	16	2	1	21	20	2	1	4
P29C	1	3	1	19	4	1	20	24	2	0	4
P29TA	0	0	1	1	0	0	19	16	2	0	4
P29TC	1	3	1	14	3	1	15	20	2	1	5
Tested	2	3	3	20	4	1	28	24	2	3	5

Table D-1

Utilization of p-coumarate by ground water bacteria.



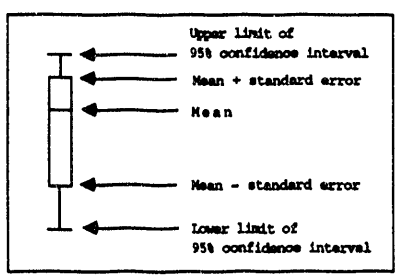
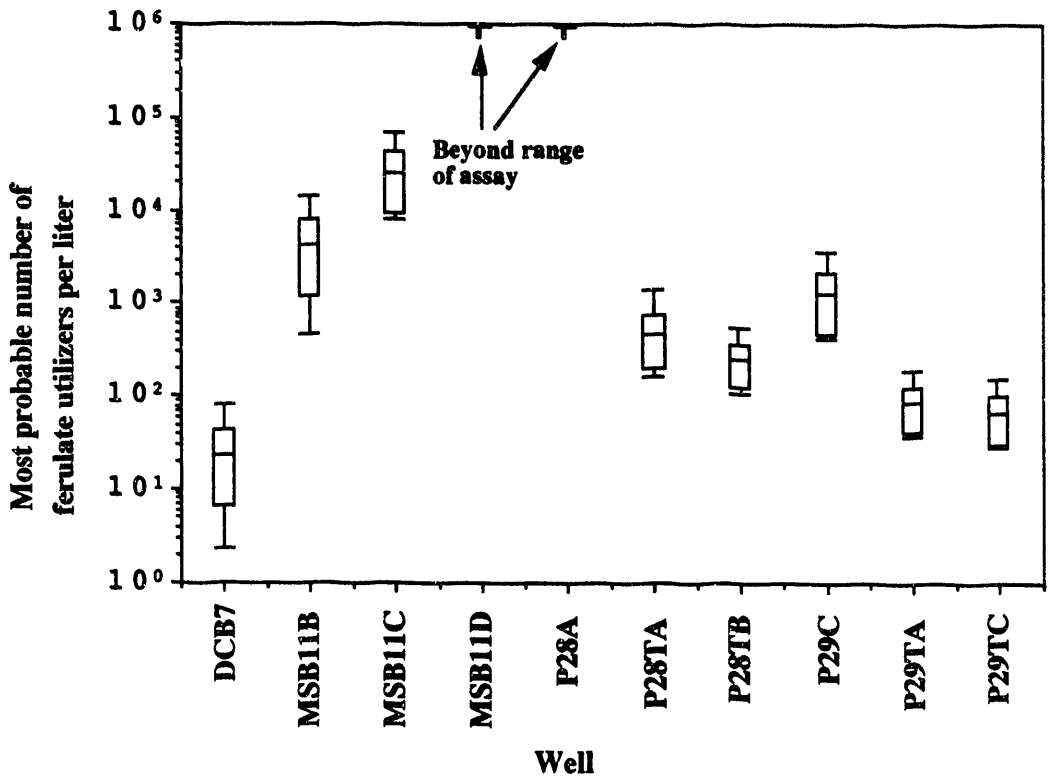


Table D-2

Utilization of ferulate by ground water bacteria.



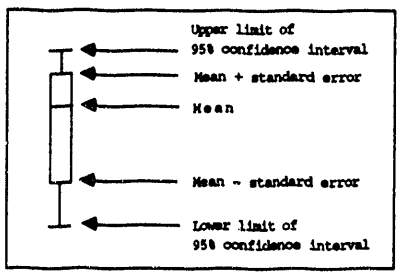
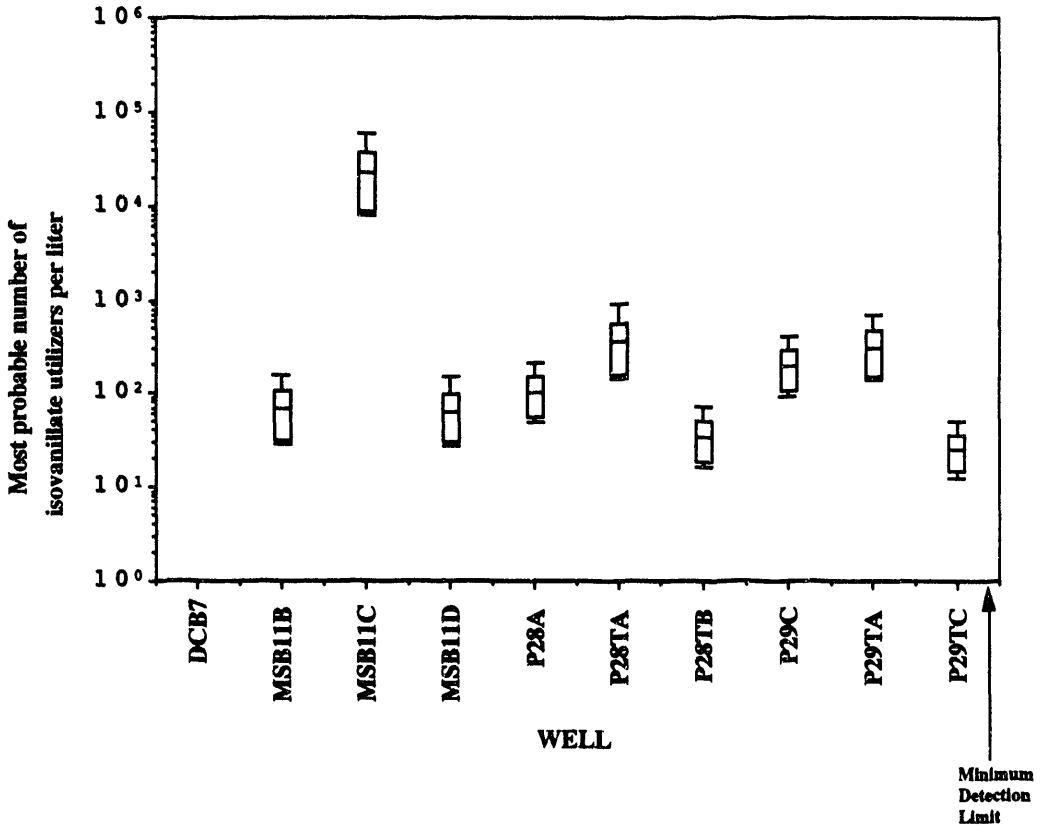


Table D-3

Utilization of isovanillate by ground water bacteria.



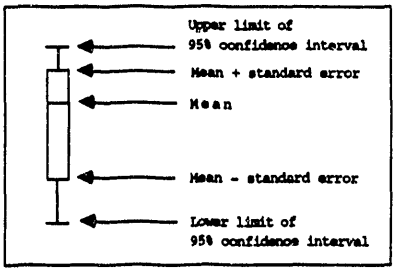
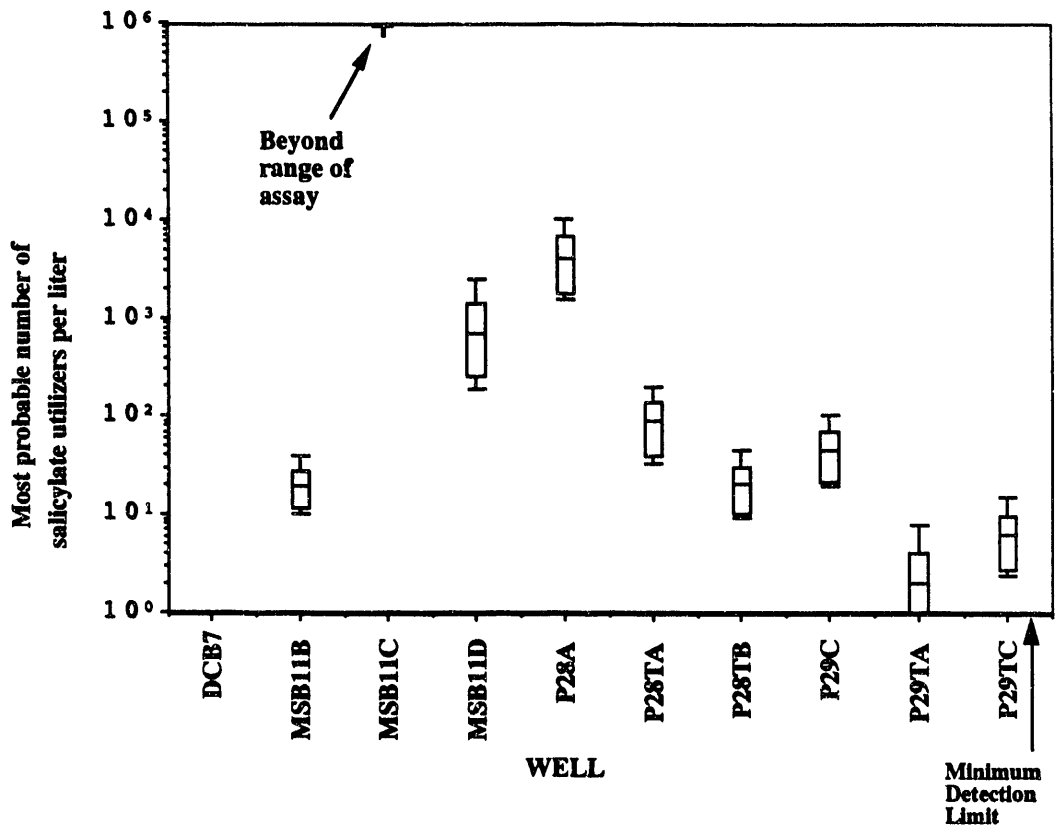


Table D-4

Utilization of salicylate by ground water bacteria.



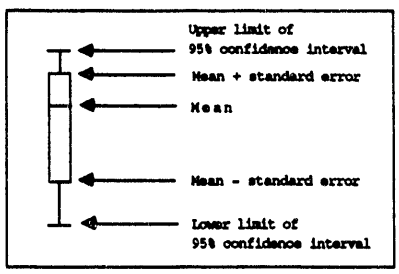
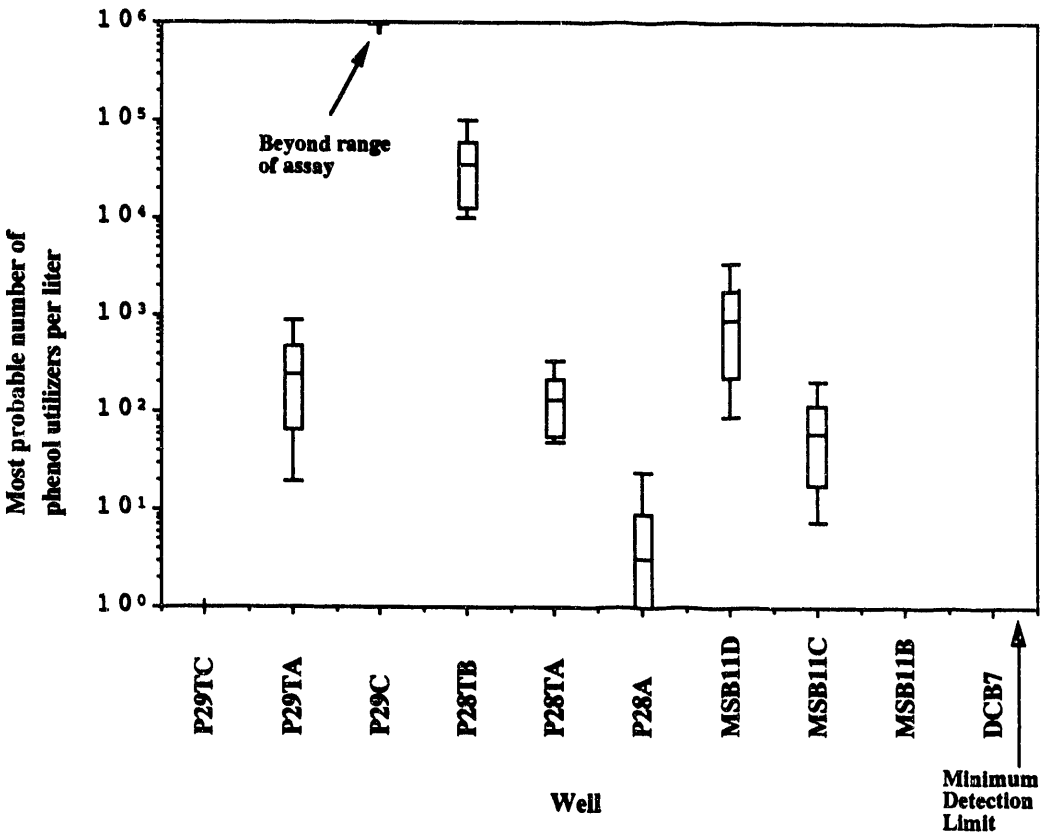


Table D-5

Utilization of phenol by ground water bacteria.



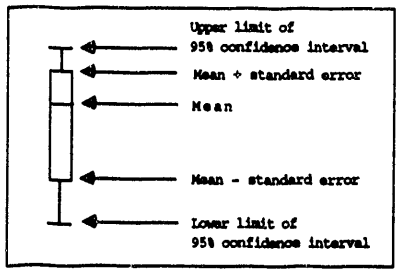
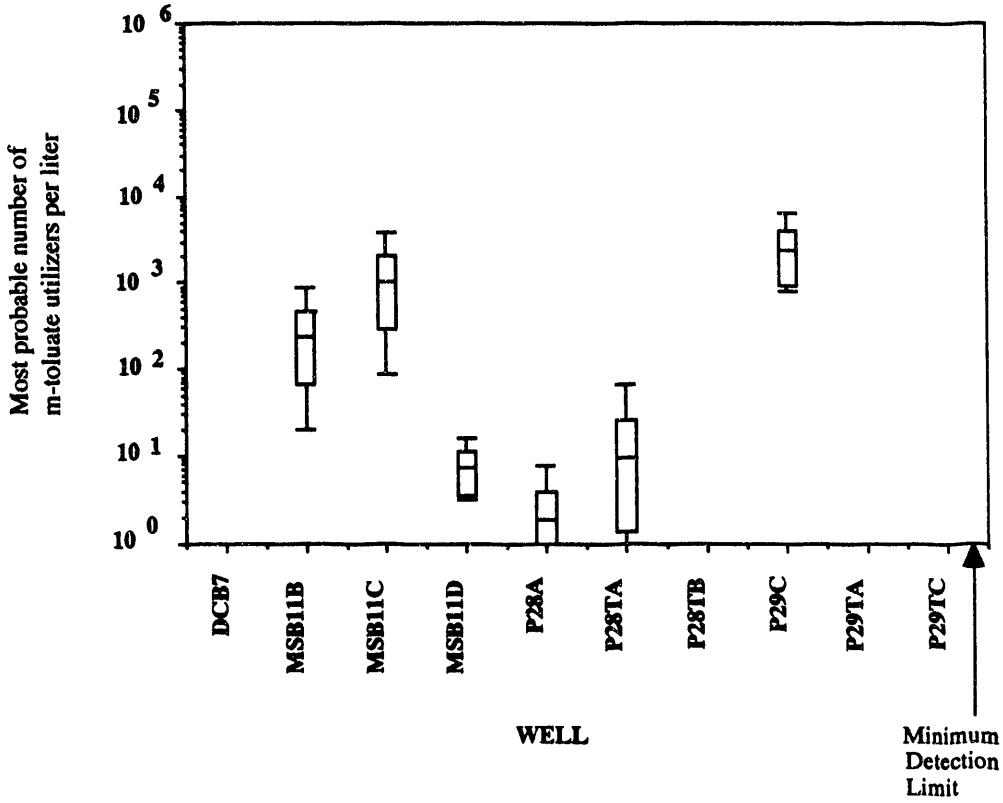


Table D-6.

Utilization of m-toluate by ground water bacteria.



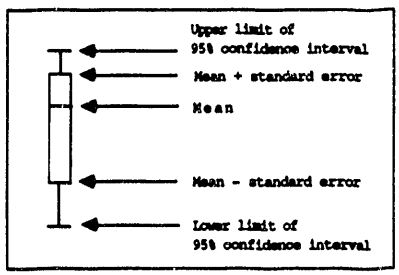


Table E.

Modification of Stanier's minimal media (Stanier et al., 1966) used for the determination of aromatic utilization by ground water bacteria in this study.

Minimal media:	
Ammonium sulfate [$(NH_4)_2SO_4$]	1.0 gm
Distilled water	bring to 930 ml
	autoclave
1 M Na_2HPO_4+KH_2PO_4 buffer pH 6.8 (filter sterilized)	40 ml
Mineral base (filter sterilized)	20 ml
Carbon source 1 g/100 ml H_2O (filter sterilized)	10 ml

Mineral base:	
1.0 M KH_2PO_4 pH 6.8	20 ml
Concentrated base	20 ml
Distilled water	to 1000 ml
	filter sterilize

Concentrated base:	
Nitrilotriacetic acid [$N(CH_2COOH)_3$]	10.0 g
Magnesium sulfate [$MgSO_4$]	14.45 g
Calcium chloride [$CaCl_2 \cdot 2H_2O$]	3.335 g
Ammonium molybdate [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$]	9.25 mg
Ferrous sulfate [$FeSO_4 \cdot 7H_2O$]	99.0 mg
Nicotinic acid (niacin) [C_5H_4NCOOH]	50.0 mg
Thiamin·HCL [$C_{12}H_{17}ClN_4OS \cdot HCL$]	25.0 mg
Biotin [$C_{10}H_{16}N_2O_3S$]	0.5 mg
Metals (see below)	50.0 ml
Distilled water	to 1000 ml
	filter sterilize

Metals:	
Zinc Sulfate [$ZnSO_4 \cdot 7H_2O$]	1095 mg
Ferrous sulfate [$FeSO_4 \cdot 7H_2O$]	500.0 mg
Manganous sulfate [$MnSO_4 \cdot 4H_2O$]	154 mg
Copper sulfate [$CuSO_4 \cdot 5H_2O$]	39.2 mg
Cobaltous nitrate [$Co(NO_3)_2 \cdot 6H_2O$]	24.8 mg
Sodium borate [$Na_2B_4O_7 \cdot 10H_2O$]	17.7 mg
Sulfuric acid [H_2SO_4]	a few drops to retard precipitation
Distilled water	to 100 ml

END

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