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IN SITU BIOREMEDIATION OF SPILLS FROM UNDERGROUND STORAGE TANKS: NEW APPROACHES FOR SITE CHARACTERIZATION PROJECT DESIGN, AND EVALUATION OF PERFORMANCE

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FOREWORD

EPA is charged by Congress to protect the Nation's land, air and water systems. Under a mandate of national environmental laws focused on air and water quality, solid waste management and the control of toxic substances, pesticides, noise and radiation, the Agency strives to formulate and implement actions which lead to a compatible balance between human activities and the ability of natural systems to support and nurture life.

The Robert S. Kerr Environmental Research Laboratory is the Agency's center of expertise for investigation of the soil and subsurface environment. Personnel at the Laboratory are responsible for management of research programs to: (a) determine the fate, transport and transformation rates of pollutants in the soil, the unsaturated and the saturated zones of the subsurface environment; (b) define the processes to be used in characterizing the soil and subsurface environment as a receptor of pollutants; (c) develop techniques for predicting the effect of pollutants on ground water, soil, and indigenous organisms; and (d) define and demonstrate the applicability and limitations of using natural processes, indigenous to the soil and subsurface environment, for the protection of this resource.

This report presents a systematic approach for the design of in situ bioremediation of hydrocarbon contamination in ground water from the determination of the total quantity of hydrocarbons in the aquifer to the utilization of that information in an actual field bioremediation demonstration.

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TABLE OF CONTENTS

INTRODUCTION

This report presents a systematic approach for the design of in situ bioremediation of hydrocarbon contamination in ground water from the determination of the total quantity of hydrocarbons in the aquifer to the utilization of that information in an actual field bioremediation demonstration.

Bioremediation of ground water contaminated with hydrocarbons such as gasoline is an on-site treatment technology that is both potentially technically feasible and more cost-effective than "pump and treat" technologies which involve pumping of contaminated ground water to the surface and removal of the contaminant by air-stripping or carbon adsorption. In situ bioremediation usually consists of modifying the environment of an aquifer by the addition of oxygen and other inorganic nutrients in order to enhance the activity of native microbial populations in degrading contaminants. Bioremediation is especially promising with hydrocarbons which are potentially biodegradable by native subsurface bacteria under the right environmental conditions to harmless byproducts.

Successful bioremediation is dependent upon a number of factors, including the hydrogeology at the site and the availability of critical nutrients in the aquifer. The primary limiting factor with hydrocarbons is the availability of oxygen. If sufficient oxygen is not present naturally, then oxygen must be provided by circulating oxygenated water through the contaminated area until degradation is complete.

The primary factor which determines how much oxygen and nutrients must be supplied to a hydrocarbon leak and how long remediation will take is the quantity of the hydrocarbon at the site. Normally, the amount of the leak is not known and available methods to determine the amount of

contaminant at the site and it's location are not acceptable.

Almost all techniques that have been applied for the analysis of oily contaminants in aquifers emphasize the compounds of regulatory interest, and few are appropriate for both solids and water. All too frequently, the only information available from a leak site is the concentration of selected organic contaminants in water from wells. Such information is inadequate for determining the total quantity of hydrocarbons in the aquifer. Therefore, it is impossible to determine how much oxygen and nutrients must be delivered to the aquifer to support sufficient microbial activity to degrade all of the contaminant to harmless byproducts.

This report explains why the total quantity of hydrocarbons in an aquifer can only be determined by collecting cores. A procedure to acquire cores from a contaminated aquifer is described. Before the procedure was developed, it was very difficult to recover good-quality cores of unconsolidated sandy material from below the water table. The report also describes two procedures to determine how much contamination the cores contain. Results of the two procedures are in good agreement, even though they are based on different principles.

The two techniques were developed and evaluated by scientists at the Robert S. Kerr Environmental Research Laboratory as part of a large bioremediation research program. An oil-and-grease method was adapted to estimate total hydrocarbons in core samples. A second method was adapted from techniques for the analysis of fuels that determines the total content of hydrocarbons as well as the specific content of individual compounds of interest.

Basically, the oil-and-grease method uses infrared spectroscopy to measure the absorbance of carbon-hydrogen chemical bonds. Quantitation is sensitive to the type of hydrocarbon but is relatively insensitive to the particular organic constituents of the fuel. In the fuel carbon technique the hydrocarbons are extracted into methylene chloride, then separated and quantified by gas chromatography. Representative peaks are selected, and the quantity of total hydrocarbons is calculated by comparing the area of the representative peaks in a standard sample of the fuel to the area of the same peaks in the extract. The method works well if the standard is representative of the material being analyzed. If the proper calibrations are done, the concentrations of compounds of regulatory interest, such as the alkylbenzenes, can be determined in the same analytical run. The techniques for core analysis and their performance is discussed in Section III of this report.

The procedures described in the report were field-tested in designing a demonstration of the bioremediation of an aviation gasoline leak. The performance of the demonstration was consistent with the expected performance based on the preliminary site characterization using the described procedures.

SECTION I. SITE CHARACTERIZATION FOR IN SITU BIOREMEDIATION OF HYDROCARBON LEAKS FROM UNDERGROUND STORAGE TANKS

Underground storage tanks have been installed in almost every possible geological lithology; however, many of the known leaks from underground storage tanks occur in unconsolidated material.

There are several reasons for this. Many of our inland cities are built on floodplains or river terraces because they are flat and near water. Major portions of our coastal cities are built on old beaches or glacial outwash. Because these materials are transmissive, releases from underground storage tanks drain readily into the water table. Ground-water flow in these areas is usually rapid, and plumes of contamination can spread over wide areas in a short period of time. Unconfined aquifers in sandy unconsolidated materials are commonly used for domestic water supply. When there is a high density of wells, detecting a release is much more likely.

The pattern of contamination from a leak is complex (Figure 1). As the release drains through the unsaturated zone, a portion is left behind trapped by capillary forces. If the released material is volatile, a plume of vapors soon forms in the soil air in the vadose zone. If the release is a light hydrocarbon, it will drain down to the water table, and then spread laterally. Ground water moving through the aquifer comes in contact with the release, and leaches out the more water-soluble components. As a result there are three distinct regions or "plumes" formed at the leak site: a plume of volatile fumes in the soil air, a ground-water plume, and the region primarily in the unsaturated zone that contains the oily-phase material which serves as a source area for both plumes.

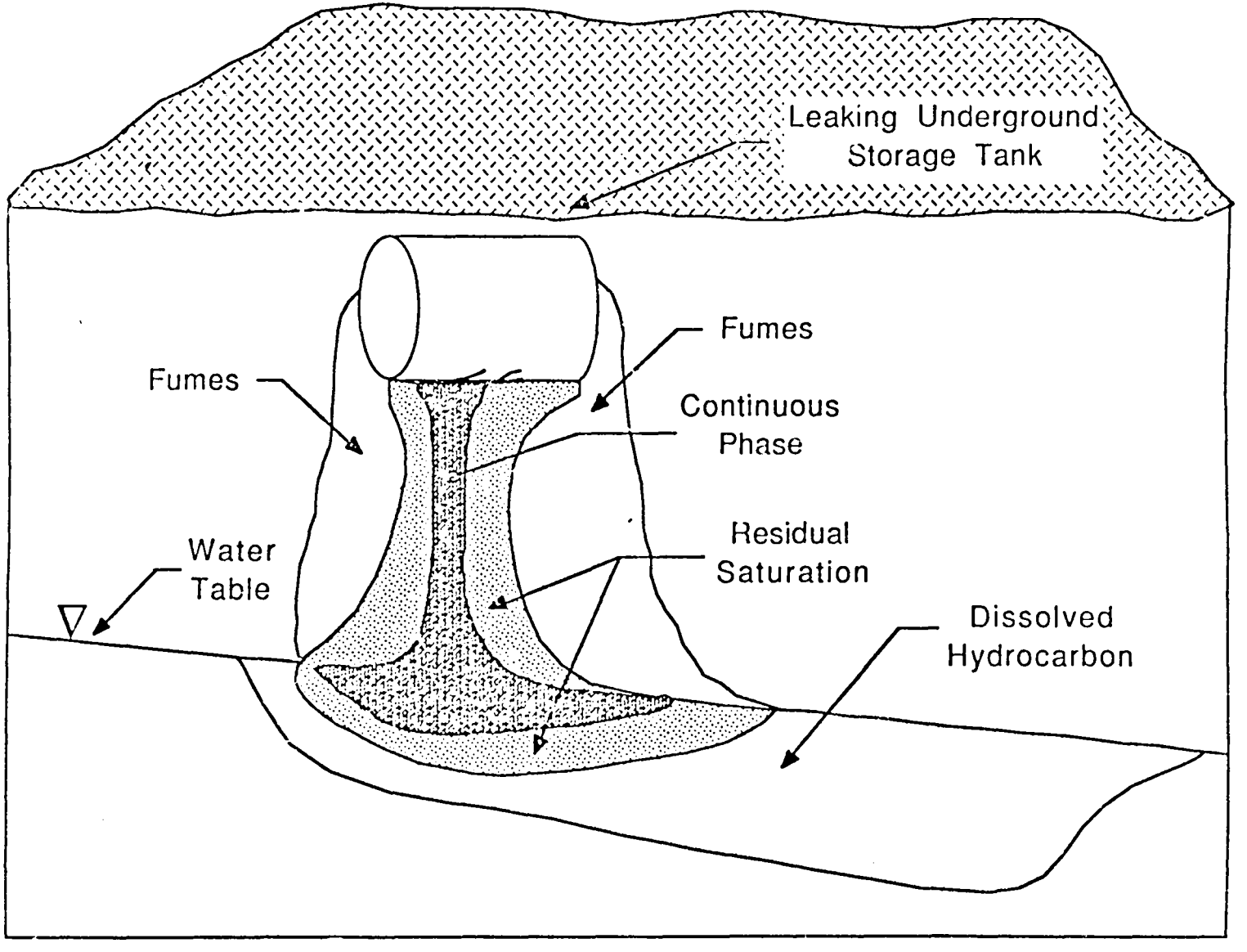


Figure 1. Regions of Contamination in a Typical Release from an Underground Storage Tank.

In practice the source area is usually the object of remedial activities. There is little point in treating the ground water or vapors if the source area is left to spread more contamination. Therefore, the first step is to remove any leaking tanks, transmission pipes, and the most visibly contaminated fill-material around the tank. Although necessary, such practices usually do not remove all of the source. The material trapped in the earth solids beneath the tank will remain and will serve as a continuous source of leaching contaminants for many years.

To intelligently remediate such a site using in situ bioremediation requires a detailed understanding of the three-dimensional distribution of the source area in the subsurface and good information on the quantity of contaminant in the source area.

Unless we know how much contaminant has escaped into the subsurface, and where it is located, there is no sensible way to locate injection and extraction wells, or to optimize pumping rates and concentrations of any amendments. Further, there is no way to determine how much time a remedial action will take, or how much it will cost.

Conventional monitoring wells can accurately define the geometry of the ground-water plume, but often they cannot distinguish the source area from the rest of the plume. In fresh spills, differential sorption of individual components of the plume to the aquifer solids can result in chromatographic separation of the components and alter the ratio of their concentrations in water from wells distant from the source area. However, in older spills, whose plumes have come to sorptive equilibrium with the aquifer, the concentration of contaminants dissolved in the ground water is similar in the source area and in the plume, although the total amount of contaminant in the source area is much greater.

For example, Section IV of this report demonstrates how comparisons of ground water analyses vs. core analyses at an aviation gasoline spill site in Michigan showed that the ground water analyses underestimated the amount of toluene in the aquifer significantly. Further analysis showed that the core contained petroleum hydrocarbons that sorbed most of the toluene. If the data from the monitoring well had been used to design a remedy, the effort and expense required to restore the aquifer would have been underestimated by a factor of six.

Obviously, the distribution of the source area and the extent of contamination can only be characterized by collecting and analyzing cores, because they sample the entire aquifer, not just the ground water. Very precise information is needed on the vertical extent of contamination, particularly for in situ biorestoration. The injected waters are very expensive, and water injected into a clean part of the aquifer is wasted (Figure 2). If injected water moves underneath the contaminated interval and breaks through in a monitoring well, it can also give the false impression that the region of aquifer between the two wells is clean.

Accurate techniques for analyzing cores to determine the total quantity of petroleum hydrocarbons in the aquifer and the concentration of individual compounds of regulatory concern are necessary not only for estimating the ultimate demand for oxygen, but also for documenting at the end of the remediation that the clean-up is complete.

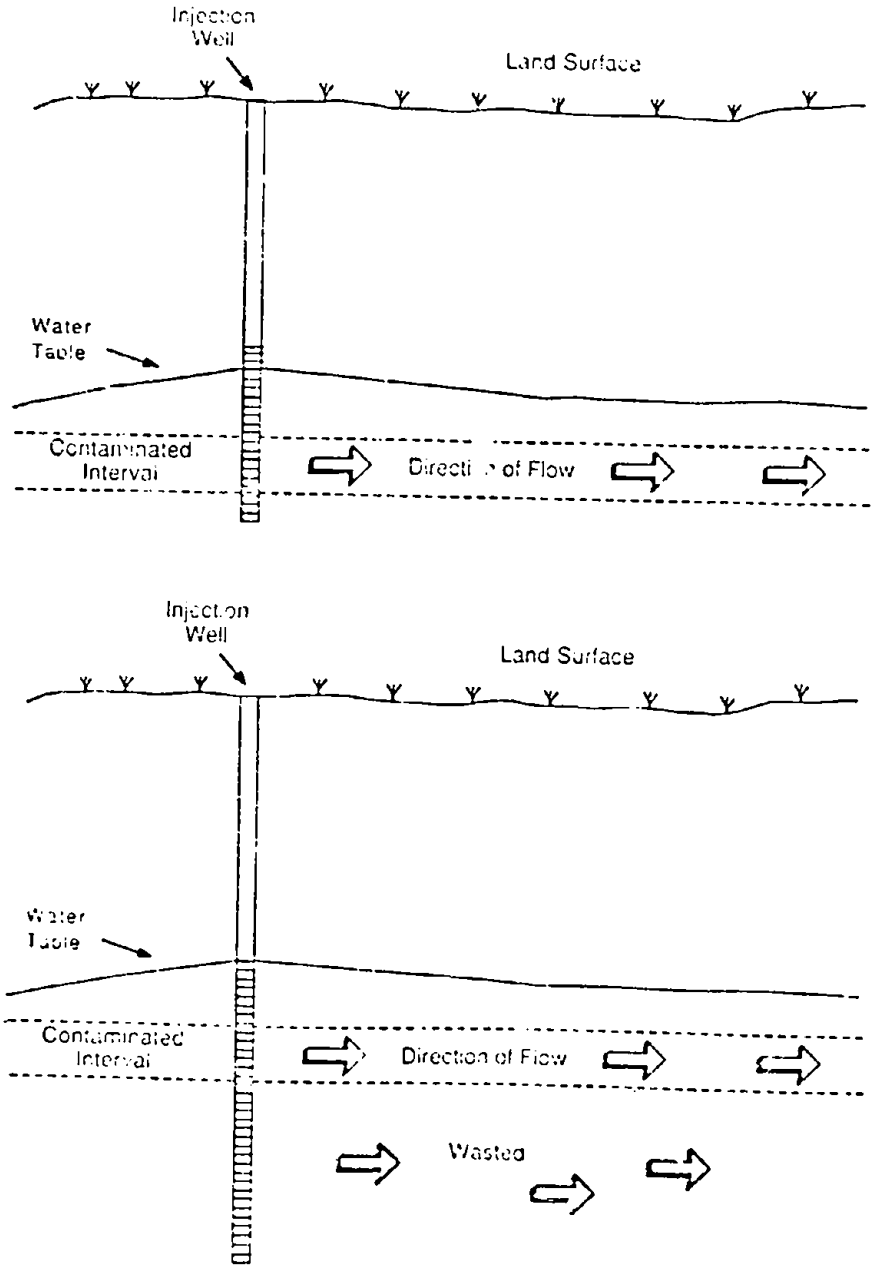


Figure 2. The Value of Accurately Locating the Contaminated Interval.

SECTION II. PROCEDURE FOR ACQUIRING CORE SAMPLES

PROBLEMS WITH UNCONSOLIDATED SEDIMENTS

Traditionally, unconsolidated soils or sediments are sampled through a hollow-stem auger with a split-spoon core barrel or a conventional thin-walled sample tube (Figure 3). The hollow-stem auger acts as a temporary casing to keep the borehole open until a sample can be acquired. A borehole is drilled down to the depth to be sampled. Then the core barrel is inserted through the annular opening in the auger and driven or pushed while rotating the auger into the earth to collect the sample. These tools work extremely well in both unsaturated and saturated cohesive materials. Unfortunately, they work poorly in noncohesive aquifer materials, such as unconsolidated sands.

There are two technical challenges to sampling noncohesive material below the water table. The first challenge is to keep aquifer material out of the annular area of the hollow stem auger. During augering, the annular area of the hollow-stem auger is plugged with a solid drill head that pushes the sand out onto the auger flights. To sample, the drill head is removed and replaced with a core barrel. When the drill head is pulled out of the auger in consolidated sands, pressure on the aquifer sediment is reduced, and water and fluidized sand rush into the annular area of the auger. This inconvenient phenomenon is commonly referred to as "heaving." The core barrel must push through (and sample) this heaved material inside the auger before it reaches the undisturbed sediment underneath. When the core is recovered, it is usually impossible to determine how much of the core is the fluidized material and how much is an authentic sample of the aquifer. Occasionally the amount of sediment in the auger is so great that the core barrel cannot be pushed, and no sample can be acquired.

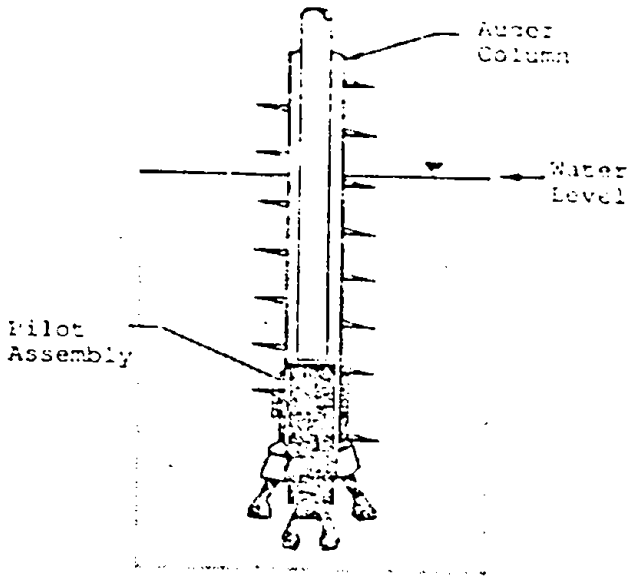


Figure 3. Hollow-stem auger containing a pilot assembly.

The second challenge is to keep the sample in the core barrel while it is being retrieved to the surface. When the sampling tool is pulled out of the aquifer, the pressure holding the sample in the tool is reduced. Noncohesive sediment will often fluidize and dribble out of conventional core barrels.

SPECIAL PISTON SAMPLING

Conventional practice to keep sediments out of the hollow-stem of an auger is to fill the hollow annular column with drilling mud. As the borehole is advanced, the weight of the mud stabilizes the hydraulic pressure of the aquifer. The use of drilling mud is not acceptable in geochemical assessments because fluids or chemicals introduced into the borehole can drain into the aquifer and alter the geochemistry of the pore water or contaminate the sample with foreign microorganisms. Such compromised samples cannot be used to assess prospects for bioremediation, and there is a strong possibility of microbial alteration of the sample during shipment or storage.

The staff of RSKERL have developed and tested new tools and protocols that consistently provide samples of the quality needed to characterize spills from underground storage tanks (Leach et al., 1988). The tools and protocols are modifications of techniques pioneered by others, principally researchers at the Institute for Ground Water Research, University of Waterloo, Ontario, Canada (Zapico et al., 1987).

Instead of drilling mud, the RSKERL protocol protects the annular opening of the auger with a hinged cap (commonly called a clam-shell) that folds down and covers the open face of the auger (Figure 4). When the auger has been advanced to the desired depth, the sampling tool is inserted into the hollow auger supported by the attached drill rods until it makes contact with the clam-shell. As the sampler is lowered, a wireline cable attached to

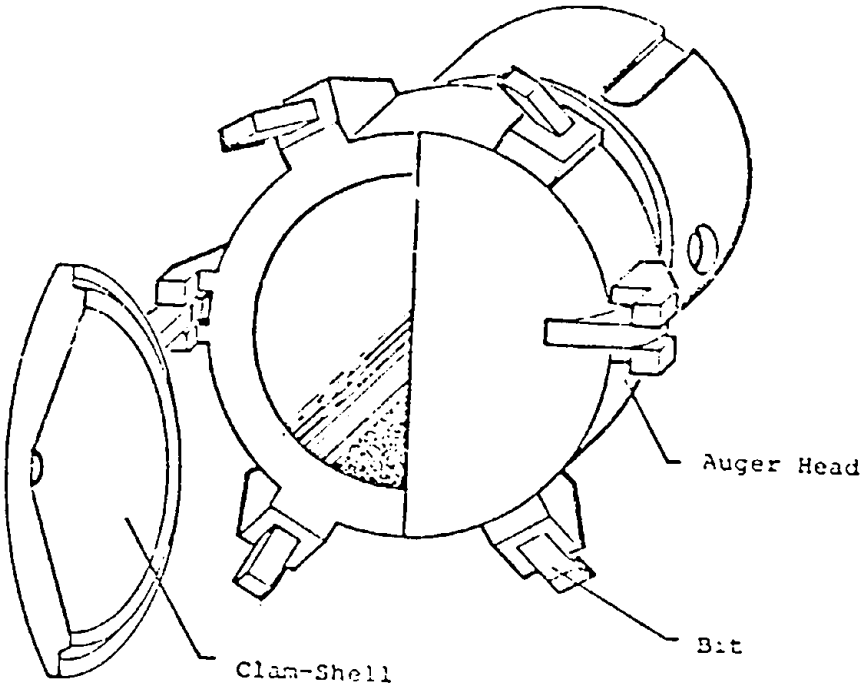


Figure 4. Clam-Shell Fitted Auger Head.

an internal piston in the sampling tool is kept slack so the piston will remain in its starting position. The augers are then lifted vertically with a separate wireline about 25 cm to open the clam-shell doors, allowing the sampler to fall into contact with the sediment to be sampled before heaving can occur. The augers are held in place with an auger fork to keep them from slipping back down the borehole and binding the sampler.

It is not presently possible to close the clam-shell doors once they have been opened in the subsurface; therefore, if deeper samples are desired, the entire flight of augers is carefully removed from the borehole. If the augers are rotated after the clam-shell is opened, the device will be destroyed. After retrieval, the augers and the clam-shell are thoroughly cleaned before reuse. The borehole can be backfilled to the surface with cuttings or clean sand and then redrilled to the next desired sampling depth. In some situations it is better to move the drilling rig a few feet and start a new borehole. This process is slower than conventional sampling, however, it is necessary to remove the augers in order to clean all heave material from the interior of the augers, properly close the clam-shell doors, and backfill the borehole. If the borehole is not backfilled, and a deeper sample is attempted in the same borehole, the clam-shell will open prematurely during augering and be destroyed.

Zapico et al. (1987) recently described a sampling device that effectively retains unconsolidated sands inside a cannister fitted inside a core barrel. A sliding piston inside the cannister maintains an air-tight seal on the core. Vacuum and friction keep the core in place. This device was modified to meet the special requirements of the RSKERL protocol (Figure 5).

The piston contains a series of neoprene seals which are mechanically compressed, creating a positive seal of the piston inside a standard thin

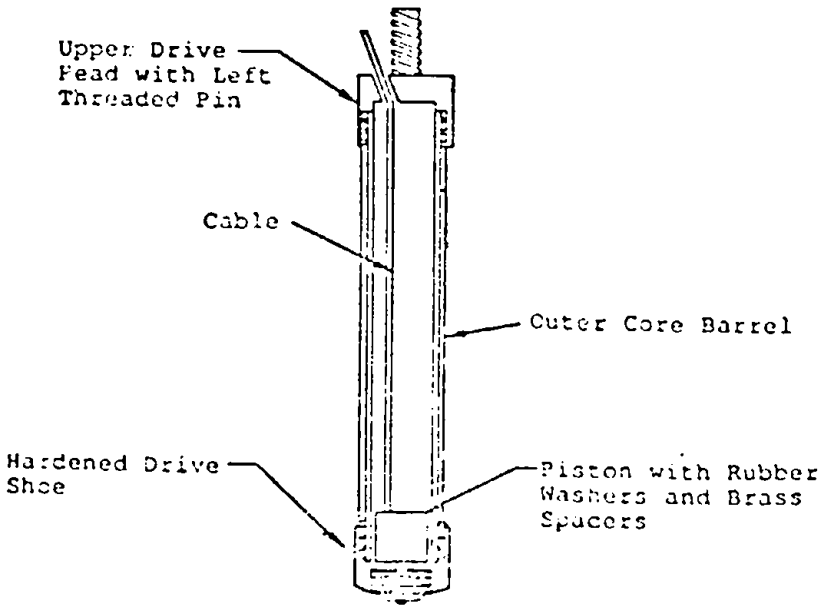


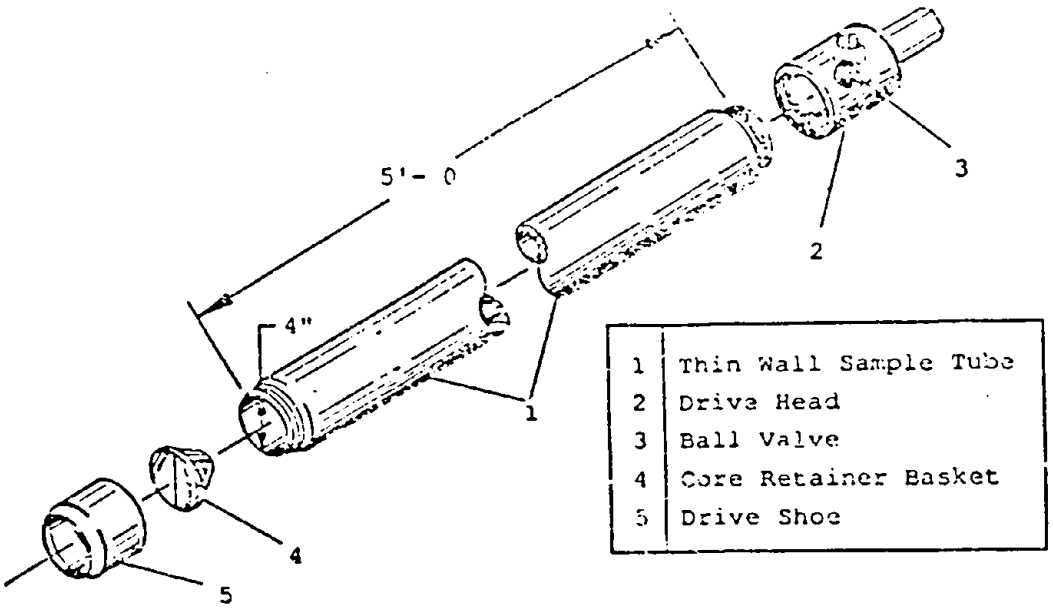
Figure 5. Waterloo Aquifer Piston Core Barrel-Schematic (Zapico, 1987).

walled core barrel (Figure 6). The wireline attached piston is positioned at the end of the core barrel that will be in contact with the sediment. The wireline is pulled taut after the piston equipped core barrel has been lowered to the bottom of the borehole. The cable holds the internal piston stationary while the core barrel is driven into the sediment, creating a vacuum on the sample.

The core barrel is driven by reciprocal percussion. A trip hammer mounted on the drill rig strikes a heavy steel rod that extends from the top of the core barrel to the surface. This rod is installed in sections as the augers are drilled into the subsurface. Driving by percussion is preferable to pushing the core barrel with a hydraulic ram. Percussion uses the inertia of the sample to force it into the core barrel while a hydraulic ram forces the sample into the tube against its natural mechanical resistance. A core barrel driven by a ram tends to push unconsolidated materials out of the way instead of into the barrel.

The conventional tool for retaining cores in a barrel is a core retainer basket. This device consists of a series of flexible steel tabs that fold flat against the core barrel while the barrel accepts the sample, then fold out and intercept the core if it starts to slip out during retrieval.

During field evaluation on the difficult, unconsolidated, sandy material at Traverse City (SECTION IV), the piston core barrel worked very well, but only when a core retainer basket was used. The piston core sampler without a core retainer basket often lost half or more of the sample before it could be recovered. A conventional core barrel with a core retainer basket recovered no sample at all. The combination of the two consistently recovered more than 95% of the cored interval (12 boreholes, more than 50 cores).



CME Standard Thin Wall Sample Tube

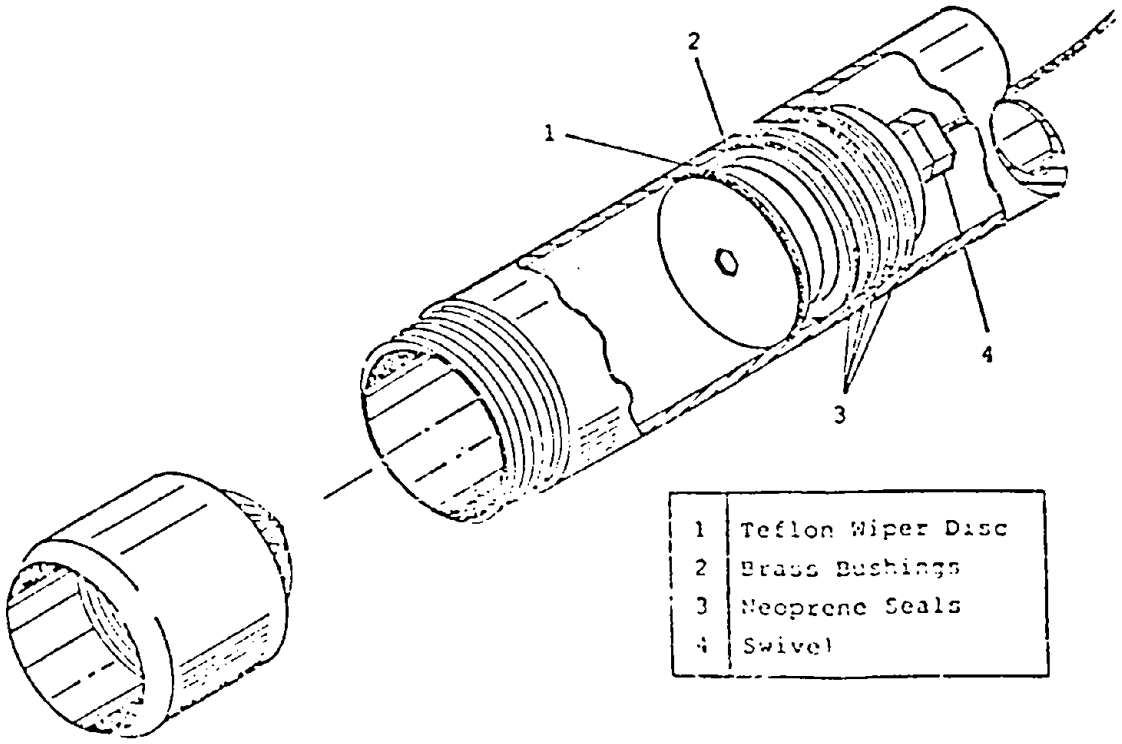


Figure 6. Modified Wireline Piston Design.

If the piston moves while the sample is being recovered, there is a significant chance of pulling air or water through the sample and spoiling it. All samples are retrieved using the center rod; no tension is placed on the wireline to the piston during retrieval.

After the piston core barrel is brought to the surface, the end of the sampler is quickly covered with a plastic bag and tightly sealed to minimize aeration of the exposed core. The sampler is then quickly disassembled by removing the drive cap and manually pulling the piston free from the top of the sample tube. Then one end of the core barrel is connected to a hydraulic ram mounted on the rig, and the core is extruded. The cores are collected in wide-mouth canning jars. If possible, each jar is entirely filled with sample. The seal on the lid of the canning jar effectively excludes oxygen and prevents loss of volatiles.

FIELD GLOVE BOX SAMPLING

If the cores are to be used for treatability studies to evaluate the prospects for bioremediation, they must be protected from contamination by foreign microorganisms. If naturally-occurring microbial processes are to be evaluated, they must also be protected from the atmosphere because many anaerobic microorganisms are killed by oxygen.

To protect from foreign microorganisms, a core is collected by extruding a small portion of the core, breaking off a small section to reveal an uncontaminated face, then installing a sterile paring device onto the end of the sample tube. This tool peels away the outer contaminated wall of the core as the material is extruded (Figure 7).

To protect the sample from the atmosphere, the sample is extruded inside a nitrogen-filled glove box (Figure 8). The core barrel is introduced into the glove box through an iris port that makes a tight seal around

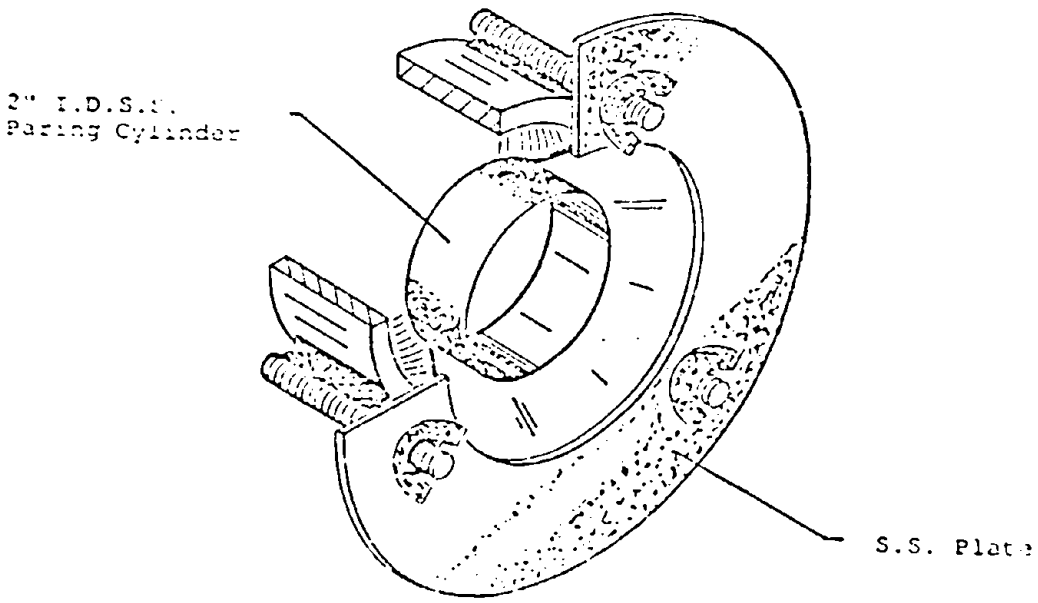


Figure 7. Core Paring Tool.

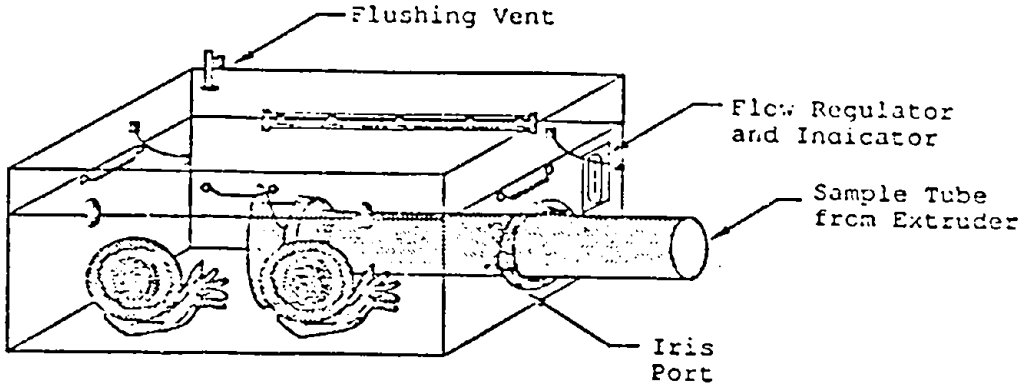


Figure 8. Field Sampling Glove Box.

the barrel. The dimensions of the box are 60 X 90 X 120 cm. The box is flushed with 1200 liters of nitrogen over a thirty minute period. Quality assurance tests were conducted by analyzing a series of 1.0 ml samples of the gas vented from the box with a Varian Model 90-P gas chromatograph equipped with a thermal conductivity detector. The concentration of oxygen fell below 0.02%.

The glove box is prepared for sample collection by filling it with the desired number of sterile canning jars and sterile paring devices, sealing the box, and then purging it with nitrogen gas. To prevent oxygen contamination when the jars are opened to receive the core in the field glove box, the jars are filled with nitrogen before they are brought to the field. They are passed into a laboratory anaerobic glove box, opened, then sealed air-tight. A slight positive pressure of nitrogen is maintained in the box during extrusion and collection of the cores.

SECTION III PROCEDURES TO DETERMINE THE CONCENTRATION OF CONTAMINANTS

OIL AND GREASE METHOD

EXTRACTION OF ANALYTICAL SAMPLE FROM A CORE FOR OIL-AND-GREASE ANALYSIS

Cores are stored in glass jars with an inner diameter that is very close to the diameter of the core. The depth of sediment in the jar is very similar to the length of core it contai ·. In the laboratory, subsamples for analysis are taken from the sample jar with a paste sampler (American Scientific Products, McGaw Park, Illinois) modified with a teflon gasket to prevent sample loss (Figure 9). The paste sampler takes a composite of all the material from the top to the bottom of the jar, and is representative of the depth interval in the aquifer from which the core was extracted. Depending on the depth interval sampled, the subsamples weigh from 5 to 12 grams.

Each subsample is extruded into a tared 50-ml culture tube with a teflon-lined screw cap. Freon-113 is used to extract the petroleum hydrocarbons. Because it has no carbon - hydrogen bonds, it is transparent at the wavelengths of infra-red light used for spectroscopic analysis of the petroleum hydrocarbons. Freon-113 is added to cover the sample. Anhydrous magnesium sulfate (an amount equal to the weight of the subsample) is added to bind any free water. Heat is given off when water combines with anhydrous magnesium sulfate. Sometimes there is enough heat to boil the Freon-113 which may cause loss of some volatile organics. After mixing, the culture tube is completely filled with Freon-113 and the cap is screwed on tightly. After ten to twenty tubes have been prepared, they are secured in a rolling mill and tumbled slowly end over end for 1(to 24 hours. The tumbling action of the tube provides the agitation necessary to efficiently extract hydrocarbons from the sediment.

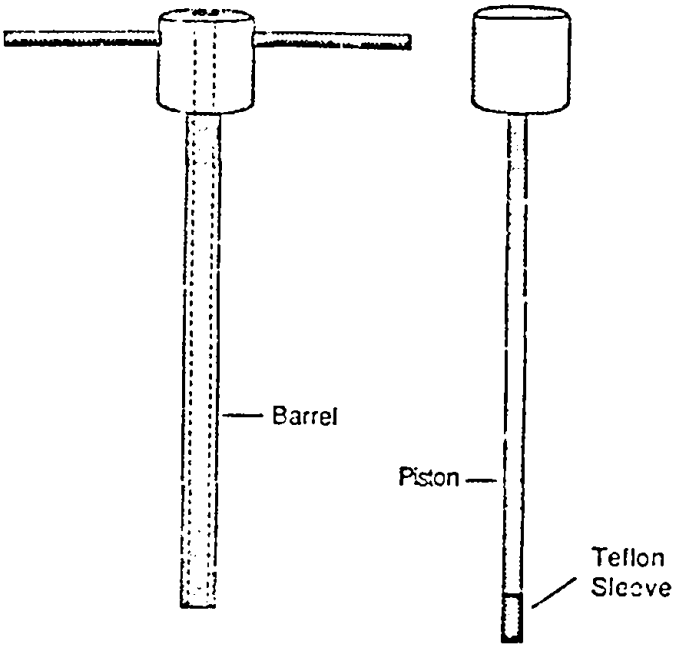


Figure 9. Paste Sampler Used to Subsample Cores.

Then the samples are centrifuged at 2,000 rpm for 10 to 15 minutes. The volume of the Freon-113 extract is measured in a graduated cylinder, to allow calculation of the quantity of petroleum hydrocarbons in the subsample from the concentration of fuel hydrocarbons in the extract, as determined by infrared spectroscopy. If the extract cannot be analyzed immediately, it is stored in a vial in a refrigerator.

INFRARED SPECTROSCOPY (IR)

A portion of the extract is transferred into a 10 mm calcium fluoride IR cell. The sample cell and a reference cell containing Freon-113 are placed into the appropriate cell holders of an IR Spectrophotometer (Perkin Elmer Model 521). The instrument is scanned from 3200 cm^{-1} to 2600 cm^{-1} wavenumber (see Figure 10). The spectrum for aviation gasoline has one strong absorption peak at 2955 cm^{-1}, while that for JP-4 jet fuel has two strong peaks at 2955 and 2925 cm^{-1}. The absorption peaks at 2955 and 2925 cm^{-1} correspond to C-H stretching vibrations in $-CH_3$ and $-CH_2$ respectively. The one absorption peak at 2955 cm^{-1} is indicative of aviation gasoline which consists mostly of branched alkanes, while the two absorption peaks at 2955 and 2925 cm^{-1} are characteristic of JP-4 jet fuel, which consists of branched and straight-chained alkanes.

If an extract is concentrated enough to deflect beyond 1.0 absorbance units, it is rescanned using a 1 mm calcium fluoride IR cell or diluted with Freon-113 until the absorbance is below 0.6 units.

QUANTITATION

Standards are prepared by adding measured aliquots of pure aviation gasoline or JP-4 jet fuel obtained from a refinery to Freon-113 in a 100 ml volumetric flask, concentrations ranging from 0 to 3500 mg/L for the

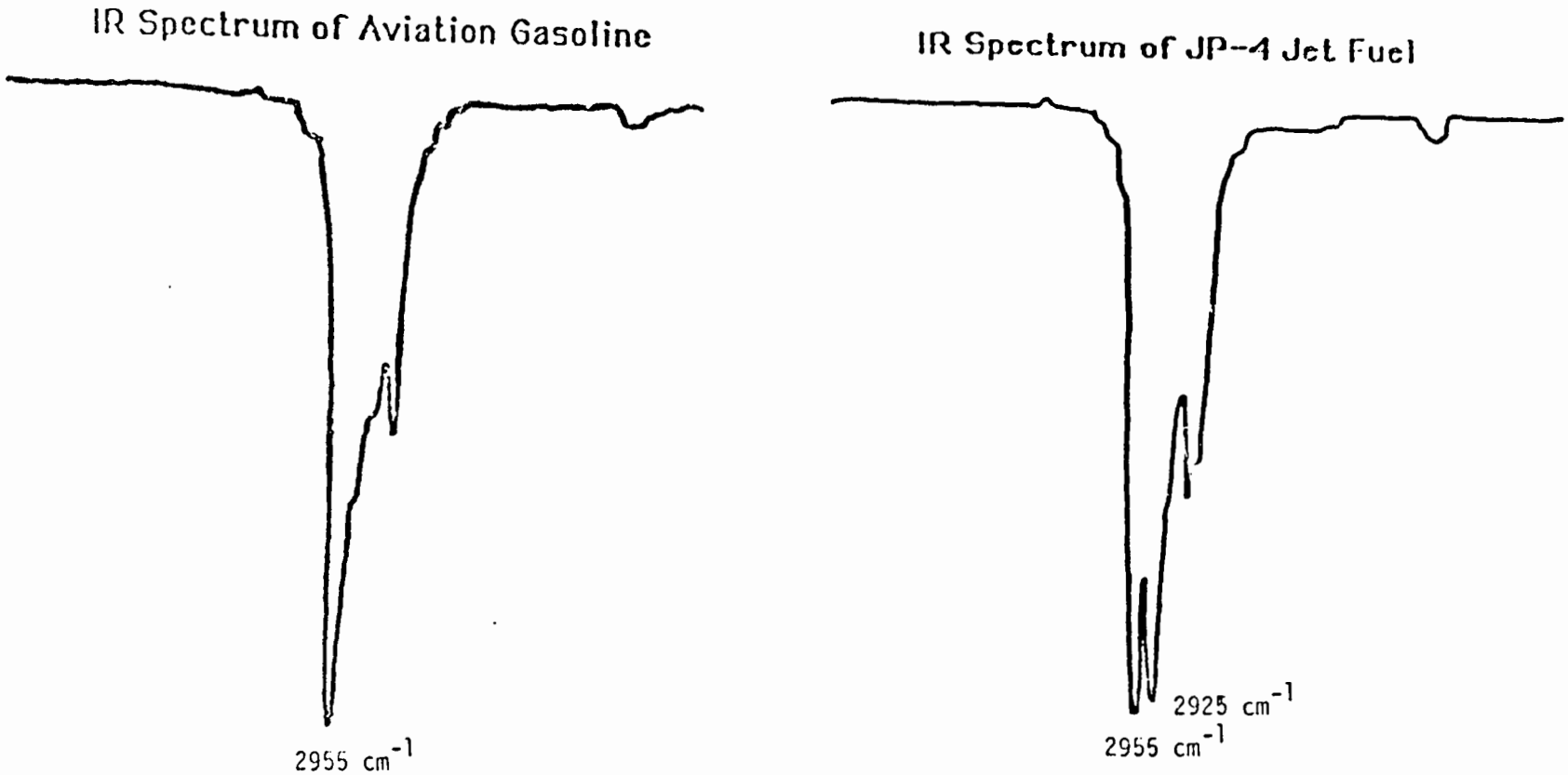


Figure 10. Infra-Red Spectrum of Aviation Gasoline and JP-4 Jet Fuel.

1 mm IR cell. Calibration curves for absorbance versus concentration are prepared using the standards. Two sets of calibration curves are developed for JP-4 jet fuel; one for absorbance at 2955 cm^{-1} and the other at 2925 cm^{-1}. Sample calibration curves are shown in Figure 11.

The fuel content is calculated as follows:

$$\text{Fuel Content} = \frac{C(mg/L) \times V(L)}{Wt(g)} \times (1000\ g/kg) = \frac{\text{mg of extractable material}}{\text{kg of wet aquifer material}}$$

where C(mg/L) is the concentration of fuel in extract (determined from absorbance and calibration curve),
V(L) is the volume of extract in liters, and
Wt(g) is the weight of wet sample in grams.

FUEL CARBON ANALYSIS

EXTRACTION OF ANALYTICAL SAMPLE FROM A CORE FOR FUEL CARBON ANALYSIS

In the laboratory, core subsamples for analysis are taken from the sample jar with a paste sampler (Figure 9) modified with a teflon gasket to prevent sample loss. The subsample is extruded into a tared 20-ml headspace vial that contains 5 ml of organic-free water. The vial is sealed with a teflon-lined septum cap. Three milliliters of pesticide-residue grade methylene chloride is injected through the septum. The vial is shaken on a rotary vibrator for 15 minutes, then sonicated in an ultrasonic bath for several minutes to break-up any emulsion. At least one milliliter of the solvent phase is passed through a micro-column of anhydrous sodium sulfate. The eluant is collected in a 2 ml vial, capped, and stored at 4°C for subsequent analysis.

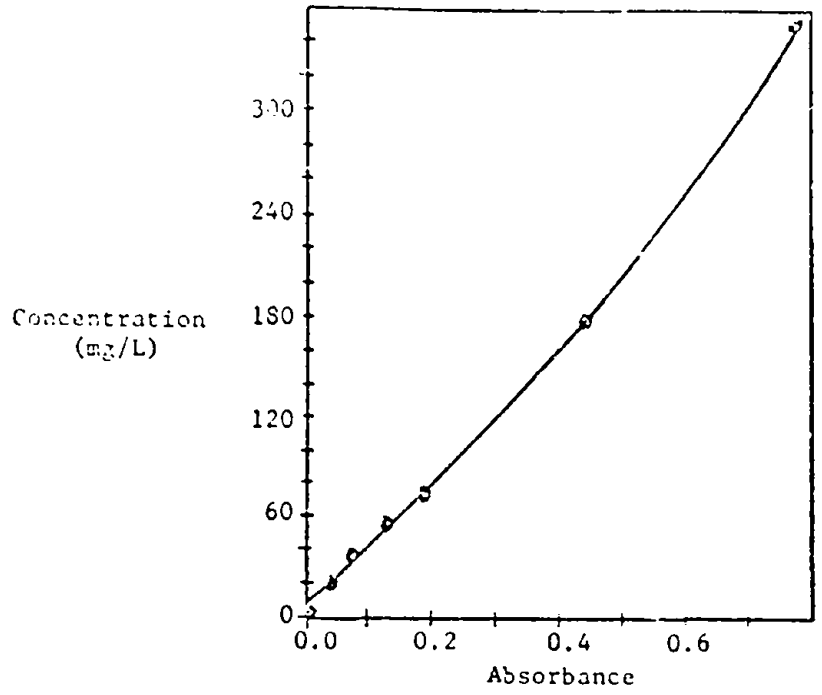


Figure 11a. Aviation Gasoline in Freon (2955 cm^{-1}), 0-350 mg/l.

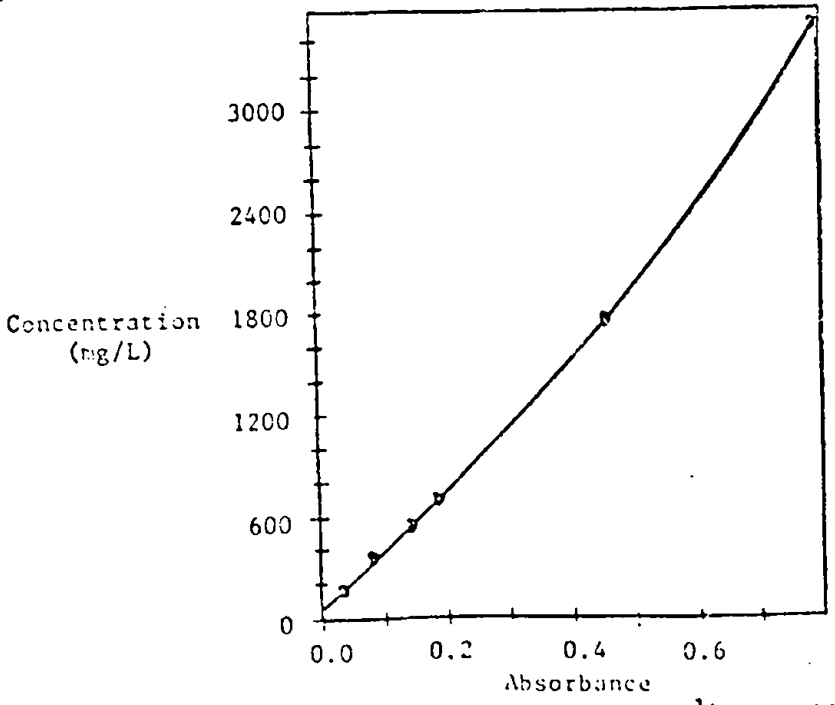


Figure 11b. Aviation Gasoline in Freon (2955 cm^{-1}), 0-3500 mg/l.

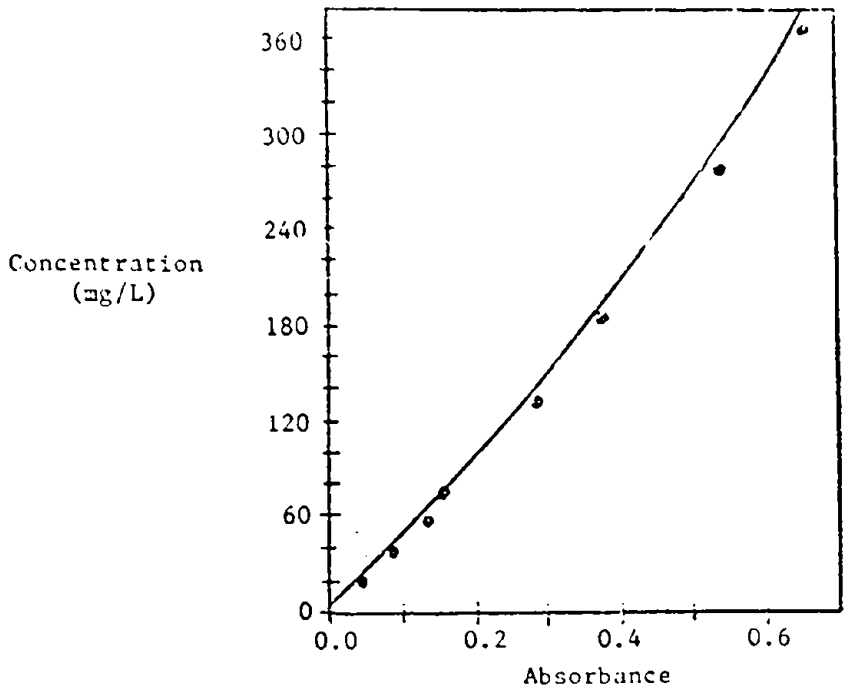


Figure 11c. Jet Fuel in Freon-113 (2955 cm^{-1}), 0-350 mg/l.

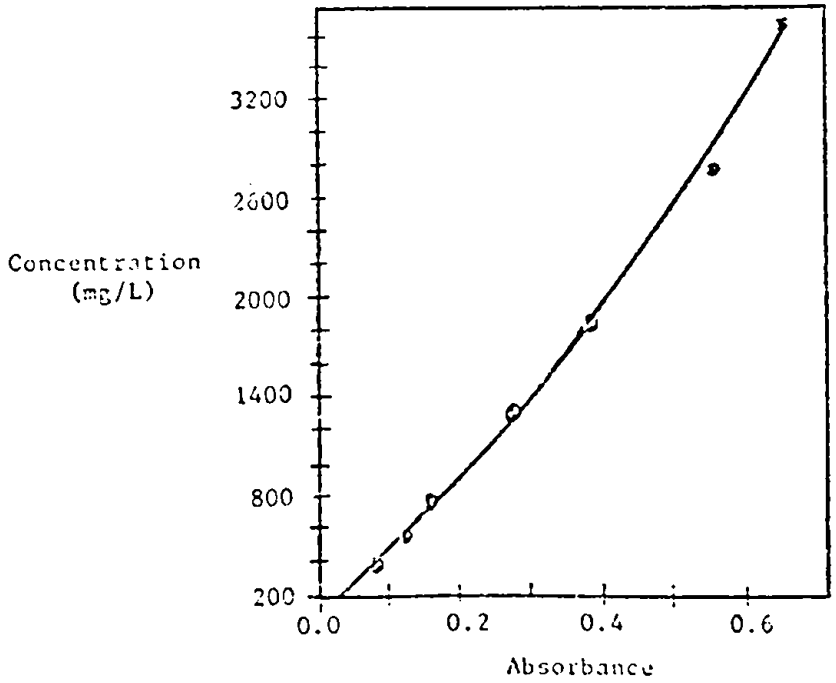


Figure 11d. Jet Fuel in Freon-113 (2955 cm^{-1}), 200-3500 mg/l.

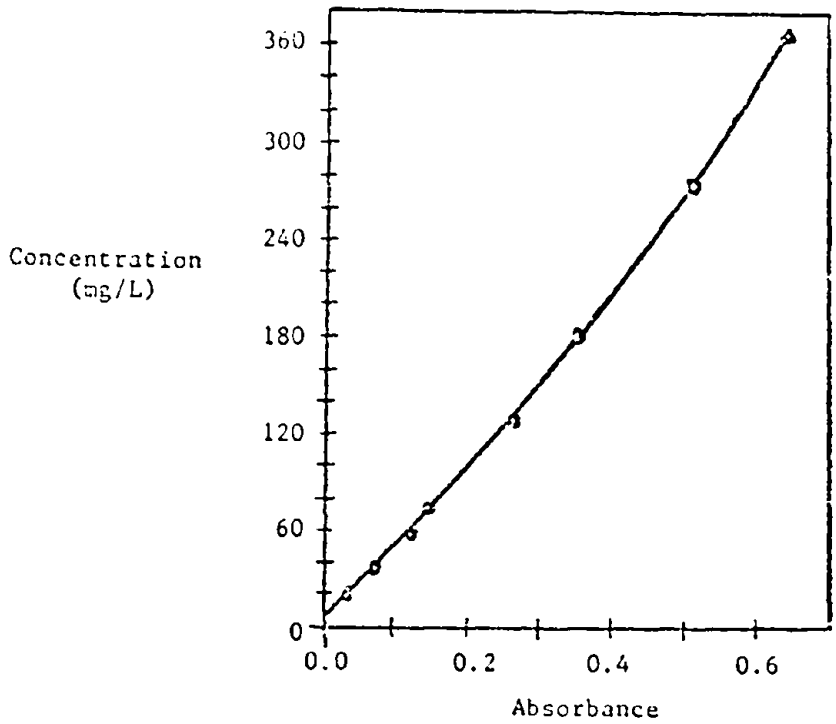


Figure 11e. Jet Fuel in Freon-113 (2925 cm^{-1}), 0-350 mg/l.

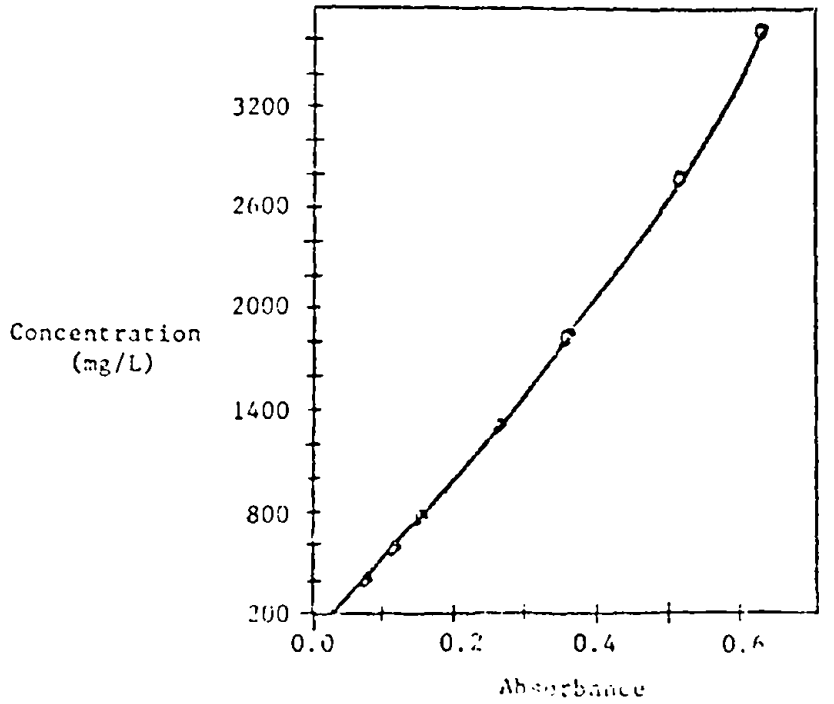


Figure 11f. Jet Fuel in Freon-113 (2925 cm^{-1}), 200-3500 mg/l.

GAS CHROMATOGRAPHY

One microliter of the dried extract is injected into a gas chromatograph equipped with a wide-bore capillary column (J & W Scientific DB-5, 15 m x .53 mm i.d., 1.5 mm film thickness). The injection is done in a splitless-mode with a solvent purge at 0.7 minutes. Both the injector and the flame ionization detector (FID) are kept at 300°C. The carrier gas is high-purity helium supplied at 9 ml/minute. The make-up gas for the FID detector is high-purity nitrogen supplied at 21 ml/minute. The GC oven is cooled cryogenically by liquid nitrogen. The temperature program is 10°C for 3 minutes, then a linear increase of 10°C/minute to 225°C, then 225°C for 2 minutes.

QUANTITATION

JP-4 jet fuel and aviation gasoline obtained from a refinery are used to prepare standards by adding measured aliquots of JP-4 jet fuel or aviation gasoline to methylene chloride. A calibration curve is prepared by analyzing the standards and summing the areas of the major peaks for each standard concentration. Sample curves are shown in Figures 12 and 13.

Retention times of the major peaks used for each calibration standard or sample analyzed are determined.

COMPARISON OF THE METHODS

The fuel carbon method and the oil and grease method compare favorably, even though they are based on entirely different principles (Powell et al., 1988). The fuel carbon analysis is preferred at R.S. Kerr Laboratory because it also provides information on the concentration of alkylbenzenes in waste oils.

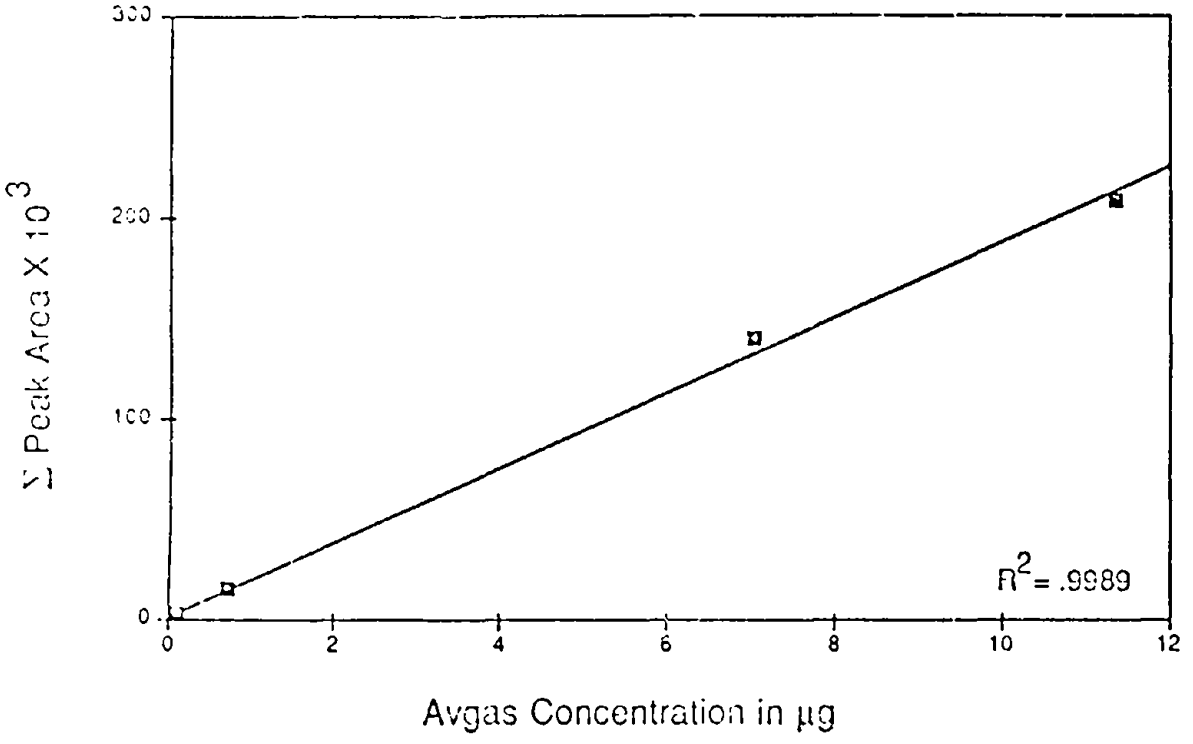


Figure 12. Standard Calibration Curve for Aviation Gasoline

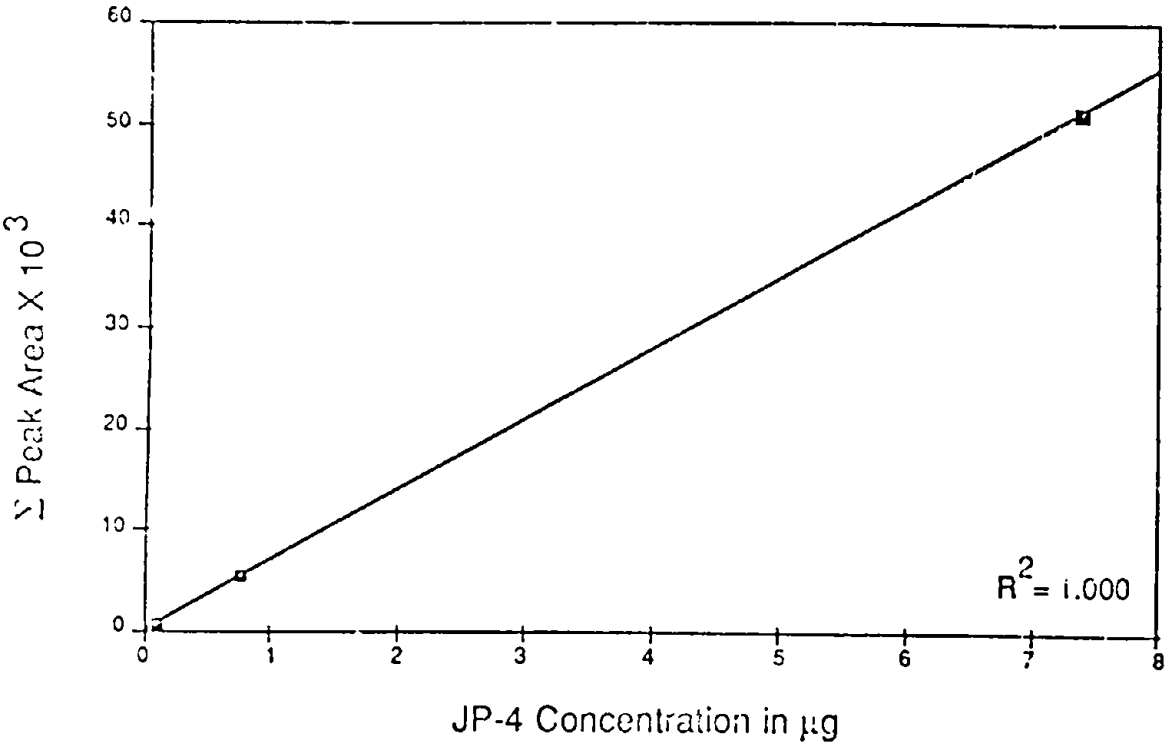


Figure 13. Standard Calibration Curve for JP-4 Jet Fuel.

SECTION IV. FIELD DEMONSTRATION OF SAMPLING AND ANALYTICAL PROCEDURES IN DESIGNING A BIOREMEDIATION

In 1969, a spill of aviation gasoline from an underground storage tank at the U.S. Coast Guard Air Station at Traverse City, Michigan, contaminated a shallow, sandy, water-table aquifer. Ground water moving through the spill produced a large plume that eventually moved off the base and ruined a large number of domestic water wells in a residental area (Figure 14). The spill contained at least 25,000 gallons of aviation gasoline, which drained to the water table 16 feet below land surface, then spread laterally in the capillary fringe to contaminate a section of aquifer about 80 yards in diameter (Figure 15).

DESIGN OF THE EXPERIMENT

In 1988 the U.S. Coast Guard and the U.S. EPA installed a pilot scale study of bioremediation in the area of the original spill. The alkylbenzenes are the object of the regulatory concern, and the bioremediation will be finished when their concentration is brought below 5 ug/liter, as specified in a consent decree between the Michigan Department of Natural Resources and the U.S. Coast Guard.

Cores were acquired from the source area to determine the vertical and horizontal extent of contamination, the concentration of total hydrocarbons in the contaminated interval, and concentrations of individual alkylbenzenes. The aviation gasoline was composed primarily of branched-chain alkanes. The material spilled at Traverse City was 38% 2,2,4-trimethylpentane; 15% 2,2,5-trimethylhexane; 14% 2,3-dimethylpentane; 13% 2,4-dimethylhexane; 7% 2,3-dimethylhexane; and 5% 2,4-dimethylpentane. Only 10% of the original spill was alkylbenzenes.

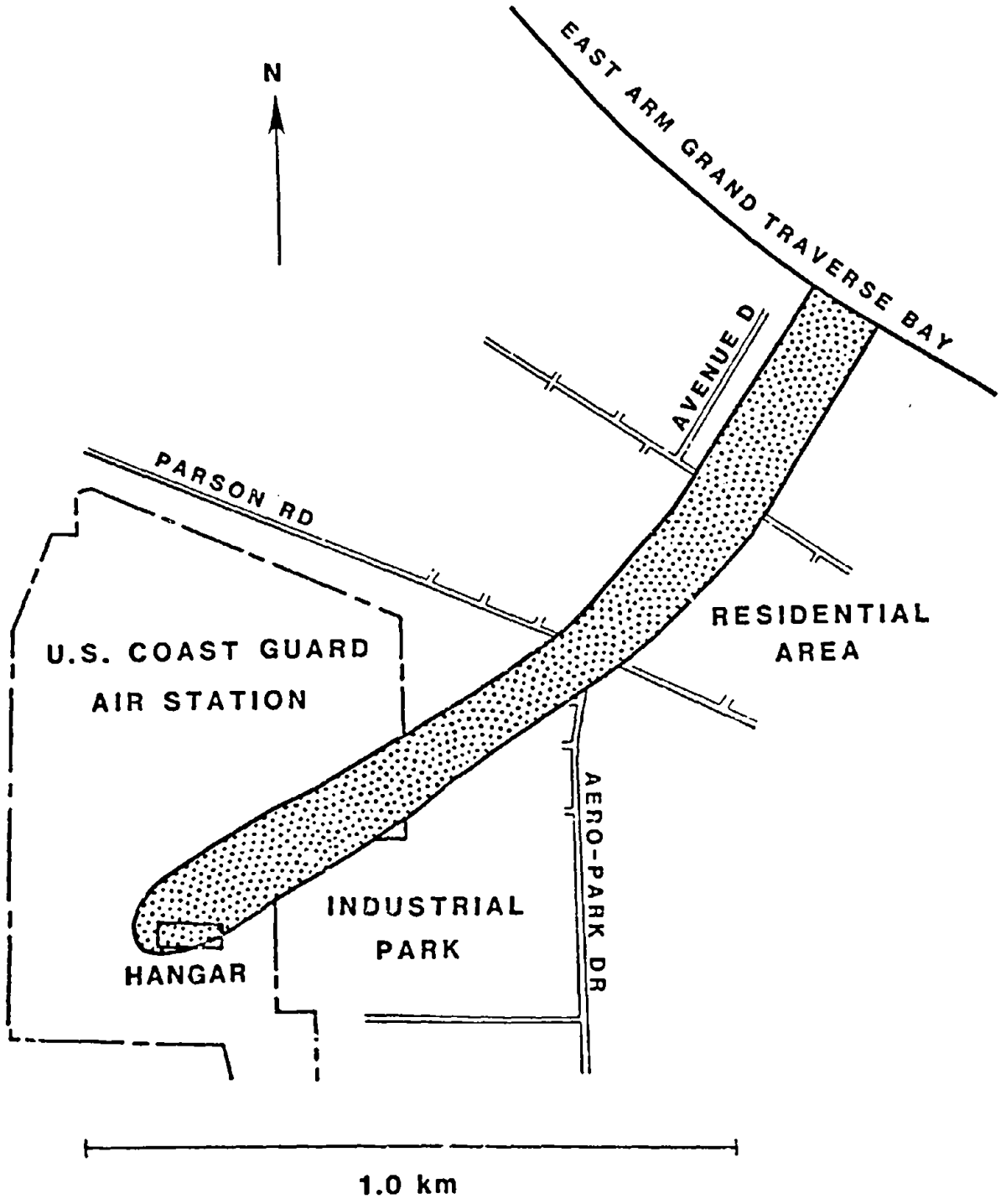


Figure 14. Former Extent of a Plume of Contamination Produced by a Spill of Aviation Gasoline on the U.S. Coast Guard Air Station at Traverse City, Michigan.

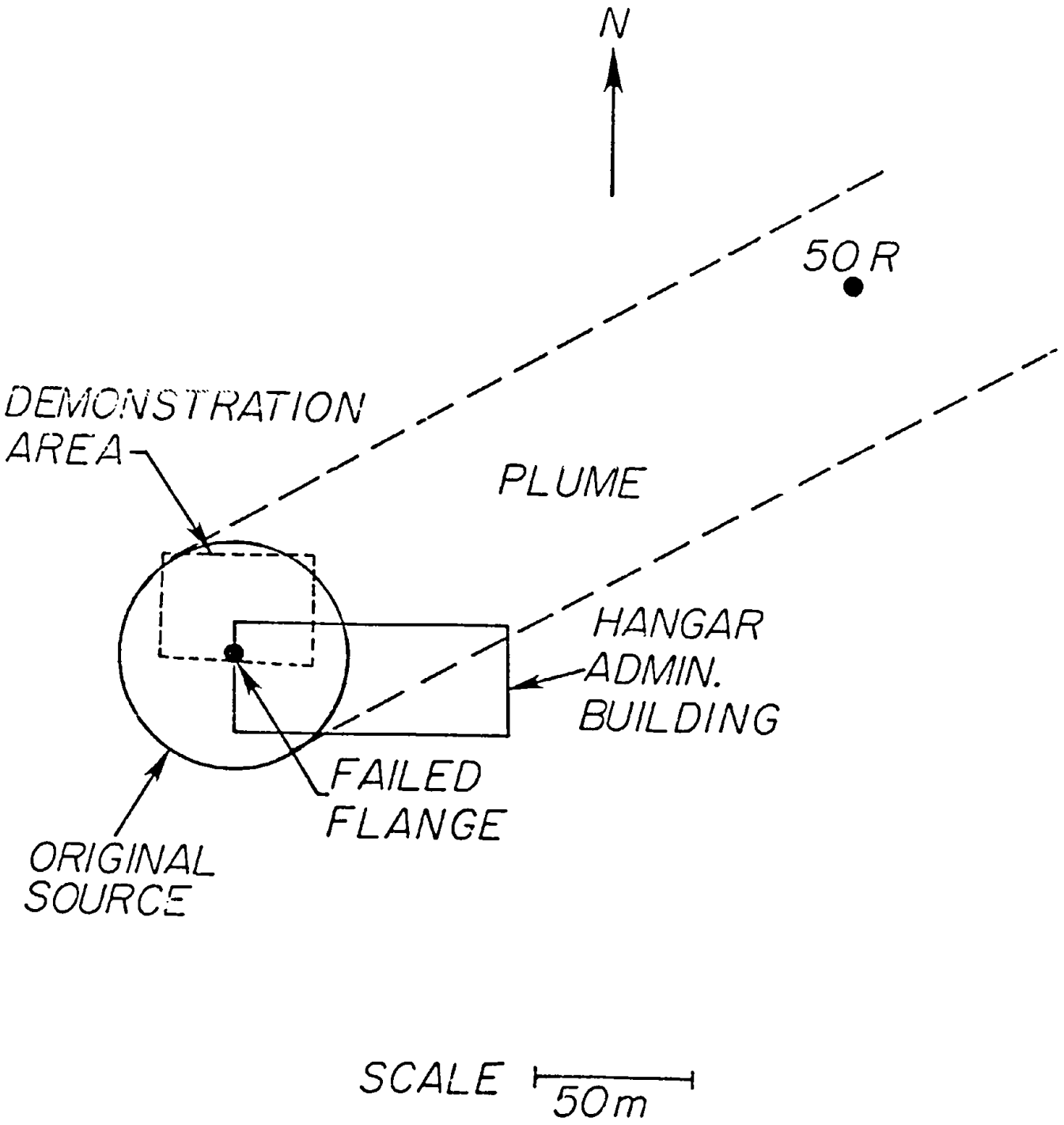


Figure 15. The Area in a Spill of Aviation Gasoline Selected for a Field Demonstration of In Situ Bioremediation.

The gasoline was confined to a narrow interval between 15 and 17 feet below the land surface (Table 1). This interval corresponds closely with the seasonal high and low water table at the site.

Table 1. Vertical distribution of contamination 50 feet down gradient from the injection wells

Depth interval (feet below surface)	Fuel Hydrocarbons (mg/kg aquifer)
15.1 - 15.5	<11
15.5 - 15.8	39
15.8 - 16.2	2370
16.2 - 16.5	8400
16.5 - 17.2	524
17.2 - 17.5	<13
18.0 - 18.3	<13

This information was used to identify the most-contaminated flow path through the spill. A series of miniature monitoring wells was installed along and below the most-contaminated flow path (Figure 16). These wells were constructed of 3/8 inch stainless steel tubing connected to a stainless-steel screen that was 6.0 inches long. The screens were constructed from stainless steel wire with a mesh width of 0.5 mm. The wells were connected to cylindrical sample traps with a volume of 300 ml. During sampling, a vacuum was applied to the traps. At least 2.0 liters of water were extracted through the well and trap to completely flush them, then a valve was closed between the well and trap, and the trap drained into the sample container through a second valve.

A set of infiltration wells was installed to perfuse the contaminated area with mineral nutrients, and oxygen or hydrogen peroxide. This water contained 380 mg/liter ammonium chloride, 190 mg/liter disodium phosphate,

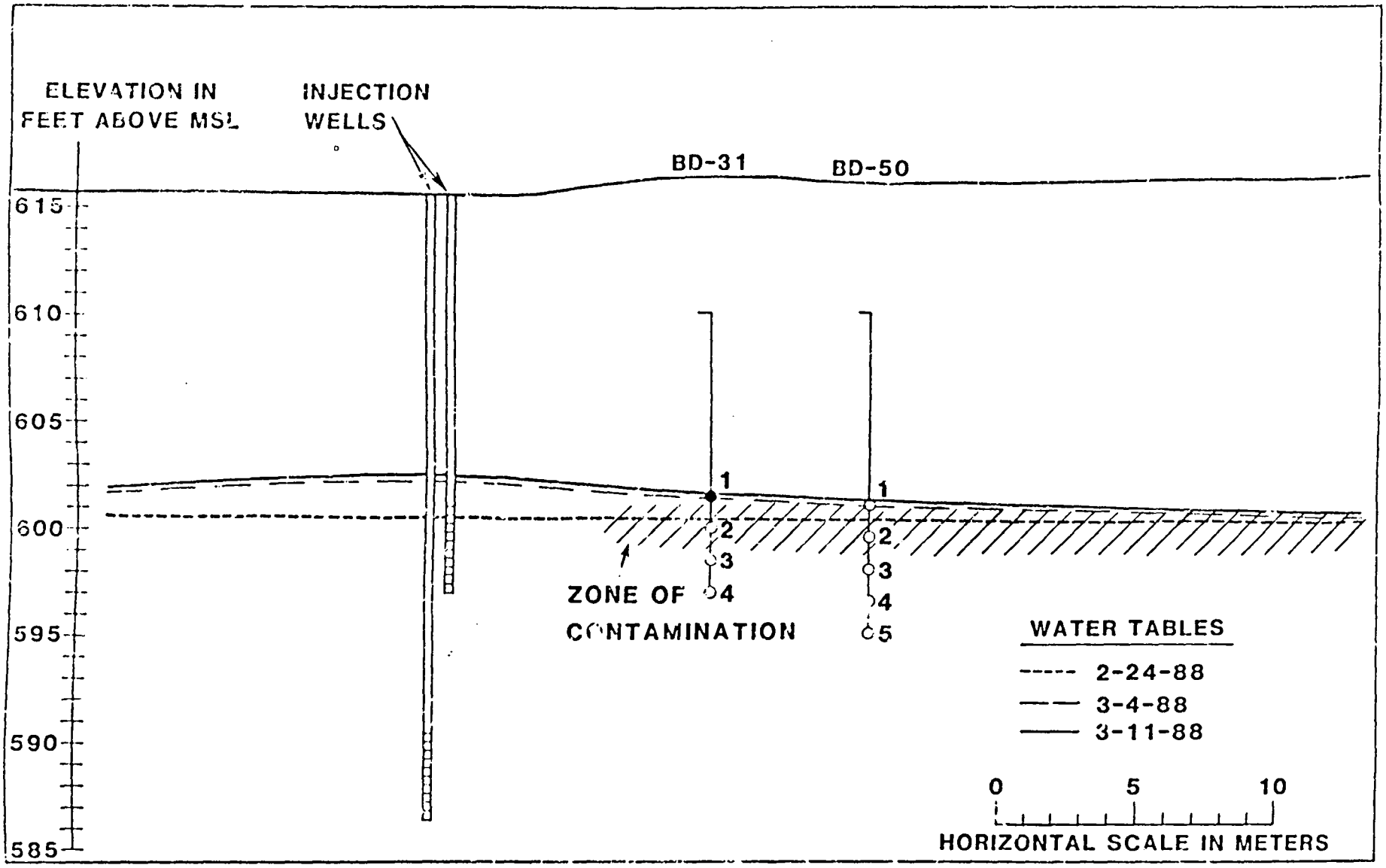


Figure 16. Vertical Cross Section of the Bioremediation Demonstration Area.

and 190 mg/liter potassium phosphate. The temperature was 11-12° C, and the pH near neutrality. The flow of chemically-amended water was 10 gallons/minute. Clean water from another part of the aquifer was infiltrated at 30 gallons/minute in a deeper set of wells. This water was not amended with nutrients or oxygen, it merely served to steepen the hydraulic gradient and increase the seepage velocity of the amended water through the contaminated interval.

The seepage velocity of the injected water in the aquifer averaged 5 to 9 feet per day (Table 2). Tracer tests were conducted for each monitoring well to determine the actual seepage velocity along the flow path to that particular well (see Figure 17 for typical breakthrough data of chloride as a tracer).

Table 2. Seepage velocity of oxygen, ammonium ion, phosphate, and chloride to monitoring wells.

Well Tracer	Depth on Figure 7. Depth #2 is in the most contaminated interval.				
	1	2	3	4	5
	---------- Apparent velocity (ft/day) ----------				
BD31- 31 feet from infiltration wells					
Chloride (03/88)		5.5	5.5	8.9	
Chloride (12/88)		8.6	8.0	not done	
Oxygen (03/88)		NBT*	4.4	9.2	
Ammonia (03/88)		3.9	3.9	6.2	
Phosphate(03/88)		3.6	3.7	6.2	
BD 508- 50 feet from infiltration wells					
Chloride (03/88)	4.3	6.0	5.5	9.2	12.6
Chloride (10/88)	NBT	7.5	8.9	16.0	18.4
Oxygen (03/88)	NBT	NBT	NBT	9.2	12.6
Ammonia (03/88)	2.3	2.1	3.3	6.0	10.0
Phosphate(03/88)	1.5	2.1	2.9	6.0	9.2

* Breakthrough not observed during the tracer test

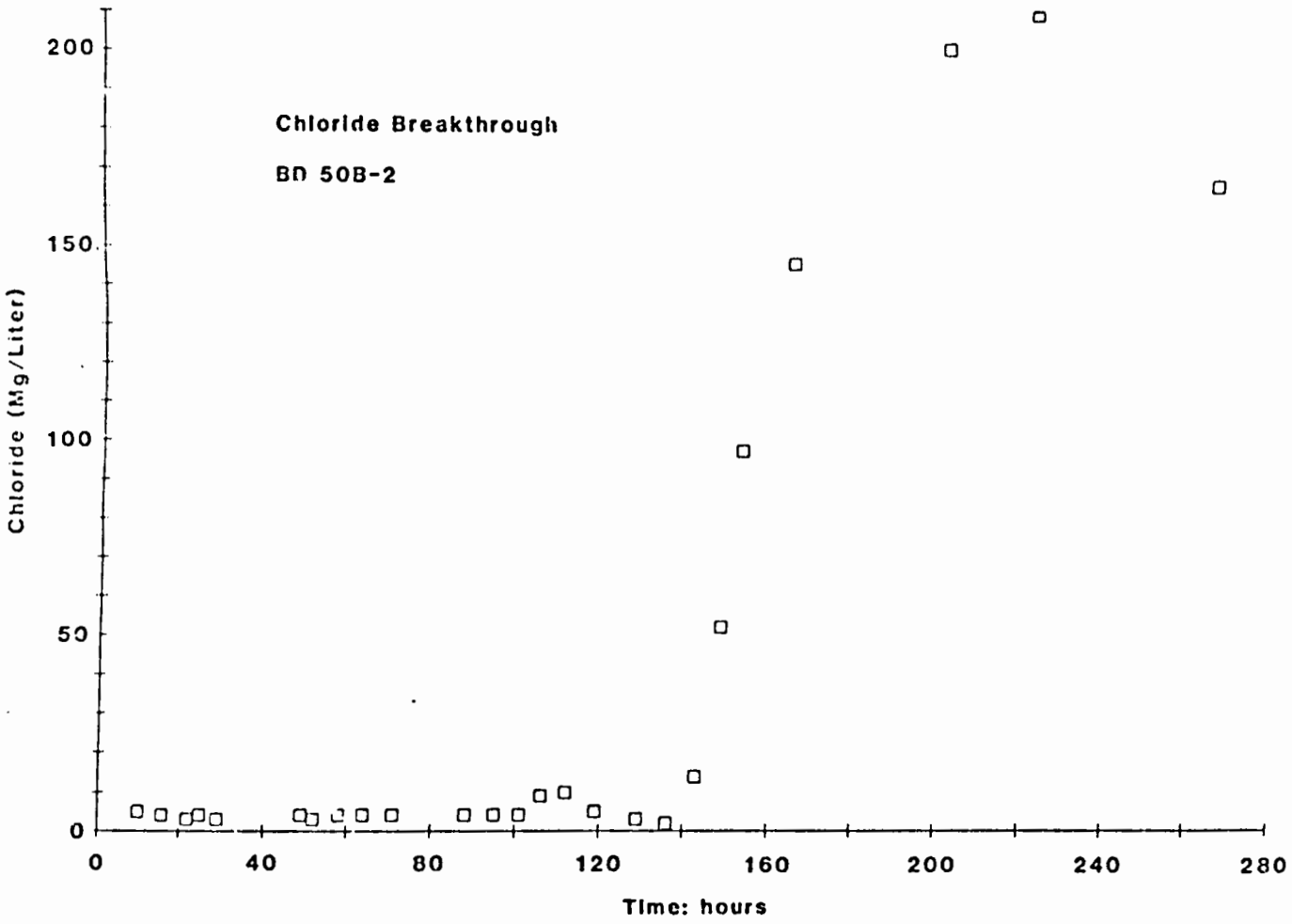


Figure 17. Breakthrough Curve for Chloride in the Flow Path from the Injection Wells to Monitoring Well BD 50B-2 in the Bioremediation Demonstration Area.

Notice that the velocity of water in the most contaminated interval (level 2) is much less than the velocity in the cleaner part of the aquifer only a few feet beneath (level 4 and 5). Also notice that ammonium ion and phosphate move at about half the velocity of water in this aquifer.

Injection began the first week of March, 1988. The system was first acclimated to oxygen, then switched to hydrogen peroxide. The schedule of application of oxygen and hydrogen peroxide is presented in Figure 18. The concentration of hydrogen peroxide was increased slowly, to allow time for microbial acclimation to concentrations of hydrogen peroxided that are generally toxic to most heterotrophic bacteria.

ESTIMATE OF OXYGEN DEMAND REQUIRED FOR REMEDIATION

The concentration of total petroleum hydrocarbons in the most contaminated interval near the infiltration wells was near 300 mg/kg. The highest measured concentration of total hydrocarbons near a monitoring well 31 feet down gradient from the injection wells is 8,400 mg/kg (core 50AE4 in figure 10 and 11). The highest measured concentration 60 feet down gradient is 6,500 mg/kg (core 50I14 in figure 19 and 20). The average of cores 50AE4 and 50I14 (7,500 mg/kg) was taken as the best estimate of the concentration of total petroleum hydrocarbons in the most-contaminated interval between the monitoring wells at 31 and 50 feet. The interval between the injection wells and the monitoring wells could not be cored because access was blocked by a sanitary sewer line. The most conservative estimate would consider the entire interval between the injection wells and the monitoring well at 31 feet to be contaminated at 7,500 mg/kg. The most liberal estimate would consider the interval to be contaminated at 300 mg/kg. An arbitrary intermediate estimate would average 7,500 and 300 mg/kg. The

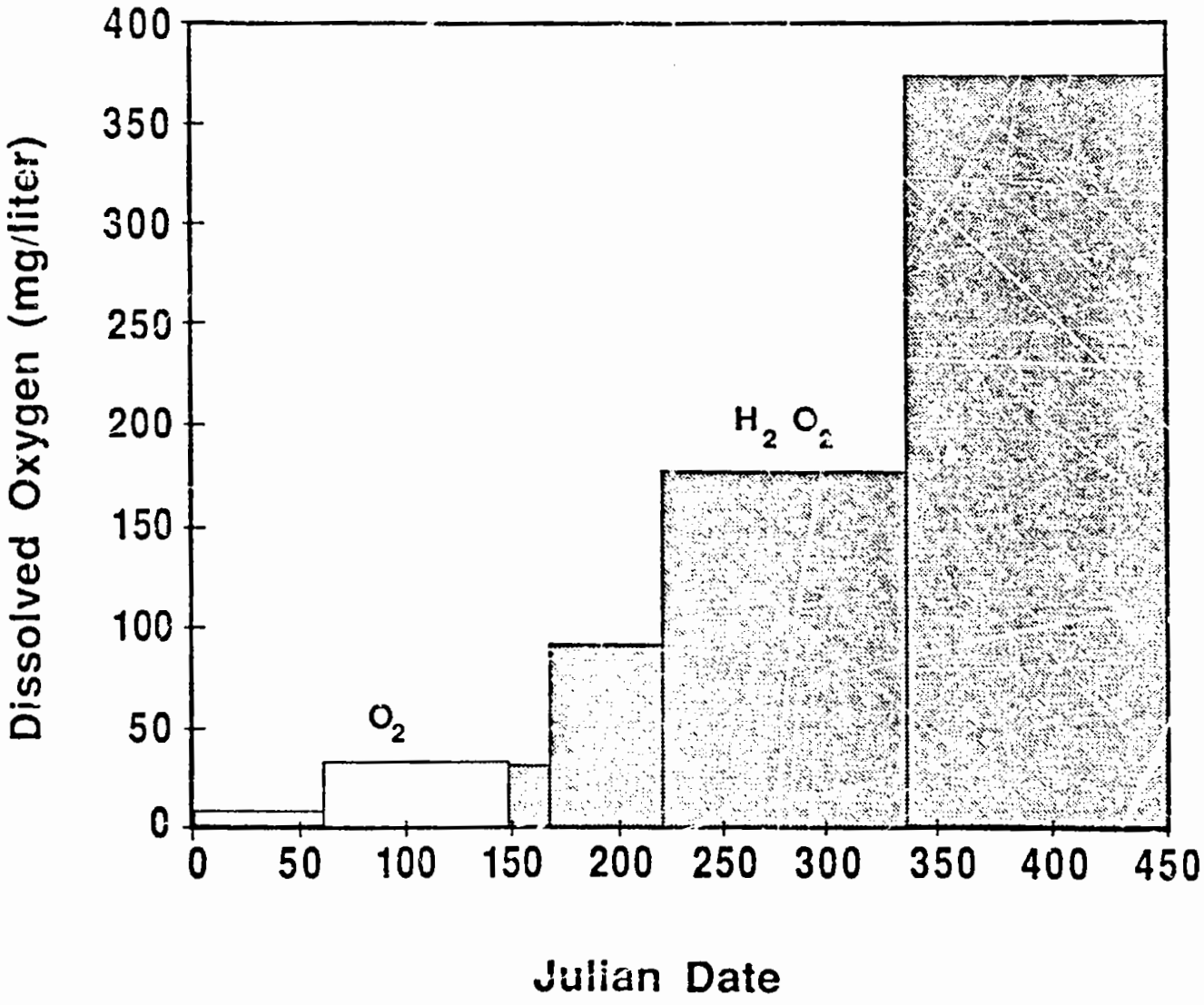


Figure 18. Schedule of Application of Oxygen and Hydrogen Peroxide. Julian Date 1 is January 1, 1988.

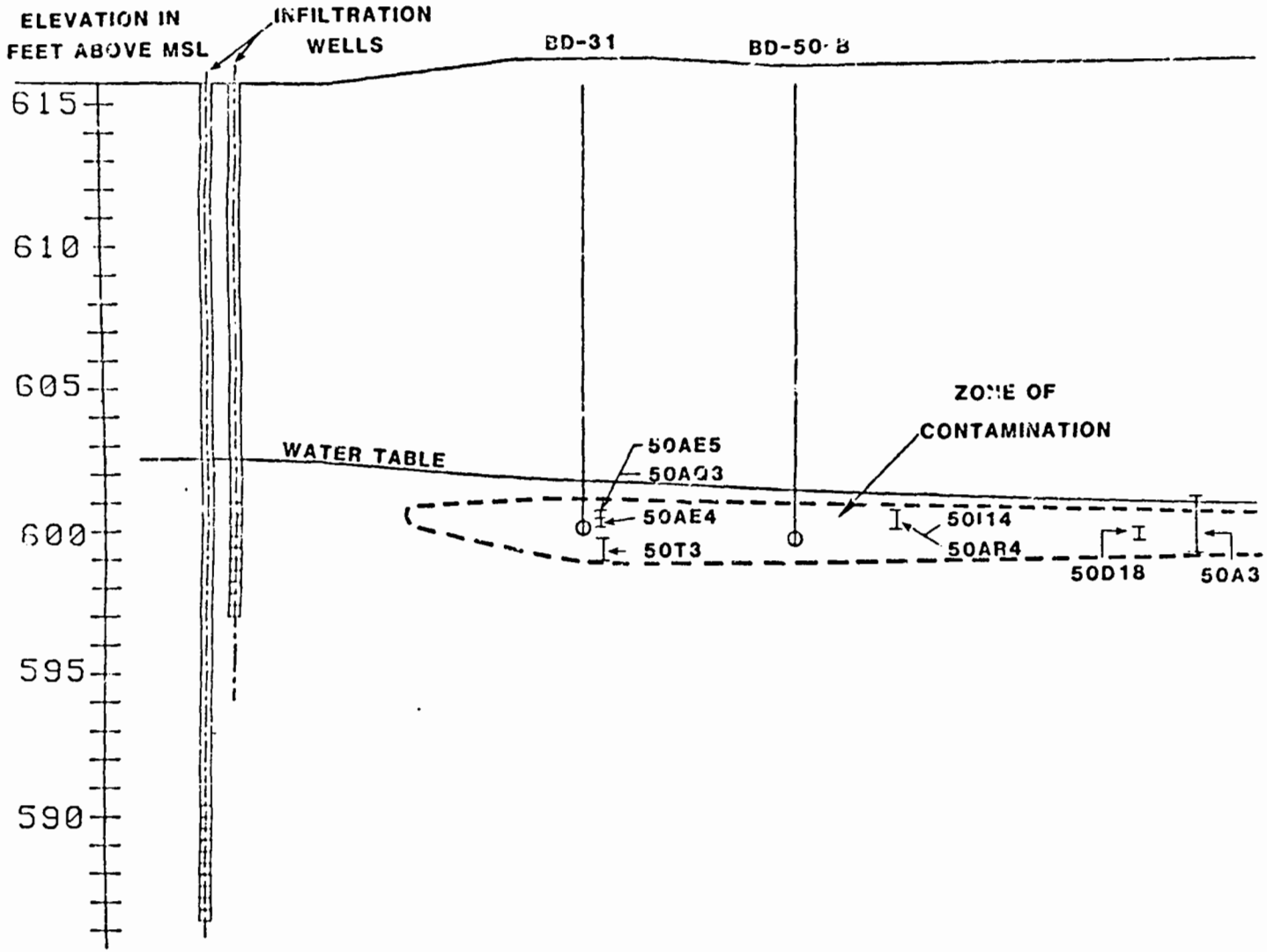


Figure 19. Relationship Between Core Samples and Monitoring Wells in the Bioremediation Demonstration Area.

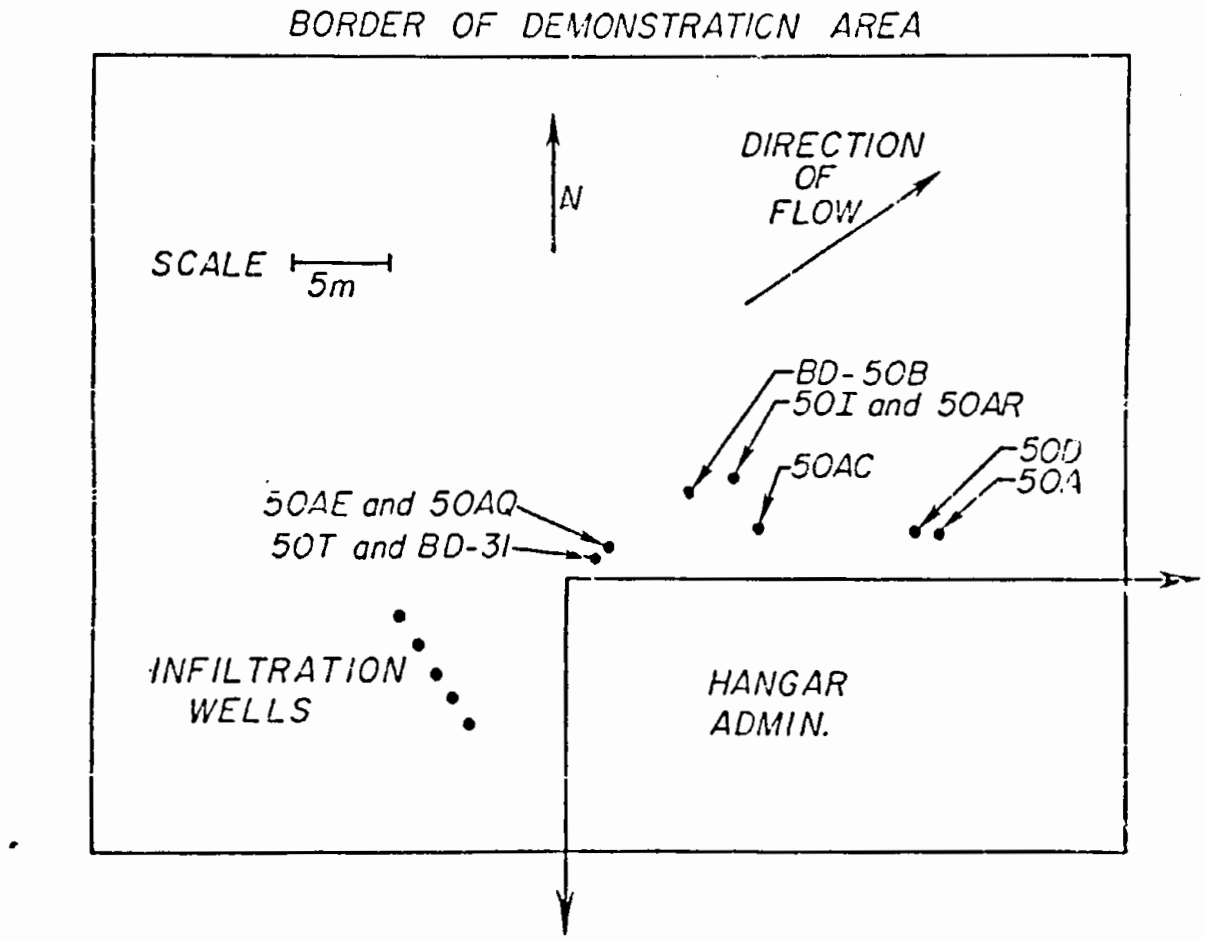


Figure 20. Map of the Relationship Between Core Samples and Monitoring Wells in the Bioremediation Demonstration Area.

oxygen demand along the most contaminated interval was calculated for all three estimates.

The empirical chemical formula for aviation gasoline is $CH_{2.2}$ (Powell et al., 1988). The empirical formula for the alkylbenzene fraction is $CH_{1.1}$. The oxygen demand for microbial respiration of total fuel hydrocarbons was estimated assuming the following stoichiometry:

$$CH_{2.2} + 1.55\ O_2 \rightarrow CO_2 + H_{2.2}O_{1.1}$$

The oxygen demand of the alkylbenzene fraction alone was estimated from:

$$CH_{1.1} + 1.28\ O_2 \rightarrow CO_2 + 0.55\ H_2O$$

The theoretical oxygen demand for aviation gasoline is 3.5 mg/mg, the demand of the alkylbenzene fraction is 3.1 mg/mg.

To calculate the theoretical oxygen demand of the hydrocarbons in a segment of a flow path, the hydrocarbon content (mg hydrocarbon/kg aquifer) was multiplied by the bulk density of the sediment (2.0 kg/liter) and divided by the porosity of the aquifer (0.4 liter pore space/liter total volume) to determine the quantity of hydrocarbon exposed to each liter of pore water in the segment. The quantity of hydrocarbon was multiplied by its oxygen demand to estimate the quantity of oxygen that must be delivered to each liter of pore water in the segment.

The interval from the injection wells to the monitoring well 31 feet down gradient was considered one segment. The demand in the flow path to the monitoring well 50 feet down gradient was estimated as the weighted average of the demand in the segment from the injection wells to 31 feet, and in the segment from 31 to 50 feet.

PERFORMANCE OF THE DEMONSTRATION

The interval between the injection wells and the monitoring wells was considered remediated when detectable oxygen broke through and alkylbenzenes disappeared. Compare Figures 21 and 22. The interval to the monitoring well at 31 feet was remediated after 220 days (Julian Date 281), and the interval to the monitoring well at 50 feet was remediated after 270 days (Julian Date 331).

The seepage velocity (as determined by the tracer tests) was multiplied by the concentration of oxygen or hydrogen peroxide in the injection wells (Figure 18) to determine the instantaneous flux of oxygen or hydrogen peroxide along the flow path. The cumulative flux at the time of remediation was considered the actual oxygen demand for remediation (Table 3).

The aquifer was purged of alkylbenzenes very quickly. Aviation gasoline is composed primarily of branched-chain alkanes. Only 10% of the original spill was alkylbenzenes. The quantity of oxygen and hydrogen peroxide required to remove alkylbenzenes from the wells agreed closely with the projected oxygen demand of the alkylbenzenes alone (Table 3).

This may, to some extent, be fortuitous. Some of the alkylbenzenes must have been washed from the source area by simple physical weathering. Some of the alkylbenzenes may have been removed by anaerobic biological processes before the front of oxygen swept through. Water from anaerobic regions of the demonstration contained significant concentrations of volatile fatty acids and was visibly turbid with microorganisms. In any case, the the flow paths to the monitoring wells at 31 and 50 feet from the injection wells were remediated when a small fraction of the oxygen demand of the spill had been supplied.

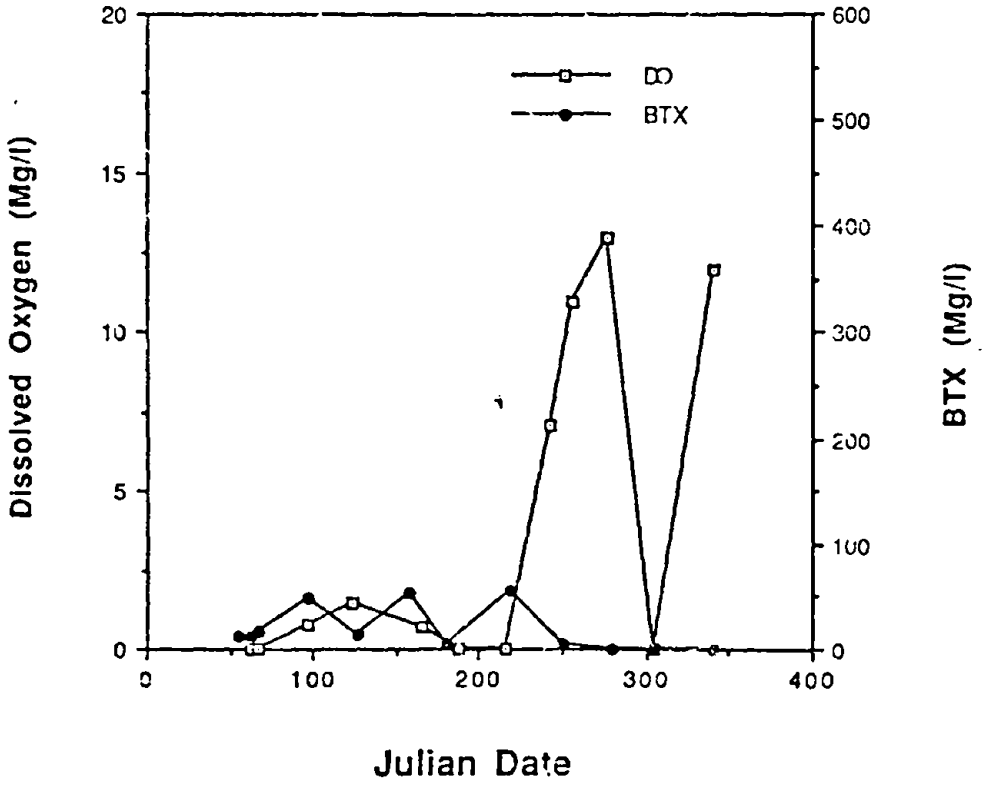


Figure 21. Changes in Concentration of Alkylbenzenes and Oxygen in Monitoring Well BD 31-2 During Bioremediation.

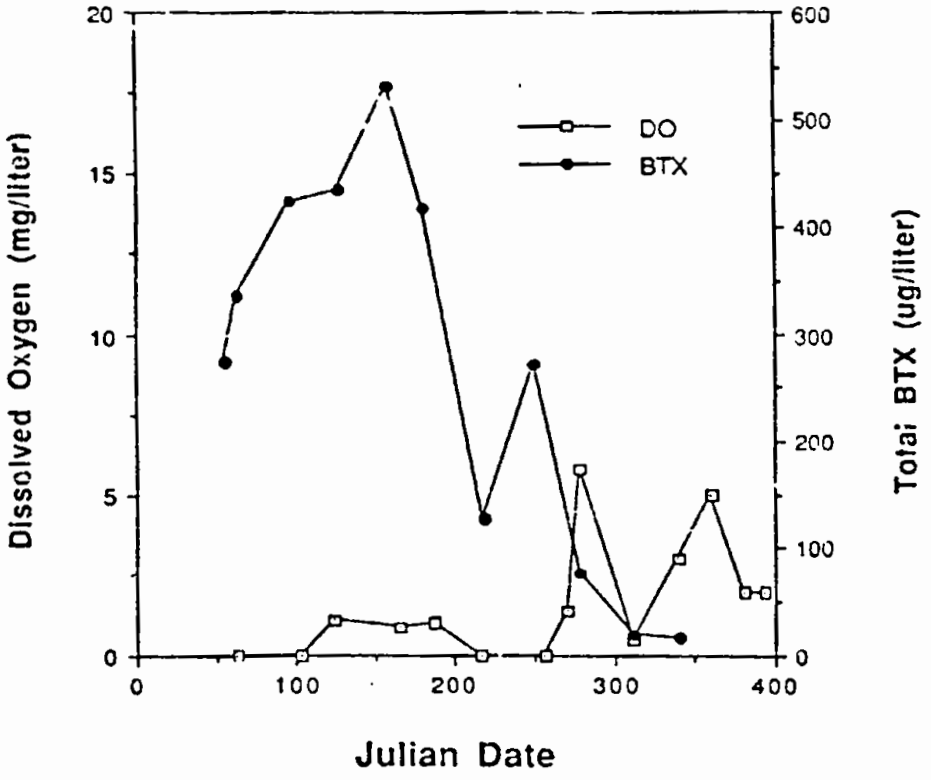


Figure 22. Changes in Concentration of Alkylbenzenes and Oxygen in Monitoring Well BD 508-2 During bioremediation.

Table 3. Estimated and actual oxygen demands of the most contaminated interval in the aviation gasoline spill at Traverse City, Michigan.

	Oxygen or Hydrogen Peroxide Demand along Flowpaths to Monitoring Wells 31 and 50 feet down gradient of the infiltration wells					
	Conservative Estimate		Moderate Estimate		Liberal Estimate	
	31 feet	50 feet	31 feet	50 feet	31 feet	50 feet
	---(mg oxygen/liter pore water)----					
Estimated demand based on:						
Total Fuel Hydrocarbons	130,000	130,000	68,000	92,000	5,000	53,000
Alkylbenzene content only, when sampled in 8/87.*	10,000	10,000	5,000	7,000	400	4,000
Alkylbenzene content only, when sampled in 3/88 just before the start of the demonstration.**	1,100	1,100	593	800	45	460
Actually delivered by 10/88. Corresponds to Julian Date 300	3,000	3,000				

*based on analysis of core 50I14 (Table 5) assuming 7,500 mg/kg total hydrocarbon
**based on analysis of core 50T3 (Table 5) assuming 7,500 mg/kg total hydrocarbon

This selective removal of alkylbenzenes may result from their relatively high water solubility. If the system follows Raoult's Law, the expected concentration of an individual hydrocarbon in water in equilibrium with the gasoline can be estimated by multiplying its water solubility by its mole fraction in the gasoline.

The expected concentration of toluene in water in equilibrium with the fuel was 15 mg/liter. As shown in Figure 23 the measured concentration of toluene has been as high as 32 mg/liter. The expected concentration of 2,2,4-trimethylpentane is only 0.2 mg/liter. Actual measured concentrations are in the anaerobic zone of the demonstration area range from 0.06 to 0.07 mg/liter. The alkylbenzenes may have been more available to the microorganisms.

CONTRIBUTION OF WATER WASHING

A significant fraction of the alkylbenzenes may simply be washed out of the demonstration area by the flow of water, instead of being destroyed by biodegradation. The significance of this physical weathering can be evaluated by comparing the retardation factor of each alkylbenzene in the most-contaminated interval to the number of pore volumes of water that have been delivered to a particular point.

The ratio of the seepage velocity of water to the apparant seepage velocity of an individual alkylbenzene is termed the retardation ratio. This retardation ratio is equal to 1.0 plus the ratio of the mass of the alkylbenzene in immobile gasoline to the mass in the flowing water. The distribution of the alkylbenzene between gasoline and water in the aquifer is estimated from Raoult's Law, by dividing the distribution of the alkylbenzene between the pure compound and water (its specific gravity

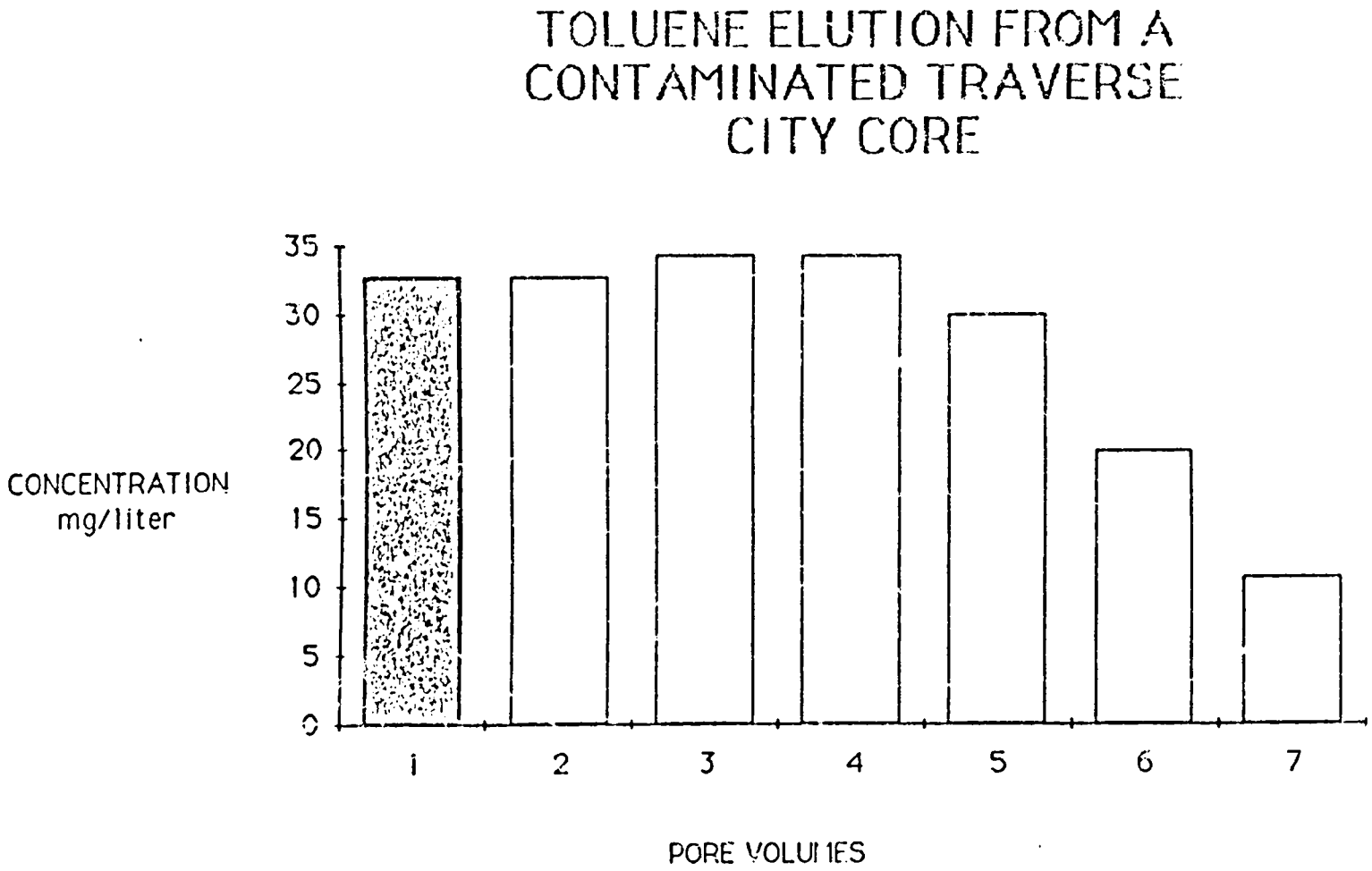


Figure 23. Comparison of the Concentration of Toluene Leached From Contaminated Aquifer Material in the First Pore Volume to Concentrations Leached in Subsequent Pore Volumes.

divided by its water solubility) by the ratio of water to gasoline in the aquifer. These calculations can be done a number of different ways. For convenience, we will express units as mass of organic compound per unit volume of the phase that contains it.

If the most-contaminated interval contains 7,500 mg/kg total petroleum hydrocarbons, and its bulk density is 2.0 kg/liter, then the most-contaminated interval contains 15,000 mg petroleum hydrocarbons per liter of aquifer. (Note: The proper unit for volume should be cubic decimeter. In formal usage the liter is a unit for capacity). The specific gravity of the gasoline is 0.76 (Smith et al., 1981), therefore the most contaminated interval of the aquifer contains 20 ml gasoline per liter of aquifer. The porosity of the aquifer, as determined by weighing cores and then measuring the weight loss on drying, is 380 ml pore space per liter of aquifer. If 20 ml of the pore space in each liter of aquifer is gasoline (Figure 24), the remaining 360 ml must be occupied by water. The volumetric ratio of water to gasoline is 360 to 20, or 18 to 1.

This approach for comuputing retardation can be evaluated with data from a column test (Bouchard et al., 1989). Core material from the demonstration area was packed into a column, washed with water to remove all the alkylbenzenes, and then a pulse of alkylbenzenes in solution was flushed through the column. The core material used to construct the column contained 1,340 mg/kg total petroleum hydrocarbons, corresponding to a water to gasoline ratio of 112 (vol/vol).

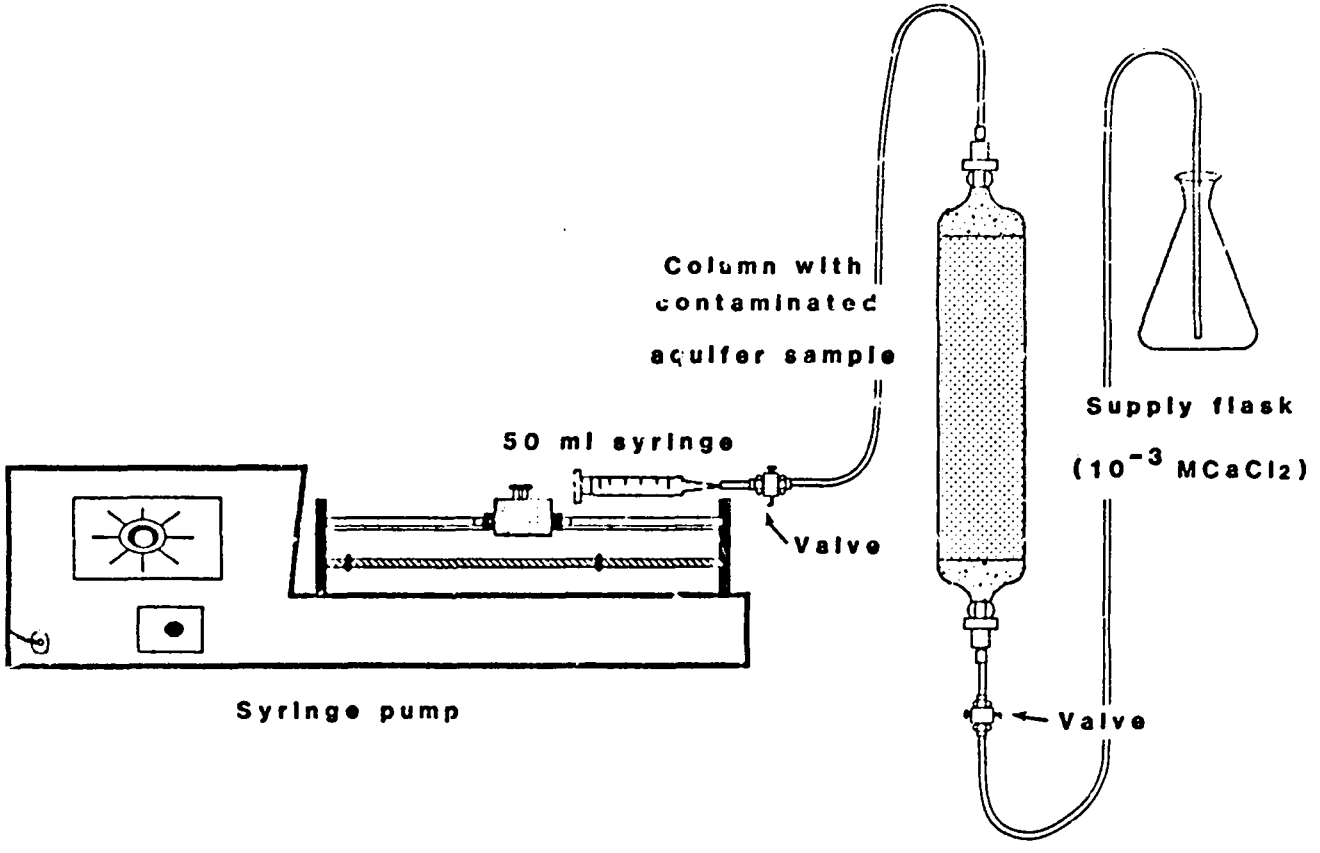


Figure 24. Laboratory Column Used to Estimate the Leaching of Alkylbenzenes from Contaminated Aquifer Material.

Table 4. Predicted retardation ratios for selected hydrocarbons in a column study (Figure 25) and in the field demonstration.

Compound	[Specific Gravity (g/liter) ÷ Solubility (g/liter) ÷ Volume Water / Volume Gasoline] + 1.0 = Predicted Retardation Ratio					
			in Aquifer	in Column	in Aquifer	in Column
Benzene	878	1.78 @ 20°C	18	112	28	5.4
Toluene	867	0.47 @ 16°C	18	112	103	17.4
o-Xylene	880	0.175 @ 20°C	18	112	280	46
p-Xylene	864	0.167 @ 25°C	18	112	290	47
m-Xylene	860	0.198 @ 20°C	18	112	240	40
Ethylbenzene	867	0.142 @ 15°C	18	112	340	56
1,2,4-Trimethylbenzene	880	0.057	18	112	860	140

Specific Gravity and Solubility from Verschueren (1983) and Smith et al. (1981).

The retardation ratios (Table 4) predicted for the column study (17.4 for toluene 46 for o-xylene, 56 for ethylbenzene, and 140 for 1,2,4-trimethylbenzene) are in acceptable agreement with the laboratory data (Figure 25). There is some justification to using the predicted retardation ratios to estimate the relative contribution of water washing and biorestoration in the field scale demonstration.

Based on the chloride tracer test, 3.6 days were required to move one pore volume of water from the injection wells to the monitoring well 31 feet down gradient, and 6.7 days to move one pore volume to the monitoring well 50 feet down gradient. By October of 1988 (Julian Date 300 in Figures 21 and 22), 67 pore volumes had moved past the monitoring well 31 feet down gradient, and 35 pore volumes had moved past the monitoring well 50 feet down gradient.

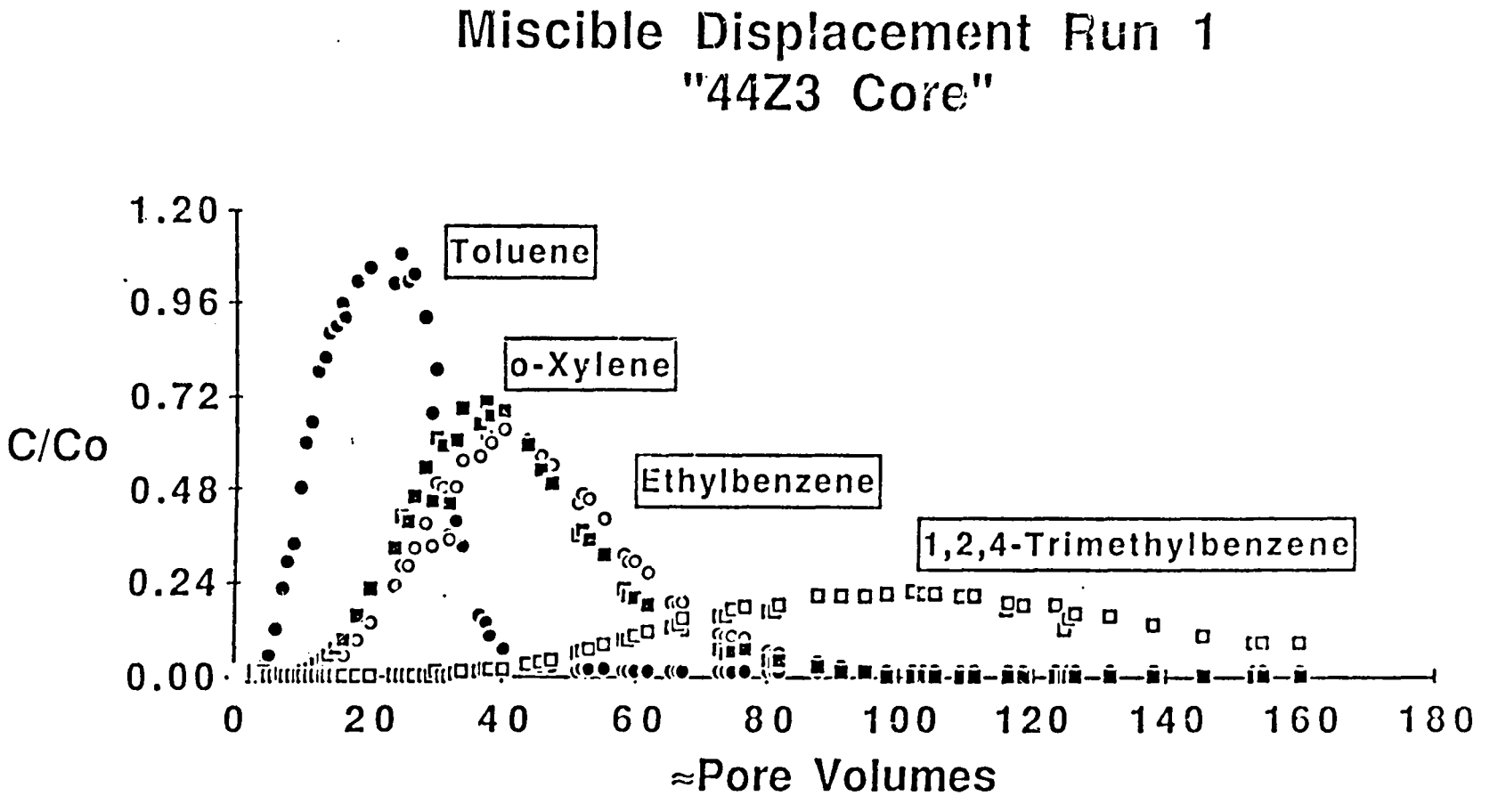


Figure 25. Retardation of Toluene, O-Xylene, Ethylbenzene, and 1,2,4 Trimethylbenzene in a Laboratory Column Constructed with Core Material from the Bioremediation Demonstration Area.

After comparing the number of pore volumes of water delivered along the most contaminated interval to the predicted retardation ratios of individual alkylbenzenes in the field demonstration (Table 4), it is evident that benzene could easily have been removed by water washing, and that a fraction of the toluene may have been removed, but hardly any removal of the xylenes, ethylbenzene, or trimethylbenzene can be expected.

CONFIRMATION OF REMEDIATION

The spill was cored in August 1987 to provide information to design the demonstration, then cored again in March 1988, just before the demonstration began, to define the initial conditions. Compare cores 50I14 and 50T3 in Table 5 and Figure 19. The proportion of alkylbenzenes in the spill declined modestly over the time interval. This was probably due to anaerobic microbial degradation as discussed earlier.

Shortly after the breakthrough of oxygen in monitoring well BD 31-2, the area near the monitoring well was cored and analyzed for alkylbenzenes and total fuel hydrocarbons. Compare cores 50AE4 and 50AF5 in Table 5 and Figure 19 to Cores 50T3 and 50I14. The aliphatic hydrocarbons remained at their initial concentration, but the alkylbenzenes were below the analytical detection limit (Table 5). It is not surprising that the non-aromatic fraction of the spill remained in the aquifer. A very minor fraction of their oxygen demand had been supplied when the aquifer was cleansed of alkylbenzenes (Table 3).

When the region near BD31-2 was cored in March of 1989, almost all the petroleum hydrocarbons had been removed, including the branched-chain alkanes. Compare core 50AQ3 to 50T3 and 50I14 in Table 5 and Figure 19.

Table 5. Changes in concentrations of alkylbenzenes and total fuel hydrocarbon in core material during bioremediation of an aquifer contaminated with aviation gasoline.

Date Core Number	Oil and Grease	Fuel Hydro-Carbon	Benzene	Toluene	Ethyl-benzene	Xylenes
	---mg/kg wet sample---					
Background conditions in an unweathered part of the spill area. June, 1988. See Figure 3 for location.						
50R6		12,150	1.0	107	57	218
50R7		5,220	1.0	170	24	100
Preliminary sampling used to design the bioremediation project near monitoring well 50-31-2, August 1987. See Figures 19 and 20 for location.						
50A3	4,310	5,590	0.6	235	33	121
50I14	4,130	6,500	0.3	544	12	48
50D18	1,130	2,500*	0.7	112	11	39
Sampled after four months of perfusion with mineral nutrients and oxygen, June, 1988.						
50T3		3,330*	1.4	1	7.3	23
Sampled after eight months of perfusion with mineral nutrients and oxygen, October, 1988.						
50AE4		8,400	<0.3	<0.3	<0.3	<0.3
50AE5		2,370*	<0.3	<0.3	<0.3	<0.3
Sampled after 12 months of perfusion with mineral nutrients and oxygen, March, 1989						
50AQ3		9	<0.3	<0.3	<0.3	0.1
Sampled after 12 months of perfusion with oxygen and mineral nutrients March, 1989. Oxygen had not reached this part of the aquifer.						
50AR4		3,100*	1.5	<0.3	9.2	36

*these cores included some uncontaminated material.

A core taken from a region in the demonstration area where oxygen was depleted showed an interesting pattern. Toluene is depleted in 50AR4, even though significant quantities of benzene and ethylbenzene remain. It is difficult to rationalize the selective removal of toluene through some purely physical mechanism.

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16. ABSTRACT

This report presents a systematic approach for the design of in situ bioremediation of hydrocarbon contamination in ground water from the determination of the total quantity of hydrocarbons in the aquifer to the utilization of that information in an actual field bioremediation demonstration. This report explains why the total quantity of hydrocarbons in an aquifer can only be determined by collecting cores. A procedure to acquire cores from a contaminated aquifer is described. The procedures described in the report were field-tested in designing a demonstration of the bioremediation of an aviation gasoline leak. The performance of the demonstration was consistent with the expected performance based on the preliminary site characterization using the described procedures.

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