Remediation of soils contaminated by leaking underground storage tanks by vapor extraction and in situ biostimulation. [DNR-096] 1994?

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Remediation of Soils Contaminated by Leaking Underground Storage Tanks by Vapor Extraction and *In Situ* Biostimulation

Final Report

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Introduction

Vapor extraction is widely used to remediate unsaturated subsurface soils contaminated by petroleum hydrocarbons. While primarily a physical remediation technique, soil vapor extraction may also enhance hydrocarbon biodegradation *in situ* by introducing O_2 into subsurface soils. Aeration may enhance microbial activity as subsurface O_2 levels are typically low in hydrocarbon-contaminated soils.

Several field studies, most involving jet fuel-contaminated soils, have examined the effects of ventilation on *in situ* biodegradation. Hinchee et al. (1991), monitored a vapor extraction system (VES) applied to a jet fuel-contaminated soil. In four months, 11,300 kg of fuel were volatilized and an additional 2,200 kg were believed to be biodegraded. Biodegradation rates (based on zero order O_2 consumption rates) ranged from 3 mg fuel kg^{-1} soil d^{-1} to 8 mg fuel kg^{-1} soil d^{-1}. There were no consistent stimulatory effects of VES operation on microbial activity. Microbial activity levels, as indicated by O_2 consumption rates, in some cases increased following VES application but in other cases decreased (Hinchee et al., 1991). Insufficient information was presented to ascertain whether or not VES effects could be detected in microbial population densities. Prior to VES operation, hydrocarbon-degraders numbered from less than 10^2 CFU g^{-1} to almost 10^6 CFU g^{-1} soil; no information was given on population densities following VES operation (Hinchee and Arthur, 1991).

Miller et al. (1991) injected air at low flow rates to maximize jet fuel removal by biodegradation while minimizing volatilization (e.g., bioventing). These investigators showed that biodegradation could be supported by extracting as few as two subsurface pore volumes d^{-1}. Biodegradation rates, initially as high as 7 mg fuel kg^{-1} soil d^{-1}, dropped to as low as 1 mg fuel kg^{-1} soil d^{-1} after bioventing started. As bioventing continued, these rates tended to increase. Yet, after seven months of bioventing, the initial levels of microbial activity were not regained. Miller et al. (1991) found no enhancement in

biodegradation rates or levels following moisture or nutrient addition. The results of Miller et al. (1991) and Hinchee et al. (1991) indicated that ventilation may not enhance microbial activity *per se*, but rather allow greater quantities of fuel to be biodegraded by ensuring adequate O_2 supplies.

Few studies have examined the effects of ventilation on biodegradation in gasoline-contaminated soils. The lack of information probably results from the assumption that, when a VES is applied, contaminant volatilization is extensive and the biodegradation potential is negligible. But, gasoline volatilization rates usually decrease rapidly, thus making biodegradation a potentially important remediation process. Also, the residual gasoline left following ventilation may be remediated largely or solely by *in situ* biodegradation. There are at least two other unknowns regarding the effect of VES operation on microbial activity. First, the relatively high subsurface air-flow rates used by VESs have been postulated to enhance microbial activity by warming the subsurface. Second, the large volumes of air removed may inhibit microbial activity by extracting moisture.

This study was undertaken to determine immediate and long-term effects of VES operation on subsurface environments. In particular, we wanted to assess the changes occurring in microbial activity during and after VES operation. The ultimate goal was to gain a better understanding of 1.) the level of site remediation achieved by biodegradation during vapor extraction and 2.) how stimulatory effects -if any- of VES operation on stimulating microbial activity might be capitalized in follow-up bioremediation efforts.

Materials and Methods

Site history and description. The research site was the former location of an underground storage tank (UST) in Northwestern Wisconsin (Fig. 1). The UST, installed in 1963, contained leaded and unleaded gasoline until deactivated in 1989. Gasoline contamination in the surrounding soil, originating from unknown causes over an undetermined period, was detected during subsequent site investigations. The most heavily contaminated soil was excavated and replaced with clean fill. The remaining contaminant plume was 40 ft long and 25 ft wide at a depth of 10 to 18 ft (Fig. 1), and estimated to contain 900 Mg of contaminated soil. Contaminant concentrations ranged from 5,000 mg total petroleum hydrocarbons (TPH) kg^{-1} soil near the former tank bed to 20 mg TPH kg^{-1} (or less) on the plumes' periphery.

The subsurface soils at the site were primarily glacial outwash sands (sand 85%, silt 5%, clay, 10%) with the following characteristics: organic matter, 3.7 g kg^{-1}, bulk density 1.26 Mg m^3; hydraulic conductivity 1.9 x 10^{-2} cm s^{-1}; pH, 5.

VES components and operation. A Rotron model DR303 positive displacement blower (EG&G, Saugerties, NY) was used to extract subsurface atmospheres. The vacuum pump was connected by a PVC header (2 inch diam., schedule 80) to two extraction wells, one located near the site former tank bed (RW1, Fig. 1) and the other on the periphery of the contaminant plume (RW2, Fig. 1). The extraction wells (2 inch diam., schedule 80 PVC) were installed to a depth of 19 ft, with the bottom 10 ft sceened (0.020 slot) across the contaminated region. A four-inch-thick asphalt layer over most of the plume likely enhanced air-flow through the contaminated region and minimized extraction well short-circuiting from the surface.

During VES operation, only one well was vented at a time; a ball valve installed in the header was used to switch the operational well every 14 d. The pump was operated at -10 inches of water, producing a flow rate of 33 cfm, and a 50 ft-radius of influence for

each well. Operating at 33 cfm, the VES extracted one subsurface pore volume within an 18 ft-radius of each well every 1.3 h.

VES monitoring. Samples were taken from the ports in the header to analyze off-gases for gasoline, benzene, CO_2, O_2, and N_2. Monitoring clusters were installed across the site to analyze "local" conditions. These clusters included nested vadose zone piezometers, thermocouples, and soil moisture sensors. Sampling/measurement devices in these clusters were placed at depths of 10 ft, 13.5 ft, and 17 ft. Five clusters (points 3 to 7; Fig. 1) were located in plume, while two (points 1 and 2; Fig. 1) were installed in non-contaminated area. Pressure readings taken during VES operation confirmed that locations 1 and 2 were not influenced by the VES. Thermocouples (type T; Omega Engineering, Stamford, CT) and soil moisture sensors (Irrometer, Riverside, CA) were read using a hand-held thermometer (Omega Engineering model HH-21) and a model 200-X Watermark sensor (Irrometer), respectively.

Subsurface atmosphere sampling. A glass Y-tube was inserted into the piezometers and sealed by a Parafilm™ wrapping. The Y-tube provided an air-tight attachment from which subsurface atmospheres were taken. A Sensidyne (Sensidyne, Clearwater, FL) model BDX 530 portable air sampling pump was connected to one arm of the Y-tube; the other arm was sealed with a septum to provide a port for syringe sampling. The portable sampling pump was operated at two l min^{-1} for three min to develop the wells, and then at one l min^{-1} during gas sampling.

Gasoline and benzene were first collected from 10 l of subsurface air with charcoal adsorption tubes. Following gasoline and benzene sampling, 10 ml of air was withdrawn from the Y-tube with a 10 ml Pressure-lok® syringe (Precision sampling, Baton Rouge, LA). The gas samples were then injected into "glycerin tubes" for storage until analyzed by gas chromatography. The glycerin tubes were glass tubes (0.3 cm i.d. x 325 cm l.) that stood upright in 500 ml of glycerin contained in a one liter beaker. One end of the tubes was sealed with a septum, the other was open to allow filling (3 ml) from the glycerin

reservoir. Gas samples were injected to completely displace the glycerin from the tubes as indicated by bubbling from the open end; field blanks (ambient air samples) were also injected into the glycerin tubes.

Soil sampling. A hand auger fitted with a 9 cm bucket was used to obtain vadose zone samples from depths of 10 to 17 ft. Before taking soil samples for microbial population analysis, the bucket was cleaned with dilute household bleach (1:100, v:v, bleach:water) and rinsed with sterile water. Soil samples were transferred to sterile glass jars by using a sterilized spatula, and stored on ice for transport to the laboratory. Soil samples analyzed for gasoline-range organics (GRO) were placed in to 110 ml glass vials without headspace; samples analyzed for petroleum volatile organic chemicals (PVOCs) were stored in 55 ml bottles containing 5 ml methanol.

Contaminant analysis. Contaminant analysis was done by certified laboratories. Carbon adsorption tubes were analyzed for gasoline and benzene by NIOSH method 1500. Soil GRO analysis was done according to WDNR publication SW-141; PVOCs were determined by EPA method 602.

Analysis of CO_2 O_2, N_2, and CH_4 by gas chromatography. A Fisher (Itasca, IL) model 1200 gas partitioner fitted with dual thermal conductivity detectors was used. Gases were separated isothermally (50°C) by sequential passage over 80/100 Poropak Q and 60/80 Molecular Sieve 13X columns with helium as the carrier gas. The partitioner was standardized by making double injections (400 µl each) of room air and then double injections (400 µl each) of a gas standard with the following composition: CO_2, 25.11%; N_2, 23.15%; H_2, 2.04%; CH_4, 49.70%.

Microbial population enumerations. Soil microbial population densities were determined by serial dilution plating. Soil (10 g) was added to 90 ml of a sterile dispersing agent (1% sodium pyrophosphate, pH 7) and mixed with a magnetic stir-bar for 30 min. Serial dilutions were made by inoculating one ml of soil suspension into nine ml of fresh dispersing agent; appropriate dilutions were then plated (100 µl) onto a mineral salts

medium (MSM). The MSM pH was adjusted to 5 to approximate that of the soil; gasoline and benzene were supplied in the vapor phase. Colonies were counted after 21 d incubation at 26 °C. Oligotrophic populations were determined by counting colonies growing on either MSM without a hydrocarbon substrate or 1% peptone/trypticase/yeast-extract/glucose (1% PTYG; Balkwill and Ghiorse, 1985) medium.

***In situ* respirometry and biodegradation calculations.** Rates and amounts of gasoline biodegradation were estimated using *in situ* respirometry (ISR) tests. In the ISR tests, the soil was ventilated so that the subsurface atmosphere's O_2, CO_2, and N_2 composition approximated that of the background area. The VES was then shut-down, and changes in the subsurface gases monitored by analyzing piezometer air samples.

Oxygen depletion was converted to fuel biodegradation according using the following equation (Hinchee et al., 1992):

$$K_b = -K_o A D_o C_o / 100 \quad (1)$$

Where K_b is the fuel biodegradation rate (mg fuel kg^{-1} soil d^{-1}); K_o is the oxygen depletion rate (% d^{-1}), A is the volume air per mass soil (m^3 air kg^{-1} soil); D_o is the density of O_2 at 10 °C (1.38 g dm^{-3}); and C_o the O_2 / hydrocarbon mass ratio described below. Site-specific soil parameters used in these calculations were: bulk density, 1,260 kg dm^{-3}; volumetric water content, 7.6%; porosity, 0.52; air-filled pore space, 0.44. Equation 1 was also used to calculate gasoline biodegradation based on N_2 enrichments. In this case, K_o, D_o, C_o were replaced by K_N (N_2 enrichment, % d^{-1}), D_N (d_{N2} at 10 °C; 1.21 g dm^{-3}), and C_N, respectively. The values for C_o and C_N in (1) were calculated based on *n*-hexane fuel equivalents as follows:

$$C_6H_{14} + 3.3\ O_2 + NH_4^+ \quad \text{----> } C_4H_7O_{1.5}N \text{ (biomass)} + 2\ CO_2 + 5.5\ H_2O \quad (2)$$

$$C_o = 1.21$$

$$C_6H_{14} + 3.7\ NO_3^- + NH_4^+ \text{ ----> } C_4H_7O_{1.5}N + 1.85N_2 + 2\ CO_2 + 5.5\ H_2O \quad (3)$$

$$C_N = 0.60$$

Based on the above, equation 1 simplified to $K_b = 3.8K_o$ or $7.3K_N$. But, the number of moles CO_2 determined in the samples (corrected for the *in situ* temperature at the time of sampling) were on average 47% lower than the number predicted from the O_2 / CO_2 and N_2 / CO_2 ratios in equations 2 and 3. Aerobic and anaerobic biodegradtion estimates were therefore adjusted down by 23.5%, giving $K_b = 2.8K_o$ or $5.6K_N$.

Results

Contaminant volatilization. Gasoline concentrations in extracted atmospheres at RW1 ranged from 4,200 to 700 mg m^{-3} and 4,700 to 700 mg m^{-3} during the first and second operation cycles, respectively (Fig. 2A). The rapid depletion of subsurface gasoline vapors was also measured at the piezometers (Fig. 3). Initial benzene concentrations in off-gases at RW1 and RW2 were 60 mg m^{-3} and 11 mg m^{-3}, respectively (Fig, 2B). As expected, benzene concentrations in the off-gases decreased more rapidly than gasoline as a whole. The two week "down" periods for the extraction wells allowed gasoline vapors to re-accumulate in the ventilated pore spaces and thus increase the efficiency of VES operation. After four extraction cycles (150 days of operation), gasoline and benzene concentrations in RW1 and RW2 off-gases had decreased to levels near or below detection limits (Fig. 2A,B). Despite increasing the pump flow rate to 60 cfm for the fifth operation cycle, off-gas gasoline concentrations remained low (Fig. 2A,B). The sharp decrease in extraction efficiency indicated that the process had become diffusion-limited, and the VES was shut-off. Approximately 400 kg of gasoline was extracted during 180 d of VES operation (Fig. 4).

During the next 350 days, re-equilibration in subsurface contaminant vapors was monitored. Gasoline accumulations were not apparent until approximately 50 d following VES shut-down. Maximum fuel accumulations did not occur until 100 to 200 d following VES shut-down (Fig. 3). Gasoline vapors then steadily declined from days 325 to 400.

The decrease in gasoline levels coincided with an increase in microbial activity as measured by O_2 consumption and N_2 production rates (see below). Benzene vapor accumulations were also detected but occurred more sporadically than gasoline vapor accumulations.

After 230 days off the VES was re-started. Off-gas gasoline concentrations were low, ranging from 6 mg m^{-3} when the system was first started to non-detectable when shut down 30 days later. Contaminant levels in samples taken from the piezometers were also low (Fig. 3). The GC analysis indicated that the residual contaminant resembled stoddard solvent or naphtha more than gasoline.

Temperature and moisture. Subsurface temperature variations in the VES-affected regions were similar to those occurring in the background area and followed a normal annual pattern (Fig. 5). The VES had no measurable effect on this pattern: Temperatures at the monitoring points were the same whether or not the VES was operating.

During VES operation, there was a slight drying in some areas as indicated by an increase in soil moisture tension (Fig. 6). In most cases, the cessation of this drying trend coincided with VES shut-off. During the subsequent 230 days, soil moisture tensions fluctuated widely and were likely the result of unusually heavy precipitation in the late winter and early spring of 1993. The precipitation negated any drying effect of the VES.

Subsurface atmospheres as indicators of microbial activity. Prior to VES operation, O_2, CO_2, N_2 levels in the contaminated area differed substantially from the background soil (Table 1). Oxygen concentrations were as low as 2%, while CO_2 and N_2 levels were increased up to 14% and 88%, respectively. There was a linear correlation between oxygen depletion and CO_2 production (Fig. 7A), while the N_2 / O_2 and N_2 / CO_2 relations were curvilinear (Fig. 7B,C). While N_2 enrichments indicated the occurrence of denitrification, there was no evidence of more highly anaerobic processes: Traces of CH_4 or H_2S were not detected.

After six days of VES operation, the subsurface atmosphere composition approximated that of the background soil (Fig. 8A-M). In a short ISR test conducted on

day six, O_2 consumption rates ranged from 6.2 to 1.4 % d^{-1}. But, by the second ISR test (started after 63 days of VES operation), the highest O_2 consumption rate measured was 0.16% d^{-1}; as a whole, microbial activity in the plume was less than that measured at monitoring point two in the non-contaminated area. Subsurface N_2 levels approximated ambient concentrations for the duration of ISR tests 1 and 2.

Subsurface atmospheres stabilized for the remainder of VES operation. In most cases, CO_2 and O_2 levels approximated that of the non-contaminated soil and the N_2 levels that of the surface (Figs. 8A-M). After VES shut-down, there was a "lag period" of at least 70 d before significant depletions of O_2 and concomitant N_2 and CO_2 enrichments occurred (Fig 8A-M). Monitoring from days 245 to 409 constituted a long-term ISR test (ISR test 3) during which time O_2 consumption rates at the monitoring points ranged from non-detectable to 0.02 % d^{-1}. The corresponding biodegradation rates were 0 to 0.6 mg fuel kg^{-1} soil d^{-1} (Fig. 9). Aerobic biodegradation rates during ISR test 3 were intermediate between those measured in ISR tests 1 and 2.

The results of ISR test 3 also provided the first indications of anaerobic activity (denitrification) since the VES was started. Rates of N_2 production ranged from 0.02 to 0.05 % d^{-1}. Calculated anaerobic biodegradation levels were about ten times lower than the corresponding aerobic rates and ranged from 0.05 to 0.2 mg kg^{-1} soil d^{-1} (Fig. 9). There was no indication of a "threshold" O_2 level below which denitrification occurred. Instead, O_2 consumption and N_2 production occurred simultaneously (Fig. 8B-M).

Locations with higher microbial activity levels were usually also those with higher residual gasoline concentrations. But, enhanced microbial activity occurred in the plume even in locations were gasoline vapors where non-detectable. Oxygen levels did not drop to concentrations measured prior to VES start-up (e.g., 2%) and biodegradation should not have been O_2-limited. Yet, on day 409, the VES was restarted and run for 30 d to replenish the subsurface atmospheres in preparation for a fourth ISR test.

Aerobic activity measured during ISR test 4 was at least two times greater than that measured in ISR tests 2 and 3, respectively. Denitrification levels had also greatly increased. In fact, the denitrification levels had increased to a point where, in many cases, anaerobic biodegradation rates were greater than the corresponding aerobic degradation rates (Fig. 9). Again, "hot spots" were biodegradation rates were the greatest were also those with the highest residual contaminant level.

Plate-count analysis of microbial populations. The impact of gasoline contamination on the soil microbial populations was readily apparent in the plate counts. Densities of hydrocarbon degraders in the plume were higher than in the non-contaminated soil (Fig. 10). But, as indicators of VES effects on microbial populations, plate count results were ambiguous. There were no clear trends in total aerobic heterotroph densities or hydrocarbon degraders over time that appeared to correlate to VES operation or soil gas analysis.

Soil nutrient and contaminant analysis. Soil nutrient levels varied across the site and with time (Table 2). Given this variability, it was difficult to discern nutrient depletion trends attributable to microbial activity. Although nitrate was, in general, lower at the later sampling dates, amounts of nitrate consumed by microbial activity vs. those removed by leaching could not be determined. Soil contaminant levels were substantially reduced, particularly the BTEX fraction, following VES operation (Table 3). The elimination of the BTEX fraction was apparent as early as 63 d after the start of VES operation. Yet, heavier gasoline constituents (GRO; Table 3) persisted even after 180 d of VES treatment and 260 d of biodegradation .

The total amount of gasoline eliminated from the soil was estimated to be 538 l (Table 4). Of the 538 l, 409 l (76%) was achieved by volatilization (Table 4). Biodegradation accounted for the elimination of 123 l of gasoline, mostly during the pre- (81 l) and post-VES (34 l) periods. Of the 123 l biodegraded, approximately 65 l occurred aerobically while 50 l anaerobically.

Discussion

The VES worked effectively with the level of gasoline contamination substantially reduced within the first 60 d of operation. The trends and rates of contaminant removal indicated that this system was functioning in a manner typical of VES operations.

Gasoline vapor re-accumulation was expected following VES shut-down. But, months passed before gasoline vapors reached maximal concentrations. The re-accumulation period was likley a function of diffusional constraints and not simply a reflection of increasing temperatures: Subsurface temperature increases during the re-accumulation period ranged from 4 to 9 °C, but these increases lagged behind the periods of maximum fuel accumulations. These results indicate that monitoring only a few weeks past the termination of VES operations is insufficient to accurately assess soil remediation levels. A more appropriate monitoring period may be two to three months following VES operation; even longer periods may be required depending on soil characteristics.

Soil analysis indicated that VES operation resulted in substantial clean-up. While some contamination remained, further VES operation would probably not be productive as the residual contaminant was sufficiently non-volatile and/or dilute to be measurable in the VES off-gas. The change in the overall character of the soil contaminant from light to heavy constituents was confirmed by the GC analysis. Hydrocarbon profiles in the final gas samples taken from piezometers resembled heavier hydrocarbon mixtures (stoddard solvent or naptha) rather than gasoline. Further remediation of the residual contaminant will be dependent on *in situ* biodegradation.

It has been speculated that warmer surface air introduced into the soil by VES operation may aid the remediation process by heating the subsurface, thereby enhancing volatilization and biodegradation. We found no evidence supporting this theory. Seasonal temperature changes in the plume were similar whether or not the VES was operating.

A concern often raised is that the high air extraction rates used in VES operations may inhibit biodegradation by drying the subsurface. While subsurface drying occurred during VES operation, the degree of moisture loss was probably insufficient to induce moisture stress. Microbial activity did appear to increase in the subsurface during the re-wetting period. But, attributing this effect to moisture levels was confounded by changes in subsurface temperature and possibly substrate (contaminant) availability.

A primary project goal was to assess the effects of VES operation on microbial populations/activity in the subsurface. The increased number of hydrocarbon degraders in samples taken from the plume provided a clear indication of the impact of gasoline contamination on the soil population. Yet, plate counts were ambiguous as indicators of VES operation effects: Densities of total heterotrophic bacteria or hydrocarbon degraders did not display trends indicating positive -or negative- VES effects. It is likely that microbial population growth occurred during VES operation (see below), but the amount of growth was probably insufficient to be detected by plate counts. Another factor limiting the resolution and accuracy of the plate counts was the soil heterogeneity and the difficulty in obtaining replicate vadose zone samples. Growth did not appear to be limited by nutrient levels; inorganic N, P and S did not drop significantly during the course of the study. As indicated above, moisture levels also appeared adequate to support microbial activity.

Soil ventilation is generally postulated to increase microbial activity levels by maintaining aerobic conditions in the subsurface. Yet, our results, as well as those of other investigators (Hinchee et al., 1991; Miller et al., 1991), indicate that microbial activity actually decreases during the initial phases of soil ventilation. This short term effect of soil ventilation is most likely attributable to substrate removal. It was unlikely that increases in subsurface O_2 inhibited aerobic activity. Furthermore, our monitoring showed that the subsurface moisture levels had not been substantially reduced, and that subsurface temperatures were near maximum during the period that activity levels decreased.

More difficult to explain are the long-term effects, specifically the slow increase in microbial activity following several months of ventilation. One explanation for this "lag effect" is a diffusion-controlled increase in substrate availability. In this case, microbial population densities are probably the greatest in larger pores, which are also the channels from which substrate will be preferentially removed by ventilation. Thus initial decreases in microbial activity might reflect the reduction in substrate available to the majority of the microbial population. Microbial activity might increase following VES shut-down (or perhaps several months of ventilation at low air-flow rates) as gasoline diffuses back into the larger pores from capillaries that were not effectively ventilated. While this hypothesis is generally consistent with our monitoring data, it is at odds with the observed increase in microbial activity levels between ISR tests 3 and 4. In this case, VES operation between ISR tests 3 and 4 should have eliminated any accumulated vapors, and thus resulted in lower microbial activity levels in ISR test 4. While higher subsurface temperatures could partially account for the increased activity in ISR test 4, there are likely additional factors involved that are not accounted for in the diffusion hypothesis.

Another possibility is that the delayed microbial activity increase could be attributed to a VES-induced shift in hydrocarbon metabolism. In this case, the microbial population may initially be dominated by bacteria metabolizing the lighter hydrocarbons (e.g., aromatics and short-chain alkanes). As these are extracted, the microbes are then required to produce a different set of enzymes that attack the heavier constituents (e.g., long-chain alkanes, polynuclear aromatic hydrocarbons). As the spectrum of hydrocarbons degraded by any given bacteria is typically limited (Ridgeway et al., 1990), this process might lead to the proliferation of a different subset of the bacterial population. This population would theoretically "specialize" in degrading the residual contaminant by the production of a different class of oxygenases and/or solubilizing agents.

The occurrence of denitrification at high subsurface O_2 levels indicated that microbial O_2 utilization rates were sufficiently high so that O_2-limitation occurred

regardless of VES operation. Aerobic biodegradation rates appeared to be limited by factors other than O_2 diffusion through the soil solution to the microbial population (discussed below); Thus percent level soil O_2 contents should be considered as providing an O_2 pool that allows a greater mass of hydrocarbon to be degraded rather than increasing degradation rates.

Biodegradation estimates based on gas production are subject to numerous inaccuracies. In addition to the sampling and measurement errors, there is the problematic aspect of determining the stoichiometric relation between gas consumption / production and fuel biodegradation. The use of stoichiometries omitting cell growth have been used because they 1.) are simple to derive and 2.) give conservative estimates of fuel biodegradation relative to values based equations including cell growth. We found these approaches to inadequately model biodegradation. This is because, the number of moles CO_2 produced (calculated based on the *in situ* temperature at the time of sampling) were on average 80% lower than the number of moles CO_2 expected (based on the moles O_2 consumed plus the moles N_2 produced). By incorporating biomass production, the estimates were still on average 47% higher than the measured amounts of CO_2.

It could be argued that, given the reactivity of CO_2 in soils, predicted CO_2 production should overestimate measured amounts. We therefore considered physical-chemical processes that might affect soil CO_2 levels. At pH 5 (the soil pH), CO_2 will be primarily in equilibrium with H_2CO_3 (which represents $CO_{2(g)}$, $CO_{2(l)}$, and H_2CO_3). Given a Henry's constant for this equilibrium of $10^{-1.5}$, the soil solution CO_2 concentration would be approximately 3% of the partial pressure of CO_2 in the soil atmosphere. Thus, the error introduced by CO_2 dissolved in the soil solution should be negligible in these estimates. Differential diffusion rates may also contribute to inaccuracies in the relative amounts of CO_2, N_2, and O_2 measured. But, as gas diffusion rates are inversely proportional to the square root of their molecular weight, a slight CO_2 enrichment would be expected relative to N_2 and O_2. Again, the magnitude of this enrichment is negligible for

the estimates made here. A third physical process that may affect CO_2 levels is sorption onto soil surfaces. The magnitude of this occurrence is difficult to estimate, however, Wood et al. (1993) successfully modeled soil CO_2 production while ignoring this processes. Sorption was, therefore, also ignored in the present study.

It is apparent from the above that soil physical-chemical processes are inadequate to account for the discrepancy between measured and predicted CO_2 levels. This means that the error was associated mainly with inaccuracies in the stoichiometric relation. Agreement between the predicted and measured CO_2 levels could be improved by including biomass production in the stoichiometric relations and/or by using fuel equivalents with a higher carbon oxidation level than *n*-hexane (e.g., benzene). These adjustments also result in higher fuel biodegradtion estimates. We believe that the discrepancy between predicted and measured CO_2 was a strong indicator that the initial stoichiometric relations examined (*n*-hexane equivalents, no biomass production) underestimated biodegradation. Therefore, we believe the partial adjustment of these calculations by the inclusion of biomass production was justified.

The N_2 enrichment, which was indicative of denitrification, has many implications. In a practical sense, the increased N_2 levels mean that additional biodegradation coupled to denitrification occurred that was not accounted for by O_2 consumption measurements. Denitrification is typically overlooked by investigators quantifying fuel biodegradation in the vadose zone. Yet, our results suggest denitrification supported a substantial amount of the gasoline biodegradation. Furthermore, given the soil pH of 5, it is possible that the N_2 production provided a conservative estimated the process: At low soil pH, N_2O may account for half or more of the denitrification end-product. The GC method we used did not detect N_2O. Thus, denitrification (as measured by N_2, N_2O analysis) should be considered to more accurately assess amounts and rates of biodegradation.

The occurrence of high denitrification levels raises several basic scientific questions. The most fundamental issue regards the mechanisms/conditions allowing

relatively high denitrification rates in an environment that is essentially O_2-saturated. The role of O_2 in repressing synthesis of proteins mediating dissimilatory nitrate reduction is well-established, and the O_2 threshold below which denitrification occurs is low (7.6 μM, 0.24 ppm; Tiedje, 1989). Still, there are many reports of denitrification in aerobic soils (Burford and Stefanson, 1973; Stefanson 1973). Anaerobic microsites could be used to explain denitrification under the aerobic conditions examined here: Although the soils appeared aerobic based on pore space O_2 content, O_2-limiting conditions might develop in the immediate vicinity of bacterial microcolonies metabolizing gasoline. In this case, nitrate-reducing enzymes might be de-repressed if O_2 diffusion to the microcolonies lags behind O_2 consumption.

The question that arises is whether denitrification could be attributed to anaerobic microsites or whether the soil population as a whole may be O_2-limited. At an O_2 consumption rate of 1.06 mg O_2 kg^{-1} soil (the highest rate measured in ISR test 4) the supply of dissolved O_2 would be exhausted in approximately 12 h (assuming an O_2 solubility of 9 mg l^{-1} and soil water content of 60 ml kg^{-1}). Maintenance of aerobic activity will be thereby be dependent on adequate O_2 diffusion rates. The issue, then, is whether O_2 diffusion rates are sufficient to sustain intracellular O_2 concentrations at levels suppressing production of denitrification enzymes.

As a first approximation, we can estimate the maximal O_2 flux to bacterial colonies by assuming that microbial activity levels are sufficiently high as to drive O_2 concentrations at the cell surfaces to 0.24 x 10^{-3} mg cm^{-3} (e.g., the dissolved O_2 concentration at which denitrifying enzymes are presumed to be de-repressed). Oxygen flux rates may then be calculated using the approach of Molz et al. (1986):

$$d_o = D_o [O - o / \sigma] \pi r^2$$

where d_o = the O_2 flux to a bacterial colony (mass colony^{-1} time^{-1}); D_o = the diffusion coefficient for O_2 in water (cm sec^{-1}), O = O_2 concentration in the pore space (mg cm^{-3}); o = water film O_2 concentration at the cell surface (mg cm^{-3}); σ = thickness of the overlaying soil water film (cm); r = radius of bacterial microcolony (cm). Assuming a soil water film thickness of 5 x 10^{-3} cm, an O_2 diffusion coefficient of 0.71 cm^2 sec^{-1}, a bacterial colony radius of 5 x 10^{-3} cm, and O_2 concentrations in the pore space and colony surface of 0.248 mg cm^{-3} (18%; v/v) and 0.24 x 10^{-3} mg cm^{-3}, respectively, the calculated O_2 flux is 8.8 x 10^{-4} mg O_2 colony^{-1} d^{-1}. If there are 10^7 colonies kg^{-1} (10^9 cells kg^{-1}, 100 cells colony^{-1}), the measured O_2 flux is 1.06 x 10^{-5} mg colony^{-1} d^{-1}, which is 83 times less than the amount theoretically supplied by diffusion. These greatly simplified calculations indicate that cellular O_2 supplies should not be limited by O_2 diffusion rates through the soil solution.

A second possibility is that, although O_2 levels at the cell surface may be high, O_2 levels to which the enzymes are exposed may be drastically decreased. This might occur if intracellular O_2 diffusion rates are substantially lower than those through the soil water films. This possibility can be evaluated by calculating the intracellular O_2 concentration drop from the cell surface to the site of enzyme (oxygenase) activity as follows (Johnson, 1967):

$$Ms - Me = 3.12\, r^2\, \mu\, [(1 / n) - 1 / D_c\, Y]$$

Where Ms = the O_2 concentration at the cell surface (moles l^{-1}); Me = the O_2 concentration at the site of enzyme activity (moles l^{-1}); r = cell radius (cm); μ = cell growth constant (sec^{-1}); n = the distance from cell center to the enzyme site (fraction of r); D_c = diffusivity of O_2 in the cell (cm^2 sec^{-1}); Y = cell yield based on O_2 (g cells formed / g O_2 consumed). It is assumed that the site of enzyme activity (n) is half way from the center of the cell, r = 2.5 x 10^{-4} cm, μ = 0.19 h^{-1}, D_c = 1.08 x 10^{-5} cm^2 sec^{-1}, and Y = 0.278. This gives a very slight O_2 concentration drop of 0.83 μM (0.027 ppm). Collectively, the two foregoing calculations indicate that the intracellular O_2 concentration should be relatively high and

approximate that of the surrounding soil solution. It is interesting to note that threshold O_2 levels for de-repression of nitrate-reducing enzymes are in the region predicted to be sensitive to intracellular O_2 concentration drop.

The foregoing calculations indicate that "aerobic denitrification" was not adequately explained by limited O_2 diffusion to the microbial population as a whole. Thus, the alternative hypothesis, the occurrence of anaerobic microsites, appears to be borne-out. These microsites may occur via the combined effects of "locally" high hydrocarbon degrader densities (e.g., 10^7 to 10^8 cells g^{-1} soil) and fuel concentrations. An increase in water-film thicknesses would also contribute to the establishment of anaerobic microsites, but less so than cell density and substrate level. Other mechanisms that impede O2 diffusion to cell surfaces may also influence the shift to anaerobic metabolism (e.g., extracellular polymers that impede O_2 diffusion to the cell cytoplasm). Another possibility is that cells residing in overlying colony layers act as O_2 sinks and deplete the dissolved O_2: Cells deeper within the colony are thereby deprived of O_2 and may then shift to anaerobic metabolism.

Conclusions and Recommendations

Subsurface gasoline contamination at the Fairchild Ranger Station was substantially reduced and, in many cases, approached levels warranting site closure. While most of the remediation was achieved via VES-enhanced volatilization, substantial contributions were made by biodegradation. The residual contamination, which is relatively resistant to removal by VES operation, will likley serve as a substrate for the microbial population and be reduced over time by on-going biodegradation.

Applying "passive" bioremediation to the residual contaminant is the most reasonable approach. The alternative, active bioremediation involving nutrient addition, and perhaps pH adjustment, would probably be of limited value. This is because 1.) there

was no evidence that nutrients and/or pH were limiting biodegradation and 2.) a clay layer occurring above the region where most of the residual contaminant is located would impede the introduction of biostimulants by standard approaches (e.g., infiltration galleries). The site will probably be most efficiently managed by operating the VES for relatively short periods (7 to 10 d) every 100 d to maintain adequate subsurface O_2 supplies.

VES operation had both positive and negative effects on microbial activity. The negative effect was apparently the removal of substrate, which resulted in reduced microbial activity levels. The positive effect was the resupply of O_2 that allowed a greater amount of residual contaminant to be degraded. Although the causes were unclear, the increase in O_2 consumption rates following VES operation may also have been a positive VES effect (e.g., the lag effect).

To quantify VES effects on microbial activity, ISR tests conducted over a period of weeks rather than days, as previously suggested (Hinchee et al., 1992), are recommended. ISR tests should be done at piezometers -not extraction wells- whenever possible and the air samples should be analyzed for O_2, CO_2, N_2 and N_2O. Quantification of N_2 and N_2O (denitrification) is strongly recommended because of the fuel biodegradation associated with the production of these gases and the potential to use the occurrence of denitrification as an indicator of O_2 limitations. While biodegradation estimates are most easily based on O_2 / N_2 (N_2O) consumption / production rates, the accuracy of these should be checked by comparison of the measured CO_2 levels to those predicted based on the assumed stoichiometric relationships. Plate counts of aerobic hydrocarbon degraders do not appear suitable for monitoring VES effects.

In a permeable soil, VES operation is clearly the method of choice for achieving maximum gasoline removal in the shortest time. While the large amounts of substrate removed by VES operation will likely reduce subsequent microbial activity, VES operation is unlikely to exert true inhibitory effects (e.g., desiccation). While amounts and rates of biodegradation are much less than those achieved by volatilization, the process can be

significant over time. Biodegradation will be particularly important for removal of the heavier gasoline fractions that persist as soil residues following VES.

Acknowledgments

Thanks are due to Kurt Jacobsen for assistance in designing, installing, and monitoring the VES and to Fred Madison and the Wisconsin Geological and Natural History Survey for instrumenting the research site. The help of many graduate students from the Department of Soil Science in sampling the site was greatly appreciated. Thanks are also due to Laura Chern, John Grump, and Greg Marks of the WDNR for making this project a reality. The assistance and cooperation of Jonathan Stone and Fairchild Ranger Station Staff was greatly appreciated.

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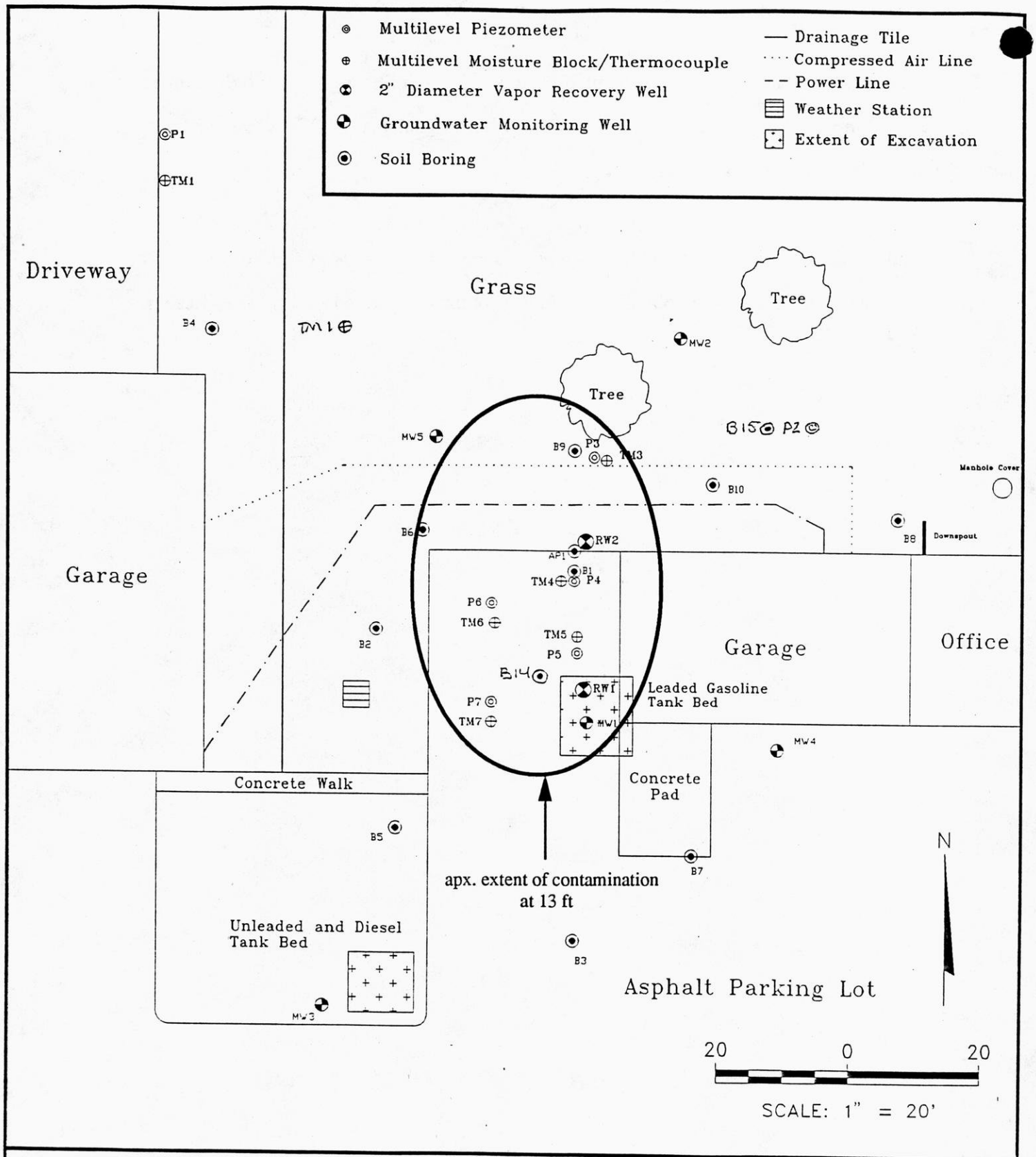
Wood., B.D., C.K. Keller, and D.L. Johnstone. 1993. *In situ* measurement of microbial activity and controls on microbial CO_2 production in the unsaturated zone. Water Resour. Res. 29:647-659.



Monitoring Point Locations

Fairchild Ranger Station

Fig. 1.

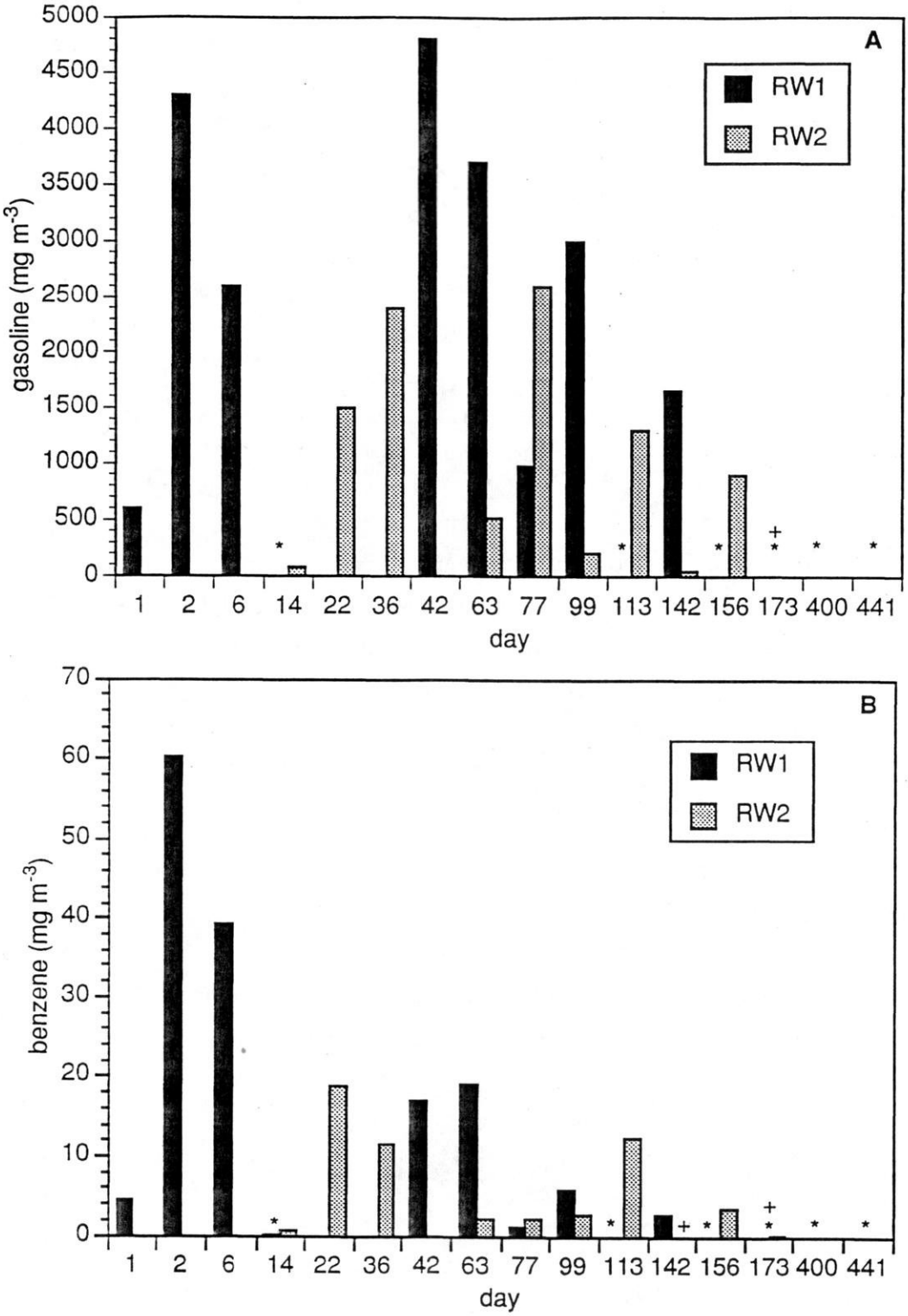
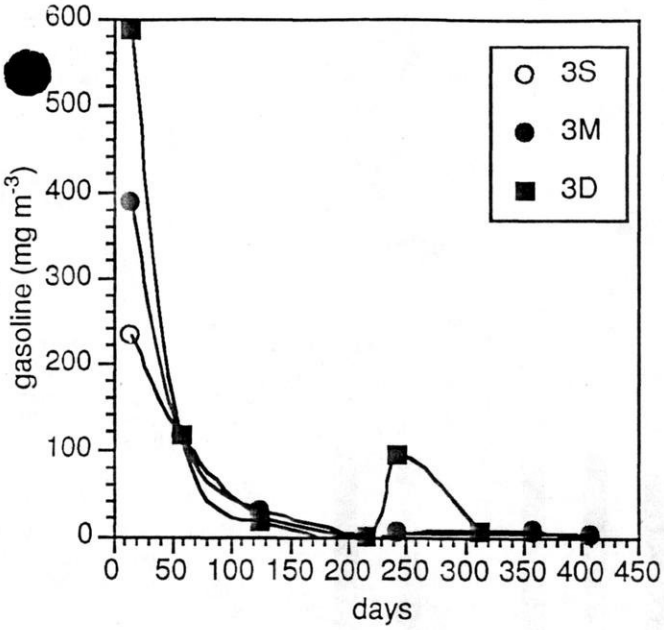
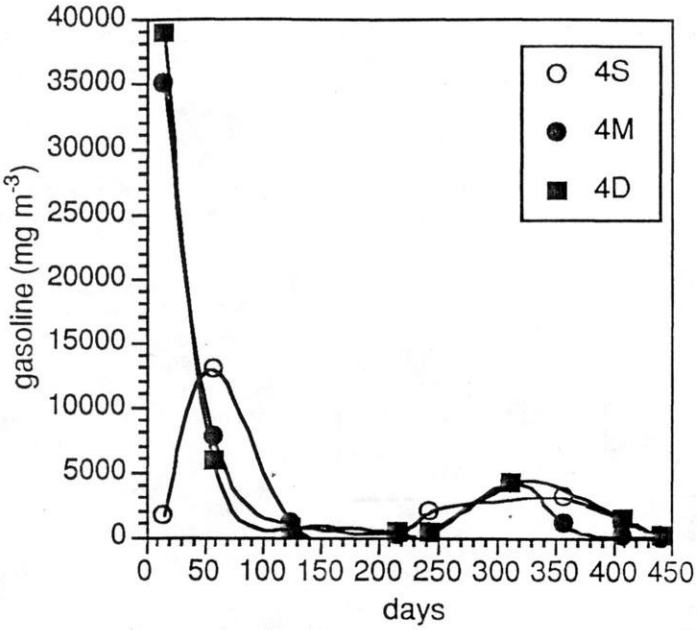


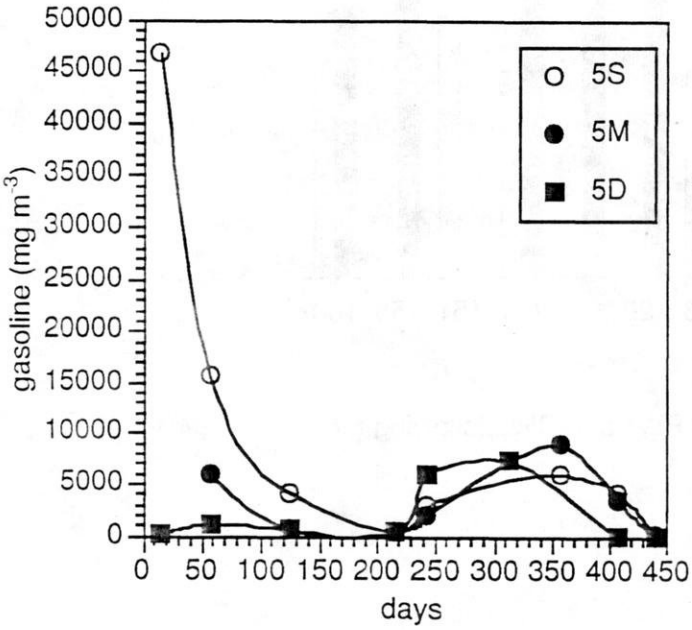
Fig. 2. Gasoline (A) and benzene (B) vapor concentrations in extraction well off-gases. In A, low level gasoline detections at RW1 (*) were (day); 0.7 (14), 4 (113), 0.5 (156), 1.5 (173), 6 (400), 0 (410). Low level gasoline detection at RW2 (+) was 1.0 (173). In B, low level benzene detections at RW1 (*) were (day); 0.04 (14), 0 (113), 0 (156), 0.03 (173), 0 (400) and 0 (410). Low level benzene detections at RW2 (+) were (day); 0 (142), 0.03 (173).

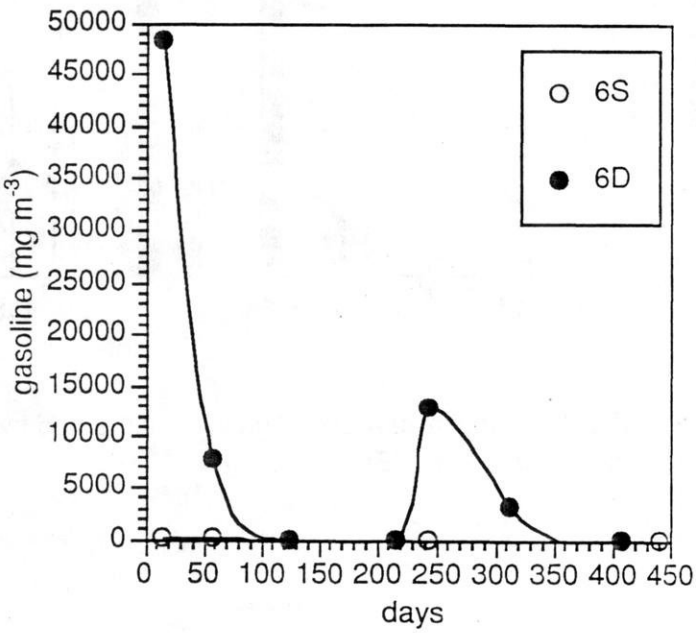
Overleaf

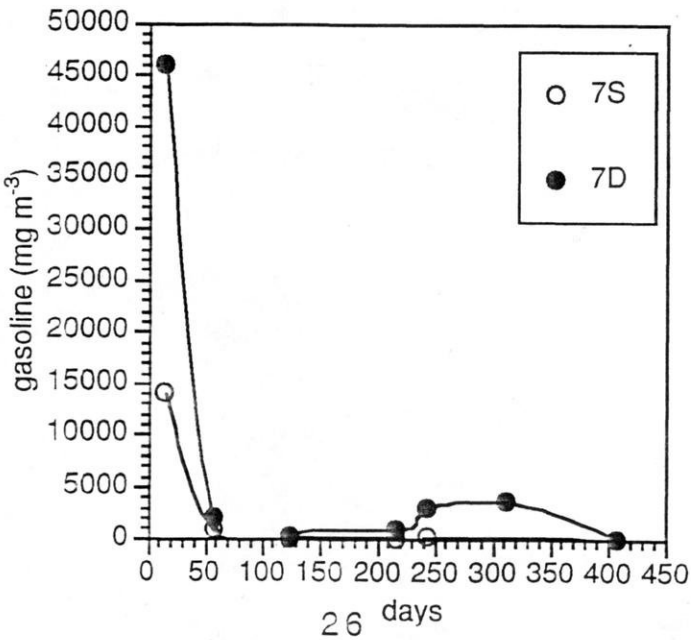
Fig. 3. Variations in subsurface gasoline vapor concentrations measured at the piezometers. Legend; 3S = shallow depth at monitoring point 3 (see Fig. 1 for monitoring point location). Sampling depths; S = 10 ft., M = 13 ft., and D = 18 ft.


3S
3M
3D
gasoline (mg m-3)
days
0
100
200
300
400
500
600
0 50 100 150 200 250 300 350 400 450


4S
4M
4D
gasoline (mg m-3)
days
0
5000
10000
15000
20000
25000
30000
35000
40000
0 50 100 150 200 250 300 350 400 450


5S
5M
5D
gasoline (mg m-3)
days
0
5000
10000
15000
20000
25000
30000
35000
40000
45000
50000
0 50 100 150 200 250 300 350 400 450


6S
6D
gasoline (mg m-3)
days
0
5000
10000
15000
20000
25000
30000
35000
40000
45000
50000
0 50 100 150 200 250 300 350 400 450


7S
7D
gasoline (mg m-3)
days
0
5000
10000
15000
20000
25000
30000
35000
40000
45000
50000
0 50 100 150 200 250 300 350 400 450

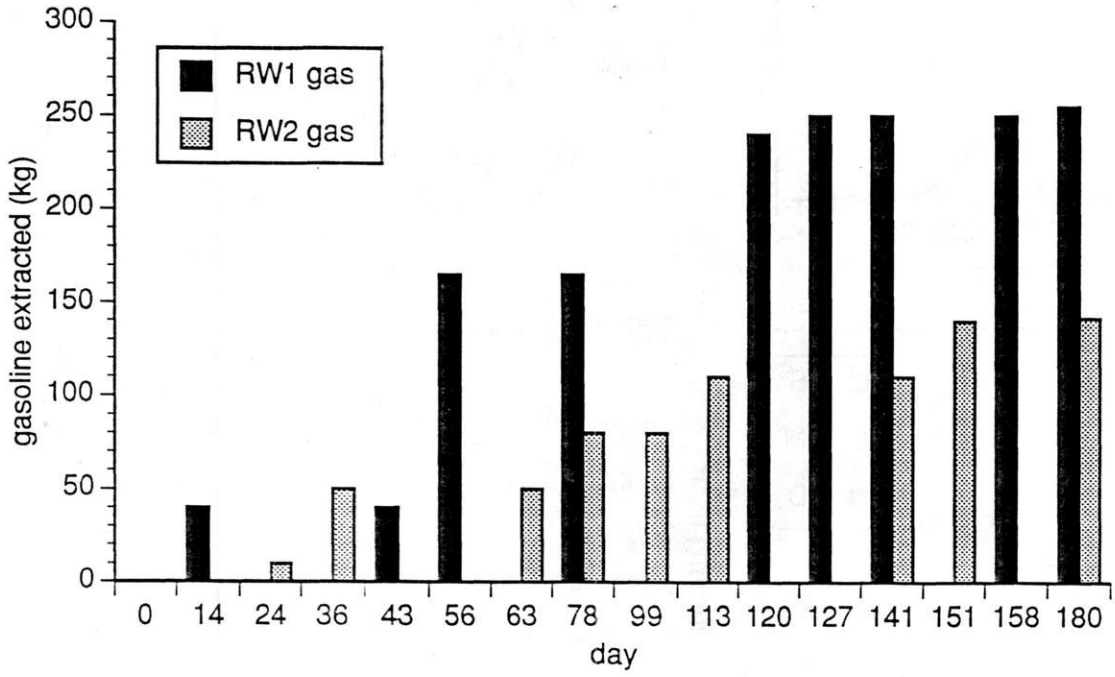


Fig. 4. Cumulative mass of gasoline extracted by RW1 and RW2 following the indicated time of VES operation.

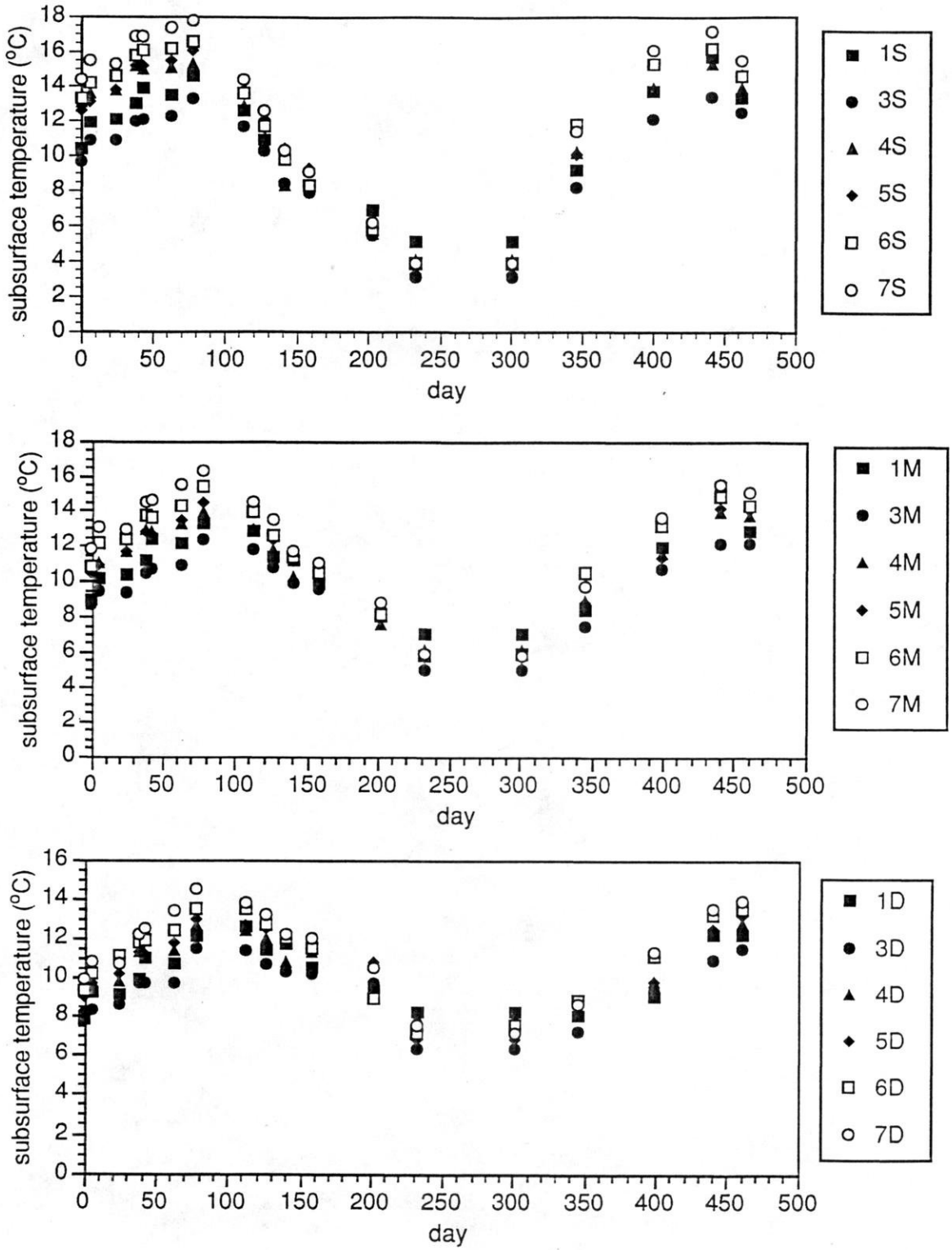
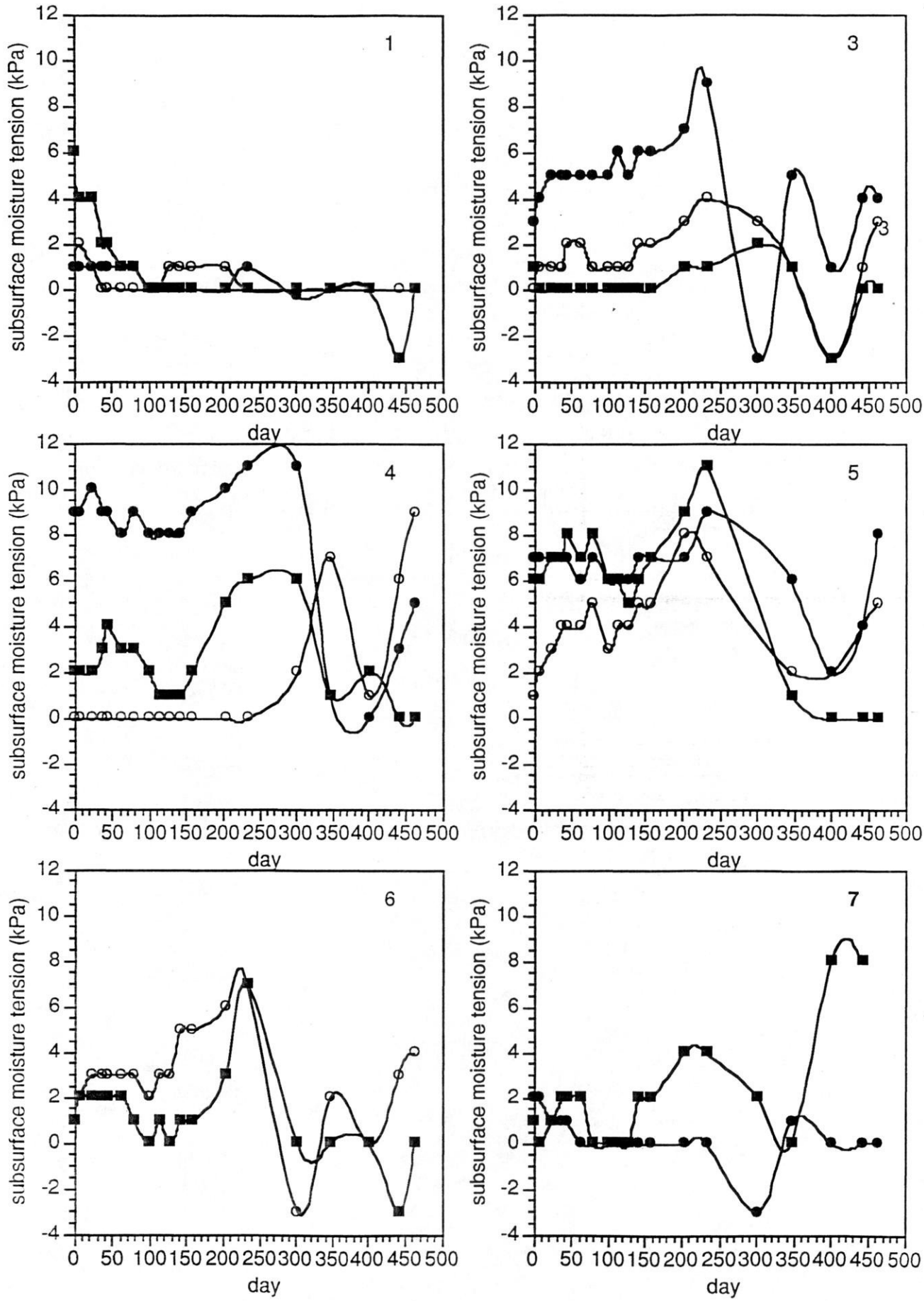


Fig. 5. Subsurface temperature variations at seven monitoring points and three depths; S 10 ft., M =13 ft., and D = 18 ft.

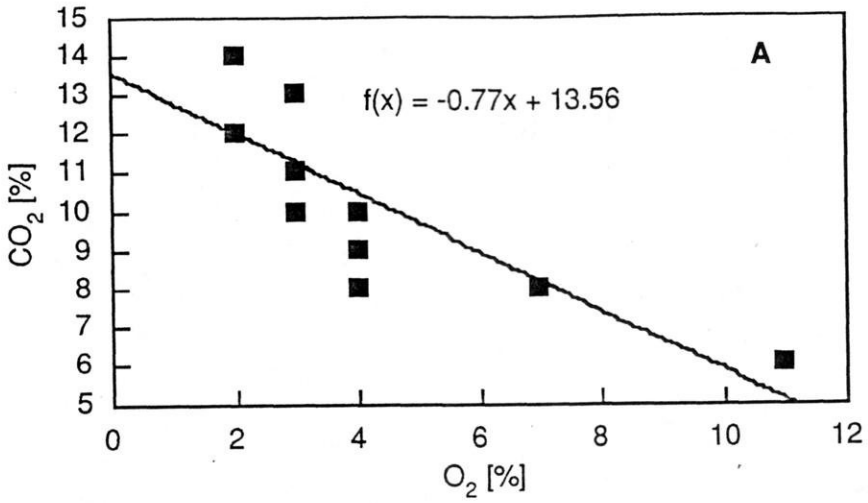
Overleaf

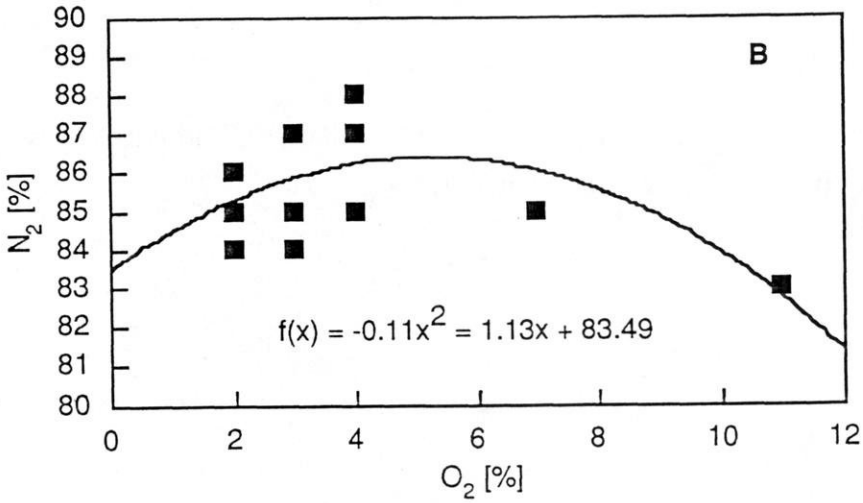
Fig. 6. Variations in subsurface moisture levels measured at the indicated monitoring location (see Fig. 1 for monitoring point location). The VES was in operation until day 180. Measurment depths; O = S (10 ft.), ● = M (13 ft.), and ■ = D (18 ft.).

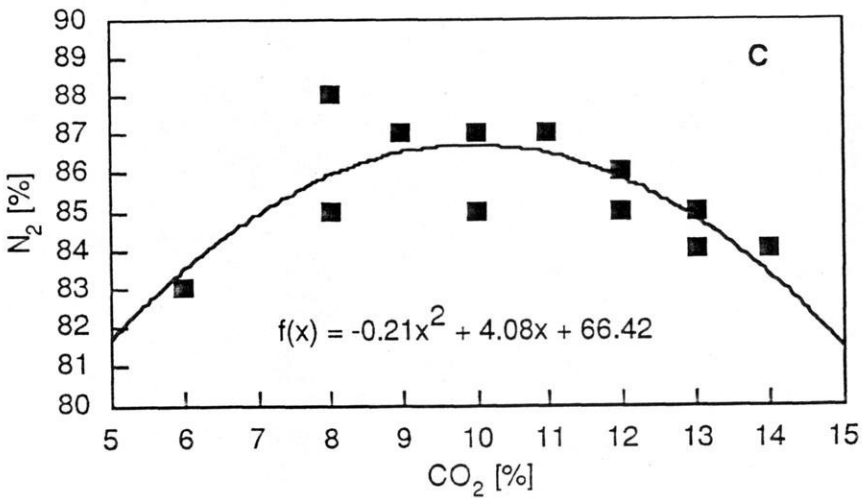

1
3
4
5
6
7
subsurface moisture tension (kPa)
day
12
10
8
6
4
2
0
-2
-4
0 50 100 150 200 250 300 350 400 450 500

Overleaf

Fig. 7. Relationships between subsurface gas concentrations measured prior to VES start-up. p-Values for the fitted curves were 0.0006 (A), 0.09 (B), and 0.0037 (C).


A
f(x) = -0.77x + 13.56
CO2 [%]
O2 [%]


B
f(x) = -0.11x2 = 1.13x + 83.49
N2 [%]
O2 [%]


C
f(x) = -0.21x2 + 4.08x + 66.42
N2 [%]
CO2 [%]

Overleaf

Fig. 8. Variations in subsurface O_2, CO_2, and N_2 levels measured at the indicated monitoring location and depth).

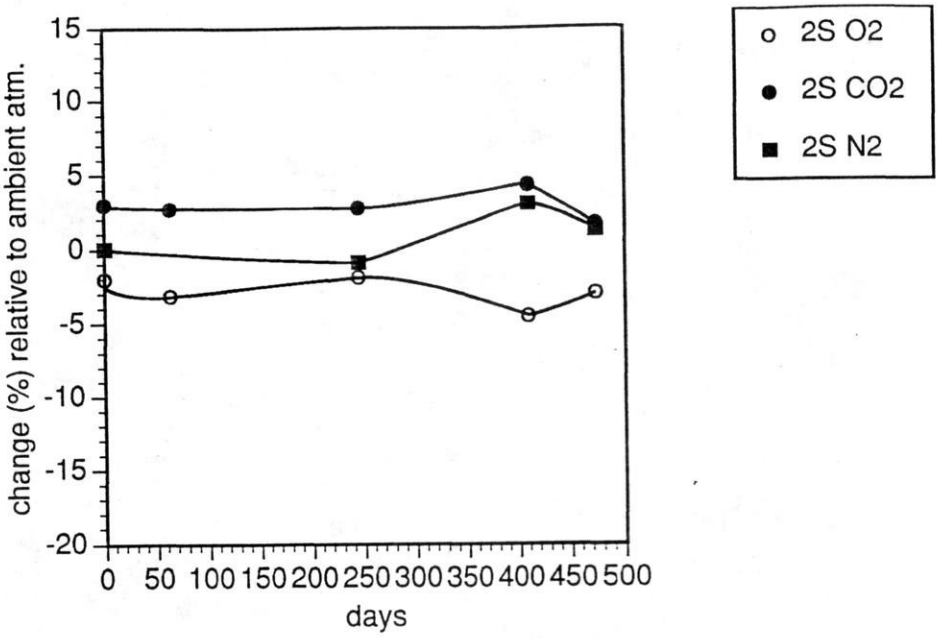


Fig. 8A

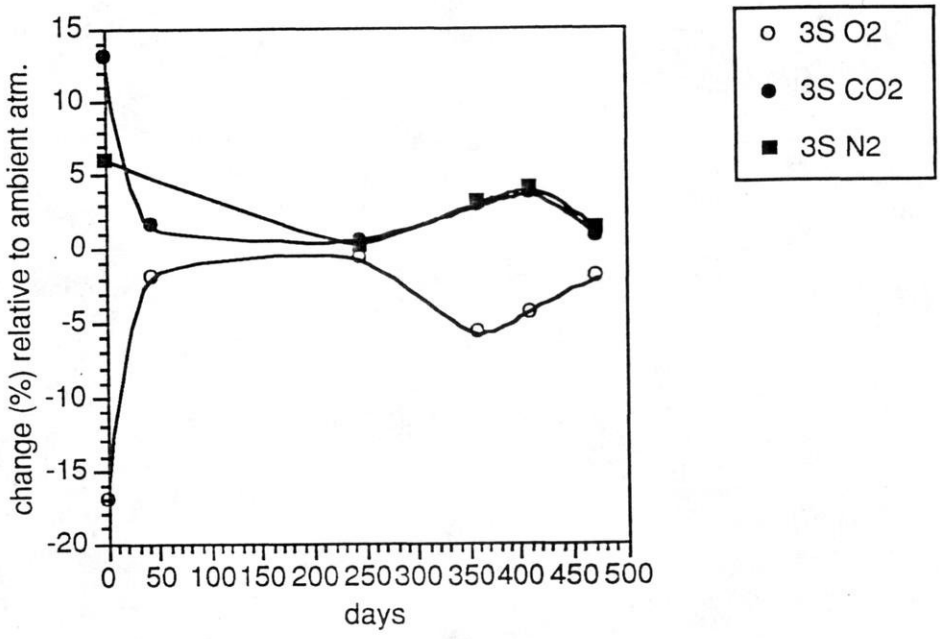


Fig. 8B

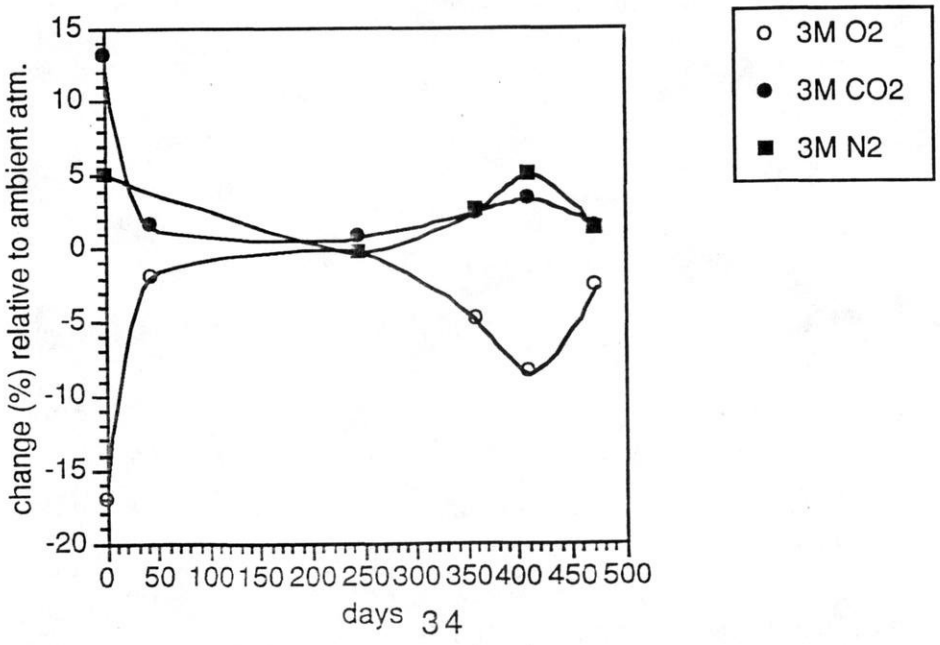


Fig. 8C

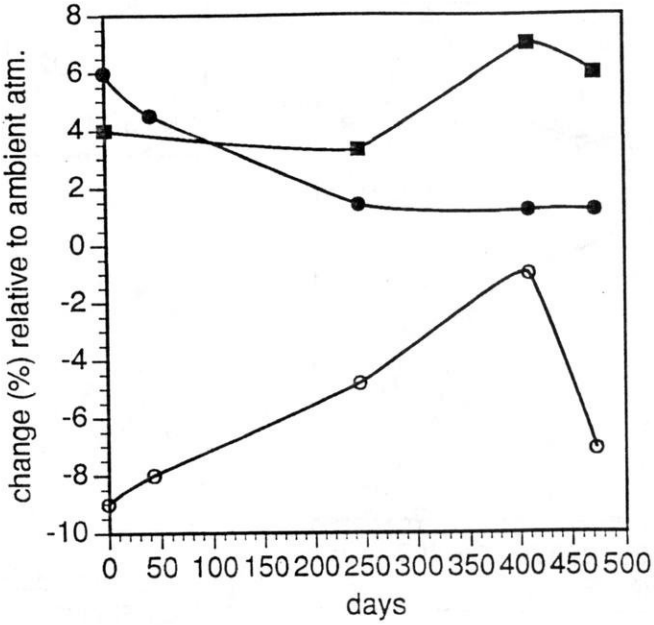


Fig. 8D

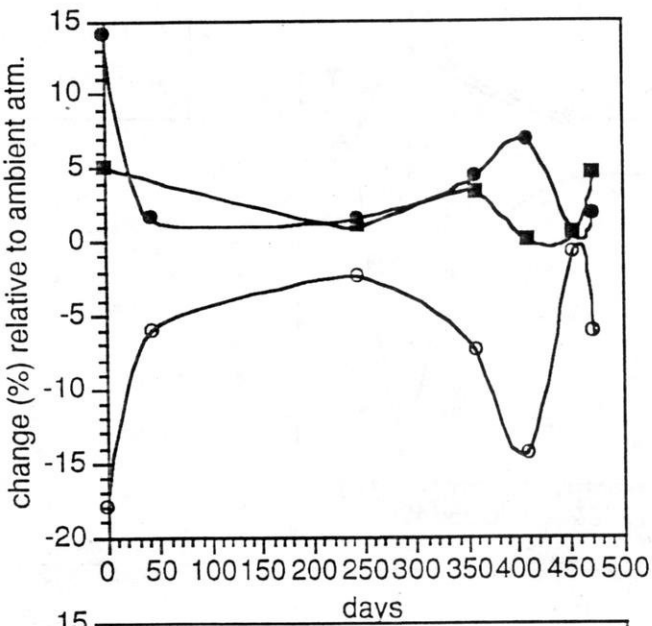


Fig. 8E

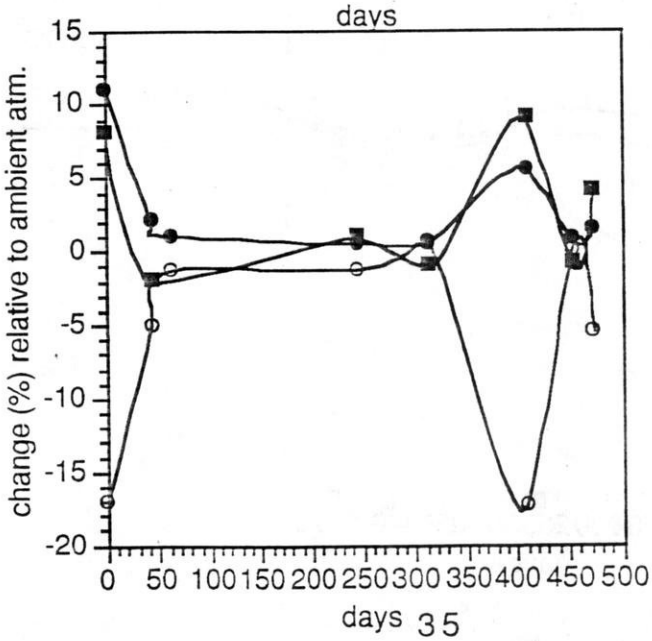


Fig. 8F

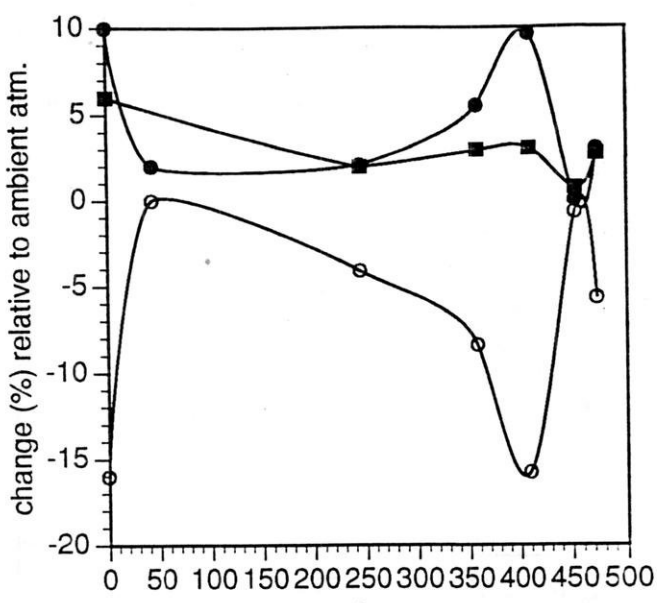


Fig.8G

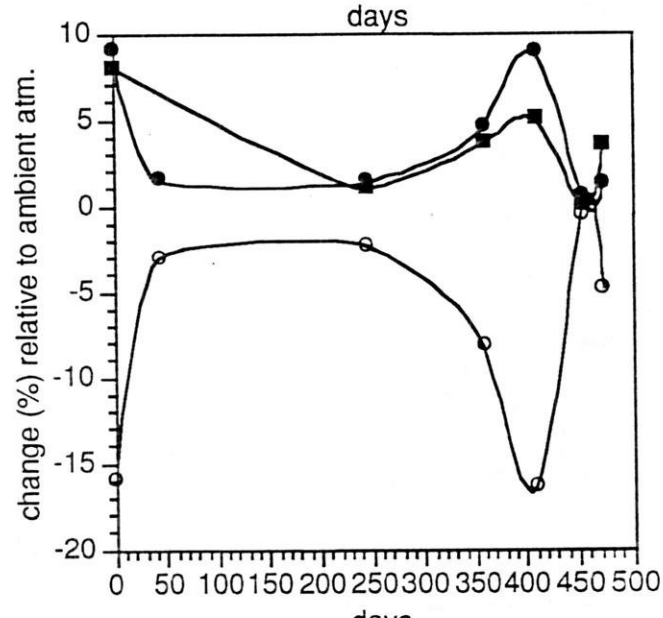


Fig. 8H

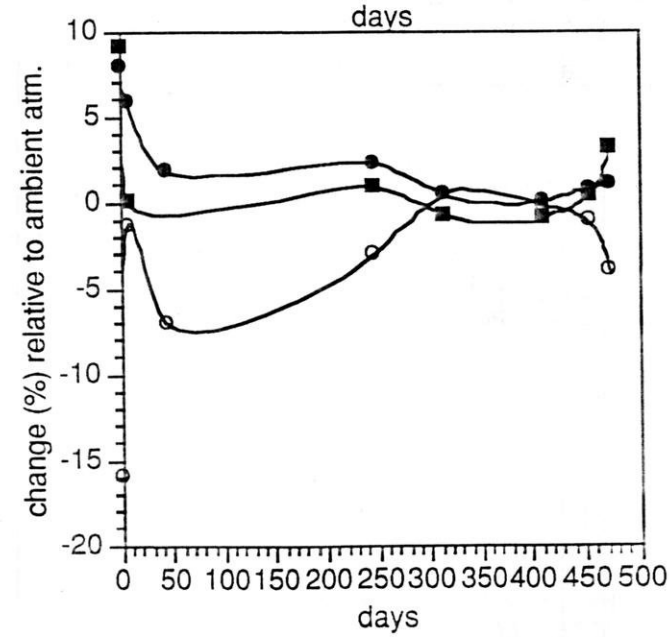


Fig. 8I

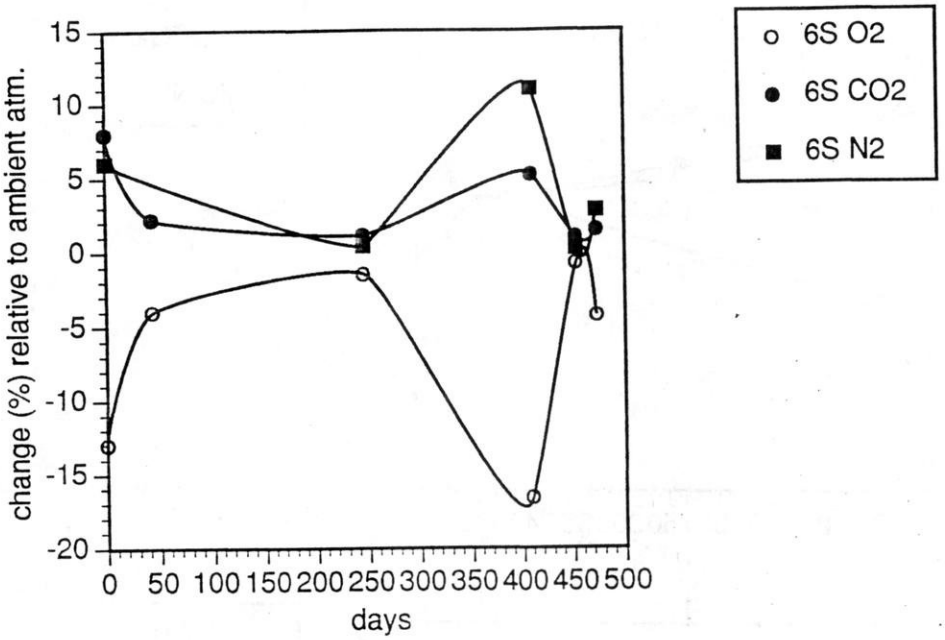

15
10
5
0
-5
-10
-15
-20
change (%) relative to ambient atm.
0 50 100 150 200 250 300 350 400 450 500
days
6S O2
6S CO2
6S N2

Fig. 8J

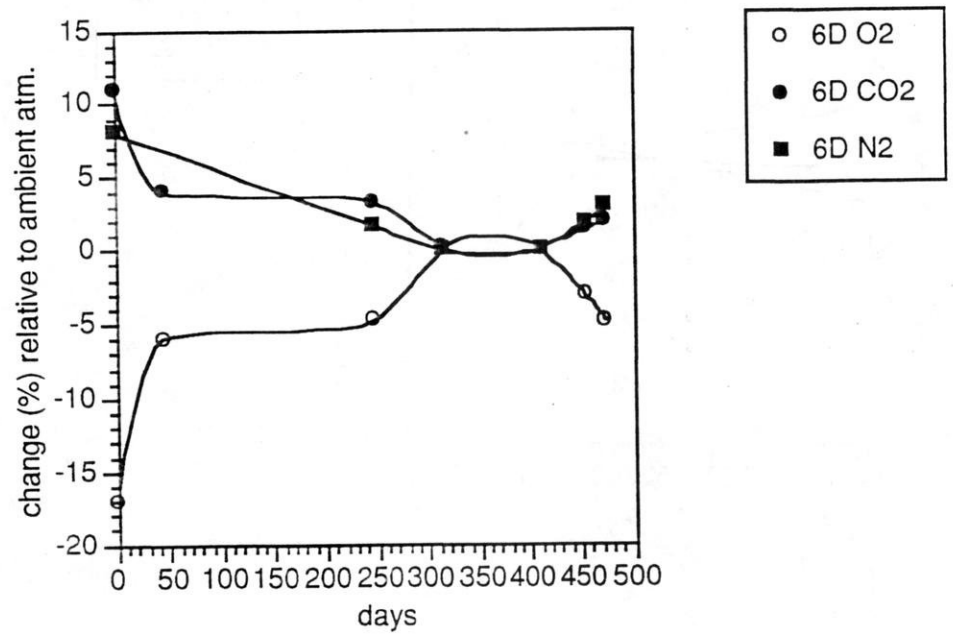

15
10
5
0
-5
-10
-15
-20
change (%) relative to ambient atm.
0 50 100 150 200 250 300 350 400 450 500
days
6D O2
6D CO2
6D N2

Fig. 8K

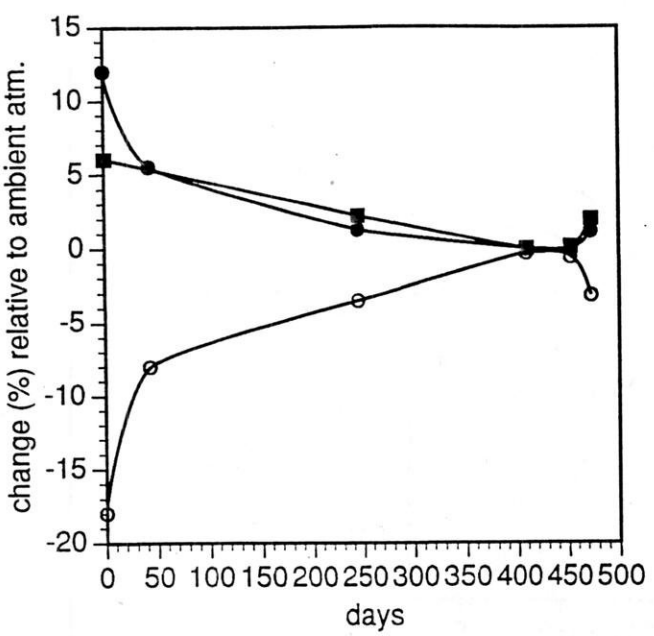

15
10
5
0
-5
-10
-15
-20
change (%) relative to ambient atm.
0 50 100 150 200 250 300 350 400 450 500
days
7S O2
7S CO2
7S N2

Fig. 8L

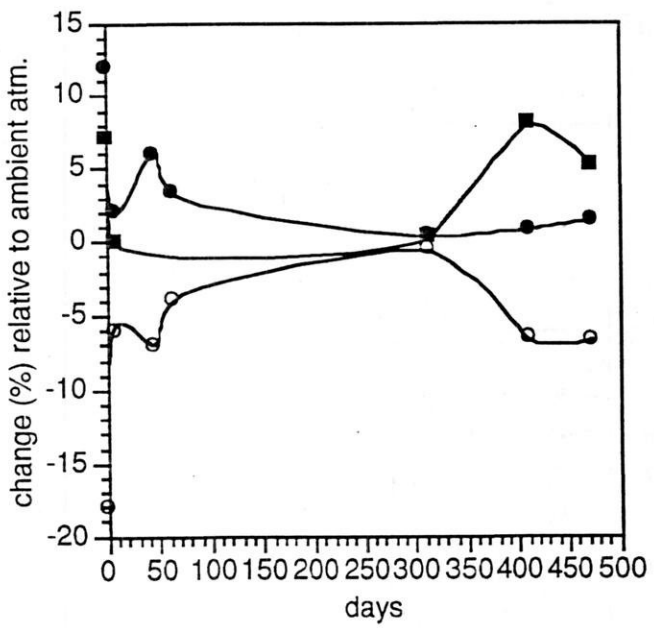

15
10
5
0
-5
-10
-15
-20
change (%) relative to ambient atm.
0 50 100 150 200 250 300 350 400 450 500
days
7D O2
7D CO2
7D N2

Fig. 8M

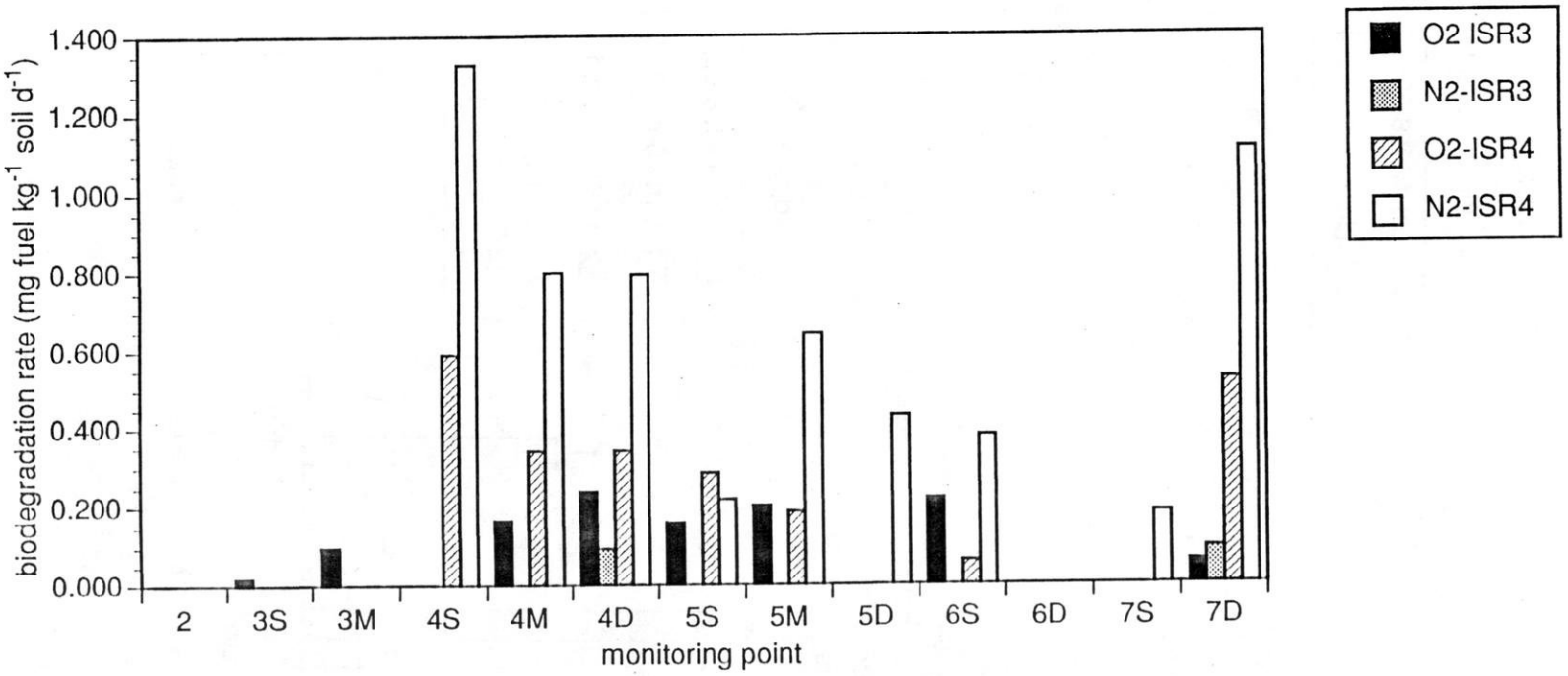


Fig. 9. Estimated aerobic(O2) and anaerobic (N2) biodegradation rates in ISR tests 3 and 4.

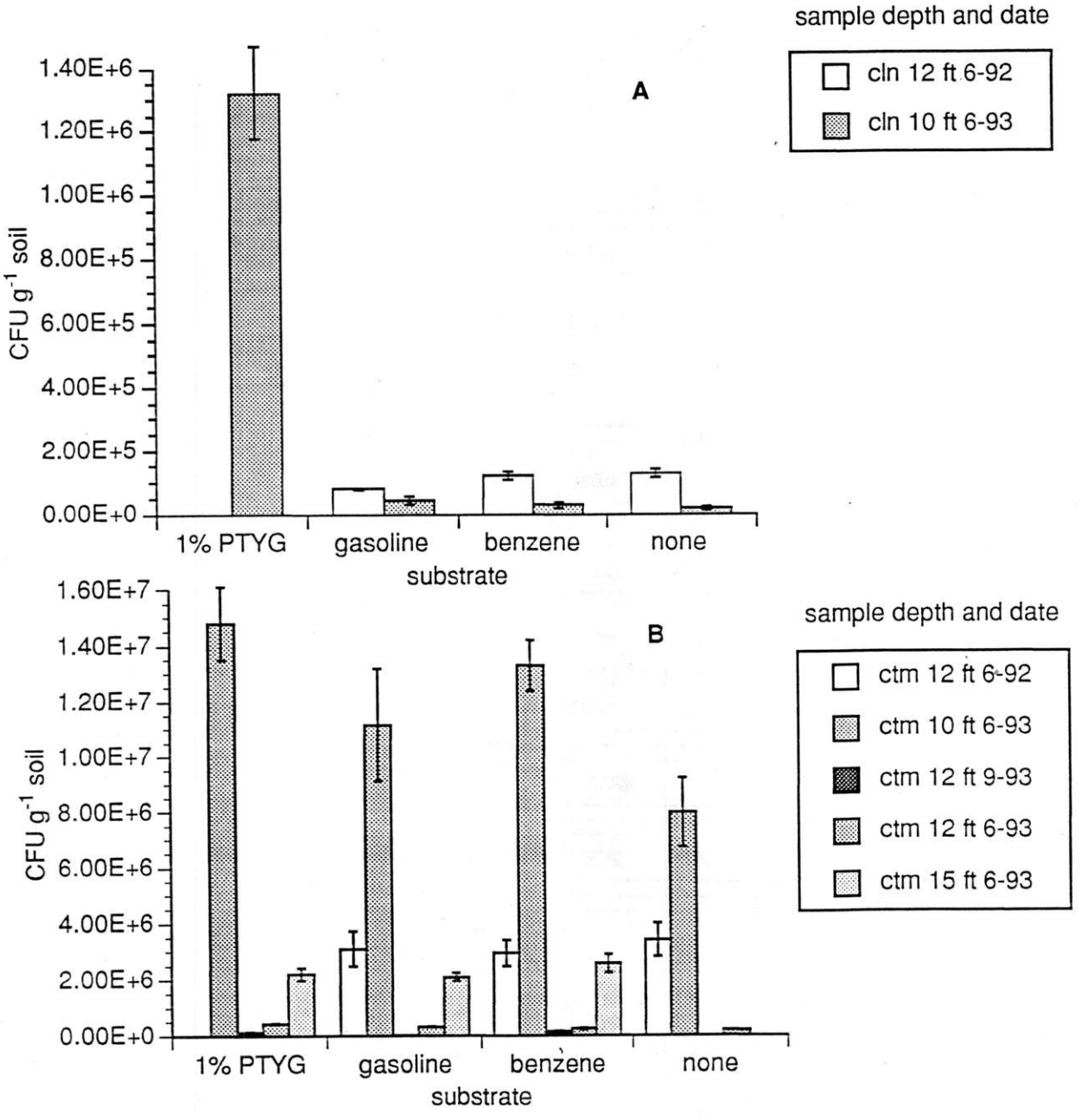


Fig. 10. Microbial populations enumerated from (A) clean (cln) and (B) contaminated (ctm) soil using the indicated growth substrate. Bars indicate standard deviations.

Table 1. Subsurface atmosphere composition determined in samples from the monitoring points and the extraction well prior to VES operation.

location[a]	depth (ft)	CO_2	O_2	N_2	CH_4	PID[b] instrument units
		%				
P2 (background)	7	3	18	79	0	0
P3	9	13	3	85	0	214
	13	13	3	84	0	239
	20	10	3	87	0	278
P4	9	6	11	83	0	246
	13	14	2	84	0	188
	20	11	3	87	0	174
P5	9	10	4	85	0	136
	13	9	4	87	0	140
	20	8	4	88	0	154
P6	9	8	7	85	0	21
	20	11	3	87	0	318
P7	9	12	2	85	0	488
	20	12	2	86	0	226
RW1	9-19[c]	3	16	81	0	ND[d]
Ambient	NA[e]	0.03	20	79	0	ND

[a] see Fig. 1 for locations

[b] reading from field photoionization detector

[c] screened interval

[d] not determined

[e] not applicable

Table 2. Soil nutrient measurements.

----------sample----------			total N	NH_4-N	NO_3-N	SO_4-S	P
date	location	depth (ft)	----------mg kg^{-1} soil----------				
6-91	B7	13	nd[a]	4.0	6.5	5.0	15
		17	nd	7.5	7.0	14.5	22
		21	nd	5.0	8.5	6.5	25
6-91	B9	13	nd	5.0	9.0	2.5	21
		17	nd	5.0	9.0	1.0	12
		21	nd	5.5	9.0	9.0	17
7-93	B14	10	78	2.0	2.5	3.0	10
		12	56	4.0	5.0	3.0	10
		15	56	2.5	5.0	4.5	11
7-93	B15	10	64	4.5	5.5	1.8	14

[a] not determined

Table 3. Subsurface contaminant concentrations before, during and after VES operation.

date	depth[a]	GRO	B[b]	T	E	X
	(ft)	(mg Kg^{-1} soil)	----------(μg Kg^{-1} soil)----------			
4/29/91[c]	10-12	5,000	310	7,500	3,100	16,000
9/29/92[d]	10.5	<10	<10	<10	16	18
6/19/93[e]	10	21	<1.2	<1.2	<1.2	<1.2
6/19/93	12	2,500	<5.4	<5.4	<5.4	260
6/19/93	15	1,600	<7.3	<7.3	<7.3	32

[a]all samples acquired within 1.5 m of the former tank bed

[b]benzene, toluene, ethylbenzene, xylenes

[c]pre-VES operation

[d]following 2 months of VES operation

[e]following 5 months of VES operation and subsurface reequilibration.

Table 4. Amounts (l) of gasoline removed by the indicated process.

	Biodegradation							
	Pre-VES[b]		During VES[c]		Post-VES[d]			
Volatilization[a]	Aerobic	Anaerobic[e]	Aerobic	Anaerobic	Aerobic	Anaerobic	total	TOTAL
409	45	36	8	0	20	14	128	538

[a] VES operation from days 0-180.

b assuming: average 13% O_2 depletion and 6.2% N_2 enrichment; 900,000 kg contaminated soil.

[c] assuming: average biodegradation rate of 0.087 mg soil d^{-1}; 675,000 kg contaminated soil.

[d] assuming: average aerobic biodegradation rates of 0.13 mg kg^{-1} soil d^{-1} (d 245 - 409) and 0.24 mg kg^{-1} soil d^{-1} (d 453-473); average anaerobic biodegradation rates of 0.06 mg kg^{-1} soil d^{-1} (d 245 - 409) and 0.59 mg kg^{-1} soil d^{-1} (d 453-473); 675,000 kg of contaminated soil.

[e] biodegradation coupled to denitrification