

Alfalfa Plants and Associated Microorganisms Promote Biodegradation Rather Than Volatilization of Organic Substances from Ground Water

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Bacteria have the capacity for bioremediation of both volatile and non-volatile organic compounds. Plants support a rhizosphere microflora, enhance soil microbial populations and may also be able themselves to metabolize some hazardous organic compounds. Plants move large amounts of water by transpiration and for volatile compounds, intersystem transfer by transpiration might occur when plants are exposed to such materials. These possibilities were tested experimentally. A tank with a channel width of 10 cm and a depth of 35 cm was used for plant growth. Alfalfa plants were supplied with a subsoil water source saturated (ca. 500ppm) with toluene or containing ca. 500 ppm phenol. Sampling wells were used to monitor the depth of the water table and concentrations of toluene and phenol in the ground water.

The toluene concentration in the ground water remained constant or decreased slightly during passage of water through the tank although there was an average net input of >300 mg toluene per day to the system. To measure toluene losses through volatilization or transpiration, the tank was covered with an aluminum and glass enclosure and monitored using Fourier transform infra-red (FTIR) spectroscopy. The gas phase toluene was below the limit of detection by FTIR (ca. 250 ppb v/v) while the expected accumulation of toluene based on water evapotranspiration rate was >50 ppm v/v increase in the gas phase concentration per hour. Thus, only a small to nil fraction of the input toluene arrived in the gas phase.

With phenol, the concentration in the aqueous phase decreased during passage through the tank. Phenol is much less volatile than water and it was undetectable in the gas phase. The amount of input contaminants was about 100 fold above the amount that could be physically adsorbed to soil organic matter in this system. The observed mass balance suggests that effective degradation of toluene and phenol occurs in this system and that the potential intersystem transfer of volatile organics by plant transpiration is not a problem, at least with adapted plants.

Bioremediation efforts have generally depended on microorganisms alone but plants may make significant additional contributions to the process. Plants are just beginning to be used specifically in this role (1-3), although they have been used in mine waste reclamation for decades. We are particularly interested in remediation of ground water contaminated by organic compounds. A recent review (4) describes the current status of and potential applications of plant assisted bioremediation. Plants provide a cost-effective way to "pump" contaminated water out of the ground, at least in less humid regions where potential evapotranspiration exceeds precipitation. They also provide input of readily available supplemental organic carbon to enhance the growth of root-associated microorganisms. This, in turn, increases the total population of microorganisms in the soil.

We previously modeled the plant-assisted bioremediation strategy for benzene and atrazine (5,6). Plants reduce the off-site transfer of the hazardous substance, primarily by reducing downward percolation of water, but also by withdrawing ground water. The relative withdrawal of ground water depends on the water demand of the plant species chosen, precipitation, and potential evapotranspiration. Degradation of organic contaminants depends on microbial biomass, which, in turn, is strongly dependent on carbon input from plant root exudate. In the model (5,6), as in real systems (7), total microbial biomass in the soil is strongly correlated with root density in the soil. To validate the model, we have constructed a system that allows direct measurement of toluene or phenol moving into and out of the saturated soil phase, into the vadose zone and potentially into the atmosphere.

Earlier work by McFarlane et al. (7) had shown that soil could serve as a sink for benzene from the vapor phase. In their studies, it was concluded that microorganisms in the soil, associated with the plant root systems, were responsible for the disappearance of benzene. Sterilized soil was not a sink for benzene and unplanted soil was much less effective than soil which had plant roots present. When a highly active rhizosphere microbial population is present, degradation of the hazardous organic substrate may be relatively rapid. Walton and Anderson (2) found that degradation of trichloroethylene was greater in soil from the rhizosphere of plants growing in a contaminated site than with contaminated soil in which plants were not growing. Microorganisms, either closely or loosely associated with plant roots, are expected to enhance degradation of toluene and phenol, as there are many bacteria capable of degrading these compounds. Microbially enhanced bioremediation of these compounds is considered a standard technique (8).

The use of plants is an extension and enhancement of the intrinsic bioremediation capabilities of the microbial community as discussed in a recent National Research Council book (6), rather than a fully engineered approach. A crucial question in plant assisted bioremediation is whether there is significant transfer of organic contaminants from soil to the plant and atmosphere. Briggs et al. (9) showed in short term tests (2-4 days) that barley plants will take up dissolved organics into their transpiration stream. Compounds in homologous series (phenylureas and methylcarbamoyloximes) with varied octanol/water partition coefficients were tested. There was considerable variation between compounds, but those with a partition coefficient of about 100 ($\log K_{ow} = 2$) had a maximum transpiration stream concentration factor (TSCF) of about 0.8. That is, such compounds were about 80% as concentrated in the transpiration stream as outside the root of the plant. Greater or lesser hydrophobicity yielded less effective uptake. The phenylureas consistently had lower TSCF values than the O-methylcarbamoyloximes.

McFarlane et al. (10) using an elegantly designed hydroponic system, showed that compounds in different chemical classes but having similar octanol/water partition coefficients may behave in quite different fashion depending on the species of plant tested. Phenol appears to be almost entirely immobilized

in the roots of soybeans, while bromacil is rapidly translocated to and accumulates in the leaves. Similarly, Briggs et al. (9) cited a number of examples from the literature where the TSCF deviated greatly, to the low side, from their relatively simple predictive curve. We may not be able to predict *a priori* whether a particular compound is translocated or immobilized by a particular species of plant. In addition, use of a non-sterile planted soil as the support medium may give different results from those observed in hydroponic systems because there are many more potential compartments for contaminant to partition into and there may be degradative losses in the soil (11).

An active plant consumes relatively large amounts of water as an inescapable consequence of photosynthesis (4-6). Adapted microorganisms associated with the roots may carry out degradation of dissolved contaminants. Thus the plant may compete with its associated microorganisms by taking up contaminants from the ground water. Or, if the microbial community is large enough and effectively adapted to the contaminant in question, the plant may serve primarily to enhance active transport of the contaminant to the microorganisms. The relative importance of these competing processes is addressed in the present research.

Boersma et al. (11) showed that accumulation of bromacil in plants increased in proportion to transpiration rate. In the absence of precipitation, an active plant significantly increases the net water flux from water table through the vadose zone to air. Removal of water increases the volume of the gas phase, which allows increased oxygen diffusion through the soil, potentially enhancing aerobic processes. In saturated soils, the root systems of some species may also supply some oxygen for microbial consumption (4). Plants thus may expose microorganisms to increased fluxes of both gas and water, which contain the volatilized or solubilized contaminant. Adapted microbial populations will probably consume most of the contaminant before it is taken up by the roots, because typical K_m values for microbial metabolism of compounds such as phenol are fairly low, in the range of 100 $\mu\text{g/L}$ (12), several thousand times below the concentrations being used in the experiments described here.

The work of Briggs et al. (9), and Boersma et al. (11) followed uptake of contaminants for relatively short periods of time. Soil microorganisms able to degrade a compound increase following exposure (13). Thus the potential uptake of organic compounds by a plant and transfer to the gas phase which is predicted from their work, might be decreased in an adapted system, where associated microorganisms could degrade the contaminant prior to uptake by the plant. This is an important consideration because intermedia transfer of contaminants is a concern in any remediation process. The present research directly addresses that question.

Materials and Methods

Apparatus for treatment of plants. A tank divided into two identical halves with inlet and outlet ports was used for plant growth. The U-shaped folded channel in each half of the tank was 1.8 m long, 10 cm wide and 35 cm deep. It was filled with Kansas river sand/silt from the Sweet tract, near the Riley County landfill where transport of organics in ground water is a concern. Details of construction and soil packing were previously described (14). Four sampling wells were placed in each half chamber at the time of soil packing. Each well consisted of a coarse glass frit on a gas dispersion tube with the tube lengthened to 38 cm. Concentrations of toluene and phenol in the ground water were monitored through these sampling wells which also allowed determination of the depth of the water table. With reasonable flow rates and a water table depth of 25 cm from the surface, the 1.8 m path would provide a mean residence time of 1-3 weeks for

input liquid. Evaporation rate greatly influences the mean residence time of the liquid effluent. The limiting case is when inlet flow just equals evapotranspiration and residence time becomes indefinitely long. The surface area of soil for each half chamber was about 0.18 m^2 , while the area available for plant top growth was slightly larger ($\approx 0.2 \text{ m}^2$) because plants could grow above the dividing walls. The entire chamber was placed within a fume hood to insure that any possible escape of volatiles did not violate regulations for air quality within the laboratory.

To monitor possible toluene or phenol output through volatilization or transpiration, the tank was covered with a gas-tight enclosure 26 cm high. On three sides and part of the fourth, steel covered with aluminum flashing material was used to provide a light reflecting surface that would not adsorb organic compounds. The top of the enclosure was covered by ordinary window glass and sealed with clear tape around the edges. Illumination for the plants was provided by six 40 watt fluorescent lights 40 cm above ground level. During monitoring periods, the front open space, about 43 cm wide, was closed with a piece of window glass and sealed with tape. Internal air circulation was assured by using a small fan inside the enclosure.

Soil analysis and plant growth. The soil used in these studies consists of medium and fine sand with a little silt and low organic matter (0.3 to 1.3 % depending on depth)(14). Effectively nodulated alfalfa seedlings were planted in pairs at 10-cm intervals along the length of the flow path and the whole tank was top watered once with 10 L Waeck's medium minus N (15) to give the plants essential nutrients. A subsurface flow of water was established using water saturated with toluene or having ≈ 500 ppm (w/v) of phenol (500 $\mu\text{L/L}$ of 93% liquified, phenol). The water table was maintained at 20-30 cm and the gradient in the central 1 m of the flow path was about 1 cm/m.

Analysis of aqueous phase organics. Fresh representative samples of the ground water were taken from the sampling wells and the concentration of phenol or toluene at the entry and exit ports was also measured. After drawing out all the liquid in a well and allowing it to refill for 5-10 minutes, a measured quantity of liquid was withdrawn into a long nylon tube attached to a 1-mL syringe. The 1-mL liquid sample was added to organic extractant in a 50-mL tube closed with a ground glass stopper. For toluene extraction, 2 mL of heptane was used. For phenol, 2 mL n-octanol was used with 5 mL of 0.1 M phosphate buffer added to assure that the phenol remained unionized. A vortex mixer was used to facilitate partitioning of the toluene or phenol into the organic phase. After separation of phases, a portion of the organic phase was transferred to a quartz cuvette. The absorbance, at 262 nm for toluene or 265 nm for phenol, was determined in a DU-2 spectrophotometer. Calibration standards were prepared, and extracted as for the unknowns. For some samples, spectra of the entire UV region from 200 - 300 nm were determined using a Hitachi Model U-3210 recording spectrophotometer.

Analysis of gas phase organics. Details of instrumentation are given in Davis et al. (14). Gas phase concentrations of toluene were monitored after closing the growth chamber to give an aboveground gas volume of 147 L. The walls of the upper enclosure had two mirrors mounted near the top to allow a beam of light to enter from the front of the chamber, pass to the back where it was reflected to the opposite end, return to the first mirror and exit at the front. Total path within the chamber was 2.44 m. Entry and exit apertures, covered by KBr plates, were slightly offset to align with the source and entry of a MIDAC F-TR. Chilling coils with refrigerant at a nominal -10°C circulated near the base of the upper enclosure to increase transpiration and decrease humidity near the KBr windows. Methane (10 mL) was used as a freely diffusible inert gas for measuring leakage out of the

upper enclosure. In earlier experiments, the half-time for equilibration was about 1 hour, while in later experiments it was three hours or greater.

FTIR Instrument calibration. Several methods were used to test the sensitivity of the MIDAC detection system. In one case, toluene was applied to a piece of paper within the chamber via a long syringe needle. It was allowed to evaporate and the spectrum measured. In another case, water saturated with toluene was siphoned from the inlet reservoir into a container and then directly applied rapidly to the surface of the chamber soil. The chamber was then immediately sealed, and the increase in toluene level in the gas phase was monitored. In a third case, water similarly saturated with toluene was applied to dry soil from the same site placed in four 23 x 33-cm pyrex baking trays which had been arranged in the chamber after the plants had been cut back to 5 cm. Finally, the potential evaporation of toluene was enhanced by flooding the chamber from below to within 10 cm of the surface with water from the inlet reservoir, simply by elevating the pressure head for a few hours. For phenol, only the direct addition of liquid phenol on filter paper was used in sensitivity tests.

Respiration rate estimation. Two different methods were used to measure respiratory CO_2 . The simpler method was to place closed cylindrical containers between the plants, with their lower edge pressed into the soil 1-2 cm. Seven containers of 7-cm diameter and 850-mL volume were placed at intervals between the plants. Gas samples of 1 mL were taken by syringe, through septa placed in the tops of the containers. Samples were analyzed for carbon dioxide by gas chromatography with a thermal conductivity detector. Samples were analyzed at times ranging from 2 to 64 hours. The second method was to cut the plants back close to the soil, close the chamber and monitor the increase of CO_2 in the gas phase using the MIDAC system. In this case, spectra were taken at 10- or 15-min intervals for two hours. Then known amounts of CO_2 , generated from K_2CO_3 , were added and spectra recorded. This provided an internal calibration. A CO_2 band in the region of 2250 cm^{-1} and a line at 720 cm^{-1} were used for estimation of CO_2 .

Molar absorptivities in the gas phase were determined for toluene, phenol and CO_2 by use of a gas cell of known pathlength. The same MIDAC instrument was used for this calibration as for the experimental measurements. For toluene and phenol, which are normally absent from the ambient air, a 5-cm pathlength cell was used. For CO_2 , it was necessary to use a 50-cm cell because fluctuations of atmospheric CO_2 in the path between source, cell, and detector interfered with calibration runs in the short pathlength cell.

Results and Discussion

Flow regime and ground water toluene levels. Conditions varied slightly throughout the period of operation, but initially the inflow of water was 1 L per day when the growth chamber was not enclosed. After metal sides and a glass lid were added, the rate of water use decreased, presumably because the glass lid reduced the air circulation which was normally induced by operation of the hood exhaust fan. If the small fan within the chamber was not run, the surface of the soil was damp whereas when it was run, the surface remained relatively dry. Spider mites were an occasional problem and were controlled by spraying with malathion as needed. During very rainy weather, the water usage decreased noticeably. We have not attempted a precise correlation of water usage with relative humidity or plant growth stage.

Usually the chamber was operated with only minimal outflow of water, although for some experiments the rate was increased so that more than 0.5 L per

day was exiting the tank. In Table I, some typical concentrations of toluene and phenol in the aqueous phase are shown for different days of similar water flow.

TABLE I Concentration of toluene and phenol in the saturated zone at several sampling wells

Substance	100 x Apparent absorbance in organic extract					
	Inlet	Port 1	Port 2	Port 3	Port 4	Outlet
toluene	38 ± 3	32 ± 2	29 ± 4	30 ± 2	27 ± 4	32 ± 1
phenol	76 ± 5	70 ± 7	56 ± 7	25 ± 3	5 ± 3	4 ± 2

Experiments were done on 5 consecutive days, with results shown as the mean \pm standard deviation. Inlet flow was water saturated with toluene or having a fixed concentration of phenol. The inlet concentration of toluene was 515 mg/L while that of toluene was 0.5 mL/L of 93% liquidified, phenol.

The concentration of the organic chemical appears to stay the same (toluene) or decrease (phenol) as the water passes through the tank. If there were selective partitioning and exclusion of the compound at the surface of the plant root as described in the studies of Briggs et al. (9), one would expect the concentration to increase at the downstream end of the chamber. For toluene and phenol, the predicted partition coefficient, based on the K_{ow} near 100, is about 0.8. Thus if 2 L of solution enter the tank and 1 L is transpired, the concentration in the 1 L that exits as ground water should be elevated $\approx 20\%$. If the outflow is a smaller fraction of the input, say 1 L for every 5 L entering which is typical for the conditions that we used, the concentration of the compound in the output water should be considerably higher, approaching twice the input concentration. Preferential evaporation of water compared to phenol, if it occurred, would also have given an increase in phenol concentration in the ground water. The extent of increased concentration would depend on relative input and outflow rates and the extent of water loss by evaporation vs. transpiration. The relevant vapor pressures at room temperature are 25 mm for water and $<1 \text{ mm}$ for phenol; thus preferential evaporation of water to concentrate the phenol is quite plausible.

For toluene, the concentration in the ground water stayed relatively constant throughout the course of flow through the chamber. This suggests that a degradation pathway for toluene was not induced in the saturated zone even after nearly 1 year of exposure. To verify that the UV-absorbing material observed in ground water was in fact toluene and not a degradation product, the entire UV spectrum of organic extracted material was determined. The spectrum could be directly superimposed on that of toluene, which has several characteristic features. Thus, the UV-absorbing material in the ground water remains as toluene.

On the other hand, the decrease in phenol concentration is quite striking, suggesting that either there is an anaerobic degradation system induced, or the ground water is being made aerobic during passage through the chamber. The extent of decrease in phenol concentration with distance of passage was much less obvious in results obtained two months earlier (14) than in the present results, indicating a time dependent induction of a degradative process. More recent studies using trichloroethylene and trichloroethane in place of phenol, have shown production of methane and chloride in the saturated zone of the latter half of the flow path, indicating that it is anaerobic.

Losses of input organic chemicals by adsorption to soil in this system are unlikely to be significant over the long term. Typical adsorption values for soil are in the range of 100 µg organic solute per g soil organic C (16). The chamber has been in operation for more than a year at high levels of input organic chemicals (ca. 500 mg/L). Adsorption sites on the soil having only ca. 1% organic matter (ca. 1500 g for the entire chamber) should be fully saturated within a few days of operation; adsorption is not likely to account for the continued disappearance of these organics. The simplest explanation is biodegradation in the soil.

The results shown in Table I were obtained with a mean water flow-through time of ca. 4-7 days. This was estimated from the cross-sectional area below the water table available for flow (ca. 1/3 of 10 x 10 cm), the length of the channel (1.8 m), the input rate of ca. 1.0 L/day and output of < 400 mL/day. These estimates have been verified using KBr as a conservative tracer (unpublished). Mean flow-through time was usually greater than 7 days but could be made indefinitely long (i.e. no outflow) by controlling water input rate. From the water consumption rate (input minus output) of ca. 600 mL/day an average net input of >300 mg toluene per day was calculated. Toluene has a boiling point of 110°C and a vapor pressure of 30 mm at 25°C compared to water with a vapor pressure of 25 mm at 25°C. Toluene should not be significantly lost to the atmosphere by preferential volatilization in the capillary fringe and vadose zone, and it ought to be present in the vapor phase at about the same relative concentration as in the water phase, if it is not degraded.

Gas-phase toluene. Some examples of spectra obtained with this system are shown in Figures 1 & 2. The steady-state toluene concentration in the gas phase was below the level of detection (ca. 250 ppb v/v) while the expected level based on water input rate was >50 ppm v/v per hour, in the gas phase. This is calculated using the input rate of 1 mL/min at 500 µL/L (ca. 300 µmol/hr). This may be seen in Figure 1B, spectrum (17/18).

The primary IR absorption band of toluene is observed at 729 cm⁻¹ as seen in Figure 1. A control experiment with toluene-saturated water applied to the surface of unadapted soil and immediately monitored in the same chamber accumulated gas-phase toluene levels of over 50 ppm v/v within two hours, as seen in spectrum (26/25). The enclosure was calibrated by direct release of known amounts of toluene into the gas phase (in theory 150 ppm v/v), as shown in spectrum (119/12). The prominent feature at 720 cm⁻¹ is a CO₂ line. This line is an excited state, which is temperature dependent and so is probably not a good line for reliable monitoring of respiration rate. The plants were harvested prior to collecting spectrum (25/26) so that there was net accumulation of CO₂ in the chamber, whereas with the plants present and the lights on for spectra (17/18) and (119/12) the plants were actively photosynthesizing and depleted CO₂.

A second toluene band at 1033 cm⁻¹ having about five-fold less intensity, was used to confirm the behavior of the system and to verify the identification of the toluene. Spectra shown in Figure 2 are the same as those of Figure 1. This latter spectral region was also monitored through windows of CaF₂. Windows of CaF₂ are much more water resistant than KBr, but energy transmission in the region of 730 cm⁻¹ is insufficient for use with that spectral feature. We found the inconvenience of potential water damage to windows of less importance than the difference in sensitivity between spectral regions.

Similar gas phase studies were attempted with phenol. A calibration spectrum obtained in a 5-cm pathlength cell showed good detection of a concentration of less than 10⁻⁵ M (250 ppm v/v), indicating that with a 2.44-m path one would obtain good spectra with 5 ppm (v/v). The estimated limit of detection at 2.44-m pathlength was ca. 0.15 ppm (v/v). If phenol volatilized at the same rate as water, the expected accumulation per hour would be at least 30 ppm (v/v).

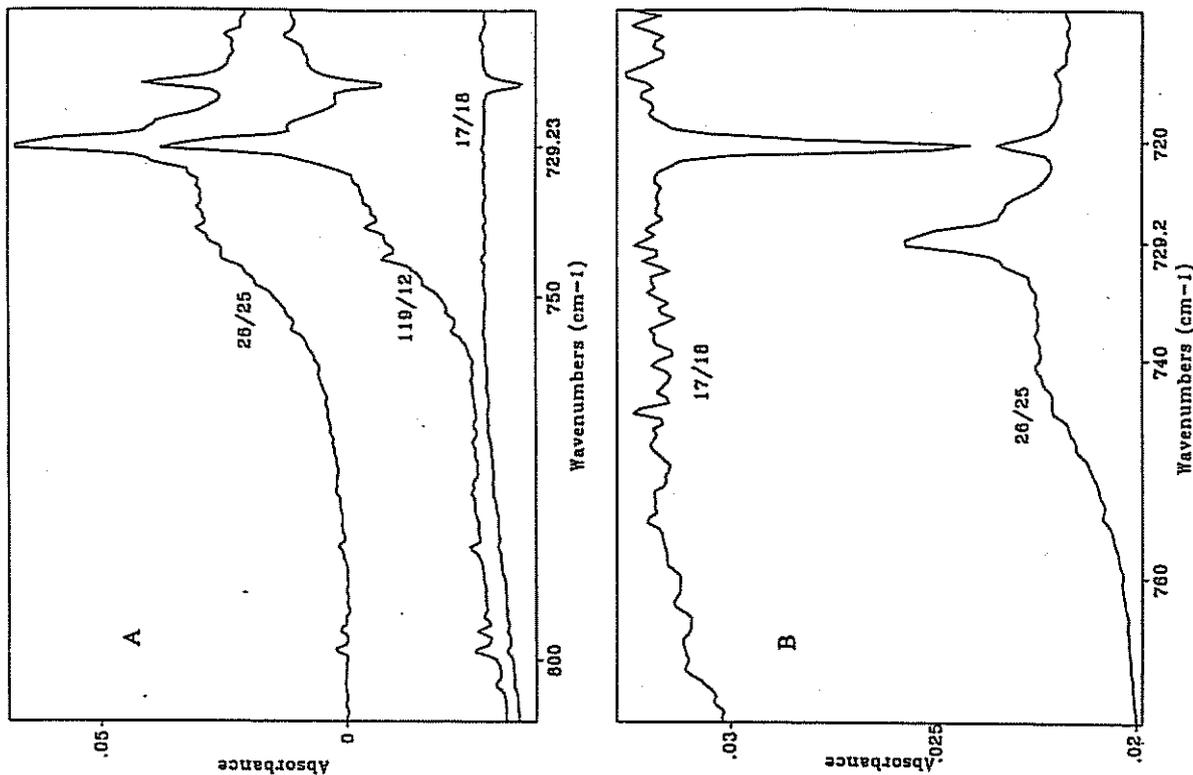


Figure 1. Main peak of toluene absorbance at 729 cm⁻¹. Part A: spectra of 15 hr equilibration vs open chamber (17/18); known amount of toluene (100 µL) evaporated in chamber (119/12); One L water saturated with toluene applied to surface of dry soil (26/25). All are displayed on same scale as indicated on left axis. Part B: (17/18) with the indicated scale; (26/25) at 10 fold less sensitivity.

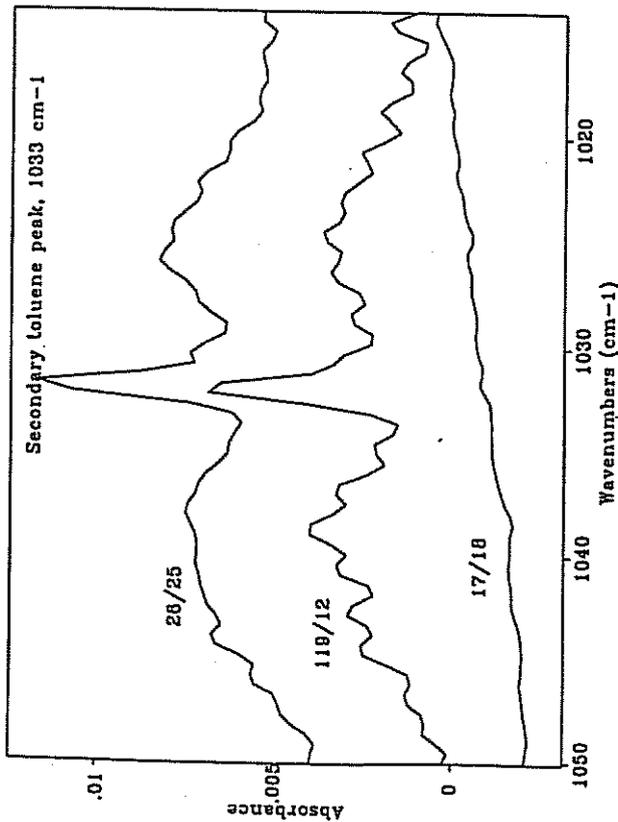


Figure 2. Secondary peak of toluene absorbance at 1033 cm^{-1} . Spectra described in Figure 1A. Note the absorbance scale which indicates about five fold lesser intensity for this peak than that at 729 cm^{-1} .

Phenol concentrations were below the limit of detection, even when 2 mL of phenol was left in the chamber for 2 hours with the fan in operation. Thus, although 1 mm partial pressure of phenol in the gas phase would be easily detected, the rate of evaporation is too low for practical detection of phenol accumulation in a chamber that has a half-time for gas exchange on the order of 1-3 hours, or else the phenol adsorbed to hydrophobic surfaces rather than remaining in the gas phase. If phenol is taken up by the plants, it appears either to be volatilized much less rapidly than water, or to be metabolized to concentrations below the detection limit of the system. We prefer the latter explanation because if the phenol did not depart the leaves at a rate comparable to that of water, it would accumulate to toxic levels over the course of days. There was no evidence of toxicity to the plants (14).

More recently, experiments have been done with trichloroethane (TCA) and trichloroethylene (TCE) in the ground water. Accumulation of TCA, which is not easily biodegraded, is readily observable in the gas phase, under experimental conditions identical to those described here. Thus, a "conservative tracer" indicates that toluene would also be observable in the gas phase if it was not being degraded.

Respiration rate. Respiratory activity in the soil + rhizosphere was initially estimated from CO_2 evolution in the soil between plants as describe in Methods. The CO_2 levels detected tended to be higher near the inlet port than further along the flow channel, but the sum of all amounts, extrapolated to the total surface area of the chamber, was only a few mmol/day , considerably less than the amount of toluene or phenol carbon entering the chamber. When accumulation of CO_2 was measured in the entire chamber by FTIR, much higher estimates were obtained.

This is presumably because a large fraction of total respiration occurs within, or in close proximity to the plants. Based on the initial rate of CO_2 accumulation and the internal calibration of the chamber, 70-100 mmol CO_2 was released per day. The input of dissolved organic C was 50 mmol/day or less. Turnover of photosynthetically fixed carbon in the rhizosphere, or via plant respiration, obviously contributes a significant fraction of the total respired CO_2 . When CO_2 accumulation was monitored after withdrawal of toluene and phenol from the feed solutions, the apparent respiration rate decreased to about 2/3 of that previously observed, consistent with a significant contribution from the input organic chemicals.

For CO_2 accumulation measurement by FTIR, the plants were cut to 5 cm so that photosynthesis or dark respiration by the plant leaves and stems would not complicate the measurements. However, cutting may have stimulated root respiratory activity for initiation of regrowth (17). In addition, the plants are totally dependent on nitrogen (N) fixation for their source of nitrogen and this process requires a significant amount of respiratory activity. Plants accumulating 1 g dry matter per day need about 1/3 mmol N fixed per day. According to Herridge and Pate (18), this may require consumption of 8 mmol carbon per day by the root system for fixation of 1/3 mmol N , about 10% of the observed total respiration. Maxwell et al. (17) noted that roots may shut down nodular activity when the tops are removed so that the contribution of N fixation to respiratory demand may decrease upon cutting. They reported a general root respiratory rate of 0.9 mg CO_2/kg root matter/sec which may be converted to about 1.8 mmol/g root/day. If the mass of roots equals that of the harvested tops (47.5 g at one harvest), a respiratory rate of 70 mmol/day from a plot of 0.4 m^2 seems plausible. As an annual rate per hectare, it is also consistent with reports of soil respiration worldwide as cited by Glinski and Stepniowski (19). They cite values for a range of crops and climates from very small up to 28 L/day/m^2 while our estimate is about 4.5 L/day/m^2 , near the median of the range quoted by Glinski and Stepniowski.

Conclusions

The work described here shows that with an adapted system plants are able to grow actively for one year in the presence of water saturated with toluene, or containing ≈ 500 ppm phenol. There is little intermedia transfer of toluene, presumably because there is little uptake by the plants or direct evaporation from the soil. Total system respiratory activity, measured as carbon dioxide evolution, is more than adequate to account for complete mineralization of the input toluene. Only a small portion of the toluene leaves the system, in the exiting ground water. Phenol, a less volatile compound which could not be detected in the gas phase, in part because of its low volatility, appears to be effectively degraded also. None of it leaves in the exiting ground water.

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Chapter 11

Volatilization and Mineralization of Naphthalene in Soil-Grass Microcosms

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The potential for vegetation-enhanced biodegradation of naphthalene in artificially contaminated soil was studied in laboratory microcosms. Microcosms containing soil without plants and soil supporting two-month old Bell Rhodesgrass were treated with naphthalene and spiked with [^{14}C]naphthalene. Compressed air was continuously passed through each microcosm, through a trap to collect volatile organics, and through a trap for CO_2 . The microcosms were incubated under artificial lighting with a 16 h photoperiod for 25 days. After incubation, soil was solvent extracted and combusted to recover bound radiolabel. Volatilization losses during operation and analysis prevented reaching a mass balance of the radiolabel. Naphthalene volatilization was enhanced by vegetation but mineralization was decreased in vegetated microcosms in comparison to those without vegetation.

Polycyclic aromatic hydrocarbon (PAH) compounds may be toxic and carcinogenic. Soils can become contaminated with PAH compounds from many industrial sources including wood preservatives, coal gasification wastes, and petrochemical wastes. Many PAH compounds, especially those with two and three ring structures, are biodegradable and can serve as growth substrate for microorganisms. Higher molecular weight PAH compounds may be cometabolized by soil microorganisms, with soil half-lives of several hundred days (1). Plant-enhanced biodegradation of PAH compounds is a recent area of research and is based on the hypothesis that increased microbial activity associated with plant roots will accelerate biodegradation. Aprill and Sims (2) showed a statistically significant increase in disappearance of benz(a)anthracene, benzo(a)pyrene, chrysene and dibenz(a,h)anthracene from soils vegetated with eight different prairie grasses compared to unvegetated soils. Wheat straw (3), trichloroethylene (4), surfactants (5), parathion (6, 7), and diazinon (7) have all been shown to degrade faster in soil/plant systems when compared with soil degradation alone.

Naphthalene is the smallest PAH and has the physicochemical properties listed in Table I. The partition coefficients in Table I were calculated from structure-

Uptake and Biodegradation of Volatile Petroleum Hydrocarbons in Planted Systems

Post-It[®] Fax Note 7671

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Date	6/12	# of pages	1
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Several field-scale phytoremediation trials are now in progress; in some cases, plants have been established on soils contaminated with toxic, volatile compounds such as benzene. An initial concern was that plants may take up harmful levels of these compounds and possibly create an ecotoxicological hazard (e.g., by contaminating an insect-based food chain). Another concern was that, in soils contaminated with volatile organic compounds (VOCs), plants might facilitate volatilization and contribute to air pollution: This problem might arise if VOCs were taken up by the roots, translocated to the shoots, and volatilized from the foliage via the stomatal pathway to air. The aim of this research was to investigate these areas of concern.

Established alfalfa plants growing in soils, or unplanted soils, were subirrigated with [¹⁴C]benzene solutions ranging in concentration from 1-15 mg/L; final benzene concentrations in soils ranged from 40-620 µg/Kg. A high-flow sealed test system enabled us to maintain a mass balance for the radiolabel, i.e., account for all of the ¹⁴C-label initially added to the system. The duration of the experiments were 7-10 days. During this time period we monitored the efflux of ¹⁴C-labeled VOCs, and ¹⁴CO₂ resulting from mineralization. At the end of the experiment, we measured radiolabel and benzene parent compound in soils and plant tissue.

Materials and Methods.

Figure 1. High-Flow sealed test system; Simplified diagram. Bell jars housed glass columns which contained the planted/unplanted soils. A rapid flow of air through the bell jars was necessary to remove transpirational water vapor as well as ¹⁴C-labeled VOCs and ¹⁴CO₂. Rapid removal of radiolabeled materials minimized uptake by the plant leaves. Arrows indicate the direction of air flow through the system. The loss of volatilized ¹⁴C-label from the system was minimized in order to maintain a budget for the radiolabel. Leaks were reduced by keeping the pressure in the bell jars close to atmospheric pressure. The low pressure-differential in the bell jars was achieved by pumping air into and out of the jars. Flow-rates from the pumps were matched using pairs of rotometers, and pressure in the bell jars was monitored using manometers. Radiolabeled benzene solutions were injected deep into the soil (~12 cm below the surface) using long "subirrigation" syringe needles.

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Figure 2. High flow sealed test system; Diagram showing the system of condensers.

Transpirational water vapor in the air effluent from the bell jars was removed with water-jacketed condensers. The air then passed over CO₂ traps (containing monoethanolamine), and traps to remove VOCs (containing 2-methoxyethanol). Bell jars stood in a growth chamber, and plants (or soil controls) were kept at 23° C, with a 16-hour photoperiod. Other condensers and dessicant columns placed at various points throughout the system served to remove vapors from the air stream which would otherwise condense in the lines and block the air-flow.

Figure 3. Soil/plant holders. Soils were packed into glass columns with porous bottoms to support the soils. Scintered glass fittings were used to connect the columns to flasks. The flasks received leachate. Side arms on the flasks and the soil holders were connected via flexible teflon tubing; this connection released pressure in the flasks which resulted when the soils were irrigated.

Table 1. Radiolabeled benzene subirrigation solution. In the various experiments, the dose of benzene was either "low" or "high". [¹⁴C]benzene was injected deep into the soil via a long syringe needle. Leaching of radiolabeled added during subirrigation did not occur because benzene solutions had minimal volumes.

Figure 4. Behavior of [¹⁴C]benzene in the high-flow sealed test system; Time course for the efflux of VOC and ¹⁴CO₂. A "low" dose of benzene was injected into a planted system. The dose was injected in three equal portions at t = 0, 2h, and 4h. The efflux of [¹⁴C]VOC and ¹⁴CO₂ was continuously monitored. Efflux of VOC reached a plateau at 1.5 days; ¹⁴CO₂ efflux reached a plateau after 7 days.

Results.

Table 2. Behavior of [¹⁴C]benzene in planted systems (Trials 1 and 2). The final distribution of radiolabel in the various compartments (VOC, Soil ¹⁴CO₂, plant tissue) was measured at the end of each experiment. In these experiments, each bell jar contained two plants as described below (see photo):

a) Experimental plants were injected with [¹⁴C]benzene. Only data for the experimental plants are shown in the Table.

b) Control plants were not injected. The shoots of these plants were exposed to [¹⁴C]benzene only in the air of the bell jar, and served as controls for foliar uptake.

Table 3. Radiolabel in plant shoots. Results are shown for radiolabel in experimental plants and control plants (Trials 1 and 2). Note that the *total* radiolabel in the experimental shoots was derived from the following two routes: a) root uptake and translocation, and; b) foliar uptake. The radiolabel in the control plants was derived only from foliar uptake. It is therefore possible to calculate the amount of radiolabel in the experimental shoots that originated from root uptake and translocation.

Table 4. Analysis of benzene parent compound. Purge and trap GC was used to analyze intact benzene molecules in soils, root fractions, and shoots from the high dose experiment (Trial 2). No benzene was found in any fraction.

Table 2. Volatilization of [¹⁴C]benzene in planted and unplanted systems (Trial 3). Rates of volatilization were approximately the same for the two types of systems. This result suggested that alfalfa plants do not facilitate volatilization of benzene, i.e., plants do not act as a conduit for VOCs.

Table 5. "Parafin" experiment. The tentative conclusion that plants do not act as a conduit for VOCs was strengthened by an experiment in which the surfaces of planted soils were covered with parafin. In this trial, only the stems of the alfalfa plants protruded through the parafin. Although the parafin did not make a perfect seal, rates of volatilization and mineralization were *both* reduced after subirrigation with [¹⁴C]benzene. (If plants acted as a conduit for [¹⁴C]VOCs, the rates of VOC efflux would presumably not have been greatly reduced by the parafin seal.)

Tables 2 and 6. Mineralization of [¹⁴C]benzene in planted and unplanted soils. Trial 3 was a comparison of the two types of systems. Data in Table 2 suggested that the extent of mineralization was slightly higher in unplanted system. In Table 6, the ¹⁴C-label remaining behind after VOC efflux was considered "100%". This calculation simplifies the analysis of the fate of benzene in planted v.s. unplanted systems. The data in Table 6 suggested the following: 1) the amount of radiolabel remaining on the soil was about the same for the planted and unplanted systems; 2) more mineralization probably occurred in the unplanted systems; and 3) the decreased mineralization in the planted systems was balanced by plant uptake. The time-course for mineralization was the same in planted and unplanted soils (i.e., plants did not accelerate the rate of mineralization; data not shown).

Summary.

1. **Test system.** A system was developed to study the fate of [¹⁴C]benzene in soils planted with alfalfa and in unplanted soils. Radiolabeled benzene was injected ("subirrigated") beneath the surface of the soils. The test system allowed us to maintain a mass-balance for the radiolabel. Efflux of VOCs occurred immediately after subirrigation, and the percentage of radiolabel recovered as VOCs was quite variable.

2. **Plant uptake.** Less than 10% (3-9%) of the recovered radiolabel was associated with the plant material: 0.5-2% in the shoots and 2-8% in the root fraction (root tissue plus rhizosphere soil).

3. **Gas chromatographic analysis of benzene.** No benzene parent compound (intact molecules) was recovered in the soils or plant tissue, even in the relatively "high dose" experiments (620 µg benzene added/Kg soil).

4. **Volatilization by plants.** Comparisons of planted and unplanted soils subirrigated with [¹⁴C]benzene indicated that plants did not facilitate volatilization (i.e., did not act as a conduit for the volatilization of radiolabeled material).

Ari M. Ferro obtained a Ph.D. degree in 1973 from the Department of Biochemistry, University of Utah, and received post-doctoral training at the University of Hamburg, and the University of California at San Francisco. During the 1980's, he served on the Research Faculty in the Biology Department at the University of Utah. His interest in environmental concerns prompted him in 1989 to switch the focus of his work from basic biochemical research, and in the early 1990's he collaborated with workers at Utah State University on phytoremediation research. Realizing the potential importance of commercializing this technology, he and Jean Kennedy started Phytokinetics in 1994.

Phytokinetics, Inc. has been providing phytoremediation services to a variety of clients for the past two years. The company designs and implements site-specific phytoremediation strategies, which may include a partial site characterization, laboratory/greenhouse studies, small-scale field trials, and full-scale site remediation. Clients have included major oil companies and the U.S. Environmental Protection Agency. Phytokinetics' focus has been the phytoremediation of surface soils and groundwater impacted with organic contaminants. The company's low cost approach is an attractive option for many who need environmental services.

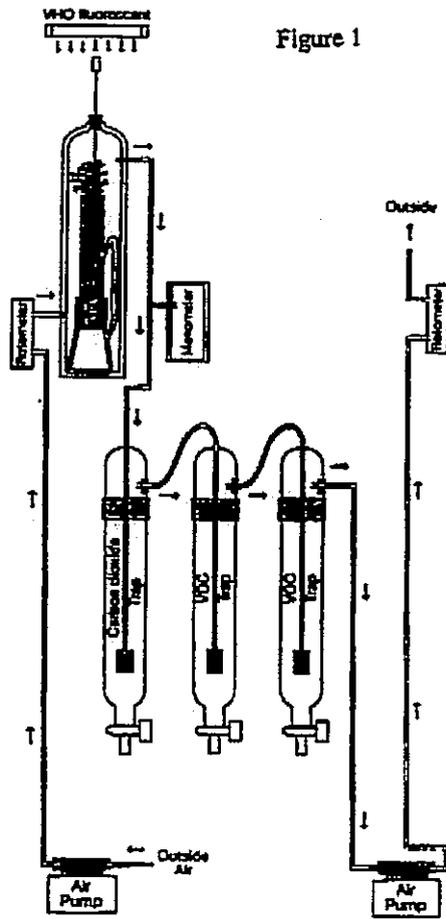


Figure 1

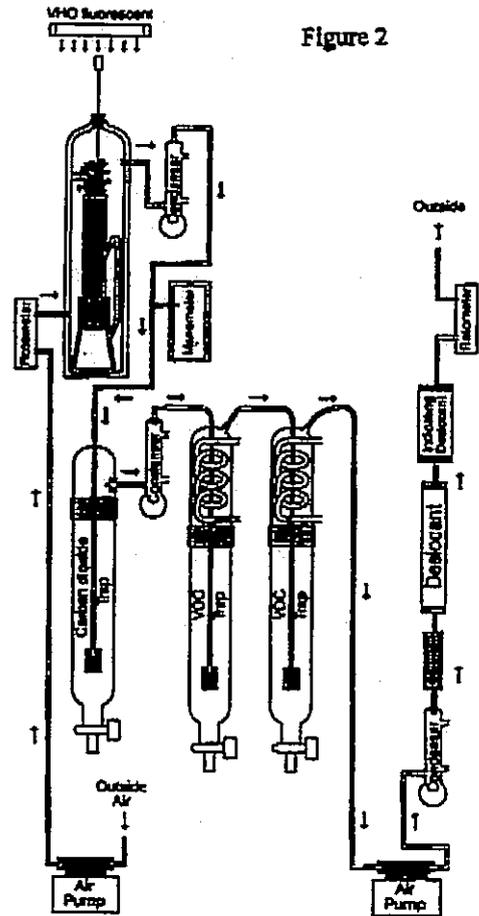


Figure 2

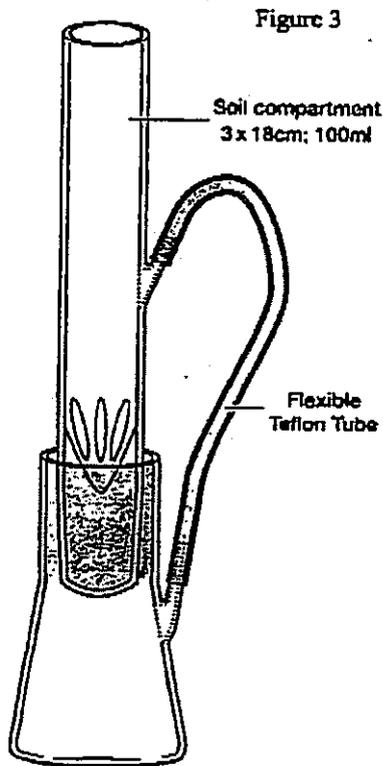


Figure 3

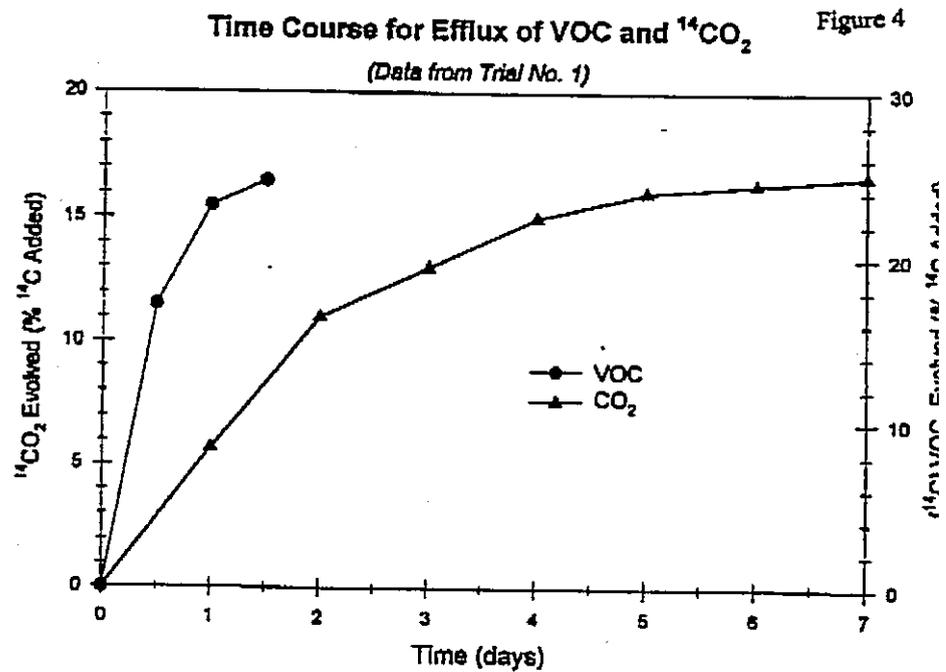


Figure 4

Table 2

Trial	Dose ($\mu\text{g}/\text{kg}$)	System	Mass Balance ¹ (%)	Distribution of Radiolabel (% Recovered)			
				VOC	Soil	¹⁴ C ₀	Plant Tissue Root Fraction
1	39	Planted (n=3)	93 (91-94)	17 ± 5	48 ± 7	26 ± 3	1.7 ± 1.2 [†] 7.5 ± 2
2	622	Planted (n=2)	119 (109-130)	56 ± 5	26 ± 2	13 ± 3	0.5 ± 0.1 [†] 4.0 ± 1.1
3	41	Planted (n=3)	107 (96-127)	61 ± 5	28 ± 3	6.3 ± 3	1.2 ± 0.4 2.0 ± 0.5
		Unplanted (n=3)	106 (79-123)	70 ± 8	21 ± 3	8.5 ± 5.2	-- --

[†] Percent Recovery of Added Radiolabel
[‡] Root → Shoot translocation

Table 3

Trial	Dose ($\mu\text{g}/\text{kg}$)	Radiolabel in Plant Shoots (% Recovered)	
		Experimental Plants (Total* uptake)	Control Plants (Foliar uptake)
1	39	2.9 ± 1.1	1.2 ± 0.03
2	622	0.8 ± 0.3	0.3 ± 0.1

* Total = foliar uptake + root uptake

Figure 5

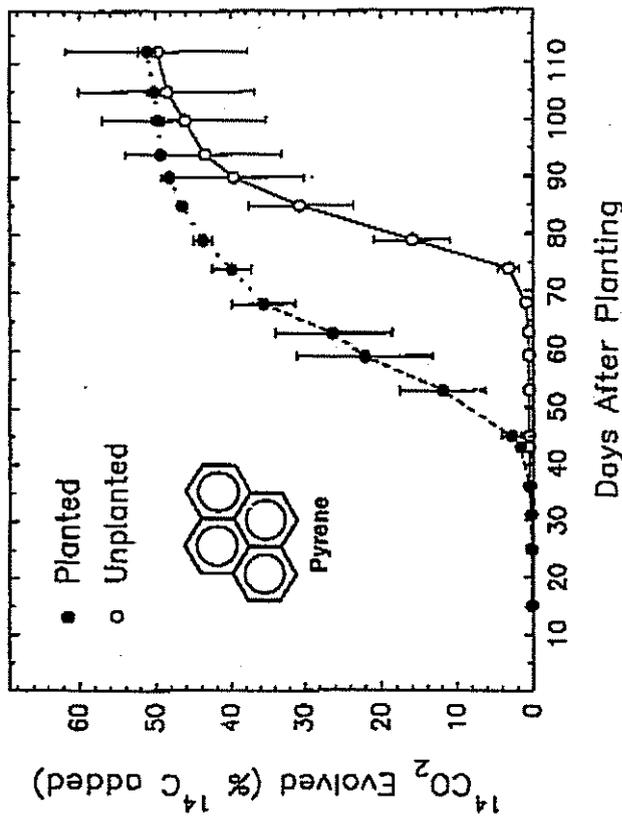


Table 1

¹⁴ C]Benzene Subirrigation Solution			
Dose Concentration (mg/L)	Vol. added [†] (ml)	Total addition (μg)	Total addition (μg/kg)
Low	1 [‡] 6	5	~40
High	15 [§] 6	90	~620

[†] 2 ml injected at 0, 2h, 4h.
[‡] Specific activity ~ 110 x 10³ dpm/μg
[§] Specific activity ~ 28 x 10³ dpm/μg

Analysis of Benzene Parent Compound
Purge and trap gas chromatography
Detection limit (FID) = 0.014 µg

Trial: 2 (planted systems)
Dose: 89.6 µg benzene / 144 g soil (0.62 µg/g)
Result: no detectable benzene parent compound in soil, roots, shoots

Fraction	Analysis	Degradation* (%)
Soil	< 0.04 µg/g	> 75
Root	< 0.04 µg/g	> 80
Shoots	< 0.25 µg/g	> 50

* Assuming 100% extraction efficiency for radiolabeled compound

Table 5

"Parafin" Experiment

Distribution of Radioactivity
 (% Recovered)

Trial	Distribution of Radioactivity (% Recovered)	
	VOC	¹⁴ CO ₂
1	17 ± 5	26 ± 3
2	56 ± 5	13 ± 3
3 (Planted)	61 ± 5	6.3 ± 3
"Parafin"	3.6 ± 3.6	2.1 ± 0.5

Table 6

Distribution of Radiolabel
Remaining after VOC Efflux

Trial	Dose (µg/Kg)	System	% Recovered		
			Soil	¹⁴ CO ₂	Plants
1	39	Planted (n=3)	57 ± 6	32 ± 5	11 ± 2
2	622	Planted (n=2)	58 ± 1	30 ± 0.3	10 ± 1
3	43	Planted (n=3)	73 ± 9	16 ± 7	8 ± 3
		Unplanted (n=3)	72 ± 12	25 ± 12	—

EVALUATION OF THE USE OF PRAIRIE GRASSES FOR STIMULATING POLYCYCLIC AROMATIC HYDROCARBON TREATMENT IN SOIL

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ABSTRACT

A research project was conducted to evaluate enhanced treatment of toxic organic chemicals in soil using deep rooted grasses. Eight types of prairie grasses were evaluated in the treatment of four polycyclic aromatic hydrocarbons (PAHs) in a sandy loam soil. The extent of PAH disappearance in vegetated soil was significantly greater than in unvegetated soil.

INTRODUCTION

The use of deep rooted prairie grasses to stimulate the degradation and detoxification of toxic and recalcitrant organic chemicals at low soil concentrations represents a potential low-cost, effective, and low-maintenance alternative for waste management. However, no significant information concerning specific applications of plants, chemicals, and soils has been published describing either laboratory or field results. Stimulation of treatment of recalcitrant toxic compounds could be brought about through several mechanisms of plant/soil interaction, including: 1) improvement of physical and chemical properties of a contaminated soil, 2) increase in soil microbial activity, and 3) increase in contact between microbes associated with the root and toxic compounds in a contaminated soil.

Polycyclic aromatic hydrocarbons (PAHs) were used in this study due to the recalcitrant nature of these compounds (Sims and Overcash, 1983). Also, the propensity for bioaccumulation and possible adverse health effects of PAH parent compounds as well as intermediates poses special problems in designing a soil treatment system that will effectively reduce the concentration of these compounds.

PAH compounds are generally hydrophobic and non-volatile (Fig. 1). The non-ionic, non-polar structure of PAH compounds leads to partitioning out of the polar water phase and onto hydrophobic surfaces in a soil matrix (Fig. 1). Lipophilic soil organic matter acts as an adsorbent and immobilizes hydrophobic PAHs (Sims and Overcash, 1983). As a result, PAH compounds have low leaching potentials (U.S. EPA, 1986 b, Sims *et al.*, 1987). With the exceptions of naphthalene, acenaphthylene and acenaphthene, PAH compounds are relatively

root systems growing through a soil offer a means of improving aeration and other soil characteristics conducive to the treatment of toxic chemicals.

Much of the gel secreted by epidermal root cells is thought to be composed of low molecular weight carbohydrates (Floyd and Ohirogbe, 1970). Usually this gel is colonized and utilized by soil bacteria (Foster and Rovira, 1976, 1978). Studies of the rhizoplane and rhizosphere with the aid of electron microscopy have demonstrated that bacterial populations in these areas may be two to three orders of magnitude higher than in the outerlying soil environment, where the rhizoplane and the rhizosphere are defined as the epidermal root surface and the immediate soil around the root, respectively (Bowen and Rovira, 1976). In addition, growth of fungi and actinomycetes are also enhanced by the presence of a root system (Bowen and Rovira, 1976). In many cases, a symbiotic relationship is formed between the fungi and the plant root. When this symbiosis takes place, a complex entangled web of fungal mycelium and epidermal root cells is formed. This symbiotic complex is referred to as a mycorrhiza, and is thought to play a role in plant mineral nutrition (Cooke, 1977). Thus, the rhizosphere is a region of intense and complex interactions between the host root system, soil microorganisms, and the soil environment with an increase in total soil microbial activity as the result.

The presence of growing root systems in the soil environment can be viewed as an effective means of increasing and distributing soil organic matter throughout the soil. The proliferation of plant roots also serves as a means of distributing soil microorganisms through the soil as they are carried with growing root tips. Therefore, the probability of contact between microbes and a toxic compound is enhanced.

Plant root systems can be grouped into two main categories; tap root and fibrous root systems. Tap root systems are characterized by an enlarged central root that penetrates down into the soil, with lateral roots branching off this central axis. Fibrous root systems, being finer and more profuse, offer a superior means of increasing the total rhizoplane surface area m^{-3} of soil when compared to a tap root system. The larger rhizoplane surface area of a fibrous root system would be advantageous in the establishment of an active microbial population.

The most intensely characterized fibrous root systems belong to the grass family. Grasses can be categorized into two main groups based on growth habit; 1) bunch grasses which are identified by erect growth of all stems, and 2) sod-forming grasses which produce horizontal stems that may grow above (stolons) or below (rhizomes) the surface of the soil. Of the two growth habits, sod-forming grasses are known for their excellence in stabilizing soil surfaces against erosive forces. Because of the fibrous root system and the capacity to effectively stabilize surface soils, sod-forming grasses are an ideal vegetative cover for *in situ* treatment of contaminated soil.

Sod-forming grasses are by definition perennials. Unlike most agriculturally important grasses (*i.e.*, wheat, barley, corn), perennial grasses do not require reestablishment on a yearly basis. The seeding of perennial grasses leads to the establishment of an easily maintained, highly competitive plant community capable of displacing undesirable or noxious plant species.

Therefore, prairie grasses are superior candidates to test the hypothesis that the presence of plant root systems may enhance removal of PAH compounds by stimulating chemical and biological processes based on the following characteristics:

- 1) The fibrous root systems of grasses provide the maximum root surface area of any vegetation m^{-3} of soil that would be conducive to the enhancement of microbial activity.
- 2) The unmanipulated genetic diversity of prairie grasses may give these species the necessary genetic advantage enabling their establishment in an unfavorable soil environment.
- 3) The deep roots of prairie grasses can penetrate and influence treatment up to 10 feet below the surface (Weaver, 1954).

The objective of this research project was to observe the apparent disappearance of four PAH compounds

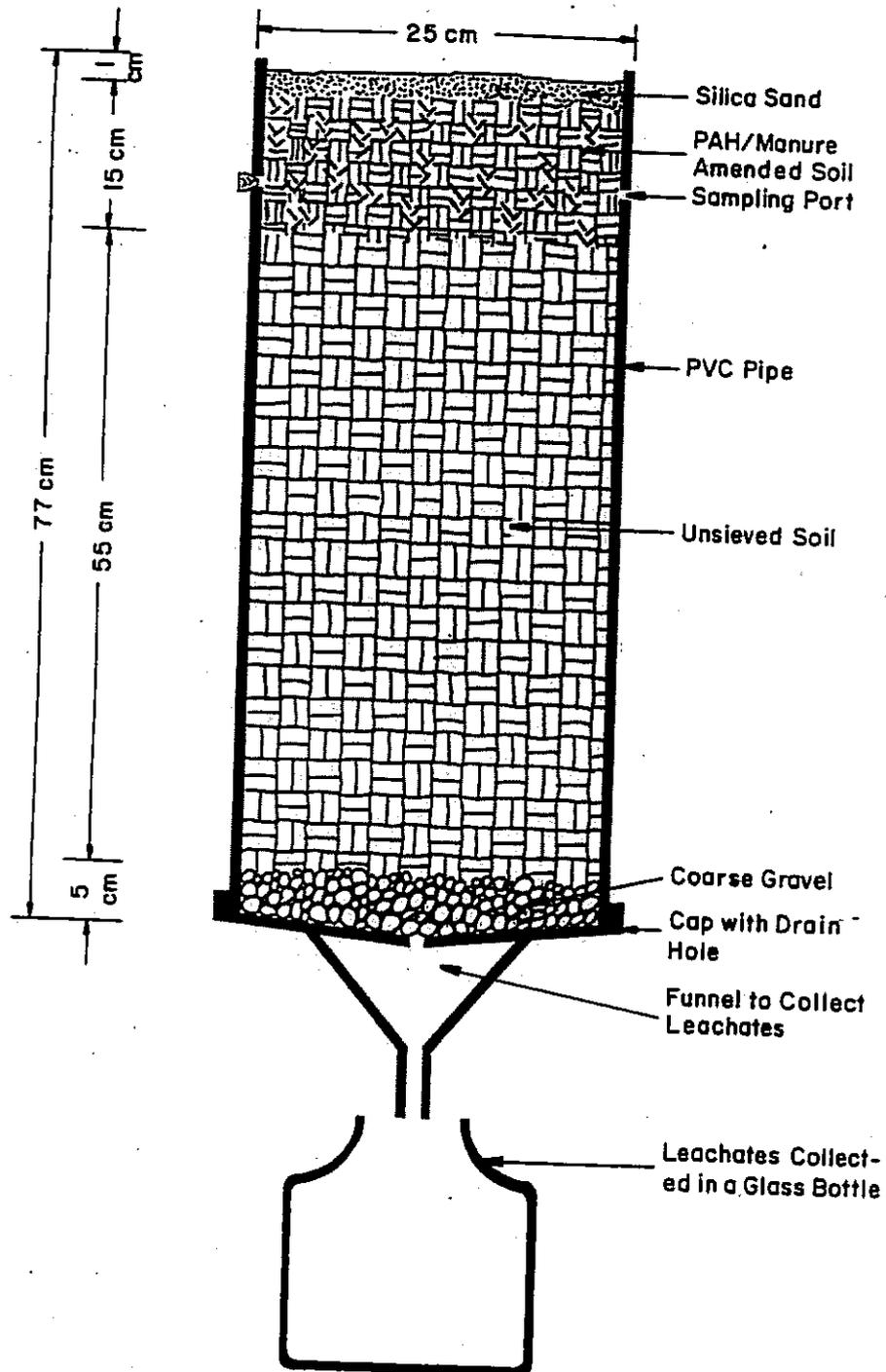


Figure 2. PVC reaction unit showing sampling ports, leachate collection apparatus, and different soil layers.

used to ensure collection of any breakthrough from the first cartridge. The flow rate for concentration was 7 to 8 mL min⁻¹. Tandem Sep-Pak cartridges were eluted using two solvents in a sequential process: 10 mL of methanol, followed by 10 mL of methylene chloride. Each solvent eluent was collected and analyzed individually for PAHs. HPLC analysis of leachate samples followed the PAH quantitation method presented above.

RESULTS AND DISCUSSION

A. PAH Disappearance From Soil

For all four PAHs evaluated, the extent of PAH disappearance was consistently greater in vegetated units compared to unvegetated controls using the Tissumizer™ extraction method after 59 days incubation, and was statistically significantly greater after 151 days incubation (Table 2).

Among the four PAHs evaluated, apparent disappearance was greatest to least for benz(a)anthracene > chrysene > benzo(a)pyrene > dibenz(a,h)anthracene, respectively. This PAH apparent disappearance ranking order was consistent through time of incubation (219 days) and for both vegetated and unvegetated units. The ranking of disappearance correlated with the water solubility of the PAH compounds (Fig. 1), *i.e.*, the more water soluble the compound, the greater the apparent disappearance.

A comparison between the efficiencies of soxhlet and Tissumizer™ soil extraction methods was performed at the beginning and at the conclusion of the experimental period (Table 3). At the beginning of the incubation period, percent recovery of each PAH with the Tissumizer™ method was relatively low compared to soxhlet extraction efficiency. This difference in extraction method performance remained the same or decreased in disparity after 219 days incubation in the unvegetated reactors. However, in vegetated units the ability of the soxhlet method to recover more of the PAHs from soil compared to the Tissumizer™ extraction method increased with time of incubation in soil.

These results lead the authors to hypothesize that the extraction of PAH constituents with the Tissumizer™ apparatus using a hydrophobic solvent system (methylene chloride) was hindered by the sorptive affinity of these compounds for manure organic matter. The affinity for the solid phase was more effectively overcome using the soxhlet method. Because the sorptive interference in PAH extractability using the soxhlet method did not increase over time in the control units but did, however, increase in the vegetated units, it is hypothesized that PAHs were being sorbed to the matrix by some other mechanism than physical adsorption (Van der Waals) alone in vegetated units. Humification processes influenced by the grasses in the vegetated units may account for these observations in the sandy loam soil used in this experiment.

Since the rates and extents of humification in grassland soils is well established as being greater than non-vegetated soils, or even forested soils (Stevenson, 1982), it is plausible that PAH incorporation into soil humus in a grassland system would continue as soil organic content increased and the supply of potential humic building blocks increased as the grass root systems proliferated in a PAH impacted soil environment. Hansen and Schnitzer (1969) recovered a host of large (4-6 ring) PAH compounds from humic acid under chemically aggressive extractive procedures (zinc distillation and fusion). Their contention was that significant amounts of PAHs occur in the "nuclei" of soil humus material. Root growth not only provides exudates that are readily available for humus synthesis, but root senescence also supplies substrates for microbial metabolism which indirectly contributes to humification.

Table 3. A comparison of extraction efficiencies using Tissumizer™ and Soxhlet extraction techniques at the initial time and after 219 days of incubation.

Compound	% Recovery at Initial Time		Average % Increase In Extraction Efficiency of Soxhlet vs. Tissumizer		Average % Increase In Extraction Efficiency of Soxhlet vs. Tissumizer After 219 Days Incubation	
	Tissumizer §	Soxhlet ¶	Initial Time	Unvegetated ¥	Vegetated ¥	Vegetated ¥
Benz(a)anthracene	54.0	91.7	70	56	137	
	±6.4	±10.3				
Chrysene	61.0	98.8	62	41	71	
	±6.8	±11.0				
Benzo(a)pyrene	53.1	102.5	93	91	157	
	±6.7	±16.5				
Dibenz(a,h)anthracene	64.6	96.0	49	49	77	
	±6.1	±10.4				

§ - average and standard deviation of a total of eight samples.

¶ - average and standard deviation of a total of six.

¥ - average of a total of eight samples.

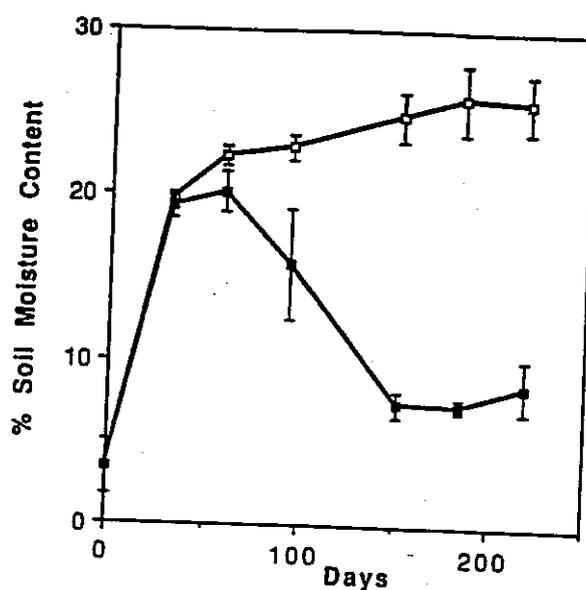


Figure 4. Average and standard deviation of percent (wt/wt) soil moisture content of vegetated (■) and unvegetated (□) units.

CONCLUSION

Results of this study indicate the potential beneficial function of plants in enhancing treatment of PAH-impacted soil at low PAH concentrations. Management applications include *in situ* remediation of uncontrolled waste sites containing PAHs and closure of soil/waste treatment systems containing low soil-PAH levels.

Results of this preliminary study also indicate the need to further investigate the role and mechanisms of plants in the ability to extend PAH disappearance in soil systems containing complex wastes, for example, petroleum and creosote wastes. Possible mechanisms of plant treatment include 1) increased microbial interaction with hazardous constituents due to rhizosphere effects, 2) increased abiotic incorporation of biologically generated intermediate hazardous constituents (*i.e.* humification), and 3) direct incorporation of hazardous compounds into humic material. Direct incorporation would occur as humic building blocks supplied by root exudates and microbial by-products aggregate and humify about the hazardous compound, thus sequestering the compound.

Finally, the function of plants in controlling soil water status should be considered in a soil/waste treatment system, especially one containing more water soluble constituents than used in this study.

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(Accepted 22 December 1989)

MEMORANDUM

Richmond, California
March 4, 1996

**Summary of Phytoremediation
Processes**

The following is a brief review of the processes of phytoremediation.

The various aspects of phytoremediation, or plant-based remediation processes, may be defined as the following: (a) the enhancement of bioremediation of impacted soil and groundwater by the introduction of vegetation, (b) the engineered uptake and subsequent disposal of high concentrations of inorganic compounds (eg. metals) by plants, (c) stabilization of inorganic constituents in soil by the addition of vegetation and/or organic material, (d) inhibiting leaching by the utilizing the water uptake ability of plants, (e) surface water/effluent remediation by wetland vegetation.

1) Published results of preliminary field tests and laboratory experiments suggest that degradation of hydrocarbon compounds by bacteria and mycorrhizae fungi, in both the saturated and unsaturated zone, are accelerated by the presence of the appropriate species of plant. The specific compounds studied have included fuel hydrocarbons, several types of pesticides, and chlorinated hydrocarbons.

1) The plant is believed to enhance the rate of in-situ biodegradation by the following processes:

(a) providing a physical substrate for bacterial growth. The roots also provide a favorable medium for the growth of mycorrhizae fungi, which are found in symbiotic association with the plant. The rhizosphere is the portion of the subsurface characterized by complex fungal/root interaction (Aprill, et al, 1990). The enzymatic activity of the fungi apparently degrade organic compounds which cannot be transformed solely by the bacteria (Schnoor, et al, 1995). Bacterial colonies may cover up to 4-10% of the area of the plant root surface area (Shimp, J.F., et al, 1993).

Rhizosphere soil was shown to be characterized by microbial numbers at least one to two orders of magnitude higher than non-vegetated soil (Anderson, et al, 1993; Aprill, et al, 1990; Schwab, et al, 1993). Soil planted with alfalfa and alpine bluegrass, were noted

in an experimental setting to have elevated numbers of bacteria, compared to non-rhizosphere soil (Roger, et al, 1995). The initial respiration rates in unimpacted planted soil were 1.6 to 2.4 times greater than the respiration rates in unvegetated soil, indicating more microbial biomass in the rhizosphere soil (Anderson, et al, 1993).

(b) oxygenating the subsurface by passive movement of air along the root-caused microfractures (Davis, L.C., et al., 1993), and active transport of oxygen to the root zone within the plant phloem (Schnoor, et al, 1995).

(c) providing a bacterial food source of discarded and dead root cells, and exuded carbohydrates, amino acids, sugars, alcohols and other organic compounds from the roots. The composition of the exudate is primarily a carbohydrate in nature (Aprill, et al, 1990). The leakage of exudates have been estimated to possibly amount to 10-20% of the total plant photosynthate produced on an annual basis (Schnoor, et al, 1995); accounting for the apparent importance of the growing plant in maintaining a viable microbe population. The shedding of organic material by the plant roots increase the bulk organic carbon content of the soil, which has the additional potential benefit of retarding the transport of dissolved organic compounds in groundwater.

(d) in addition to the enhancement of microbial activity, a component of the solute may be directly taken up by the plant and metabolized. The extent of incorporation of various compounds into nonphytotoxic metabolites within the plant tissue is currently being investigated (Cunningham, et al, 1995; Bedell, 1992). Degradation of the target compounds within the plant are believed to take place primarily by intracellular metabolism, followed by incorporation of the conjugates into stable plant structural molecules (such as lignin or cellulose), or sequestered in subcellular compartments called vacuoles (Bedell, 1992).

Cunningham, et al, (1995) characterizes the possible plant pathways of organic and inorganic constituents as follows:

- (i) storage within plant tissue unchanged
- (ii) bound to plant structural constituents
- (iii) metabolized within the plant
- (iv) passed through the plant as the parent compound, and volatilized

A mass balance project designed to quantify the extent and fate of dissolved benzene uptake by alfalfa and fescue, is presently being conducted by Ari Ferro (Phytokinetics), and funded by CRTC.

Work with whole plants and cell cultures in the degradation of trinitrotoluene (TNT), polychlorinated biphenyls (PCBs), various pesticides suggest that a plant based metabolism strategy has

advantages over a bacterial based approach (Bedell, 1992). Bedell cites the following potential advantages of a whole plant/cell culture versus an enhanced bacterial-based remediation approach, based on his work using cell culture material to degrade TNT:

(a) plants have the ability to withstand exposure to greater concentrations of pollutants.

(b) plants have the capacity to accumulate xenobiologic compounds rapidly inside the cell. Bacteria tend to metabolize the target compounds on the surface of the cell.

(c) plants are apparently able to metabolically convert compounds of interest to less toxic forms at a rate faster than either bacteria or fungi.

Accelerated rates of TNT degradation was documented within a cell culture environment by Bedell (1992).

e) The plant root zone, the rhizosphere, is the active region of the subsurface where increased microbial activity may be utilized to degrade the soluble and residual hydrocarbons in-situ. The characteristic root structure of the individual plant species would be one of the critical factors determining the site specific plant to be used in the remediation project. In general, a fibrous root structure is viewed as most advantageous for (a) the encouragement of bacterial growth, (b) improved aeration of the soil, and (c) increasing the contact area between the microbes and the residual contaminants.

In most grasses, for example, the bulk of the root structure is generally within the upper 20-30 cm of the soil. Direct soil remediation using plants is likely limited to the upper six feet, which is the reasonable lower limit of the rhizosphere (Schwab, et al, 1993).

2) Phreatophytic plants, such as Cottonwoods, Poplars and Willows, possess an active root system, which can exist at least partially submerged within the generally anaerobic saturated zone. Field projects to determine the applicability of using Poplar trees (*Populus* spp.) as hydraulic barriers, rely on the documented presence of approximately 35% of the Poplar root mass able to be exist within the saturated zone (Paterson, et al, 1992). At a particular test site in Iowa, the viable Poplar root system, in healthy plants, was noted to extend at least one meter below the water table. Part of phreatophytic trees' adaptability to drawing water directly from below the water table lies in the ability to transport oxygen to the immediate vicinity of the submerged roots; therefore encouraging biologic activity and associated biodegradation in the saturated zone.

The Cottonwoods, Poplars, Willow, and others, also remove

significant quantities of water from the subsurface. Limited data suggests that the water removal may generate a groundwater cone of depression which may be applied to minimize solute migration (Gatliff, E.G., 1994). The intended purpose of tree installation at a site would be to both enhance local biodegradation of the dissolved and residual phase, and to establish a hydraulic barrier to inhibit migration.

(a) An operating leachate collection enclosure at the Sanifill Landfill facility in McMinnville, Oregon, utilizes approximately 40,000 Poplar hybrid trees to inhibit the introduced leachate water from impacting the local water table. The evapotranspiration ability of the planted trees has been shown to be sufficient to maintain an upward hydraulic gradient, preventing the contamination of groundwater (verbal communication - Sanifill; and Schnoor, et al, 1995).

The trees are planted 10 feet apart, in rows separated by a 2 foot spacing. The entire planted area is 14.3 acres, within a 100 acre facility. The Poplars were planted in 1992 and 1993. During the 1995 growing season (May-October), approximately 12.5 million gallons of leachate water were irrigated into the planted area.

The compound of concern in the leachate water is soluble nitrate, probably in the form of ammonium nitrate. The presence of dissolved hydrocarbons, metals, solvents, etc. are minimal in the leachate.

(b) The presence of Poplar hybrid trees (Imperial Carolina hybrid, Populus deltoides nigra, DN34) densely planted along stream margins in Iowa, are believed responsible for reductions in measured nitrates in excess of 90%; presumably as compared to pre-planted concentrations (Schnoor, et al, 1993).

(c) An appropriate series of groundwater monitor wells should be present both upgradient and downgradient of the installed line of trees, to establish the effectiveness of the system to inhibit off-site migration. A series of monitor wells or piezometers oriented perpendicular to the line of trees will also help determine to what extent the activity of the trees creates a hydraulic barrier and locally depressed water table.

3) Uptake of water and associated solute also likely involves the unmetabolized transpiration of a portion of the mobilized compound. While mass balance studies are minimal, plant uptake is generally maximum for compounds with a log octanol-water partition coefficient (K_{ow}) in the range of 1 to 2 (eg. volatile aromatic hydrocarbons). Movement of the solute from the root to the above ground portions of the plant, via the plant circulatory system, is also optimal when the compound is characterized by a log K_{ow} in the range of 1 to 2 (Ferro, et al., 1994; McFarlane, et al., 1987; Cunningham, S.D., et al., 1993). The results of hydroponic studies using several plant species exposed to nitrobenzene ($K_{ow} = 1.85$),

suggest that more than 50% of the nitrobenzene taken up by the plants were transpired as non-metabolized nitrobenzene (McFarlane, et al., 1990). It should be noted that a hydroponic study eliminates the effects of the soil microbial population, and therefore is not a realistic simulation of a naturally occurring field situation.

Preliminary results of a CRTC funded mass balance study using alfalfa in soil exposed to benzene solution indicates minimal transpiration and uptake of the solute (A. Ferro - written communication, September 1995). Elevated concentrations of carbon dioxide was also noted associated with the introduction of the dissolved benzene, suggesting increased levels of biodegradation by the rhizosphere microbe community. The extent of solute metabolism by the plant has not as yet been defined.

4) A significant amount of experimental and literature review analyses concerning the uptake and incorporation of hydrocarbon compounds by plants, has recently been sponsored by the Electrical Power Research Institute (EPRI, 1992, and unpublished). The polynuclear aromatic hydrocarbons (PNAs) have generally been considered for plant uptake analyses, due to their common presence as residual hydrocarbon soil components. The compounds are characterized by relatively low water solubility and volatility, and a high affinity to adsorb to clays and organic carbon in the soil matrix.

PNA compounds of high molecular weight (4-5 or more rings, eg. pyrene) and high lipophilicity, have an affinity to sorb to the outer root surface, without crossing the cell wall into the root structure. The high molecular weight PNAs would not be expected to be transported to the foliage or other above ground portion of the plant in amounts greater than trace quantities. The chemical and physical properties of the high ring number PNAs suggest that bioconcentration of the compounds or their metabolites within the plant tissue would not be expected to occur.

PNAs of low molecular weight (2, 3 and 4 rings) have the greater potential for plant uptake. While the bioconcentration of the compounds or their metabolites was not noted, some uptake of naphthalene (2 rings), anthracene (3 rings) and benzo(a)anthracene (4 rings) has been indicated for root tissue.

Preliminary results of the Phase II portion of the EPRI study has indicated the following degree of incorporation of PNA compounds into plant material, during controlled experimental procedures (verbal communication, Douglas Munnucke, ABATE Program coordinator of EPRI).

(a) after five days of contact with the compound, less than 0.8% of the available naphthalene (2 rings) in the test plot was incorporated into the analyzed plants.

(b) less than 0.02% of the available C¹⁴ tagged phenanthrene (3 rings), fluorene (3 rings) and pyrene (4 rings) were incorporated as plant tissue, during a test of unspecified length.

The results of the literature review portion of the Phase I EPRI study, other published works (eg. Schwab, et al, 1993), and the preliminary experimental results of the Phase II EPRI study, suggest that the incorporation of PNA compounds within the soil by plants is negligible. The degree of apparent uptake of PNAs by plants from a soil medium has also been noted to be species specific.

5) Degradation of PNAs, in association with plants and rhizosphere microbes, was shown to be accelerated as compared to unplanted samples (Schwab, et al, 1993; Sims and Overcash, 1983; and Qiu, et al, 1993). The Schwab (1993) study also documents that the observed reduction in the concentrations of PNAs in the test soil is the result of biodegradation, rather than adsorption, abiotic degradation and leaching.

A comparison of degradation and volatilization rate of C¹⁴ labeled naphthalene, between soil and grass laboratory environments, showed the following results (Watkins, et al, 1994):

(a) increased rates of volatilization within the grass planted, as compared to unplanted environment. The mechanism explaining the apparent increase in the volatilization rate is unknown.

(b) decreased rates of mineralization of the supplied naphthalene in the planted versus unplanted samples.

The anomalous results were not explained by the authors.

6) Accelerated degradation rates of pentachlorophenol in soil was documented in experimental work by Ferro, et al, 1993 with Crested wheatgrass. In 155 days the mineralization of PCP was 22% in the planted system, versus 6% for the unplanted control.

7) A study by Sims and Overcash (1983) states that background levels of PNAs in plant tissue are not uncommon. The authors believe that atmospheric deposition is generally the primary source of PNA compounds in vegetation, rather than uptake from an impacted soil source, based on the following:

(a) the general presence of higher concentrations of PNAs in the above ground portion of the plant, compared to the plant roots. Their study cites a range of measured PNA concentrations in tree leaves as 22-88 ppb, in cereals as 48-66 ppb, in leafy vegetables as 0.05-50 ppb, and in fruits as 0.02-0.04 ppb. By contrast, the range of PNA concentrations in analyzed underground vegetables,

including potatoes, carrots, onions and radishes measured 0.01-6 ppb.

(b) the degree of PNA concentration within the above ground portion of vegetables may often be explained by surface area/kg, location to an airborne source, and exposure time.

Support for a primarily atmospheric coating mechanism for the presence of PNAs on the leaves of plants is expressed in pre-print data present in Reilly, et al, and Schwab, et al, 1993. The Reilly, et al, pre-print cites typical background concentrations of PNAs in plant tissue, in remote regions, within the range of 50-80 ug PNA/kg plant.

8) Species specific work involving the degradation of the herbicides atrazine, metachlor and trifluralin by the presence of the plant Kochia sp., document increased rates of biodegradation in planted versus unplanted samples (Anderson, T.A., et al, 1993). The amount of plant exudation was observed to increase when various test plants were exposed to xenobiotic chemicals, such as herbicides. The impacted plant/soil samples showed a corresponding increase in microbial colony forming numbers of two to three orders of magnitude when compared to the planted, unimpacted samples. The increased exudation by the plant may provide the means to sustain the increased microbial community, apparently responsible for the measured increased rate of biodegradation.

Experimental data involving petroleum hydrocarbons, similarly showed an increase in the populations of organic chemical degrader species of bacteria in test plots containing both the plants and the subject chemical (Roger, 1995).

Insecticides, which are usually not toxic to plants, are likely cometabolized by the enhanced microbial community of the rhizosphere (Anderson, et al, 1993).

Published work, cited in the Anderson (1993) paper, describe the results of biodegradation of atrazine, 2,4-D, diazinon, parathion, benthocarb, endosulfan, dieldrin and pentachlorophenol using plant associated microorganisms.

9) Increased rates in the degradation of C¹⁴-labelled TCE were noted in the rhizosphere of several species in whole plant studies, where planted versus unplanted environments were compared (Walton, et al, 1990). The response was noted to be species specific of the type of plant employed in the test (Anderson, et al, 1993; Schwab, et al, 1993).

10) A plant screening experiment involving varying concentrations of soil hydrocarbons was conducted to determine the germination and seedling viability of the legumes alfalfa, red clover (Trifolium pratense), white clover (Trifolium repens), birdsfoot trefoil

(Lotus corniculatus), and the grasses alpine bluegrass, tiley sage (Artemisia tilesii), Bering hairgrass (Deschampsia beringensis), reed canarygrass (Phalaris arundinacea), and quackgrass (Elytrigia repens) (Rogers, 1995). The response to the presence of the applied hydrocarbons in the soil was species specific, but germination rate and plant survivability were severely reduced at concentrations of hydrocarbons at, and above, 4000 ppm.

11) Minimal data is available to date regarding the uptake of water by trees.

(a) A study by Hinckley, et al, 1994, conducted on a four year old stand of Poplar hybrids (Populus) indicated the following:

Trees - 33-45 feet tall, 3-6 inch diameter

Water use = 20-26 kg water/day to 39-51 kg water/day
= 5.3-6.8 to 10.3-13 gallons water/day

It should be noted that a dense, broadleaf canopy will create a micro-environment which will tend to decrease the water usage/uptake of the individual plant. Ari Ferro (Phytokinetics) believes that the water uptake ability of an individual plant (Poplar), removed from a stand, is likely at least two times the value cited above.

The water consumption within a thinner canopy, and needle leaved tree will apparently exhibit a greater coupling to local atmospheric conditions, such as wind speed and humidity. Conditions within a dense stand are characterized by increased local humidity, decreased ground warming, and decreased influence of wind; all factors which will result in diminished water uptake.

The Hinckley, et al, study also showed maximum transpiration in the upper portions of the subject trees' canopies.

(b) An estimate of water use for Bald Cypress (Taxodium distichum) and Cabbage Palm (Sabal palmetto) was calculated based on the stomatal conductance of the two species, and meteorological conditions in their native growing area, southwest Florida (Roger Kjellgren, Utah State University, written communication). Water use during the Cabbage Palm's twelve month growing season varied seasonally, but was estimated at 21,700 gallons (0.04 gpm). Water uptake during the Bald Cypress nine month growing season was estimated to be 49,300 gallons (0.13 gpm). It should be noted that the water uptake estimates do not distinguish moisture originating in either the shallow unsaturated zone or the deeper saturated zone.

(c) The water uptake rate of grasses may also be estimated, which would be useful in determining whether a planted field could inhibit the leaching of rain water.

12) Pole planting and whip planting techniques are designed to encourage root development within the saturated zone, by the placement of a portion of the hardwood cutting directly below the water table (USDA - Soil Conservation Service, 1993).

Whip planting is commonly used for the revegetation of riparian areas. Pole planting, or the planting of very large hardwood cuttings, have successfully been used in more upland environments with relatively deeper water tables than a riparian situation.

Installation involves the drilling of holes to a depth below the water table, with the bottom portion of the pole installed within the saturated zone. A minimum shoot length of approximately 1 foot should be continually submerged during the first 1 - 2 growing seasons, but submerged lengths in excess of 6 feet have been documented (verbal communication Jay Banta - Fish Springs Wildlife Refuge, UT, Wes Martin - Lower Colorado River Refuge Complex, CA/AZ, and Mark Pater - Natural Resources Conservation Service/Tucson Plant Materials Center, AZ). Roots developing within the saturated soil have direct access to groundwater, both eliminating the need for irrigation and further encouraging the development of deep root growth. Root development and success rates will in part depend on the availability of oxygen in the subsurface; a situation which can be at least in part addressed by the installation of 3/4 inch PVC breather tubes installed along side the planted cutting.

The associated advantage of pole planting is that the high energy stores in these large hardwood cuttings leads to the rapid development of relatively large trees.

Pole planting Cottonwood has proven to be successful in revegetating riparian and stream marginal environments at various US Wildlife Refuges in southern California and Arizona (verbal communication - Banta, Martin and Pater). Survival rates in excess of 80% to 85% were noted in planted environments characterized by water tables in the range of 2 to 8 feet BGL, with projects described as successful when groundwater was 15 feet BGL. Tree growth of 15 to 20 feet within the first growing season was noted accompanying the planting of 1500 Cottonwoods at the Bill Williams Wildlife Refuge, AZ (?) (verbal communication - Pater). The Cottonwoods at the Bill Williams Refuge were planted with approximately 1 to 5 feet of the cutting submerged below the water table.

Wes Martin (verbal communication) believes that the success of his planting projects is in part due to the drip watering systems installed with the trees. The watering is gradually diminished for the purpose of encouraging root growth to the water table/capillary fringe. It should be noted that Mr. Martin believes in planting the cuttings to a depth immediately above the water table, rather than

initially submerging a portion of the cutting.

The use of water soaking and growth hormonal treatment prior to planting is recommended as essential by the Wildlife Refuge specialists, cited above.

13) The metabolism of the PNA compound benzo(a)pyrene to oxygenated derivatives within the plant cell, was documented using a cell suspension culture of Chenopodium rubrum (Harms, et al, 1977). The quantity of radiolabelled metabolite was observed to increase as a function of incubation time.

14) Uptake of inorganics - metals

Trace amounts of metals are taken up, translocated within the plant, and in some cases accumulated in many species of plants. The ability exists for some plant species (hyperaccumulators) to uptake and accumulate metals within the roots, or in the above ground shoots, at concentrations exceeding those in the surrounding soil and/or water. The tendency to hyperaccumulate apparently developed as a survival adaptation to soil rich in metals, as evidenced by the presence of hyperaccumulator species often restricted to former/present mining areas and mafic, metaliferous bedrock.

An example of a hyperaccumulator is the tree species Sebertia acuminata, which grows on nickel ore outcropping in New Caledonia. The sap of the tree has been analyzed to contain up to 25% dry weight nickel. Other examples of metal hyperaccumulators are provided in Cunningham, et al, (1995b), and Gowthaman, unpublished, (1995).

The utilization of hyperaccumulator species to remove metals bound to the soil, or dissolved in pore water forms the basis of the concept of phytoextraction (as it is termed in Cunningham, et al, 1995b). It is estimated that the subject plant should be able to accumulate the metal in question to a concentration of >1-3% of the plant dry weight, for phytoextraction to be a viable remediation strategy (Cunningham, et al, 1995b). The plant should also ideally have sufficient mass to enhance the metal removal rate, and the ability to translocate the dissolved metals into the shoots for easier removal and disposal.

The phytoextraction process also requires the bioavailability of the metal. For example, metal bound within the interstitial clays or organic matter in the subsurface may be unavailable for plant uptake.

(a) Chromium - the different chemical properties of the two common species of chromium, Cr(III) and Cr(VI), influence their respective ability to be taken up from the subsurface, and translocated and accumulated by plants.

Cr(III), the less toxic valence state, is the predominant form under reducing conditions and is relatively immobile in the subsurface. In contrast, Cr(VI), is significantly more toxic, is the predominant form under oxidizing conditions, and is relatively mobile in aqueous solution (Davis, et al, 1995). Cr(VI) has been shown to reduce to the less mobile Cr(III), augmented by the presence of Fe^{+2} . The reverse reaction, the oxidation of Cr(III) to Cr(VI), is apparently enhanced in the presence of dissolved manganese oxide (verbal communication, Bill Berti - DuPont).

Experimental work has shown that the movement of an acidic solution of dissolved Cr(VI) through an organic matrix, results in the reduction of the metal species to Cr(III) (Makos, et al, 1995). The complexing of the chromium by organic matter results in the binding of formerly mobile Cr(VI) to the soil as Cr(III). Of the two common valence states of chromium, relatively immobile Cr(III) would be the less likely to be potentially taken up and translocated within a hyperaccumulator plant.

Remediation of chromium in soil by utilizing the uptake and translocation ability of specific types of plants requires that the metal be in a biologically available form. Cr(III), which would likely be the more common form in a reducing wetland soil, is essentially unavailable for plant uptake (Vajpayee, et al, 1995). Chelating the Cr(III) in the presence of organic acids, such as citric acid, fulvic acid, diethylenetriaminepentacetic acid (DTPA), and water-soluble organic matter, has been found to significantly increase the solubility range of the organically complexed Cr(III) (James, et al, 1983a, Davis, et al, 1994). The organically complexed, and soluble Cr(III), is also likely to be in a form which may be more readily available for plant uptake (Andy Davis - Geomega, verbal communication).

It may be possible to induce the organic complexing of Cr(III) in-situ by the addition of organic acids, in order to encourage the plant uptake and translocation of chromium present in soil. The candidate acids which may be used (eg. citric acid, fulvic acid) are compounds which are naturally occurring components of many wetland environments; therefore the potential impact of the chelating step to the local environment may be minimal.

The potential creation of Cr(VI) from Cr(III), possibly caused by chelation induced oxidation, has been noted in laboratory procedures (James, et al, 1983b). The presence of zero valence state iron (eg. iron filings) may have the effect of inhibiting the formation of Cr(VI) during chelation of the Cr(III) (Andy Davis - verbal communication).

In spite of a general reluctance of plants to uptake chromium (Baker, et al, 1989), several field laboratory studies indicate notable hyperaccumulation of chromium from solution, by several wetland and water plant species (Lee, et al, 1981; Zaranyika, et

al, 1995; Vajpayee, 1995; Gowthaman, UC Berkeley, 1995, written communication, David Morrey, Golder Associates, verbal communication). Uptake of chromium was noted in the following common wetland/aquatic species: *Phragmites communis*, *P. australis*, *Spartina patens*, *S. alterniflora*, *S. foliosa*, *Scirpus validus*, *S. robustus*, *Distichlis spicata*, *Triglochin maritima*, and water hyacinth. Many of these species are found in temperate climates, suggesting a possible application for chromium remediation of US sites.

While uptake of chromium was measured in the above studies, it should be noted that the metal was concentrated entirely in the plant root. It is not stated in the above stated literature whether Cr(III) or Cr(VI) is the valence state being accumulated by the plant.

David Morrey (Golder Associates, verbal communication) believes that any Cr(VI) in solution taken up by a plant will be rapidly converted to Cr(III) within the plant system.

The effect of induced chelation on the solubility, valence state formation, and plant uptake ability should be confirmed in a laboratory/greenhouse study prior to application at a field site.

15) The potential effectiveness of plants to act as capping agents may be estimated by calculating the anticipated water uptake rate of particular species under specific climatic conditions. For example, Ari Ferro and Roger Kjelgren (Utah State University) have estimated that a field of tall fescue (12 inches high) growing in the Seattle, Washington area, will transpire between 41 and 173 gallons/acre, depending on the season (written communication). The transpiration rate is calculated by the relationship of the estimated stomatal conductance of the plant species, and the seasonal temperature/humidity of the site.

16) Potential applications of a phytoremediation strategy are as follows:

(a) accelerate the rate of the degradation of residual hydrocarbons and pesticides in the shallow soil horizon by planting vegetation characterized by an aggressive, fibrous root system (eg. grasses, alfalfa). The realistic depth for remediation is likely the upper two feet (optimal), to approximately six feet below ground level. Enhancing biodegradation may be applied either in-situ or to excavated hydrocarbon impacted soil.

(b) inhibit groundwater flow with the installation of a line(s) of closely spaced phreatophytic trees, oriented perpendicular to the direction of groundwater flow. The concept is to use the trees to establish a hydraulic barrier.

The ability of the trees to influence groundwater flow is

dependent, in part, on (a) the transpiration ability of the plants, (b) climate, and (c) depth to groundwater. A depth to groundwater in excess of approximately 10 feet is likely too deep to realistically expect the tree root system to impact groundwater flow.

(c) dewatering of a shallow perched water interval by planting phreatophytic trees. It must be assured that the roots of the plants are in contact with the perched water.

(d) uptake, translocation and accumulation of metals from soil or surface water. The metal must be present in a form which is available to the hyperaccumulator species.

(e) the water uptake ability of many plant species may be used to inhibit downward water migration, as a mechanism to inhibit leaching.

(f) the use of plants as a means of stabilizing metals in soil is being investigated. Research at DuPont (Cunningham, Berti, and others) suggests that the presence of organic material in the subsurface tends to bind many metals in an insoluble form, therefore inhibiting migration.

Please call me at (510) 242-1383 if you would like to discuss this matter further.

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Beneficial Effects of Plants in the Remediation of Soil and Groundwater Contaminated with Organic Materials

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ABSTRACT: The use of plants in remediation of soil and unconfined groundwater contaminated with organic materials is appealing for a variety of reasons: (1) plants provide a remediation strategy that utilizes solar energy; (2) vegetation is aesthetically pleasing; (3) plant samples can be harvested and tested as indicators of the level of remediation; (4) plants help contain the region of contamination by removing water from soil; (5) rhizosphere microbial communities are able to biodegrade a wide variety of organic contaminants; and (6) many plants have mechanisms for transporting oxygen to the rhizosphere. However, before effective plant remediation strategies can be developed, an understanding is needed of the physical, biological, and chemical relationships that determine the fate of each organic contaminant in the rhizosphere. This review presents an overview of some factors required to understand and model the complex processes that determine the fate of the organic contaminants in plant remediation strategies. In addition, some planning and management criteria for the development of practical plant remediation strategies are presented.

KEY WORDS: bioremediation, rhizosphere, transport, plants, uptake, soil, groundwater.

I. INTRODUCTION

There are a number of techniques available to remediate soil and groundwater contaminated with organic compounds. These include physical containment, withdrawal and treatment, and *in situ* treatment.^{1,31,91,110,143,146,159} Physical containment techniques may consist of excavation and removal to a secure site, installation

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of physical barriers, and extraction by wells or subsurface drains. The contaminated soil and groundwater can be withdrawn and treated by microbial, chemical, and/or physical processes. *In situ* treatment involves the microbial, chemical, and physical processes that effect remediation of contaminated soil and groundwater in place, e.g., vapor extraction, biodegradation, and soil flushing. The selection of the appropriate technology depends on the hydrologic and geologic characteristics of the affected site. Because of their simplicity, pump-and-treat methods have been widely used.¹¹³ Conventional soil and groundwater remediation techniques are very expensive for the clean-up of large sites contaminated with organic substances such as herbicides, petroleum hydrocarbons, or chlorinated solvents.

A possible cost-effective bioremediation technology was suggested by Licht⁹² and Schnoor and Licht,¹⁴¹ which involves the use of deep-rooted poplar trees planted as a buffer zone at the edge of the contaminated site. Plants may stimulate the removal of the hazardous organic substances by uptake and accumulation, by metabolism, and by microbial biotransformation in the rhizosphere. This could prove to be an efficient treatment technique to remediate soil and groundwater if effective planting and management strategies can be developed. In a recent book by Chambers et al.,³¹ plants received very little attention under the topic of *in situ* treatment methods. Yet almost all terrestrial ecosystems subject to contamination have significant plant populations that have a vast range of microorganisms associated with their roots.

The following chemical balance for microbial growth, with the organic contaminant as the substrate, defines the primary nutritional requirements for aerobic biodegradation as it may occur in the root zone of plants.



Contaminant (substrate)	Biomass	Extracellular product
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In addition, phosphorus and other inorganic nutrients, including trace quantities of several minerals, in an aqueous environment are essential for microbial growth and biodegradation. The rate of contaminant utilization also depends on soil temperature.

The microorganisms that degrade contaminants at low concentration levels in the soil or groundwater may be limited by any one or a combination of these factors. Usually, microbial growth is substrate/energy limited; less frequently, it may be limited by nitrogen, phosphorus, or trace minerals; and in saturated soils, it may be oxygen limited. Plant-aided, *in situ* biodegradation depends on (1) the composition of the rhizosphere microbial communities; (2) root exudates that act as supplemental substrates; (3) nitrogen present in the water, supplied by the decaying roots, or fixed by symbiotic or free-living bacteria; (4) oxygen transfer to the soil; and (5) the kinetics of microbial degradation, which is, in turn, dependent on temperature, nutrient concentrations, and the presence of water.

The first part of this review examines how plant roots are able to support a viable microbial population, which is, in turn, available to biodegrade contaminants.

Some of the contaminants may be taken up by the roots into the plant. The movement of the contaminants in the soil and groundwater is affected by the flow of groundwater to the roots. A soil and root water transport model and a plant uptake model are presented later in this review.

II. THE BASIS FOR *IN SITU* BIODEGRADATION

A. Soil Microbial Populations

A useful textbook treatment of soil ecology is found in Foth.⁵¹ The three major types of soil microorganisms in numerical terms are bacteria, fungi, and actinomycetes. Prokaryotic single-celled bacteria are the most numerous, with 1 g of soil containing 10^7 to 10^{10} individuals.⁵¹ Most are heterotrophs that feed on the carbon in organic compounds. Some types of bacteria are anaerobes, or facultative aerobes, but most species of bacteria of interest for the proposed bioremediation strategy are aerobic, requiring oxygen for respiration. Under favorable conditions of temperature, pH, and substrate concentration, some bacteria may reproduce every 20 min. A $>10^7$ increase of bacteria could be produced in 1 day if each bacterium and its descendants were to reproduce every hour; but growth quickly becomes limited by lack of substrate and the accumulation of self-inhibitory substances.

Fungi are eukaryotic heterotrophs that generally grow from spores and form threadlike structures called mycelia. Whereas bacteria grow on a localized site, the fungal mycelium grows out into the surrounding environment. A gram of soil may have 10 to 100 m of mycelial threads; however, the live weight of eukaryotic fungus per gram of soil is approximately the same as that of bacteria.

Actinomycetes are a group of prokaryotic soil bacteria that are superficially similar in structure to fungi. They are single celled, but form filaments resembling mycelia and often produce spores. Their numbers typically vary from 1 to 36 million per gram of soil.⁵¹ When in competition with bacteria and fungi, actinomycete numbers are low. In uncontaminated soils, they do not play a very important role in degradation. However, they are able to degrade compounds resistant to decomposition by other bacterial and fungal species (see pp. 115-120 in Reference 51). This class of microbes may be able to degrade many of the complex chemicals often found in contaminated soil.

Although microbial populations are sensitive to factors such as temperature, pH, and various ions, they also are adaptable both within species and through changes in the relative species population. It is likely that in most situations the plant partner is more sensitive than the associated microorganisms. Densities of different microbial species may vary with conditions, but little is known of how this might influence degradation of particular compounds in soil.

B. Microbial Metabolic Diversity

1. Soils

Soil microorganisms are able to degrade a wide variety of toxic substances, but the time to effect remediation varies. Over a decade ago, McFarlane et al.¹⁰⁴ showed that soil planted with alfalfa (*Medicago sativa* L.) or bermudagrass (*Cynodon dactylon* [L.] Pers.) could serve as a strong sink for benzene vapor at 0.11 ppm v/v in air. A portion was rapidly released as CO₂ and then reassimilated. Unplanted soil depleted the benzene at half the rate of planted soil; sterilized, unplanted soil did not affect it at all. In the tested configuration, half-times were a few hours and removal of the aboveground portions of the plant immediately before testing had no effect on rates. Thus, it was primarily the root-associated microbes, or the roots of the plants, that enhanced the degradation process.

The literature on bioremediation of petroleum-contaminated soil has been reviewed by Lee et al.,⁹¹ Sims et al.,^{144,145} Erickson et al.,⁴⁵ Khondaker et al.,⁸¹ Mercer and Cohen,¹¹² and Testa and Winegardner.¹⁵³ The proceedings of several conferences on petroleum-contaminated soils have been published.^{29,85,86,120} Research on the biodegradation of organic compounds is reviewed in Davis,³⁹ Atlas,^{12,13} Gibson,⁵⁵ Rochkind-Dubinsky et al.,¹³⁷ Erickson and Fan,⁴⁴ and Sayler et al.,¹⁴⁰ research on bioremediation supported through the Robert S. Kerr Environmental Research Laboratory is reviewed in its annual reports;⁷⁹ and several of the hazardous substance research centers are conducting bioremediation research.¹⁶⁰

Microbes may take more than 10 years to remediate crude oil spills and more than 6 years to decontaminate retorted oil shale.¹⁴³ The hydrocarbon-degrading microbes present in most soils survive oil spills, but the toxic components of petroleum reduce populations of species that do not degrade hydrocarbons.²¹

In general, pesticides designed specifically to kill some organism(s) are remediated in a 2- to 16-week period. Extremely high doses of pesticides can reduce soil microorganism populations, but under normal applications microbes can remediate pesticides.¹⁴³ Smith and Mayfield¹⁴⁹ found that bacterial and fungal populations increased during a 2-week period following application of paraquat. However, individual microbe populations can be adversely affected; the treatment of soil with glyphosphate or diquat and paraquat increased the occurrence of take-all disease in wheat by reducing populations of antagonistic microbes.¹¹¹

Both the disturbance produced by surface mining for coal and heavy metals in mine tailings or exposed overburden from coal or mineral mines have a huge detrimental effect on microbes and it may take 50 to 100 years following mining activity before microbe populations return to their original state. Although some metals are essential for plant growth, many heavy metals also are toxic to the plants that may support microbial populations in their rhizosphere (see Section II.D.1). This further impoverishes the microbial flora. Uptake and sequestration of metals by plants are subjects that are not reviewed in this article. One should

be aware that the presence of toxic levels of heavy metals may make it difficult in some locations to implement the plant-based organic substance remediation strategies described herein.

2. Groundwater

The study of unconfined groundwater microbiology has generally focused on microbes as an indicator of pollution from sources such as septic tanks, landfills, agricultural uses, or land disposal of sewage byproducts.^{101,124,171} Although most work concerns aerobic degradation, several groundwater microbiology studies point out the importance of anaerobic bacteria in the transformation of some compounds.^{44,137} For example, in laboratory experiments, aerobic bacteria completely degraded aromatic compounds such as naphthalene, styrene, chlorobenzene, and 1,4-dichlorobenzene within 11 weeks, whereas 1,3-dichlorobenzene was more resistant to degradation. These compounds were not degraded under anaerobic conditions with a methanogenic inoculum.¹³⁵

On the other hand, anaerobic bacteria successfully degraded some halogenated aliphatic compounds that were unaffected by aerobic bacteria.^{75,136} Chemicals that were significantly degraded under anaerobic conditions in a 4-week period included chloroform, dichloromethane, bromodichloromethane, dibromodichloromethane, and 1,1,1-trichloroethane. Trichloroethylene and tetrachloroethylene were degraded 21 to 53% in a 16-week test.

In a field test at Palo Alto, CA, where reclaimed municipal wastewater was injected into the water table, naphthalene and heptaldehyde were degraded within 12 h and within 8 m of the wellbore. No reduction in concentration levels for chlorinated benzenes and trihalomethanes was noted after 3 months. After injection ceased, anaerobic activity continued and concentrations of chloroform, trihalomethanes, trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane continued to decrease.¹³⁶ Recent research at several field sites is summarized in the Kerr Laboratory annual report.⁷⁹

C. Soil Horizons and Microbial Distribution

Microbial population distribution is related to the soil horizons. Starting at the surface, there is the A horizon, followed by the B and C horizons underlain by the R layer or bedrock.* Ninety-nine percent of the world's soils are derived from parent material that comes from weathered bedrock. As plants begin to establish themselves, the surface soil A horizon is formed. The A horizon is characterized by an abundance of humified organic material, relatively high po-

* For simplicity, the O and E horizons and the more complex classification systems are not discussed. The O horizon lies above the A horizon and is an organic soil layer. The E horizon is an eluvial transition zone between the A and B horizons.

rosity, and good soil aeration. At this point in the soil formation process, the C horizon, consisting of relatively unaltered parent material, underlies the A horizon above the bedrock. As the soil develops, a B horizon appears between the A and C horizons and is distinguished by the presence of colloidal particles and low amounts of humus that have been leached from the A horizon. In general, the deeper the soil horizon, the lower the organic matter concentration and the lower the porosity and soil aeration (see pp. 11–18, 33 in Reference 51).

It is in the surface A horizon where all the components required for microbial growth are present: humus acting as substrate, high soil porosity allowing for adequate oxygen diffusion, an aqueous environment coming from rainfall, and warming from solar radiation. Of the total numbers of microbes that live in the soil, most live in the A horizon. For example, in cultivated grassland soil, there were approximately 5×10^6 bacteria, 9×10^5 actinomycetes, and 1.7×10^3 fungi per gram of air-dry soil from the A horizon, while the B horizon had 9×10^2 bacteria, 1×10^2 actinomycetes, and 10^3 fungi per gram of air-dry soil. Comparatively few microbes were found in the C horizon (see pp. 115–120 in Reference 51).

An unconfined aquifer, also called a phreatic or water table aquifer, is bounded at its base by impermeable bedrock or the R layer. Because there is no upper permeability barrier, the water level is free to fluctuate up or down into the A, B, and C layers depending on recharge and discharge factors. The soil above the water table, or saturated zone, is called the unsaturated zone, aeration zone, or the vadose zone.⁷¹ The manner in which flooding of the upper soil layers affects soil and groundwater microbiology has not been extensively studied, but it is well known that aerobic microbes may be replaced by anaerobic ones during flooding.

Many bioremediation techniques take advantage of the relative abundance of microbes in the A horizon. In the pump-and-treat method, groundwater, which has a low microbial population environment and low organic matter, is applied to the soil surface where microbial populations and organic matter are high. A disadvantage of pump and treat is that contaminants often adsorb to soil constituents. Rogers et al.¹³⁴ and Brusseau and Rao²⁶ showed that sorption to the mineral portion of the soils and to sterilized soils was minimal. Sorption to the organic matter in the soil occurred and the sorbed material was metabolized by resident microbial populations. In the study of Rogers et al.,¹³⁴ nearly one fourth of the added benzene (1 ppm) was sorbed and metabolized in 6 days.

Another bioremediation technique, land treatment, injects or mixes the organic components into the soil where microbial degradation can occur in the A horizon. This method works well unless metal concentrations, which may be high in sewage sludge and some other materials, increase to the point that the soil becomes sterilized.

When contamination is limited to the A horizon, current bioremediation technologies may be adequate. However, by selecting appropriate plants, bioremediation may be enhanced in the A horizon and made possible in the B and

C horizons with more favorable economic prospects. As a passive system, plants are much less costly to manage than other technologies.

D. Rhizosphere and Microbial Communities

The rhizosphere is the region immediately surrounding the root of a plant and serves as an enrichment zone for increased growth of certain bacteria. The surfaces and surroundings of plant roots provide specialized habitats for soil microorganisms.^{54,169} Bacteria live in colonies that cover 4 to 10% of the root surface (see p. 126 in Reference 51). Different species of plants support different bacterial flora via a complex interaction of growth enhancers, such as sugars, and inhibitors, such as phenolic compounds. Some approaches to understanding this complexity have been reviewed.^{16,84} Any complete model for the impact of plants on bioremediation must consider not only the plant, but also the microbial communities that it supports in different soil zones and different soil types.

The rhizosphere supports larger microbial populations than the surrounding bulk soil. For example, plants grown in a solution culture yield increased microbial populations compared to the solution alone.¹⁵ After 16 days, solution with plant roots supported a population of 97.0 (10^6) microorganisms as compared to a microbial population of 7.1 (10^6) in the solution culture without plants. On a dry root weight basis, there were 1.35 (10^6) microbes per milligram of root. The increased microbial populations in the rhizosphere also are seen in the relationship between microbial population and distance from the root surface.^{125a} For lupin (*Lupinus angustifolius* L.) seedlings in sandy loam, bacterial colonies per gram of oven-dried soil increased from 27 (10^6) colonies at 80 mm from the root, to 50 (10^6) colonies at 0 to 3 mm from the root, to 159 (10^6) colonies on the root. Streptomycetes showed a similar increase, with 9 (10^6) colonies at 80 mm, 15 (10^6) colonies 0 to 3 mm, and 47 (10^6) colonies on the root surface. Fungal populations went from 91 (10^3) colonies at 80 mm, to 176 (10^3) colonies at 0 to 3 mm, to 355 (10^3) colonies on the root. In this study, 80 mm from the root was considered to be nonrhizospheric soil.

There is a wealth of information on bacterial populations in the rhizosphere but much less regarding the way in which the rhizosphere selectively enriches populations of some microbes while inhibiting others.⁹⁹ Rhizobia provide one of the best studied bacterial associates of plants. They are known to be wind dispersed and there are few soils that do not contain at least low numbers. Strains specific for certain species of plants are enriched in the presence of the host, but the bacteria can survive for at least a decade in the absence of their host.¹²⁷ Interstrain competition often results in the loss of introduced strains, whereas indigenous strains remain and even increase in number, despite the seasonal nature of host plant presence in the case of annual legumes.¹⁰⁸ There is little understanding of the factors that determine bacterial numbers and interstrain competition either within or outside of the rhizosphere.

One group of soil-dwelling fungi that infect plant roots are called mycorrhizae, which means "fungus root." The hyphae of mycorrhizal fungi on the exterior of the root extend the effective root surface out into the soil and may improve water and nutrient uptake. There are two general types of mycorrhizae. Ectotrophic or ectomycorrhizal types initially infect the exterior of the root on the epidermal cells and then form a sheath or mantle that surrounds the root. These fungi are generally associated with trees. The second type is endotrophic or endomycorrhizal, in which the hyphae invade the root and infect both the interior and the surface of the root cells (see pp. 126–127 in Reference 51). Further distinctions have been made, dividing endomycorrhizae into the vesicular-arbuscular (VA), orchidaceous, and ericaceous types; an extensive discussion of these subdivisions is given by Moser and Haselwandter.¹¹⁹ Although most plants will have one type of mycorrhizal infection, Moser and Haselwandter¹¹⁹ note that genera such as *Eucalyptus*, *Cupressus*, *Salix*, *Malus*, *Pyrus*, *Tilia*, *Arbutus*, and *Populus* may have both ecto- and endomycorrhizal types. The symbiotic relationship of mycorrhizae with plants in improving uptake of nutrients and water is recognized, but the role of mycorrhizae in degrading organic contaminants is poorly understood.

Plant-microorganism interactions have been known and studied for more than a century. There is a vast body of literature concerning rhizosphere biology, and various aspects of root microorganisms and their interactions with plants have been reviewed.^{22,33,98,99,122} Many studies have shown that the microbial communities at the root zone have positive effects on the growth of plants, such as increasing the rate of mineral uptake by roots, improving nitrogen fixation, enhancing root elongation, etc., whereas other microbes are related to plant pathology.^{77,94,158} A full understanding of the structure, dynamics, and function of microbial communities on roots and the influence of plant deposits and exudates on rhizosphere microorganisms is regarded as a major challenge to microbiologists, biochemists, and engineers.

Microbial systems can be acclimated to degrade complex mixtures of relatively toxic materials when supplied with glucose as an energy source.¹⁶² A future area of investigation is to determine the plant species that are capable of living in soil contaminated by toxic chemicals and have the appropriate rhizosphere microbes to degrade those compounds. Little is known, but the potential benefits are very great.

Some studies have identified rhizospheric microorganisms that are capable of degrading complex compounds. For example, bacteria of the genus *Rhizobium* have been shown to be proficient in degrading the herbicide glyphosphate.⁹⁵ The bacteria are specifically associated with root nodules of legumes and their numbers in soil may be increased by the growth of legumes. In this way, legumes could enhance the degradation of glyphosphate indirectly. Rhizobia also degrade a wide range of aromatic compounds.^{126,152} If one could selectively enrich or engineer bacteria that degrade more challenging compounds, one might have a significant impact on bioremediation processes.

Recent papers by Aprill and Sims³ and Walton and Anderson¹⁴⁵ address this question. Walton and Anderson measured the rate of metabolism to CO₂ of trichloroethylene in soils. They used soil samples taken from the vicinity of a former chlorinated solvent disposal site, which had presumably enriched microbial species able to tolerate and degrade chlorinated hydrocarbons. Levels of compounds were low enough that they did not prevent revegetation of the site with plants common to the region. Four dominant plant types were examined: a grass *Paspalum notatum* var *saurae* Parodi; a legume (*Lespedeza cuneata* [Dum.] G. Don); a composite *Solidago* sp.; and a tree, loblolly pine (*Pinus taeda* L.). The most active degradation occurred in the soil sampled from around the roots of *L. cuneata*. This is probably due to the root nodule bacteria fixing nitrogen, which may be the limiting growth factor. Soil samples from areas without plant roots, either within the contaminated area or elsewhere, showed lower rates of degradation than samples from around the roots of each of the four dominant plant species that were examined. (Note also the works of Rogers et al.¹³⁸ and McFarlane et al.¹⁰⁴ cited previously for benzene.)

The work of Walton and Anderson¹⁴⁵ shows that degradation of chlorinated hydrocarbons may be enhanced in long-term enrichment cultures in nature. Kuhn et al.⁹⁰ showed that degradation of xylenes by bacteria could be intentionally enriched over a period of weeks in a soil column through which water containing xylenes was percolated. More recently, the same group isolated a bacterium, presumably a pseudomonad, able to mineralize toluene in the absence of molecular oxygen;⁴¹ nitrate and nitrous oxide served as the electron acceptors. One would not expect to find plant roots in such denitrifying conditions because they are generally intolerant of anaerobic conditions. However, at the boundary of the vadose and saturated zones, there may be a transfer of carbon from plant roots to anaerobic soil microbial communities, particularly as the level of the water table rises and then falls after rain.

1. Root Exudates and Enhanced Microbial Activity

Rhizodeposition and root exudation are two means by which substrate is provided to the rhizosphere. Rhizodeposition, the organic substances resulting from the decay of dead root hairs and fine roots, serves as an important carbon substrate for rhizosphere microorganisms.^{22,122} Annual plants, of course, leave the entire root system at their death. Exudation can occur over the entire length of the root, but the primary area of release is at the root tip.¹⁵⁰ The composition of root exudates varies with the species of plant and rhizosphere microbes, light, temperature, water, nutrition, and competition. Also, the rates of exudation and composition of exudates vary with the age of the plant. Root exudates are classified as leakages, which are compounds that leak from the epidermal or cortical cells; secretions, which are compounds that move across membranes as a result of metabolic activity; mucilages, which are materials contributed by root cap cells,

primary cell walls, root hair secretions, and dead cells; and lysates, which consist of sloughed cells and released microbial metabolites. Exudates include sugars, amino acids, organic acids, fatty acids, sterols, growth factors, nucleotides, flavanones, enzymes, and numerous other compounds.⁸⁴ Smith¹⁵⁰ classifies exudates as carbohydrates, amino acids/amides, organic acids, anions, and cations.

Estimates for the annual amount of rhizodeposition and root exudates vary from 7 to 27% of the total plant mass (see p. 405 in Reference 119). Smith¹⁵⁰ presents reviews that estimate the amount of exudate added to the soil for various crop and tree species on a plant, dry root, and hectare basis. A study of grassland and forest ecosystems in south-central Wisconsin showed that forests added 184 metric tons per hectare of organic material to the top 105 cm of soil, whereas grassland prairie added 345 metric tons per hectare. These amounts correspond to about 18 to 34 g/kg/year or roughly one tenth to one fifth mole of glucose equivalent per kilogram in the top meter of soil. Barber and Lynch¹⁵ tabulated bacterial mass, substrate flow, and calculated maintenance energies for grassland, forest, and silt loam.

In both ecosystems, the quantity of organic matter decreased with depth. Forests added 81 metric tons per hectare in the top 15 cm, 25 metric tons per hectare from 15 to 30 cm, and then gradually decreasing to 9 metric tons per hectare at a depth of 90 to 105 cm. Grassland contributed 112 metric tons per hectare in the top 15 cm, 83 metric tons per hectare from 15 to 30 cm, declining to 11 metric tons per hectare at a depth of 90 to 105 cm (see pp. 263-264 in Reference 51). Thus, in the uppermost layers, energy input may be up to one half mole glucose equivalent per kilogram per year, which supports a significant microbial biomass.

The difficulty in measuring root exudate and rhizospheric microbial colonies should be noted. The process of separating the roots from the soil for analysis may stimulate a burst of microbial growth on exudates, small root fragments, and sloughed off root surface coverings. Thus, we do not know the extent to which the observed results reflect the *in situ* functioning of the microbial community. For instance, it is well documented that the numbers of the associative nitrogen-fixer *Azospirillum* increase dramatically when washed root pieces are incubated under low oxygen tensions.¹⁵⁴ In this case, there is proliferation of the bacteria at the expense of soluble carbon compounds present in root pieces. Activities obtained without such disturbance are much lower and probably more accurately reflect the *in situ* biodegradation potential of the bacteria.

The input of organic matter into humus-poor soil may be important to facilitate biodegradation of low concentrations of contaminants. In laboratory studies, higher concentration samples of chlorobenzenes and chloroethylenes degraded faster than samples with initially low concentrations, which were not affected in some cases presumably because there was insufficient carbon substrate to support significant microbial populations.¹⁰¹ With the large amount and wide variety of exuded compounds and depositions, roots should support a varied population of microbes, of which some might not be functional if they depended solely on the contaminant

for substrate. That is, plants may provide essential cosubstrate for the pollutant-degrading microbes.

2. Nitrogen Fixation by Rhizosphere

Although N_2 is abundant in the atmosphere, combined nitrogen is generally a limiting factor in plant growth. Nitrogen is introduced into the soil system by several processes. Biological dinitrogen fixation is the process whereby atmospheric nitrogen is converted to ammonia and then into amino acids that are useable by plants and microbes.¹⁵¹ Some nitrogen is fixed to oxides by lightning and enters with rain (see pp. 127–128, 186–192 in Reference 51), some is transported by water contaminated by sources such as agricultural runoff, and some may enter as amino acids and amides in root exudates (see p. 199 in Reference 150 and p. 197 in Reference 84). The largest contributor of “new” nitrogen is probably symbiotic or nonsymbiotic biological nitrogen fixation. The enzyme, nitrogenase, is the critical factor in this process.

Legume plants provide the environment for symbiotic nitrogen fixation with the heterotrophic bacteria of the tribe Rhizobiaceae (see pp. 30–34 in Reference 151). When a root hair of a legume encounters a rhizobial cell, a “recognition event” occurs. The bacterial cell penetrates the root and ultimately a nodule containing bacteroids is formed. The plant provides carbon, nutrients, and perhaps growth compounds for the modified bacteria, whereas the bacteria supply the plant with nitrogen. Forage legumes when grown as cover crops can provide 100 to 600 kg nitrogen per hectare per year (see p. 94 in Reference 151). Horticultural legumes also can add nitrogen to the soil but at generally lower rates. Many trees, such as the black locust (*Robinia pseudoacacia* L.) and the tropical tree *Leucaena leucocephala*, also can fix nitrogen. The *Leucaena* tree adds enough nitrogen to the soil that it is planted with crops to increase food yields (see pp. 127–128, 186–192 in Reference 51). *R. pseudoacacia* is reported to enhance growth of black walnut (*Juglans niger*) on poor soils (see p. 141 in Reference 151).

Some nonlegume plants also can fix nitrogen symbiotically through actinomycetes in their root nodules. Red alder (*Alnus rubra* Bong.) is a pioneer species because of its nitrogen-fixing capabilities in soils that are burned over or have mostly parent material. Actinomycetes associated with *Ceanothus* species, a flowering shrub of the U.S. Pacific Northwest, are capable of fixing more nitrogen in natural ecosystems than some legumes used in agricultural fields (see pp. 127–128, 186–192 in Reference 51). Russian olive (*Eleagnus angustifolia*) and *Casuarina equisetifolia* also fix nitrogen in significant quantities (see p. 134 in Reference 151).

The bacteria *Azotobacter* and *Clostridium* are two of several dozen genera capable of nonsymbiotic nitrogen fixation. *Azotobacters* have been found in every soil that has a pH > 6, good aeration, and abundant organic material. *Clostridium* is an anaerobe that can thrive in soil too acidic for *Azotobacter*. Both bacteria

are usually limited by lack of carbon substrate and contribute little nitrogen to cultivated fields (see pp. 127–128, 186–192 in Reference 51). There are many species of associative nitrogen-fixing bacteria that contribute significant levels of nitrogen to the rhizosphere of plants in low nitrogen soils (see p. 122 in Reference 151).

Instances where rhizospheric bacteria were able to fix nitrogen, thus providing for degradation of contaminants, were discussed in Section II.D.^{3,95,165}

A possible use of plants supporting nitrogen-fixing bacteria is in the remediation of oil spills. Partial degradation of petroleum products has been accomplished by the addition of urea and phosphate fertilizer, which increased microbe numbers. The use of nitrogen-fixing species may further enhance clean-up of oil-contaminated soils without the need for addition of urea.¹⁴⁶

3. Phosphorus Accessibility

Phosphorus in soil is generally unavailable for plant uptake or microbe consumption. The rhizosphere is able to solubilize phosphorus by the chemical activity of root exudates and the biological activity of rhizosphere bacteria and mycorrhizal fungi. The processes are not clearly understood, but the presence of bacteria such as *Bacillus*, *Pseudomonas*, and *Agrobacterium* improved the uptake of phosphorus by plants. Mycorrhizal roots were shown to be more efficient than nonmycorrhizal roots in plant phosphate uptake (see pp. 211–212 in Reference 150). Just as plants benefited from improved phosphorus availability, rhizosphere bacterial populations were higher when VA-infected roots were inoculated with phosphate-solubilizing bacteria (see p. 406 in Reference 119). Some nitrogen-fixing species have mycorrhizal species associated with their roots (see pp. 183–185 in Reference 151).

4. Trace Mineral Transfer

Roots also may be able to sustain a healthier microbial population by providing trace minerals and nutrients (vitamins and growth substances). This may be important as the microbes can be stressed by the toxicity of the contaminant. Because the root exudates and dead roots were once part of a living plant, they probably contain trace nutrients. This reasoning is parallel to an observation from agriculture. A farmer noted that hogs fed on corn grown in manure-fertilized soil remained healthy while those fed on corn planted on soil fertilized with manufactured products contracted swine dysentery. It was speculated that the manure provided trace elements not available in the commercial fertilizer. Likewise, rhizodepositions and roots exudates may supply trace substances to the microbes.¹³⁰

5. Extent of Root Development and Magnitude of the Rhizosphere

The linear extent and surface area of the root system is a factor in the degradation of contaminants in the soil. This is illustrated by some examples in Kramer.⁸⁹ Grasses produce many fibrous roots. For instance, crested wheatgrass (*Agropyron cristatum* L. Gaertn.) has over 200,000 m of roots per cubic meter of soil. A 100-year-old Scotch pine (*Pinus sylvestris* L.) was reported to have 50,000 m of roots with 5,000,000 root tips. If each tip supported 1 mg of bacteria, it would provide 5 kg of bacterial mass to carry out metabolism. The crucial factor is of course the volumetric distribution of the root tips and the associated microorganisms. Even younger trees can have extensive root development: 3-year-old coffee trees (*Coffea arabica* L.) have 28,000 m of roots. Root structure is species specific — a 6-month-old dogwood (*Cornus florida* L.) had over 5 m of roots as compared to 0.38 m of roots for a loblolly (*P. taeda* L.) seedling.

Root depth also can be extensive in deep, nonsaturated soils, with *M. sativa* L. roots growing to a depth of at least 10 m. Even "shallow-rooted" corn (*Zea mays* L.) and sorghum (*Sorghum vulgare* Pers.) penetrate to depths of 2 m. Eighteen-year-old apple trees (*Malus* spp.) had roots to depths of 10 m and had filled in the 10-m rows between trees. Root spread has been measured as far as 17 m in longleaf pine (*P. palustris* Mill.) and 16.5 m in turkey oak (*Quercus laevis* Walt). Fruit trees in a California study penetrated to 5 m, with most of the roots at depths of 0.6 to 1.5 m. In heavier soils, root development becomes restricted to the upper soil. For example, 90% of pear (*Pyrus* spp) roots in heavy adobe soil were in the top meter (see pp. 147–149 in Reference 89).

Based on these examples, it might be expected that sandy soils would yield deeper rooted plants; however, precipitation and depth to the water table also are factors. In the southeastern coastal plain of the U.S., with sandy soil and a water table at a depth of at least 40 to 50 ft, plants form mats of roots near the surface in order to take advantage of nutrients and summer showers.¹⁷² In the sand hills of the high plains where precipitation is less, root development is deeper. Of these plant species, 9% had roots limited to the top 2 ft of soil, 18% had primarily deeper roots, and 73% had roots in both the shallower and deeper soil horizons (see pp. 125–129 in Reference 166).

Plants that live in dry environments often depend on groundwater for their primary water source. As a result, their roots will penetrate into the water table. In dry years, as the water table drops, these roots will penetrate further into the soil. Later, when the water table rises, the roots often continue to survive in the water-saturated environment. For instance, the osage orange (*Maclura pomifera* L.) and the American persimmon (*Diospyros virginiana* L.) are naturally found in moist lowland soils. Both are rather tolerant of drought, with the osage orange planted as a windbreak throughout the Great Plains. Yet, both are moderately tolerant of flooding, so that they have a wide range of adaptability to soil/water status (see later discussion with Table 1). This adaptability is discussed further

in the next section. It is dependent on the root morphology of a particular species; not all roots will survive saturation. Roots of some species are able to penetrate into not only the A, B, and C soil horizons, but also the unconfined groundwater table as well.

McNabb and Dunlap¹⁰⁹ reviewed earlier studies of microbial distribution in soil as a function of depth. In that paper and continuing studies, it was shown that significant numbers of bacteria may be recovered to depths of many meters. Their numbers are probably limited by nutrient (carbon) availability. Nitrogen-fixing rhizobia have been recovered from root nodules of mesquite (*Prosopis* spp.) trees at depths of 3.9 to 4.5 m,¹⁶⁴ indicating that even such aerobic, plant-associating species may be present to great depths under suitable conditions of enrichment. Whether rhizobia live in the soil at that depth in the absence of the plant or whether they colonize the root as it grows is unknown.

A difficulty faced by bioremediation technologies is delivering the limiting factor in microbial growth to the site of the contaminants. These limiting factors were listed in Section I. Pump-and-treat technology brings some contaminants to the surface where oxygen and nutrients are adequate but leaves part of the contaminant adsorbed to or at least associated with the soil particles at depth. Injecting oxygen and nutrients down the wellbore partially addresses the problem, but *in situ* remediation is limited to the soil region that is supplied with nutrients. Often some of the contaminated soil is not affected because of nonuniform flow. By selecting species appropriate to the depth of the contamination, plant roots should be able to penetrate the entire contaminated area and supply or help distribute the limiting nutrients for microbial growth.

6. Oxygen Transfer

Plants have several internal gas transport pathways. Gases are transported through intercellular gas spaces in the parenchyma (see p. 273 in Reference 6). It has been shown that bog plants are able to transport oxygen to anaerobic soil.⁴ Some trees, such as baldcypress (*Taxodium distichum* [L.] Rich), swamp tupelo (*Nyssa sylvatica* L.), and green ash (*Fraxinus pennsylvanica* Marsh.) also oxidize the rhizosphere (see p. 312 in Reference 74). The pathways in plants of oxygen transport through the roots and into the soil are not well understood; however, plants and trees seem to fall into three general categories with respect to their varying oxygen-ventilating efficiencies: (1) nonwetland herbaceous and woody plants with poor ventilating capabilities; (2) wetland woody plants with moderate ventilating capacities; and (3) wetland herbaceous plants (see p. 312 in Reference 7).

a. Nonwetland Plants

Nonwetland plants use two pathways to move oxygen to their roots. As seen in Luxmoore et al.,⁹⁷ oxygen is supplied to the root through a soil pathway and

through a plant pathway. Upon soil flooding, fine root growth is reduced or stopped for flood-intolerant plants. When soil is flooded, the water fills the soil pores previously occupied by air; any remaining oxygen is quickly consumed by aerobic microbes and any oxygen transport is limited to aqueous phase diffusion. The aerobic organisms are replaced by anaerobic organisms whose byproducts are often harmful to nonwetland plants and trees. Inhibitory compounds produced include carbon dioxide, methane, hydrogen, hydrocarbons, alcohols, carbonyls, volatile fatty acids, nonvolatile organic acids, phenolic acids, and volatile sulfur compounds. Most nonwetland trees and plants will die when subjected to an oxygen-depleted, chemically hostile root environment (see pp. 307-325 in Reference 88).

Gliński and Stepniewski⁹⁹ present a thorough examination of the soil pathway of oxygen transfer. Usually, it is via passive diffusion, although changes in the water table or water extraction by plants will induce convective flow.

The movement of oxygen within the plant appears to be diffusion controlled under some conditions. The simplest model assumes diffusion in a tubular structure

$$\text{Diffusion Rate (gs}^{-1}\text{)} = \frac{DA(C_2 - C_1)}{L}$$

where D = the diffusion coefficient (cm²/s); A = cross-sectional area (cm²); C₂ = gas source concentration (g/cm³); C₁ = gas sink concentration (g/cm³); and L = length of tube or diffusion path (cm).

Because the gas spaces in plants are not simple tubes but follow irregular pathways, the diffusional impedance is rewritten to include the porosity and tortuosity (see p. 271 in Reference 6).

Mathematical models for oxygen diffusion in roots were presented by Armstrong and Beckett.^{10,11} Both gas-phase and liquid-phase transport of oxygen were modeled by those authors for nonwetland and wetland plants.

Armstrong et al.⁹ and Armstrong and Armstrong⁸ reported evidence of convective gas flows in plants. Temperature differences and humidity-induced diffusion appear to create pressure differences that cause convective flow in plants. Armstrong et al.⁹ reported greater rates of oxygen transfer in reeds (*Phragmites australis*) when light was present to provide both heat and a reduced relative humidity in the gas phase around the plant.

b. Wetland Plants

Research on oxygen transfer in wetland plants is discussed by Licht.⁷² The ability of a tree to provide oxygen to water-logged roots is related to the adaptability of the tree to flooding.⁷⁸ Flood-tolerant or wetland plants and trees have adaptations that enable them to maintain an aerobic environment in the rhizosphere and detoxify the soil. Wetland plants growing in unsaturated soil use the two oxygen pathways previously described. However, upon flooding, several phys-

iological changes occur and more oxygen is transferred through the plant pathway. Many wetland plants are able to absorb oxygen through their leaves, lenticels of twigs, stems, bark, and even the unflooded roots. Oxygen diffuses out of the roots into the rhizosphere. A study of lodgepole pine (*Pinus contorta* Dougl.) indicated that their deeper root penetration into waterlogged soil was due to more efficient oxygen transport (see pp. 328–335 in Reference 88).

However, there are conflicting reports and species variations regarding these generalizations. One study showed that most of the oxygen that diffused from the roots entered the plant through the bark immediately above the water table in seedlings. In willow (*Salix* spp.) seedlings, gas intake for root leakage was within the bottom 3 cm of the shoot.⁵

Resistance to air movement across the bark or the cambium varies with species. Water tupelo (*N. aquatica* L.) and green ash (*F. pennsylvanica* L.) allow air to move easily through the cambium, whereas sweet gum (*Liquidambar styraciflua* L.) and cottonwood (*Populus deltoides* Bartr. ex Marsh.) present a high resistance to air movement through the bark (see pp. 305–306 in Reference 74).

In addition to their normal physiological adaptations, many flood-tolerant plants adapt by forming lenticels and adventitious roots. Many flood-tolerant plants, including *P. deltoides*, produce hypertrophied lenticels on submerged roots and stems, which may increase aeration of the stem and permit release of toxic metabolites such as ethanol, acetaldehyde, and ethylene. The adaptation of releasing toxic products through lenticels is species specific. Willow (*Salix alba* L.) lenticels are used for both the diffusion of air into the tree and the excretion of ethanol, acetaldehyde, and ethylene. The poplar (*P. petrowskiana* Sch.), on the other hand, is unable to release these internal toxics (see p. 305 in Reference 74). The lenticels of flooded plants have larger intercellular spaces than non-flooded plants, which allows for better gas exchange.

Adventitious roots may grow on the submerged portion of the stem and/or the root. When the root hairs and secondary roots die back during flooding, they are replaced by adventitious roots, which enable them to maintain aerobic conditions (see pp. 328–335 in Reference 88). The epidermis becomes unsuberized with little evidence of Casparian strips, which would serve as a barrier to gas diffusion (see p. 311 in Reference 74). These adaptations increase the oxygen supply to the soil.

Another characteristic of wetland plants and trees is that their normal metabolism enables them to control or tolerate anaerobic metabolism. Even when grown in unsaturated soil, some wetland plants operate under anaerobic conditions and have higher levels of anaerobic byproducts than nonwetland plants. For example, *N. sylvatica* var *biflora* (Walt.) Sarg. produces the same amount of lactic acid whether it is flooded or not. Therefore, when wetland plants are flooded, they easily adapt to the higher levels of toxic metabolites, while non-wetland plants die when they produce anaerobic byproducts. It has been hypothesized that the relative flood tolerance is inversely related to the alcohol dehydrogenase (ADH) activity in the roots (see pp. 313–314 in Reference 74). A recent study showed that *P. deltoides* seedlings had a lower initial ADH activity than did soybean (*Glycine max* [L.] Merr.) seedlings. Within 3 days of flooding,

G. max ADH activity decreased exponentially, while *P. deltoides* ADH activity remained constant.⁴²

The various adaptative processes of tree species to flooding are summarized by Hook and Scholtens (see p. 325 in Reference 74) in a flow chart format. A good compilation of early references on plant adaptations to flooding and oxygen transport in plants can be found in the article by Kawase.⁷⁸ Variations in flood tolerance of some common trees are listed in Table 1. As stated earlier, the ability of a plant to oxidize the rhizosphere is related to its flood tolerance; however, there are exceptions. It is important to note that different tree species within a genus may show wide variations in adaptation and flooding tolerance, e.g., compare the relative positions of different species of single genera in Table 1. A more comprehensive review of response to flooding can be found in Gill,³⁶ who noted vast differences between published reports for presumably identical species grown in different locations or tested by different methods. There may be different ecotypes within a single species as well as effects of the prior experience of the trees.

Wetland herbaceous plants have a more efficient air transport system than do nonwetland plants. For example, the yellow water lily (*Nuphar luteum*, Sibth & Sm.) and the white water lily (*Nymphaea alba* L.) have air flows of 260 ml/h/leaf to the rhizosphere. Liquid-phase diffusion does not account for these gas transfer rates. It was found in the study on water lilies that thermoosmosis of gases and Knudsen diffusion contributed to the improved gas flow. Thermoosmosis affects oxygen transfer because of the mass diffusion that results from thermal gradients. When the pore diameters are $<1 \mu\text{m}$, Knudsen diffusion occurs from the cooler side of a porous partition to the warmer side. The leaves of many wetland plants meet this criterion in sunlight. Thermoosmosis also extends to wetland trees, with the seedlings of the nitrogen-fixing black alder (*Alnus glutinosa* [L.] Gaertn) having an air flow rate of approximately 1 ml/h.⁶² The partition in *A. glutinosa* was in the cambial layer or the phellogen of the lenticels.²⁷

Armstrong and Armstrong⁸ provided evidence for a transpiration-driven oxygen diffusion as well as a Venturi effect on broken stalks of reeds (*Phragmites australis* [Ca.] Trin. ex Steud.). They showed how this leads to increased oxidation of the rhizosphere in saturated soils and used a simple mathematical model to predict the magnitude of the oxygenation of up to one half mole O_2 per square meter per day. Wetland plants and trees are able to oxidize the rhizosphere at much higher rates than expected from liquid-phase diffusion alone. In areas with a high or fluctuating water table, such processes will be important in successful bioremediation efforts.

Trees also may increase degradation by two other mechanisms of soil oxygenation. If sufficient numbers of trees are planted, the groundwater level may be lowered, which increases the amount of unsaturated soil and the oxygen supply by the soil pathway. As a result, trees can increase microbial activity. Many trees in a closed canopy stand will transpire more than a precipitation equivalent of 1 m of water per year, if it is available. Their effect on the water table may thus be considerable in subhumid and semiarid climates, where there is a large deficit of precipitation over transpiration.

TABLE 1
Examples of Tree Species with Differing Degrees of Flooding Tolerance

Genus and species	Common name
Highly Intolerant	
<i>Acer saccharum</i> Marsh.	Sugar maple
<i>Carya ovata</i> (P. Mill.) K. Koch	Shagbark hickory
<i>Fagus grandifolia</i> Ehrh.	American beech
<i>Fraxinus americana</i> L.	White ash
<i>Juglans nigra</i> L.	Black walnut
<i>Juniperus virginiana</i> L.	Eastern red cedar
<i>Liriodendron tulipifera</i> L.	Yellow poplar (tulip tree)
<i>Quercus stellata</i> Wang.	Post oak
<i>Robinia pseudoacacia</i> L.	Black locust
<i>Sassafras albidum</i> (Nutt.) Nees.	Sassafras
<i>Tilia americana</i> L.	American basswood
<i>Ulmus pumila</i> L.	Siberian elm
Moderately Tolerant	
<i>Acer negundo</i> L.	Boxelder
<i>Ainus rugosa</i> (Ehrh.) Spreng.	Black alder
<i>Betula niger</i> L.	River birch
<i>Carya illinoensis</i> (Wang.) K. Koch	Pecan
<i>Celtis</i> spp.	Hackberry
<i>Diospyros virginiana</i> L.	American persimmon
<i>Maclura pomifera</i> (Raf.) Schneid.	Osage orange
<i>Nyssa sylvatica</i> var. <i>biflora</i> (Walt.) Sarg.	Swamp tupelo
<i>Pinus taeda</i> L.	Loblolly pine
<i>Platanus occidentalis</i> L.	Sycamore
<i>Quercus macrocarpa</i> Michx.	Bur oak
<i>Q. michauxii</i> Nutt.	Swamp chestnut oak
<i>Ulmus americana</i> L.	American elm
Highly Tolerant	
<i>Acer nigrum</i> Michx.	Black maple
<i>A. rubrum</i> L.	Red maple
<i>A. saccharinum</i> L.	Silver maple
<i>Carya aquatica</i> (Michx. f.) Nutt.	Water hickory
<i>Fraxinus pennsylvanica</i> Marsh.	Green ash
<i>Gleditsia triacanthos</i> L.	Honey locust
<i>Liquidambar styraciflua</i> L.	Sweet gum
<i>Nyssa aquatica</i> L.	Water tupelo
<i>Populus deltoides</i> Bartr.	Eastern cottonwood
<i>Populus</i> × <i>euroamericana</i>	Hybrid poplars
<i>Quercus lyrata</i> Walt.	Overcup oak
<i>Salix nigra</i> Marsh.	Black willow
<i>Taxodium distichum</i> (L.) L.C. Richard	Bald cypress

Derived from several sources including Hall et al.,⁶⁴ Gill,⁶⁵ Hook and Brown,⁷³ Loucks and Keen,⁶⁶ and Kozlowski et al.⁶⁸ (see p. 325). Species are given as listed by original authors, except in cases of obvious synonymy.

An important effect of plant roots on the growth of microbes is that they may increase the oxygen availability as well as the solute transport by increasing the hydraulic conductivity and diffusivity in soil.⁵⁸ Oxygen is essential for the growth of many microbes that live in the soil subsurface where oxygen availability is limited. The continuously dying root hairs produce tiny channels and pores in the soil, which facilitates the diffusion of oxygen from the top surface. In addition, various nutrients move more rapidly due to the increased soil porosity so that the growth of microbes is enhanced. This is the basis of the general tillth effect in agriculture.

In situ degradation of contaminated groundwater is generally limited by the lack of oxygen and nutrients. One way of overcoming this limitation is by pumping oxygen and nutrients into the groundwater. Oxygen can be pumped directly into the wellbore, but it has the disadvantage of biofouling at the wellbore, which decreases flow rates. A method to bypass this problem is the use of hydrogen peroxide, which decomposes into water and oxygen once it is past the wellbore. However, this method has the drawback of being potentially toxic to the bacteria. The current use of wetlands for degrading contaminants points to a possible use of wetland trees to remediate contaminated soil, in a more passive system.

Mitsch and Gosselink¹¹⁴ reviewed wetlands as an ecosystem, and the volume edited by Hammer⁶⁵ presents a broad overview of the use of wetlands for treatment of municipal, agricultural, and industrial wastes. While most studies to date have examined herbaceous wetland species such as reeds (*Phragmites* spp.), rushes (*Scirpus* spp.), and cattails (*Typha latifolia* L.),^{25,65,131} there are some forested swamps used for nutrient removal of municipal wastewater discharges.^{132,134}

Wetland herbaceous plants are able to promote decomposition of the organic components of municipal sewage because they provide the limiting factor for microbial growth, oxygen, and may increase the surface area for microbial colonization. Other components, such as nitrogen and substrate, are already present in the wastewater. The use of wetlands is now being extended to water pollution control.³⁵ Litchfield⁹³ describes the use of an unstructured wetland for wastewater treatment at an oil refinery in North Dakota. In addition to ponds and vegetated channels, 50,000 trees were planted at the site, which is now a wildlife sanctuary. In British Columbia, a marshland is used to treat low-strength landfill leachate.¹⁸

Because wetland trees have been shown to oxidize the rhizosphere, it appears that contaminated soil that is oxygen deficient can be remediated. Instead of water being the planting medium, as for wetland herbaceous plants, soil is the planting medium for wetland trees. Therefore, the potential use of wetland or flood-tolerant upland trees as a means of decontaminating soil is worthy of additional investigation.

7. Water and Solute Transfer among Roots

Trees and other plants also can change the soil and surface environment. In dry climates, most of the feeder roots of a tree are deep in the soil. As water is

drawn up to nourish the tree, water also is transported to the oxygen-rich, dry soil near the surface where transported pollutants are more susceptible to biodegradation. Water and mineral transfer between *Populus* spp. seedlings has been observed.⁶⁶

The transfer of water has been seen from deep-rooted alfalfa (*Medicago sativa* L.) to shallow-rooted maize (*Zea mays* L.).³⁶ Habben and Blevins⁶³ showed that *M. sativa* also is able to transfer non-N-minerals to shallower rooted plants. These transport processes may enhance the environment for *in situ* bioremediation. In desert ecosystems, there is significant water transfer from lower to upper soil levels via a process known as hydraulic lift.^{30,133} This may make available to associated plants both water and pollutants, which can be further metabolized in the rhizosphere. Transpiration may be enhanced 25 to 50% by such nighttime lift and daytime reabsorption.

8. Temperature

The relationship between bacterial growth and temperature is described by the Arrhenius equation in which:

$$k = Ae^{-(E_a/RT)}$$

where k = reaction rate constant (s^{-1}); E_a = activation energy (J/mol); R = gas law constant (J/mol·K); A = frequency factor (s^{-1}); and T = absolute temperature (K).

Although different microbes can grow at temperatures between -5 and 95°C (23 to 203°F), the typical growth rate for mesophiles shows a rapid decline as the temperature decreases below 15°C . The optimum temperature for mesophile growth is 30 to 45°C (86 to 113°F) and for obligate psychrophiles it is 15 to 18°C (59 to 65°F) (see pp. 132–135, 392–394 in Reference 14). Soils containing plants and adequate water for microbial growth rarely exceed 45°C , so high-temperature limitation is of little concern.

Soil temperature regimes in the top meter have been classified (see p. 279 in Reference 51). At the surface, microbial growth is enhanced by solar radiation in most climates. Growth is less in deeper soil layers as soil temperature drops dramatically with depth in temperate climates. In eastern Kansas where the annual air temperature variation is 51°C , under grass cover at 1 ft, the variation in soil temperature was 27°C ; at 3 ft, 21°C ; at 6 ft, 16°C (see p. 219 in Reference 167). At some depth, the soil temperature will not be significantly affected by heating from the surface. This ambient soil temperature might be equated with groundwater temperatures. Groundwater temperatures from wells 50 to 150 ft deep range from 27°C in south Texas to 13°C in Kansas to 7°C in North Dakota (see p. 252 in Reference 161). It is expected that rates of microbial metabolism will be less at greater depth than on the surface during the warm season, while in winter it may be relatively higher at greater depth, although low on an absolute scale.

Plant roots and the associated microorganisms that penetrate the deeper, cooler soil horizons may provide the means to increase the temperature in the rhizosphere slightly. As microbes consume the substrate, heat is given off, which provides energy for further metabolic activity.

The heat of combustion is proportional to the consumption of oxygen. The heat of combustion per equivalent of oxygen was found to be fairly constant for a wide variety of compounds.¹²⁸ Assuming that ammonia, water, and carbon dioxide are in a physiologically dead state and given the microbial growth formula provided in Section I, the heat generated is⁴³

$$\Delta H = 4bQ_o$$

where b = moles of oxygen consumed; ΔH = heat of combustion (kJ); Q_o = heat of reaction per equivalent of available electrons = 26.5 kcal/equiv. a.e. = 111 kJ/equiv. a.e.

The rate of degradation of contaminants may be enhanced by the exothermic heat of reaction associated with the microbial activity in the rhizosphere. However, except during degradation of large amounts of organic matter, the rate is low and the temperature is not altered appreciably by microbial processes.

E. Microbial Degradation Models

Conley et al.³⁴ modeled the reduction in biological oxygen demand (BOD) of wastewater treated by the root zone method, which uses wetland plants to oxidize organic contaminants in the rhizosphere and promote microbe growth. First-order kinetics and plug flow were assumed:

$$\frac{C_e}{C_i} = \exp(-K_T\theta)$$

where the hydraulic retention time, θ , is defined as

$$\theta = \frac{V_v}{Q} = \frac{\epsilon V}{Q}$$

The required volume of the bed, derived from the previous equations is

$$V = \frac{Q(\ln C_i - \ln C_e)}{K_T\epsilon}$$

where C_i = influent concentration (mg/l); ϵ = porosity of media bed; C_e = effluent concentration (mg/l); V = volume of root zone bed (m^3); K_T = temperature-dependent rate constant (day^{-1}); V_v = void volume in root zone bed (m^3); and Q = wastewater flow rate (m^3/day).

In another degradation model for the contaminants in the saturated root zone,¹⁴² a tanks-in-series approach was used where the groundwater flows through a network of roots. The microbial growth follows the Monod kinetic model and depends on the concentration of oxygen (C_o), biomass (C_b), organic contaminants (C_s), and root exudates (C_r). The microbes live in the pore spaces (ϵV) and on the soil particles ($\rho q_{b,i}$).

Under steady-state flow conditions

$$Q_{i-1} = Q_u + Q_i$$

The contaminant balance is

$$\epsilon V_i \frac{dC_{s,i}}{dt} + \rho V_i \frac{dq_{s,i}}{dt} = Q_{i-1}C_{s,i-1} - Q_i C_{s,i} - Q_u C_{s,i} - \frac{\mu_m(\epsilon C_{b,i} + \rho q_{b,i})}{Y_s} \left(\frac{C_{s,i}}{K_s + C_{s,i} + C_{r,i}} \right) \left(\frac{C_{o,i}}{K_o + C_{o,i}} \right) V_i$$

where the last term includes biodegradation in the pore space and biodegradation on soil particles. The notation is K = kinetic constant (mg/l); t = time (day); ρ = density (g/l); μ_m = maximum specific growth rate (day⁻¹); q = adsorbed concentration (mg/g). The subscripts are s = contaminant; r = root exudates; b = biomass; o = oxygen; and u = uptake.

III. TRANSPORT IN SOIL AND PLANTS

A. Water Transport

The root-soil water transfer process is a major component of the subsurface hydrologic system. A quantitative understanding of water movement in the root-soil environment is needed. Microscopic analyses of the root extraction process have been presented by Cushman,³⁸ Gardner,⁵² Molz,¹¹⁵ and Molz et al.¹¹⁸ Each of these studies considered the radial flow of water to a single root, which was modeled as an infinitely long cylinder of constant radius that absorbs water from the soil matrix. However, studies with this degree of detail are impractical for use in field-scale agricultural studies of soil moisture transport. Thus, many investigators have used a macroscopic representation to describe the water extraction process by the root system of a crop. Some recent efforts have been presented by Afshar and Mariño,² Bresler,²³ Bresler and Hoffman,²⁴ Feddes et al.,⁴⁷⁻⁴⁹ Gardner,⁵³ Gish and Jury,^{57,58} Herkelrath et al.,^{69,70} Hillel et al.,⁷² Jury,⁷⁶ King and Hanks,⁸³ McCoy et al.,¹⁰² Molz,¹¹⁶ Molz and Remson,¹¹⁷ Neuman et al.,¹²¹ Nimah and Hanks,¹²³ Rowse et al.,¹³⁹ and Whisler et al.¹⁷⁰

Each of the above-mentioned models has been verified against field data, with a reasonable degree of accuracy. However, a major drawback in using any of these models is that the extraction of soil water by a root system is simulated

by using a sink term in the soil water flow equation. Thus, the vertical movement of water through the root system is neglected. However, the resistance to the vertical flow of water through a root system can affect the distribution of soil water extraction by the roots. Mariño and Tracy¹⁰⁰ and Tracy and Mariño¹⁵⁵ developed a coupled root-soil water flow model that has been shown to provide a more realistic and accurate description of the movement of water through the root-soil environment.

In their study, the soil water movement in the vertical and horizontal direction of a nonhomogeneous variably saturated soil can be described as

$$\frac{\partial}{\partial x_i} \left[K_{si} \frac{\partial}{\partial x_i} (\psi_s + x_2) \right] - S_w R_d \Gamma (\psi_s - \psi_r + \psi_o) = \left(\beta S_s + S_y \frac{dS_s}{d\psi_s} \right) \frac{\partial \psi_s}{\partial t}$$

Water movement in the root in the vertical and horizontal directions can be described as

$$\frac{\partial}{\partial x_i} \left[K_{ri} R_d \frac{\partial}{\partial x_i} (\psi_r + x_2) \right] + S_w R_d \Gamma (\psi_s - \psi_r + \psi_o) = R_d \frac{\partial WC_r}{\partial t} + WC_r \frac{\partial R_d}{\partial t}$$

where x_1, x_2 = horizontal and vertical direction, respectively (m); K_{si} = hydraulic conductivity of the soil in the x_i direction (m/day); K_{ri} = hydraulic conductivity of the root in the x_i direction (m/day); R_d = root density (m^3/m^3); S_s = effective saturation (m^3/m^3); S_y = specific storage of a soil (m^{-1}); S_w = degree of soil water saturation (m^3/m^3); S_y = specific yield of a soil (m^3/m^3); t = time (day); WC_r = water content in a root (m^3/m^3); Γ = root permeability factor ($m\text{-day}^{-1}$); ψ_o = osmotic pressure head of soil water (m); ψ_r = root-water pressure head (m); ψ_s = soil water pressure head (m); $\beta = 0$ if $\psi_s \leq 0$; and $\beta = 1$ if $\psi_s > 0$.

The equations can be solved numerically using a Galerkin finite element method with appropriate boundary and initial conditions.^{100,155}

B. Uptake by Plants

Experimental work with plant uptake of organic xenobiotics has been limited mostly to studies of herbicides and pesticides. Relatively little is known about plant uptake and metabolism of hydrocarbon and chlorinated hydrocarbon compounds. A summary of experimental studies on plant uptake is given in Table 2. Although Hatzios and Penner⁶⁷ discuss metabolism and accumulation of herbicides in plants, there is little evidence as to whether plants can metabolize a wide variety of hydrocarbon compounds.

A starting point for understanding uptake of organic compounds by plants is found in plant physiology. Background information on the current theories of transport in the phloem and xylem is described herein. Plants generally translocate water through the xylem and solutes through the phloem. The phloem is near the

TABLE 2
Selected Investigations on Uptake of Chemicals by Plants

Compound	Plant	Ref.
Sulfonamides	Broad bean (<i>Vicia faba</i> L.)	37
Dichlobenil	Bush bean (<i>Phaseolus vulgaris</i> L.)	163
Organochlorine insecticides	Soybean (<i>Glycine max</i> [L.] Merr.)	17
Oxamyl	Potato (<i>Solanum tuberosum</i> L.)	157
PCBs	Many species, wild and cultivated	125
<i>o</i> -Methylcarbamol, phenylureas	Barley (<i>Hordeum vulgare</i> L.)	25
Polynuclear aromatic compounds	Many species, wild and cultivated	147
Asulam, bromacil	Maize (<i>Zea mays</i> L.), bush bean (<i>P. vulgaris</i> L.)	61
PCBs	Purple loosestrife (<i>Lythrum salicaria</i> L.)	28
Anthracene	Bush bean (<i>P. vulgaris</i> L.)	42
Benzene, substituted benzenes	Soybean (<i>G. max</i>)	103
Bromacil, phenol, nitrobenzenes, dichlorobenzonitrile	Soybean (<i>G. max</i>), barley (<i>H. vulgare</i>)	105, 106
2,3,7,8-TCDD	Many species, mostly cultivated	80
Nitroguanidine	Soybean (<i>G. max</i>), fescue (<i>Festuca</i> spp.), bromegrass (<i>Bromus inermis</i> Leyss)	68
Nitrobenzene	Includes soybean (<i>G. max</i>), barley (<i>H. vulgare</i>), hybrid poplar (<i>Populus</i> × <i>rubusta</i> C.K. Schneid.), Russian olive (<i>Eleagnus angustifolia</i> L.), green ash (<i>Fraxinus</i> <i>pennsylvanica</i> Marsh.), and three others	107
Dieldrin, heptachlor, chlordane	Eight species of field crops	148
Atrazine, PCB, DDT, chlorobenzenes, dieldrin	Barley (<i>H. vulgare</i>)	156

Abbreviations: PCBs, polychlorinated biphenyls; TCDD, tetrachlorodibenzo-*p*-dioxin.

inner bark while the xylem is the woody part of a dicotyledenous tree. In herbaceous dicots such as *M. sativa*, the phloem is generally found toward the outside, with the xylem toward the center of the stem. In monocots such as prairie grass, the vascular bundles are clustered throughout the stem. A general review is given by Devlin and Witham (see pp. 53-71, 296-314 in Reference 40).

1. Phloem

The distance from the leaves, where photosynthesis occurs, to the roots of the plant is vast in a tree. The phloem transports sugars through tissues called sieve tube elements. Although sugars, mostly sucrose, are the main solutes transported, amino acids and amides also are translocated through the phloem. Transport is bidirectional, so that a sieve tube can transfer material either up or down. In a study of bean (*Phaseolus vulgaris* L.) plants,⁴⁰ it was found that the metabolites from the lower leaves are transported to the roots. The leaves near the top supply the growing stems, while the middle leaves provide metabolites, which are transported to locations both above and below.

Reported solute translocation rates varied within a single species, from 40 cm/h for pumpkin (*Cucurbita pepo* L.) to 290 cm/h for straight-necked squash (*Cucurbita* spp.). The only tree listed by Devlin and Witham (see p. 305 in Reference 40) was the willow (*Salix* spp.), which had a phloem translocation rate of 100 cm/h; for a metabolite to travel 16 cm, it had to go through 1600 to 2000 sieve plates.

One theory of phloem transport is the Munch pressure flow hypothesis, which is based on the assumption that a turgor (osmotic) pressure gradient exists between a source and a sink. In this model, metabolites are carried by diffusion from a higher to a lower concentration (see pp. 296-314 in Reference 40).

2. Xylem

The main role of the xylem is to transport water. The plant absorbs water through the roots because of an osmotic pressure gradient; the osmotic pressure of the soil is often higher than it is in the roots because of the higher salt concentration in the soil. If the osmotic pressure of the soil is higher than the root, the plant will have difficulty obtaining water. Water absorbed in this manner does not require energy input from the plant, but the internal solute concentration must be higher than the soil solute concentration to drive the uptake process.

As the water moves into the root, the relatively impermeable Casparian strip filters the water before it goes into the xylem. The transfer of water through the plant is explained by the cohesion-tension theory, which states that the leaves of the tree act like a sponge on top of a tube. As water evaporates from the sponge, the cohesive forces of water maintain the water column and pull water up.

The xylem consists of vessel elements that are open ended, tracheids that are elongated and have open pits on the sides, and fibers. It is through the tracheids

and vessels that the plant is able to move large amounts of water (see pp. 53–71 in Reference 40).

C. Modeling Uptake and Metabolism

Although extensive research has examined the uptake of chemicals by plants, as cited in Table 2, modeling this behavior is a newer development. Currently, there are two approaches to the modeling of plant uptake. One is from the soil science perspective in which the concern is the uptake of pesticides. The other approach originated from an attempt to understand solute flow in plants. The article of Trapp et al.¹⁵⁶ includes a mathematical model based on fugacity: chemical movement is modeled throughout the entire system — soil, plant, and air.

The development of solute flow models is described in several articles, which are reviewed here. One of the first articles modeling phloem flow was written by Eschrich et al.,⁴⁶ who attempted to determine the mechanism of solute movement in sieve tubes. In this experiment, unsteady-state diffusion was measured in a tube where half was filled with water and the other half was filled with sucrose solution. The measured rate of movement of the diffusion front gave support to the Munch pressure-flow theory as being the method of solute transport from a source to a sink in the phloem. From this simple experiment, modeling improvements were made.

Christy and Ferrier³² noted that phloem flow was closer to a steady-state process rather than a one-time equalization between a source and sink. Also, a sieve tube in the phloem is more complex than a glass tube in the lab. In Model I of these authors, the solute was assumed to be loaded directly into the sieve tube. An active role of phloem parenchyma cells in loading was assumed in Model II. Other factors included in this model are hydrostatic pressure and membranes that affect loading and unloading between sieve tubes.

Later, Ferrier and Christy⁵⁰ were able to calculate the effect of lowered temperature on solute transport. They found that the translocation recovery from low temperature depended primarily on sieve pore blockage and not on viscosity changes. Model II of the 1973 paper was used and successfully matched data from sugar beet experiments.

The model of Ferrier and Christy^{32,50} was critiqued by Goeschl et al.⁶⁰ as being thermodynamically inconsistent in having at least one more variable than equations. This was resolved by adding an equation that defined the rate of loading and unloading across the sieve plates as a function of the solute concentration. One shortcoming of this model and others as noted by those authors was that the phloem was treated as a single sieve tube.

A subsequent paper by Weir¹⁶⁸ examined sucrose flow over long distances. This article concluded that the Munch pressure-flow theory was adequate to describe solute flow over short distances. However, over longer distances, gravitational forces should be included because they are important in tall trees.

In the early work, only the phloem was considered as a pathway for solute transport and the only solute movement analyzed was sucrose. The article by

Tyree et al.¹⁵⁷ included the role of the xylem in transport and the behavior of pesticides in plants. Some chemicals move in an apoplastic pattern, which is transport outside the cells, through the xylem. Symplastic movement is chemical translocation through the phloem and the protoplasts of the parenchyma cells. Chemicals that move by both patterns are called ambimobile. An ambimobile pesticide can be distributed throughout the plant. Still another behavior is called pseudoapoplastic movement, in which the chemical is absorbed into the plant cells. This divides apoplastic chemicals into two classes, those that do not absorb appreciably into the cell walls and those that do. It is believed that the high permeability of pseudoapoplastic chemicals allows them to absorb from the symplast into the apoplast where they are transpired.

Chemical volatility and solubility are critical variables in transport and accumulation studies. The xenobiotic pesticide oxamyl has been studied because it is ambimobile and has a high enough permeability to have pseudoapoplastic behavior. The model of Tyree et al.¹⁵⁷ includes a single xylem vessel and a single phloem vessel running the length of the plant and includes a leaf, stem, and root section. The pesticide source is assumed to be on the leaf, from which the phloem initially transports it to the rest of the plant. In addition, the cells lining the phloem wall in the leaf, stem, and root are assumed to be a sink so that pseudoapoplastic pesticides can be absorbed and leaked into the xylem.

Further investigation of chemical behavior in plants and further refinement of modeled plant anatomy were made by Boersma et al.²⁰ One refinement is that their model includes interaction of the root with the soil, which allows for coupling with existing models for chemical behavior in the soil. Chemical uptake is further modeled by using relationships that include the appropriate partition coefficients and the plant uptake rate. The root concentration factor (RCF) and the transpiration stream concentration factor (TSCF) are defined as partitioning ratios at different stages of transfer. The transfer of exogenous substances into and through a plant is primarily a function of lipophilicity of the compound of interest. For optimal transfer, it must have both water solubility and, to pass the membrane barriers of the root, a moderate level of lipid solubility. Estimates of lipid solubility are usually derived from the log of the octanol-water partition coefficient (K_{ow}), with the most mobile compounds having a log K_{ow} in the range from 1 to 3.²⁵ The model, of Boersma et al.²⁰ also takes into account biodegradation in the reactive environment of plasmalemma cells of the symplastic system.

G. max was modeled with compartments assigned to the soil, the phloem and xylem of root, stem, and leaf.²⁰ The root had a storage compartment assigned to it. The leaf and root phloem compartments were designed to interact with their respective apoplast and symplast compartments. The compartments were separated by physical and chemical barriers designated by reflection coefficients, partition coefficients, and hydraulic conductivity. The compartments were defined by volume, contact area with other compartments, sorption coefficient, and a coefficient for first-order loss processes.

UTAB (uptake, translocation, accumulation, and biodegradation) is a three-leaf, three-stem, one-root adaptation of the model described by Boersma et al.²⁰

Here, Boersma et al.¹⁹ made further refinements, with three-leaf, three-stem, and one-root compartments. With these modifications, each compartment has two transport compartments, one each for the phloem and the xylem and a storage compartment. The soil was assigned two compartments for the root outside the Casparian strip: one for the free space in the soil and another for the cell volume. In the leaves and the root, the xylem and phloem are allowed to interact, while in the stem the xylem and phloem are separated by the storage compartment.

With this latest model of Boersma et al.,¹⁹ the fate of contaminants in water taken up by vegetation can be modeled whether the chemical is transpired, accumulated in plant tissue, or biodegraded. This approach also has an advantage over the soil science-based model in that the transport of oxygen from the stem through the roots to the soil can be included in the model.

For those compounds not transpired or biodegraded to nontoxic molecules, complete analysis of remediation efforts requires consideration of accumulation within the plant (e.g., see Reference 19) and possible accumulation in the food chain.¹²⁹ Compounds with a high K_{ow} , such as polynuclear aromatic hydrocarbons, can scarcely enter the plant root.²³ These are the type of compounds most strongly bioaccumulated through the food chain because of their high lipophilicity. Compounds with very low K_{ow} are generally rejected by the plant root unless there is a specific transport mechanism (e.g., methylamine). Nonvolatile compounds of intermediate K_{ow} (e.g., some herbicides) are the most troublesome and must be considered on a case-by-case basis. The primary role of plants described in this article is to supply the needs of microbes, not to serve directly in the remediation process in many cases. Much more analysis is needed in the area of bioaccumulation of substances in plants and the possible transfer up the food chain.

Remediation strategies must be developed for specific locations. For instance, the present authors are specifically concerned with a landfill built without liners on a river floodplain, <20 ft above the average groundwater depth. The detectable contaminants in groundwater are very low levels of organics, such as benzene and vinyl chloride. In this instance, the contaminants would volatilize rather than accumulate in the plants, if not degraded in the rhizosphere. Trees, perhaps those ultimately used for firewood, are good candidate species.

In other situations, e.g., a "land-farming" site associated with a refinery, the clean-up of recalcitrant compounds may be enhanced by providing drought-tolerant herbs and grasses, which can supply cosubstrates in a water-limited environment. In still another example, there are many coal gasification sites where the planting of oak or walnut trees would stabilize the site, reduce groundwater infiltration and enhance the degradation of recalcitrant complex organic substances. Here, the ultimate product, many decades in the future, might be valuable timber. Research is needed to determine whether the compounds in soil would accumulate to measurable levels in the trees, precluding ultimate harvest but not their use in remediation.

IV. CONCLUSIONS

Plants and trees are capable of assisting in the bioremediation of contaminated soil and groundwater. They provide a favorable subsurface environment by supplying oxygen and additional substrates that promote microbial growth. Although much is yet to be learned about microbial degradation in the rhizosphere and plant transport mechanisms, the models derived for groundwater movement, plant uptake, and microbial growth should provide the basis for the design of shelter belts capable of decontaminating soil and groundwater. With advances in our knowledge, it may be possible to prevent the movement of contaminants beyond the contaminated area by utilizing vegetation to remove water and by nurturing microorganisms in the rhizosphere that can degrade the contaminants.

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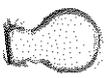
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Appendix H
Post-Closure Notices



Chevron

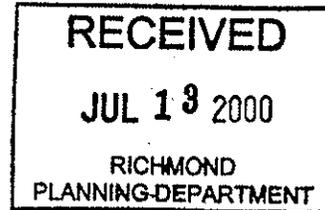
JUL 20 2000

July 5, 2000

Chevron Products Company
P. O. Box 1272
Richmond, CA 94802-0272

Jeff Hartwig
Environmental and Safety Division Manager
510 242 1400

Mr. Martin Jacobsen
City of Richmond Planning Department
2600 Barrett Ave. 2nd Floor City Hall
Richmond, CA 94804



Record of Landfarm Closure at the Richmond Refinery

Dear Mr. Jacobsen:

Chevron is submitting the enclosed record of hazardous wastes disposal within the recently closed Landfarm Treatment Facility that was operated by Chevron in the 1970's and 80's at the Richmond Refinery.

This submission is made for purposes of complying with 22CCR 66265.119 (a). This regulation requires the owner or operator of a closed hazardous waste disposal unit to submit to the local zoning authority (or authority with jurisdiction over local land use) a record of the type, location, and quantity of the hazardous wastes disposed of with each cell or area of the facility. This submission has also been provided to the California Environmental Protection Agency-department of Toxic Substances Control, per 22CCR 66265.119(a).

We are providing two sets of attachments. Would you please stamp one copy as having been received and filed and return it to me in the enclosed, stamped, self-addressed envelope.

If you have any questions regarding this letter, please contact Mr. John MacLeod at (510) 242-2295.

Sincerely,

Attachments

RECORD OF HAZARDOUS WASTES DISPOSED OF IN CLOSED LANDFARM TREATMENT FACILITY AT THE RICHMOND REFINERY

The Chevron Products Company currently is the owner and operator of the closed Landfarm Treatment Facility. The Landfarms are located on the property which is described in Exhibit A to this Record.

The Landfarms has been closed pursuant to a the *Revised Landfarm Closure Plan* (1997) which was approved by the California Environmental Protection Agency, Department of Toxic Substances Control (DTSC). A certificate of closure was submitted to the DTSC on September 30, 1999.

Pursuant to 22 CCR 66265.119, Chevron is providing this record of the type, location and quantity of hazardous wastes disposed of in the Landfarm Treatment Facility.

Prior to closure, the Landfarms consisted of 5 Landfarm units. The Landfarms were in operation for the biological treatment of oily wastes generated from on-site petroleum processing from the mid 1970's to 1987. The principle wastes applied were oil/water separator sludge (at Nos. 1, 2, 4 and 5 Landfarms), non-leaded tank bottoms (at Nos. 1,2,3 and 4 Landfarms), oil/water mixtures, algae water, pond sediments and oily dirt. When in operation, wastes were applied to the surface of the Landfarms and tilled into the top six to 12 inches. Prior to 1980, no data are available on waste application rates to the Landfarms. Since 1980, a total of 188,000 tons of waste were applied to the Landfarms. The location and dimensions of the units as closed is indicated in the survey plat which is Exhibit B to this Record. The wastes, which remain in the Landfarm after closure, are described in "*Revised Landfarm Closure Plan*". The relevant sections of this plan are Sections 2.2.3 and 2.2.4.

This Record is based on available records and investigations, which have been conducted into the environmental conditions at the Landfarms. The information contained in the Record is, to the best of Chevron's current knowledge and belief, true, accurate and complete.

EXHIBIT "A"

PROPERTY DESCRIPTION

Landfarm 1

Tract 1 , Parcel 3, of the San Pablo Rancho. Recorded March 8, 1918, Book 315, Page 168.

Also known as Assessor Parcel 561-100-013

Landfarms 2-5

Tract 5, Parcel 3, of the San Pablo Rancho. Recorded February 28, 1917, Book 294, Page 255

Also known as APN 561-100 037

Tract 5, Parcel 1, of the San Pablo Rancho. Recorded November 16, 1953, Book 2245, page 538

Also known as APN 561-100-038

RECORDING REQUESTED BY
CHICAGO TITLE COMPANY

RECORDING REQUESTED BY
AND WHEN RECORDED MAIL TO:

c/o Chevron Real Estate Management Company
Robert Vanderlaan, 2613 Camino Ramon Rm 2278
San Ramon, CA 94583

133787-1

CONTRA COSTA Co Recorder Office
STEPHEN L. WEIR, Clerk-Recorder
DOC- 2000-0144183-00

Acct 2- Chicago Title
M, JUL 10, 2000 08:00:00
MIC \$1.00 MOD \$31.00 REC \$35.00
TCF \$30.00 NCP \$93.00
Ttl Pd \$190.00
Nbr-0000037897
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DECLARATION

THIS DECLARATION (the "Declaration") is made as of July 6, 2000, by CHEVRON U.S.A. INC., a Pennsylvania corporation ("Declarant").

1. Declarant is the owner of that certain real property located in Contra Costa County, State of California (the "Property"), which is more particularly described on Exhibit "A" attached hereto.
2. The Property is subject to the terms and conditions of a Revised Landfarms Closure Plan dated December 30, 1996 and revised May 28, 1997, filed with the California Environmental Protection Agency, Department of Toxic Substances Control, Region 2, 700 Heinz Ave., Berkeley, CA 94710. (Closure Plan).
3. In accordance with the Closure Plan, Declarant hereby declares and agrees that the plat attached as Exhibit "B" shall be placed of record with respect to the Property. Additionally, Exhibit "C" is the Post Closure Notice of Hazardous Waste, and Exhibit "D" is the Record of Hazardous Waste.

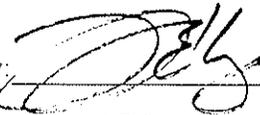
IN WITNESS WHEREOF, the undersigned Declarant has executed these presents by its officer thereunto duly authorized, this 6 day of July, 2000.

DECLARANT:

CHEVRON U.S.A. INC.,
a Pennsylvania corporation

By:

Title:


ASSISTANT SECRETARY



CHICAGO TITLE COMPANY HAS FILED THIS INSTRUMENT FOR
RECORD AS AN ACCOMMODATION ONLY. IT HAS NOT BEEN
EXAMINED AS TO ITS EXECUTION OR AS TO ITS EFFECT UPON TITLE.

EXHIBIT "A"

PROPERTY DESCRIPTION

Landfarm 1

Tract 1 , Parcel 3, of the San Pablo Rancho. Recorded March 8, 1918, Book 315, Page 168.
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Also known as APN 561-100 037

Tract 5, Parcel 1, of the San Pablo Rancho. Recorded November 16, 1953, Book 2245, page 538
Also known as APN 561-100-038

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EXHIBIT B

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STATION	ANGLE	DISTANCE	COORDINATES
1	120° 00'	100.00	100.00 0.00
2	120° 00'	100.00	173.21 86.60
3	120° 00'	100.00	213.14 173.21
4	120° 00'	100.00	213.14 279.82
5	120° 00'	100.00	173.21 386.43
6	120° 00'	100.00	100.00 386.43
7	120° 00'	100.00	0.00 386.43
8	120° 00'	100.00	-100.00 386.43
9	120° 00'	100.00	-173.21 386.43
10	120° 00'	100.00	-213.14 386.43
11	120° 00'	100.00	-213.14 279.82
12	120° 00'	100.00	-173.21 173.21
13	120° 00'	100.00	-100.00 86.60
14	120° 00'	100.00	0.00 0.00

LEGEND

1. THE CENTER LINE OF THE CANAL SHALL BE THE CENTER LINE OF THE CANAL AS SHOWN ON THE PLAN AND THE CENTER LINE OF THE CANAL SHALL BE THE CENTER LINE OF THE CANAL AS SHOWN ON THE PLAN.

2. THE CENTER LINE OF THE CANAL SHALL BE THE CENTER LINE OF THE CANAL AS SHOWN ON THE PLAN AND THE CENTER LINE OF THE CANAL SHALL BE THE CENTER LINE OF THE CANAL AS SHOWN ON THE PLAN.

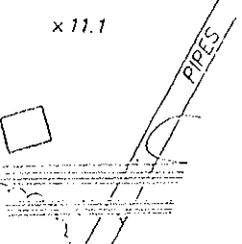
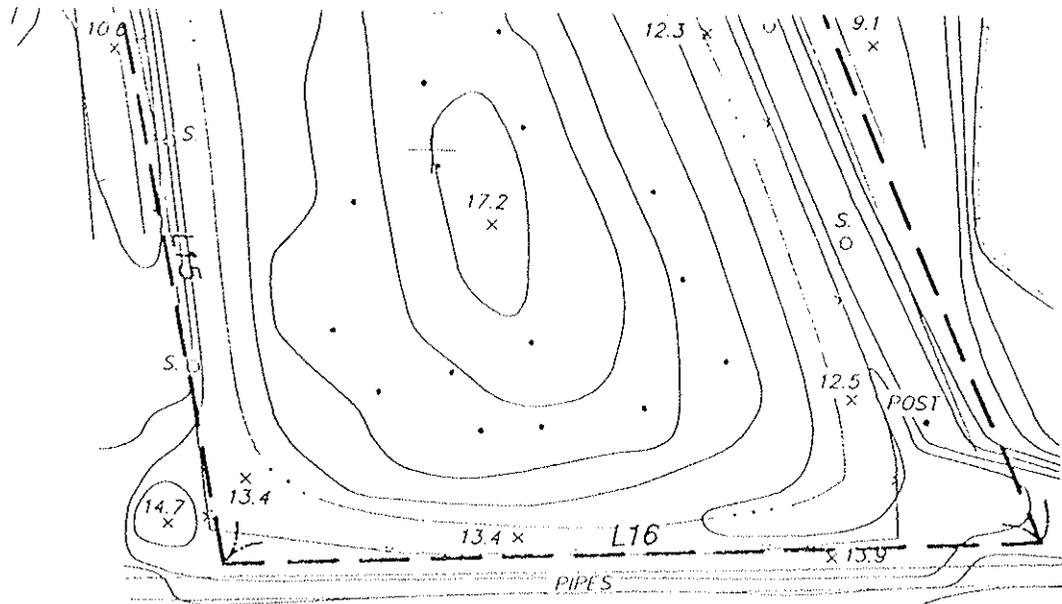
3. THE CENTER LINE OF THE CANAL SHALL BE THE CENTER LINE OF THE CANAL AS SHOWN ON THE PLAN AND THE CENTER LINE OF THE CANAL SHALL BE THE CENTER LINE OF THE CANAL AS SHOWN ON THE PLAN.



NO.	DESCRIPTION	AMOUNT
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144183



Michael P. Rei

MICHAEL P. REI
 R.C.E. No. 13810
 EXP. 3/31/01

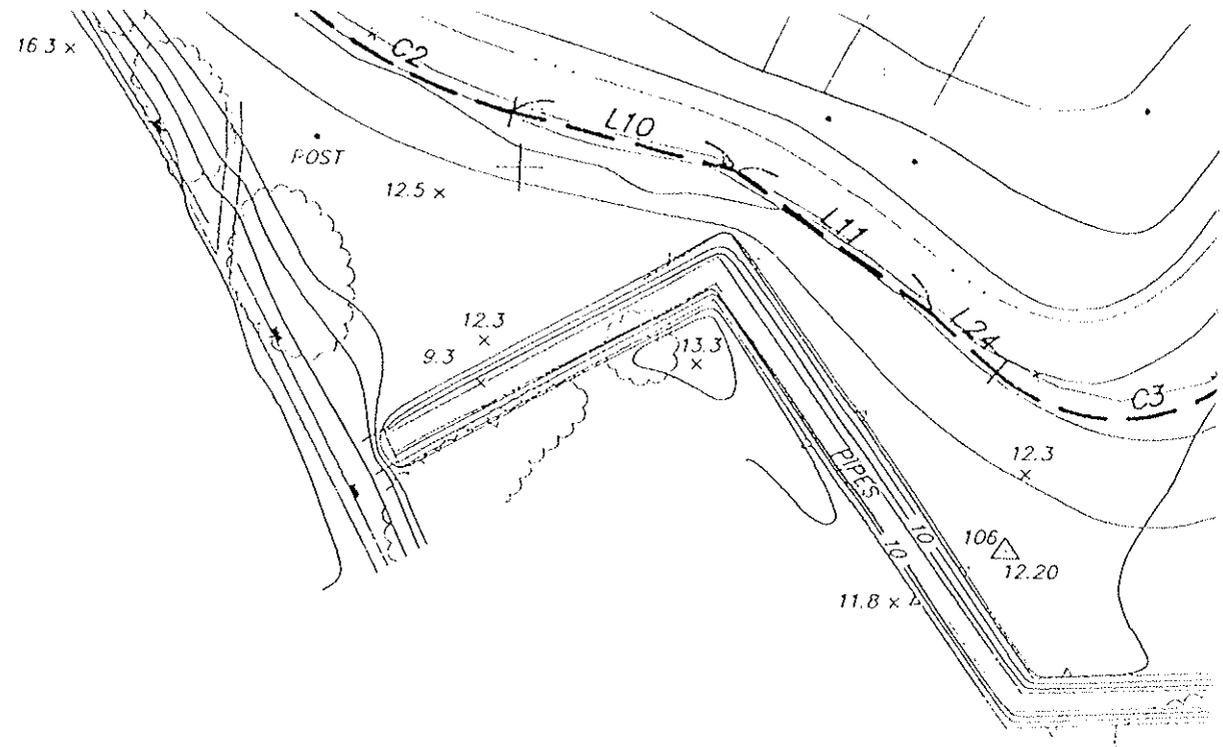


REVISIONS 10/15/99 AUTH 99 (08 ADD CLOSURE INFORMATION. 11/16/99 CHANGE TITLE 5/31/00 ADD NORTH #6	KISTER, SAVIO & REI, INC. LAND SURVEYORS - CIVIL ENGINEERS. 3095 RICHMOND PARKWAY, SUITE 214 RICHMOND, CALIFORNIA 94806 PHONE: (510) 222-4020 FAX: (510) 222-3718 E-MAIL: KSR@earthlink.net		DESCRIPTION TOPOGRAPHIC SURVEY LANDFARM CLOSURE PLAN LANDFARM No. 1 CHEVRON RICHMOND REFINERY
	FOR CHEVRON PRODUCTS CO.		
	SCALE 1" = 50'	JOB No. 9677 AUTH. 99 0015	
	DATE 7/9/99	WORK No. 0-789-1	
		RICHMOND, CALIFORNIA	

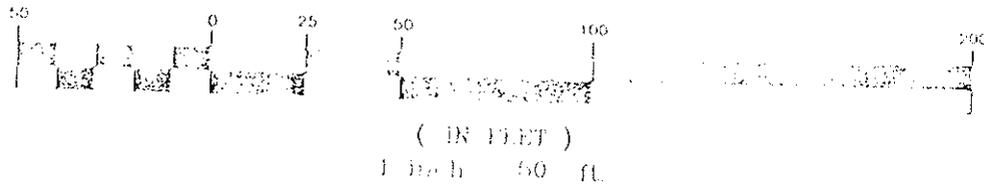
11

144183

N 2172250



GRAPHIC SCALE



LEGEND

REFERENCE

- FIELD BOOK NO. DR-967
- FILE MAP
- FILE CO.
- FIELD No.
- GAZON (GARDEN) REED.
- POST (POST)

BY JOHN W. GARDNER
2172250

10

144183

L4	N 05°22'4" W	63.99'
L5	N 71°10'59" W	74.41'
L6	N 77°26'17" W	85.83'
L7	N 73°51'45" W	105.54'
L8	S 54°19'1" E	216.05'
L9	S 52°36'53" E	332.48'
L10	N 76°27'7" W	61.44'
L11	S 55°34'14" E	63.79'
L12	S 28°1'45" W	168.89'
L13	N 37°26'21" W	25.52'
L14	N 16°31'48" W	55.42'
L15	N 12°34'15" W	161.26'
L16	N 87°15'20" E	213.60'
L17	S 24°0'48" E	322.77'
L18	N 58°6'14" W	174.62'
L19	N 53°45'38" W	357.61'
L20	N 52°39'20" W	139.99'
L21	N 50°34'47" W	167.16'
L22	N 49°33'46" W	174.13'
L23	N 45°0'45" W	209.35'
L24	S 50°27'10" E	23.29'

C4	119.94'	60.00'	114°31'54"
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CLOSURE NOTES

1. THE UPPER LAYER OF THE LANDFARM FILL IS 1.5 TO 7 FEET THICK AND CONSISTS OF DARK GRAY TO BROWN SILTY CLAY WITH BLACK MOTTLING, PLANT ROOTS AND SCATTERED GRAVEL AND SAND.
2. THE LOWER LAYER OF THE LANDFARM FILL RANGES FROM 3 TO 19 FEET THICK AND IS GENERALLY COMPOSED OF GRAVELLY TO SILTY CLAY WITH VARIABLE AMOUNTS OF GRAVEL, SAND, SILT AND MAN MADE MATERIALS SUCH AS BRICK, CONCRETE AND GLASS, AND POCKETS OF BLACK, SOFT SLUDGE (SILTY CLAY SATURATED WITH OILY AND TARRY SUBSTANCES).
3. YOUNG BAY MUD UNDERLIES THE LANDFARM FILL AND OVERLIES THE OLD BAY MUD, AND RANGES BETWEEN 5 AND 30 FEET IN THICKNESS BENEATH THE LANDFARMS. THE YOUNG BAY MUD GENERALLY CONSISTS OF DARK BROWN TO DARK GRAY, SOFT TO MEDIUM-STIFF SILTY CLAY THAT CONTAINS VARYING PERCENTAGES OF ORGANIC MATERIAL, PEAT AND OCCASIONAL SANDY UNITS. PEATY BAY MUD, WHICH HAS AN ABUNDANT AMOUNT OF PEAT, ARE PRESENT IN THE UPPER 1 TO 7 FEET OF THE YOUNG BAY MUD.
4. OLD BAY MUD RANGES FROM 1 TO 15 FEET THICK BENEATH THE LANDFARMS. THE OLD BAY MUD CONSISTS OF BLuish TO GREENISH GRAY, MEDIUM-STIFF TO STIFF SILTY CLAY THAT CONTAINS ORGANIC MATERIAL AND LOCAL TRACE AMOUNTS OF SAND.
5. THE PRESENT LOCATION OF THE NORTHWESTERN PART OF LANDFARM 1 WAS UNDERLAIN BY SEVERAL PONDS THAT WERE BACKFILLED. POCKETS OF SLUDGE FOUND WITHIN THE LOWER FILL PROBABLY REPRESENT OIL FILL MATERIAL AND OILY WASTES WITHIN THE PONDS.
6. AS REQUIRED BY 22 CCR §66265.116, THE FOLLOWING NOTE IS PROVIDED: THE OWNER OR OPERATOR HAS AN OBLIGATION TO RESTRICT DISTURBANCE OF THE HAZARDOUS WASTE DISPOSAL UNIT SHOWN ON THIS SURVEY PLAT IN ACCORDANCE WITH THE APPLICABLE REGULATIONS OF ARTICLE 7 OF CHAPTER 10 OF TITLE 22 OF THE CALIFORNIA CODE OF REGULATIONS [22CCR § 66265.110 et seq.]

NOTE: THE PRECEDING INFORMATION WAS TAKEN FROM BECHTEL ENVIRONMENTAL, INC. DAMES & MOORE, "REVISED LANDFARM CLOSURE PLAN", FOR CHEVRON PRODUCTS COMPANY, DECEMBER 30, 1996.

GENERAL NOTES

1. AERIAL SURVEY PROVIDED BY KELLLOGG AERIAL SURVEYS 4200 N. FREEWAY BLVD. SUITE 3, SACRAMENTO, CA.
2. CONTOUR INTERVAL 1'
3. COORDINATES AND ELEVATIONS ARE BASED ON THE CHEVRON RICHMOND METRIC DATUM.



144183



08

144183

POST



N. 2172250

ABOVE GROUND

12.3

11.7

11.20

12.5

POST

C2

C3

L10

L11

L24

L9

12.9

14.2

17.2

14.3

13.5

16.3

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21.4

21

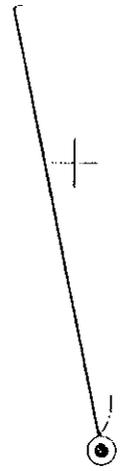
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08

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N 2172500

144183



CHEVRON MONUMENT No. B-42
N 2171707.04
E 6015478.32

LANDFARM CELL LIMIT LINE AND CURVE TABLES

LINE TABLE		
LINE	BEARING	LENGTH
L1	S 52°16'5" W	320.34'
L2	S 47°1'31" W	236.36'
L3	N 51°56'48" W	45.41'
L4	N 65°22'4" W	63.99'
L5	N 71°10'59" W	74.41'
L6	N 77°26'17" W	85.83'
L7	N 73°51'45" W	105.54'
L8	S 54°19'1" E	216.05'
L9	S 52°36'53" E	332.48'
L10	N 76°27'7" W	61.44'
L11	S 55°34'14" E	63.79'
L12	S 28°1'45" W	168.89'
L13	N 57°26'21" W	25.52'
L14	N 16°5'45" W	55.42'
L15	N 12°34'15" W	161.26'
L16	N 87°15'20" E	213.60'
L17	S 24°6'48" E	322.77'
L18	N 18°6'14" W	174.62'
L19	N 55°41'38" W	157.61'
L20	N 52°15'27" W	155.63'
L21	N 55°34'47" W	167.16'
L22	N 49°1'36" W	174.13'

CURVE TABLE			
CURVE	LENGTH	RADIUS	DELTA
C1	112.28'	65.00'	98°58'19"
C2	62.41'	150.00'	23°50'14"
C3	88.59'	50.00'	101°31'05"
C4	119.94'	60.00'	114°31'54"

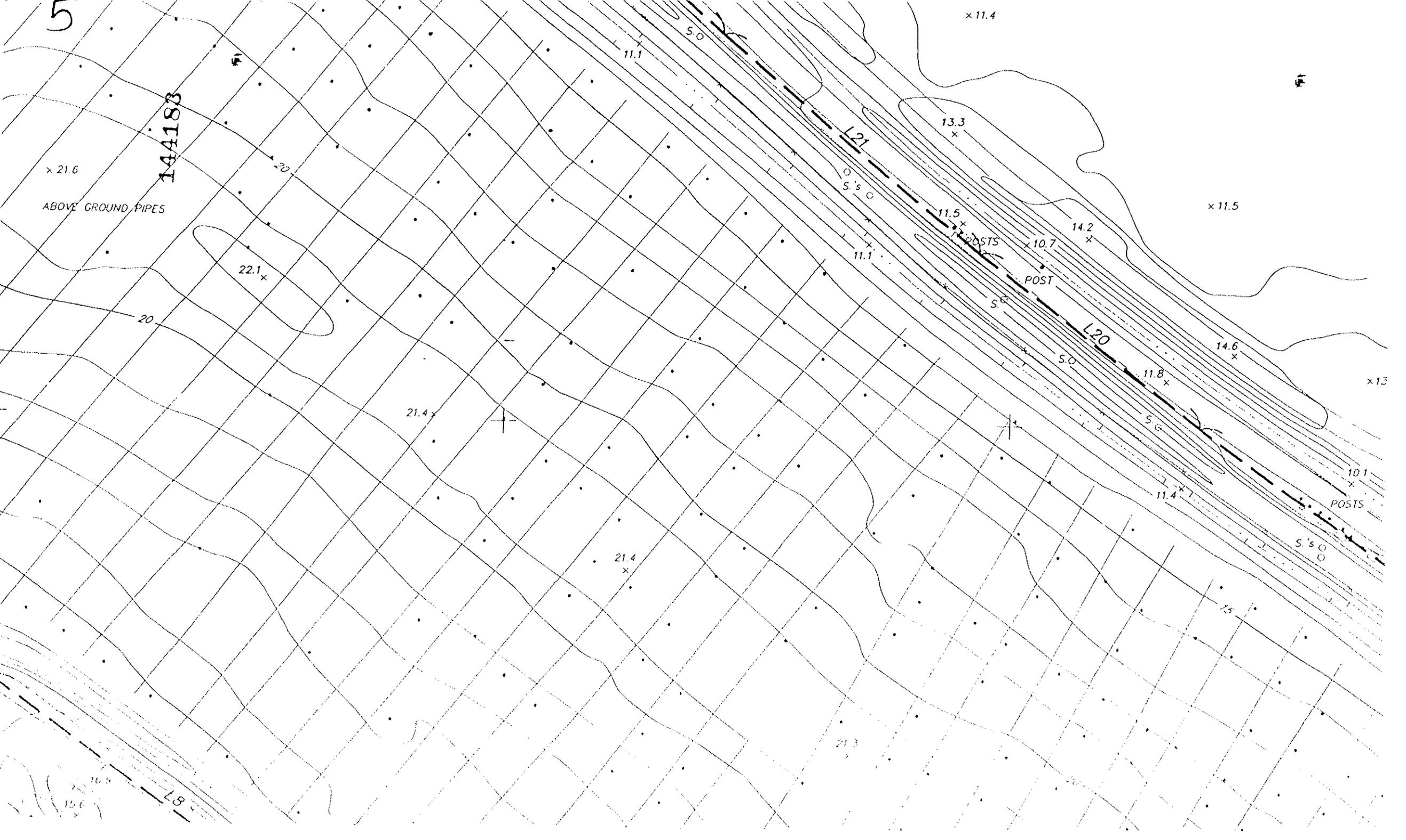
CLOSURE NOTES

1. THE UPPER LAYER OF THE LANDFARM FILL IS 1.5 TO 7 FEET THICK AND CONSISTS OF DARK GRAY TO BROWN SILTY CLAY WITH BLACK MOTTING, PLANT ROOTS AND SCATTERED GRAVEL AND SAND.
2. THE LOWER LAYER OF THE LANDFARM FILL RANGES FROM 5 TO 15 FEET THICK AND IS GENERALLY COMPOSED OF GRAVELLY TO SILTY CLAY WITH VARIABLE AMOUNTS OF GRAVEL, SAND, SILT AND MAN MADE MATERIALS SUCH AS BRICK, CONCRETE AND GLASS, AND PORTS OF BRACK, SOFT SLUDGE (SILTY CLAY UNREACHABLE WITH OIL AND TALET CONTAINERS)
3. TO A CLAY MUD UNDERLIES THE LANDFARM FILL AND OVERLIES THE RED BAY MUD, AND EXTENDS BETWEEN 1 AND 50 FEET DEEP. THIS RED BAY MUD IS A CLAY MUD WHICH GENERALLY CONSISTS OF 75% TO 90% DARK GRAY TO BROWN MEDIUM GRAIN SILTY CLAY WITH CONTAINING VARYING PERCENTAGES OF SAND AND MAN MADE MATERIALS OF VARIOUS SIZES AND OCCASIONAL SANDY BRICKS. THE RED BAY MUD, WHICH IS AT THE BOTTOM OF THE LANDFARM CELL, IS NOT REACHABLE BY THE

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x 21.6
ABOVE GROUND PIPES



E 6016750

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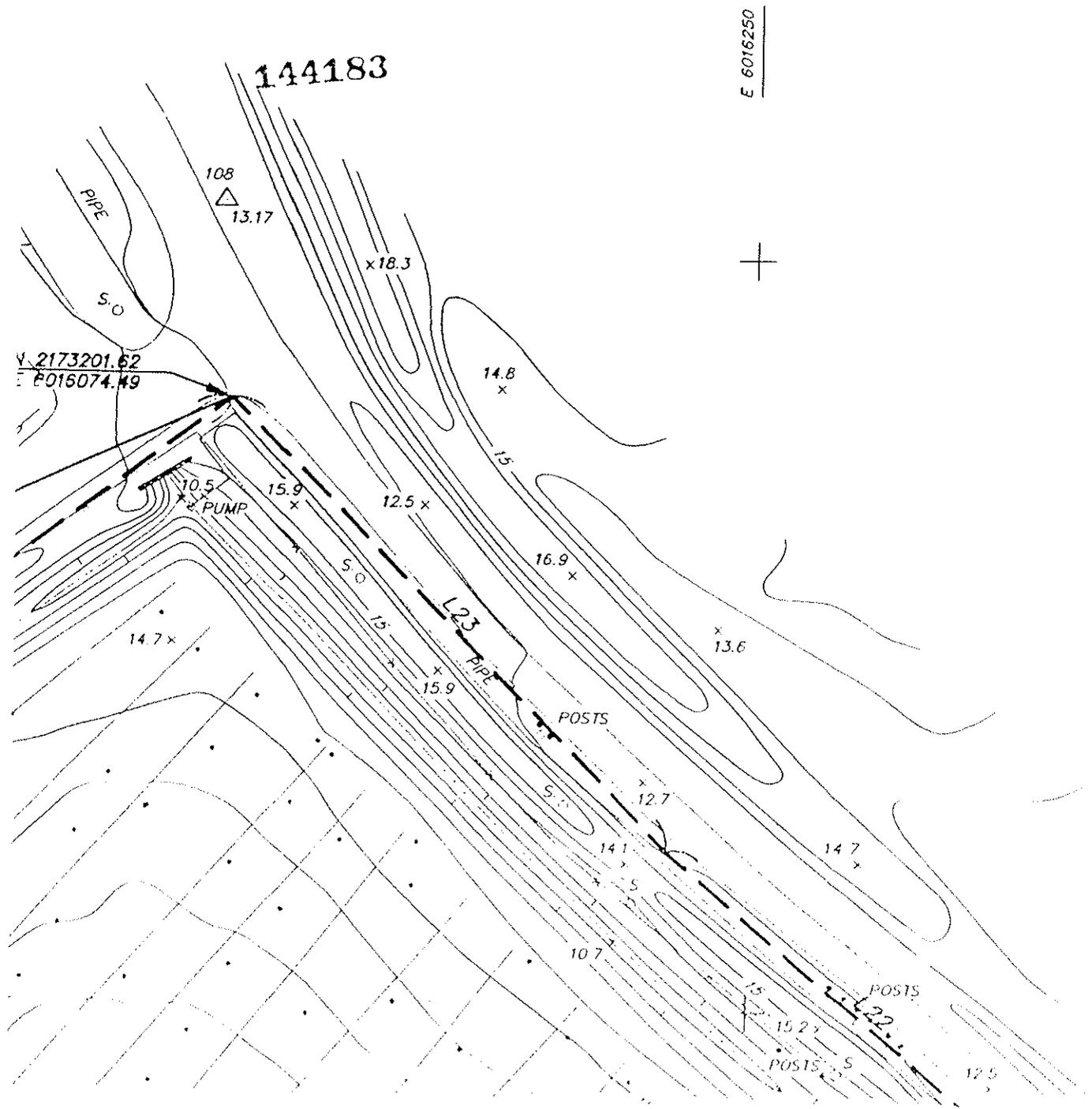
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144183

N 2173250

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S64°55'26"W

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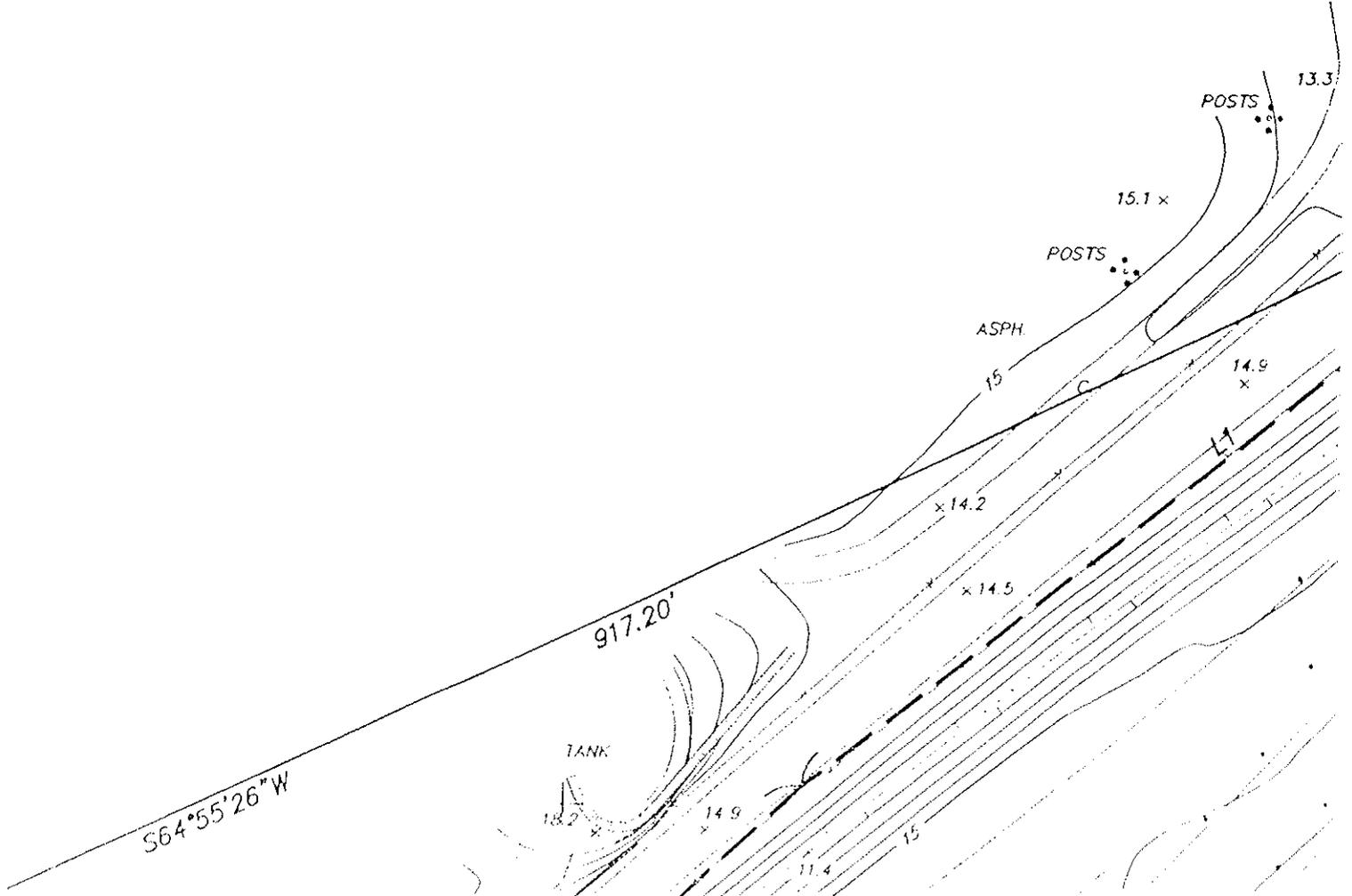
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POSTS

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144183

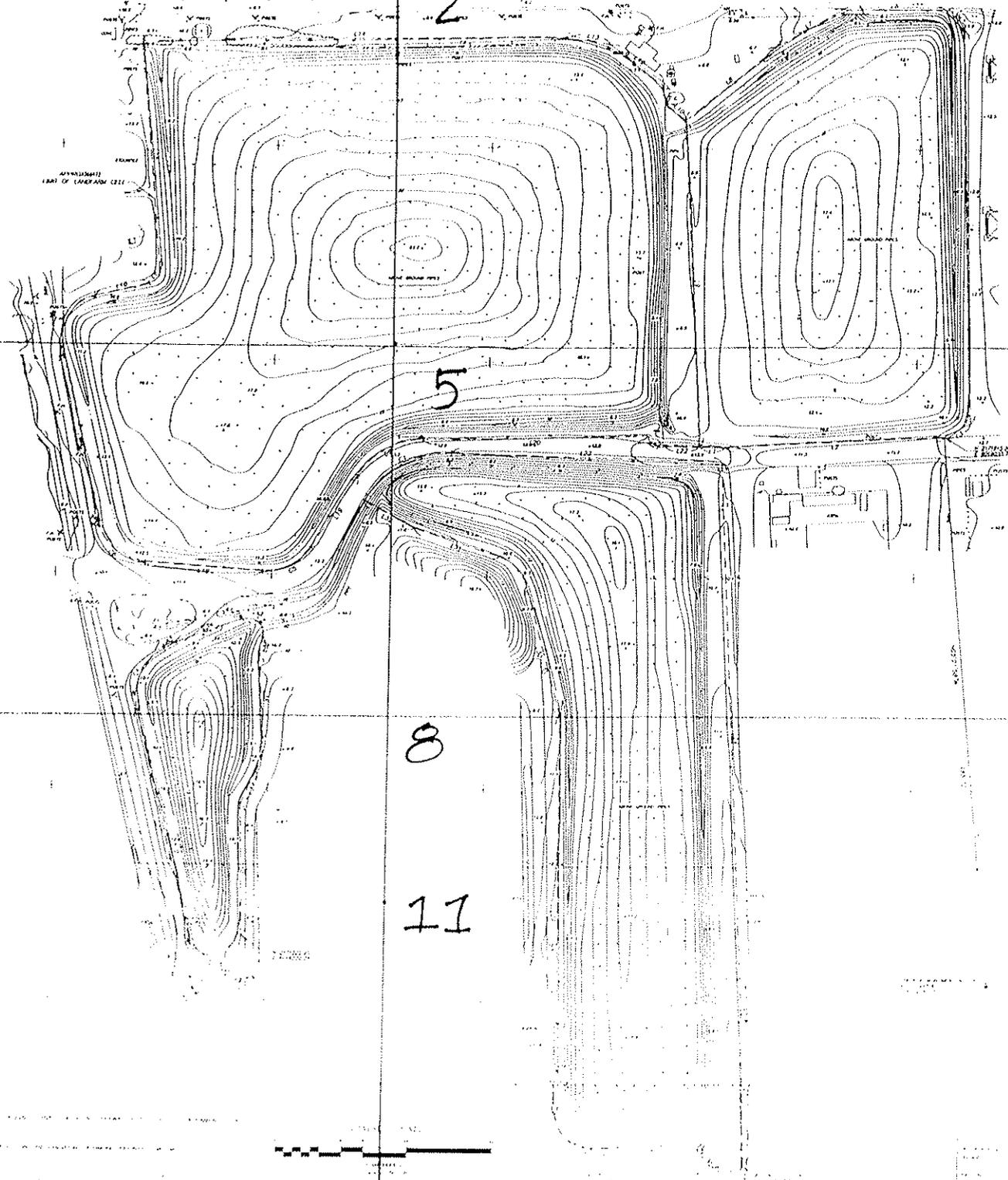


TABLE OF ELEVATIONS

POINT	ELEVATION	POINT	ELEVATION
101	115.00	111	115.00
102	115.00	112	115.00
103	115.00	113	115.00
104	115.00	114	115.00
105	115.00	115	115.00
106	115.00	116	115.00
107	115.00	117	115.00
108	115.00	118	115.00
109	115.00	119	115.00
110	115.00	120	115.00
121	115.00	131	115.00
122	115.00	141	115.00
123	115.00	151	115.00
124	115.00	161	115.00
125	115.00	171	115.00
126	115.00	181	115.00
127	115.00	191	115.00
128	115.00	201	115.00
129	115.00	211	115.00
130	115.00	221	115.00
131	115.00	231	115.00
132	115.00	241	115.00
133	115.00	251	115.00
134	115.00	261	115.00
135	115.00	271	115.00
136	115.00	281	115.00
137	115.00	291	115.00
138	115.00	301	115.00
139	115.00	311	115.00
140	115.00	321	115.00
141	115.00	331	115.00
142	115.00	341	115.00
143	115.00	351	115.00
144	115.00	361	115.00
145	115.00	371	115.00
146	115.00	381	115.00
147	115.00	391	115.00
148	115.00	401	115.00
149	115.00	411	115.00
150	115.00	421	115.00
151	115.00	431	115.00
152	115.00	441	115.00
153	115.00	451	115.00
154	115.00	461	115.00
155	115.00	471	115.00
156	115.00	481	115.00
157	115.00	491	115.00
158	115.00	501	115.00
159	115.00	511	115.00
160	115.00	521	115.00
161	115.00	531	115.00
162	115.00	541	115.00
163	115.00	551	115.00
164	115.00	561	115.00
165	115.00	571	115.00
166	115.00	581	115.00
167	115.00	591	115.00
168	115.00	601	115.00
169	115.00	611	115.00
170	115.00	621	115.00
171	115.00	631	115.00
172	115.00	641	115.00
173	115.00	651	115.00
174	115.00	661	115.00
175	115.00	671	115.00
176	115.00	681	115.00
177	115.00	691	115.00
178	115.00	701	115.00
179	115.00	711	115.00
180	115.00	721	115.00
181	115.00	731	115.00
182	115.00	741	115.00
183	115.00	751	115.00
184	115.00	761	115.00
185	115.00	771	115.00
186	115.00	781	115.00
187	115.00	791	115.00
188	115.00	801	115.00
189	115.00	811	115.00
190	115.00	821	115.00
191	115.00	831	115.00
192	115.00	841	115.00
193	115.00	851	115.00
194	115.00	861	115.00
195	115.00	871	115.00
196	115.00	881	115.00
197	115.00	891	115.00
198	115.00	901	115.00
199	115.00	911	115.00
200	115.00	921	115.00
201	115.00	931	115.00
202	115.00	941	115.00
203	115.00	951	115.00
204	115.00	961	115.00
205	115.00	971	115.00
206	115.00	981	115.00
207	115.00	991	115.00
208	115.00	1001	115.00

NOTES

1. THE SPHERE OF THE SURVEY IS TO BE KEPT OPEN AND UNOCCUPIED AT ALL TIMES TO ALLOW FOR THE CONDUCT OF FURTHER SURVEYS AND FOR THE INSTALLATION OF NEW EQUIPMENT.
2. THE SPHERE OF THE SURVEY IS TO BE KEPT OPEN AND UNOCCUPIED AT ALL TIMES TO ALLOW FOR THE CONDUCT OF FURTHER SURVEYS AND FOR THE INSTALLATION OF NEW EQUIPMENT.
3. THE SPHERE OF THE SURVEY IS TO BE KEPT OPEN AND UNOCCUPIED AT ALL TIMES TO ALLOW FOR THE CONDUCT OF FURTHER SURVEYS AND FOR THE INSTALLATION OF NEW EQUIPMENT.
4. THE SPHERE OF THE SURVEY IS TO BE KEPT OPEN AND UNOCCUPIED AT ALL TIMES TO ALLOW FOR THE CONDUCT OF FURTHER SURVEYS AND FOR THE INSTALLATION OF NEW EQUIPMENT.
5. THE SPHERE OF THE SURVEY IS TO BE KEPT OPEN AND UNOCCUPIED AT ALL TIMES TO ALLOW FOR THE CONDUCT OF FURTHER SURVEYS AND FOR THE INSTALLATION OF NEW EQUIPMENT.
6. THE SPHERE OF THE SURVEY IS TO BE KEPT OPEN AND UNOCCUPIED AT ALL TIMES TO ALLOW FOR THE CONDUCT OF FURTHER SURVEYS AND FOR THE INSTALLATION OF NEW EQUIPMENT.
7. THE SPHERE OF THE SURVEY IS TO BE KEPT OPEN AND UNOCCUPIED AT ALL TIMES TO ALLOW FOR THE CONDUCT OF FURTHER SURVEYS AND FOR THE INSTALLATION OF NEW EQUIPMENT.
8. THE SPHERE OF THE SURVEY IS TO BE KEPT OPEN AND UNOCCUPIED AT ALL TIMES TO ALLOW FOR THE CONDUCT OF FURTHER SURVEYS AND FOR THE INSTALLATION OF NEW EQUIPMENT.
9. THE SPHERE OF THE SURVEY IS TO BE KEPT OPEN AND UNOCCUPIED AT ALL TIMES TO ALLOW FOR THE CONDUCT OF FURTHER SURVEYS AND FOR THE INSTALLATION OF NEW EQUIPMENT.
10. THE SPHERE OF THE SURVEY IS TO BE KEPT OPEN AND UNOCCUPIED AT ALL TIMES TO ALLOW FOR THE CONDUCT OF FURTHER SURVEYS AND FOR THE INSTALLATION OF NEW EQUIPMENT.

THE SPHERE OF THE SURVEY IS TO BE KEPT OPEN AND UNOCCUPIED AT ALL TIMES TO ALLOW FOR THE CONDUCT OF FURTHER SURVEYS AND FOR THE INSTALLATION OF NEW EQUIPMENT.



GENERAL NOTES: THIS MAP IS A REPRODUCTION OF THE ORIGINAL SURVEY MAP AND IS NOT TO BE USED FOR ANY OTHER PURPOSE.

ADDITIONAL NOTES: THE SPHERE OF THE SURVEY IS TO BE KEPT OPEN AND UNOCCUPIED AT ALL TIMES TO ALLOW FOR THE CONDUCT OF FURTHER SURVEYS AND FOR THE INSTALLATION OF NEW EQUIPMENT.

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144183

NOTE: THE PRECEDING INFORMATION WAS TAKEN FROM BECHTEL ENVIRONMENTAL, INC. DAMES & MOORE, "REVISED LANDFARM CLOSURE PLAN", FOR CHEVRON PRODUCTS COMPANY, DECEMBER 30, 1996.



Michael P. Rei

MICHAEL P. REI
R.C.E. No. 13810
EXP. 3/31/01



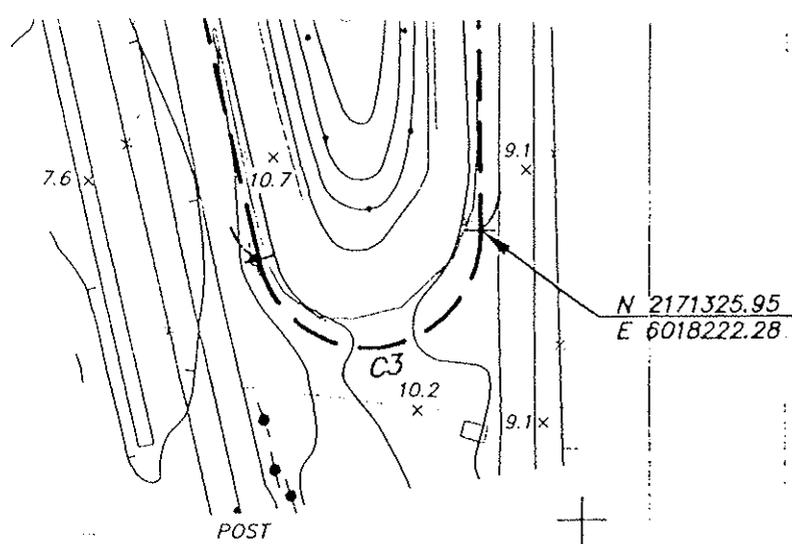
E 6019250

REVISIONS	KISTER, SAVIO & REI, INC. LAND SURVEYORS - CIVIL ENGINEERS		DESCRIPTION	
/15/99 AUTH. 99-008 AD. CLOSURE INFORMATION.	3095 RICHMOND PARKWAY, SUITE 214 RICHMOND, CALIFORNIA 94806 PHONE: (510) 222-4020 FAX: (510) 222-3718 E-MAIL: ksinc@pacbell.net		TOPOGRAPHIC SURVEY LANDFARM CLOSURE PLAN LANDFARM No. 2-5 CHEVRON RICHMOND RETINERY	
31/00 ADD NOTE #6				
	SCALE 1" = 50'	PHONE: 0-789-2	PROJECT AREA:	CALIFORNIA

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144183

N 2171250

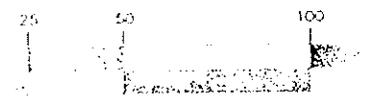


GENERAL NOTES

1. AERIAL SURVLY PROVIDED BY KELLOGG AERIAL SURVEYS 4200 N. FREEWAY BLVD. SUITE 3, SACRAMENTO, CA.
2. CONTOUR INTERVAL = 1'
3. COORDINATES AND ELEVATIONS ARE BASED ON THE CHEVRON RICHMOND REFINERY DATUM



GRAPHIC SCALE



(IN FEET)
1 inch = 50 ft

E 6018222

10.2

E 6018222

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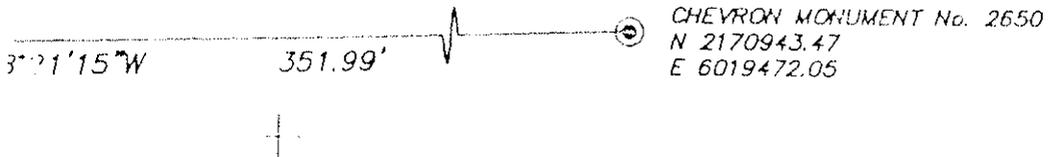
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CLOSURE NOTES

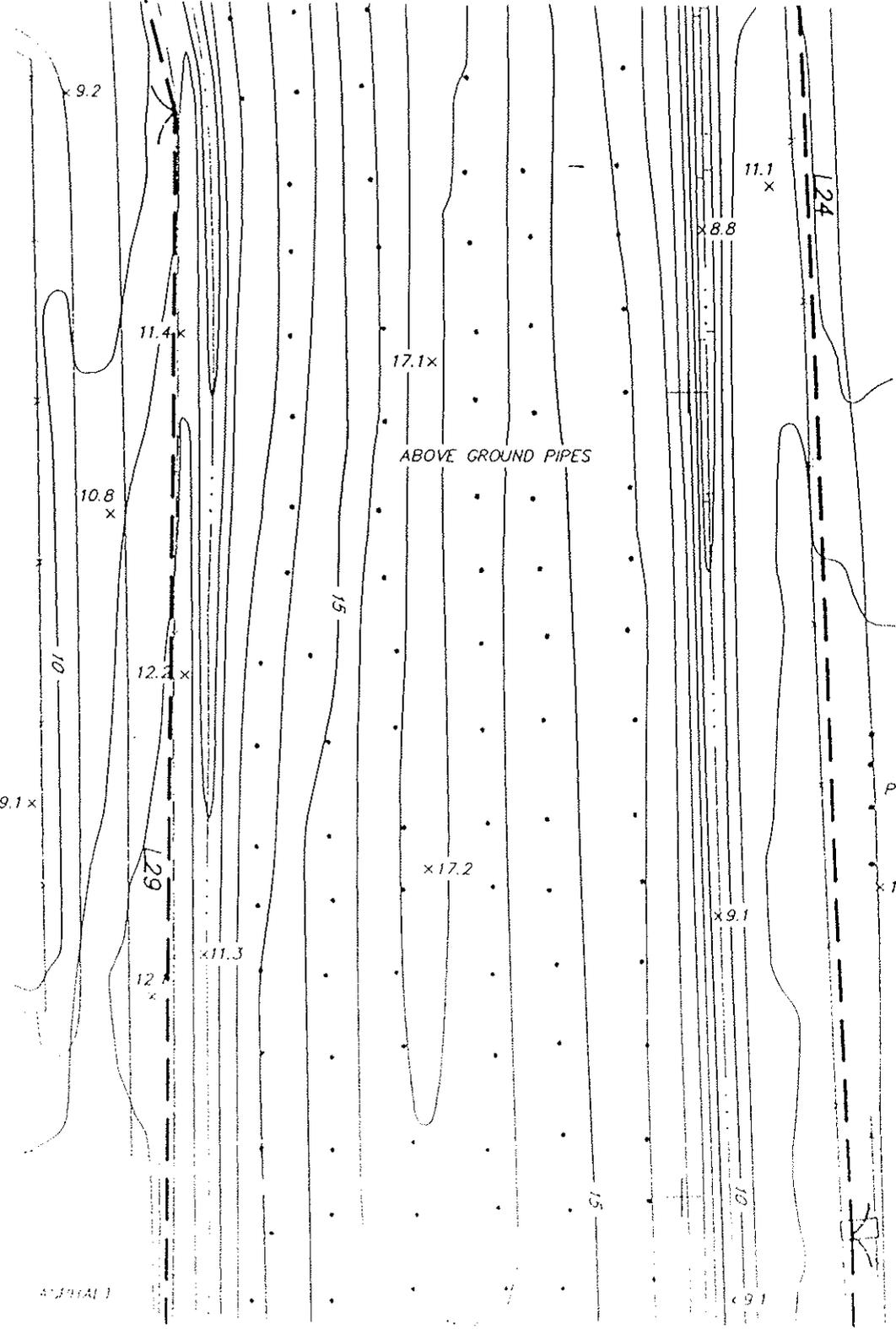
1. THE UPPER LAYER OF THE LANDFARM FILL IS 1.5 TO 7 FEET THICK AND CONSISTS OF DARK GRAY TO BROWN SILTY CLAY WITH BLACK MOTTILING, PLANT ROOTS AND SCATTERED GRAVEL AND SAND.
2. THE LOWER LAYER OF THE LANDFARM FILL RANGES FROM 3 TO 19 FEET THICK AND IS GENERALLY COMPOSED OF GRAVELLY TO SILTY CLAY WITH VARIABLE AMOUNTS OF GRAVEL, SAND, SILT AND MAN MADE MATERIALS SUCH AS BRICK, CONCRETE AND GLASS, AND POCKETS OF BLACK, SOFT SLUDGE (SILTY CLAY SATURATED WITH OILY AND TARRY SUBSTANCES).
3. YOUNG BAY MUD UNDERLIES THE LANDFARM FILL AND OVERLIES THE OLD BAY MUD, AND RANGES BETWEEN 5 AND 30 FEET IN THICKNESS BENEATH THE LANDFARMS. THE YOUNG BAY MUD GENERALLY CONSISTS OF DARK BROWN TO DARK GRAY, SOFT TO MEDIUM-STIFF SILTY CLAY THAT CONTAINS VARYING PERCENTAGES OF ORGANIC MATERIAL, PEAT AND OCCASIONAL SANDY UNITS. PEATY BAY MUD, WHICH HAS AN ABUNDANT AMOUNT OF PEAT, ARE PRESENT IN THE UPPER 1 TO 7 FEET OF THE YOUNG BAY MUD.
4. OLD BAY MUD RANGES FROM 1 TO 15 FEET THICK BENEATH THE LANDFARMS. THE OLD BAY MUD CONSISTS OF BLuish TO GREENISH GRAY, MEDIUM-STIFF TO STIFF SILTY CLAY THAT CONTAINS ORGANIC MATERIAL AND LOCAL TRACE AMOUNTS OF SAND.
5. THE PRESENT LOCATION OF THE NORTHWESTERN PART OF LANDFARM No.s 2 THROUGH 4 WERE UNDERLAIN BY SEVERAL PONDS THAT WERE BACKFILLED. POCKETS OF SLUDGE FOUND WITHIN THE LOWER FILL PROBABLY REPRESENT OIL FILL MATERIAL AND OILY WASTES WITHIN THE PONDS.
6. AS REQUIRED BY 22 CCR §66265.116, THE FOLLOWING NOTE IS PROVIDED: THE OWNER OR OPERATOR HAS AN OBLIGATION TO RESTRICT DISTURBANCE OF THE HAZARDOUS WASTE DISPOSAL UNIT SHOWN ON THIS SURVEY PLAT IN ACCORDANCE WITH THE APPLICABLE REGULATIONS OF ARTICLE 7 OF CHAPTER 10 OF TITLE 22 OF THE CALIFORNIA CODE OF REGULATIONS [22CCR § 66265.110 et seq.]

NOTE: THE PRECEDING INFORMATION WAS TAKEN FROM BECHTEL ENVIRONMENTAL, INC. DAMES & MOORE, "REVISED LANDFARM CLOSURE PLAN", FOR CHEVRON PRODUCTS COMPANY, DECEMBER 30, 1996.



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144183



985.72'

CHEVRON MONUMENT No. 2651
 N 2171933.36
 E 6015120.21

LANDFARM CELL LIMIT LINE AND CURVE TABLE

144183

LINE TABLE		
LINE	BEARING	LENGTH
L1	S87°33'12"E	29.98'
L2	N86°52'39"E	258.59'
L3	N62°31'18"E	33.83'
L4	N01°37'16"W	458.19'
L5	N44°16'13"W	33.92'
L6	S89°49'22"W	119.08'
L7	S60°42'40"W	91.22'
L8	S50°37'07"W	134.63'
L9	N03°11'07"W	380.89'
L10	N50°27'08"W	29.27'
L11	N08°44'04"W	26.01'
L12	N55°10'24"W	74.72'
L13	N77°49'17"W	44.18'
L14	S89°09'58"W	502.33'
L15	S05°04'26"E	269.42'
L16	S76°18'19"W	64.91'
L17	S12°41'13"E	209.01'
L18	S86°19'32"E	135.70'
L19	N31°35'26"E	99.91'
L20	N88°57'33"E	264.22'
L21	S58°52'18"E	23.51'
L22	N89°48'18"E	39.40'
L23	N88°03'28"W	316.77'
L24	N02°46'24"W	647.23'
L25	N01°19'47"W	180.26'
L26	N87°40'05"E	34.24'
L27	S21°02'27"E	28.87'
L28	N87°44'33"E	131.30'
L29	N00°08'00"W	469.06'
L30	S15°25'49"E	157.86'
L31	S72°14'06"E	105.98'
L32	S61°22'22"E	66.63'
L33	S59°25'53"W	15.57'
L34	S70°05'57"W	81.84'
L35	N11°33'58"E	21.19'
L36	N03°34'40"W	98.15'
L37	N24°59'44"E	40.84'
L37	S13°32'15"E	279.27'
L38	N01°05'57"W	155.65'

CURVE TABLE			
CURVE	LENGTH	RADIUS	DELTA
C1	113.54'	110.00'	59°08'25"
C2	38.16'	18.00'	121°28'01"
C3	87.74'	30.00'	167°33'42"
C4	82.78'	65.00'	72°58'08"
C5	65.01'	60.00'	62°05'02"
C6	77.11'	60.00'	73°38'19"
C7	77.66'	50.00'	88°59'32"
C8	21.31'	15.00'	81°22'45"
C9	90.11'	90.00'	57°22'07"

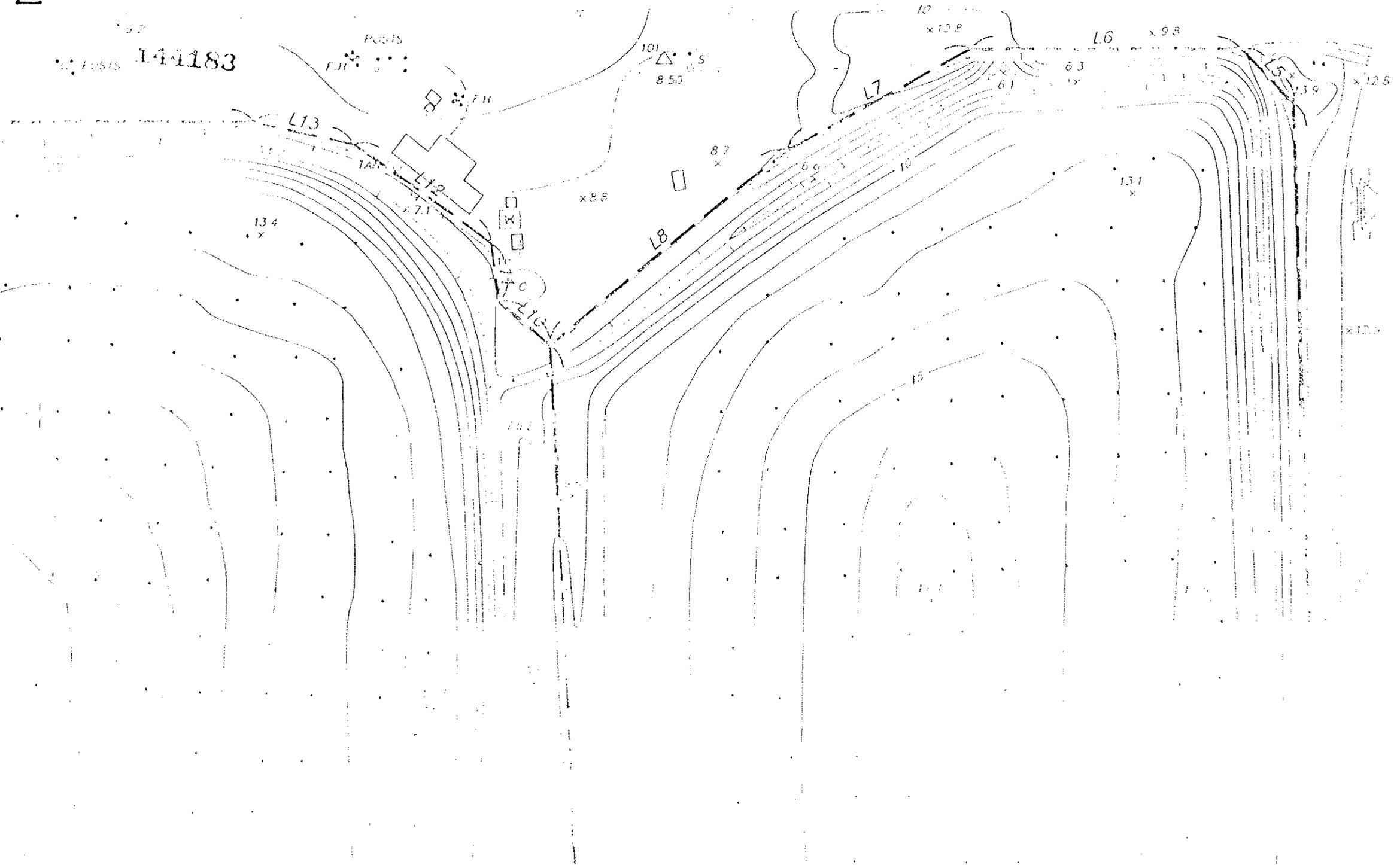
100' Δ 12.28

144183

3

140

144183



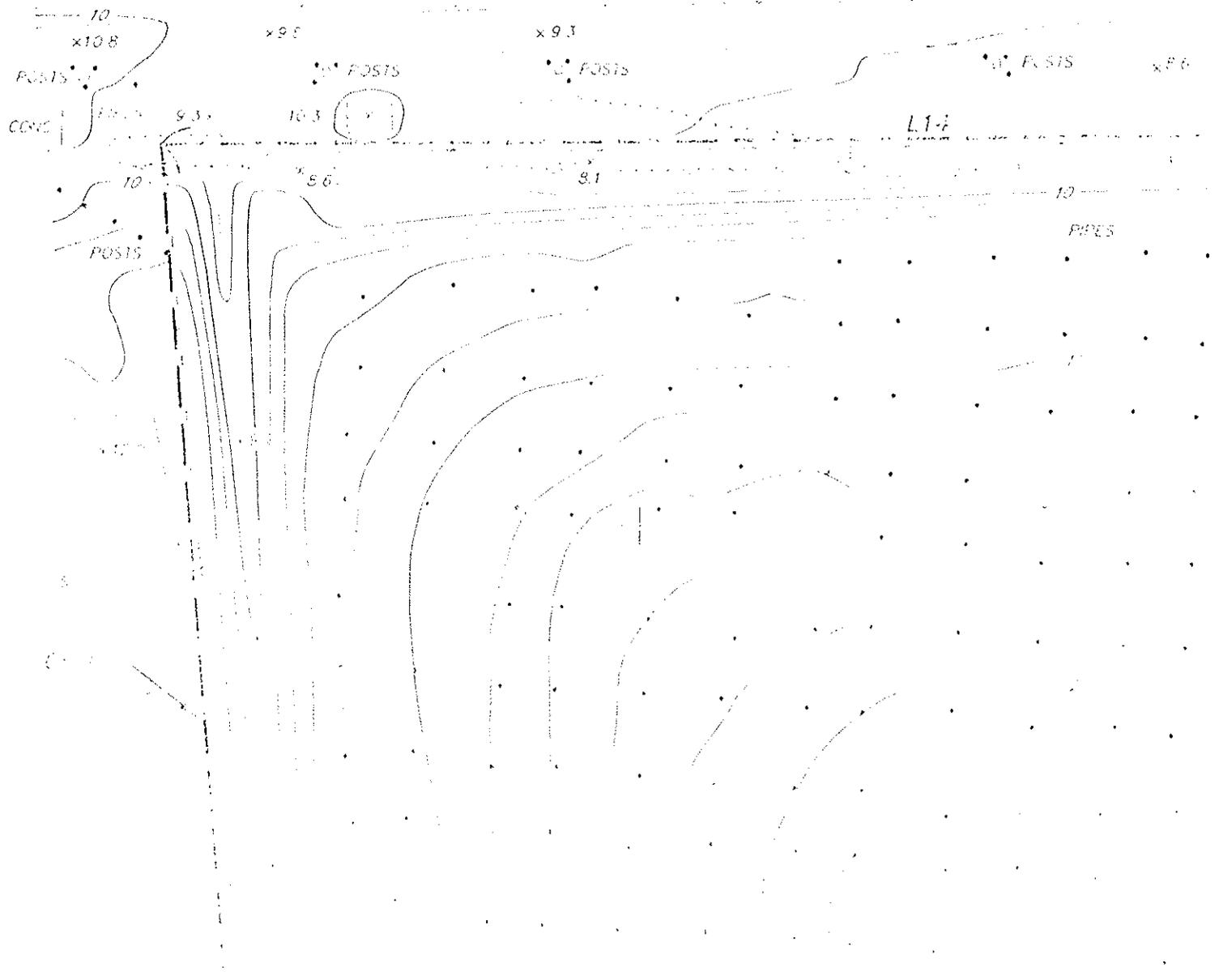
1

100° 13 5'

1641153



B 217



100° 13 5'

EXHIBIT C

POST CLOSURE NOTICE OF HAZARDOUS WASTE DISPOSAL UNIT

The Chevron Products Company currently is the owner and operator of the Landfarm Treatment Facility. The Treatment Facility area is located on the property which is described in Exhibit A to this Post-Closure Notice (the "Property").

The Landfarms has been closed pursuant to the *Landfarm Closure Plan* (1997) which was approved by the California Environmental Protection Agency, and Department of Toxic Substances Control (DTSC). A certificate of closure was submitted to the DTSC on or about September 30, 1999.

Pursuant to 22 CCR 66265.119(b), the Chevron Product Company provides the following notification:

1. The property has been used to manage hazardous wastes.
2. The use of the Property is restricted under Title 22 of the California Code of Regulations [22CCR 66265.110]
3. The survey plat and record of the type, location, and quantity of hazardous wastes disposed of within the Landfarms required by 22 CCR 66265.116 and 66265.119 (a) have been filed or contemporaneously with the recording of this Post-Closure Notice are being filed with the City of Richmond Planning Department on July 5, 2000.

144183

EXHIBIT D

RECORD OF HAZARDOUS WASTES DISPOSED OF IN CLOSED LANDFARM
TREATMENT FACILITY AT THE RICHMOND REFINERY

144183

The Chevron Products Company currently is the owner and operator of the closed Landfarm Treatment Facility. The Landfarms are located on the property which is described in Exhibit A to this Record.

The Landfarms has been closed pursuant to a the *Revised Landfarm Closure Plan* (1997) which was approved by the California Environmental Protection Agency, Department of Toxic Substances Control (DTSC). A certificate of closure was submitted to the DTSC on September 30, 1999.

Pursuant to 22 CCR 66265.119, Chevron is providing this record of the type, location and quantity of hazardous wastes disposed of in the Landfarm Treatment Facility.

Prior to closure, the Landfarms consisted of 5 Landfarm units. The Landfarms were in operation for the biological treatment of oily wastes generated from on-site petroleum processing from the mid 1970's to 1987. The principle wastes applied were oil/water separator sludge (at Nos. 1, 2, 4 and 5 Landfarms), non-leaded tank bottoms (at Nos. 1,2,3 and 4 Landfarms), oil/water mixtures, algae water, pond sediments and oily dirt. When in operation, wastes were applied to the surface of the Landfarms and tilled into the top six to 12 inches. Prior to 1980, no data are available on waste application rates to the Landfarms. Since 1980, a total of 188,000 tons of waste were applied to the Landfarms. The location and dimensions of the units as closed is indicated in the survey plat which is Exhibit B to this Record. The wastes which remain in the Landfarm after closure are described in "*Revised Landfarm Closure Plan*". The relevant sections of this plan are Sections 2.2.3 and 2.2.4.

This Record is based on available records and investigations which have been conducted into the environmental conditions at the Landfarms. The information contained in the Record is, to the best of Chevron's current knowledge and belief, true, accurate and complete.

CALIFORNIA ALL-PURPOSE ACKNOWLEDGMENT

State of California

County of Contra Costa } ss.

On July 6, 2000 before me, Carrie Ho, Notary Public
Date Name and Title of Officer (e.g., "Jane Doe, Notary Public")

personally appeared J. J. Ely
Name(s) of Signer(s)

- personally known to me
- proved to me on the basis of satisfactory evidence

to be the person(s) whose name(s) is/are subscribed to the within instrument and acknowledged to me that he/she/they executed the same in his/her/their authorized capacity(ies), and that by his/her/their signature(s) on the instrument the person(s), or the entity upon behalf of which the person(s) acted, executed the instrument.



Place Notary Seal Above

WITNESS my hand and official seal.
Carrie Ho
Signature of Notary Public

OPTIONAL

Though the information below is not required by law, it may prove valuable to persons relying on the document and could prevent fraudulent removal and reattachment of this form to another document.

Description of Attached Document
Title or Type of Document: Declaration

Document Date: July 6, 2000 Number of Pages: _____

Signer(s) Other Than Named Above: _____

Capacity(ies) Claimed by Signer

- Signer's Name: _____
- Individual
 - Corporate Officer — Title(s): _____
 - Partner — Limited General
 - Attorney in Fact
 - Trustee
 - Guardian or Conservator
 - Other: _____



Signer Is Representing: _____

144183

END OF DOCUMENT

Appendix I
Compliance with Other Federal Laws Determination



January 3, 2002

Chevron Products Company
P. O. Box 1272
Richmond, CA 94802-0272

J.W.Hartwig
Manager
Environment, Health and Safety
Phone 510 242 1400

Ms. Cherry Padilla
Department of Toxic Substances Control
700 Heinz Avenue, Suite 200
Berkeley, Ca 94710-

**Richmond Refinery Landfarm
Certification per 22 CCR §66270.3**

Dear Ms. Padilla:

Closure of the Chevron Richmond Refinery Landfarm is being pursued by the Chevron Environmental Management Company (EMC) on behalf of Chevron Products Company. Pursuant to Chevron's application for a post-closure permit relative to said closure, we have taken note of the requirements of 22 CCR §66270.3, which requires certain certifications of compliance with federal law. After due inquiry, and based on our best knowledge and good-faith beliefs, the undersigned certifies the following:

1. **Wild and Scenic Rivers Act, 16 USC §§1273 *et seq.*** Chevron knows of no wild, scenic or recreational river within the vicinity of the proposed project; therefore, we believe the statute is inapplicable in our circumstances.
2. **National Historic Preservation Act of 1966, 16 USC §§470 *et seq.*** Chevron knows of no properties listed or eligible for listing within the vicinity of the proposed project. We believe that the old Richmond Refinery administration building is so listed; however, it is considerably distant from the proposed project and will be unaffected by it. Consequently, we believe the statute has no applicability in this situation.
3. **Endangered Species Act, 16 USC §§1531 *et seq.*** Chevron does not know or have reason to know of endangered or threatened species for which the proposed project acts as habitat. To the contrary, we believe closure of the Landfarm will diminish or eliminate any threat to transient life forms in the area. Again, we believe this statute to be inapplicable.
4. **Coastal Zone Management Act, 16 USC §§1451 *et seq.*** According to our reading of the definition of the "coastal zone" contained in Cal. Pub. Res. §30103(a), the proposed project does not lie in the coastal zone. Again, we believe the statute is inapplicable.

Ms. Cherry Padilla

January 3, 2002

Page 2

5. **Fish and Wildlife Coordination Act, 16 USC §§661 *et seq.*** The proposed project does not contemplate the impoundment, diversion, or other modification of any body of water. Consequently, this last statute is inapplicable as well in our opinion.

We trust that this letter will meet the Department's needs in this matter. Please feel free to contact John MacLeod @ (510) 242-2295 should you have any additional questions.

Sincerely

A handwritten signature in cursive script that reads "J.W. Hartwig". The signature is written in dark ink and is positioned below the word "Sincerely".

J.W. Hartwig
Environment, Health and Safety Manager
Chevron Products Co.

Appendix J
Revised Landfarms Closure Construction Completion Certification Report
(March 27, 2000)



DAMES & MOORE

A DAMES & MOORE GROUP COMPANY

**REVISED
LANDFARMS CLOSURE
CONSTRUCTION COMPLETION
CERTIFICATION REPORT
WASTE DISCHARGE ORDER
CHEVRON RICHMOND
REFINERY
RICHMOND, CALIFORNIA
Prepared For:
CHEVRON PRODUCTS COMPANY**

D&M Job No. 42100-001-043

Revised: March 27, 2000

Original: September 30, 1999

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Plate 1-1 Vicinity Map of Landfarm Nos. 1 through 5

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Table 1 List of Vegetation Planted on the Landfarms

LIST OF APPENDICES

- Appendix A Bechtel Environmental and Dames & Moore (BEDM), Revised Closure Plan, December 30, 1996 and Revised on May 28, 1997
- Appendix B As-Built Drawings of the Landfarm Closure Construction
- Appendix C As-Built Drawings of the Landfarm Drip Irrigation System
- Appendix D As-Built Drawings of the Landfarms Storm Water Piping Plan, No. 1 Landfarm
- Appendix E As-Built Drawings of the Landfarm Groundwater Protection System – Shallow Extraction Trenches and Extraction Sumps

1.0 INTRODUCTION

Dames & Moore is pleased to present the Landfarm Closure Certification Report for Landfarm Nos. 1 through 5 at the Chevron Richmond Refinery located in Richmond, California (Plate 1-1). The original Landfarm Closure Plan was submitted to the U.S. Environmental Protection Agency (US EPA) and Department of Toxic Substances Control (DTSC). This certification report was prepared to comply with the Revised Landfarm Closure Plan, herein referred to as "Revised Closure Plan," dated December 30, 1996 (Bechtel Environmental Inc., and Dames & Moore [BEDM]), and revised on May 28, 1997. The DTSC approved the Revised Closure Plan in their letter dated March 19, 1998. The primary focus of this report is to document the closure construction activities that were performed at Landfarms Nos. 1 through 5. The Landfarms are located within Chevron's Richmond Refinery. The Richmond Refinery is situated on 2,800 acres roughly bounded by Castro Street to the East, Interstate Highway 580 to the South, and San Pablo Bay to the West and North.

Implementation of the Closure activities was conducted under the technical supervision of Mr. Patrick Kaspari, P.E., and Mr. Thomas Sweet, P.E. This report was prepared under the supervision of Mr. Thomas M. Sweet, California Registered Civil Engineer No. C-58265, and documents the Landfarms closure activities.

2.0 SITE HISTORY

Landfarm Nos. 1 through 5 cover approximately 29 acres. A vicinity map showing the location of the five Landfarms are shown on Plate 1-1. All of the Landfarms areas are internal to the Refinery and are not adjacent to the perimeter of the Refinery. The approximate size of each Landfarm is as follows:

No. 1 Landfarm	13.5 acres
No. 2 Landfarm	8 acres
No. 3 Landfarm	3.5 acres
No. 4 Landfarm	3 acres
No. 5 Landfarm	1 acre

Access to the Landfarms facility is controlled by the Environmental Operations Section of the Refinery's Utilities/Environmental Area Business Unit (U/E ABU). Security measures utilized by the Environmental Section are designed to prevent unknowing or unauthorized access to the Landfarms facility. Areas of the Refinery that do not border the Bay are secured by a 6-foot-high chain-link fence, which is generally topped by three rows of barbed wire. The only

unfenced land ward access to the Refinery is across Herman Slough from the property of Chevron Chemical Company, to which the public does not have access.

Prior to the landfarm operation, which began in the 1970s, the Landfarm area was comprised of imported fill placed at the site. The thickness of the fill ranged from 10 to 25 feet and was referred to as the, "landfill under the Landfarms." The perimeter of each fill area was encircled by an above-grade berm designed to minimize access and surface water run-on/runoff. The interior surface of each bermed area was graded to control runoff. Refer to the Revised Closure Plan, Plates 2-1, 2-2, and 2-3, for the typical cross section of the aboveground berm, former topography for Landfarm No. 1 and former topography for Landfarms Nos. 2 through 5, respectively.

Between the 1970s and 1987, Chevron conducted landfarming operations at five locations within the bermed areas. The landfarming was designed to promote biodegradation of oily soils that had been generated within the Refinery from various operations. Between 1980 and 1984, Chevron submitted a hazardous waste permit application for Landfarms Nos. 1 through 5, as requested by the U.S. EPA. On February 10, 1987, Chevron was notified that the Landfarms did not qualify for a hazardous waste permit because some of the permit conditions could not be met. In response to this condition, Chevron entered into a Consent Agreement with the U.S. EPA and DTSC to close the landfarm units. On March 31, 1988, Chevron submitted the original *Landfarms Closure Plan*, and initiated closure of the Landfarms under that plan in the first quarter of 1988. The Closure Plan included initial biodegradation activities prior to final closure.

In accordance with the Closure plan, between July 1990 and 1993, BEDM submitted a series of bioremediation reports presenting modifications, evaluations and sampling results of biodegradation activities at the Landfarms. On August 10, 1993, BEDM submitted the *Landfarms Sampling and Analysis Report*, based on the results included in the report, it was concluded that the Landfarms had been operating as active biodegradation units and had been effective in reducing petroleum wastes applied to the ground surface before 1987. However, the degree to which constituents had been degraded suggested that future biodegradation activities may provide limited benefit. Therefore, BEDM recommended that landfarming activities be discontinued and the Landfarms be permanently closed.

Starting in March 1995, Chevron began a series of discussion with the DTSC, RWQCB, and U.S. EPA to identify agency concerns and special site conditions at Landfarm Nos. 1 through 5. On February 27, 1996, Chevron presented their revised conceptual plan to close the Landfarms to the DTSC in a meeting with Ms. Wei Wei Chui and Mr. Tony Morales of the DTSC, Elizabeth Christian of the RWQCB, San Francisco Bay Region, and Mr. Ron Leach of the U.S. EPA. After reaching tentative consensus with the DTSC on the Revised Closure Plan elements,

Chevron requested BEDM prepare a Revised Closure Plan. DTSC approved the Revised Closure Plan in their letter dated March 19, 1998.

In addition to the activities outlined in the Revised Closure Plan, Chevron and BEDM developed and constructed a Groundwater Protection System (GPS) for the Refinery, including the Landfarms, in response to the Regional Water Quality Control Board (RWQCB), San Francisco Bay Region's *Order No. 89-175, Revised Waste Discharge Requirements for: Chevron U.S.A., Inc., Richmond Refinery, Richmond, Contra Costa County*. The objective of the GPS for the entire Refinery, including the Landfarms, is to establish and maintain a physical or hydraulic barrier to prevent the off-site movement of potentially contaminated near-surface groundwater. Constructions of GPS elements in the Landfarm vicinity began in 1990 and were completed in 1999 (see Section 3.2.6).

3.0 LANDFARMS CLOSURE ACTIVITIES

3.1 GENERAL

The landfarm closure was designed to allow the continued passive biodegradation of oily soil, to reduce surface water infiltration through the surface of landfarm fills and soils, and to prevent lateral off-site migration of "A" zone groundwater from landfarm sites. The performance goals for the closure construction are outlined in Title 22 California Code of Regulation (22 CCR), Section 66265.111, which require that facilities be closed in a manner that:

- Minimizes the need for further maintenance; and
- Controls, minimizes, or eliminates to the extent necessary, post-closure escape of hazardous waste, hazardous constituents, leachate, contaminated rainfall or runoff, or waste decomposition products to the ground or surface waters or to the atmosphere to protect human health and the environment.

The landfarm closure was constructed to conform to the specifications presented in the Revised Closure Plan (Appendix A), which was developed to address the performance goals.

In compliance with applicable regulations and in conformance with the Revised Closure Plan, Chevron elected to close the Landfarms with a final vegetative cover. As shown on the enclosed as-built drawings D-767512 and D-767515 (Appendix B), the Landfarms were regraded into low mounds to facilitate surface drainage and improve the visual appearance of the sites. The landfarm soils were lightly compacted with light tracked equipment to support the construction of the final cover while allowing penetration of vegetation roots. The final grades were designed

to divert rainfall runoff away from the Landfarms sites. A layer of clean soil covers the entire landfarm area and serves to prevent surface run-off from direct contact with waste material and prevent wind erosion of the wastes. Lined perimeter drainage ditches were constructed to collect surface run-off from the Landfarms. The drainage ditch for Landfarm No. 1 is routed to the Refinery's clean water impoundment system and the drainage ditches for Landfarm Nos. 2 through 5 are routed to the Richmond Refinery Water Enhancement Wetland. All storm water is discharged from the Refinery in accordance with Chevron's NPDES permit.

The improved surface grading and the surface vegetation are intended to reduce water infiltration into the Landfarms soil and promote water uptake by evapotranspiration. Spray and drip irrigation systems were designed and installed to provide water to ground cover grasses, shrubs, plants, and trees that were planted on the Landfarms cover surface. Steep grades were covered with erosion control mats with vegetation to minimize erosion potential. Additional GPS shallow extraction trench and extraction sumps were constructed in the vicinity of the Landfarms to control local groundwater in the vicinity of the landfarm drainage ditches. Also, in the event that light non-aqueous phase liquid (LNAPL) hydrocarbon is present, it would be captured by the additional GPS features installed. The final cover, irrigation systems, and drainage control features are described in the following sections.

Independent contractors hired by Chevron performed the construction of various elements of the cover. Designated Chevron and Dames & Moore field representatives monitored the contractor's work and to assist the contractor in meeting the specifications and conditions of the Revised Closure Plan. Dames & Moore coordinated the quality assurance and quality control programs outlined in the specifications.

Following the completed construction, construction drawings were revised to reflect the "as-built" conditions. These as-built drawings are included in Appendix B. The following sections discuss the physical details of the cover and the significant variations in the as-built configuration from those previously submitted with the Revised Closure Plan.

3.2 FINAL COVER CONFIGURATION

3.2.1 Perimeter Berms and Interior Soils

Historic perimeter berms at the Landfarms were regraded to serve as a foundation for drainage ditches and provide lateral support for the Landfarm soils. The soils were evaluated to locate areas that were soft or loose. Placed soil was tested in accordance with ASTM D-1557 to confirm that the materials were compacted to at least 90 percent of maximum dry density to a depth of 2 feet.

The fill within the closure unit was graded and lightly compacted to support the construction of the final landfarm cover. Fill spreading and compaction were performed in 8-inch lifts by tracked vehicles. Use of heavy equipment and trucks was minimized where practical.

3.2.2 Vegetated Layer

The final vegetative cover consists of a minimum of six-inches of topsoil and six-inches of imported clean fill. The 6-inch-thick lower clean fill layer provides a firm base suitable for supporting shrubs and trees, and the 6-inch upper layer of relatively loose topsoil with higher nutrient content will facilitate plant growth. The vegetation specified for the final cover include native grasses, plants, and trees, which should result in a low-maintenance cover. Before establishing vegetation, erosion control mats were placed on steeply sloped areas that were identified being more susceptible to erosion.

3.2.3 Vegetation

The purpose of establishing vegetation on Landfarm Nos. 1 through 5 is to reduce water infiltration and erosion, to increase microbial populations in the vadose zone, and to enhanced biodegradation of remaining petroleum hydrocarbons.

A variety of plant and tree species was used in order to draw from the largest population of species adapted to the soils and climatic conditions of the Landfarms. Vegetation types planted on the Landfarms is listed in Table 1. The planting of grasses, shrubs, and trees was completed in summer 1999 rather than vegetating the landfarms with grasses in the first year and planting shrubs and trees in subsequent years, as specified in the Revised Closure Plan. Faster growing grasses and herbaceous plants (shrubs) were planted on the landfarms to minimize wind and water erosion. Trees were planted in a closely spaced grid pattern with the shrubs placed between the rows of trees. The three different vegetation types, grasses, shrubs and trees, were selected and planted with the intent that the associated rooting depths will inhabit the entire vadose zone. The grasses were selected for their shallow (0-6 inches) depth penetration; the shrubs were selected for their medium (6-12 inches) depth penetration; and the trees were selected for their deep (greater 12 inches) depth penetration. Once colonized by a relatively high density of roots, the soil microbial populations will increase and hydrocarbon degradation in the subsoils will be greatly enhanced.

3.2.4 Vegetation Maintenance and Monitoring

In order to maintain the shrubs and trees that were planted in the summer of 1999, spray and drip irrigation systems were designed and installed to provide water to the grasses, shrubs and trees.

As-built drawings of the landfarm spray irrigation systems are included in Appendix B. As-built drawings with the approximate locations of the trees and shrubs, and the drip irrigation system are included in Appendix C. Although the drip irrigation system was not included in the Revised Closure Plan, construction of the drip irrigation system was necessary to support the planting of the trees and shrubs in the early years of plant root growth. The irrigation systems are temporary and will not be necessary once the plants are established.

Consistent with the approval letter from DTSC for the Revised Closure Plan (March 19, 1998), tensiometers have been installed in each landfarm to monitor soil moisture. Two tensiometers were installed in Landfarm No. 1 and one tensiometer was installed in each of the remaining landfarms. Twelve-inch long tensiometers were installed at low-points of the vegetative caps as shown on drawings D-767512 and D-767515 in Appendix B. Soil moisture information will be obtained from the tensiometers and will be used to adjust the irrigation schedule, as necessary, to minimize the amount of water that may infiltrate through the landfarm soils during the summer months. Vegetative maintenance and monitoring, including collection of data from the tensiometers is detailed in the Revised Landfarm Post Closure Plan.

3.2.5 Surface Drainage Control Facilities

The final surface of the vegetative portions of the landfarms were graded with 4 percent minimum slopes to accommodate long-term settlement and are intended to retain 3 percent minimum slopes after settlement. The surface drainage control facilities are shown in plan view on as-built drawing D-767512 for Landfarm No. 1 and drawing D-767515 for Landfarms No. 2 through 5 (Appendix B). The areas surrounding the closure units were graded to drain away from the closure units such that runoff from the surrounding areas will flow away from the landfarm surfaces.

Surface runoff from the exterior slopes of the closure units is collected in perimeter drainage ditches. More steeply sloped ditch areas were lined and covered with erosion control mats and vegetation to minimize potential erosion. Ditch areas above the GPS shallow extraction trenches (as described in Section 3.2.6) were lined with a 40-mil High-Density Polyethylene (HDPE) liner to elevation +10 (Richmond Refinery Datum). To control erosion, HDPE liners and erosion control mats were used to replace concrete swales referenced in the Revised Closure Plan.

Runoff from the Landfarms is collected within the ditches and routed to the Refinery's clean stormwater impoundment. Runoff collected in the drainage ditches at Landfarm No. 1 flows via gravity through the slide gate and headwall located at the northeast corner of the landfarm and is discharged to the first pass of the No. 1 Oxidation Pond. Runoff from Landfarm Nos. 2 through 5 drains to the slide gate and headwall north of Landfarm No. 3 and is discharged to Pass No. 3

of the Richmond Refinery Water Enhancement Wetlands (Richmond Wetlands). As-built drawings of the storm water piping plan from Landfarms No. 1 are included in Appendix C and the storm water discharge piping plan from Landfarm No. 3 is shown on drawing D-318549 of the Landfarms Closure Construction Plans included in Appendix B. Runoff generated from the Landfarms, as described above, will be managed under the NPDES (National Pollution Discharge Elimination System) permit of the Refinery.

3.2.6 Groundwater Protection System

As part of the overall Refinery-wide GPS, groundwater extraction trenches were installed along the downgradient perimeter of the Landfarms between 1990 and 1993. As described in the Revised Closure Plan, the major GPS groundwater control features provide hydraulic control and in some areas physical control on the downgradient and cross gradient sides of the Landfarms. The GPS units around the Landfarms began operation between 1991 and 1994.

During the landfarm closure construction, additional GPS features were added to Landfarm Nos. 1 through 4 to recover "A" zone hydrocarbon from the sites. Specifically, shallow extraction trenches were installed at: 1) the northeast and northwest side of Landfarm No. 1; 2) the south side of Landfarm No. 2; 3) the south, southeast, and north side of Landfarm No. 3; and 4) the north side of Landfarm No. 4. The shallow extraction trenches consist of 8-inch diameter Polyvinyl Chloride (PVC) pipes that were installed below parts of the landfarm perimeter ditches. The extraction trenches drain into 12-inch diameter PVC pipes that were installed at low points of the respective landfarm ditches. The 12-inch PVC pipes serve as groundwater collection sumps. Pneumatically controlled extraction pumps with a 20 gallon-per-minute capacity were installed in the sumps to recover "A" zone groundwater from under the landfarms and discharge to existing GPS collection pipes located to the north of Nos. 2 and 3 Landfarms and to the northeast border of No. 1 Landfarm. As-built drawings of the additional GPS features are included in Appendix E.

3.3 GROUNDWATER QUALITY MONITORING PROGRAM

The Post-Closure Landfarms Groundwater Monitoring Program is outlined in the Revised Landfarms Post Closure Monitoring Plan dated March 11, 1998 (Dames & Moore), which was submitted separately from the Revised Closure Plan.

Per the Revised Closure Plan, wells 654C, 655C, 656C, 657A, 658A, and 659A were installed and their boring logs and completion diagrams can be found in the Revised Landfarms Post Closure Monitoring Plan.

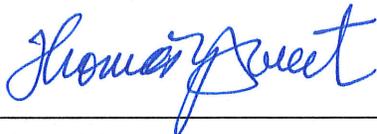
To accommodate construction activities, wells 103A, 192A, 194A, 246A, 503A, 504A, 507A, 579A, and 580A were abandoned per Contra Costa County Health guidelines before construction of the landfarm caps commenced.

3.4 POST-CLOSURE NOTICE SUBMITTALS

In accordance with 22 CCR Sections 66265.116 and 66265.119, Chevron will prepare a survey plat indicating the locations and dimensions of the Landfarms cells. The survey plat will contain notes describing the nature and quantities, to the best of Chevron's knowledge, of the waste within the closure cells. This plat will be filed with the City of Richmond's Planning Department and the DTSC. A copy of the plat will also be attached to the deed for the property.

4.0 SUMMARY

It is Dames & Moore's opinion that Landfarm Nos. 1 through 5 were completed in substantial conformance with the approved Revised Closure Plan, construction drawings, and specifications. Implementation of the activities completed to date and preparation of this document was prepared under the supervision of Mr. Thomas M. Sweet, California Registered Civil Engineer No. C-58265.



Thomas M. Sweet
California Registered Engineer No. C-58265



5.0 REFERENCES

BEDM, *Proposed Interim Vegetation Plan for Nos. 1 through 5 Landfarms*, for Chevron Products Company, May 23, 1996.

BEDM, *Revised Landfarms Closure Plan*, for Chevron Products Company, December 30, 1996.

Dames & Moore, *Revised Landfarms Post Closure Monitoring Plan*, for Chevron Products Company, March 11, 1998

Department of Toxic Substances Control, 1998. Correspondence with Chevron USA Products Company, "Approval of the Closure and Post-Closure Plan for the Landfarms Units 1 through 5 at Chevron Richmond Refinery, EPA ID No. CAD 009 114 919" dated March 19, 1998.

**Table 1. List of Selected Plant Species
Landfarms Closure Construction
Chevron Richmond Refinery**

SHALLOW ROOTED SPECIES (GRASSES)

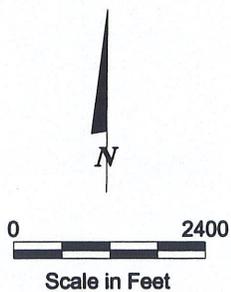
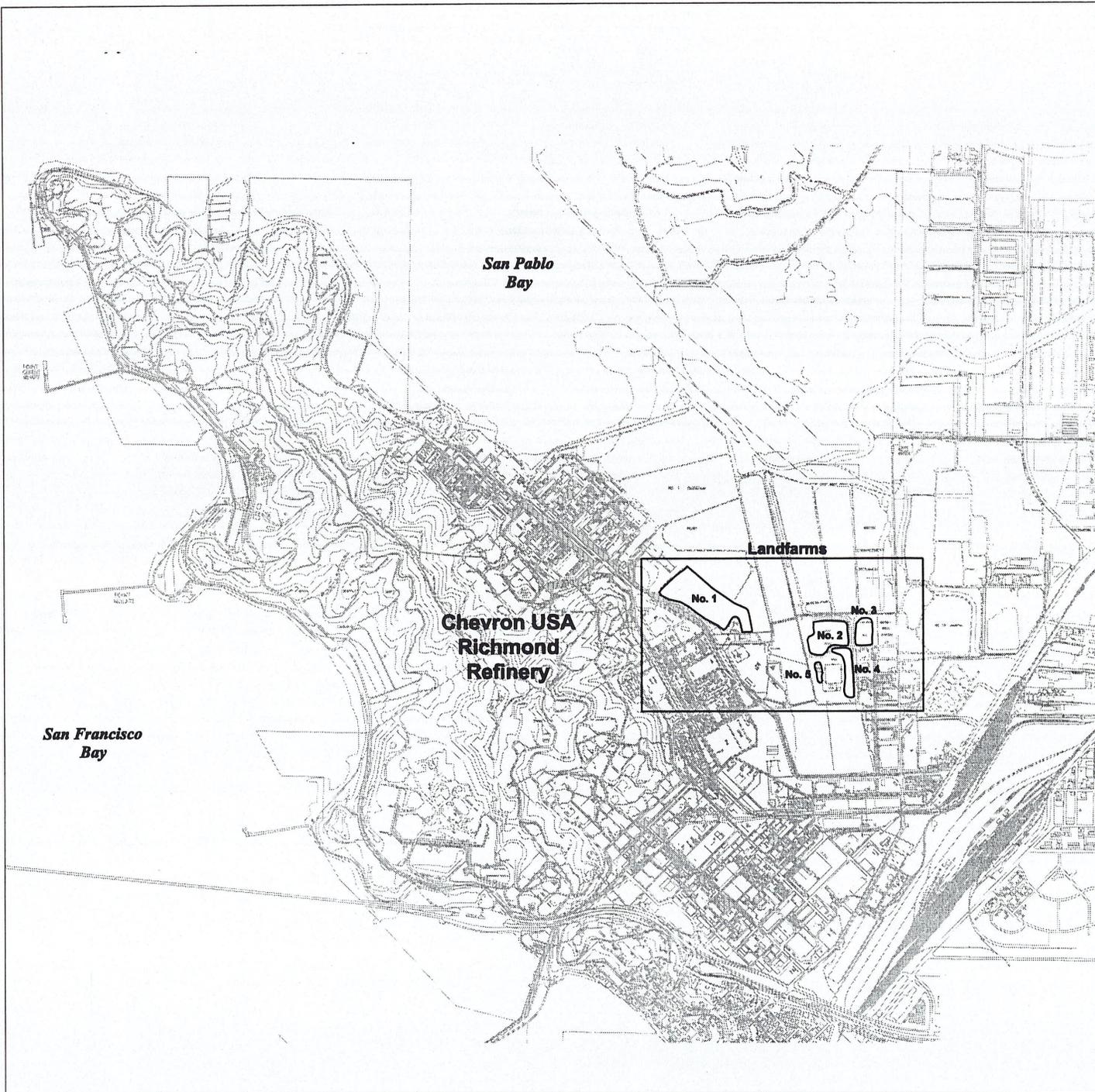
- Tall fescue (*Festuca arundinacea*)
- Bermuda grass (*Cynodon dactylon*)
- Creeping wild rye (*Elymus triticoides*)
- California brome (*Bromus carinatus*)
- Alfalfa (*Medicago sativa*)
- White clover (*Trifolium repens*)
- Saltgrass (*Distichlis spicata*)
- Annual fescue (*Vulpia myuros*)

INTERMEDIATE ROOTED SPECIES

- Coyotebrush (*Baccharis pilularis*)
- Toyon (*Heteromeles Arbutifolia*)
- Wax Myrtle (*Myrica Californica*)
- Wild Rose (*Rosa Californica*)

DEEP ROOTED SPECIES

- Cottonwood (*Populus fremontii*)



**VICINITY MAP - CHEVRON USA
RICHMOND REFINERY LANDFARMS**

September 1999
42100-001-043

CHEVRON USA
Landfarms Closure Plan
Richmond, California