BIOREMEDIATION OF POLLUTED SUBSOIL FOR PROTECTION OF GROUNDWATER SUPPLIES

M. C. Templeton and Lynn Prewitt
Mississippi Forest Products Laboratory
Mississippi State, MS 39762

Introduction

Movement of agricultural and industrial chemicals through soils, streams, and lakes and their resulting effects on the quality of groundwater is a matter of increasing concern throughout Mississippi and the entire south Atlantic-Gulf region. The use of chemicals is a necessary evil; however, many of these chemicals are used without full knowledge of their fate in the environment.

One widely used class of agricultural and commercial chemicals are the polycyclic aromatic hydrocarbons (PAHs) and chlorinated phenols. PAHs and chlorinated phenols are abundant in many industries, including the petroleum, wood preservation, waste incineration, and road construction industries. According to the Environmental Protection Agency, hydrocarbons such as the polycyclic aromatic hydrocarbons have polluted more of the U.S. groundwater drinking supply by volume than has any other class of chemicals. Cleanup efforts have failed to keep pace with this toxic accumulation, in part because many physical and chemical properties of groundwater, subsoil, and the aquifers--major influences on the success of cleanup strategies--remain poorly understood. The objective of this study is to better understand the movement of these types of chemicals in the subsoil, to determine what factors are important for bioremediation in the subsoil, and to determine if biological barriers can be formed in subsoil to stop movement of these chemicals into groundwater.

The proposed study is expected to yield basic information about the feasibility of adding microorganisms to subsoil by injection, the influence of native microorganisms on the added microorganisms, and the effect of pressurized air being added to the subsoil.

If the bioremediation of subsoils is successful, it would provide a cost effective method for cleaning up large areas of contaminated soils at wood treating sites and refineries, as well as soils contaminated with gasoline and oils and from leaking underground tanks.

The objective of this study is to determine techniques to increase the microorganism populations in the subsoil in order to maximize decomposition of organic pollutants in the subsoil. Polynuclear aromatic hydrocarbons and chlorinated phenols will be studied since these compounds are very common environmental pollutants and offer a wide range of chemical properties. Therefore, the results of the proposed study will be applicable to a variety of cleanup scenarios.

Groundwater is one of our most important resources. It accounts for over 95% of the world's supply of freshwater. In the United States approximately 50% of our drinking water comes from groundwater. In Mississippi over 90% comes from this source (1). Groundwater is replenished by surface water that infiltrates and percolates through the soil down to the groundwater aquifer. This can be a natural cleaning process that purifies the surface water and makes it suitable for human consumption providing the soil and subsoil are relatively clean; however, the opposite effect occurs when the soil and subsoils are contaminated.

Every day hundreds of hazardous chemicals move through the soil into groundwater. These materials come from both contaminated runoff from surface contamination and from soil contamination. Sources include waste products from industrial processes, spills, agricultural processes (e.g. fertilizer and pesticide additions to soil), mining operations, and surface runoff from city streets.

The major contaminants in the earth's subsurface reservoirs are hydrocarbons, such as crude oil, gasoline, and creosote. These materials which have come from leaking storage tanks, industrial processes, and spilled from vehicles have polluted more of the United States groundwater drinking supply by volume than has any other class of chemicals (2). Cleanup efforts have failed to keep pace with this toxic accumulation, in part, because of our lack of

understanding of the physical, chemical, and biological processes that occur during movement of water through the soil and subsoil into the groundwater and the naive belief by many that groundwater contamination can be cleaned up simply by pumping and treating groundwater. In most cases the soil and subsoil are contaminated with hydrocarbons which have a limited solubility in water. As water slowly percolates through the soil, it removes small amounts of the hydrocarbons into the water phase and ultimately into the groundwater. If the soil itself is not cleaned, the groundwater below it will continue to be contaminated for many years by pollutants leaching from the soil.

In recent years, a cleanup technology for contaminated soils has been developed which uses the microscopic life in the soil to decompose organic pollutants (3). By adding food, nutrients, and other materials to the soil, large populations of microorganisms can be built up that will rapidly decompose organic pollutants. This technology, called bioremediation, has proven to be very successful for cleanup of contaminated topsoil.

Similarly, biological techniques have been successfully used to clean up contaminated groundwater in situ (1,2,4,5). Nutrients, microorganisms, and oxygen can be injected into the aquifer to build up the population of microorganisms necessary to break down pollutants in reasonably short time spans. Both land farming and in situ groundwater bioremediation have been shown to be practical, economical methods for cleanup of topsoil and aquifers, respectively. However, this still leaves the contaminated subsoil. Subsoil pollutants with low water solubility or high affinity for soil will continue to leach into the aquifer, necessitating continuation of groundwater cleanup efforts for many years. If the bioremediation technologies already proven successful for topsoil and aquifer cleanup could be appropriately modified for use in cleaning up contaminated subsoil, groundwater pollution could be greatly reduced and substantial amounts of time and resources could be saved.

Technology transfer: Currently our laboratory is working on clean-up at seven hazardous waste sites in the United States. The initial work involves surface cleanup and groundwater remediation. A later phase will involve sub-surface aerobic bioremediation. The results from this study should provide the necessary conditions to start subsoil remediation.

The first phase of the study involved testing the effect on remediation of several combinations of inorganic nutrients applied to three distinct types of subsoil. The subsoils were mixed with five PAHs and PCP. The results of the first phase provided the data necessary to choose a nutrient application regime best suited to the conditions in each soil type. The selected nutrient solutions were used in the second phase of the study.

The second phase of the study was conducted in soil microcosms, consisting of soil uniformly mixed with the model pollutants, packed in sections of pipe. The microcosms were provided with inlets and outlets for pressurized gas, water, and nutrient solution. Pressurized gas (air or nitrogen) were used to push water or nutrient solution through the microcosms, and gas pressure was maintained between nutrient solution applications. Water draining from the microcosms was collected for analysis. After 90 days the microcosms were sectioned and each section analyzed for the model pollutant compounds.

Oxygen was supplied to the microcosms in two ways--as O_2 in the pressurized air and as hydrogen peroxide in the nutrient solution. This study was done using two separate groups of cores in order to determine the effectiveness of the two different oxygen delivery systems. Half the microcosms used air as the pressurizing gas, and half used nitrogen. The use of nitrogen gas allowed the study of microbial breakdown under anaerobic conditions.

The results of the second phase indicated that the subsoil microbial population can be stimulated by appropriate additions of nutrients and oxygen. Most of the microcosms had some reduction of PCP in the top few inches of the microcosm, but only the microcosm with air, but no hydrogen peroxide, had reduced PCP throughout most of the microcosm. This same microcosm gave the best reduction of PAHs, also. Microcosms with N2 as the pressurizing gas generally showed little or no reduction in PAHs or PCP.

The third phase of the study focused on problems concerning "maximum contaminant levels" (MCLs) set by regulatory agencies. Regulatory agencies overseeing cleanup efforts at pollution sites often require that contaminant concentrations be reduced to a given level or less. For example, the agency might require that PAH levels in soil at a particular site be reduced to 100 ppm or less. When biological methods are used for cleanup, pollutant breakdown often is rapid at higher concentrations but slows down as pollutant levels are reduced. PAHs in soil at 5000 ppm might be rapidly degraded until PAH levels reach 200-300 ppm, then further breakdown might be much slower. This phenomenon could be due to the

dependence of rate on concentration (kinetic effect), a buildup of inhibitory byproducts, strong bonding of the hazardous compound to the soil particle, or a lack of some essential nutrients. The third phase focused on a potential method of improving the breakdown rates at lower concentrations. Strains of bacteria selected for ability to transform the pollutant compounds of interest were applied to three different soils that had been contaminated with PAHs and PCP. Breakdown rates of the pollutants were compared to rates in soils mixed with autoclaved cultures of the same bacteria and to soils mixed with fresh nutrient broth similar to that in which the bacteria were grown.

Methods and Materials

The soil from the phase I study was mixed with all three soil types (Wiggins, MS; Wilmington, NC; and Atlanta, GA) and divided into three portions of 1250 grams each. Each soil type was loaded with 1000 ppm total PAHs and 250 ppm PCP. Each portion was mixed in a ball mill for at least an hour. Each treatment (bacterial culture, autoclaved culture, or nutrient broth) was added to each soil portion to bring soil up to 70% of field capacity. This is 123 ml in Wiggins soil, 180 ml in Atlanta soil, and 43 in Wilmington soil. Each portion was again mixed for one hour.

Each soil portion (1250 grams) was divided into three 400 gram portions (based on soil dry weight) and put into jars to make reps 1, 2, and 3. The jars were capped with Whatman #1 filter paper to allow air and water transfer. Moisture balance in each jar was maintained respectively as determined by weight loss.

Analysis of total PAHs and PCP occurred at day 0, 30, 60, and 90. PCP analysis was by HPLC and total PAHs was by GC/Mass Spectrometry.

Results

Transformation of PCP occurred most rapidly in the Atlanta soil type, due possibly to very active and acclimated indigenous microorganisms occurring in that particular soil type. Atlanta soil showed a depletion of PCP to below detection limits after thirty days in all three treatments. PCP levels in the Wilmington soil type reached below detection limits at sixty days while PCP levels in Wiggins soil remained at about 100 ppm in all three treatments. The results showed that the JP bacterial culture added had little effect on PCP transformation in all three soil types.

Transformation of total PAHs occurred most dramatically in the Wilmington and Atlanta soils. Total PAH levels were reduced to about 70 ppm in both the Wilmington and Atlanta soils after 90 days in all three treatments. Total PAH levels in the Wiggins soil remained between 350 and 450 ppm in the three treatments after 90 days.

It seems that the added bacterial culture did not noticeably enhance the remediation of PAHs or PCP in any of the soil types. What possibly facilitated remediation was the added nutrients provided by the media that was added to each treatment.

References

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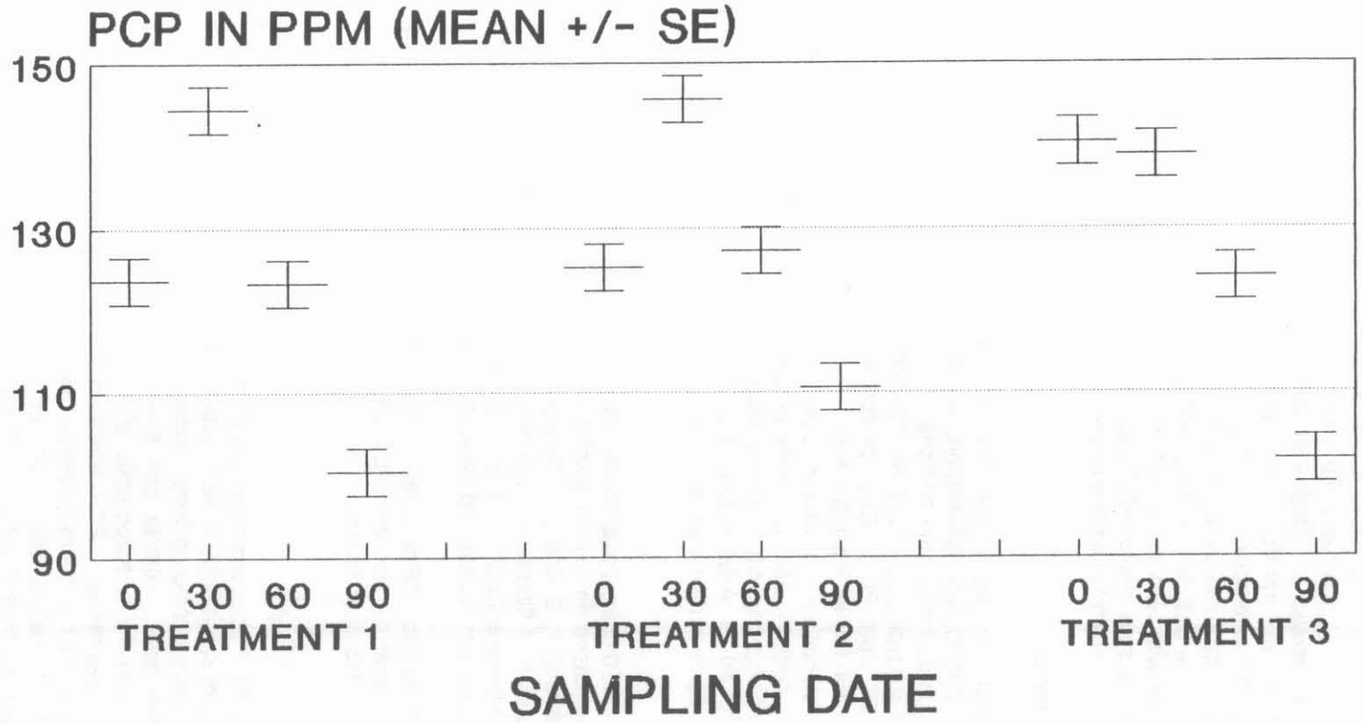
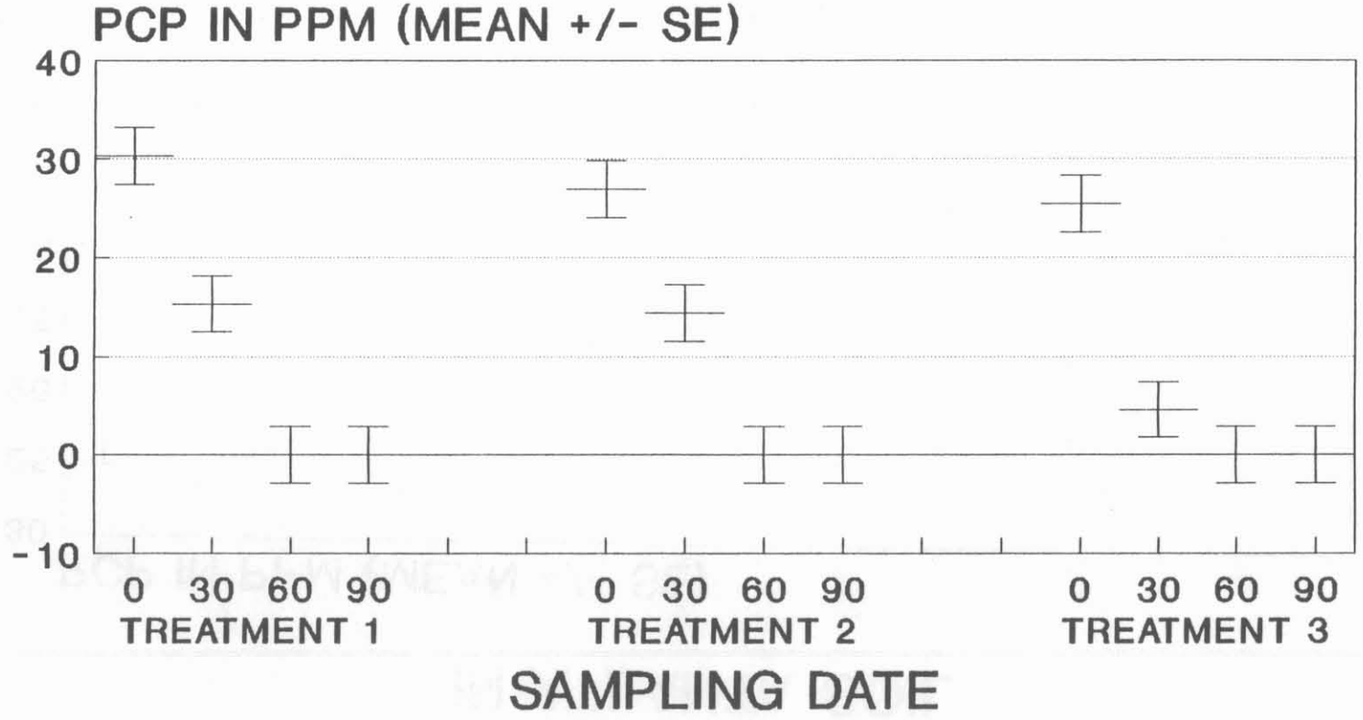
WRRI - LOW LEVEL REMEDIATION
PCP TRANSFORMATION
IN WIGGINS SOIL



TREATMENT # 1 - JP BACTERIA
TREATMENT # 2 - AUTOCLAVED JP BACTERIA
TREATMENT # 3 - NUTRIENT BROTH

WRRI - LOW LEVEL REMEDIATION
PCP TRANSFORMATION
IN WILMINGTON SOIL



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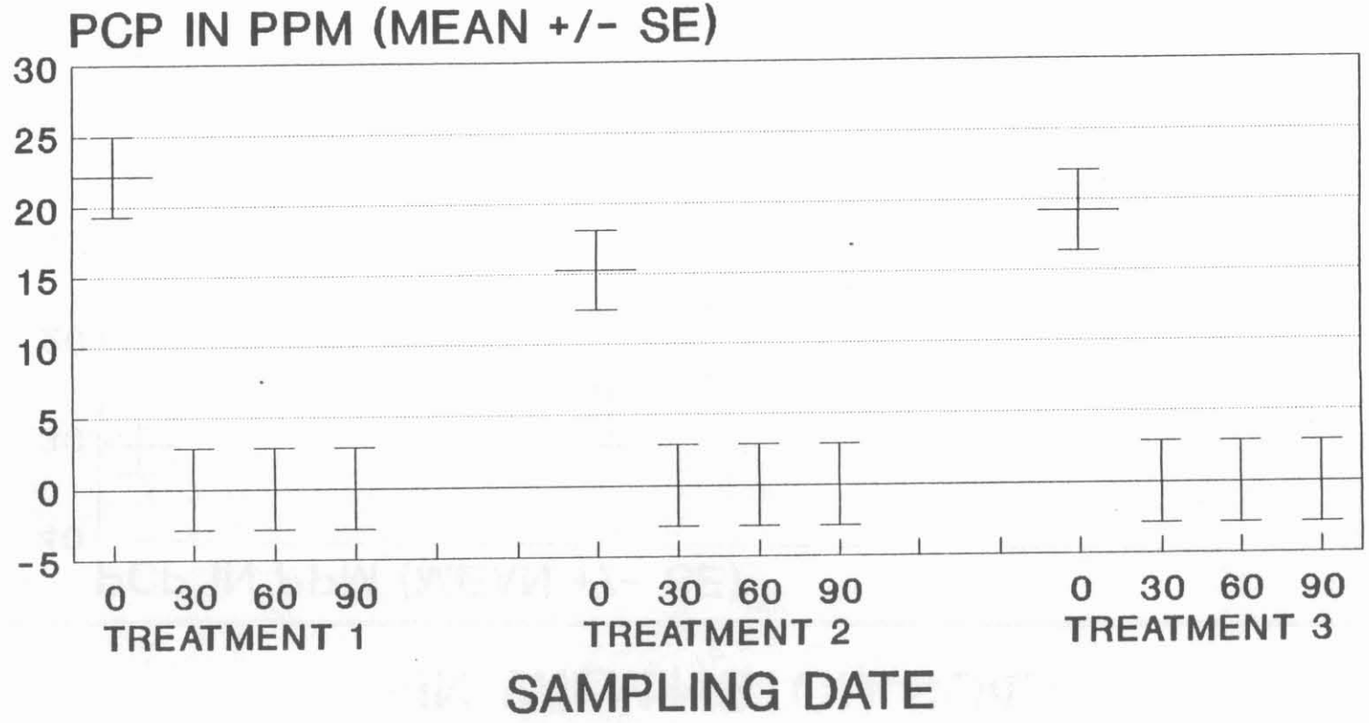
PCP TRANSFORMATION
IN ATLANTA SOIL



TREATMENT # 1 - JP BACTERIA
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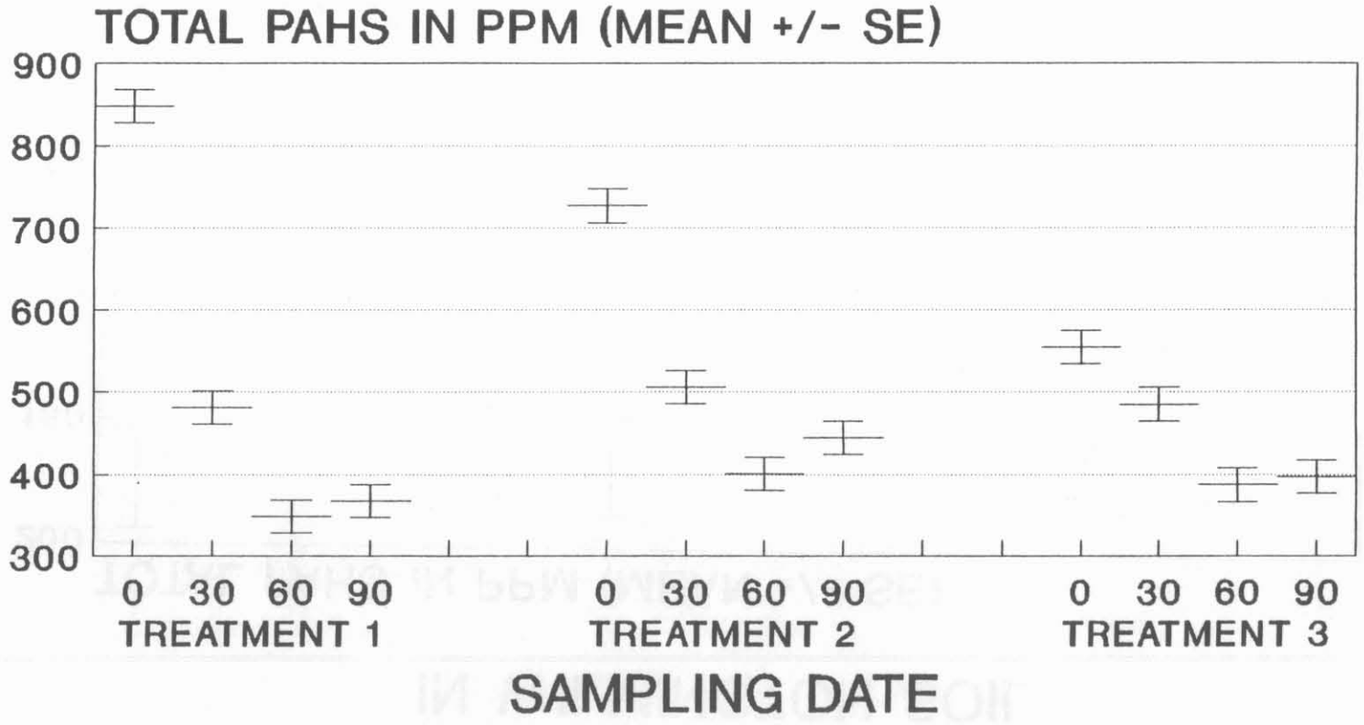
WRRI - LOW LEVEL REMEDIATION
TOTAL PAH TRANSFORMATION IN WIGGINS SOIL



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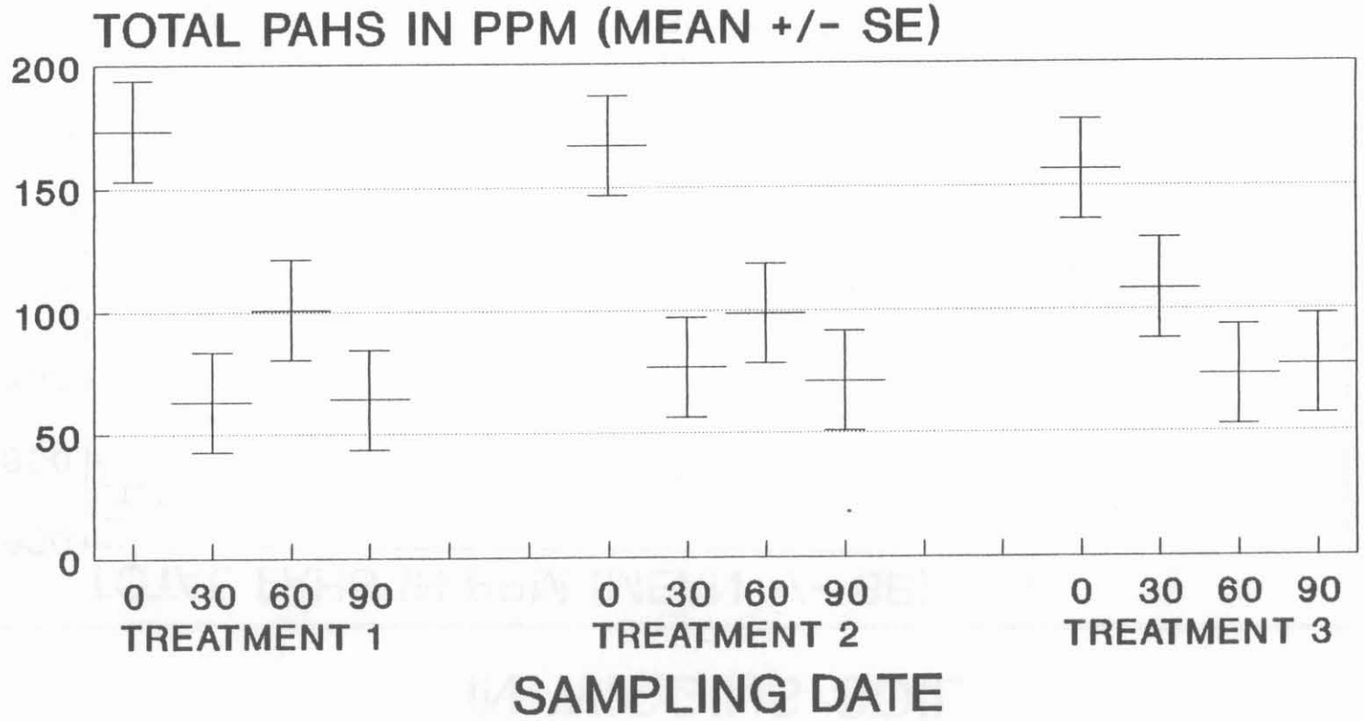
TREATMENT # 3 - NUTRIENT BROTH

WRRI - LOW LEVEL REMEDIATION
TOTAL PAH TRANSFORMATION IN WILMINGTON SOIL



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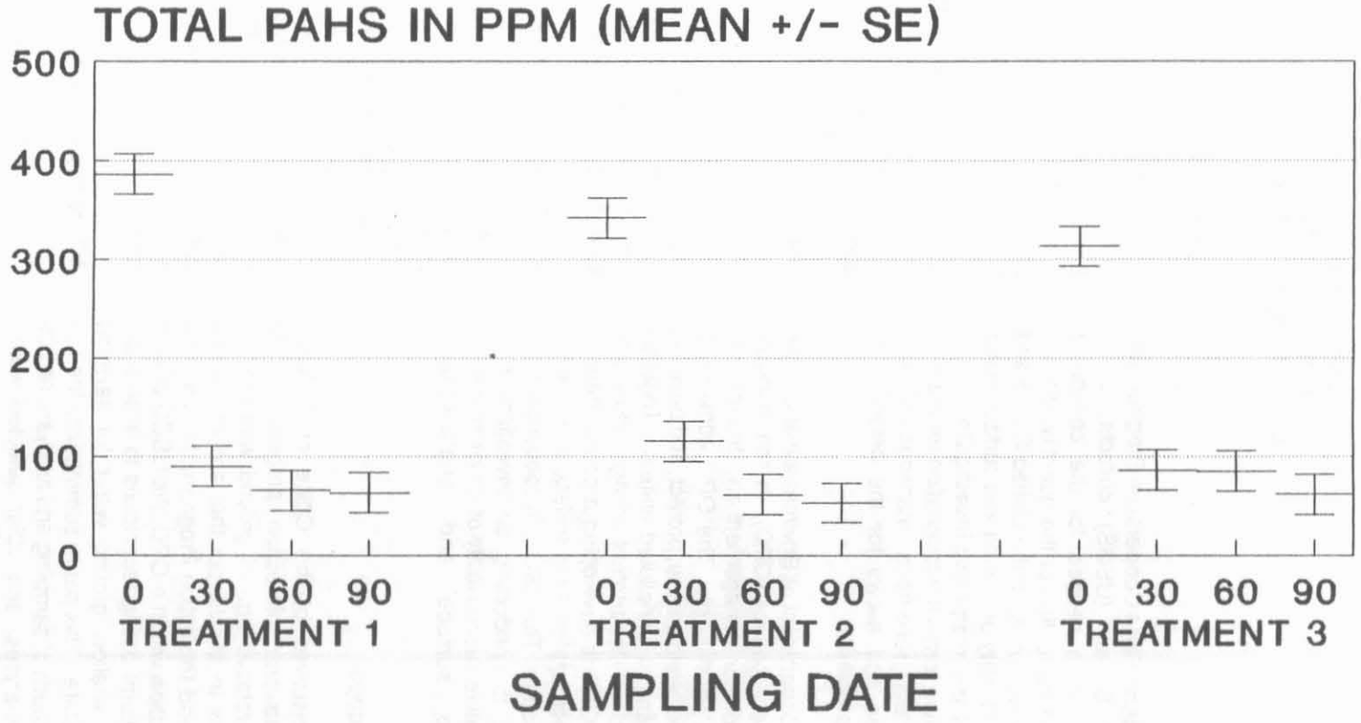
TREATMENT # 3 - NUTRIENT BROTH

WRRI - LOW LEVEL REMEDIATION
TOTAL PAH TRANSFORMATION IN ATLANTA SOIL



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