Presented at the Conference on Hazardous Waste Research, Kansas State University, Manhattan, Kansas, May 23-24, 1989.

DETERMINATION OF A METHOD FOR OPTIMIZATION OF IN SITU BIODEGRADABILITY OF SUBSURFACE SOIL CONTAMINANTS

Warren L. Jones[1]
Karen E. Bucklin[2]
Anne K. Camper[3]

Institute for Biological and Chemical Process Analysis
Montana State University
Bozeman, MT

ABSTRACT

Contaminant removal from subsurface locations poses an unique problem in that the target area is not accessible. A method is proposed for determination of the in situ capabilities of the existing microbial populations to biologically degrade certain target contaminants. Samples for this study have been procured from a site in Butte, Montana which has recently been the target of an EPA Superfund response action, where over 10,000 gallons of 5% industrial grade pentachlorophenol wood preservative in No. 6 fuel oil were removed from a layer floating on top of the groundwater. Core samples were taken at several locations and used as inocula into a variety of inorganic nutrient media. Incubations were carried out to determine the effect of removing nutrient limitations on the response of the indigenous microbial population and the kinetics of pentachlorophenol degradation. Ammonia nitrogen was found to be more effective at increasing microbial growth than other additives. The microbial population following incubation was different among media and from the original sample, as characterized by colony morphology, but the difference was one primarily of predominance rather than appearance or complete disappearance of a particular type.

---

[1]Assistant Professor, Department of Civil and Agricultural Engineering

[2]Graduate Research Assistant, Department of Civil and Agricultural Engineering

[3]Research Microbiologist, Institute for Biological and Chemical Process Analysis

INTRODUCTION

Pentachlorophenol, also known as PCP or penta, has been used widely as a biocide in the US since the 1940s. Approximately 22,000 tonnes of the chemical are produced each year, with the bulk being used in the wood-preserving industry. It has only been recently that the magnitude of groundwater and subsurface soil contamination from spillage and leaking storage containers has been recognized underneath facilities using this chemical. In Montana, a response action was taken by the EPA in 1985 at the Montana Pole site near Butte, Montana, to remove approximately 10,000 gallons of fuel oil/penta product from the surface of the groundwater to prevent its running unchecked into Silver Bow Creek (Weston, 1986). Thousands of cubic meters of surface soil were excavated, and are stored onsite in four steel buildings. A large quantity of contaminated sub-surface soil remains on the site, and continued contamination of the groundwater in the area is strongly suspected. It has been estimated that nearly 1200 similar facilities exist in the US, some of which are suspected of being in a condition similar to the Montana Pole facility. While contamination of this magnitude clearly represents a "substantial threat" (sic) to public health if untreated, the remedial action to be taken under CERCLA or Superfund has not yet started. This delay is in part because the exact nature of the required treatment has not yet been determined.

Research on pentachlorophenol degradation to date appears to have focused on identification of particular microbial species which can degrade reagent grade (i.e., pure) pentachlorophenol, on treatability studies in activated sludge systems subjected to shock loads, and on fate and mechanisms of PCP degradation in surface waters. The ultimate goal of this project is to develop methods for identifying the applicability of in situ biodegradation in a subsurface contaminated soil. As the methods used are not dependent on a particular site, the general approach may be useful for performing similar types of studies at reasonable cost on subsurface contaminated soil environments which cannot be brought wholesale to the surface for remedial action.

The purpose of this research study is to define the capability of the existing microbial population in situ to accomplish this removal action. In particular, two short-term objectives have been identified which relate to this overall goal:

I. Enumerate and identify the dominant species of microorganisms in the soil layer saturated with product and in the layers above it, with particular regard to those types of organisms which have been noted to accomplish degradation of chlorinated aromatic compounds in other studies.

II. Determine the degradation rates which might be attained under optimum conditions, i.e., determine the maximum growth and contaminant removal rates with an enrichment culture.

The results of the studies described here will be applied to laboratory testing of soil columns under controlled conditions. The combination of these two studies will allow application of predictive models using laboratory enrichment culture data to in situ conditions for remedial action.

BACKGROUND

Pentachlorophenol degradation by microorganisms has been recorded in a variety of environments and circumstances. These vary from activated sludge wastewater treatment systems at wood-preserving facilities and attached microorganisms in artificial stream systems (Dust and Thompson, 1973; Edgehill and Finn, 1983; Etzel and Kirsch, 1974; Pignatello, et al, 1983,1986) to pure cultures of microorganisms isolated from such sites (Watanabe, 1973; Stanlake and Finn, 1982; Chu and Kirsch, 1973; Klecka and Maier, 1988; Saber and Crawford, 1985; Steiert et al, 1987). Biodegradation rate and extent were found to depend on temperature, pH, inorganic nutrient sources, the nature and amount of additional substrates, and the presence of strong toxicants such as the poly-chlorinated dibenzo-dioxins and dibenzo-furans found in the original product and in groundwater contamination at contaminated sites (Jenkins, 1985; Johnson, et al, 1973). Further, different strains and even mixed cultures showed different responses from each other to adjustment of these parameters (Topp, et al, 1988; Watanabe, 1973). Clearly, a single enrichment procedure based on the results of a single study would not be expected to apply to all situations.

Pentachlorophenol is a weak acid, losing a proton from the hydroxyl group, with a pK of around 5.0. Thus, studies performed at pH 7.0 with 100 mg/L of PCP were in fact working with only 1 mg/L of free PCP; the remainder was in the form of pentachlorophenate. Stanlake and Finn (1982) found that increasing the non-ionic PCP concentration from 0.4 mg/L to 2.0 mg/L resulted in a loss of microbial activity. Growth was not observed if the non-ionic form was present in concentrations above 2.0 mg/L, regardless of the pH or total PCP dosage in the medium. The solubility of PCP has been reported as 14 mg/L (Weast, 1975), and the compound is only slightly volatile, with a vapor pressure of 0.12 mm Hg at 100°C. A log octanol/water partition coefficient has been measured at 5.01 (Leo, et al, 1971). The presence of significant quantities of free compound in aqueous solution would favor the accumulation of PCP into lipid bi-layers and similar hydrophobic environments. Conversely, there is little driving force for dissolution of PCP into groundwater from an organic phase such as fuel oil.

A number of mechanisms for dechlorination and eventual degradation of PCP have been suggested. During anaerobic degradation, chlorine atoms around the ring are progressively removed by reductive dechlorination until only phenol remains, whereupon the phenol is degraded (Mikesell and Boyd, 1986). Aerobically, the dechlorination step appears to be a replacement of chlorine with a hydroxide group. Suzuki, et al (1977) found hydroxy-dechlorination to occur at the para and ortho

positions (2 and 4), and almost no initial reaction at the meta position (3). These findings are substantiated by the findings of Etzel and Kirsch (1974) from their work with various tri- and tetra-chlorophenols. Steiert and Crawford (1986) found that a *Flavobacterium* species catabolized PCP by replacement of the *para* chlorine with a hydroxyl group that originated from water, not from molecular oxygen. Since no anaerobic activity was found, the additional energy generated by aerobic metabolism may be required to carry out catabolism of PCP (Crawford, 1989). Further, the next two reactions were reductive dechlorinations at the same end of the molecule, resulting in production of 2,6-dichlorohydroquinone, which was then observed to go rather rapidly to mineral end products (CO_2, H_2O, and HCl). It also appears that few researchers have measured significant phenol oxidation by those bacteria which actively degrade PCP, indicating that phenol itself is not a likely intermediate, nor would it be used as a co-substrate.

These studies have contributed significantly to the understanding of the mechanisms of PCP degradation, and some have even resulted in the development of a successful above ground treatment process (Crawford, 1989). However, there has been a lack of research directed at developing methodologies to be applied in the field to optimize the degradation of PCP by autochthonous microbial soil communities. This research seeks to combine the existing knowledge with practical, applicable strategies for bioremediation of contaminated sites.

MATERIALS AND METHODS

Sampling Procedures and Locations

An aerial map of the Montana Pole site is shown in Figure 1. Monitoring wells are shown in this figure, along with the locations of recovery and recharge trenches. Wells 1, 3, 9, 14, 16 and 17 were found free of product at the time they were drilled, so probably show the extent of the contamination by free product (Weston, 1986). Core samples were taken about six feet from Wells 5 and 8 (one each) on April 7, 1988, in conjunction with a sampling effort being carried out by the Montana College of Mineral Science and Technology. A three-inch split-spoon sampler was fitted with polybutylene plastic liners, and placed inside an 8-inch auger bit to obtain intact soil cores. Grab samples from the neighborhood of the product layer were also obtained when soil cores lost integrity and leaked from the liners during removal from the split spoon sampler. Wherever possible, cores were taken so as to obtain a continuous sample from the vadose zone down through the product layer (upper product) to below the surface of the groundwater table (lower product). While the sampler could not be adequately field-sterilized between operations, core barrels were handled to minimize possible contamination of the interior of the core.

Protocols are given below for batch shake-flask experiments carried out in the laboratory using the samples obtained April 7, 1988. Methods and procedures for analysis are also presented following the experimental protocols.

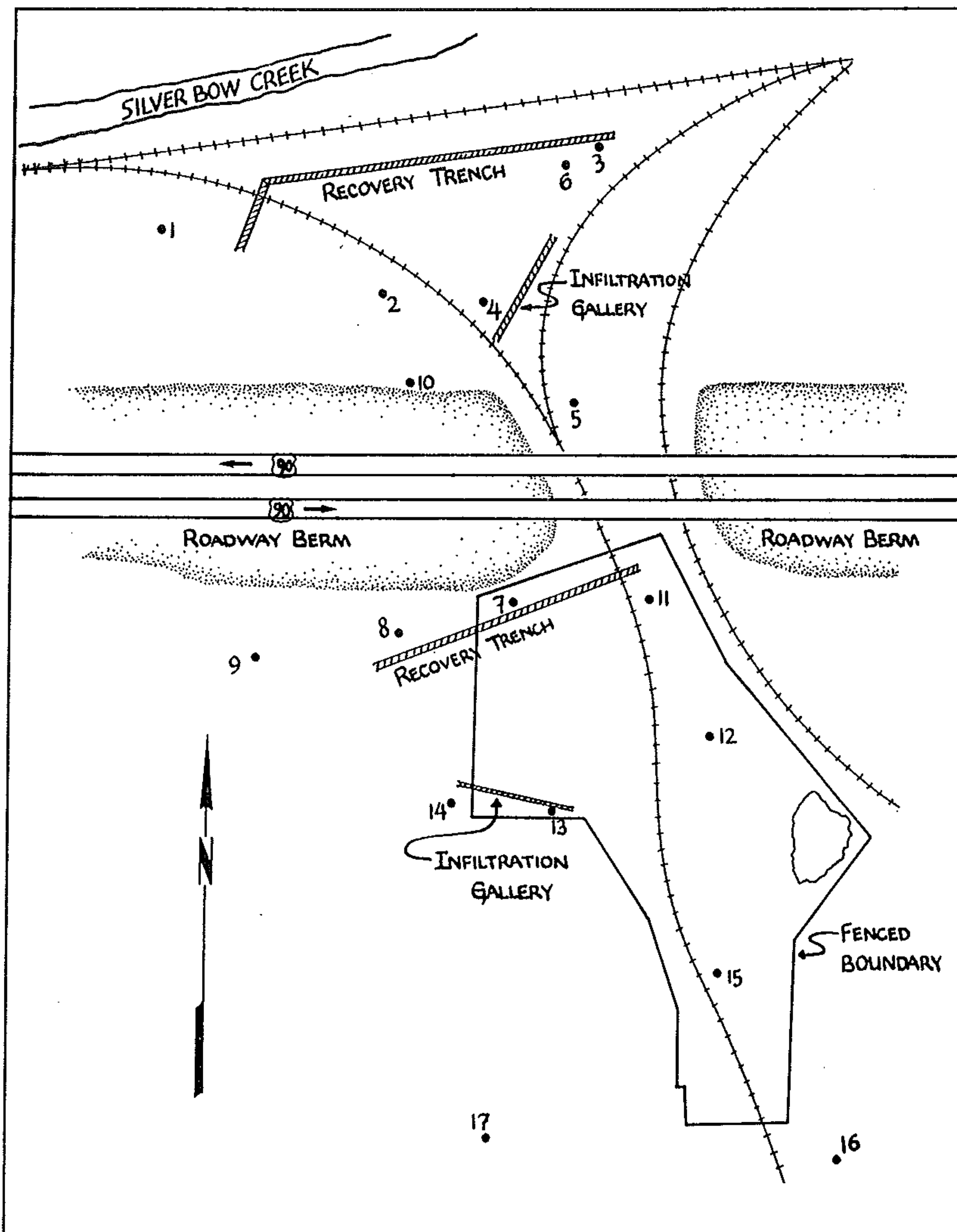

Figure 1. Site plan of the Montana Pole site, Butte, Montana

Experiment One

Twenty gram (wet weight) soil samples were taken from three grab samples representing three soil depths (vadose, upper product, lower product) from near Well 8 and added to 500 ml sterile mineral salts medium with ammonia and nitrate and 500 ml sterile phosphate buffer in one liter erlenmeyer flasks. The flasks were covered, placed on a shaker table, and agitated several times daily. After one week, samples were removed from the flasks for enumeration on R2A medium. Then two 125 ml amounts from each of the six original flasks were placed in sterile 250 ml erlenmeyer flasks. One of each of these duplicates was spiked with PCP to give a final concentration of approximately 25 ppm based on absorbance measurements at 320 nm. The 250 ml flasks were placed on the shaker table at room temperature, and agitated continually. Following one week incubation, samples from the flasks which had been spiked with PCP were plated on R2A medium plus 25 ppm PCP for bacterial enumerations, and aliquots from flasks which had not received PCP were enumerated with R2A without PCP. The samples taken the following week were enumerated on both R2A and R2A plus 25 ppm PCP agars. As no significant difference was noted in the bacterial counts, all later samples were incubated on R2A medium containing 25 ppm PCP. After eight weeks of incubation, 25 ml of the suspension was added to 250 ml flasks containing 100 ml of the same sterile medium. Sampling was continued as described above on the diluted samples.

Experiment Two

The soil inoculum was provided by aseptically removing a subcore from a larger core taken of the soil profile at Well 15. The subcore was well mixed, and five gram aliquots were removed for incubation in a broad variety of media. The soil samples were placed in triplicate 250 ml erlenmeyer flasks containing 100 ml of one of the following media: distilled water, phosphate buffer, mineral salts medium with no added nitrogen, mineral salts medium with nitrate, mineral salts medium with ammonia, or mineral salts medium with both nitrate and ammonia. All media contained 25 ppm added PCP. A control was prepared by autoclaving 5 g soil in distilled water in a tightly sealed container. The resulting 19 flasks (six media in triplicate plus one control) were incubated at room temperature on the rotary shaker. Initial samples were removed for total organic carbon, nitrogen, and PCP analyses, and for microbial enumerations. Enumerations utilized R2A agar with 25 ppm PCP. For the first four weeks, samples were removed for enumerations, PCP and nitrogen analyses three times weekly. Total organic carbon analyses and pH determinations were made weekly. After week four, enumerations also were performed weekly.

Analytical Procedures

Incubation media. Two basal media were used for both experiments. One was a phosphate buffer consisting of 0.65 g l^{-1} K_2HPO_4 and 0.17 g l^{-1} KH_2PO_4. The second was a mineral salts medium which contained the same phosphates as above, with the addition of 0.1 g l^{-1} $MgSO_4$, a trace $FeSO_4$, and a nitrogen source. The nitrogen sources were 0.5 g l^{-1} $NaNO_3$, 0.2 g l^{-1} NH_4Cl, or both nitrate and ammonia. When stated, 25 ppm reagent grade pentachlorophenol (Sigma Chemical Co.) was also

added. The basal media and PCP were autoclaved, and separately autoclaved nitrogen sources were added to give the final desired concentrations. The media were aseptically dispensed into sterile erlenmeyer flasks prior to the addition of the soil inoculum.

Microbial enumerations. Bacterial enumerations were performed after appropriate dilution in sterile blanks with the spread plate technique in triplicate on either R2A medium (Difco) or R2A medium plus 25 ppm PCP. Initial experiments showed that R2A medium yielded significantly higher bacterial counts than soil extract agar. Incubation was at room temperature for one week. Numbers of colonies as well as a description of the dominant colony morphologies for each sampling date and incubation medium type were recorded.

Total organic carbon analysis. Total organic carbon analyses were performed with a Dohrmann model DC-80 carbon analyzer. Samples from flasks were placed in acid washed vials, frozen and then acidified to a pH of < 2. Samples were then stripped with oxygen to remove dissolved CO_2. Analyses were done in triplicate.

Pentachlorophenol analysis. Samples were removed from the incubation flasks, placed in hexane rinsed vials, and frozen. Samples were extracted according to the method of Borsetti and Thurston (1984), a four-step procedure involving (1) acid-phase digestion, (2) extraction into hexane, (3) extraction into strong base followed by acidification, and (4) extraction into hexane. Analyses reported here were performed with a Hewlett-Packard model 5710A gas chromatograph equipped with an electron capture detector, but all future analyses will be using a Hewlett-Packard model 5790A GC with a model 3390A integrator/controller and an autosampler.

Nitrogen analyses. Nitrogen analyses were performed on aliquots taken from the experimental flasks by a Technicon Autoanalyzer II which combines automated wet chemistry steps with final colorimetric analysis for nitrite via spectrophotometry. Thus, total oxidized nitrogen (nitrate plus nitrite) and ammonia were determined, but nitrate and nitrite were not measured separately. Samples were stored frozen prior to testing.

RESULTS AND DISCUSSION

From the history of this site, one might expect that the high levels of toxic organics (particularly a broad-spectrum biocide like PCP) would have resulted in near-complete die-off of the indigenous population. As will be shown below, this was not the case. The results of two experiments will be presented in chronological order: 1) a comparison of the growth of organisms from three layers of soil from Well 8 (directly downgradient from the site), and 2) a comparison of the effects of various nutrient addition schemes on the growth of organisms from a combined sample from Well 15 (downgradient from the site, in an area where product pooled against a flow barrier).

There seemed to be significant shifts in the dominant colony morphologies observed as the incubation time progressed. It is interesting to note that while the phosphate buffer samples exhibited similar dynamics within a particular soil sample following the eighth week, the mineral salts samples did not. That is, within a particular soil sample, the B- and B* samples (phosphate buffer with and without additional 25 ppm PCP) were identical or similar (in dominant morphology) for each time period, while the MS- and MS* samples generally became less similar. Not surprisingly, the dominant morphologies for the phosphate samples were different from the mineral salts samples, with the exception of weeks 6 and 8 in the vadose zone samples.

Two of the colony morphologies observed -- bright yellow glossy colonies and large glistening colonies which produce a yellow-green diffusible pigment -- are recognizable as being distinctive to two particular genera of commonly occurring soil microorganisms: *Flavobacterium* and *Pseudomonas*. The common occurrence of the *Flavobacterium*-type colonies is of particular interest, as Saber and Crawford (1985) have isolated a *Flavobacterium* species which is capable of complete mineralization of pentachlorophenol under very severe conditions. While we are presently unaware of specific *Pseudomonas* species which degrade PCP, numerous reports of xenobiotic degradation by members of this genus have been reported.

A brief experiment was also conducted to determine the possible effects of inhibition of other extractable compounds present from the site. A 1:1 slurry of soil and boiling water was prepared, filter sterilized and infused into filter discs which were then placed onto plates where isolates had been streaked. No inhibition of colony growth was observed in any of the 60 isolates tested in this manner; colonies were observed to grow as large and as rapidly in direct proximity to the disc as on opposite ends of the plate.

Samples were taken from two samples during the first experiment to determine the range of PCP concentrations which would be encountered for PCP analysis. A sample from the vadose zone sample incubated in phosphate buffer with PCP added was found to contain 24 ppm of PCP, while a sample from the vadose zone incubated in phosphate buffer with no PCP added was found to contain 2 ppm of PCP following the 5:1 dilution (week 8). Thus, some disappearance of PCP may have occurred, even in the absence of an additional nitrogen source.

<u>Experiment Two</u>

Growth curves from the second experiment on a composite soil sample from the site are shown in Figure 6. As in Experiment One, it appears that the majority of the growth of the organisms in these flasks occurred in the first two days of the incubation. However, the amount of growth appeared to be a strong function of the medium used in the incubation. In particular, the top three curves reflect the effects of adding nitrogen in some form to the incubation media; those samples receiving ammonia, nitrate, or both exhibit nearly identical behavior, while those receiving no additional nitrogen are consistently 1 to 2 orders of magnitude lower in total cells. It is also

interesting to note that the addition of phosphate buffer or mineral salts medium (without nitrogen) had little to no effect compared to distilled water on the total cell yield from these samples. As an additional qualitative observation, the oily phase originally present in all of the samples disappeared gradually with time until the soil incubations appeared nearly chalk-white in color. The oil sheen was observed to disappear most rapidly in the samples which were supplemented with ammonia and/or nitrate.

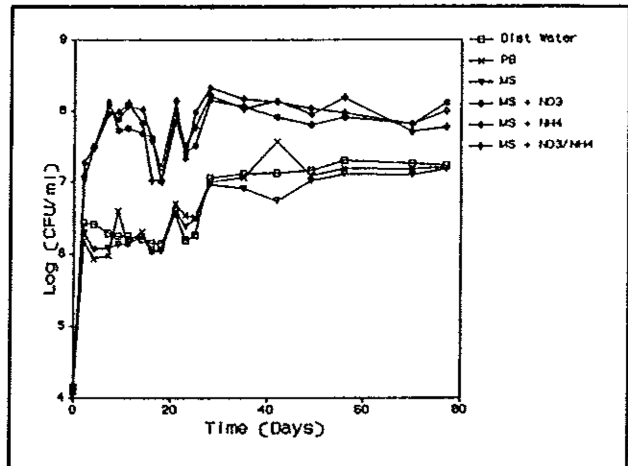

Figure 6. Viable cell counts from Experiment Two.

Data from the analysis of the samples for nitrogen content are shown in Figure 7. In the case of samples which received both ammonia and nitrate nitrogen, it appears that ammonia was preferentially removed, but in the flasks receiving only nitrate, nitrate was consumed. Overall, the consumption of nitrogen in those flasks which received it was about 15 mg/L of N, or about 0.3 mg N per gram wet weight of soil.

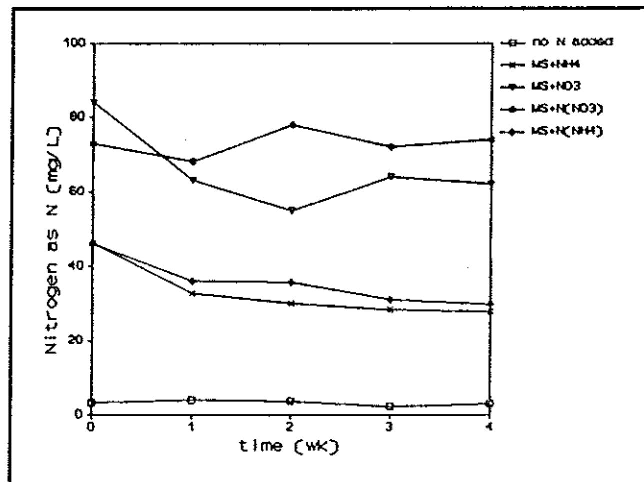

Figure 7. Nitrogen content of slurries from Experiment Two.

The progression of the dominant colony morphologies for the first four weeks of the experiment are shown in Figure 8. Not surprisingly, colony morphologies similar to the previous experiment appeared in these samples. It is interesting to note that while the colony counts from the non-N-supplemented samples were nearly identical, the dominant colony *morphologies* were not the same. The same observation is true of those samples which were supplemented with nitrogen. That these shifts in population were reflected in all three of the triplicates is a further indication that population dynamics were rather complex.

Weekly samples were taken for total organic carbon (TOC) analysis. It can be seen from Figure 9 that some TOC was observed to be removed from the system, but that complete removal was not observed. This is not surprising, as some of the organic material would be tied up in the microorganisms. Samples were not taken past the ninth week due to a high degree of scatter in the data from those samples (not shown in Figure 9). As analyses were done by high-temperature incineration methods on slurry samples from the flasks, with TOC measured by CO_2 evolved, it may be that

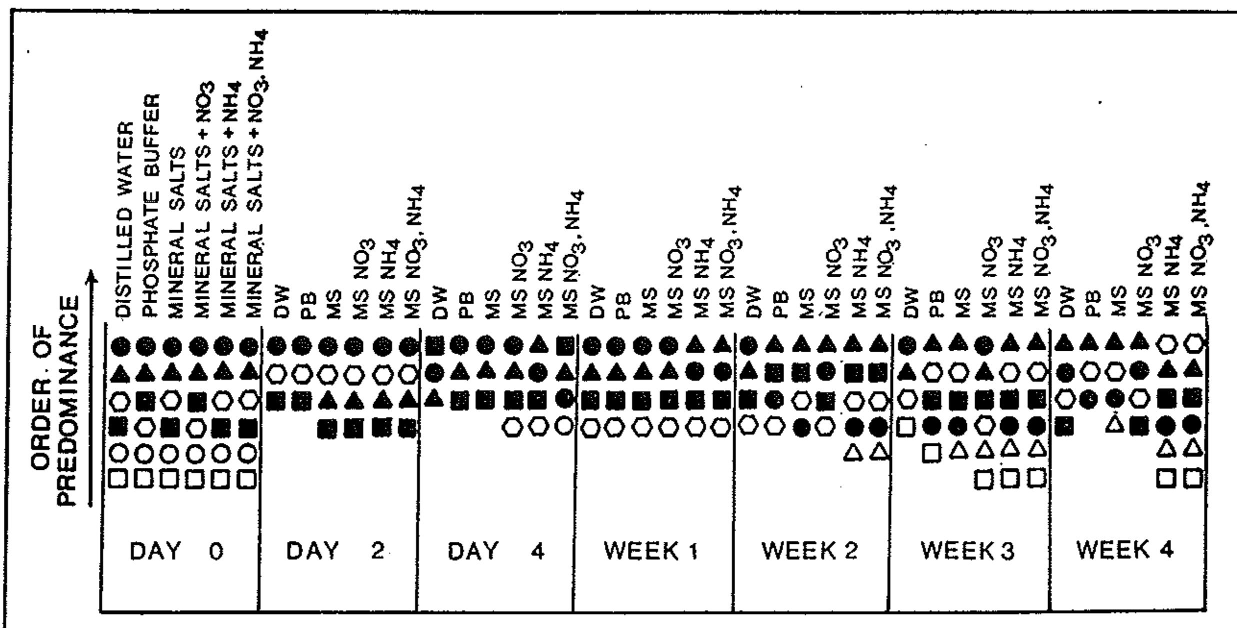

Figure 8. Dominant colony morphologies from Experiment Two. Key to morphologies given in Figure 5 and in text.

the carbonate content of the soil began to be observed as the volume of the flasks was depleted and a higher fraction of more dense material was obtained in the samples. Even though samples were acidified and aerated prior to analysis, particularly large or dense soil particles could have retained their carbonate content until the incineration step, whereupon the released carbon dioxide would be measured as though it were organic carbon.

While samples were taken at regular intervals for PCP analysis during the course of this experiment, equipment problems prevented a complete analysis of the PCP content of these samples. However, some initial screening of selected samples has been performed, and the results of these are shown in Figure 10. It appears from this figure that the addition of ammonia nitrogen resulted in the greatest amount of pentachlorophenol removal. The onset of pentachlorophenol disappearance in a supposedly sterile control coincided with the appearance of microbial contamination in that control. Since the control was incubated on the same shaker table in close proximity to the other samples, it is entirely possible that the contamination resulted from airborne

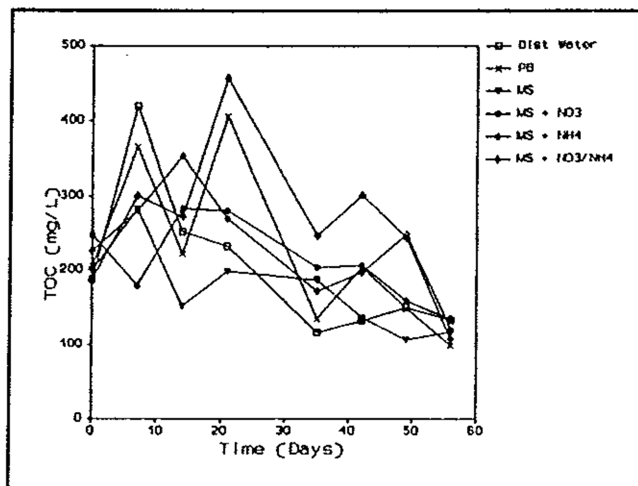

Figure 9. Total Organic Content of whole soil slurries from Experiment Two.

transport of bacteria from one sample to another. While all samples were kept capped with aluminum foil during incubation, shaking was rather vigorous, and some gas exchange was theoretically possible (note: the entire flask/shaker table apparatus was kept under a hood to prevent contamination of the laboratory environment). Ongoing experiments will utilize sealed containers with 0.22 μm filters to prevent cross-migration of airborne organisms. Gas exchange to maintain aerobic conditions will be allowed only through these sterile filters.

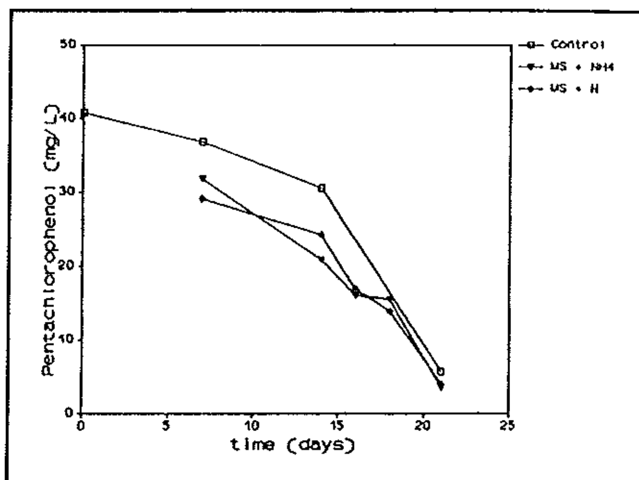

Figure 10. Pentachlorophenol concentrations from screening of soil slurry samples, Experiment Two.

CONCLUSIONS

1. For the samples tested, nitrogen appears to be the limiting nutrient to microbial growth.

2. A variety of microorganisms are capable of growth in this environment, and the dynamics of the population will be dependent on the media or nutrients used to encourage growth.

3. A significant lag phase may be expected in PCP degradation, but subsequent removal rates may be high.

4. While carrier oil constituents were visually observed to be removed, it is not known whether volatilization processes or microbial degradation were dominant.

Acknowledgements

This work was supported in part by grants from the Montana State University Water Resources Center and the US Geological Survey, the US Environmental Protection Agency Superfund Grant Program (Grant # R815249-01-0), and the US Environmental Protection Agency Hazardous Substance Research Center for Regions VII and VIII (Grant # R-815709). Their support of this work is gratefully acknowledged. Sampling efforts were largely made possible by Dr. Rick Appleman of the Montana College of Mineral Science and Technology and the staff of the Montana Bureau of Mines. Their participation in this work is also greatly appreciated.

Note: The contents of this report do not necessarily represent the views and policies of the Environmental Protection Agency or other federal agencies.

REFERENCES

Borsetti and Thurston, 1984. J. Assoc. Off. Anal. Chem., 67, 275-277.

Chu, J. and E.J. Kirsch, 1973. Utilization of Halophenols by a Pentachlorophenol Metabolizing Bacterium. Dev. Ind. Microbiol., 14, 264 - 273.

Crawford, R.L. (1989) Personal Communication.

Dust, J.V. and W.S. Thompson, 1973. Pollution Control in the Wood-Preserving Industry. Part IV. Biological Methods of Treating Wastewater. Forest Products J., 23:9, 59-66.

Edgehill, R.U. and R.K. Finn, 1983. Activated Sludge Treatment of Synthetic Wastewater Containing Pentachlorophenol. Biotechnol. Bioeng., 25, 2165-2176.

Etzel, J.E. and E.J. Kirsch, 1974. Biological Treatment of Contrived and Industrial Wastewater Containing Pentachlorophenol. Dev. Indust. Microbiol., 16, 287-295.

Jenkins, C. Alternate Concentration Limits for Dioxin Ground Water Contamination at Wood Preserving Sites. USEPA internal Memorandum, May 8, 1986 (Unpubl.)

Johnson, et al, 1973. Chlorinated Dibenzodioxins and pentachlorophenol. Env. Health Perspect. 5, p 171.

Klecka, G.M., and W.J. Maier. 1988. Kinetics of Microbial Growth on Mixtures of Pentachlorophenol and CHlorinated Aromatic Compounds. Biotech. Bioeng., 31, 328 - 335.

Leo, et al. 1971. Partition Coefficients and their Uses. Chem. Rev., 7, p 525.

Mikesell, M.D. and S.A. Boyd. 1986. Complete Reductive Dechlorination and Mineralization of Pentachlorophenol by Anaerobic Microorganisms. Appl. Environ. Microbiol., 52:4, 861-865.

Pignatello, J.J., M.M. Martinson, J.G. Steiert, R.E. Carlson, R. L. Crawford. 1983. Biodegradation and Photolysis of Pentachlorophenol in Artificial Streams. Appl. Environ. Microbiol., 46:5, 1024-1031.

Pignatello, J.J., L.K. Johnson, M.M. Martinson, R.E. Carlson, R. L. Crawford. 1985. Response of the Microflora in Outdoor Experimental Streams to Pentachlorophenol: Compartmental Contributions. Appl. Environ. Microbiol., 50:1, 127-132.

Pignatello, J.J., L.K. Johnson, M.M. Martinson, R.E. Carlson, R. L. Crawford. 1986. Response of the Microflora in Outdoor Experimental Streams to Pentachlorophenol: Environmental Factors. Can. J. Microbiol., 32:1, 38-46.

Saber, D.L. and R.L. Crawford, 1985. Isolation and Characterization of *Flavobacterium* Strains that Degrade Pentachlorophenol. Appl. Env. Microbiol., 50:6, 1512-1518.

Stanlake, G.J. and R.K. Finn, 1982. Isolation and Characterization of a Pentachlorophenol-degrading Bacterium. Appl. Env. Microbiol., 44:6, 1421-1427.

Steiert, J.G. and R.L. Crawford, 1986. Catabolism of Pentachlorophenol by a *Flavobacterium* sp. Biochem. Biophys. Res. Comm., 141:2, 825-830.

Steiert, J.G., J.J. Pignatello, and R.L. Crawford, 1987. Degradation of Chlorinated Phenols by a Pentachlorophenol-degrading Bacterium. Appl. Env. Microbiol., 53:5, 907-910.

Suzuki, T. 1977. Metabolism of Pentachlorophenol by a Soil Microbe. J. Env. Sci. Health, B12, 113-127.

Topp, E., R.L. Crawford, and R.S. Hanson, 1988. Influence of Readily Metabolizable Carbon on Pentachlorophenol Metabolism by a Pentachlorophenol-degrading *Flavobacterium* Species. Appl. Env. Microbiol., 54:10, 2452-2459.

Watanabe, I. 1973. Isolation of a Pentachlorophenol-degrading Bacterium from Soil. Soil Sci. Plant Nutr., 19:2, 109-116.

Weast, R.C. (ed) 1975. Handbook of Chemistry and Physics. 5th edition, Chemical and Rubber Company Press, Cleveland, OH.

Weston, Roy F., Inc. 1986. "Immediate Removal Action at Montana Pole, Butte, Montana". prepared for USEPA Region VIII - Emergency Response Branch. TDD#8-8510-7.