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FAIRBANKS INTERNATIONAL AIRPORT BIOREMEMDIATION DEMONSTRATION REPORT: MICROBIOLOGY

by

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16. Abstract AKDOT&PF, in conjunction with the Construction Productivity Advancement Research Program of the U. S. Army, is conducting a multi-agency bioremediation demonstration project at the Fairbanks International Airport (FIA). The demonstration project utilizes two different systems to bioremediate petroleum-contaminated soil and water: an experimental landfarm and an infiltration gallery/trickling filter. UAF was given the responsibility for monitoring the microbiology of environmental samples with and without nutrient amendments. The purpose of the monitoring program was to quantify two general parameters before and during the soil and water treatments: to monitor biological activity during soil and water treatment and to monitor microbial numbers in soil and water during treatment.

Bioremediation (biological cleaning of contaminated soil and water) uses naturally occurring microorganisms, such as bacteria and fungi, to degrade and/or detoxify harmful chemicals into less toxic or nontoxic compounds. Biological remediation of soil and water contaminated with organic chemicals has been demonstrated as an effective alternative treatment that can often meet the goal of achieving a permanent clean-up remedy. The treatment of petroleum-contaminated soil and water at the FIA research site consisted of bioenhancement: the addition of nutrient amendments, oxygen and tilling to enhance the growth of naturally occurring hydrocarbon-degrading microorganisms. The activity of these microorganisms are often limited by the supply of readily available nutrients such as nitrogen, phosphorous, or oxygen. Microorganisms break down a wide variety of organic compounds (including petroleum-hydrocarbons) in nature to acquire energy for growth, transforming these compounds into cell material, mineral carbon (carbon dioxide), and water. This is referred to as mineralization.

Removal of hydrocarbon contaminants from nature ultimately depends on the activity of the microorganisms present. In this study we tested the activity of the microorganisms to determine their potential to degrade and/or detoxify the contaminants present to carbon dioxide and water. These hydrocarbon-oxidizing activities (mineralization potentials) were measured in the laboratory with the UAF protocol developed and routinely used in our laboratory.

In conjunction with activity measurements, the number of hydrocarbon-degrading microorganisms were determined using the Sheen Screen technique also developed and routinely used in our laboratory.

The results from these measurements taken during the 1992 sampling season showed: significant increases of microbial activity in the landfarm due to nutrient addition, irrigation, and tilling; maintenance of a large population of hydrocarbon degrading microorganisms in the landfarm; and after startup of the infiltration gallery, a decline in both measures.

The significant increase in microbial activity and the large populations of hydrocarbon-metabolizing microorganisms maintained in the landfarm would indirectly indicate increased biodegradation rates *in situ*. Cleaning times may be further increased by maintaining a vigilant schedule for the irrigation and nutrient amendment regime. Allowing the landfarm to dry completely does not enhance biodegradation. From the microbiological data, there was no indication of any biodegradation taking place in the infiltration gallery. Due to the design, the nutrient levels were not being maintained within the system. Nutrient limitations in and of themselves are enough to shut down a bioremediation project.

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FAIRBANKS INTERNATIONAL AIRPORT BIOREMEDIATION DEMONSTRATION REPORT: MICROBIOLOGY

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ABSTRACT

The Alaska Department of Transportation and Public Facilities, in conjunction with the Construction Productivity Advancement Research Program of the U. S. Army, is conducting a multi-agency bioremediation demonstration project at the Fairbanks International Airport (FIA). The demonstration project utilizes two different systems to bioremediate petroleum-contaminated soil and water: an experimental landfarm and an infiltration gallery/trickling filter. The University of Alaska was given the responsibility for monitoring the microbiology of environmental samples with and without nutrient amendments. The purpose of the monitoring program was to quantify two general parameters before and during the soil and water treatments:

- to monitor biological activity during soil and water treatment,
- to monitor microbial numbers in soil and water during treatment.

Bioremediation (biological cleaning of contaminated soil and water) uses naturally occurring microorganisms, such as bacteria and fungi, to degrade and/or detoxify harmful chemicals into less toxic or nontoxic compounds. Biological remediation of soil and water contaminated with organic chemicals has been demonstrated as an effective alternative treatment that can often meet the goal of achieving a permanent clean-up remedy. The treatment of petroleum-contaminated soil and water at the FIA research site consisted of

bioenhancement: the addition of nutrient amendments, oxygen and tilling to enhance the growth of naturally occurring hydrocarbon-degrading microorganisms. The activity of these microorganisms are often limited by the supply of readily available nutrients such as nitrogen, phosphorous, or oxygen. Microorganisms break down a wide variety of organic compounds (including petroleum-hydrocarbons) in nature to acquire energy for growth, transforming these compounds into cell material, mineral carbon (carbon dioxide), and water. This is referred to as mineralization.

Removal of hydrocarbon contaminants from nature ultimately depends on the activity of the microorganisms present. In this study we tested the activity of the microorganisms to determine their potential to degrade and/or detoxify the contaminants present to carbon dioxide and water. These hydrocarbon-oxidizing activities (mineralization potentials) were measured in the laboratory with the UAF protocol developed and routinely used in our laboratory.

In conjunction with activity measurements, the number of hydrocarbon-degrading microorganisms were determined using the Sheen Screen technique also developed and routinely used in our laboratory.

The results from these measurements taken during the 1992 sampling season showed:

- significant increases of microbial activity in the landfarm due to nutrient addition, irrigation, and tilling;

- maintenance of a large population of hydrocarbon degrading microorganisms in the landfarm;
- after startup of the infiltration gallery, a decline in both measures.

The significant increase in microbial activity and the large populations of hydrocarbon-metabolizing microorganisms maintained in the landfarm would indirectly indicate increased biodegradation rates *in situ*. Cleaning times may be further increased by maintaining a vigilant schedule for the irrigation and nutrient amendment regime. Allowing the landfarm to dry completely does not enhance biodegradation. From the microbiological data, there was no indication of any biodegradation taking place in the infiltration gallery. Due to the design, the nutrient levels were not being maintained within the system. Nutrient limitations in and of themselves are enough to shut down a bioremediation project.

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INTRODUCTION

The Alaska Department of Transportation and Public Facilities (ADOT&PF), in conjunction with the Construction Productivity Advancement Research Program (CPAR), is conducting a multi-agency demonstration project at the Fairbanks International Airport (FIA) in which bioremediation is being used to treat petroleum-contaminated soil and water. Bioremediation is the process of microbial oxidation of the hydrocarbon contaminants to carbon dioxide, water and various other oxidized ligands which may then be utilized in the formation of new biomass and in biochemical assimilation (King et al., 1992). Many naturally occurring microorganisms have the ability to utilize hydrocarbon substrates as sole sources of carbon and energy and in the process convert these contaminants to less harmful products. The detoxification of the parent compound to products that are no longer harmful to human health and the environment is the goal of on-site bioremediation (Sims et. al., 1990). This reaction may be written as follows (Cole, 1994; King et. al., 1992):

$$\text{Substrate} + \text{Nutrients (N, P, K)} + O_2$$

$$\rightarrow \{\text{Acid intermediates}\}$$

$$\rightarrow CO_2 + H_2O + \text{Biomass} + \text{Other Organics} + \text{Energy}$$

Up to 60% of the hydrocarbons may be used by the bacteria for reproduction and cell material (Cole, 1994) while the remainder is converted to carbon dioxide and water. This is referred to as mineralization.

A number of factors may affect the biodegradative process and can subsequently be altered to enhance bioremediation. This may include the addition of mineral nutrients, the addition of an electron acceptor, tilling, and the adjustment of temperature, pH, and moisture. All of these factors influence petroleum biodegradation and ultimately microbial activity. The manipulation of these factors will help to minimize Liebig's law of the minimum which states that the rate of a biological process such as growth or metabolism is limited by that factor which is present at its minimum level (Calabrese and Kostecki, 1993). During the summer of 1992, the bioremediation of petroleum-contaminated soil and water at the Fairbanks International Airport consisted of bioenhancement: the addition of nitrogen (ammonium nitrate), phosphorous (triple super phosphate), and potassium (muriate of potash) to enhance the growth of naturally occurring hydrocarbon-degrading microorganisms. Bioremediation techniques can generally be grouped into two categories: *in situ*, which treats the wastes in place, and above ground (EPA Publication 540/2-9/002). Two different operating systems were tested at the Airport: an infiltration gallery which utilized *in situ* techniques and a landfarm which utilized above ground techniques. Before, during, and after the nutrient additions, two different microbiological parameters were measured as a function of irrigation and tillage (for the landfarm) and oxygen addition (for the infiltration gallery). The Water Research Center at the University of Alaska

Fairbanks was given the responsibility for monitoring and assessing the microbiology of environmental samples with and without nutrient amendments. The purpose of the monitoring program was to quantify three parameters before and during the soil and water treatments:

- whether biodegradation was stimulated by the addition of the nutrient amendments;
- increases or decreases in biomass measurements due to fertilization as compared to nonfertilized samples;
- microbial activity measurements: assaying mineralization potentials in samples collected.

Two laboratory bioassays were used (Table 1) for the monitoring of the microbial community, radiorespirometry and the Sheen Screen most probable number technique. The goal of the monitoring program was the determination of whether the application of nutrient amendments would stimulate the *in-situ* biodegradation of the petroleum contaminates. Past bioremediation studies (Lindstrom et al., 1991) have shown an indirect correlation between increased biodegradation of organic contaminants and elevated numbers of hydrocarbon-degrading microorganisms combined with high mineralization potentials. The purpose of this study was to evaluate the efficacy of the FIA bioremediation treatments using these proven techniques.

Bioremediation ultimately depends on the activity of microorganisms. Testing may be used to establish the potential of microorganisms to degrade

Table 1. Monitoring Techniques in Biodegradation Assessment

Technique	Process examined
Sheen Screen	Biomass/enumeration assay to determine number of surfactant-producing microorganisms
Radiorespirometry	Mineralization potential: activity assay used to determine hydrocarbon oxidation potential of hexadecane

and/or detoxify the contaminants present to carbon dioxide and water. These hydrocarbon-oxidizing potentials (mineralization potentials) were measured in the laboratory using the UAF protocol described by Brown et al. (1991). For this radiorespirometry assay, radiolabeled hexadecane (1-^{14}C labeled) was used as a paradigm of aliphatic hydrocarbons. The underlying assumption of radiorespirometry is that the radiolabeled compound will biodegrade at a rate that is representative for the petroleum or certain classes of hydrocarbons in the petroleum. One has to keep in mind that this is an *in vitro* assay. Most laboratory studies are unable to simulate the constantly changing conditions that are found in the field.

In conjunction with activity measurements, the number of surfactant-producing hydrocarbon-degrading microorganisms was determined using the Sheen Screen most probable number technique (Brown and Braddock, 1990). The Sheen Screen uses the disruption of an oil film to indicate the presence of hydrocarbon-metabolizing microorganisms in varying dilutions of sample. Though MPN's are not appropriate for producing absolute counts, they do offer consistent results in comparing relative numbers, especially between different sites or different treatments (Lindstrom, 1991).

This report summarizes the microbiological assays of the petroleum-contaminated soils and water receiving bioremediation treatment. The goal of this portion of the study was to determine whether the application of nitrogen, phosphorous and potassium would stimulate the *in situ* biodegradation of the petroleum contaminates. The hydrocarbon mineralization potential of microorganisms and the number of hydrocarbon-degrading microorganisms in soil and water were used to make this assessment. Determining an actual rate of hydrocarbon biodegradation is very difficult, but elevated numbers of hydrocarbon-degrading bacteria coupled with a high potential for mineralizing hydrocarbons will provide strong evidence of *in situ* hydrocarbon biodegradation (Lindstrom, 1991).

MONITORING SITES

Two systems were utilized as part of the pilot project to develop and demonstrate cost effective bioremediation techniques at the Fairbanks International Airport.

Landfarm

Soils contaminated with #2 diesel and unknown petroleum wastes were excavated and placed in a lined excavation approximately one acre in size. The top 8 inches (approximately 1200 cu. yd.) were targeted for treatment which consisted of (1) nutrient amendments consisting of 270 lb of nitrogen, 34 lb of phosphorous and 26 lb of potassium (Hinchee et al., 1994); (2) tilling; and (3) moisture addition. Nutrient additions took place on June 22 and July 22 of 1992.

Infiltration Gallery

Some soils and groundwater at the test site had been contaminated by a leaking storage tank. The soils beneath the tank were excavated and an infiltration gallery was constructed in its place. Seven wells were installed in direct association with the infiltration gallery while four remaining wells were either upgradient or downgradient; one well (the control) was off site. Treatment of the soils and water consisted of nutrient amendments and oxygenation. Fertilization was achieved by injecting the nutrients into a water stream which was then allowed to percolate into the infiltration gallery. Oxygen was added through an aeration system.

SAMPLING STRATEGIES

Spatial Sampling

Landfarm

The contaminated soils deposited in the landfarm were a result of excavations from various locations at the test site. As a consequence, the soils exhibited heterogeneity in hydrocarbon loading. To account for this heterogeneity, the landfarm was partitioned into a grid. During the 1991 season, the landfarm was divided into twenty-five equal sections (five rows of five each), with each section being 46 x 55 ft. Composite samples were taken from each row, for a total of five soil samples collected each sampling period. To increase statistical sensitivity during the 1992 season, the landfarm was divided into a grid of nine equal sections (see Figure 1), each section being 63 x 76 ft. Four soil samples from each grid were taken randomly, with each sample analyzed separately. This resulted in a total of thirty-six soil samples collected each sampling period.

Infiltration Gallery

The infiltration gallery is a system designed to treat soil and groundwater simultaneously. For this test site, only water samples were evaluated for microbiological activity and biomass. Samples were collected from a total of eleven wells during the operating period. Seven wells are associated with the infiltration gallery: B-3, IG-1, IG-2, IG-3, IG-WW, DEC-1, and DEC-2. Three wells are on site but not associated with the infiltration gallery: B-1, B-2, and B-4. One well, P-TAN, is off site and acts as a control. Water samples were collected by the Alaska Division of Geological and Geophysical Surveys for chemical analyses. Part of each sample was then transferred to the Water Research Center for microbiological measurements.

Temporal Sampling

Landfarm

On June 16, 1992 the first thirty-six samples were taken from the landfarm prior to nutrient amendments and prior to the first tillage (Week 0). Samples were collected approximately every two weeks (Weeks 2, 5, 7 and 9) after the initial addition of nitrogen and phosphorous until the beginning of

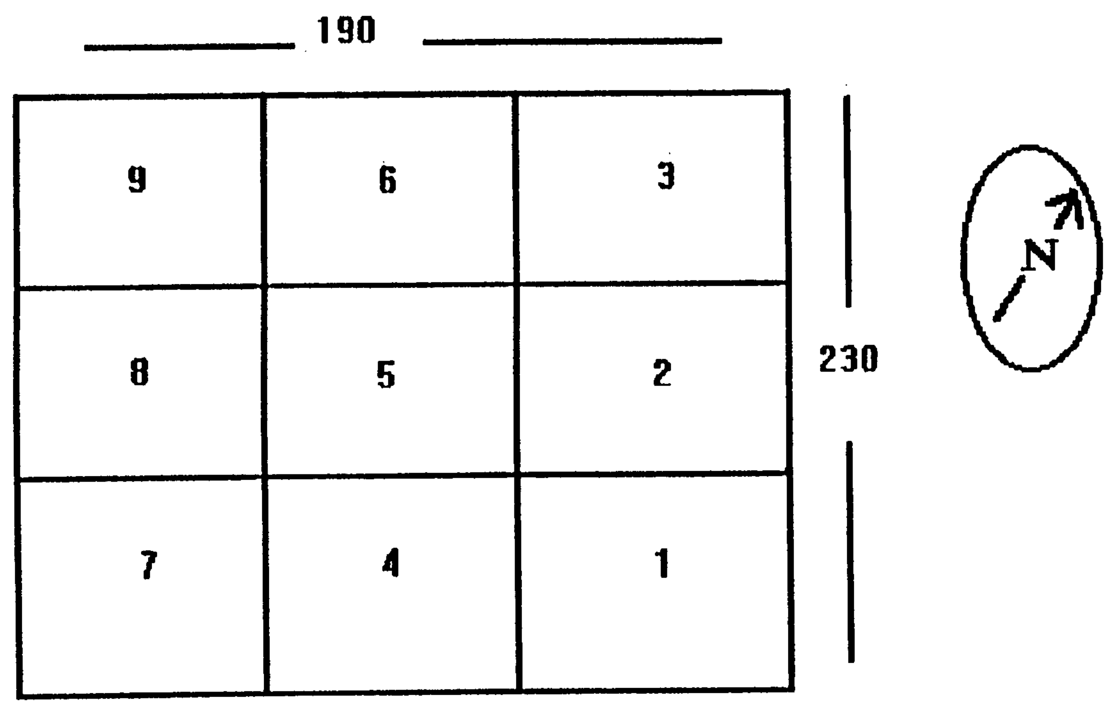


Figure 1: Diagram of landfarm sampling grid. Four random samples were taken from each grid for a total of 36 samples each sampling period.

September, 1992, for a total of 180 samples.

Infiltration Gallery

Once a month all eleven wells on the site (and the control) were sampled and subsequently analyzed microbiologically. Twice a month the DEC monitoring wells (1 and 2) and well IG-1 received microbiological examination, IG-WW was sampled each week (see Table 2). Approximately 45 water samples were taken during the 1992 season.

LABORATORY METHODS

Sample Analysis

Upon receipt of the samples into the laboratory, a 1:10 dilution was made with Bushnell-Haas (BH) medium (Difco, Detroit, MI). These dilutions were then used for the biomass/enumeration assays and the radiorespirometry activity assays.

Sediment Dry Weight

Dry weight determinations were made for each soil sample by weighing approximately 20 g of soil in a container. The sample was then dried at 100^0 for 24 hours, cooled and then reweighed. Since the moisture content of soils vary, the microbial numbers were standardized to dry weight for each of the samples taken.

Table 2. Water Samples Collected for Microbiological Assays

Week 1	Week 2	Week 3	Week 4
DEC1		DEC1	
DEC2		DEC2	
IGWW	IGWW	IGWW	IGWW
IG1		IG1	
IG2			
IG3			
B1			
B2			
B3			
B4			
P-TAN			

Microbial Population Enumeration

Surfactant-producing hydrocarbon-degrading microorganisms were enumerated using the Sheen Screen most-probable-number (MPN) method (Brown and Braddock, 1990). For each sample, duplicate "five tube" MPN's were made in which sterilized Prudhoe Bay Crude oil was used as a carbon source and as an indicator. This method uses disruption of an oil film to indicate the presence of hydrocarbon-metabolizing microorganisms in various dilutions of the sample. After inoculation, the plates were allowed to incubate for three weeks at room temperature before the plates were scored for disruption of oil sheen. Duplicate sets of plates were prepared for each sample and the scores averaged. The values were then corrected to dry weight sediment. Though MPN's are not appropriate for producing absolute counts, they do offer consistent results in comparing relative numbers, especially between different sites or different treatments (Lindstrom, 1991).

Hydrocarbon Mineralization Potential

Testing may be used to establish the potential of microorganisms to degrade and/or detoxify the contaminants present to carbon dioxide and water. To measure this substrate mineralization potential of the soil and water samples collected, the UAF protocol described by Brown et al. (1991) was followed. The hydrocarbon degradation potential assays were used to determine the ability of the soil and water microbes to utilize selected substrates. Triplicate vials were prepared for each sample taken from the landfarm and the infiltration gallery. These samples were then purged on the radiorespirometry line and the radioactivity counted using a liquid scintillation counter. This yielded a number (disintegrations per minute, dpm) for each sample and control. To obtain the % mineralization, the control dpm values for each hydrocarbon were averaged. This mean was then subtracted from the averaged dpm values of each sample to give a corrected dpm. This corrected dpm was then divided by the total dpm of the hydrocarbon used then multiplied by 100 to give the % mineralization of each sample. For this particular radiorespirometric assay, radiolabeled hexadecane (a 1-^{14}C labeled linear alkane substrate) was employed. Choice of individual hydrocarbons for a study will give a qualitative approximation of the fate of a particular oil (Atlas, 1979). The underlying assumption of radiorespirometry is that the radiolabeled compound will biodegrade at a rate that is representative for the petroleum. A variety of nutrient amendments were evaluated: (1) with nitrogen and phosphorous (BH), and (2) without nitrogen and phosphorous (-N,P). This allowed for the determination of

- whether the addition of nutrients in the field increased activity in the slurries in the lab;
- mineralization potentials of chosen substrates in the lab under varying conditions;
- whether the soil and water microbes are metabolically acclimated to the hydrocarbon that was released into the environment.

Samples were incubated for 48 hours before stripping. Radiorespirometry is a short term assay. It is designed to determine the metabolic activity of the sample at the time the sample was collected. Because of this, incubation time is a critical factor. If incubated too long, the microbial community will become acclimated *in vitro* to the hydrocarbon that has been introduced, resulting in higher mineralization potentials. Radiorespirometry is an *in vitro* assay and can not be transferred to a rate in the field.

RESULTS

Overview

Appendix A contains hydrocarbon oxidation potentials (% dpm) for the landfarm soil samples. Appendix B contains the replicate most probable numbers of hydrocarbon-oxidizing bacteria for the landfarm soil samples. Appendix C contains the mineralization potentials for the infiltration gallery water samples. Appendix D contains the replicate mpn's of surfactant-producing hydrocarbon-oxidizing microorganisms for the infiltration gallery.

Landfarm

Enumeration of Hydrocarbon-Oxidizing Microorganisms

Averaged numbers of surfactant-producing hydrocarbon-degrading microorganisms estimated by the most probable technique are summarized in Table 3.

Table 3. Summary of Microbial Enumeration Data-Soil

Time	Average Cell Numbers (Cells/g dry wt.)	Grid Number
Pre-Fertilization	10^5 10^6	1 2, 4, 5, 6, 7, 8, 9
Week 2	10^6 10^7	1, 2, 3, 9 4, 5, 6, 7, 8
Week 5	10^6 10^7	1, 3, 6, 9 2, 4, 5, 7, 8
Week 7	10^6 10^7	All Grids except Grid 7 7
Week 9	10^5 10^6 10^7	6, 8, 9 1, 2, 3, 5, 7 4

Figure 2 shows the number of hydrocarbon-oxidizing microorganisms for each grid over the whole sampling season. Figures 3, 4, and 5 compare the temporal relationship of microbial numbers over the sampling period. As can be seen, week 2 of fertilization resulted in an increase in the microbial numbers in all grids except grids 2 and 3. Samples collected during week 5 began a general decline that continued all the way to week 9. Week 5 microbial numbers were lower than week 2, but still higher than the prefertilization samples. All samples collected from week 7 are lower than week 5. Grids 1, 6, and 7 are

Figure 2: Comparison of Hydrocarbon-Degrading Microorganism Numbers for the Landfarm Soil Samples

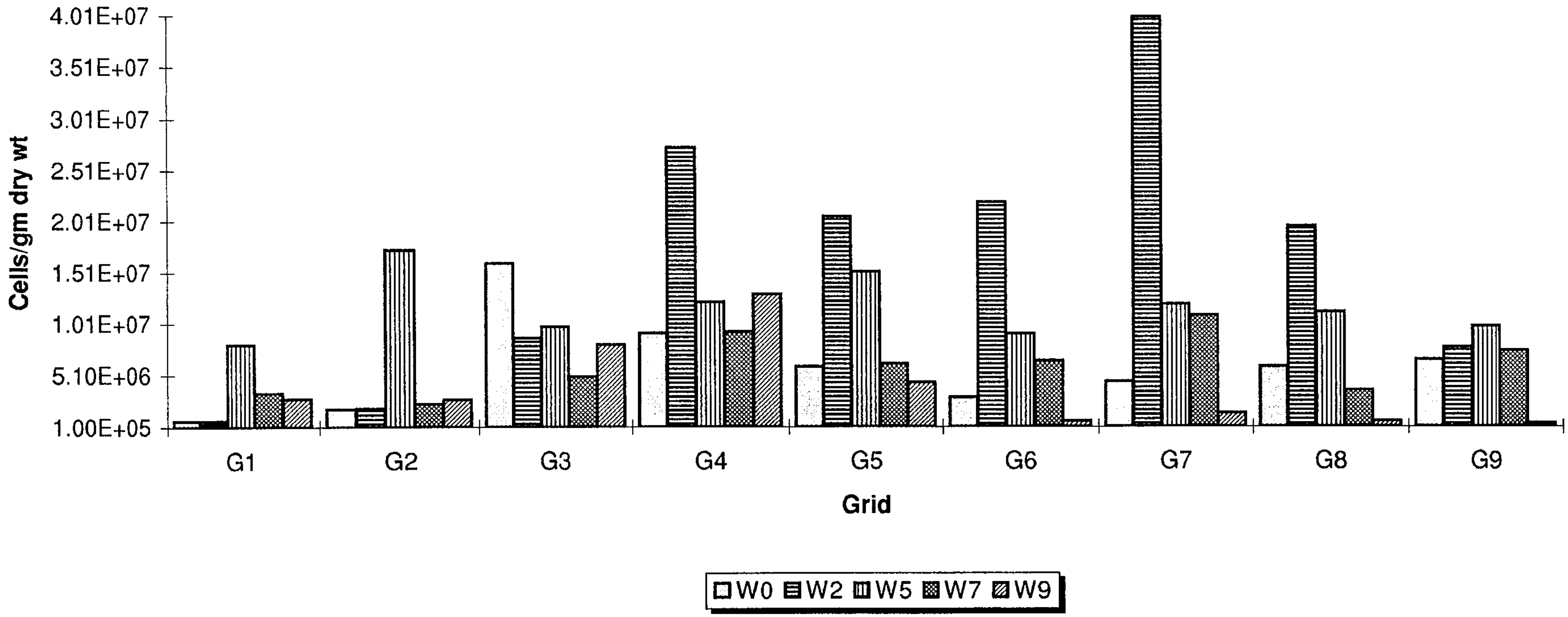


Figure 3: Comparison of Hydrocarbon-Metabolizing Microorganisms Over Time: Landfarm Grids 1-3

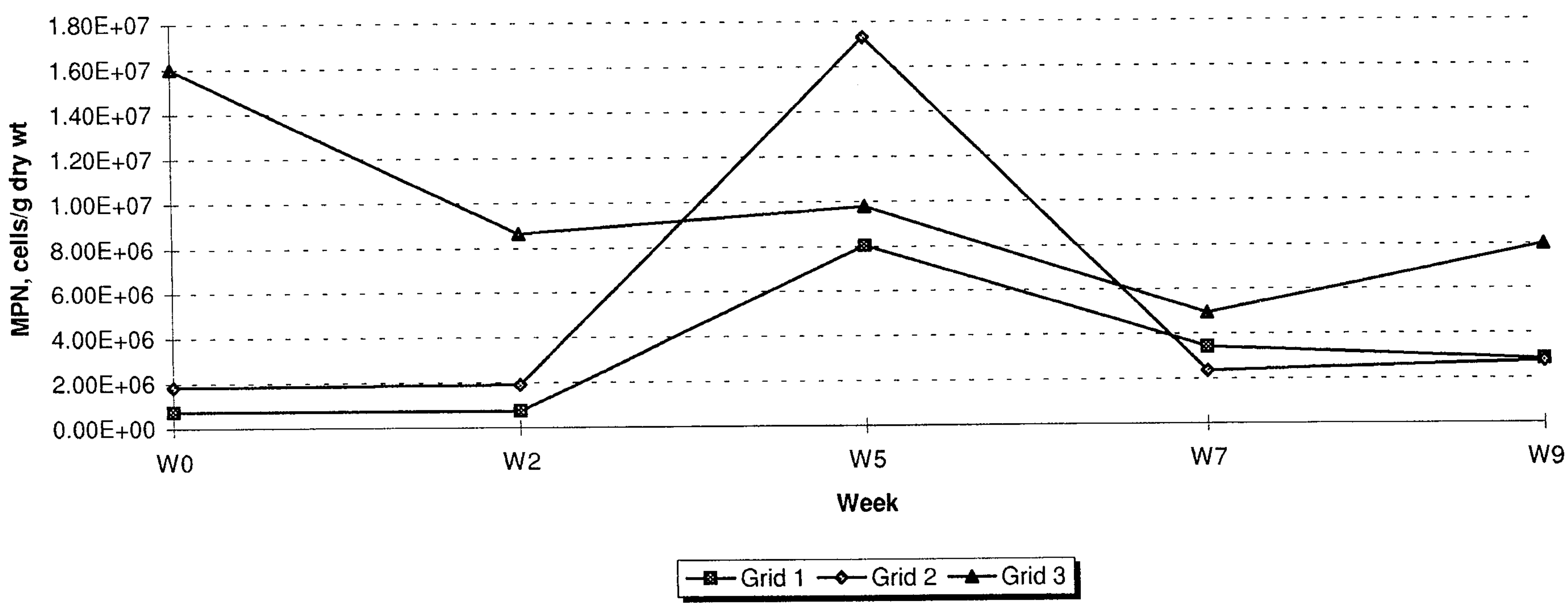


Figure 4: Comparison of Hydrocarbon-Metabolizing Microorganisms Over Time: Landfarm Grids 4-6

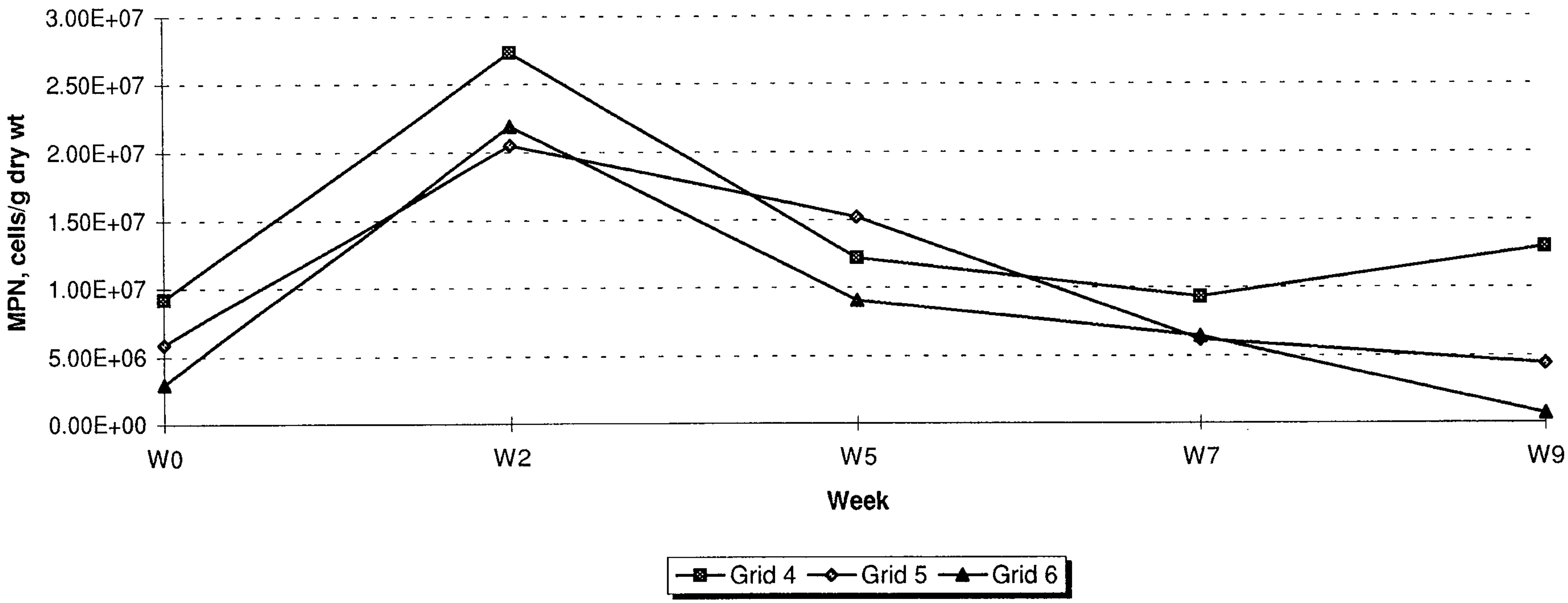
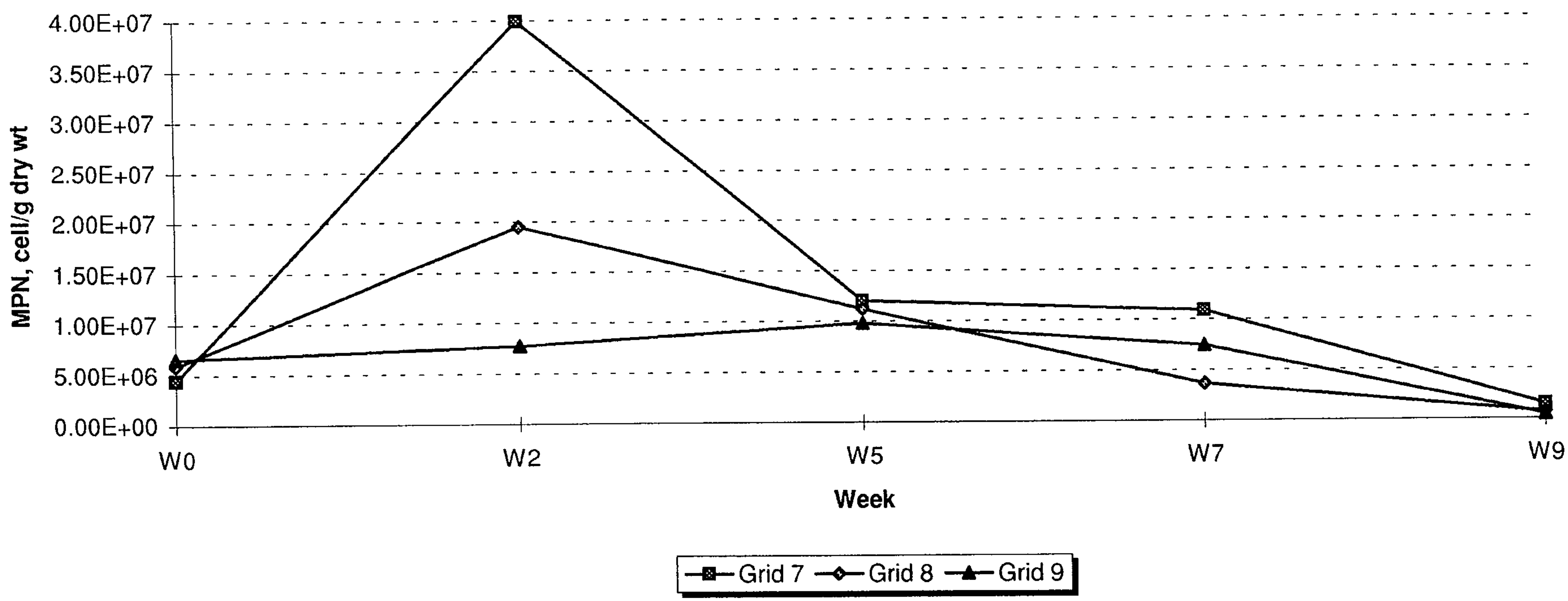


Figure 5: Comparison of Hydrocarbon-Metabolizing Microorganisms Over Time: Landfarm Grids 6-9



still higher than the prefertilization samples, but the remaining 6 grids are lower. Week 9 continued the general decline with all grids except 3 and 4 being lower than week 7.

Hydrocarbon Oxidizing Potential

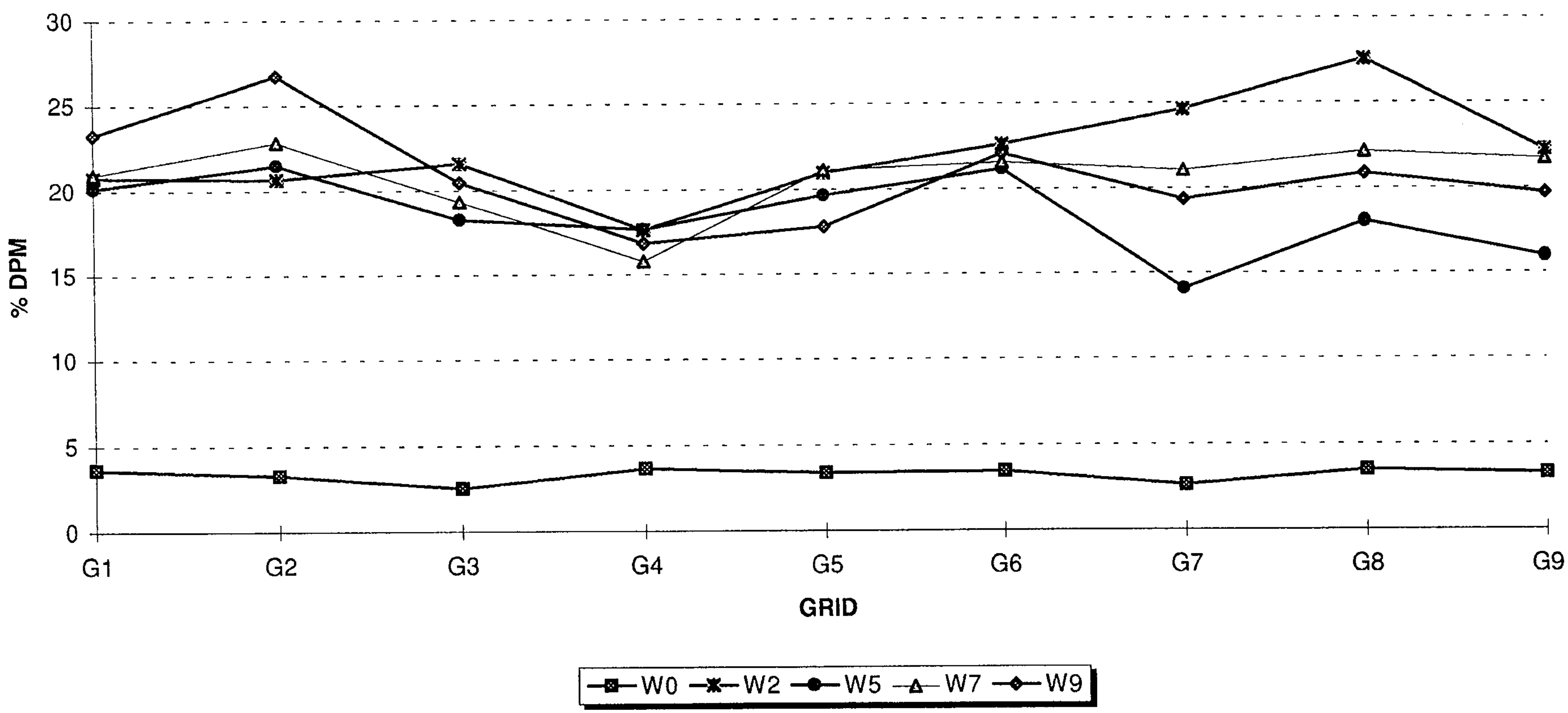
Hexadecane mineralization potentials for soil samples are summarized in Table 4.

Table 4. Hexadecane Mineralization Rates for Soil Samples (-NP)

Time	Average Hexadecane Mineralization (%)	Grid Number
Pre-Fertilization	>3%	1, 2, 4, 5, 6, 8, 9
	>2.5%	3, 7
Week 2	>25%	8
	>20%	1, 2, 3, 5, 6, 7, 9
	>15%	4
Week 5	>20%	1, 2, 6
	>15%	3, 4, 5, 8, 9
	>10%	7
Week 7	>20%	1, 2, 5, 6, 7, 8, 9
	>15%	3, 4
Week 9	>25%	1, 2, 3, 6, 8
	>15%	4, 5, 7, 9

Using ANOVA, all grids had increases in activity measurements that were significantly higher than the prefertilization samples (week 0). Figure 6 illustrates this difference by comparing the samples incubated in medium that lacked nitrogen and phosphorous (-NP). Prefertilization samples remained below 5% mineralization indicating that activity was low. Immediately after fertilization, activity measurements increased to 15% mineralization and above for the remainder of the sampling season. All samples incubated in

Figure 6: Comparison of Hexadecane Mineralization Potentials for Landfarm Soil Samples Incubated in (-N,P) Medium



Bushnell-Haas Medium (see Figure 7) maintained high mineralization potentials. Figures 8, 9, 10, 11, and 12 show the correlation between mineralization potentials and most probable numbers of hydrocarbon-oxidizing microorganisms. As stated above, microbial numbers and activity measurements increased after the first fertilization (week 2). Activity measurements remained high during the sampling season while the MPN numbers slowly declined.

Infiltration Gallery

Enumeration of Hydrocarbon-Degrading Microorganisms

Table 5 summarizes the microbial enumeration data of samples taken from the infiltration gallery. During weeks 0, 4, and 8 all wells at the FIA bioremediation demonstration project were sampled. These three sampling weeks were used to track the progress of bioenhancement. Figure 13 compares the MPN numbers with the samples from individual wells over weeks 0, 4, and 8. Figures 14, 15, 16, and 17, trace the temporal relationship of microbial numbers over time. Prefertilization samples indicate that the microbial numbers range from 10^3 to 10^4 except for wells B4 and IGWW. Once the infiltration gallery was operating, these numbers generally declined over the sampling season.

Figure 7: Comparison of Hexadecane Mineralization Potentials for Landfarm Soil Samples Incubated in BH Medium

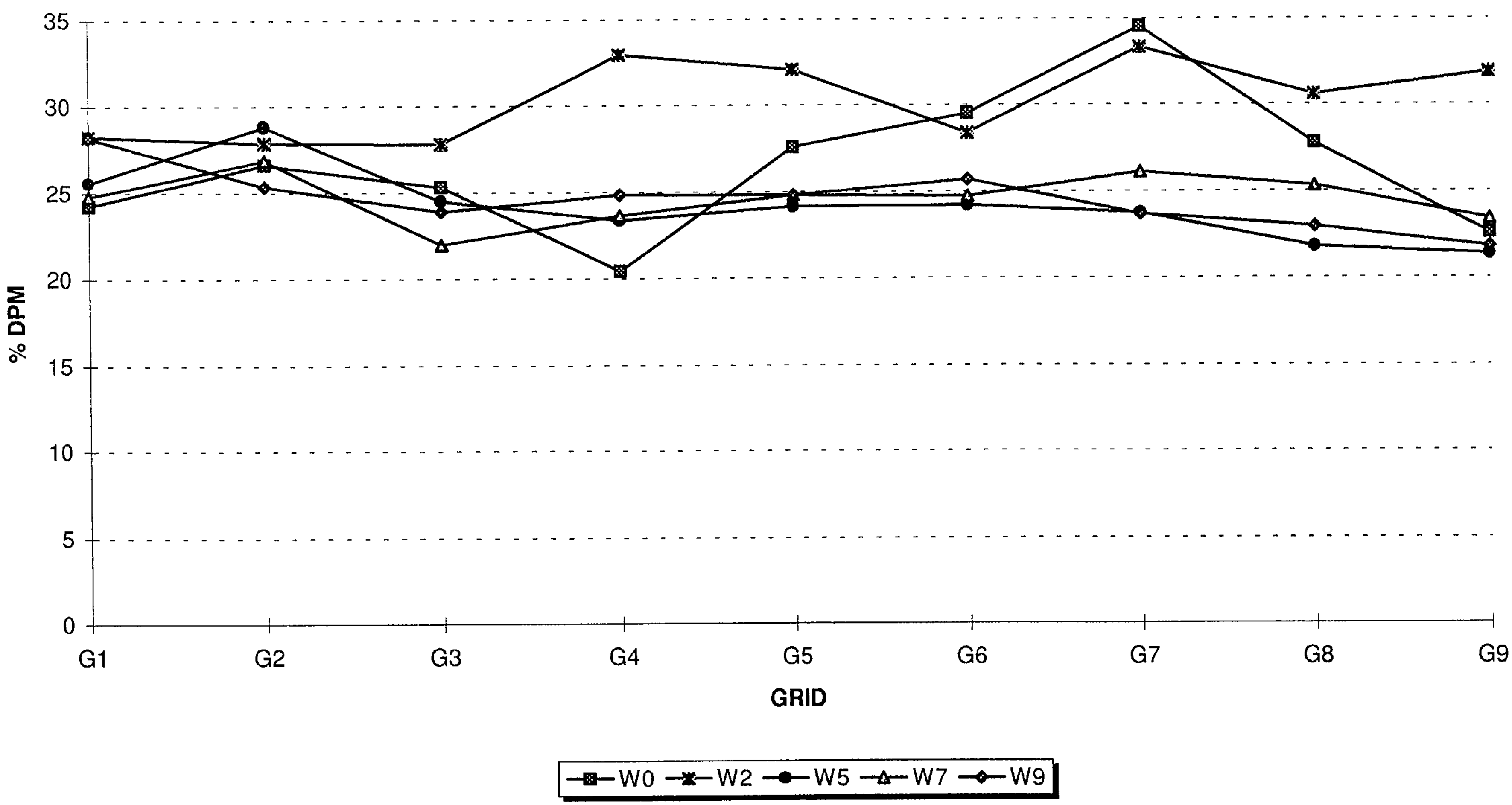


Figure 8: Comparison of Landfarm MPN Numbers with Activity Measurements Over Sampling Period-Grids 1 and 2

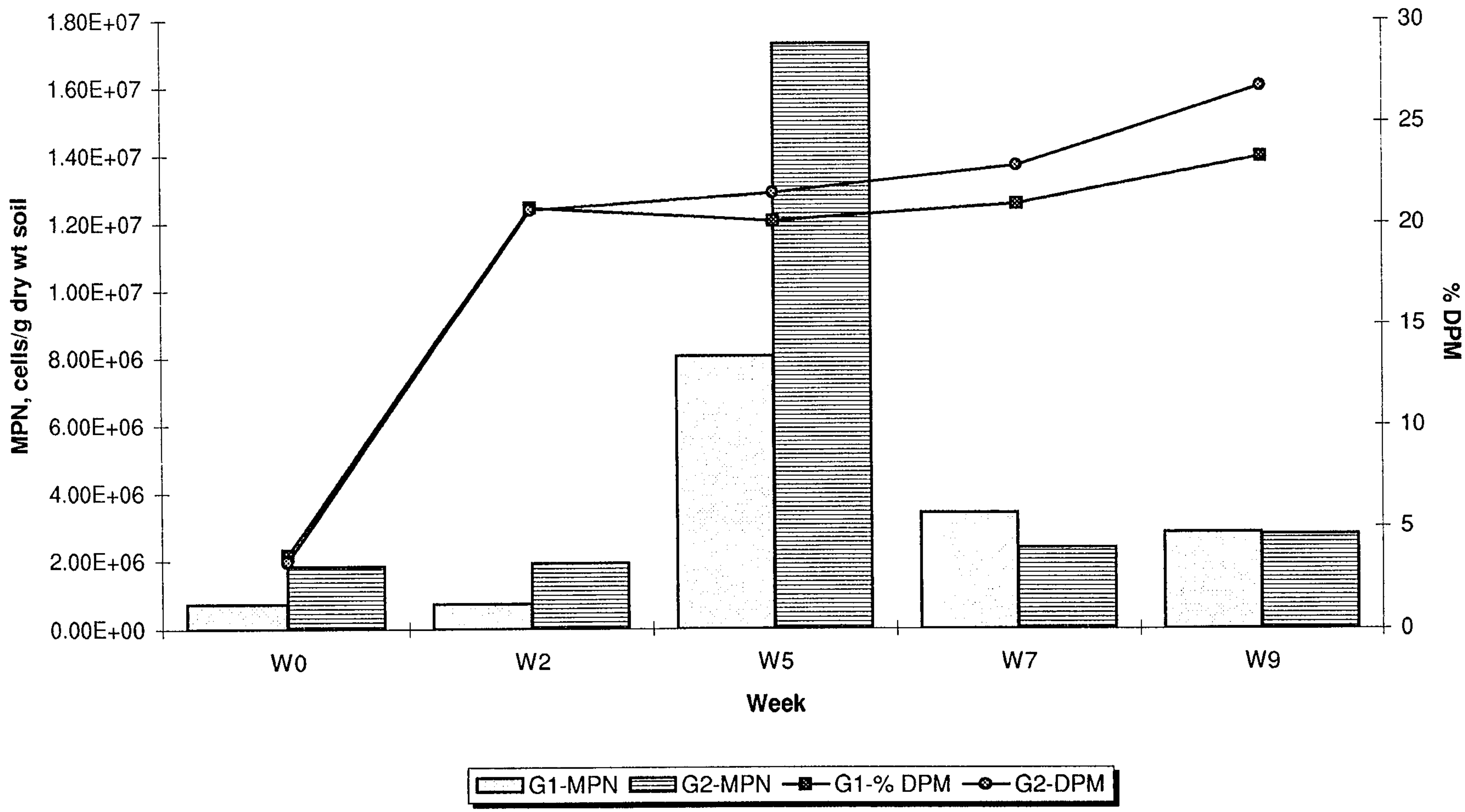


Figure 9: Comparison of Landfarm MPN Numbers and Activity Measurements Over Sampling Period-Grids 3 and 4

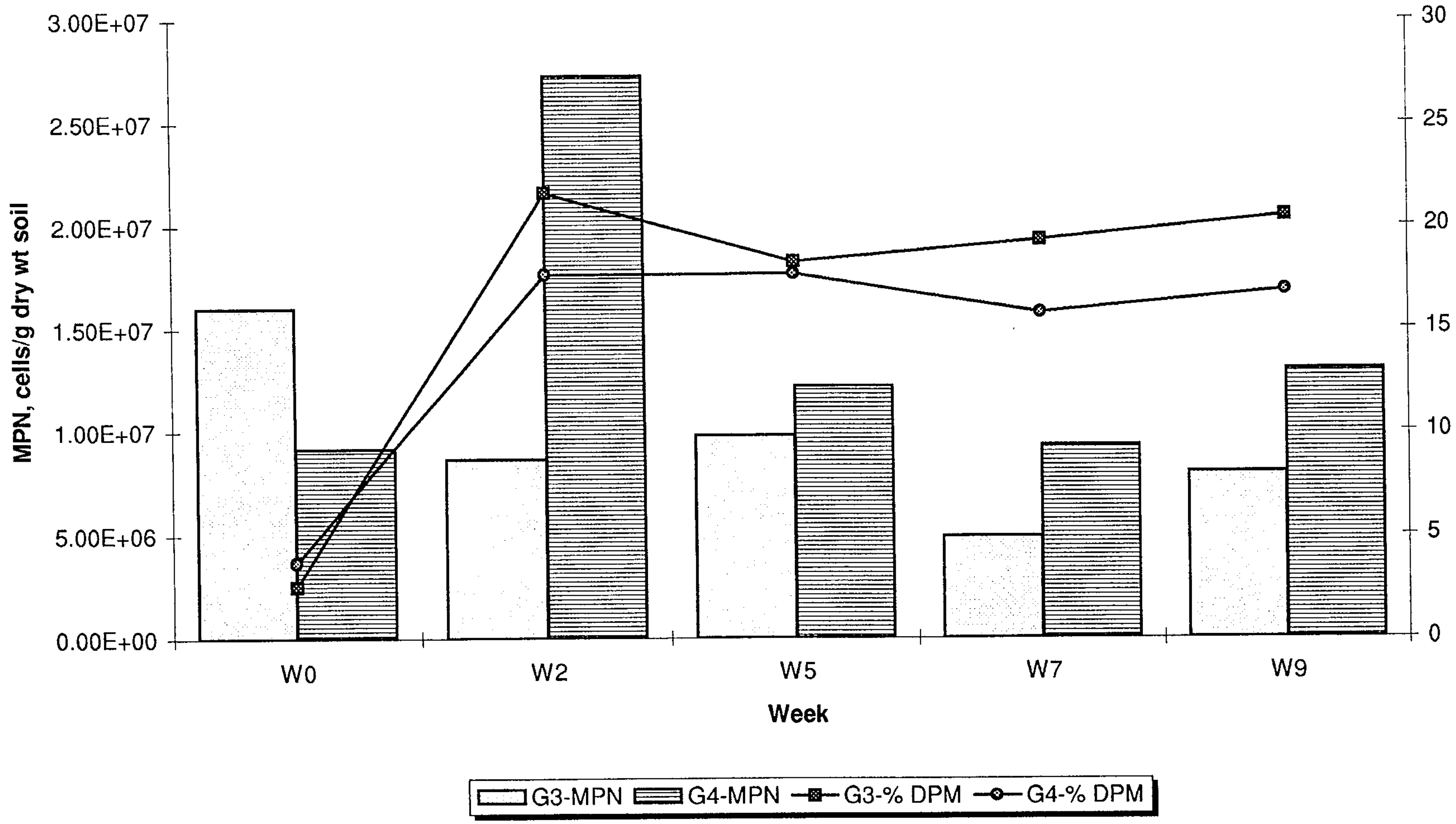


Figure 10: Comparison of Landfarm MPN Numbers and Activity Measurements Over Sampling Period-Grids 5 and 6

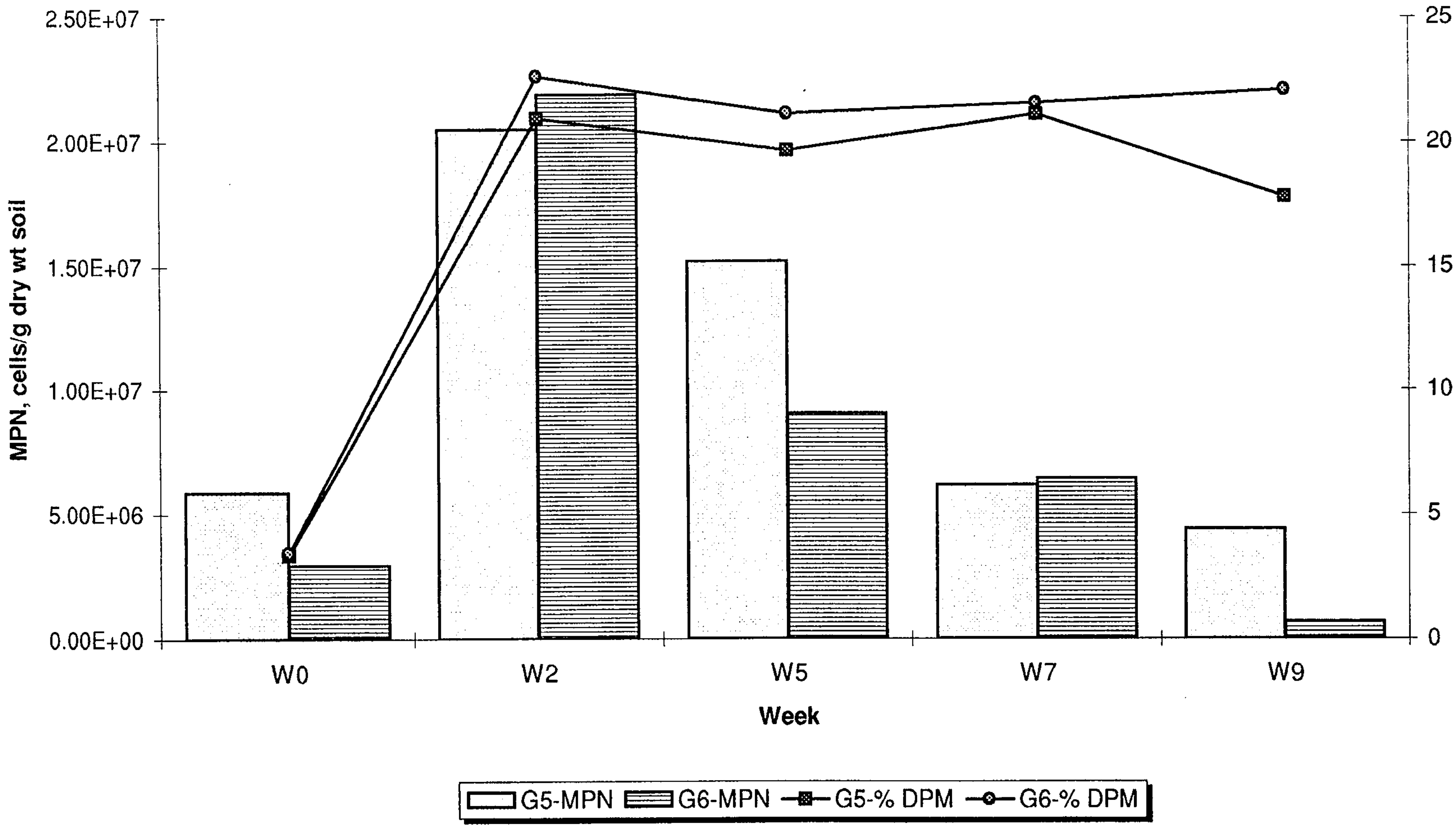


Figure 11: Comparison of Landfarm MPN Numbers and Activity Measurements Over Sampling Period-Grids 7 and 8

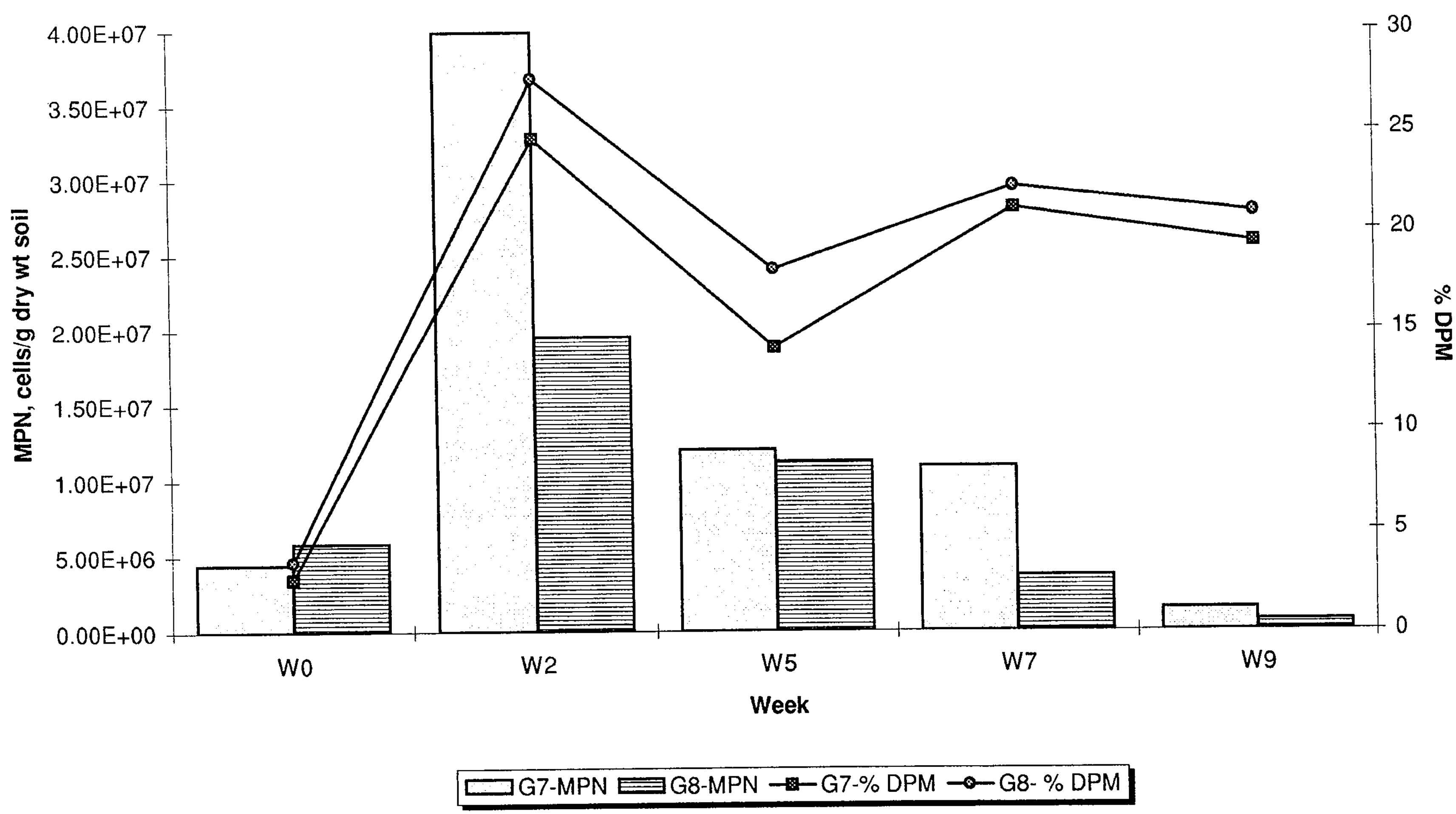


Figure 12: Comparison of Landfarm MPN Numbers and Activity Measurements Over Sampling Period-Grid 9

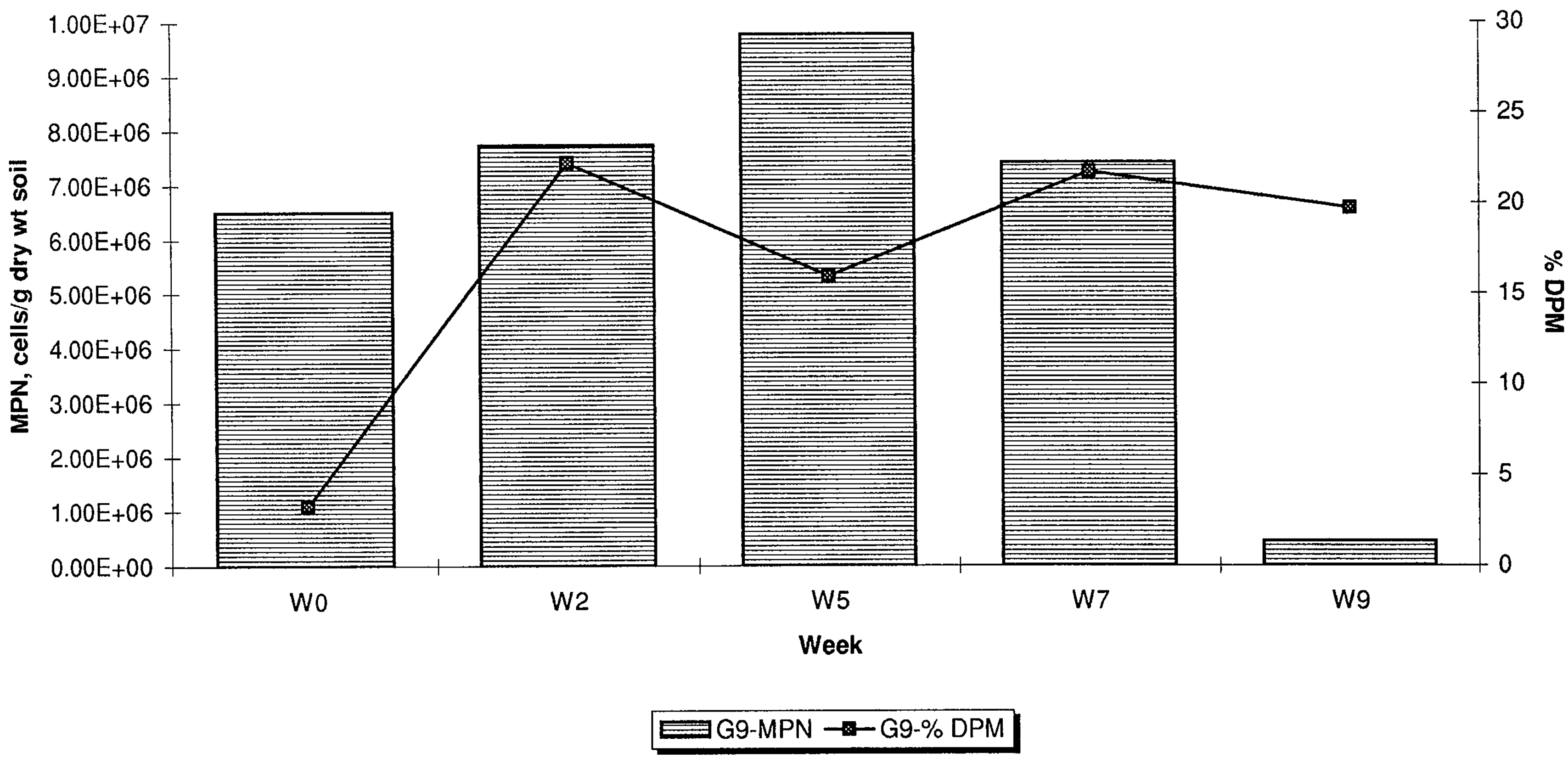


Table 5. Summary of Microbial Enumeration Data-Water

Well	Cell Numbers (cells/ml water)	Week Sampled
IG-1	10^3 10^2 10^1	Prefert, 8 2, 4 6
IG-2	10^3	Prefert, 4, 8
IG-3	10^3 10^2	Prefert, 4 8
IG-WW	10^3 10^1 10	1, Prefert, 6, 8 2, 3, 4
B-1	10^3 10^2	Prefert 4, 8
B-2	10^3 10^2	Prefert 4, 8
B-3	10^4 10^3 10^2	Prefert 4 8
B-4	10^1 10	Prefert 4, 8
DEC-1	10^4 10^3 10^2	Prefert 2, 4 6, 8
DEC-2	10^3 10^2 10^1	Prefert, 8 4, 6 2
PTAN	10^3 10^2	Prefert, 8 4

Figure 13: Comparison of Hydrocarbon-Metabolizing Microorganism Numbers for Infiltration Gallery Water Samples-Weeks 0, 4, and 8

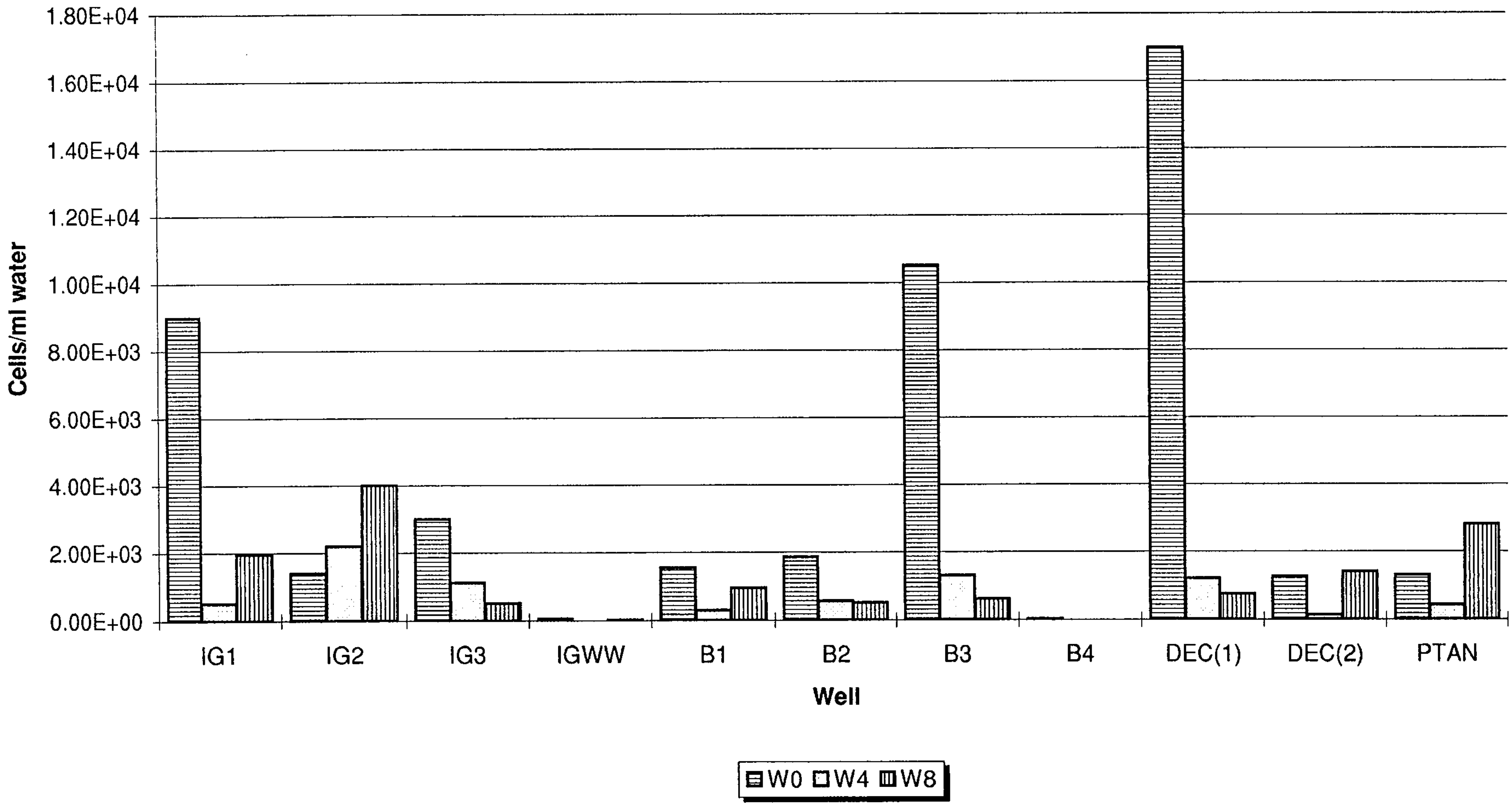


Figure 14: Comparison of Hydrocarbon-Metabolizing Microorganism Numbers Over Time-Infiltration Gallery Well Water Samples

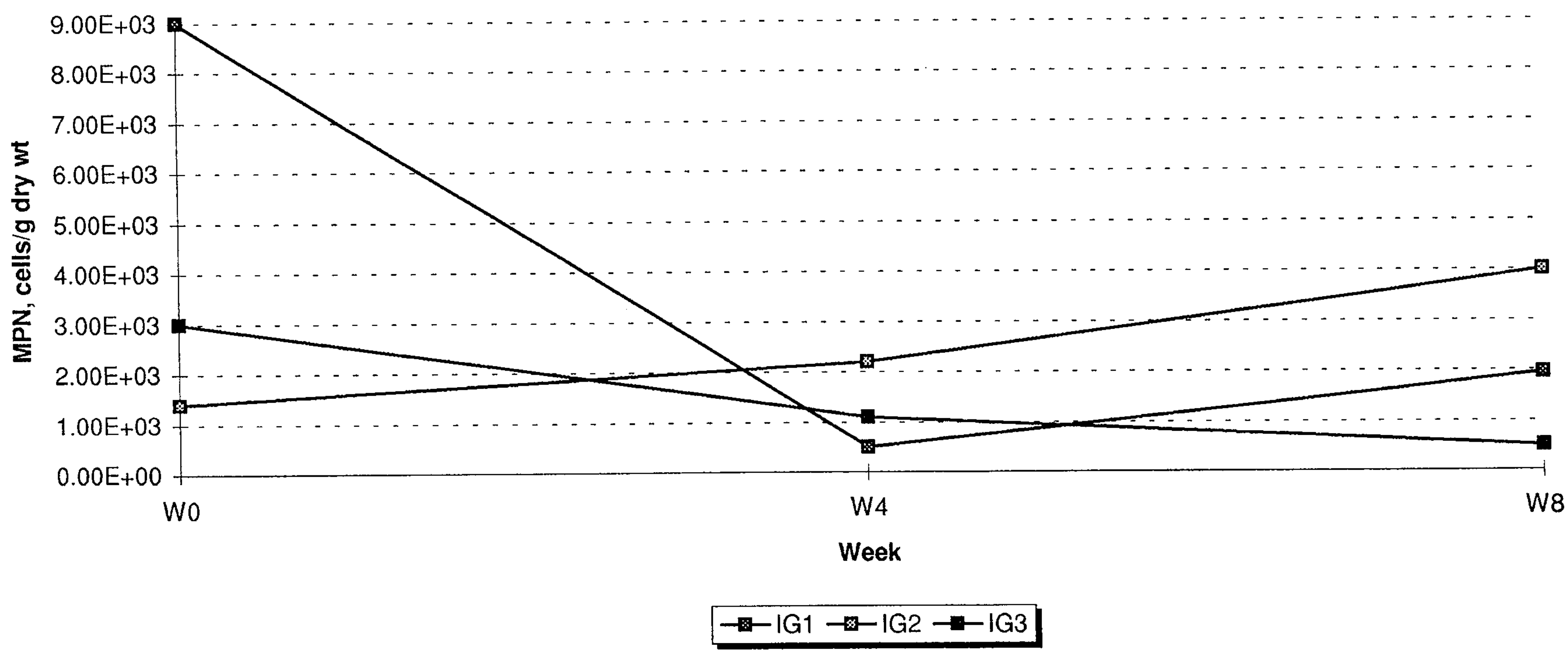


Figure 15: Comparison of Hydrocarbon-Metabolizing Microorganism Numbers Over Time-Infiltration Gallery Well Water Samples

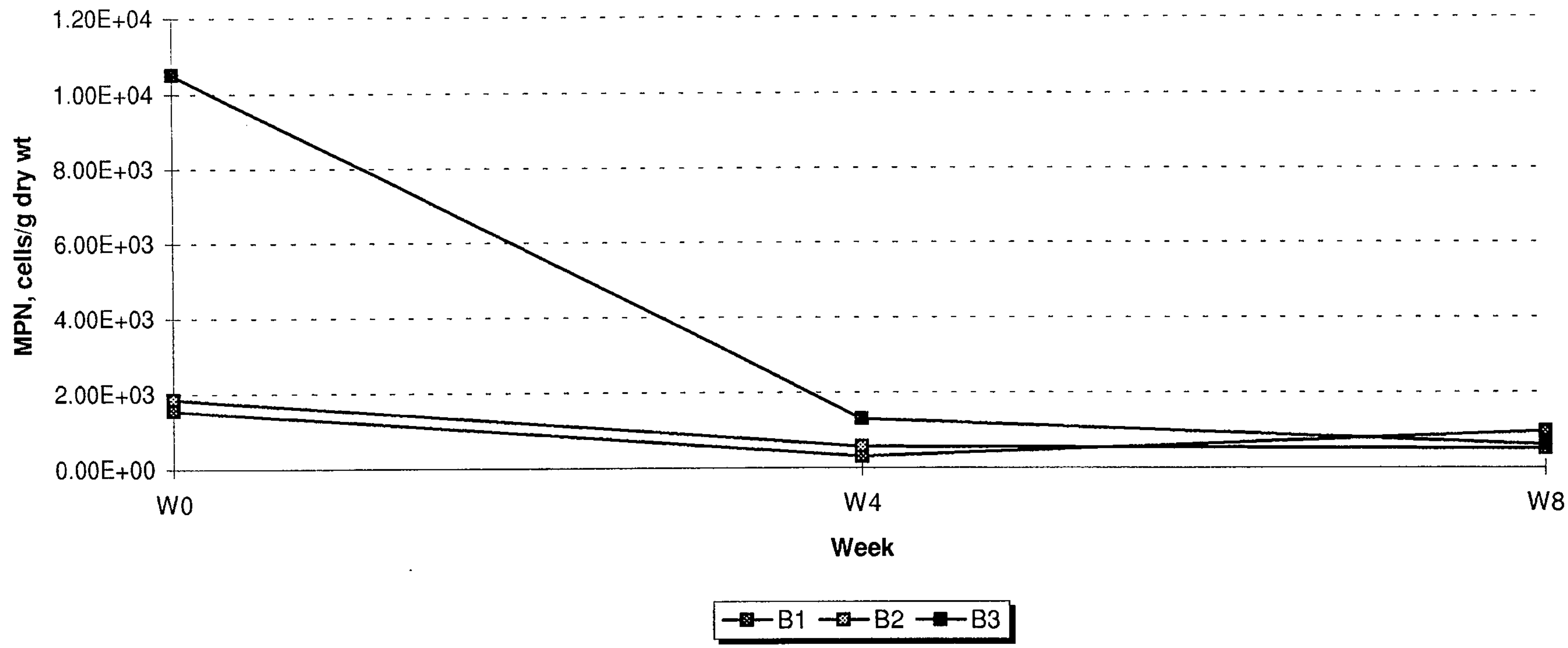


Figure 16: Comparison of Hydrocarbon-Metabolizing Microorganism Numbers Over Time- Infiltration Gallery Well Water Samples

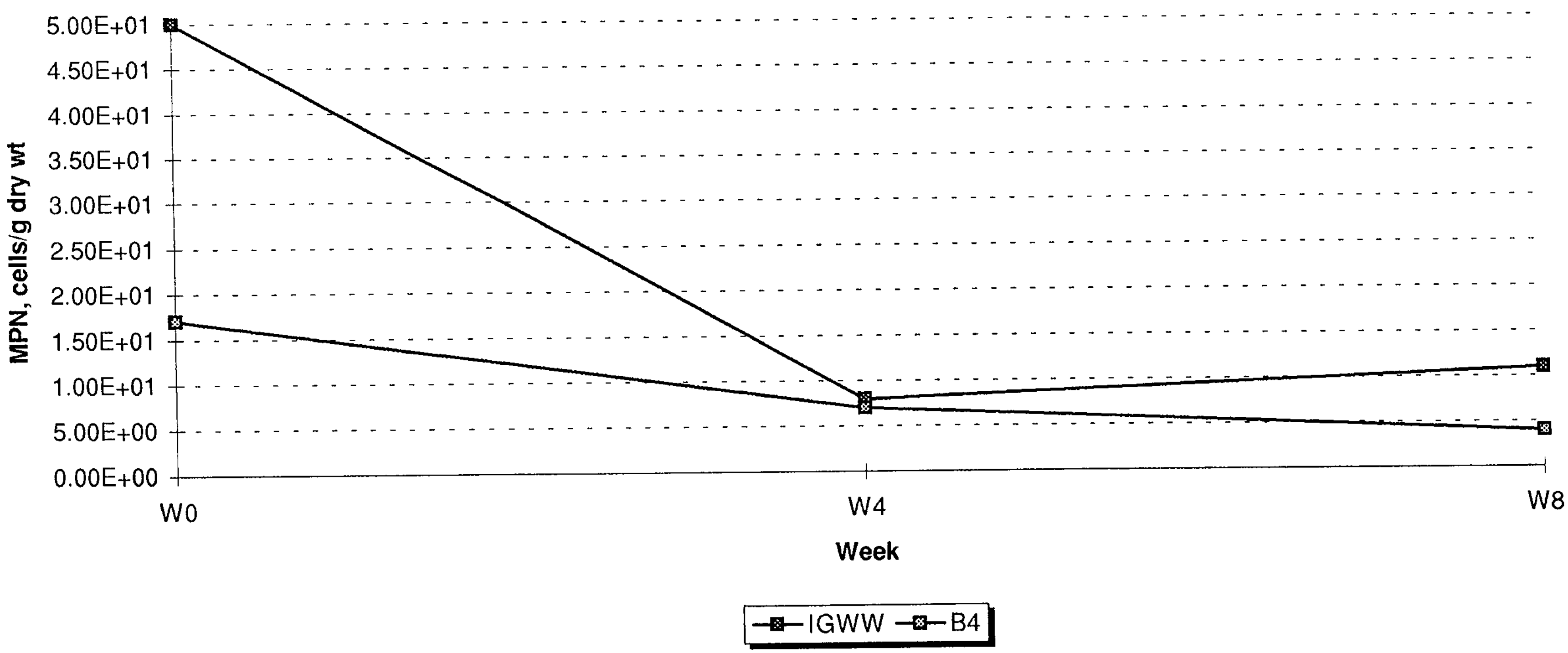
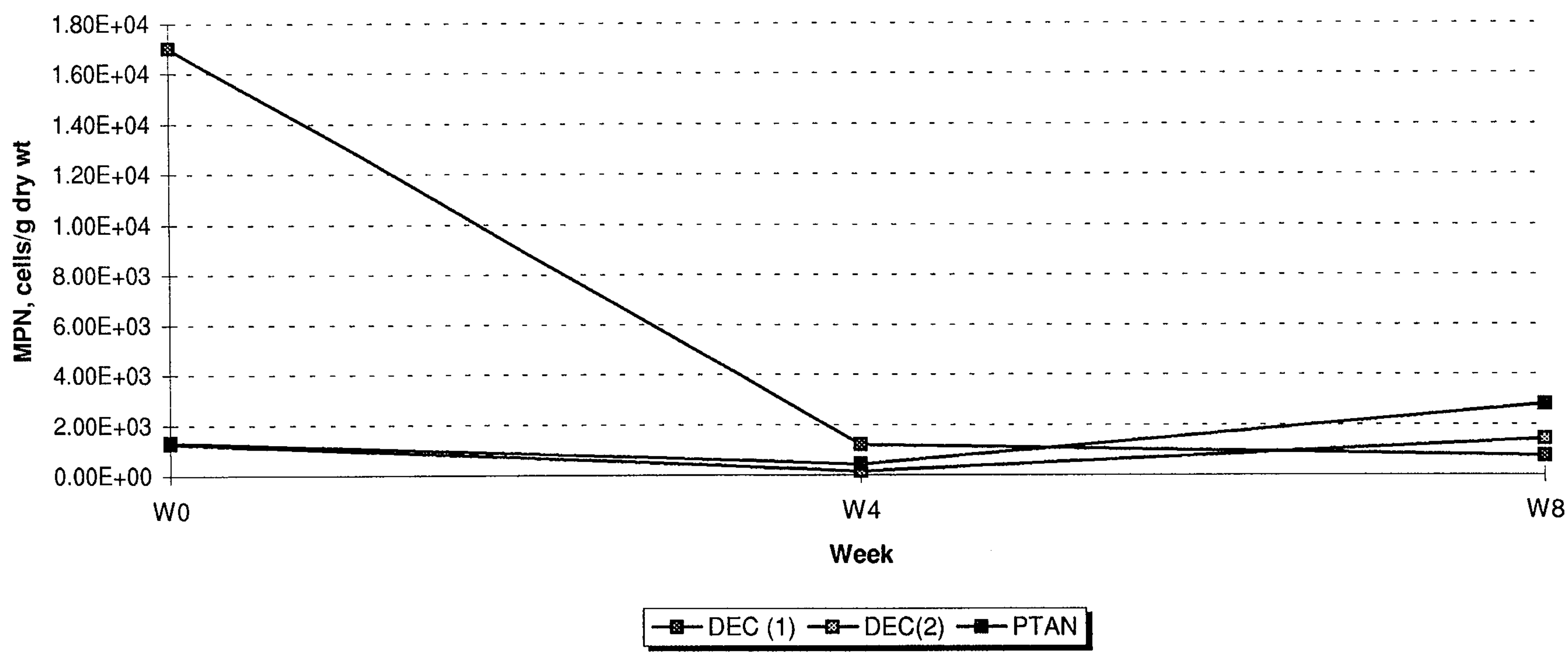


Figure 17: Comparison of Hydrocarbon-Metabolizing Microorganism Numbers Over Time-Infiltration Gallery Well Water Samples



Hydrocarbon-Oxidizing Potentials

Hexadecane mineralization rates for the infiltration gallery water samples are summarized in Table 6. Figure 18 compares the samples incubated in

Table 6. Hexadecane Mineralization Rates for Water Samples (-NP)

Well	Mineralization Potential	Week
IG-1	>3.0% >0.1% >0.05%	Prefert 2, 6, 8 4
IG-2	>0.5% >0.1%	Prefert 4, 8
IG-3	>1.0% >0.1%	Prefert 4, 8
IG-WW	>2.5% >1.0% >0.5% >0.01%	3 2, 4 1 6, 8
B-1	>0.2% >0.02%	Prefert 4, 8
B-2	>2.5% >0.1%	4 Prefert, 8
B-3	>3.0% >0.01%	Prefert 4, 8
B-4	>0.2% 0.01%	4 Prefert, 8
DEC-1	>2.5% >0.5% >0.01%	Prefert 2, 4 6, 8
DEC-2	>3.0% >1.0% >0.01%	Prefert 2 4, 6, 8
PTAN	>1.5% >0.05%	Prefert, 4 8

Bushnell-Haas medium before fertilization with weeks 0, 4, and 8 samples incubated in N and P limiting medium. Figure 19 compares mineralization potentials of the samples incubated in Bushnell-Haas medium with samples

Figure 18: Comparison of Infiltration Gallery Hexadecane Mineralization Potentials Over Time: Prefertilization Samples Incubated With Bushnell-Haas Medium vs (-N,P) Incubated Samples

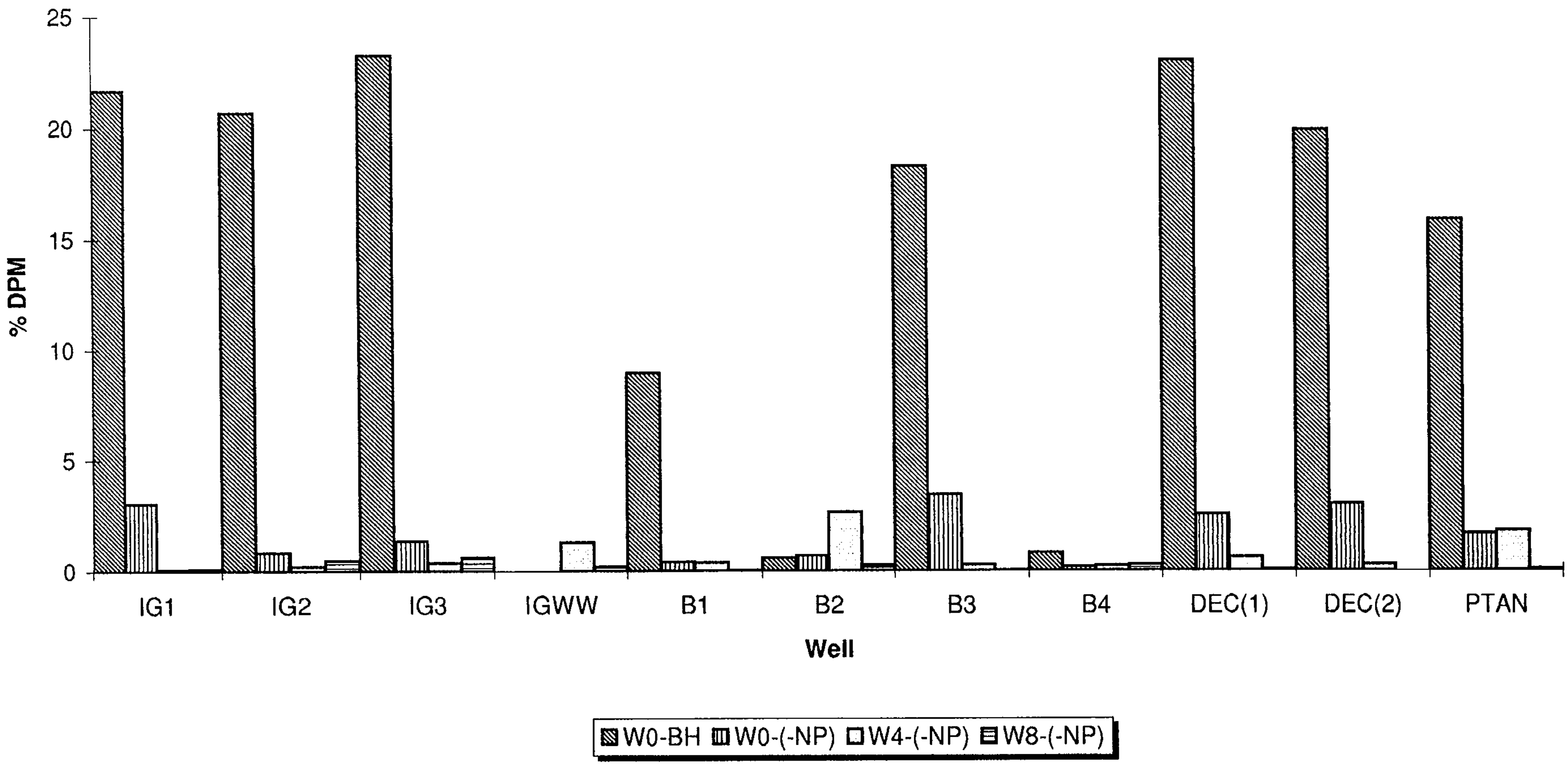
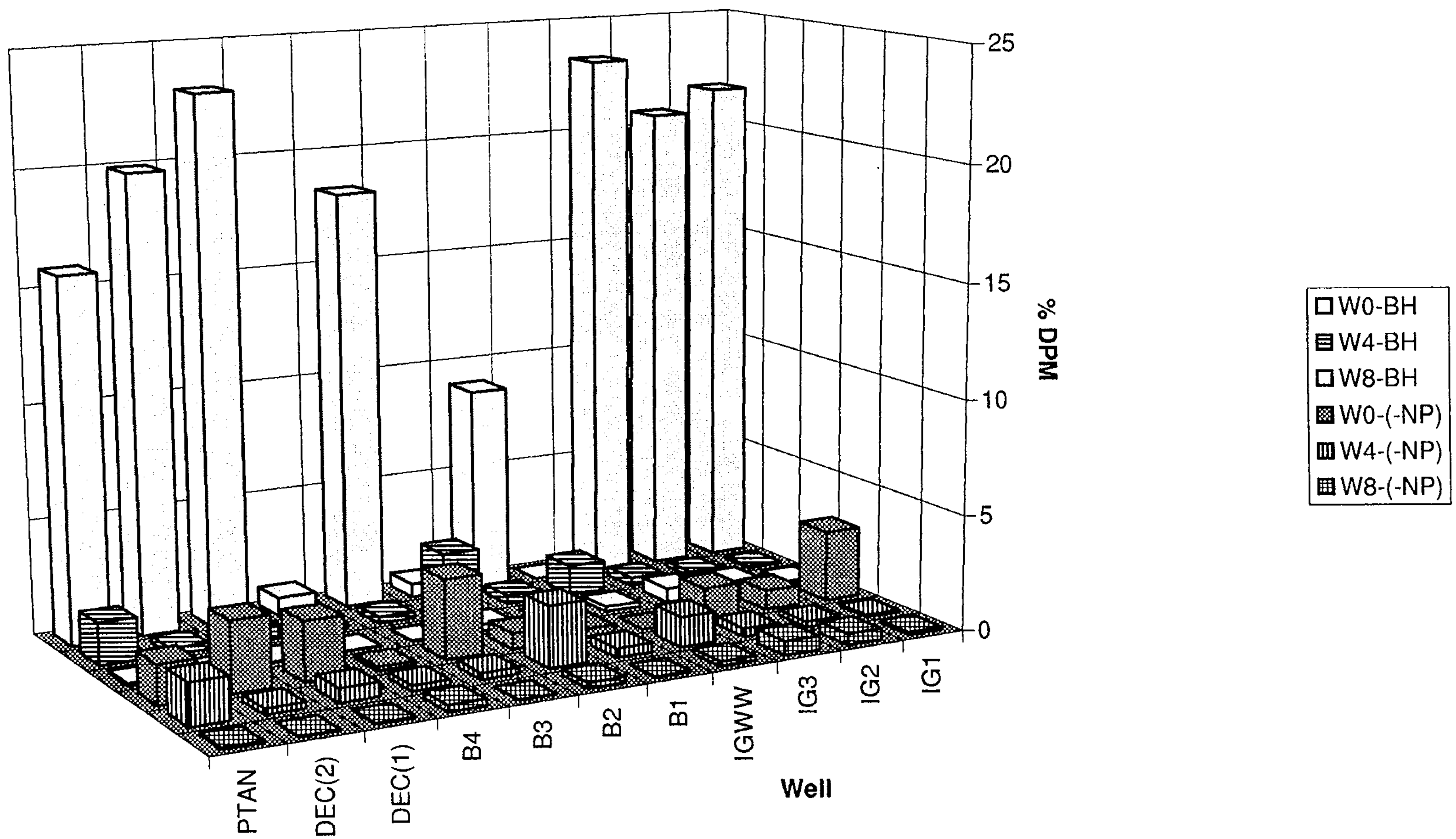


Figure 19: Comparison of Infiltration Gallery Hexadecane Mineralization Potentials-Bushnell-Haas and (-N,P) Medium



incubated in N and P limiting medium. Prefertilization samples incubated in Bushnell-Haas medium showed significant mineralization potentials except for wells B2 and B4 (see Appendix D).

DISCUSSION

Bioremediation is a technique used for cleaning hydrocarbons from soils and water. Its effectiveness may be evaluated from several perspectives. For this portion of the FIA bioremediation project, microbial populations and their metabolic activity were chosen to evaluate the efficacy of bioremediation. Hydrocarbon-degrading microorganisms are ubiquitous in nature and are capable of degrading a wide variety of petroleum hydrocarbons (Leahy and Colwell, 1990).

It has been shown that the application of oil to soils and water results in increased numbers of bacteria and fungi, with bacteria being primarily responsible for the mineralization of the petroleum-hydrocarbons (Leahy and Colwell, 1990). It is important to know whether microorganisms capable of degrading petroleum-hydrocarbons are present in soil and water samples since they are the first line of defense against environmental oil spills (Walker and Colwell, 1976). Hence, the enumeration of hydrocarbon-degrading microorganisms and the determination of hydrocarbon-oxidation potentials will reveal the ability of the indigenous microbial populations to remove petroleum contaminates. These techniques will provide valuable information over the course of treatment and will help determine if the site is suitable for bioremediation.

A variety of factors affect the rate of hydrocarbon transformation: hydrocarbon concentration, oxygen, mineral nutrient availability, temperature, pH, biomass, and acclimation of the microbial population to a particular hydrocarbon. The rates of biodegradation of hydrocarbons is a complex process that is dependent on local environmental conditions which have a great influence on the fate of the petroleum contaminate (Atlas, 1981). The factors affecting hydrocarbon degradation that were investigated in this study included microbial biomass, acclimation of that biomass to a particular hydrocarbon, mineral nutrient availability, and lastly the hydrocarbon mineralization potential of that particular biomass. It has been shown that by augmenting microbial populations and/ or metabolic activity hydrocarbon removal will be enhanced (Lindstrom et al., 1991). The initial biotreatability study prior to full scale operation indicated that the indigenous populations on site were capable of degrading the petroleum-hydrocarbons but that the nutrient levels in the soil and water were not capable of supporting growth and complete degradation of the petroleum-contaminants. This was evidenced by the high mineralization potentials for all the baseline samples incubated in Bushnell-Haas medium, which contains the major mineral nutrients needed for biological metabolism. *In Vitro* incubations in nitrogen and phosphorous limited medium resulted in significantly lower mineralization potentials, indicating that the systems were N and P limited, thereby needing nutrient amendments to accomplish enhanced biodegradation *in situ*.

Landfarm

Over the course of the measurement period, the number of hydrocarbon-degrading microorganisms showed a gradual decline but remained within the range expected for a site with a history of pollution. Tilling and moisture application (60% saturation being optimum) were not consistent over the summer and may have participated in the decrease. The reduction of available hydrocarbons due to its conversion to carbon dioxide and water may also be a contributing factor. As hydrocarbon levels drop, microbial numbers will also be expected to drop. Hydrocarbon mineralization potentials remained high over the course of the sampling period. This is an indication that nitrogen and phosphorous were not limiting and that the microbial numbers were sufficient to enhance biodegradation. The measurement of hydrocarbon biodegradation potentials is useful in assessing the relative biodegradability of the hydrocarbons within the context of that spill, but it does not measure overall petroleum biodegradation (Atlas, 1979).

Overall, the bioenhancement of the landfarm was a success.

Infiltration Gallery

The infiltration gallery still seemed to be plagued with problems. The number of hydrocarbon degrading microorganisms declined over the treatment period. This correlated to a decline in the mineralization potentials in samples incubated in Bushnell-Haas medium. Mineralization potentials for samples incubated in medium lacking N and P did not show any increase indicating that the infiltration gallery and the microorganisms therein were still nutrient limited. This would seem to indicate that the residence time of the nutrient laden water was not sufficient to support growth. The drop in MPN cell numbers and respiration potentials would indicate that the microbial populations are still being diluted by water infiltration into the gallery. Any decrease in the hydrocarbon load was probably the result of volatilization or adhesion to the soil matrix.

CONCLUSIONS

1. In the Landfarm, high numbers of hydrocarbon-degrading microorganisms coupled with high hydrocarbon mineralization potentials in Bushnell-Haas and N, P limited medium indicate an acclimated population ready and willing to degrade the petroleum contaminants throughout the sampling period. These increases were due to nutrient addition, irrigation, and tilling.

2. In the infiltration gallery, there were no substantial increases in the microbial populations or in the mineralization potentials over the sampling period.

3. A decline in mineralization potentials and microbial numbers were witnessed in the infiltration gallery indicating that biodegradation was not taking place.

4. The infiltration gallery most likely has a design flaw that is preventing any significant microbial oxidation of the petroleum contaminants, thus

preventing any subsequent biological treatment of the water and surrounding soils.

The significant increase in microbial activity and the large populations of hydrocarbon-degrading microorganisms maintained in the landfarm would indirectly indicate increased biodegradation rates *in situ*. Cleaning times may be further increased by maintaining a vigilant schedule for the irrigation and nutrient amendment regime. From the biological data, there was no indication of any biodegradation taking place in the infiltration gallery. Due to the design, the nutrient levels were not being maintained within the system. Nutrient limitations in and of themselves are enough to shut down a bioremediation project.

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1992 SAMPLING DATA TAKEN FROM THE FIA BIOREMEDIATION DEMONSTRATION PROJECT

APPENDIX A

MINERALIZATION POTENTIALS (% DPM)-SOIL

TABLE1: PRE-FERTILIZATION OF SOIL

GRID 1: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	3.43	3.45	3.80	3.66
Average 3.59				

GRID 1: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	25.99	21.12	24.99	24.85
Average 24.24				

GRID 2: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	3.40	4.08	2.11	3.51
Average 3.28				

GRID 2: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	25.47	27.34	26.90	26.65
Average 26.59				

GRID 3: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	3.11	2.11	2.40	2.36
Average 2.50				

GRID 3: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	23.37	26.04	25.36	26.50
Average 25.32				

GRID 4: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	3.53	2.80	5.52	2.70
Average 3.64				

GRID 4: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	29.78	27.25	22.00	2.70
Average 20.43				

GRID 5: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	4.41	2.91	2.99	3.15
Average 3.37				

GRID 5: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	22.91	27.64	28.78	30.93
Average 27.57				

GRID 6: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	2.35	3.99	4.25	3.16
Average 3.44				

GRID 6: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	27.58	31.06	30.01	29.44
Average 29.52				

GRID 7: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	2.80	2.20	1.78	3.60
Average 2.60				

GRID 7: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	37.67	32.83	35.06	32.59
Average 34.54				

GRID 8: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	2.72	4.06	3.66	3.43
Average 3.47				

GRID 8: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	28.18	27.29	27.35	28.29
Average 27.78				

GRID 9: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	2.24	2.24	3.84	4.73
Average 3.26				

GRID 9: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	25.92	22.02	18.93	23.43
Average 22.58				

TABLE 2: WEEK 2 FERTILIZATION OF SOIL

GRID 1: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	26.34	16.26	15.34	25.03
Average 20.74				

GRID 1: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	26.37	28.72	27.16	30.69
Average 28.24				

GRID 2: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	21.23	22.98	19.06	19.34
Average 20.65				

GRID 2: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	25.68	29.78	27.51	28.53
Average 27.88				

GRID 3: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	17.40	25.64	23.28	19.98
Average 21.58				

GRID 3: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	26.58	29	28.16	27.86
Average 27.80				

GRID 4: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	19.69	16.98	13.21	20.50
Average 17.60				

GRID 4: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	30.86	31.08	33.47	36.35
Average 32.94				

GRID 5: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	14.77	19.67	26.04	23.28
Average 20.94				

GRID 5: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	34.34	33.78	29.33	30.78
Average 32.06				

GRID 6: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	23.25	22.64	26.22	18.31
Average 22.61				

GRID 6: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	26.93	29.83	30.80	25.90
Average 28.37				

GRID 7: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	22.31	17.13	21.03	37.91
Average 24.60				

GRID 7: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	34.53	32.20	32.38	34.15
Average 33.32				

GRID 8: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	20.74	32.17	26.44	30.94
Average 27.57				

GRID 8: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	30.13	29.74	31.42	31.03
Average 30.58				

GRID 9: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	25.77	23.26	16.97	22.97
Average 22.24				

GRID 9: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	27.26	36.37	33.80	30.07
Average 31.88				

TABLE 3: WEEK 5 FERTILIZATION OF SOIL

GRID 1: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	21.98	24.70	15.10	18.64
Average 20.11				

GRID 1: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	22.96	28.38	25.07	25.80
Average 25.55				

GRID 2: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	22.42	23.68	18.01	21.83
Average 21.49				

GRID 2: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	24.63	27.41	26.92	36.32
Average 28.82				

GRID 3: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	15.95	15.33	20.71	20.96
Average 18.24				

GRID 3: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	25.89	24.71	26.88	20.46
Average 24.49				

GRID 4: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	16.12	12.28	21.32	20.89
Average 17.65				

GRID 4: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	25.00	20.97	20.77	26.65
Average 23.35				

GRID 5: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	18.86	20.71	18.98	19.95
Average 19.63				

GRID 5: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	23.38	24.92	23.69	24.54
Average 24.13				

GRID 6: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	22.35	21.09	20.15	20.92
Average 21.13				

GRID 6: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	23.98	23.02	24.75	25.04
Average 24.20				

GRID 7: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	13.65	17.48	12.84	12.37
Average 14.09				

GRID 7: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	23.51	22.35	25.40	23.65
Average 23.73				

GRID 8: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	22.14	17.85	14.64	17.44
Average 18.02				

GRID 8: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	22.15	20.26	21.84	22.81
Average 21.77				

GRID 9: (-NP)

Sample	1	2	3
Mineralization Potential (% dpm)	15.59	17.00	15.33
Average 15.97			

GRID 9: (BH)

Sample	1	2	3
Mineralization Potential (% dpm)	17.94	23.62	22.49
Average 21.35			

TABLE 4: WEEK 7 FERTILIZATION OF SOIL

GRID 1: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	22.59	19.54	16.32	25.25
Average 20.93				

GRID 1: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	25.46	25.03	17.55	31.02
Average 24.77				

GRID 2: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	27.72	22.82	22.32	18.50
Average 22.84				

GRID 2: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	30.26	28.19	24.5	24.44
Average 26.85				

GRID 3: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	23.69	17.95	15.42	20.04
Average 19.28				

GRID 3: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	22.18	17.3	24.18	24.21
Average 21.97				

GRID 4: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	13.89	13.78	17.18	18.13
Average 15.75				

GRID 4: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	22.38	27.21	24.87	20.13
Average 23.65				

GRID 5: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	14.73	23.14	26.92	19.60
Average 21.10				

GRID 5: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	20.96	25.29	26.35	26.65
Average 24.81				

GRID 6: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	20.51	19.77	25.11	20.75
Average 21.54				

GRID 6: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	25.35	27.13	26.20	20.18
Average 24.72				

GRID 7: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	16.14	18.60	25.55	23.94
Average 21.06				

GRID 7: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	26.45	23.56	26.73	27.71
Average 26.11				

GRID 8: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	21.22	22.00	23.76	21.6
Average 22.15				

GRID 8: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	25.29	25.13	23.42	27.44
Average 25.32				

GRID 9: (-NP)

Sample	1	2	3
Mineralization Potential (% dpm)	25.56	19.83	19.77
Average 21.72			

GRID 9: (BH)

Sample	1	2	3
Mineralization Potential (% dpm)	26.04	24.06	20.03
Average 23.38			

TABLE 5: WEEK 9 FERTILIZATION OF SOIL

GRID 1: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	16.97	22.69	27.54	25.83
Average 23.26				

GRID 1: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	23.99	36.12	27.38	25.28
Average 28.19				

GRID 2: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	28.38	27.93	28.52	22.07
Average 26.73				

GRID 2: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	25.97	29.68	24.13	21.57
Average 25.34				

GRID 3: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	18.11	16.14	24.1	23.47
Average 20.46				

GRID 3: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	21.80	24.73	24.53	24.53
Average 23.90				

GRID 4: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	18.06	16.64	15.68	16.93
Average 16.83				

GRID 4: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	25.53	25.31	24.11	24.43
Average 24.85				

GRID 5: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	19.03	19.03	17.74	15.35
Average 17.79				

GRID 5: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	25.60	24.93	24.05	24.60
Average 24.80				

GRID 6: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	19.03	22.96	21.76	24.54
Average 22.07				

GRID 6: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	23.06	24.64	31.57	23.48
Average 25.69				

GRID 7: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	17.92	15.43	19.66	24.29
Average 19.33				

GRID 7: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	23.98	23.57	25.63	21.53
Average 23.68				

GRID 8: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	20.86	18.82	22.47	21.26
Average 20.85				

GRID 8: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	26.43	23.90	20.39	21.03
Average 22.94				

GRID 9: (-NP)

Sample	1	2	3	4
Mineralization Potential (% dpm)	19.58	21.64	21.77	15.82
Average 19.70				

GRID 9: (BH)

Sample	1	2	3	4
Mineralization Potential (% dpm)	20.00	24.70	22.51	19.91
Average 21.78				

APPENDIX B

MICROBIAL ENUMERATION DATA-SOIL

TABLE 1: HYDROCARBON DEGRADER NUMBERS-PRE FERTILIZATION

GRID 1

Sample	1	2	3	4
MPN Numbers	7.00E+05	3.50E+05	8.00E+05	7.00E+05
(cells/gram) wet wt.	8.00E+05	3.50E+05	3.00E+05	7.00E+05
Average	7.50E+05	3.50E+05	5.50E+05	7.00E+05
% Dry Wt.	0.78	0.83	0.83	0.80
Corrected MPN	9.62E+05	4.22E+05	6.63E+05	8.75E+05

GRID 2

Sample	1	2	3	4
MPN Numbers	1.10E+06	7.00E+05	3.00E+06	1.40E+06
(cells/gram) wet wt.	1.10E+06	7.00E+05	3.00E+06	1.10E+06
Average	1.10E+06	7.00E+05	3.00E+06	1.25E+06
% Dry Wt.	0.80	0.83	0.83	0.82
Corrected MPN	1.38E+06	8.43E+05	3.61E+06	1.52E+06

GRID 3

Sample	1	2	3	4
MPN Numbers	1.70E+06	1.70E+06	8.00E+07	1.10E+06
(cells/gram) wet wt.	1.40E+06	2.20E+06	1.80E+07	1.10E+06
Average	1.55E+06	1.95E+06	4.90E+07	1.10E+06
% Dry Wt.	0.80	0.80	0.84	0.81
Corrected MPN	1.94E+06	2.44E+06	5.83E+07	1.36E+06

GRID 4

Sample	1	2	3	4
MPN Numbers	3.00E+06	3.00E+06	2.20E+07	5.00E+06
(cells/gram) wet wt.	3.00E+06	3.00E+06	1.70E+07	5.00E+06
Average	3.00E+06	3.00E+06	1.95E+07	5.00E+06
% Dry Wt.	0.82	0.85	0.83	0.83
Corrected MPN	3.66E+06	3.53E+06	2.35E+07	6.02E+06

GRID 5

Sample	1	2	3	4
MPN Numbers	5.00E+06	5.00E+06	7.00E+06	5.00E+05
(cells/gram) wet wt.	5.00E+06	3.00E+06	1.30E+07	3.00E+05
Average	5.00E+06	4.00E+06	1.00E+07	4.00E+05
% Dry Wt.	0.82	0.83	0.83	0.83
Corrected MPN	6.10E+06	4.85E+06	1.20E+07	4.82E+05

GRID 6

Sample	1	2	3	4
MPN Numbers	5.00E+06	1.30E+06	1.30E+06	1.30E+06
(cells/gram) wet wt.	5.00E+06	1.30E+06	1.70E+06	2.40E+06
Average	5.00E+06	1.30E+06	1.50E+06	1.85E+06
% Dry Wt.	0.82	0.80	0.82	0.82
Corrected MPN	6.10E+06	1.63E+06	1.83E+06	2.26E+06

GRID 7				
Sample	1	2	3	4
MPN Numbers	3.00E+06	5.00E+06	3.00E+06	3.00E+06
(cells/gram) wet wt.	5.00E+06	5.00E+06	2.30E+06	3.00E+06
Average	4.00E+06	5.00E+06	2.65E+06	3.00E+06
% Dry Wt.	0.83	0.85	0.80	0.80
Corrected MPN	4.82E+06	5.88E+06	3.31E+06	3.75E+06

GRID 8				
Sample	1	2	3	4
MPN Numbers	3.00E+06	3.00E+06	7.00E+06	5.00E+06
(cells/gram) wet wt.	5.00E+06	2.30E+06	5.00E+06	7.00E+06
Average	4.00E+06	2.65E+06	6.00E+06	6.00E+06
% Dry Wt.	0.81	0.79	0.79	0.80
Corrected MPN	4.94E+06	3.35E+06	7.59E+06	7.50E+06

GRID 9				
Sample	1	2	3	4
MPN Numbers	1.30E+06	7.00E+06	5.00E+06	8.00E+06
(cells/gram) wet wt.	1.30E+06	5.00E+06	5.00E+06	8.00E+06
Average	1.30E+06	6.00E+06	5.00E+06	8.00E+06
% Dry Wt.	0.82	0.77	0.78	0.78
Corrected MPN	1.59E+06	7.79E+06	6.41E+06	1.03E+07

TABLE 2: HYDROCARBON DEGRADER NUMBERS-WEEK 2

GRID 1

Sample	1	2	3	4
MPN Numbers	1.70E+06	8.00E+05	3.00E+06	3.00E+06
	1.10E+06	5.00E+05	5.00E+06	3.00E+06
Average	1.40E+06	6.50E+05	4.00E+06	3.00E+06
% Dry Wt.	0.83	0.81	0.83	0.80
Corrected MPN	1.69E+06	8.02E+05	4.82E+06	3.75E+06

GRID 2

Sample	1	2	3	4
MPN Numbers	1.30E+06	5.00E+05	7.00E+06	5.00E+05
	1.30E+06	8.00E+05	5.00E+05	7.00E+05
Average	1.30E+06	6.50E+05	3.75E+06	6.00E+05
% Dry Wt.	0.80	0.84	0.83	0.81
Corrected MPN	1.63E+06	7.74E+05	4.52E+06	7.41E+05

GRID 3

Sample	1	2	3	4
MPN Numbers	8.00E+06	1.70E+07	1.30E+06	5.00E+06
	1.10E+07	1.10E+07	1.30E+06	3.00E+06
Average	9.50E+06	1.40E+07	1.30E+06	4.00E+06
% Dry Wt.	0.84	0.83	0.82	0.82
Corrected MPN	1.13E+07	1.69E+07	1.59E+06	4.88E+06

GRID 4

Sample	1	2	3	4
MPN Numbers	5.00E+07	5.00E+06	1.30E+07	5.00E+07
	2.30E+07	5.00E+06	1.30E+07	2.30E+07
Average	3.65E+07	5.00E+06	1.30E+07	3.65E+07
% Dry Wt.	0.85	0.83	0.83	0.82
Corrected MPN	4.29E+07	6.02E+06	1.57E+07	4.45E+07

GRID 5

Sample	1	2	3	4
MPN Numbers	1.30E+07	1.30E+07	1.30E+07	3.00E+06
	1.70E+07	1.30E+07	1.30E+07	5.00E+07
Average	1.50E+07	1.30E+07	1.30E+07	2.65E+07
% Dry Wt.	0.83	0.82	0.83	0.82
Corrected MPN	1.81E+07	1.59E+07	1.57E+07	3.23E+07

GRID 6

Sample	1	2	3	4
MPN Numbers	5.00E+06	1.70E+07	3.00E+07	1.70E+07
	5.00E+06	2.20E+07	3.00E+07	1.70E+07
Average	5.00E+06	1.95E+07	3.00E+07	1.70E+07
% Dry Wt.	0.81	0.80	0.83	0.82
Corrected MPN	6.17E+06	2.44E+07	3.61E+07	2.07E+07

GRID 7

Sample	1	2	3	4
MPN Numbers	8.00E+07	1.70E+07	5.00E+07	8.00E+06
	5.00E+07	1.70E+07	3.00E+07	1.10E+07
Average	6.50E+07	1.70E+07	4.00E+07	9.50E+06
% Dry Wt.	0.84	0.83	0.80	0.80
Corrected MPN	7.74E+07	2.05E+07	5.00E+07	1.19E+07

GRID 8

Sample	1	2	3	4
MPN Numbers	3.00E+06	5.00E+06	3.00E+06	5.00E+07
	5.00E+06	5.00E+06	5.00E+06	5.00E+07
Average	4.00E+06	5.00E+06	4.00E+06	5.00E+07
% Dry Wt.	0.78	0.79	0.80	0.81
Corrected MPN	5.13E+06	6.33E+06	5.00E+06	6.17E+07

GRID 9

Sample	1	2	3	4
MPN Numbers	8.00E+06	1.70E+06	5.00E+06	1.10E+07
	8.00E+06	3.00E+06	5.00E+06	8.00E+06
Average	8.00E+06	2.35E+06	5.00E+06	9.50E+06
% Dry Wt.	0.82	0.79	0.79	0.80
Corrected MPN	9.76E+06	2.97E+06	6.33E+06	1.19E+07

TABLE 3: HYDROCARBON DEGRADER NUMBERS-WEEK 5

GRID 1

Sample	1	2	3	4
MPN Numbers	1.70E+06	1.30E+07	1.10E+07	1.40E+06
	1.30E+06	1.30E+07	1.10E+07	1.10E+06
Average	1.50E+06	1.30E+07	1.10E+07	1.25E+06
% Dry Wt.	0.79	0.83	0.84	0.80
Corrected MPN	1.90E+06	1.57E+07	1.31E+07	1.56E+06

GRID 2

Sample	1	2	3	4
MPN Numbers	2.20E+07	1.30E+07	7.00E+06	1.70E+07
	1.70E+07	1.10E+07	5.00E+06	2.20E+07
Average	1.95E+07	1.20E+07	6.00E+06	1.95E+07
% Dry Wt.	0.83	0.83	0.80	0.82
Corrected MPN	2.35E+07	1.45E+07	7.50E+06	2.38E+07

GRID 3

Sample	1	2	3	4
MPN Numbers	5.00E+06	1.70E+07	5.00E+06	8.00E+06
	5.00E+06	1.10E+07	5.00E+06	8.00E+06
Average	5.00E+06	1.40E+07	5.00E+06	8.00E+06
% Dry Wt.	0.83	0.82	0.81	0.81
Corrected MPN	6.02E+06	1.71E+07	6.17E+06	9.88E+06

GRID 4

Sample	1	2	3	4
MPN Numbers	7.00E+06	1.30E+07	5.00E+06	1.70E+07
	5.00E+06	8.00E+06	5.00E+06	2.20E+07
Average	6.00E+06	1.05E+07	5.00E+06	1.95E+07
% Dry Wt.	0.86	0.84	0.84	0.83
Corrected MPN	6.98E+06	1.25E+07	5.95E+06	2.35E+07

GRID 5

Sample	1	2	3	4
MPN Numbers	2.40E+07	1.10E+07	8.00E+06	2.40E+07
	1.30E+07	5.00E+06	3.00E+06	1.30E+07
Average	1.85E+07	8.00E+06	5.50E+06	1.85E+07
% Dry Wt.	0.85	0.86	0.82	0.81
Corrected MPN	2.18E+07	9.30E+06	6.71E+06	2.28E+07

GRID 6

Sample	1	2	3	4
MPN Numbers	3.00E+06	5.00E+06	8.00E+06	5.00E+06
	5.00E+06	1.60E+07	1.10E+07	5.00E+06
Average	4.00E+06	1.05E+07	9.50E+06	5.00E+06
% Dry Wt.	0.82	0.82	0.78	0.79
Corrected MPN	4.88E+06	1.28E+07	1.22E+07	6.33E+06

GRID 7

Sample	1	2	3	4
MPN Numbers	8.00E+06	1.70E+07	1.10E+07	5.00E+06
	8.00E+06	1.30E+07	1.10E+07	5.00E+06
Average	8.00E+06	1.50E+07	1.10E+07	5.00E+06
% Dry Wt.	0.80	0.80	0.83	0.82
Corrected MPN	1.00E+07	1.88E+07	1.33E+07	6.10E+06

GRID 8

Sample	1	2	3	4
MPN Numbers	5.00E+06	8.00E+06	8.00E+06	1.30E+07
	1.10E+07	8.00E+06	5.00E+06	1.30E+07
Average	8.00E+06	8.00E+06	6.50E+06	1.30E+07
% Dry Wt.	0.78	0.79	0.80	0.79
Corrected MPN	1.03E+07	1.01E+07	8.13E+06	1.65E+07

GRID 9

Sample	1	2	3
MPN Numbers	1.10E+07	5.00E+06	5.00E+06
	1.10E+07	5.00E+06	8.00E+06
Average	1.10E+07	5.00E+06	6.50E+06
% Dry Wt.	0.76	0.77	0.78
Corrected MPN	1.45E+07	6.49E+06	8.33E+06

TABLE 4: HYDROCARBON DEGRADER NUMBERS-WEEK 7

GRID 1

Sample	1	2	3	4
MPN Numbers	8.00E+06	7.00E+05	8.00E+05	1.10E+06
	8.00E+06	9.00E+05	1.10E+06	1.10E+06
Average	8.00E+06	8.00E+05	9.50E+05	1.10E+06
% Dry Wt.	0.79	0.80	0.81	0.83
Corrected MPN	1.01E+07	1.00E+06	1.17E+06	1.33E+06

GRID 2

Sample	1	2	3	4
MPN Numbers	2.60E+06	1.10E+06	3.00E+06	1.10E+06
	2.10E+06	1.10E+06	3.00E+06	1.70E+06
Average	2.35E+06	1.10E+06	3.00E+06	1.40E+06
% Dry Wt.	0.82	0.82	0.83	0.84
Corrected MPN	2.87E+06	1.34E+06	3.61E+06	1.67E+06

GRID 3

Sample	1	2	3	4
MPN Numbers	7.00E+05	7.00E+05	5.00E+06	1.10E+07
	5.00E+05	8.00E+05	5.00E+06	8.00E+06
Average	6.00E+05	7.50E+05	5.00E+06	9.50E+06
% Dry Wt.	0.84	0.82	0.81	0.80
Corrected MPN	7.14E+05	9.15E+05	6.17E+06	1.19E+07

GRID 4

Sample	1	2	3	4
MPN Numbers	7.00E+06	1.10E+07	7.00E+06	7.00E+06
	7.00E+06	1.10E+07	7.00E+06	5.00E+06
Average	7.00E+06	1.10E+07	7.00E+06	6.00E+06
% Dry Wt.	0.84	0.83	0.84	0.82
Corrected MPN	8.33E+06	1.33E+07	8.33E+06	7.32E+06

GRID 5

Sample	1	2	3	4
MPN Numbers	3.30E+06	5.00E+06	5.00E+06	5.00E+06
	2.60E+06	8.00E+06	5.00E+06	5.00E+06
Average	2.95E+06	6.50E+06	5.00E+06	5.00E+06
% Dry Wt.	0.80	0.79	0.78	0.79
Corrected MPN	3.69E+06	8.23E+06	6.41E+06	6.33E+06

GRID 6

Sample	1	2	3
MPN Numbers	8.00E+05	8.00E+06	8.00E+06
	5.00E+05	5.00E+06	8.00E+06
Average	6.50E+05	6.50E+06	8.00E+06
% Dry Wt.	0.80	0.79	0.78
Corrected MPN	8.13E+05	8.23E+06	1.03E+07

GRID 7

Sample	1	2	3	4
MPN Numbers	3.00E+06	7.00E+06	8.00E+05	2.20E+07
	3.00E+06	1.10E+07	5.00E+05	2.20E+07
Average	3.00E+06	9.00E+06	6.50E+05	2.20E+07
% Dry Wt.	0.80	0.80	0.81	0.79
Corrected MPN	3.75E+06	1.13E+07	8.02E+05	2.78E+07

GRID 8

Sample	1	2	3	4
MPN Numbers	5.00E+06	3.00E+06	1.30E+06	5.00E+05
	8.00E+06	3.00E+06	1.30E+06	5.00E+05
Average	6.50E+06	3.00E+06	1.30E+06	5.00E+05
% Dry Wt.	0.78	0.77	0.79	0.80
Corrected MPN	8.33E+06	3.90E+06	1.65E+06	6.25E+05

GRID 9

Sample	1	2	3	4
MPN Numbers	8.00E+06	5.00E+06	5.00E+06	5.00E+06
	5.00E+06	5.00E+06	8.00E+06	5.00E+06
Average	6.50E+06	5.00E+06	6.50E+06	5.00E+06
% Dry Wt.	0.76	0.78	0.78	0.79
Corrected MPN	8.55E+06	6.41E+06	8.33E+06	6.33E+06

TABLE 5: HYDROCARBON DEGRADER NUMBERS-WEEK 9

GRID 1

Sample	1	2	3	4
MPN Numbers	8.00E+05	5.00E+05	3.00E+06	3.00E+06
	1.10E+06	1.10E+06	3.00E+06	5.00E+06
Average	9.50E+05	8.00E+05	3.00E+06	4.00E+06
% Dry Wt.	0.78	0.79	0.76	0.78
Corrected MPN	1.22E+06	1.01E+06	3.95E+06	5.13E+06

GRID 2

Sample	1	2	3	4
MPN Numbers	8.00E+05	7.00E+06	1.10E+06	5.00E+05
	1.70E+06	5.00E+06	8.00E+05	5.00E+05
Average	1.25E+06	6.00E+06	9.50E+05	5.00E+05
% Dry Wt.	0.78	0.79	0.75	0.79
Corrected MPN	1.60E+06	7.59E+06	1.27E+06	6.33E+05

GRID 3

Sample	1	2	3	4
MPN Numbers	3.00E+06	2.20E+06	1.10E+07	1.10E+07
	1.70E+06	2.60E+06	1.10E+07	8.00E+06
Average	2.35E+06	2.40E+06	1.10E+07	9.50E+06
% Dry Wt.	0.80	0.81	0.79	0.78
Corrected MPN	2.94E+06	2.96E+06	1.39E+07	1.22E+07

GRID 4

Sample	1	2	3	4
MPN Numbers	1.40E+07	8.00E+06	8.00E+06	3.00E+06
	2.20E+07	1.10E+07	1.30E+07	3.00E+06
Average	1.80E+07	9.50E+06	1.05E+07	3.00E+06
% Dry Wt.	0.79	0.78	0.80	0.78
Corrected MPN	2.28E+07	1.22E+07	1.31E+07	3.85E+06

GRID 5

Sample	1	2	3	4
MPN Numbers	3.00E+06	3.00E+06	2.30E+06	2.30E+06
	2.30E+06	1.10E+07	1.30E+06	3.00E+06
Average	2.65E+06	7.00E+06	1.80E+06	2.65E+06
% Dry Wt.	0.79	0.80	0.81	0.81
Corrected MPN	3.35E+06	8.75E+06	2.22E+06	3.27E+06

GRID 6

Sample	1	2	3	4
MPN Numbers	5.00E+05	5.00E+05	8.00E+05	3.00E+05
	8.00E+05	3.00E+05	8.00E+05	3.00E+05
Average	6.50E+05	4.00E+05	8.00E+05	3.00E+05
% Dry Wt.	0.80	0.80	0.79	0.78
Corrected MPN	8.13E+05	5.00E+05	1.01E+06	3.85E+05

GRID 7

Sample	1	2	3	4
MPN Numbers	7.00E+05	1.70E+06	1.10E+06	8.00E+05
	5.00E+05	1.70E+06	1.40E+06	1.10E+06
Average	6.00E+05	1.70E+06	1.25E+06	9.50E+05
% Dry Wt.	0.76	0.78	0.77	0.80
Corrected MPN	7.89E+05	2.18E+06	1.62E+06	1.19E+06

GRID 8

Sample	1	2	3	4
MPN Numbers	5.00E+05	3.00E+05	3.00E+05	8.00E+05
	5.00E+05	3.00E+05	5.00E+05	8.00E+05
Average	5.00E+05	3.00E+05	4.00E+05	8.00E+05
% Dry Wt.	0.75	0.74	0.79	0.77
Corrected MPN	6.67E+05	4.05E+05	5.06E+05	1.04E+06

GRID 9

Sample	1	2	3	4
MPN Numbers	5.00E+05	3.00E+05	3.00E+05	3.00E+05
	5.00E+05	3.00E+05	2.30E+05	3.00E+05
Average	5.00E+05	3.00E+05	2.65E+05	3.00E+05
% Dry Wt.	0.79	0.78	0.75	0.77
Corrected MPN	6.33E+05	3.85E+05	3.53E+05	3.90E+05

APPENDIX C

MICROBIAL ENUMERATION DATA-WATER

TABLE 4: HYDROCARBON DEGRADER NUMBERS-WATER

WEEK 0

	MPN numbers	Average
IG-1	1.10E+04	9.00E+03
	7.00E+03	
IG-2	1.10E+03	1.40E+03
	1.70E+03	
IG-3	3.00E+03	3.00E+03
	3.00E+03	
IG-WW	5.00E+01	5.00E+01
	5.00E+01	
B1	1.70E+03	1.55E+03
	1.40E+03	
B2	2.40E+03	1.85E+03
	1.30E+03	
B3	8.00E+03	1.05E+04
	1.30E+04	
B4	1.70E+01	1.70E+01
	1.70E+01	
DEC (1)	1.70E+04	1.70E+04
	1.70E+04	
DEC (2)	1.10E+03	1.25E+03
	1.40E+03	
PTAN	1.30E+03	1.30E+03
	1.30E+03	

WEEK 1

	MPN numbers	Average
IG-WW	1.10E+03	1.10E+03
	1.10E+03	

WEEK 2

	MPN numbers	Average
DEC (1)	5.00E+03	6.50E+03
	8.00E+03	

DEC (2)	1.10E+02	9.50E+01
	8.00E+01	
IG-WW	4.00E+00	4.00E+00
	4.00E+00	
IG-1	5.00E+02	5.00E+02
	5.00E+02	

WEEK 3

	MPN numbers	Average
IG-WW	9.00E+00	6.50E+00
	4.00E+00	

WEEK 4

	MPN numbers	Average
IG-1	5.00E+02	5.00E+02
	5.00E+02	
IG-2	2.20E+03	2.20E+03
	2.20E+03	
IG-3	1.10E+03	1.10E+03
	1.10E+03	
IG-WW	8.00E+00	8.00E+00
	8.00E+00	
B1	3.00E+02	3.00E+02
	3.00E+02	
B2	8.00E+02	5.50E+02
	3.00E+02	
B3	1.30E+03	1.30E+03
	1.30E+03	
B4	7.00E+00	7.00E+00
	7.00E+00	
DEC (1)	1.30E+03	1.20E+03
	1.10E+03	
DEC (2)	1.30E+02	1.30E+02
	1.30E+02	
PTAN	3.00E+02	4.15E+02
	3.00E+02	

WEEK 6

	MPN numbers	Average
IG-WW	5.00E+01	5.00E+01
	5.00E+01	
IG-1	1.70E+01	1.95E+01
	2.20E+01	
DEC (1)	8.00E+02	6.50E+02
	5.00E+02	
DEC (2)	1.10E+02	1.10E+02

WEEK 8

	MPN numbers	Average
IG-1	1.70E+03	1.95E+03
	2.20E+03	
IG-2	5.00E+03	4.00E+03
	3.00E+03	
IG-3	5.00E+02	5.00E+02
	5.00E+02	
IG-WW	1.10E+01	1.10E+01
	1.10E+01	
B1	8.00E+02	9.50E+02
	1.10E+03	
B2	5.00E+02	5.00E+02
	5.00E+02	
B3	5.00E+02	6.00E+02
	7.00E+02	
B4	4.00E+00	4.00E+00
	4.00E+00	
DEC (1)	8.00E+02	7.50E+02
	7.00E+02	
DEC (2)	1.70E+03	1.40E+03
	1.10E+03	
PTAN	2.80E+03	2.80E+03
	2.80E+03	

APPENDIX D

MINERALIZATION POTENTIALS (% DPM)-WATER

TABLE 1: PRE-FERTILIZATION

IG-1	(-NP)	BH
Mineralization Potential (% DPM)	3.07	21.68
IG-2	(-NP)	BH
Mineralization Potential (% DPM)	0.846	20.69
IG-3	(-NP)	BH
Mineralization Potential (% DPM)	1.37	23.27
B-1	(-NP)	BH
Mineralization Potential (% DPM)	0.40	8.94
B-2	(-NP)	BH
Mineralization Potential (% DPM)	0.68	0.59
B-3	(-NP)	BH
Mineralization Potential (% DPM)	3.44	18.27
B-4	(-NP)	BH
Mineralization Potential (% DPM)	0.17	0.82
DEC(1)	(-NP)	BH
Mineralization Potential (% DPM)	2.55	23.0
DEC(2)	(-NP)	BH
Mineralization Potential (% DPM)	3.02	19.88
PTAN	(-NP)	BH
Mineralization Potential (% DPM)	1.68	15.85

TABLE 2: WEEK 1

IG-WW	(-NP)	BH
Mineralization Potential (% DPM)	0.63	15.15

TABLE 3: WEEK 2

	(-NP)	BH
IG-WW	(-NP)	BH
Mineralization Potential (%DPM)	1.20	18.13
IG-1	(-NP)	BH
Mineralization Potential (%DPM)	0.33	0.18
DEC(1)	(-NP)	BH
Mineralization Potential (%DPM)	0.57	0.69
DEC(2)	(-NP)	BH
Mineralization Potential (%DPM)	1.13	2.77

TABLE 4: WEEK 3

IG-WW	(-NP)	BH
Mineralization Potentials (%DPM)	2.90	2.42

TABLE 5: WEEK 4

	(-NP)	BH
IG-1	(-NP)	BH
Mineralization Potential (% DPM)	0.078	0.46
IG-2	(-NP)	BH
Mineralization Potential (% DPM)	0.22	0.38
IG-3	(-NP)	BH
Mineralization Potential (% DPM)	0.37	1.34
IG-WW	(-NP)	BH
Mineralization Potential (% DPM)	1.31	19.17
B-1	(-NP)	BH
Mineralization Potential (% DPM)	0.39	4.03
B-2	(-NP)	BH
Mineralization Potential (% DPM)	2.65	13.14
B-3	(-NP)	BH
Mineralization Potential (% DPM)	0.28	0.601
B-4	(-NP)	BH
Mineralization Potential (% DPM)	0.24	0.19
DEC(1)	(-NP)	BH
Mineralization Potential (% DPM)	0.61	0.6
DEC(2)	(-NP)	BH
Mineralization Potential (% DPM)	0.27	17.63
PTAN	(-NP)	BH
Mineralization Potential (% DPM)	1.81	3.21

TABLE 6: WEEK 6

	(-NP)	BH
IG-1	(-NP)	BH
Mineralization Potential (%DPM)	0.21	0.91
IG-WW	(-NP)	BH
Mineralization Potential (%DPM)	0.01	19.12
DEC(1)	(-NP)	BH
Mineralization Potential (%DPM)	0.014	5.34
DEC(2)	(-NP)	BH
Mineralization Potential (%DPM)	0.31	0.47

TABLE 7: WEEK 8

	(-NP)	BH
IG-1	(-NP)	BH
Mineralization Potential (% DPM)	0.11	20.05
IG-2	(-NP)	BH
Mineralization Potential (% DPM)	0.49	21.24
IG-3	(-NP)	BH
Mineralization Potential (% DPM)	0.61	1.78
IG-WW	(-NP)	BH
Mineralization Potential (% DPM)	0.21	8.56
B-1	(-NP)	BH
Mineralization Potential (% DPM)	0.02	0.95
B-2	(-NP)	BH
Mineralization Potential (% DPM)	0.26	0.15
B-3	(-NP)	BH
Mineralization Potential (% DPM)	0.022	2.55
B-4	(-NP)	BH
Mineralization Potential (% DPM)	0.028	0.72
DEC(1)	(-NP)	BH
Mineralization Potential (% DPM)	0.039	2.91
DEC(2)	(-NP)	BH
Mineralization Potential (% DPM)	0.01	24.07
PTAN	(-NP)	BH
Mineralization Potential (% DPM)	0.08	20.84