I. Project Title and Project Number: Microbial Deterioration of Asphalt Materials and Its Prevention, ZZ-19

Program Manager: Dr. Fu-Hsian Chang
Agency Affiliation: Bemidji State University
Mailing Address: Center for Environmental Studies
Bemidji State University
Bemidji, MN 56601-2699
Telephone: (218) 755-4104

A. Legal Citation: ML 95, Chp. ____, Art. ____, Sec. ____, Subd. 7J
Total Biennial Budget: $60,000
Balance: $0
Appropriation Balance Language: This appropriation is from the oil overcharge money to the commissioner of administration for a transfer to the commissioner of transportation to survey microbial deterioration of asphalt-bituminous materials in cooperation with Bemidji State University or other research institutions.

B. Status of Match Requirement: N/A

II. Project Summary:

This research project will research the microbial deterioration of asphalt materials, the alteration of their physical properties by studying the biodegradation process through biochemical transformations. The goal is to identify a possible connection between the asphalt stripping witnessed on Minnesota highways and hydrocarbon consuming microbes indigenous to various soils around the state of Minnesota. The study would incorporate field sampling and laboratory testing to identify the presence of hydrocarbon consuming microbes and to quantify the relative numbers present.

Samples would be supplied by MN/DOT from various locations known to be deteriorating through stripping processes. These samples would be cultured and the resulting cultures would be typed and enumerated to identify bacterial species present and the relative number present. The goal of efforts at Bemidji State University would be to establish the presence and asphalt degrading capabilities of bacteria found within pavement and subgrade soil samples. This testing would be done upon samples from similar pavements from various locations in the state. The information from this effort should assist in identifying the extent of any problem and areas affected to the greatest degree. An effort will be made to identify soil or subgrade conditions most likely to develop biological action in asphalt pavements.

III. Six Month Work Program Update Summary:

Asphalt samples (and soils underneath) from the stripped pavements have more asphalt-degrading microbial population than the control non-stripped asphalt samples (and soils underneath). The composition and concentration of chemicals related to microbial activities, such as acidity (pH), alkalinity, organic acids, NH_4^+-N, NO_3^--N, NO_2^--N, total nitrogen, total phosphorus and total hydrocarbon has been analyzed in this period of research. A correlation of microbial population density and degree of pavement damage and soil characteristics were included in this report. Organic acid composition and concentration is damaged asphalt materials and degree of stripping were also reported here.

All of the samples contained primarily hydrocarbons and less amounts of nitrogen and phosphorus. Most soil samples underneath the asphalt pavements has less hydrocarbons and higher amount of nitrogen and phosphorus than those of asphalt pavement samples. Stripped and non-stripped asphalt pavements and soil samples underneath the asphalt pavements were basic. pHs were between 7.8 and 8.5 and averaged 8.2 for all samples. Bicarbonate alkalinity was predominantly in most asphalt pavements and soil samples underneath the asphalt pavements, included stripped and non-stripped. In most cases, total hydrocarbon (%) in stripped asphalt pavement was less (2-3%) than that in non-stripped asphalt pavement. However, soil samples underneath stripped asphalt pavements had more hydrocarbon than those underneath non-stripped asphalt pavement.

The presence of higher hydrocarbon content in stripped asphalt pavement stimulated the growth and activity of all types of hydrocarbon oxidizing microorganisms and inhibited the growth of heterotrophs. In general, the worse the biodeterioration in asphalt pavements the greater the asphalt degrading microbial count. Taxonomic studies show that most hydrocarbon utilizing bacteria belong to genera of Pseudomonas, Micrococcus, Flayobacterium, Mycobacterium, and Pro-actinomyces, Actinomyces, and other genera.

Seventy four asphalt degraders (39 bacteria, 19 actinomyces and 16 fungi) were isolated from thirty one asphalt and soil samples collected by five district labs around the state of Minnesota. It was found that 7 isolates belong to Pseudomonas sp., 9 isolates of Sphingobacterium sp., 3 isolates of Acinetobacter sp., 2 isolates of Agrobacterium sp. and 2 isolates of Chryseomonas sp. among 39 of asphalt-degrading isolates.

An index of hydrocarbon oxidation by microorganisms is the growth of bacterial culture on the material which serves as the sole source of carbon. The highest population densities were observed in the tank submersed with enrichment culture and Marshall Pucks. An adaptation process usually followed by logarithmic growth rate (occurred in 1.5 month) was observed after Marshall pucks submersed in three different solution for 4.5 months.

Most soil samples underneath the damaged asphalt pavement belonged to coarse loamy sand or clay or silty according to granulation test. The stripped asphalt pavement and soil samples underneath the asphalt pavement were both basic, pH was between 7.9-9.2 and 7.7-8.8, respectively. Bicarbonate alkalinity was predominant in all samples. Soil samples underneath the asphalt pavement had less hydrocarbons and nitrogen and phosphorus, especially the soil samples of Brainerd and Willmar District. It might influence microbial utilization of hydrocarbons since nitrogen and phosphorus are required for microbial activity. The lowest total hydrocarbons (%) occurred in stripped asphalt pavement collected from Bemidji District. It could be resulted from worse biodeterioration by microbial degradative activity.

Heterotrophs are significantly more abundant than asphalt-degrading microbial populations in all soil samples. However, the stripped pavement have more asphalt-degrading microbial populations than heterotrophic populations in samples collected from Bemidji, Duluth, Willmar and Rochester Districts. In general, the higher the asphalt degrading microbial count the worse the biodeterioration of asphalt pavement happened.

On the basis of different chemical-physical properties and microbial enumerations in five soil samples, the experiment of Marshall puck buried-in-soil was set up 120-140 lbs. of each soil were put in 20-liter Nalgene rectangular tank and adjusted to 30%, 60%, 90% water holding capacity. Triplicated Marshall pucks were buried in the soil of each container and incubated for four time period (3, 7, 12, 18 months). The control Marshall pucks were kept in the containers that are maintained at the same humidity. At the end of each incubation period three pucks from each soil and control container will be removed and tested for chemical-physical properties, microbial enumerations and physical strength. After Marshall pucks had been soaked for 1.5 and 4.5 months in mixed-asphalt degrading microbial broth culture or

straight lake water or autoclaved lake water revealed that Marshall pucks were relatively weaker (softening) in tensile strength than the control Marshall pucks (non-treated). Tensile strength of pucks was less weakened after treated with enrichment culture as compared to those treated with lake water or autoclaved lake water. This might be because a magnitude of microorganisms to form a thick microbial film around pucks treated in enrichment culture which prevented the water and cells from moving in and out of pucks. Also, microbial metabolites in the enrichment-culture treated might have pucks formed complex with carbonate and aggregate in the pucks, which might have shielded asphalt from dissolution and showed less weakened physical strength than those pucks soaked in lake water or autoclaved lake water. After Marshall pucks had been pre-soaked in mixed asphalt-degrading microbial culture for 1.5 months highest percentage of broken aggregate was found. It appears that some low molecular weight of constituents might have been biodeteriorated. These low molecular weight components are usually more readily absorbed onto aggregate surfaces than the higher molecular weight constituents, they have a disproportionately larger influence on bond development. Pore space provides access channels for water and bacterial cells to the asphalt-aggregate. Water has been described as the worst enemy of bituminous pavements deterioration. Water is essential for microbial activity, when water gets into a pavement crack the microorganisms become active and begin to multiply.

Effects of microbial activity on asphalt-aggregate bond by physical measurement of tensile and bond strength and microbial enumeration of heterotrophic and asphalt-degrading microbial populations, chemical properties of asphalt materials, as well as biochemicals/metabolites produced by asphalt-degrading microbes after Marshall pucks have been submersed in enriched broth culture and lake water (fresh lake water and autoclaved sterile lake water) for 10 months reveal that the highest population densities of both the heterotrophs and the asphalt degraders still appeared in Marshall puck specimens that had been submersed in the tank containing mixed asphalt-degrading microbes for 10 months. A dramatic rate of proliferation of the asphalt-degrading microorganisms was also found in Marshall pucks that had been submersed in the tank with mixed asphalt-degrading culture for 10 months. It is noted that the higher microbial growth response, the worse the stripping of asphalt materials caused by microorganisms.

Chemical properties of Marshall pucks soaked in three broth media for 10 months show that all Marshall pucks were alkaline, pHs were between 8.1 and 8.5. Bicarbonate alkalinity was predominant in all samples. Denitrification might have occurred to form NO_2^- in tank I broth medium that contained enrichment culture due to insufficient dissolved oxygen present in the broth culture. Control Marshall pucks without any degradation contain 7.36-7.95% total hydrocarbon. Asphalt materials treated with mixed enrichment asphalt-degrading culture show a fairly good degradation (1.91-2.2% hydrocarbon loss) after pucks were soaked for 10 months. Marshall pucks soaked in straight lake water or sterile lake water had 6.92 - 7.08% and 7.01-7.45% hydrocarbon remained, respectively.

Biochemical/metabolic products produced by asphalt-degraders can be fractionated into components by chromatographic techniques using high performance liquid chromatography (HPLC) and gas chromatography (GC). It reveals that significant mineralization or microbial conversion of asphalt extractable compounds to low molecular weight of compounds or to water soluble metabolites occurred in all test samples, with the greatest mineralization occurred in the puck sample treated with enrichment culture for 10 months.

Physical properties and tensile strength changed after Marshall pucks had been soaked in mixed-asphalt-degrading microbes or straight lake water or sterile lake water for 10 months showed that Marshall pucks were relatively weak (softening effect) in tensile strength ratio (TSR%) than the control Marshall pucks (non-treated). The tensile strength ratio percent of

pucks approached the same softening stage after Marshall pucks were soaked in tanks containing three different broth media for 10 months. The higher broken aggregate percent and visual stripping percent were observed after Marshall pucks had been submersed in mixed asphalt-degrading broth culture for 10 months. It was clear that significant effect of microbial action on asphalt materials after Marshall pucks had been soaked in tank containing asphalt-degrading microbes for 10 months. It also appears that the effects of microbial action on asphalt are less dramatic but are more traumatic in the long run. However, it is also possible to have substantial damage due to the cumulative effects of microbial action with other factors such as action of water and other environmental conditions.

Chemical analysis, enumerations of heterotrophic microorganisms and asphalt degraders, metabolites produced by asphalt-degraders and tensile strength test were conducted in Marshall pucks after having been buried for three months in soils collected from five Districts (Bemidji, Brainerd, Duluth, Rochester and Willmar) that were maintained at 30%, 60% and 90% water holding capacities.

All pucks buried in soils were alkaline, pHs were between 7.99-8.41. Bicarbonate alkalinity was predominant in most asphalt samples. Samples from Rochester and Duluth were with carbonate alkalinity. Denitrification might have occurred to form NH_4^+ and NO_2^- in most samples. In most cases, total hydrocarbon (%) in Marshall pucks with treated soils that contained different water holding capacities was less than that in control sample. Marshall pucks treated in soils with 60% and 90% water holding capacities had less hydrocarbons (%) than those samples with 30% water holding capacity. Total hydrocarbons (%) in asphalt samples from Bemidji District with 90% water holding capacity and Duluth District with 60% water holding capacity show a good degradation (5.19 and 5.92% hydrocarbons remained, respectively).

Heterotrophs are significantly more abundant than asphalt-degrading microbial population in all samples. The highest heterotrophic microorganisms and asphalt degraders were found in pucks buried in soils that had 60% water holding capacity. The abundance of heterotrophs and asphalt-degraders in asphalt samples buried in soil with 60% water holding capacity in decreasing order is Duluth>Rochester>Bemidji>Brainerd>Willmar.

Metabolic products produced by asphalt-degraders after Marshall pucks had been buried in soils for three months were detected for components by GC using chromatographic techniques. The control extract showed the cleanest chromatograms, with largest four sharp peaks. Significantly smaller peak areas in four major peaks were observed in Bemidji District sample with 90% water holding capacity and samples from Duluth, Rochester, Brainerd and Willmar District with 60% water holding capacity. It is also revealed that some original asphalt component has been mineralized or bioconverted to water soluble metabolites or to smaller molecular weight of compounds.

Changes of tensile strength, broken aggregate and visual stripping of buried Marshall pucks in soils with different water holding capacities for three months showed that Marshall pucks in most treated samples are weaker in tensile strength than untreated Marshall pucks. Weaker (softening) tensile strength, higher visual stripping and broken aggregate were found in Marshall pucks buried in soils, collected from Duluth and Rochester District, with 60% water holding capacity than those buried in 30% and 90% water holding capacities. Relatively weaker and no visual stripping or only small amount of stripped aggregate were found in Marshall pucks buried in soils collected from Bemidji Brainerd, Willmar Districts with 90% water holding capacity. The test of Marshall pucks -buried in soils for three months led to the consideration that microbial population density, water penetration into and out of Marshall pucks, soil water content, length of bury in soils and soil characteristics are the causes that affect asphalt material stripping.

Chemical analysis and metabolites produced by asphalt-degraders were analyzed using gas and high pressure liquid chromatographic techniques and physical strength was tested using standard Lottman Method on Marshall pucks after having been buried for seven months in soils, collected from five Districts, that were maintained at 30%, 60%, and 90% water holding capacities.

All pucks were basic, pHs were between 7.31-8.56. Bicarbonate alkalinity was predominant in most asphalt samples. After having been buried in treated soils for seven months all pucks had less nitrogen and more phosphorous contents than those pucks kept in control containers or fresh puck. This might be attributed to microbial activities during degradation of asphalt materials. Total hydrocarbon (%) in Marshall pucks buried in treated soils at different water holding capacities was less than that in control or fresh puck samples in most cases. The Marshall pucks treated in soil with 60% and 90% water holding capacities had less hydrocarbons (%) than those samples with 30% water holding capacity.

Decreasing heterotrophic densities were observed in most Marshall pucks buried in soils for seven months compared with those buried in soils for three months. However, a dramatic rate of proliferation of asphalt-degraders was observed in most Marshall pucks as a result of utilization of the hydrocarbon as substrate. The highest heterotrophs and asphalt degraders were observed in Marshall pucks buried in soils that were maintained at 60% and 90% water holding capacities.

Biochemical/metabolites produced by asphalt-degrading microbes after Marshall pucks had been buried in soil for seven months were analyzed using GC and HPLC chromatographic techniques. The major peak areas present in gas chromatograms from all puck samples buried in soils maintained at 30%, 60%, and 90% water holding capacities including control puck samples were smaller than those of fresh puck samples. Some deterioration in control samples might be due to water dissolution or water action in concert with microbial activities. Relatively small or almost absence of four major peaks were found in Marshall pucks buried in soils, collected from Brainerd, Duluth, Rochester and Willmar, for seven months with 60% and 90% water holding capacities. Apparent molecular weight changes of metabolic compounds in HPLC chromatograms were observed in extracts of asphalt samples buried in all soils for seven months, with 60% and 90% water holding capacities. It reveals that asphalt samples buried in soils maintained at 60% and 90% water holding capacities for seven months had undergone more degradation as compared to chromatograms obtained from asphalt samples buried in soils for three months with the same water holding capacities.

Physical strength test after Marshall pucks had been buried in soils with different water holding capacities for seven months showed relatively weak (softening) tensile strength (TSR% is between 68.01-82.67%) was found in pucks buried in soils maintained at 90% water holding capacity (with an exception of Duluth District) and some samples in Bemidji and Duluth Districts with 60% water holding capacity (TSR% is between 91.33-91.35%) as compared with Marshall pucks buried in soils for three months. The visual stripping and broken aggregate appeared on edges of the surface of fracture planes. The higher stripping or more white aggregate were found in Marshall pucks buried is five Districts soils maintained at 60% and 90% water holding capacities for seven months.

The test of puck-buried in soil for seven months has demonstrated that proliferation of asphalt-degraders, hydrocarbon degradation and biochemical/metabolites changes were related to pavement deterioration. Moisture is also a requirement for microbial deteriorative activities, as well as causing aggregate weakening of asphalt materials.

Chemical analysis, enumerations of heterotrophic microorganisms and asphalt degraders, metabolites produced by asphalt-degraders using GC chromatographic technique and tensile strength test were conducted in Marshall pucks after having been buried for twelve months in

soils, collected from five Districts, that were maintained at 30%, 60% and 90% water holding capacities.

All pucks were basic, pHs were between 7.68-8.61. Bicarbonate alkalinity was predominant in most asphalt samples. The pucks kept in control container had less nitrogen and phosphorus than those pucks buried in treated soils. It might be due to microorganisms adhering to the surface of Marshall pucks in the control containers utilized hydrocarbons as sole carbon source. Nitrogen and phosphorus were utilized simultaneously after Marshall pucks had been kept in control containers for 12 months. Denitrification might have occurred to form NO2- in the samples buried in soils collected from Brainerd and Rochester District. Total hydrocarbon (%) in Marshall pucks buried in various soils or kept in control containers that were maintained at different water holding capacities was less than fresh pucks. The pucks kept in control containers with 30%, 60% and 90% humidities for 12 months had less hydrocarbon than those pucks buried in soils. it might be due to insufficient inorganic nutrient available to those microorganisms adhering to Marshall pucks in control containers since only asphalt materials are the sole source of carbon and energy present in the containers.

Growth curve of heterotrophs and asphalt-degraders was observed after Marshall pucks had been buried in soils for 3, 7 and 12 months. Slow metabolic process of heterotrophs and asphalt-degraders occurred in soils that were maintained at 30% water holding capacity at the beginning because low moisture content is unfavorable to bacterial activity. The growth curve remained at optimum for about seven months in most samples that were maintained at 30% water holding capacity. The growth rose exponentially called logarithmic stage of heterotrophs and asphalt-degraders occurred in most sample buried in soils that were maintained at 30% water-holding capacity for 12 months. However, almost no lag phase of heterotrophs was observed in most samples buried in soils that were maintained at 60% and 90% water-holding capacities because population is at its biochemical optimum conditions. The heterotrophs proliferated with a second exponential phase after Marshall pucks have been buried in soils that were maintained at 60% and 90% water holding capacities for 12 months. A long adaptation period was observed during which time asphalt degraders synthesized the required enzymes. Then, the mass of asphalt-degraders increased by exponential proliferation after Marshall pucks had been buried in soils for seven months in most samples.

Gas chromatograms for biochemical/metabolites changes show absence of two major peaks that had longer retention time (higher molecular weight compounds) in most asphalt samples buried in soils that were maintained at 30%, 60% and 90% water holding capacities and one major peak with longer retention time was disappeared in the control puck samples as compared with major peaks present in gas chromatograms for fresh puck samples. The rest of the peaks for most samples were smaller than those of fresh puck samples. It was found that asphalt samples buried in soils that were maintained at 30%, 60% and 90% water holding capacities, and pucks that were kept in the control containers that had been maintained at the same humidity for 12 months had molds and bacteria adhered to the surface of those pucks and had undergone biodeterioration.

Physical strength test after Marshall pucks had been buried in soils with different water-holding capacities showed relatively more weakened in tensile strength when pucks were maintained at 90% water holding capacity. The higher visual stripping percentage and dull, rough, crumbly materials and more white aggregate appeared on edges and surface of Marshall pucks that had been buried in soils with 60% water holding capacity. It is clear that microbial activity in soils had negative impact on puck strengths and enhanced stripping process.

Chemical properties analysis, enumeration of heterotrophic and asphalt degrading microorganisms, metabolites produced by asphalt-degraders using GC chromatographic technique and tensile strength test of Marshall pucks were conducted after having been buried

for 23 months in soils collected from five Districts and maintained at 30%, 60% and 90% water holding capacities. An additional experiment was conducted to compare populations of heterotrophs and asphalt degraders capable of growing in soils adjacent to the buried Marshall pucks compared with those populations in undisturbed soil that was far apart from the Marshall pucks.

At the end of 23 months all pucks were basic, pHs were between 7.26-7.98. Bicarbonate alkalinity was predominant in most asphalt samples. Most pucks buried in treated soils had less nitrogen and phosphorus than those pucks kept in control containers. No denitrification had been observed in all asphalt samples buried in soils. Total hydrocarbon (%) in Marshall pucks that were buried in soils was less than those in control and fresh puck samples. The Marshall pucks buried in soils with 60% and 90% water holding capacities had less total hydrocarbon (%) than those samples that were maintained at 30% water holding capacity in most cases.

Two logarithmic growth phases for heterotrophs and asphalt-degraders were found in most samples buried in soils that were maintained at different water holding capacities during the 23 months incubation period. Intermediate metabolites, recycled inorganic nutrients and newly released hydrocarbon stimulated cell reproduction and resulted in a new exponential growth phase. The significant numbers of heterotrophs and asphalt degraders were found in almost all samples buried in soils that were maintained at 60% water holding capacity for 23 months. Significant numbers of heterotrophs and asphalt degraders were found in soil adjacent to the asphalt materials. There is no question concerning a considerable more asphalt degraders found in soils near asphalt materials (Marshall pucks) where microbes derived their organic carbon from.

Gas chromatograms for biochemical/metabolites changes and biodegradation of asphalt materials buried in various soils for 23 months show that major peaks almost disappeared in most asphalt samples buried in soils that were maintained at 60% water holding capacities. It could be interpreted as original asphalt materials had been mineralized or bioconverted to water soluble metabolites or transformed to microbial cellular materials. It was confirmed that asphalt samples buried in soils, especially, that were maintained at 60% water holding capacity for 23 months had undergone most severe biodegradation.

Physical strength test after Marshall pucks had been buried in soils with different water holding capacities for 23 months show that all pucks left in control containers and pucks buried in soils that were maintained at 30% water holding capacity developed some degrees of "permanent" hardening with the passage time. Mainly because conditions are unfavorable to bacterial activity such as lack of moisture and exposure to air (control containers) etc. After the utilization of high molecular weight fractions of the bitumen by significant microbial degradative activity, a softening of the asphalt occurred when Marshall pucks had been buried in soils that were maintained at 60% water holding capacity for 12 to 23 months. The higher visual stripping percentage and higher broken aggregate percentage were found in Marshall pucks that had been buried in soils for 23 months and maintained at 60% water holding capacity.

It is further confirmed that under favorable conditions such as when asphalt materials were buried in soils that were maintained at 60% water holding capacity, microorganisms caused damage to asphalt pavement and bacterial infestation are considered major mechanisms to stripping process.

IV. Statement of Objectives:

The objective of this proposed project is to survey biodeterioration/biodegradation of asphalt (bituminous) pavements under various environmental conditions. Elements to be studies include: 1. microbial enumeration and characterization of asphalt-degrading population, 2. biodegradation/biodeterioration of asphalt (bituminous) materials, and 3. asphalt strength testing. Microbiological investigations of the stripped base material would provide us the cause and effect relationship between microbial actions and asphalt stripping under different environmental conditions.

Timeline for completion of objective:

	7/95	1/96	6/96	1/97	6/97
Microbial enumeration and characterizatoin of asphalt-degrading populations	X	- - - - - - - - -	X		
Biodegradation/biodeterioration of asphalt materials			X	- - - - - - - - -	X
Asphalt strength testing				X - - - -	X
Final Report					X - - - - X

V. Objectives/Outcome:

A. Microbial Enumeration and Characterization of Asphalt-Degrading Population: Survey of Biodeterioration/Biodegradation of Asphalt (Bituminous) Pavements:

A.1. Activity:

Asphalt samples that are to be provided by the Minnesota Department of Transportation (MNDOT), will be enumerated in mineral medium using plate count method and five tube most probably number techniques. Asphalt-degrading microorganisms will be purified and each isolate will be identified and maintained in appropriate medium for future physiological/biochemical characterization.

A.I.a. Context Within the Project:

The ability to utilize hydrocarbons is widely distributed among diverse microbial populations (1). Many species of bacteria, cyanobacteria, filamentous fungi, and yeasts coexist in natural ecosystems and may act independently or in combination to metabolize aromatic hydrocarbons (2, 3, 4). In general, population levels of hydrocarbon utilizers and their proportions within the microbial community appear to be a sensitive index of environmental exposure to hydrocarbons (1).

In 1946, ZoBell (5) reported that nearly 100 species of bacteria, yeasts, and molds, representing 30 microbial genera, had been discovered to have hydrocarbon-oxidizing properties. Since that time, many other species and genera have been reported to have this ability (5) and to be widely distributed in soils (1, 6). Although many microorganisms appear limited to degradation of a specific group of chemicals, others have demonstrated a wide diversification of substrates that they are capable of metabolizing.

Some compounds appear to be degraded only under aerobic conditions, others only under anaerobic conditions, and some under either condition, while others are not transformed at all. It has been concluded that hydrocarbons are subject to both aerobic and anaerobic oxidation (7).

Numerous bacterial strains and species have been isolated that can degrade many of the petroleum-derived compounds of the greatest hazard to the environment and to health (8). The compounds than can be attacked include most of those of major environmental concern, including benzene, toluene, biphenyls, and naphthalene.

Bacteria are predominantly involved with degradation of those chemicals that have a higher degree of water solubility and are not strongly adsorbed. The binary fission-type reproductive methods of bacteria enable them to complete more successfully than fungi for readily available substrates.

Aerobic bacteria ultimately decompose most organic compounds into carbon dioxide, water, and mineral matter, such as sulfate, nitrate and other inorganic compounds (9), and do not produce hydrogen sulfide or methane as reaction products (10).

The most commonly isolated organisms in areas of hydrocarbon contamination are heterotrophic bacteria of genera Pseudomonas, Achromobacter, Arthrobacter, Micrococcus, Vibrio, Acinetobacter, Brevibacterium, Corynebacterium, Flavobacterium, (11), Mycobacterium (12, 13) and Nocardia (14).

Anaerobic decomposition of organic matter to carbon dioxide and methane involves interactions within consortia of obligate anaerobic bacteria (11).

Obligate anaerobes require not only anoxic (oxygen-free) conditions, but also oxidation-reduction potentials of less than -0.2 (11). While many soil bacteria can grown under anaerobic conditions, most fungi and actinomycetes cannot grow at all (15). Anaerobic decomposition is performed mainly by bacteria utilizing either an anaerobic respiration or interactive fermentation/methanogenic type of metabolism.

Enumeration of microorganisms can be difficult, since most subsurface bacteria exist in an ecosystem low in organic carbon and do not grow well, if at all, in conventional growth media with high organic carbon concentrations (16). In addition, many organisms attach firmly to particles (17). Dilution plating techniques yield 1 to 10% of the number of cells determined by microscopic direct counting.

The proportion of hydrocarbon-degrading organisms to total heterotrophs is now considered to be a more significant indicator of the biological activity in the subsurface, with respect to hydrocarbon contaminants.

Direct counts tend to be fairly consistent with soil depth and sampling site and were found to be on the order of 10^6 to 10^7 bacteria/g dry wt of soil. Plate counts, on the other hand, suggested highly variable and decreasing counts with depth, ranging from zero to 10^8.

The number of hydrocarbon-utilizing organisms in a soil reflects the soil's past exposure to hydrocarbons. These organisms are most abundant in places that have been chronically exposed to hydrocarbon pollution.

Hydrocarbon degraders have been measured at naturally occurring levels of 10^2 to 10^5 organisms/g. These numbers increase after biostimulation of the contaminated sites.

Fungi play an important role in the hydrocarbon-oxidizing activities of the soil (18). They seem to be at least as versatile as bacteria in metabolizing aromatics (19). Their extracellular enzymes may help to provide substrates for bacteria, as well as for themselves, by hydrolyzing polymers. They are also important sources of secondary metabolites.

Some filamentous fungi, unlike other microorganisms that attack aromatic hydrocarbons, use hydroxylations as a prelude to detoxification rather than catabolism and assimilation (20). These organisms do not degrade aromatic hydrocarbons as nutrients, but simply detoxify them. Several fungi (Penicillium and Cunninghamella) even exhibit greater hydrocarbon biodegradation than bacteria (Flavobacterium, and Arthrobacter). The ability to utilize hydrocarbons occurs mainly in two orders, the Mucorales and the Moniliales (21). Aspergillus and Penicillium are rich in hydrocarbon-assimilating strains. It has been

concluded that the property of assimilating hydrocarbons is a property of individual strains and not necessarily a characteristic of particular species or related taxa.

The genera most frequently isolated from soils are those producing abundant small conidia; e.g., Penicillium and Verticillium spp. (22). Oil-degrading strains of Beauveria bassiana, Morteriella spp., Phoma spp., Scolecobasidium obovatum, and Tolypocladium inflatum have also been isolated. Fifty-six out of 500 yeasts studied were found to be able to degrade hydrocarbons; almost all of these were in the genus Candida (23). Hydrocarbonoclastic strains of Candida, Rhodosporidium, Rhodotorula, Saccharomyces, Sporobolomyces, and Trichosporon have been identified from soil (24; 25). Cladosporium resinae has been found in soil, and has repeatedly been recovered as a contaminant of jet fuels. This organism can grow on petroleum hydrocarbons and creates problems in the aircraft industry by clogging fuel lines.

A.3. Methods:

Representative samples of broken pavement and soils underneath the destructed pavement from five districts throughout Minnesota were collected from sites that have different soil characteristics (physical-chemical properties) through the help of MN/DOT research personnel during the first three months of the project. Fresh asphalt pavement samples were requested from MN/DOT and used as a control (reference). At the initial stage, samples were collected from five districts - Bemidji, Detroit Lakes, Rochester, Mankato and Willmar.

Asphalt degrading microorganisms were isolated in mineral medium using plate count method and five-tube most probable number techniques. Asphalt-degrading microorganisms were purified and each isolate were maintained in appropriate medium for future physiological/biochemical characterization.

The medium for the enumeration of asphalt degraders is basal medium that contains 10 g of NaCl, 0.5 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of NH_4NO_3, 3 ml of 10% KH_2PO_4 and 7 ml of 10% KH_2PO_4 per liter of distilled water, concentrated PO_4^{-3} salt solution (pH 7.0) will be autoclaved separately and added to the autoclaved basal broth. Ten grams of asphalt material will be added to 100 ml of broth in 250 ml Erlenmeyer flasks. Control experiments will be carried out in the same broth with 10 g of sterile asphalt materials (100 g asphalt materials in 1 liter distilled water will be autoclaved at 121°C/15 atm pressure for 45 mins., and liquid will be discarded). Growth in broth medium will be incubated on a rotary shaker at room temperature for 3 to 7 days. Turbidity indicates growth of asphalt-degrading microorganisms in the broth medium.

Asphalt-degrading bacteria were identified by testing their physiological/biochemical characteristics. The methods and techniques follow Bergey's Manual of Systematic Bacteriology (27).

A.4. Materials:

No new equipment will be purchased or leased to carry out this research element. However, some existing equipments (such as heavy-duty shakers, incubators, colony counter, bench-top autoclave, floor model autoclave and glove box) will be used throughout the research period. Microbial growth media, chemicals, glasswares and other supplies will be purchased during the research period.

A.5. Budget:
Total Biennial LCMR Budget: $20,000
LCMR Balance: $0
Match: N/A
Match Balance: N/A

A.6. Timeline:

	7/95	1/96	6/96	1/97	6/97
Microbial enumeration and characterization of asphalt-degrading populations	X	- - - - - - - -			X
6-month status report			X		
Annual status report and product A					X

A.7. Workprogram Update:

Samples of thirty-two stripped and non-stripped asphalt pavements and soils underneath the asphalt pavement were collected by MN/DOT's five District labs (Bemidji, Detroit Lakes, Willmar, Mankato and Rochester) around the State of Minnesota and studied in the Environmental Biotechnology Laboratory at Bemidji State University. It was found that asphalt samples (and soils underneath) from the stripped pavements have more asphalt-degrading microbial population than the control non-stripped asphalt samples (and soils underneath). It was apparent that degradation of asphalt materials by microorganisms is not a simple process and may even involve a variety of different biochemical degradative mechanisms as the study of physical strength (tensile strength) change with environmental conditions. In order to proceed with an investigation of microbial action on asphalt stripping, it is important to establish certain abiotic parameters which will mediate the rate of microbial growth and degradation of the asphalt. The composition and concentration of chemicals related to microbial activities, such as acidity (pH), alkalinity, organic acids, NH_4^+-N, NO_3^--N, NO_2^--N, total nitrogen, total phosphorus and total hydrocarbon has been analysed in this period of research. Damaged asphalt (stripped pavement) material and the controlled asphalt (non-stripped asphalt) sample were extracted by HPLC grade n-hexane, the hexane-soluble material were fractionated on silica open tubular column by an HP 5890 Series II gas chromatograph. The differences of peak area of reach chemical species shown on gas chromatogram resulted from microbial degradative activities. A correlation of microbial population density and degree of pavement damage and soil characteristics were included in this report. Organic acid composition and concentration is damaged asphalt materials and degree of stripping were also reported here. Samples were homogenized by blender and hammer and passed through a 0.0469-in. or 0.0787-in. sieves, then stored at 4° C. The locations and description of those samples were described in Table 1. The fresh pavement was used as a control (reference).

Chemical-Physical properties of asphalt pavement and soil samples.

Chemical-Physical properties of asphalt pavement and soil samples include acidity (pH), alkalinity, total hydrocarbon, total nitrogen, NH_4^+-N, NO_3^--N, NO_2^--N, total phosphorus were analysed according to the methods described in "Methods of soil analysis, Part II: Chemical and Microbiological Properties" Second Edition, 1982 and "Standard Methods for the Examination of Water and Wastewater", 19th ed., 1995.

1. PH

Corning reference electrode with Corning ion analyze 250 meter using a 1:1 asphalt (or soil) - water mixture and 1/2-hour tempering time.

2. Extraction of asphalt and soil samples.

The preparation of extracts for determination of alkalinity, exchangeable ammonium, nitrate, and nitrite in asphalt and soil samples, the asphalt or soil sample is shaken with 2N KCl for 1 hour using 10 ml of this reagent per gram of soil. The KCl is filtered and filtrate analyzed for alkalinity, NH_4^+, NO_2^-, NO_3^- measurements.

3. Alkalinity

Total alkalinity was measured using 4-5 drops of phenolphthalein indicator to a 50 ml extracted sample. If sample turns pink, titrated with standardized 0.1 N HCl until colorless. Note the volume and normality of HCl used as a titrant (P ml). Add 4-5 drops of methyl orange indicator and titrated from yellow to orange with the standardized 0.1 N HCl solution. Note the volume and normality of the HCl titrant (M ml). Total titration (ml) of 0.1 N HCl solution is equal to $P_{ml} + M_{ml}$. Calculate alkalinity according to the following formula.

$$\text{Total alkalinity, mg CaCO3/L} = \frac{\text{Total ml HCl x normality HCl x 50000}}{\text{ml sample}}$$

4. Total hydrocarbon

Hydrocarbon in asphalt materials was extracted in a Soxhlet apparatus. Fifty to 100 g of finely ground sample was placed in a Soxhlet apparatus and extracted for 24 to 30 hours with ethyl ether. Evaporate and recover the ether extract using rotaevaporator to a small volume (about 5-10 ml) and dry the sample to a constant weight in an oven at 60° C.

5. Total Kjeldahl Nitrogen

In the Kjeldahl method, the N in the sample is converted to ammonium (NH_4^+) by digestion with concentrated H_2SO_4 containing catalysts which promote this conversion, and the ammonium is determined from the amount of NH_3 liberated by distillation of the digested aliquot with alkali (NaOH solution).

To calculate percent nitrogen.

$$\% N = \frac{(ml_s - ml_c) \times N_{H_2SO_4} \times 0.014 \times 100}{\text{Dry wt. of sample}}$$

Where

ml_s = ml used in titration of sample
ml_c = ml used in titration of control blank.

6. Exchangeable ammonium, nitrite and nitrate

The ammonium, nitrite and nitrate in asphalt and soil samples were extracted with 2N KCl as described above. The filtration of the asphalt or soil suspension was analyzed for ammonium by Nesslerization colorimetric technique at 425 nm. The nitrite is analyzed by a modification of the Griess-Ilosvay (the diazotization and coupling reactions) at 520 nm. The colorimetric method for nitrate is the phenoldisulfonic acid technique and measurements were made at 410 nm.

7. Total phosphorus.

To release phosphorus from combination with organic matter in asphalt and soil materials, the sulfuric acid-nitric acid digestion method was used. After digestion, liberated orthophosphate was determined by stannous chloride method of colorimetric determination at 690 nm.

Enumeration of heterotrophic and asphalt-degrading microorganisms.

Microbial numbers in asphalt and soil samples taken from 5 District Labs were enumerated using plate count method and most probable number (MPN) techniques for total heterotrophic microorganisms and asphalt-degrading microorganisms.

GC analysis

Asphalt samples taken from broken roadway are compared with samples from uncompromised roadway using analysis by gas chromatography (GC).

1 g of asphalt was mixed with 10 ml HPLC grade n-hexane in an extraction funnel and agitated for 3 minutes. Three 2 ml centrifuge tubes were filled with the liquid fraction and centrifuged at 15,000 rpm for 5 minutes. The concentrated extract was stored in centrifuge tubes at 4° C, and placed in 2 ml glass vials with screw top teflon septa caps. The supernatant from samples 1 and 4 was analyzed without dilution. The supernatants of samples 0, 2 and 3 were diluted 10:1 with HPLC grade n-hexane prior to analysis.

Samples were analyzed with a HP 5890 Series II gas chromatograph equipped with a flame ionization detector (Hewlett-Packard, Avondale, PA). A 50 m x 0.32 mm OV-1 fused silica open tubular column with 0.3 μm film coating was used (Alltech, Deerfield, IL). Nitrogen was passed through a molecular sieve and drying tube (American Scientific Products, McGraw Park, IL) and used as carrier gas at 13.6 ml/minute. Hydrogen gas and medical grade air were supplied to the FID detector at 10.8 ml/minute and 245 ml/minute respectively. Nitrogen was used as a make up gas at 5.7 ml/minute. Samples were injected using a HP 6890 injector and autosampler, and the GC signal was plotted and integrated using an HP 3396 Series II integrator (Hewlett-Packard). Injector temperature was 150°C. After an initial period of four minutes at 100° C, the GC oven temperature increased at 8°C per minute to a final temperature of 250°C and remained at 250°C for a 15 minute bake.

All experiments were set up with 3 (or 2) replicates and 3 (or 2) controls. All values are means of three (or two) replicates. The statistical analysis was conducted using student T-test. The difference is considered significant at $P<0.05$ level.

Table 2 gives the chemical-physical properties of stripped and non-stripped asphalt pavements and soil samples underneath the asphalt pavements. Results revealed that all of the samples contained primarily hydrocarbons and less amounts of nitrogen and phosphorus. Most

soil samples underneath the asphalt pavements has less hydrocarbons and higher amount of nitrogen and phosphorus than those of asphalt pavement samples. Available literature identified almost all the asphalt used in U.S. was refined from petroleum crude oil, petroleum in a complex mixture of many hydrocarbons such as gases, ether, gasoline, kerosene, furnace oil, lubricating oils, greases, and paraffins. Therefore, asphalt materials are described as the mixtures of hydrocarbons together with some compounds containing nitrogen, phosphorus, sulfur, and oxygen as well as minute quantities of metals. Table 2 shows that stripped and non-stripped asphalt pavements and soil samples underneath the asphalt pavements were basic. PHs were between 7.8 and 8.5 and averaged 8.2 for all samples. Bicarbonate alkalinity was predominantly in most asphalt pavements and soil samples underneath the asphalt pavements, included stripped and non-stripped. The broken asphalt and non-broken asphalt materials from Mankato (sample No. 1-4) and Willmar (sample no. 27) were alkali. PH was between 9.0-10.1. The alkalinity was predominantly hydroxide and carbonate types. In most cases, total hydrocarbon (%) in stripped asphalt pavement was less (2-3%) than that is non-stripped asphalt pavement with the exception of samples 21. This could be due to microbial degradative activities. However, soil samples underneath stripped asphalt pavements had more hydrocarbon than those underneath non-stripped asphalt pavement (Table 2). This could be interpreted as percolation of the biodeteriorated components of asphalt materials which deposited in soils underneath stripped pavement.

Microbial enumerations in samples of asphalt pavements and soils underneath asphalt pavements from five District Labs were listed on Table 3. Obviously, heterotrophs are significantly more abundant than asphalt-degrading microbial populations in all samples. Asphalt samples from the stripped (biodeteriorated) pavement have more asphalt-degrading microbes than non-stripped pavement samples. Soil samples underneath the stripped pavement have more asphalt-degrading microbial population and less heterotrophic populations than those underneath the non-stripped asphalt pavement. The heterogeniety of the samples make it possible to microbial degradation of asphalt materials relied on the components of asphalt and environmental conditions such as temperature, pH and oxygen tension etc. The presence of higher hydrocarbon content in stripped asphalt pavement stimulated the growth and activity of all types of hydrocarbon oxidizing microorganisms and inhibited the growth of heterotrophs (in sample 21, Table 3). In general, the worse the biodeterioration in asphalt pavements the greater the asphalt degrading microbial count. Taxonomic studies show that most hydrocarbon utilizing bacteria belong to genera of Pseudomonas, Micrococcus, Flavobacterium, Mycobacterium, and Pro-actinomyces, Actinomyces, and other genera. In some areas fungi were found to contribute to hydrocarbons degradation by their accumulation of complex metals and their production of a low pH in the media. It was found that 7 isolates belong to Pseudomonas sp., 9 isolates of Sphingobacterium sp., 3 isolates of Acinetobacter sp., 2 isolates of Agrobacterium sp. and 2 isolates of Chryseomonas sp. among 39 of asphalt-degrading isolates according to api NFT method. Laboratory experiments indicated that fungi and actinomyces and some tiny bacteria are predominant in stripped asphalt pavement samples (Fig. 1).

GC analysis of biodeteriorated asphalt pavement

Asphalt pavement samples were collected from broken and unbroken roadway at two locations in Mankato, Minnesota. Fresh Marshall pucks representing unused asphalt pavement was provided by MNDOT Bemidji District Lab.

Sample number	Description
0	Fresh asphalt pavement
1	M.P. 31.3, non broken top
2	M.P. 31.3, broken top
3	M.P. 31.5, non broken top
4	M.P. 31.5, broken top

The chromatograms for samples 0 through 4 are appended and shown in Fig. 2.

Sample 0 showed the "cleanest" chromatograms, with eight sharp peaks eluting between 17 and 24 minutes after injection. This suggest a simpler mixture in the unused asphalt sample, perhaps due to a lack of microbial degradation products.

In samples 1 and 3, both collected from intact pavement, both the number of peaks and the peak areas were greater than in samples from broken pavement of sample 2 and 4. Sample 1 had 10 more peaks than sample 4, with a total non-solvent peak area 30% greater. This indicates that a significant part of the original asphalt mass has been mineralized or transformed into water soluble compounds or might have been transformed into microbial cellular materials.

B. Biodeterioration/Biodegradation of Asphalt (Bituminous) Materials:

B.1. Activity: Fresh asphalt materials were requested from MNDOT and sterilized in an autoclave at 121°C and 15 atmospheric pressure. Single- or mixed-cultures were tested for their biodegradative capability on asphalt materials by enrichment techniques using a mineral medium to which sterile asphalt is added as the sole source of carbon. In other sets of biodeterioration/biodegradation of asphalt (bituminous materials), soils contain different levels of organic matter collected from underneath the damaged asphalt materials at various parts of Minnesota were mixed with sterile asphalt and tested for indigenous microbial degradative capabilities on asphalt materials.

B.2. Context Within the Project: There are three processes by which microorganisms can break down hydrocarbons: fermentation, aerobic respiration, and anaerobic respiration (14). In fermentation, the carbon and energy source is broken down by a series of enzyme-mediated reactions that do not involve an electron transport chain. In aerobic respiration, the carbon and energy source is broken down by a series of enzyme-mediated reactions in which oxygen serves as an external electron acceptor. In anaerobic respiration, the carbon and energy source is broken down by a series of enzyme-mediated reactions in which nitrates, sulfates, carbon dioxide, and other oxidized compounds (excluding oxygen), serve as external electron acceptors.

The ability of certain microorganisms to oxidize simple aromatic hydrocarbons has been demonstrated (28). Most of our present knowledge of the microbial degradation of aromatic hydrocarbons has been obtained with single hydrocarbon substrates and pure cultures of different microorganisms.

Petroleum is chemically very complex, consisting of hundreds of individual organic compounds (29). For microorganisms to biodegrade petroleum completely or attack even simpler refined oils, thousands of different compounds may be involved, which must be metabolized. The chemical nature of these petroleum components varies from the simple n-paraffin, monoalicyclic, and monoaromatic compounds, to the much more complex branched chains and condensed ring structures (30, 31). Many different enzymes are necessary to biodegrade these types of compounds.

Aerobic degradation in soil is dominated by a variety of organisms, including bacteria,

actinomycetes, and fungi, which require oxygen during chemical degradation (15). This process involves oxidation-reduction reactions in which molecular oxygen serves as the ultimate electron acceptor, while an organic component of the contaminating substance functions as the electron donor or energy source in heterotrophic metabolism.

Most aerobic bacteria use oxygen to decompose organic compounds into carbon dioxide and other inorganic compounds (32). In soil, oxygen is supplied through diffusion. If the oxygen demand is greater than the rate of oxygen diffused, the soil naturally becomes anaerobic. Maximum degradation rates for petroleum hydrocarbons are dependent upon the availability of molecular oxygen.

Aerobic biodegradation occurs via more efficient and rapid metabolic pathways than anaerobic reaction (3.3). Therefore, most site decontaminations involving refined oils and fuels are conducted under aerobic conditions.

Metabolic pathways have been established for the degradation of a number of simple aliphatic and aromatic structures (34). The general degradation pathway for an alkane involves sequential formation of an alcohol, an aldehyde, and a fatty acid. The fatty acid is then cleaved, releasing carbon dioxide and forming a new fatty acid two carbon units shorter than the parent molecule. This process is known as beta oxidation. The initial enzymatic attack involves a class of enzymes called oxygenases. The general pathway for degradation of an aromatic hydrocarbon involves cis-hydroxylation of the ring structure forming a diol, e.g., catechol. (In the cis configuration, the hydroxyl groups are on the same side of the molecule.) The ring is then oxidatively cleaved by oxygenases, forming a dicarboxylic acid, e.g., muconic acid.

Degradation of substituted aromatic compounds generally proceeds by initial beta oxidation of the sidechain, followed by cleavage of the ring structure. Simple alkyl substitution of benzene generally increases its rate of degradation, but extensive alkylation inhibits degradation. Branching generally retards the rate of alkane degradation. It may also change the metabolic pathway for utilization of a hydrocarbon (35). Some long chain alkanes may be degraded by different metabolic pathways, such as subterminal oxidation (36). The degradative pathway for a highly branched compound, such as pristane or phytane, may proceed by omega oxidation, forming a dicarboxylic acid instead of only monocarboxylic acids, as in normal beta-oxidation.

Anaerobic microbial transformations of organic compounds are important in anoxic environments (37, 38). Less cell material is formed under anaerobic conditions because of the lower growth yield, but organic fermentation products are likely to accumulate, unless they are converted into methane, or other hydrocarbon gases. Anaerobiosis usually occurs in any habitat in which the oxygen consumption rate exceeds its supply rate and is a common phenomenon in many natural aquatic environments receiving organic materials (39). Examples include flooded soils and sediments, eutrophic lagoons, stagnant fresh and ocean waters, and some groundwaters.

Petroleum can be microbially degraded anaerobically by the reduction of sulfates and nitrates (40). Aromatic hydrocarbons, common to many fuels, that can be biodegraded without the presence of molecular oxygen, include toluene, xylene, alkylbenzenes, and possibly benzene (41, 42, 43).

Depending upon which of the electron acceptors (e.g., NO_3^-, SO_4^{-2}, or CO_2) is dominant in an anoxic environment, anaerobic respiration may be employed to degrade organic compounds (39). This may be performed by denitrifying bacteria, sulfate reducing bacteria, or methanogens.

Many microorganisms that inhabit anoxic environments obtain their energy for growth through fermentation of organic carbon (44). Fermentation in a process that can be carried out in the absence of light by facultative or obligatory anaerobes. In fermentation, organic compounds serve as both electron donors and acceptors.

The phenomenon known as stripping has been recognized since 1935 and yet there are still some questions as to the cause (45, 46). At the onset of research on stripping, the cause was thought to be either physical or chemical in nature. In 1956 evidence that microorganisms might play a role in asphalt pavement failures was presented (47, 48, 49). It is an accepted scientific fact that a diverse group of microorganisms are capable of degrading asphalt and related hydrocarbons. Biological activity is also likely a causative agent. It may be only a minor influence and may indeed act only as a catalyst for a chemical or physical process. However, in some instances it may be of primary importance.

More evidence concerning the involvement of microbes in the process of asphalt concrete degradation has been reported (45, 50, 51, 52, 53, 54). Obviously, there are a number of causes of stripping but almost all of the causes appear to involve water. Be it physical stripping or biologically induced stripping, there is a breaking of the adhesive bonds between the asphalt and the aggregate.

Numerous studies have shown that products of microbial degradation: water, gases and surfactants, and/or emulsifying agents are detrimental to asphalt-aggregate bond which is the key to asphalt-aggregate mixture performance. The effects on asphalt-aggregate bond is particularly acute since microbial activity will be concentrated on mineral aggregate surfaces, the primary source of nutrients for microorganisms in such an environment.

A considerable amount of research as been conducted on the microbial ability to produce surfactants and emulsifiers to enhance recovery of oil from subterranean hydrocarbon deposits. In this case however, efforts are made to stimulate microbial activity and accelerate the stripping of oil from the sand and other solid material in the oil-bearing strata. If the microbe can produce compounds to strip oil from rock then the same compounds would strip another hydrocarbon, such as asphalt, from rock.

Microbial activity between asphalt layers (particularly overlays) may also contribute to pavement distress. Brown and Darnell (51) and Hironaka and Holland (55) examined similar data from an asphalt overlay that had experienced blistering and heaving. Brown and Darnell concluded that microbial activity was a major factor in the blister development while Hironaka and Holland concluded no effect.

The exact role of microbial activity in such a deterioration process in not known. However, conditions at the interface (damp anaerobic environment) are conducive to biological activity which may act to accentuate and accelerate physical and chemical processes.

A more thorough study is needed to understand the mechanisms of asphalt deterioration caused by microbial activities under various environmental conditions.

B.3. Methods:

Two different media will be used in this biodegradation study: Minimal buffered salts 1 (MBSI) will be prepared by adding the following substances to 1 liter of deionized distilled water: K_2HPO_4, 7 g; KH_2PO_4, 3g; $MgSO_4 \cdot 7H_2O$, 0.1g; $(NH_4)_2SO_4$, 1g; $NaNO_3$, 1g; and $(HOCOCH_2)3N$ (nitrilotriacetic acid), 1g. The pH of this buffer is 6.8.

Minimal buffered salts 2 (MBS2) will be prepared by adding the follwoing substances to 1 liter of deionized distilled water: K_2HPO_4, 7g; KH_2PO_4, 3g; $MgSO_4 \cdot 7H_2O$, 0.1g; $(NH_4)_2SO_4$, 1; and NaCl, 1g. This buffer is suitable for UV absorbance measurement to determine microbial population density.

The inoculum will be prepared by placing 25 g of damaged asphalt/soil collected from various sites of Minnesota highway into 90 ml distilled water. The slurry will be shaken on a wrist action shaker for two hours prior to use. One ml per 50 ml MBS will be employed as an inoculum. The samples will be mixed with MBS1, and then filtered through Whatman no. 1 filter paper. The culture will be enriched for microorganisms capable of utilizing asphalt by

the follwoing procedure. An Erlenmeyer flask will be prepared with asphalt melted on the interior, bottom surface. The flask contains approximately 0.06 g of asphalt per 1 ml of MBS1. The culture will be grown under aerobic conditions at room temperature. The concentration optical density of bacterial will be monitored with a Beckman spectrophotometer.

Biodeterioration/biodegradation of asphalt materials will be carried out using the following two sets of experiments. In the first set of test, fresh asphalt obtained from the MREL of MNDOT will be sterilized in an autoclave at 121°C and 15 atmospheric pressure. Pure- or mixed-culture will be tested for their biodegradative capability on asphalt (bituminous) materials by enrichment techniques using MBS1 and MBS2 media to which sterile asphalt is added as the sole source of carbon. In other set of experiment, soils contain different levels of organic matter that were collected from underneath the damaged asphalt pavement will be mixed with sterile asphalt and tested for indigenous microbial degradative capabilities on asphalt materials.

To determine degrees of biodeterioration of asphalt materials by pure- or mixed-culture, a series of chemical analyses will be carried out to quantify the composition and concentration of metabolites related to microbial degradative activities. Metabolic products and by-products will be analysed according to the methods described in Standard Methods (56) and Methods of Soil Analysis (57).

Biodegraded asphalt residual material will be extracted by a mixture of tolune-methanol (v/v=3/1). After de-asphalting with hexane, the hexane-soluble material will be fractionated on silica gel column and the alkane fraction obtained by elution with hexane will be further separated by reverse-phase HPLC [rp-18]; elution with methanol-chloroform (85:15 ratio). The final results will be compared with those of the controlled asphalt sample. The difference are what have been degraded by microbial activities. Detail methodology for biodegration of asphalt materials by enriched indigenous soil microorganisms will follow Chang et al. (58, 59) and Dawson and Chang (60).

B.4. Materials: No new equipment will be purchased or leased to conduct this research element. However, some new parts will be purchased for repair of existing equipments that will be used throughout this research project. Existing equipments that will be used in this research element are glove box, autoclave (both bench-top and floor models), heavy duty shakers, incubators, spectrophotometer and gas chromatograph). Besides microbial media, chemicals, glasswares and other supplies will be needed during the whole research period.

B.5. Budget:
Total Biennial LCMR Budget: $30,000
LCMR Balance: $0
Match: N/A
Match Balance: N/A

B.6. Timeline:

	7/95	1/96	6/96	1/97	6/97
Biodegradation/biodeterioration test of asphalt materials by asphalt-degrading isolates		X	- - - - - - - - -		X
Annual status report		X			
6-month status report			X		
Product B					X--X

B.7. Workprogram Update:

The effects of microbial activity on the biodeterioration/biodegradation of asphalt (bituminous) materials were conducted in two tests. The first test was microbial enumeration of heterotrophic and asphalt-degrading microbial population after Marshall pucks had been submersed in mixed asphalt-degrading culture and lake waters (fresh lake water and autoclaved lake water) for 1.5 and 4.5 months. The second test was Marshall pucks buried-in-soils in which soils were adjusted to contain 30%, 60%, 90% water holding capacity and contain indigenous asphalt-degrading microbes. These soil buried puck samples will also be tested for physical strength at various incubation periods. Strength loss vs. soaking time of puck samples in mixed broth culture of asphalt degrading bacteria and lake waters (fresh lake water and autoclaved lake water) using standard Lotmann test procedures. Results will be reported in C.7 of our next Workprogram Update.

Effects of microorganisms on asphalt (bituminous) materials after soaked in enriched broth culture and lake water for 1.5 and 4.5 months.

Mixed enriched microbial cultures were prepared and tested for their degradative capability on asphalt materials using a mineral medium (10 g of NaCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 1.0 g of NH_4NO_3, 3 ml of 10% KH_2PO_4 and 7 ml of 10% KH_2PO_4 per liter of distilled water) to which Marshall pucks were added as a sole source of carbon. In the second set of biodegradation/biodeterioration of asphalt (bituminous) test, lake water from Lake Bemidji was added to an aquarium tank that contained Marshall pucks to test for indigenous microbial degradative capabilities on asphalt materials. In the third test, autoclaved sterile lake water (without indigenous microbe) was added to submerse the Marshall pucks and used as a reference.

Seventy four asphalt degraders (39 bacteria, 19 actinomyces and 16 fungi) were isolated from thirty one asphalt and soil samples collected by five district labs around the state of Minnesota. Detailed microbial isolation and enumeration have been described in A.7. Workprogram Update. Stock culture of all isolates were maintained on mineral agar medium slants enriched with asphalt extract at 4° C refrigerator. Inocula were prepared from slants and grown on nutrient broth made of nutrient medium and lake water and incubated on a rotary shaker at room temperature for 24 hours. The mixed culture (Fig. 3) was transferred to BBL nutrient broth and incubated for 24 hours with agitation.

Three Nalgene rectangular tanks (15 gal/24X12X12 in^3) were used in this study. Tank one was filled with 4 L of enriched asphalt - degrading cultures and diluted to 24 L using autoclaved BBL nutrient medium in lake water. At biweekly interval, 12 L cultures were discarded and 12 L sterile mineral broth (components of the medium were the same as those described in the previous section) in lake water were added to the tank to submerse the Marshall pucks. The second tank was filled with 24 L of straight lake water collected from Lake Bemidji, and the third tank was filled with 24 L of autoclaved lake water as a reference (Fig.4). Triplicated Marshall pucks were added in each tank and used as a sole source of carbon (Fig. 5). Heterotrophs and asphalt degraders activities on bituminous material were carried out after submersed for 1.5 and 4.5 months. Three unsoaked fresh Marshall pucks were used as control. All tanks were aerated with purified air and incubated at room temperature (Fig. 4).

Fragments were taken from the fracture planes produced during the tensile-strength testing. These fragments were homogenized by blender and hammer and passes through a 0.0469 in. or 0.0787 in. sieve. Heterotrophic microorganisms and asphalt degraders were enumerated using plate count method and most probable number (MPN) techniques, as described previously, to confirm the presence of asphalt-degrading microbial populations and their

proliferation.

An index of hydrocarbon or bitumen oxidation by microorganisms is the growth of bacterial culture on the material which serves as the sole source of carbon. This is a valid index, since growth can occur only when the organism is capable of oxidizing the substrate. As the microorganism grows, it increases in population number on petri plates (plate count method), and the growth medium becomes more turbid by measuring the increase in tubidity of broth culture (MPN method). Heterotrophic microbial populations and microbial enumeration of asphalt degraders after Marshall pucks had been submersed in enriched broth culture and lake waters (fresh lake water and autoclaved lake water) for 1.5 and 4.5 months are presented in Table 4. It shows both the heterotrophs and the asphalt degraders gave approximately the same growth response on enrichment broth and lake water at the first sampling time (submersed for 1.5 months). The highest population densities were observed in the tank submersed with enrichment culture and Marshall Pucks. That was due to the fact that we used a complex culture medium such as BBL nutrient broth in lake water (to form nutrient broth) to enrich asphalt degraders at the beginning of this study. However, we replaced 50% of mineral broth medium (as well as 50% of lake water and sterile lake water in another two tanks) once every two weeks in order to allow organisms to synthesize their required enzymes to metabolize complex hydrocarbon components in asphalt materials. An adaptation process usually followed by logarithmic growth rate (occurred in 1.5 month) was observed after Marshall pucks submersed in three different solution for 4.5 months (Table 4). The highest population densities appeared in Marshall puck specimens submersed in enrichment mixed cultures. This test will be repeated by increasing the length of soaking time to 10 month or 1 year, soaking in the solution bath in order to establish the effects of extended exposure times. The asphalt materials soaked in water/broth medium will be analysed for chemical-physical properties and degradation by-products.

Effects of microorganisms on asphalt (bituminous) materials after soaked in enriched broth culture and lake water for 10 months

Effects of microbial activity on asphalt-aggregated bond by physical measurement of tensile and bond strength and microbial enumeration of heterotrophic and asphalt-degrading microbial population, as well as biochemicals/metabolites products by asphalt-degrading microbes after Marshall pucks have been submersed in enriched broth culture and lake water (fresh lake water and autoclaved sterile lake water) for 10 months were examined in this research. Certain abiotic parameters related to microbial action on asphalt stripping, such as acidity (pH), alkalinity, total hydrocarbon, total nitrogen, total phosphorus, NH4+-N, NO^-_2-N, NO-3-N have been analyzed.

Three replicated Marshall pucks incubated in three Nalgene rectangular tanks with different broth cultures described previously were taken after 10 months of incubation and homogenized by hammer and passed through a 0.0787-in. sieve. Samples were then stored in 4°C refrigerator for future analysis.

The analytical procedures for chemical characteristics and enumeration of heterotrophic and asphalt-degrading microorganisms of treated Marshall pucks were described previously. Tensile strength tests follow standard Lotmann test procedures through assistance provided by MnDOT Bemidji District Laboratory, the results will be reported in C. 7 of the next Workprogram Updates to LCMR.

Metabolites produced by asphalt-degrading microbes after Marshall pucks had been submersed in test solutions for 10 months were analyzed by HPLC and GC. The samples were extracted for 24 hours with 200 ml methanol by Soxhlet extraction method. Extracts were

stored at 4°C. A 2 ml aliquot from each extract was centrifuged at 15,000 rpm for 15 minutes. The supernatant was retained for metabolites analysis.

HPLC Analysis: A 70% methanol and 30% water solvent mixture was used for the HPLC analysis. Solvent flow rate was set at 1.0 ml/min. Fifty μl of extract was injected into a 20 μl loop and introduced to an Alltech Econosphere C 18 5U 250 mm column and was detected by UV absorption at 254 nm. Chromatograms were recorded and integrated using Beckman's System Gold v510 software.

GC Analysis: A HP 5890 Series II gas chromatograph (Hewlett Packard, Avondale, PA) was used for analysis. Extracts were injected into a 50 m x 0.32 mm OV-1 coated fused silica open tubular column (Alltech, Deerfield IL) using a HP 6890 Series Injector (Hewlett Packard). Elution was detected by a flame ionization detector, and chromatograms were plotted and integrated with a HP 3396 Series II integrator (Hewlett Packard).

During Analysis, the injection port and detector were held isothermally at 150°C and 250°C, respectively. The oven was set at 35°C for ten minutes, then was programmed to increase 8°C per minute to 255°C, then maintained at 255°C for twenty minutes. Nitrogen was used as a carrier gas with flow rate of 30 ml/min.

Comparable results of plate count and most probably number of heterotrophs and asphalt-degraders after Marshall puck samples had been submersed in enriched broth culture and lake waters (fresh lake water and sterile autoclaved lake water) for 1.5, 4.5 and 10 months are shown in Table 11. It shows that highest population densities were observed in Marshall pucks submersed in enrichment culture for 1.5 months. The cause was probably due to use nutrient in lake water to enrich asphalt degraders. An adaptation process gradually occurred when 50% of mineral broth medium was replenished in the tank with nutrient broth, as well as 50% of lake water or sterile lake water in another two tanks once every other week. A complete adaptation process was observed after Marshall pucks were submersed in three different liquid media for 4.5 months (Table 11). The highest population densities of both the heterotrophs and the asphalt degraders and a dramatic rate of proliferation of asphalt-degraders appeared in Marshall pucks that had been submersed for 10 months in the tank containing mixed asphalt-degrading microbes (Table 11). It is clear that the higher microbial proliferation, the worse the stripping of asphalt materials caused by microorganisms.

Chemical properties of Marshall pucks soaked in three broth media for 10 months are shown in Table 12. Marshall pucks submersed in mixed enriched culture or straight lake water (fresh lake water) or autoclaved (sterile) lake water and control pucks were alkaline, pHs were between 8.1 and 8.5. Bicarbonate alkalinity was predominant in all samples. Results reveal that all samples contain primarily hydrocarbons and less amounts of nitrogen and phosphorus. Marshall pucks soaked in mixed asphalt-degrading culture for 10 months had higher total nitrogen and inorganic nitrogen, such as $NH4^+$ and $NO2^-$. It might be because inorganic nutrient ammonium compounds that were added in the broth medium to support a magnitude of microorganisms to form cellular proteins. Denitrification might have occurred to form $NO2^-$ due to an insufficient dissolved oxygen present in tank I broth medium containing enrichment cultures. Figure 14 shows percent degradation of asphalt material in each Marshall puck by different treatments. Control Marshall pucks with zero degradation contain 7.36-7.95% hydrocarbons. Asphalt materials treated in mixed enrichment asphalt-degrading culture show a fairly good degradation (5.16-6.04% hydrocarbons remained) after soaked for 10 months. Marshall pucks treated with straight lake water or sterile lake water had 6.92-7.08% and 7.01-7.45% hydrocarbons remained, respectively.

Metabolic products produced by asphalt-degraders can be fractionated into components by chromatographic techniques using HPLC and GC.

The treatment conditions and the number of puck samples are listed as below.

Puck Number	Incubation conditions
0	Control
1	Enrichment culture in lake water
2	Straight lake water
3	Sterilized lake water

The results of HPLC analysis of asphalt extracts are shown in Fig. 15.

Four major peaks were detected for each sample with the 1.0 ml/min solvent mixture flow rate. Retention time (RT) for the major peaks were 1.972, 2.842, 2.904, and 3.821 minutes. All samples showed similar areas for the 3.821 peak, which accounted for about 14% of total area for each sample. The enrichment culture sample, Sample 1, had the largest peak area for the 1.972 minutes RT which accounts for 62% of total area. Sample 1's 1.972 peak was 50% larger than Samples 0 and 2, and 240% larger than Sample 3's. Sample 1's third peak 2.904 minutes RT was 30% smaller than Sample 0's and Sample 2's, and 41% smaller than Sample 3's. Some asphalt-degrading microorganisms can excrete an emulsifier, emulsan. Emulsan is an extracellular polysaccharide polymer with fatty acid (R-COOH) side chains. This extracellular molecular forms a complex with cellular proteins to make Marshall pucks less weakened which also happened when asphalt materials were soaked in mixed enriched asphalt-degrading culture for 1.5 and 4.5 months. It is apparent that the larger peak of retention time 1.972 minutes shown in the mixed enrichment-culture treated pucks (Fig. 15, sample 1) on HPLC analysis. When the large phenolic cementing compunds ( or R-OH) in asphalt was converted to water soluble metabolites, asphalt was stripped. Figure 15 shows a consistent result to support our hypothesis that some higher molecular weight compounds (two peaks with RT of 2.842 and 2.904 minutes on Fig. 15) with culture treated sample 1 was smaller than sample 0, 2, 3 due to asphalt-degrading microbial degradation.

The results of GC analysis of asphalt extracts are shown in Fig. 16.

In terms of number of peaks, the control extract has the most complex mixture, resolving into 7 peaks, followed by the sterilized lake water, straight (fresh) lake water, and culture treated extracts which had 4,3 and 2 peaks, respectively. The control extract also had the largest combined peak area. Samples 1, 2, and 3 had 9%, 17% and 25% of area of Sample O, respectively. This suggests that significant mineralization or microbial conversion of asphalt extractable compounds to small molecular weight of compounds or to water soluble metabolites occurred in all test samples, with the greatest mineralization occurred in the puck sample treated with enrichment culture.

Biodeterioration/biodegradation of asphalt (bituminous) materials in Marshall pucks-buried-in-soils test.

This test is to examine soils that contain different levels of organic matter which were collected from underneath the damaged asphalt pavement. Marshall pucks were buried in the soils and tested for indigenous microbial degradative capabilities on asphalt materials.

Five samples of stripped pavement and soils underneath the pavement were collected by MN/DOT's five District Labs (Bemidji, Brainerd, Duluth, Willmar and Rochester) around the

state of Minnesota (Fig. 6, 7). The locations and description of those samples were described in Table 5. Fresh Marshall pucks were prepared by the MN/DOT Bemidji District Lab and buried in the soils.

Chemical-Physical properties of damaged asphalt pavement and soil samples were analysed for acidity (pH), alkalinity , total hydrocarbon, total nitrogen, NH_4^+-N, NO_3^--N, NO_2^--N, total phosphorous according to the methods described in "Methods of soil analysis, Part II: Chemical and Microbiological Properties", Second Edition, 1982. The analytical procedures of chemical-physical properties were described in A.7. Workprogram Update. Granulation of soil samples was analysed at the MN/DOT Bemidji District Lab.

Most soil samples underneath the damaged asphalt pavement belonged to coarse loamy sand or clay or silty clay according to granulation test (Tables 5 and 6). The stripped asphalt pavement and soil samples underneath the asphalt pavement were both basic, pH was between 7.9-9.2 and 7.7-8.8, respectively. Biocarbonate alkalinity was predominant in all samples. Soil samples underneath the asphalt pavement had less hydrocarbons and nitrogen and phosphorus, especially the soil samples of Brainerd and Willmar District. It might influence microbial utilization of hydrocarbons since nitrogen and phosphorus are required for microbial activity. Table 6 depicts that total hydrocarbons (%) in stripped asphalt pavement at Rochester District was less (1%) than that in non-stripped asphalt pavement. The lowest total hydrocarbons (%) occurred in stripped asphalt pavement collected from Bemidji District. It could be resulted from worse biodeterioration by microbial degradative activity.

The hydrocarbon components of soil samples underneath asphalt pavement were fractionated by chromatographic techniques using HPLC. The soil samples were extracted with 200 ml of methanol for 24 hours by Soxhlet extraction method. A 70% methanol and 30% water solvent mixture was used for the HPLC analysis. Solvent flow rate was set at 0.5 ml/min. Fifty µl of extract was injected onto a 20 µl loop and introduced to an Alltech Econosphere C 18 5U 250 mm column and was detected by UV absorption at wavelength of 254 nm. Chromatograms were recorded and integrated using Beckman's System Gold V510 software.

The chromatograms for soil samples underneath asphalt pavements of Bemidji, Brainerd, Duluth and Rochester Districts are shown in Fig. 8. Fig. 8 shows that some smaller molecular weight compounds (RT with 2.946 and 6.100) were observed in soil sample of Rochester District. Some higher molecular weight compounds (major peak with RT of 10.710 of Brainerd District, RT of 10.890 and 11.413 of Duluth District and RT of 10.929 of Bemidji District) were obtained in soil of those Districts.

Microbial enumerations using plate count method and three-tube most probably number (MPN) techniques were described in section A. 3. The results of heterotrophs and asphalt degraders enumeration in soils underneath asphalt pavements and samples of asphalt pavements are shown Table 7. Heterotrophs are significantly more abundant than asphalt-degrading microbial populations in all soil samples. However, the stripped pavement have more asphalt-degrading microbial populations than heterotrophic populations in samples collected from Bemidji, Duluth, Willmar and Rochester Districts. Asphalt samples from stripped (biodeteriorated) pavement of Rochester District have more asphalt-degrading microbes and heterotrophs than non-stripped pavement samples. The greatest asphalt-degrading-microbial count and heterotrophs count were obtained in soil samples underneath asphalt pavement and the sample of asphalt pavement from Bemidji District. In general, the higher the asphalt degrading microbial count the worse the biodeterioration of asphalt pavement happened.

On the basis of different chemical-physical properties and microbial enumerations in five soil samples, the experiment of Marshall puck buried-in-soil was set up 120-140 lbs. of each soil were put in 20-liter Nalgene rectangular tank and adjusted to 30%, 60%, 90% water holding capacity (Fig. 9). Triplicated Marshall pucks were buried in the soil of each container

and incubated for four time period (3, 7, 12, 18 months). The control Marshall pucks were kept in the containers that are maintained at the same humidity (Fig. 10). At the end of each incubation period three pucks from each soil and control container will be removed and tested for chemical-physical properties, microbial enumerations and physical strength.

Biodeterioration/biodegradation of asphalt (bituminous) materials in Marshall pucks - buried in soil for three months with different water holding capacities.

Due to a number of causes of physical-chemical stripping appear to be mediated by water, the experimental design used three different water holding capacities for the study of Marshall pucks-buried-in-soils test.

Three pucks were removed from each treated or control container after having been buried for various period in soil that contained 30%, 60% and 90% water holding capacities and tested for chemical properties, microbial population densities, biochemicals/metabolic products and physical strength. Marshall pucks were removed from soil container and soil particles were wiped and cleaned with damp paper. It is apparent that soil microorganisms attached to the Marshall pucks (Fig. 17, 18). The highest population densities appeared in Marshall pucks that had been buried in soils maintained at 60% and 90% water holding capacities (Fig. 17, 18).

Fragments were taken from the fracture planes produced during the tensile-strength test. These fragments were homogenized by hammer and passed through a 0.0787 in. sieve. Chemical properties analysis, enumerations of heterotrophic and asphalt-degrading microorganisms, metabolites produced by asphalt-degraders and tensile strength test were conducted to confirm the effects of microbial activity on the biodeterioration/biodegradation of asphalt (bituminous) materials.

Results of chemical properties of Marshall pucks after buried in soils for three months are listed on Table 13. All pucks were basic, pHs were between 7.99 - 8.41. Biocarbonate alkalinity was predominant in most asphalt samples. Some samples from Rochester and Duluth were predominant with carbonate alkalinity. All pucks buried in treated soils had less nitrogen than those pucks left in control. It might be due to microbial assimilation of nitrogen and phosphorus since both nutrient are required for microbial activity. Denitrification might have occurred to form $NH4^+$ and $NO2^-$ in most samples due to an insufficient dissolved oxygen present in the containers. In most cases, total hydrocarbon (%) in Marshall pucks with treated soils and different water holding capacities was less than in cotnrol sample (Table 13). The Marshall pucks treated in soil with 60% and 90% water holding capacities had less hydrocarbons (%) than those samples with 30% water holding capacity. The total hydrocarbons (%) in asphalt sample from Bemidji District with 90% water holding capacity and Duluth District with 60% water holding capacity show a good degradation (5.19 and 5.92% hydrocarbons remained, respectively).

Microbial enumerations of Marshall pucks after had been buried in soil for three months are given in Table 14. Heterotrophs are significantly more abundant than asphalt-degrading microbial population in all samples. The highest heterotrophic microorganisms and asphalt degraders were found in pucks buried in soils that had 60% water holding capacity. Less heterotrophic and asphalt-degrading microbial populations were found in pucks buried in soils that were maintained at 30% water holding capacity. The abundance of heterotrophs and asphalt-degraders in asphalt samples buried in soils with 60% water holding capacity, in decreasing order, are Duluth>Rochester>Bemidji>Brainerd>Willmar. The heterogeniety of the samples make it possible to support microbial activity not only relied on its water content, but also the environmental conditions such as pH, inorganic nutrient content, organic matter

content and soil type.

Metabolites produced by asphalt-degraders after Marshall pucks had been buried in soils for three months were assayed by gas chromatographic techniques. A HP 5890 Series II gas chromatograph (Hewelett Packard, Avondale, PA) was used for biochemical components analysis. Sample extraction method and GC analysis conditions were the same as those described previously in B 7.

The chromatograms for biodegradation of asphalt materials buried in various soils for 3 months are shown in Fig. 19-23. The control pucks extract show the cleanest chromatograms, which have four sharp peaks. Retention time (RT) for the major peaks are 15.492, 18.441, 22.369, 25.848 minutes. The major peak areas of all puck samples buried in soils with 30%, 60% and 90% water holding capacities were smaller than those of control puck sample (Fig. 19-23). The smallest four major peak areas were observed in pucks buried in the Bemidji District soil with 90% water holding capacity. This result was associated with good degradation (5.19% hydrocarbons remained). Figure 20 to 23 show relatively small four major peak areas were found in Marshall pucks buried in soils, collected from Duluth, Rochester, Brainerd and Willmar, for 3 months with 60% water holding capacity. It is apparent that some higher molecular weight compounds (two peaks with RT of 25.848 and 24.409 min. on Fig. 22) in treated puck sample buried in Brainerd District soil with 60% water holding capacity has transformed to low molecular weight compounds (RT of 18.421, 20.782 and 23.039) by asphalt-degrading microorganisms. All these phenomena could be explained as significant part of the original asphalt material has been mineralized or bioconverted to water soluble metabolites or small molecular weight compounds or transformed into microbial cellular materials. This experiment led to the conclusion that microbial numbers, permeability of asphalt materials, soil moisture content, soil physical-chemical properties and length of time pucks buried in the soils must be considered in the stripping of asphalt material.

Biodeterioration/biodegradation of asphalt (bituminous) materials buried in soils for seven months with different water holding capacities.

Three Marshall pucks were removed from each treated or control soil held in container after having been buried for seven months in soils that were maintained at 30%, 60%, and 90% water holding capacities and tested for chemical properties, microbial densities, biochemical/metabolic products and physical strength. Marshall pucks were wiped and cleaned with damp paper towel. Prepared Marshall pucks were measured for physical strength using standard Lotmann Test procedures in Soil Lab, at MN/DOT Bemidji District. Fragments were taken from the fracture planes produced during the physical strength test. These fragments were homogenized by hammer and passed through a 0.0787 inch sieve. Chemical properties analysis, enumeration of heterotrophic and asphalt-degrading microorganisms using plate counts and most probable number techniques, metabolites produced by asphalt-degraders using GC and HPLC chromatographic techniques were done at Bemidji State University and tensile strength was tested at MN/DOT Bemidji District Lab, to confirm the effects of microbial activity on biodeterioration/biodegradation of asphalt (bituminous) materials and to better understand microbial activities and biochemical/metabolites changes in asphalt stripping.

Chemical properties of Marshall pucks after buried in soils for seven months are shown on Table 17. All pucks were basic, pHs were between 7.31-8.56. Fresh puck and the pucks buried in Duluth soil had a lower pH than pucks buried on other soils (pH were between 7.31-7.63). Bicarbonate alkalinity was predominant in most asphalt samples. All pucks buried in treated soils had less nitrogen that those pucks kept in control container. It might be due to microbial assimilation of nitrogen during degradation of asphalt materials. Denitrification

might have occurred to form N_2O and N_2 in some samples due to insufficient oxygen present in the containers. Total hydrocarbon (%) in Marshall pucks removed from treated soils after pucks were extracted by ethyl ether and dried at 60°C or 105°C was less than that in control and fresh puck samples in most cases (Table 17). Marshall pucks treated in soils that were maintained at 60% and 90% water holding capacities had less hydrocarbon (%) than those samples maintained at 30 % water holding capacity. Total hydrocarbon (%) in asphalt samples from Brainerd District maintained at 90% water holding capacity and Rochester District soil maintained at 60% water holding capacity show a good degradation (6.29 and 6.22% hydrocarbons remained, respectively. Table 17). It was interesting that all pucks buried in treated soils for seven months had more total phosphorus than those pucks left in moisture control chamber or fresh pucks or pucks buried in treated soils for three months (see Table 13). It might be due to mineralization of organic phosphorus in soils by microbial activities during degradation of asphalt materials and solubilized inorganic phosphorus was withheld by Marshall pucks.

Comparable results of plate count and most probable number of heterotrophs and asphalt-degraders after Marshall pucks had been buried in soils for three and seven months are shown in Table 18. It shows that decreasing heterotrophic microbial densities were observed in most Marshall pucks buried in soils for seven months as compared with those buried in soils for three months. It is possible that most heterotrophs were about using up available organic substrate present in the soils. However, a dramatic rate of proliferation of asphalt-degraders was observed in most Marshall pucks, with the exception of pucks buried in soils from Duluth District, buries in soils for seven months (Table 18). At the initial time, the majority of the hydrocarbon-oxidizing organisms prefer the easily available complex organic compounds such as carbohydrates, fats, and proteins as carbon and energy sources, result in heterotrophs that are significantly more abundant than asphalt-degrading microbial population occurred in all samples when Marshall pucks were buried in soils for three months (Table 18). As the easily available compounds were exhausted, then a lag phase of growth occurred during which time organisms synthesized adaptive enzymes. This period was then followed by a further growth response from the utilization of the hydrocarbon substrate. A dramatic proliferation rate of asphalt-degraders occurred. Table 18 also shows the highest heterotrophs and asphalt degraders observed in Marshall pucks buried in soils that were maintained at 60% and 90% water holding capacities. Less heterotrophs and asphalt-degrading microbial populations were found in pucks buried for seven months in soils that were maintained at 30% water holding capacity (except samples from Bemidji District) because of low moisture content is unfavorable to bacterial activity. The higher proliferation of the asphalt-degraders, the worse the stripping of asphalt materials response by microorganisms.

Biochemical/metabolites produced by asphalt-degrading microbes after Marshall pucks had been buried in soil for seven months were analyzed using GC and HPLC chromatographic techniques.

A 24g composite samples (eight g portion of sample from three replicates of each treatment were mixed uniformly) were extracted with 200 ml of methanol in soxhlet extraction for 24 hr. After that a 2 ml aliquot from each extract was centrifuged at 1500 rpm for 15 minutes. The supernatants were retained for metabolites analysis.

A HP 5890 Series II gas chromatograph equipped with a 50 Mx0.32 mm OV-1 coated fused silica open tubular column (Alltech, Deerfield IL) using a HP 6890 series injector (automatic liquid sampler, Hewlett Packard) was used for gas chromatographic analysis for the biochemical/metabolites. Nitrogen was used as a carrier gas. The GC oven temperature was programmed as follows: first maintained at 35° C (10 min) then increased from 35° to 255° C (at 8° C/min increment) then maintained at 255° C (for 20 min). The instrument was

equipped with a FID (flame ionization detector) and flow rate was set at 30 ml/min.

High performance liquid chromatography (HPLC) used is Beckman System Gold TM, the personalTH Chromatograph. A 70% methanol and 30% water solvent mixture was used and flow rate was set at 0.5 ml/min. Fifty ul of above methanol extract was injected into a 20 ul loop and introduced to an Alltech Econosphere C 18 5U 250 mm column and was detected by UV absorption at 254 nm. Chromatograms were recorded and integrated using Beckman's System Gold V510 software.

Evaluation of the extent of asphalt deterioration by microbial activity can be followed by chromatographic fraction using GC and HPLC chromatograms. It was usually done by looking at changes of retention time and peak areas on chromatograms. When longer retention time (higher molecular weight compounds) was changed to shorter retention time (small molecular weight compounds) it accounts for large phenolic cementing compounds (aromatic and saturated hydrocarbons) in asphalt was converted to water soluble metabolites, asphalt stripped. The areas or position of chromatographic peak numbers were reduced or changed which were interpreted as original asphalt materials has been mineralized or bioconverted to water soluble metabolites or transformed to microbial cellular materials.

Gas chromatograms for biochemical/metabolites changes and biodegradation of asphalt materials buried in various soils for seven months are shown in Fig. 27. They show four major peaks present in fresh puck extract. Retention time (RT) for the major peaks are 22.325, 18.625, 12.196, and 12.020 minutes. Four major peaks displayed retention time of 25.775, 22.297, 18.359, 11.492 minutes are present in control pucks extract. The major peak areas of all puck samples buried in soils with 30%, 60%, 90% water holding capacities including control puck samples are smaller than those of fresh puck samples (Fig. 27). The biochemical/metabolites changes and biodegradation of asphalt material are also noticed in control asphalt puck samples (Fig. 27) which were kept in the containers that had been maintained at the same humidity (30%, 60%, and 90% water holding capacities). The reason for some deterioration in control samples might be due to water dissolution or water action with microbial activities after Marshall puck had been kept in the containers with 30%, 60%, and 90% humidities for seven months. Fig. 27 shows relatively small or almost absence of four major peaks are found in Marshall pucks buried in soils, collected from Brainerd, Duluth, Rochester and Willmar, for seven months with 60% and 90% water holding capacities (sample from Bemidji District might have some problems with solvent concentration). It reveals that asphalt sample buried in soils for seven months with 60% and 90% water holding capacities had undergone more biodegradation as compared with chromatograms obtained from asphalt samples buried in soils for three months with the same water holding capacities (60% and 90% W.H.C., Fig. 28). Asphalt samples buried in soils for seven months with 30% water holding capacity in Brainerd and Willmar Districts had undergone mild biodegradation which shows higher molecular weight compound (two peaks of RT 25.775 and 22.29) had been transformed to lower molecular weight compounds (RT 19.615 and 11.648 in Willmar District and RT 17.185 in Brainerd District). Some peak areas increased in asphalt samples buried in soils for seven months with 30% water holding capacity in Duluth and Rochester Districts which might indicate that some intermediate products accumulated during microbial transformation processes.

The HPLC chromatograms for biochemical/metabolites changes and biodegration of asphalt materials after having been buried in soils for seven months are depicted in Fig. 29. Four major peaks are detected for fresh puck samples with the 0.5 ml/min solvent mixture flow rate. Retention time (RT) for the major peaks are 12.015, 21.123, 26.308, and 32.130 minutes. All treated samples and control sample show changes of metabolites components or reduced peak areas of compounds as compared with chromatograms of fresh sample. A high

molecular weight compound (major peak with a RT of 31.13) was present in the extracts of control samples, Duluth soil with 60% and 90% water holding capacity (W.H.C.), Bemidji soil with 90% W.H.C. and Rochester soil with 30% W.H.C. The rest of extracts from treated samples have changed from higher molecular weight compound (RT of 31.13) to low molecular weight compounds (shorter retention time) or water soluble metabolites that had been biodegraded (peaks disappeared). Apparent molecular weight changes of metabolic compounds in HPLC chromatograms were observed in extracts of asphalt samples buried in all soils for seven months, with 60% and 90% water holding capacities. Considerable amount of metabolic compounds had been biotransformed in all asphalt samples buried in soils for seven months with 30% water holding capacity (Fig. 29).

Biodeterioration/Biodegradation of Asphalt (Bituminous) Materials Buried in Soils for Twelve Months with Different Water Holding Capacities.

Soil burial tests have been used to assess the effects of microorganisms on the durability of bituminous materials, and also test some concern of the mechanisms of microbial degradative activity associated with asphalt stripping. Three 4" diameter and 2.55" thickness Marshall pucks were removed from each of the treated and control containers after having been buried for twelve months in soils or left in control container that were maintained at 30%, 60%, and 90% relative humidities. Removed Marshall pucks were cleaned with damp paper towel by gentle wiping. Fragments of Marshall puck were taken from the fracture planes produced during the physical strength test. These fragments were homogenized by hammer and passed through a 0.0787 inch sieve. Chemical properties analyses, enumeration of heterotrophic and asphalt-degrading microorganisms using plate counts and most probable number (MPN) techniques, metabolites produced by asphalt-degraders were analyzed by GC chromatographic techniques. Physical/tensile strength was tested at MN/DOT's Bemidji District Lab, to study the mechanism of asphalt deterioration caused by microbial activities under various environmental conditions and to better understand microbial activities and biochemical/metabolites changes as a result of asphalt stripping, as well as to find their relationships among biochemical/ metabolites changes, physical strength and degree of stripping.

Chemical properties of Marshall pucks after having been buried in soils or placed in control containers for twelve months are shown in Table 20. All pucks were basic, pHs were between 7.68-8.61. Bicarbonate alkalinity was predominant in most asphalt samples. It is different from the previous report that the pucks kept in control containers had less nitrogen and phsophorus than those pucks buried in soils. It might be due to microorganisms adhering to the surface of Marshall pucks in control containers had utilized hydrocarbon as sole carbon source and nitrogen and phosphorus utilization occurred simultaneously during the 12 months period. Total hydrocarbon (%) remained in Marshall pucks that were buried in soils or kept in control containers and maintained at different water holding capacities for 12 months was less than that in fresh pucks. The pucks kept in control containers with 30%, 60% and 90% humidities for 12 months had less hydrocarbon than those pucks buried in soils (Table 20). It might be due to the fact that microorganisms adhering to Marshall puck in control containers only use asphalt as the sole source of carbon and energy because only asphalt materials were present in the containers. Denitrification might have occurred to form NO2- in the puck samples buried in soils collected from Brainerd and Rochester Districts (Table 20).

Comparable results of plate count and MPN of heterotrophs and asphalt degraders after Marshall pucks had been buried in soils for 3, 7, and 12 months are summarized in Table 21, Fig. 34 and Fig. 35. It also shows growth curve of heterotrophs and asphalt-degraders after Marshall pucks had been buried in soils for 3, 7 and 12 months which are depicted in Fig. 34

and 35. Slow metabolic process of heterotrophs and asphalt-degraders occurred in soils that were maintained at 30% water holding capacity at the beginning time because low moisture content is unfavorable to bacterial activity. The growth curve remained at optimum for about seven months in most samples that were maintained at 30% water-holding capacity. Organisms require time to build up their new biosynthetic enzymes. Following the period of adaptation, microbial populations increased with a characteristic of constant doubling time. The growth rose exponentially called logarithmic stage of heterotrophs and asphalt-degraders occurred in most sample buried in soils that were maintained at 30% water-holding capacity for 12 months (Fig. 34 and Fig. 35). However, almost no lag phase for heterotrophs was observed in most samples buried in soils that were maintained at 60% and 90% water-holding capacities because population is at its biochemical optimum conditions, i.e. moisture content and nutrients et al are most suitable for microbial proliferation. Then, the vigor of heterotrophic population changes, the rate of reproduction slows down and some cells die off due to the use up of available organic substrate or an accumulation of a toxic waste product present in soils. The actual number of heterotrophs declines during the course of Marshall pucks buried in different soils for seven months (Fig. 34). Fig. 34 also shows that heterotrophs proliferated with a second exponential phase after Marshall pucks have been buried in soils that were maintained at 60% and 90% water-holding capacities for 12 months. It might be because the use of recycled nutrients in soil and waste products released from metabolic activity. Most asphalt-degraders actually prefer to use complex energy sources such as carbohydrates, fats and proteins in soils. It is an adaptation period when asphalt-degraders began to utilize hydrocarbons. A long adaptation period was observed in Fig. 35 during which time asphalt degraders synthesized the required enzymes. Then, the mass of asphalt-degraders increased by exponential proliferation after Marshall pucks had been buried in soils post seven months in most samples (Fig. 35).

Our earlier reports showed high asphalt-degrading population in stripped pavements (and soils underneath) than the non-stripped asphalt samples (and soils underneath). Recent data reveal that high heterotrophic and asphalt-degrading populations present in asphalt samples that had been buried in soils with 60% and 90% water holding capacities for 3, 7 months (Fig. 34 and Fig. 35). It shows more asphalt-degraders proliferated in Marshall pucks that had been buried in soils with 30% water holding capacity for 12 months after a long period of adaptation (Fig. 35).

Gas chromatograms for biochemical/metabolites changes and biodegradation of asphalt materials buried in various soils for 12 months are shown in Fig. 36. The extraction and analytical conditions for gas chromatographic analysis in a HP 5890 Series II gas chromatograph were described in our previous report. It shows (Fig. 36) five major peaks present in gas chromatograms from fresh puck extract. Retention time (RT) for the major peaks are 28.566, 28.192, 26.598, 25.528 and 22.071. Four major peaks display retention time with 29.600, 28.560, 25.519, 22.062 minutes were present in the control pucks extract.

Fig. 36 shows absence of two major peaks that had longer retention time (higher molecular weight compounds) of 28.566 (or 29.600) and 28.192 (or 28.560) minutes in most asphalt samples buried in soils that were maintained at 30%, 60%, and 90% water-holding capacities. The other two peaks with retention time of 25.519 and 22.062 minutes in most samples were smaller than those of fresh puck samples or control puck samples (Fig. 36). The areas or position of chromatogram peaks had disappeared or changed which were interpreted as original asphalt materials had been mineralized or bioconverted to water soluble metabolites or transformed to microbial cellular materials. It was also confirmed that asphalt samples buried in soils that were maintained at 30%, 60%, and 90% water-holding capacities for 12 months had undergone biodegradation to various extent by microbial action. Metabolic activities

associated with considerable level of growth rate and reproduction of asphalt degraders (see Table 21, even if the samples buried in soils with 30% water-holding capacity) are responsible for the biodeterioration of asphalt materials. It was noticed (Fig. 36) that even though the asphalt puck samples kept in the control containers that have been maintained at the same humidities (30%, 60%, and 90% water-holding capacities) for 12 months had undergone biodeterioration as compared with chromatograms obtained from fresh asphalt sample. Molds and bacteria (Fig. 37) adhering to the surface of Marshall pucks was noticed after buried in control containers with 30%, 60%, and 90% humidities for 12 months. Water is often working in concert with various populations of microorganisms. Significant numbers of asphalt degraders were present in asphalt materials that were kept in the control containers (Table 21). Those organisms present in the control container only rely on asphalt as the sole source of carbon and energy because there was only asphalt puck present in the containers. Actions of moisture and microorganisms on asphalt materials in the control containers is also detrimental to the durability of asphalt pavements.

Biodeterioration/Biodegradation of Asphalt (Bituminous) Materials Buried in soils for 23 months with different water holding capacities.

This is the last set of Marshall puck samples of the buried-in-soil test. Pucks were removed from each treated or control containers after having been buried for 23 months in soils or left in the control containers that were maintained at 30%, 60% and 90% water holding capacities. Chemical properties analyses, enumeration of heterotrophic and asphalt degrading microorganisms were assayed using plate counts and most probably number (MPN) techniques, metabolites produced by asphalt-degraders were analyzed using GC chromatographic techniques. Physical/tensile strength test was done at MN/DOT Bemidji District Lab. An additional experiment was conducted to compare populations of hetrotrophs and asphalt degraders capable of growing in soils adjacent to the buried Marshall puck compared with those populations in undisturbed soil that was far apart from the Marshall pucks (Fig. 44). Data reported here deals with the numbers and types of asphalt-utilizing microorganisms in Marshall pucks and soils adjacent to the Marsall pucks, and their biochemical/metabolites changes as a result of asphalt-degrading microbial activity relative to physical strength and degree of stripping.

Chemical characteristics of Marshall pucks after having been buried in soils or placed in control containers for 23 months are shown in Table 23. All pucks are basic, pHs are between 7.26-7.98. Bicarbonate alkalinity was predominant in most asphalt samples. Most pucks buried in treated soils had less nitrogen and phosphorus than those pucks kept in control containers. This could attribute to microbial assimilation of nitrogen and phosphorus during the degradation of asphalt materials. No microbial dentrification or chemical dissimilatory reduction had occurred to form NH_4^+ and NO_2^- in all asphalt samples. Total hydrocarbon (%) in Marshall pucks with treated soils was less than those in control and fresh puck samples (Table 23). The Marshall pucks treated in soils with 60% and 90% water holding capacities had less hydrocarbon (%) than those samples that were maintained at 30% water holding capacity in most cases (Table 23).

Plate count and MPN of heterotrophs and asphalt degraders after Marshall pucks had been buried in soils for 3, 7, 12 and 23 months are summarized in Table 24, Fig. 45 and Fig. 46. Two logarithmic phases of heterotrophs and asphalt-degraders were found in most samples buried in soils that were maintained at different water holding capacities during the 23 months incubation period (Fig. 45 and Fig. 46). The second logarithmic phase of heterotrophs and asphalt degraders (Fig. 45 and Fig. 46) was probably due to microorganisms used intermediate

metabolites, recycled inorganic nutrients or newly released hydrocarbons from asphalt materials and biodegradation of other soil organic matter. Those newly released intermediate metabolites and nutrients stimulated cell reproduction and resulted in a new exponential growth phrase. Fig. 45 and Fig. 46 show that significant numbers of heterotrophs and asphalt degraders were found in almost all samples buried in soils that were maintained at 60% water holding capacity for 23 months. The reason is probably due to microbial proliferation at their optimum conditions, i.e. moisture content, nutrients such as biodegradation products and metabolites et al are most suitable for microbial growth.

Table 2 5 and Fig. 4 7 compares populations of heterotrophs and asphalt degraders capable of growing in soil adjacent to the buried Marshall pucks with those populations in undisturbed soil that was far apart from Marshall pucks. Significant numbers of heterotrophs and asphalt degraders were found in soil adjacent to the asphalt materials. Soils taken from adjacent to the Marshall pucks, had heterotrophic counts 1.1-2.1 times higher than those soils taken from undisturbed soils far apart from the pucks. Forty-six percent of soil attached to asphalt materials had counts of 2 times, 22% of soils had counts of 4-5 times and 22% of soil had counts of 20-30 times more asphalt degrading populations than in undisturbed soil far apart from Marshall pucks. There is no question concerning a considerable numbers of asphalt degraders were in the soils near asphalt materials (Marshall pucks) where they derived organic carbon from.

Earlier reports have shown that high populations of asphalt degraders were associated with failing asphalt road and have been given extensive data reporting high microbial counts in soils near asphalt materials .

Ga s chromatograms for biochemical/ metabolite changes and biodegradation of asphalt materials buried in various soils for 23 months are shown in Fig. 48. It shows six major peaks were present in gas chromatograms of fresh puck extract. Only one peak with retention time of 13.910 minutes has changed and some peaks were smaller than those in fresh puck extract that was observed in control pucks extract. Major peaks almost disappeared in most asphalt samples buried in soils that were maintained at 60% water holding capacities (Fig. 48). The area percents or position of chromatogram peaks had disappeared or decreased could be interpreted as original asphalt materials had been mineralized or bioconverted to water soluble metabolites or transformed to microbial cellular materials. It was confirmed that asphalt samples buried in soils that were maintained at 30%, 60% and 90% water holding capacities for 23 months (Fig. 48), especially, the asphalt sample buried in soils with 60% water holding capacity had undergone most severe biodegradation.

C. Asphalt Strength Testing:

C.1. Activity: A correlation of strength loss and time in samples exposed to cultures of hydrocarbon degrading bacteria will be established using standard engineering testing procedures.

C.2. Context within the project: Asphalts are described as black to dark brown highly viscous mixtures of paraffinic, naphthenic and aromatic hydrocarbons together with heterocyclic compounds containing sulphur, nitrogen, and oxygen as well as small quantities of metals. Asphalts are primarily employed in engineering applications which make use of their adhesive quality, waterproofing ability, relative chemical inertness, and high viscosity at ambient temperatures. Being a powerful cement with the aforesaid characteristics, asphalt is of particular interest to road builders. Several million tons of asphalt are consumed annually in

the United States in road construction.

Stripping, as the term is applied to asphalt pavements, refers to the breaking of the adhesive bond between the gravel and the asphalt. There are a variety of ways in which stripping occurs but the net result is a washing away of the asphalt from the gravel. Since microbes produce surfactants and emulsifying agents that could accelerate the process, the question arises as to whether they play a meaningful role in the stripping process. Preliminary analyses of stripped asphalt samples confirmed the presence of both aerobic and anaerobic microbes. Other studies have shown that microbial growth accelerates the stripping process.

Major types of bituminous pavement distress were rutting, cracking (longitudinal, transverse, and alligator) and stripping. The transverse cracks were 1/8" to 3" wide. Cuts made perpendicular to them revealed inverted trough shaped deteriorated areas. The central portion of these were rubble-like. A soft loosely bound asphaltic concrete was present between the rubble and bound materials. The rubble and loosely bound material contained bacteria. The deterioration lessened upward from the pavement-soil interface. The soil appears to be the prime source of the bacteria (62).

Mechanical vibrations from passing vehicles and the role of stone-eating bacteria have had a disastrous effect on our civilization's proudest monuments. Some microbes rapaciously devour paintings and pesticides. Certain other species are the root cause of devastating metal corrosion and concrete deterioration .

Badgley (63), during his study of bacterial utilization of AC-7 asphalt coating on limestone observed that the aggregate itself had suffered form the biological weathering in the form of badly pitted surfaces. In laboratory investigations, Crumpton and Badgley (64, 65) found MC-O asphalt treated samples of clay fractions in the presence of bacteria, exhibited considerable change in physical properties. Samples of loess treated with 2 to 5 percent MC-O asphalt doubled in plastic index values compared to untreated samples.

During field studies of asphalt road failures associated with loess in north central Kansas. Crumpton and Badgley (65) observed a thin layer of green clayey material in the pavement subgrade just below the asphalt mat at failing locations. According to these authors the soil bacteria utilize the asphalt as an energy source and cause reducing conditions at the soil-asphalt interface, where the supply of air and oxygen is limited. The result was an underclay formation in the soil in contact with the asphalt pavement. It was already known from previous
studies (48) that aerobic bacteria were active in the same horizons. This supports Robinson's (66) suggestion that both aerobic and anaerobic process could occur simultaneously. Harris (67) found that both aerobic and anaerobic micro-environments do exist within a small space in the soil back-fill around pipelines.

Premature pavement failure attributed to moisture-induced damage has long been recognized, but solutions to the problem have been far from satisfactory. This type of damage, generally believed related to rupture of the adhesive bond at the asphalt-aggregate interface, is a complex phenomenon involving physical and chemical properties of both the asphalt and the aggregate. Moisture damage is also strongly influenced by pavement-mixture morphology and external environmental factors.

A laboratory test method for predicting moisture-induced damage in asphaltic pavements was developed by Lottman (68) during work sponsored by the National Cooperative Highway Research Program (NCHRP). Phase 2 of this program involved a field evaluation to determine test-method predictability. The field evaluation commenced in 1974 under Lottman's direction with participation by seven state and federal agencies (69.70) and was completed in 1981.

C.3. Asphalt Strength Testing Methods: The effects of microbial action on asphalt strength will be tested at the Materials, Research & Engineering Bituminous Testing Laboratory of MNDOT.

Marshall puck samples will be prepared and tested using the modified Lotmann Test (ASTMD4867) Procedures. Identical prepared samples will be treated simultaneously in three tests. One test will be the normal Lotmann procedure which involves soaking the samples in 77°F sterile water bath for 3, 6, 12 and 18 months and load tested to determine strength.

The second test will be to use identical procedures except that the water bath will be inoculated with a microbial broth culture of known asphalt degrading characteristics. The samples will also be tested for strength through load testing.

The control samples will be the third part of the samples and will be tested in the same manner except that the pucks will be held at 77°F in a dry pan for the same time period as the first two samples.

The tests will be repeated by increasing the length of time, up to 3 - 5 years, soaking in the water bath in order to establish the effects of extended exposure times. The soaked water/broth medium will be analysed for pH and degradation by-products.

C.4. Materials:

No equipment will be purchased or leased to carry out this project. However, some existing equipment will be used (as inkind matching) throughout the execution of the research project. Twenty four 5-gallon volume glass container will be purchases to carry out soaking experiment.

C.5. Budget
Total Biennial LCMR Budget: $10,000
LCMR Balance: $0
Match: N/A
Match Balance: N/A

C.6. Timeline:

	7/95	1/96	6/96	1/97	6/97
Asphalt strength testing		X	- - - - - - - - - - - - - -		X
Status reports			X	X	
Final report and Product C					X--X

C. 7. Workprogram Update.

The microbial action on asphalts is due to oxidation of certain asphalt compounds which is also important in determining the rheological characteristics of the asphalt. It will be detrimental to asphalt-aggregate bond which is the key to asphalt-aggregate mixture performance. Usually, the effects can be examined by measuring the tensile strength of asphalt-aggregate mixtures and bond strength. The effect of microbial actions was scored by changes in tensile strength, broken aggregate and visual stripping. Correlation studies to establish strength loss vs. soaking time of puck samples were conducted in mixed broth culture of asphalt degrading bacterial and lake waters (fresh lake water and autoclaved lake water) after Marshall pucks had been soaked for 1.5 and 4.5 months. The design and procedures of Marshall pucks which were submersed in mixed enriched microbial culture or lake waters were described in B. 7. Workprogram Update. Marshall pucks prepared by MN/DOT Bemidji District Lab had an average void of 8.55 percent with average maximum specific gravity of

about 2.456 g/cm^3 the preparation of Marshall pucks and their basic information are shown in Table 8, 9. Tensile strengths were applied through standard Lotmann test procedures. Changes of physical properties are summarized in Table 10. After Marshall pucks had been soaked for 1.5 and 4.5 months in mixed-asphalt degrading -microbial broth culture or straight lake water or autoclaved lake water revealed that Marshall pucks were relatively weaker (softening) in tensile strength than the control Marshall pucks (non-treated). Tensile strength of pucks was less weakened after treated with enrichment culture as compared to those treated with lake water or autoclaved lake water. It might be because a magnitude of microorganisms to form a thick microbial film around pucks treated in enrichment culture (see Fig. 11) which prevented the water and cells from moving in and out of pucks. Also, microbial metabolites in the enrichment-culture treated might have pucks formed complex with carbonate and aggregate in the pucks, which might have shielded asphalt from dissolution and showed less weakened physical strength than those pucks soaked in lake water or autoclaved lake water. Similar results were obtained by Harris et. al. (1958) using a percolation technique with two different strains of hydrocarbon-utilizing bacteria. They concluded that either a softening or a hardening of the asphalt occurred, depending on the nature of the bacteria present. The intensity of microbial action depends on several factors including the type of bacteria, presence of moisture and oxygen, pH, composition of asphalt etc. Usually the effects of bacteria action are seen only after several years. Under favorable conditions, however, bacteria can utilize hydrocarbons at a rapid rate. Bacteria are adaptive and their infestations are hard to suppress.

As table 10 shows that after Marshall pucks were soaked in mixed asphalt-degrading-microbial culture for 1.5 months resulted in highest percentage of broken aggregate. It appears that some low molecular weight of constituents might have been biodeteriorated as shown in Fig. 12. These low molecular weight components are usually more readily absorbed onto aggregate surfaces than the higher molecular weight constituents, they have a disproportionately larger influence on bond development. The higher broken aggregate % was obtained after Marshall pucks were submersed in sterile lake water and straight lake water for 4.5 months than those soaked in mixed asphalt-degrading broth culture (Table 10, Fig. 13). The reason was the fact of asphalt are subjected to water dissolution and hydrolysis action. There are no obvious formation of microbial films around pucks in sterile lake water treated pucks (Fig. 11), however, a thin microbial film was found around the pucks soaked in lake water (Fig. 11). Pore space provides access channels for water and bacterial cells to the asphalt-aggregate. Water has been described as the worst enemy of bituminous pavements deterioration. Water is essential for microbial activity, when water gets into a pavement crack the microorganisms become active and begin to multiply.

The results of changing physical properties and tensile strength after Marshall pucks had been soaked in mixed-asphalt degrading microbial culture or straight lake water or sterile lake water for 10 months are summarized in Table 15. Marshall pucks post-10 months treatments showed that pucks were relatively weak (softening) in tensile strength ratio (TSR%) than the control Marshall pucks (non-treated). Tensile strength ratio percent of pucks approached the same softening stage after Marshall pucks had been soaked for 10 months. Marshall pucks soaked in asphalt-degrading broth culture were relatively less weakened than those soaked in lake water. Tensile strength test also show higher broken aggregate percent and higher visual stripping percent (Table 15, Fig. 24) after Marshall pucks had been submersed in mixed asphalt-degrading microbial culture for 10 months. It is clear that significant effect of microbial degradative action on asphalt materials after pucks had been soaked in the tank containing asphalt-degrading microbes for 10 months. Marshall pucks soaked in sterile lake water were worst weakened due to the fact that many asphalt components are subject to water dissolution and hydroysis action. Data obtained from this study appear that the effects of

microbial degradative activity on asphalt are less dramatic but are more traumatic in the long run. However, it is also possible to have substantial damage due to the combined effects of microbial degradative action with other abiotic factors such as action of water and environmental parameters.

Changes of tensile strength, broken aggregate percent and visual stripping percent of buried Marshall pucks in soils with different water holding capacities for three months are summarized in Table 16 and Fig. 25, 26. Table 16 shows Marshall pucks in most treated samples (except samples from Duluth, Rochester, Willmar that were maintained at 30% water holding capacity) are relatively weak in tensile strength (TSR%) than untreated Marshall pucks (also with different water holding capacities) after Marshall pucks had been buried for 3 months in soils with three different water holding capacities. Weaker (softening) tensile strength was found in pucks after pucks had been buried in soils, collected from Duluth and Rochester Districts, with 60% water holding capacity than those buried in 30% and 90% water holding capacities (Table 16). It also shows that higher visual stripping and broken aggregate (Table 16, Fig. 25, 26) were found in Marshall pucks buried in soils, collected from Duluth and Rochester Districts, with 60% water holding capacity. This result was in agreement with the data of biochemical/ metabolites produced by microbial degradation as mentioned in B7. These phenomena might be due to higher numbers of asphalt-degrading microbes and higher asphalt degrading activities observed in Duluth and Rochester soil samples that had 60% water holding capacity (Table 14).

Relatively weak (softening) tensile strength was observed in Marshall pucks buried in soils, collected from Bemidji, Brainerd and Willmar Districts, with 90% water holding capacity (Table 16). Also, it reveals that no visual stripping and only small amount of white aggregate was found in these three samples (Table 16, Fig. 25, 26). The reason for puck softening might be due to water dissolution and hydrolysis mechanism or water action with microbial activities when Marshall pucks were buried in soils that were maintained at 90% water holding capacity.

Changes of tensile strength, broken aggregate percent and visual stripping percent of Marshall pucks buried for seven months in soils that were maintain at different water holding capacities are summerized in Table 19 and Fig. 30-33. Three Marshall pucks were removed from each treated or control soils after had been buried for seven months. Pucks were cleaned with damp paper towel, soaked in 77° water bath in sealed zip bags for 20 min. before conducting normal Lotmann teat. Table 19 shows control Marshall pucks that were kept in the same moisture level of containers and most treated samples that were maintained at 30% water holding capacity as well as some soil samples from Brainerd, Rochester, and Willmar with 60% water holding capacity were relatively less weakened in tensile strength (wet strength and TSR%) after Marshall pucks had been buried for seven months as compared with Marshall pucks buried in soils for three months. Although moisture level is indeed critical to affect microbial degradative activities of asphalt materials. It appears that considerable amounts of asphalt-degraders were present (Table 18) and metabolites components were produced and in some cases accumulated as evidence on chromatograms of GC and HPLC (Fig.. 27 and Fig. 29) after Marshall pucks had been buried for seven months in soil with 30% and 60% water holding capacities and in pucks left in control chambers with the same water holding capacities. Considerable evidence is obvious which shows that the net effects of these microorganisms on the viscosity of the asphalts tested and on bond weakening processes. The bond-weakening effects can, to a large extent, be due to oxidation of certain asphalt components by the microorganism. These weakening mechanisms reduce the adhesiveness related to the rheological characteristics of these asphalts and, therefor, the in-service durability of the material. This can be found in some broken aggregates which exhibited in the control samples and treated

sample that were maintained at 30% and 60% water holding capacity (Fig. 30 and 31). some metabolites show some changes on GC and HPLC chromatograms (Fig. 27 and Fig. 29) of these samples due to susceptibility of asphalt materials to bacterial actions. Relatively weak (Softening) tensile strength (TSR% is between 68..01-82.67%) was found in pucks after pucks had been buried in soils with 90% water holding capacity for seven months except the soil sample from Rochester District (probably due to different soil type) as wells as some samples from Bemidji and Duluth Districts with 60% water holding capacity (TSR% is between 91.33-91.35%). Tensile strength loss might be due to both aging under moist conditions and microbial degradative activity. moist anaerobic conditions (an insufficient oxygen present in most soil containers) accentuate strength loss. Water is the worst enemy of bituminous deterioration and water is essential for microbial activity. When water gets into a Marshall puck, the microorganisms become active and begin to multiply. It is clear (Table 18) that a significant proliferation of asphalt-degraders was observed in most Marshall pucks buried in soil for seven months with 60% and 90% water holding capacities compared with that of Marshall pucks buried in soil for three months. It was also shown (Table 17) that good degradation (less hydrocarbon %) was found in Marshall pucks treated in soil with 60% and 90% water holding capacities than those samples with 30% water holding capacity. Moreover, it is also evident that most metabolic components have been converted or mineralized to lower molecular weight of compounds or water soluble metabolites by asphalt-degrading microorganisms which can be seen on chromatograms of GC and HPLC (Fig. 27 and Fig. 29) when Marshall pucks had been buried in soils for seven months with 60% and 90% water holding capacities. It is apparent from tensile strength test that the bacterial attack is only at the surface of the asphalt sample (Fig. 32, 33) when Marshall pucks were removed from soil containers and cleaned with damp paper. In the soil burial tests, the asphalt pucks were applied as a relatively thick film, therefore, only a small overall portion of the Marshall pucks were directly exposed to microbial action. Stripped or white aggregate was only shown on edges of the surface of fracture planes (Fig. 30, 31). The higher population densities and more white aggregates on edges of the surface of Marshall pucks appeared in those that had been buried for seven months in soils that were maintained at 60% and 90% water holding capacities (Fig. 30-33).

It seems puck-buried in-soil tests can be demonstrated to show the moisture requirement for rupture of the adhesive bond at the asphalt-aggregate interface. Actually, it is a complex phenomenon involving physical, chemical and biological actions of both the asphalt and the aggregate since our study has demonstrated that proliferation of asphalt-degraders, hydrocarbon degradation, and biochemical/metabolites changes were related to pavement deterioration. Moisture is also a requirement for microbial deteriorative activities, as well as causing aggregate weakening of asphalt materials. The extent of biodegradation of asphalt materials buried in soils depends on microbial population densities, types of soil, permeability of asphalt materials, soil moisture content and length of time pucks buried in soils. Under favorable environmental conditions, microbial activities can result in rapid deterioration/transformation of asphalt materials, but any cracking/stripping in asphalt pavement would take about a few years to one decade if any preventive measures are not taken.

It is apparent a more thorough study is needed to evaluate the magnitude of the stripping problem to identify specific causes of the pavement stripping with special reference to understanding the mechanisms of metabolites formation and physical strength deterioration and stripping caused by microbial activities, and to study the inhibitory function of some effective agents in the retardation of asphalt stripping.

Changes of tensile strength, broken aggregate percent and visual stripping percent of Marshall pucks buried for 12 months in soils that were maintained at 30%, 60%, and 90%

water holding capacities are summarized in Table 22, Fig. 38-42. Table 22 and Fig. 38 show Marshall pucks buried for 12 months in various soils that are maintained at 90% water-holding capacity were relatively weakened in tensile strength (wet strength and TSR%) with an exception of sample collected from Duluth District that was relatively dry at sampling time. Crack was noticed in one Marshall puck buried in Bemidji soil for 12 months with 90% water-holding capacity (Fig. 43). It seems premature to state that pavement failure was solely attributed to moisture-induced damage. Generally, it is a complex phenomenon related to the rupture of the adhesive bond at the asphalt-aggregate interface. It involved physical and chemical properties and microbial actions on both the asphalt and the aggregate. Moisture damage is also strongly influenced by pavement-mixture morphology and external environmental factors including microbial actions. The tested asphalt is dull, rough, crumbly material and has lost its usual rheological characteristics (see Fig. 41 and Fig. 42) due to the oxidation of certain asphalt compounds by microbial action. The higher visual stripping percentage (Table 22) and higher microbial population densities of asphalt-degrader (Table 21 and Fig. 35) underwent more biodegradation and more biochemical/metabolites changes (Fig. 36) appeared on edges of the surface of Marshall pucks (Fig. 41-42) that had been buried for 12 months in soils with 60% water holding capacity. Relatively more weakened in tensile strength was noted in Marshall puck buried in various soils that were maintained at 90% water-holding capacity. It is presumed that microbes in the soils utilize maltene constituents, oils and resins in the asphalt and thereby upset the balance between asphaltenes and maltenes. The latter provide flexibility to asphalt in mixes. Loss of maltenes would make an asphalt binder less flexible and hence there could be an increase in hardening or softening and susceptible to cracking, especially at higher moisture content or at lower temperature. Another plausible reason was that microbial activities create pinholes due to the utiliation of some constituents in the asphalt film on aggregates. The pinholes provide access channels for water and microbes to the asphalt-aggregate interface. water is a strong polar liquid which acts to displace the weakly polar to non-polar asphalt from the aggregate surface. This results in stripped aggregates. Table 22 and Fig. 38 show results of some treated samples from Duluth, Rochester, and Willmar that were maintained at 30% water-holding capacity and from Rochester and Willmar that were maintained at 60% water holding capacity that had relatively hardening (TSR>100%) after Marshall pucks had been buried for twelve months. It is possible that loss of maltenes would make asphalt binder less flexible and hence resulted in an increase in hardening, or the net effect of microbial action on asphalt was an increase in the viscosity of the asphalts. The increase in viscosity is due to the oxidation of certain asphalt compounds which are also important in determining the rheological characteristics of the asphalts. This hardening effect reduces the adhesiveness and, therefore, the in-service durability of the asphalt material.

It is concluded from this research that microbial activity can affect on constituents of asphalt materials. The latter provides flexibility and rheological characteristics changes on both the asphalt and the aggregate result in stripping or cracking. Microbial activity is not the major cause to the weakening of puck strengths.

Changes of tensile strength, broken aggregate percent and visual stripping percent of Marshall pucks buried in soils for 23 months in soils that were maintained at 30%, 60% and 90% water holding capacities are summarized in Table 26 and Fig. 49-54. Fig. 49 shows wet strength (lb/in^2) of Marshall pucks that had been buried in soils or had been left in control containers that were maintained at 30%, 60% and 90% water holding capacities or moisture contents. Strength of control puck can be used to calculate tensile strength ratio (TSR%), TSR % from all pucks buried in soils that were maintained at different water holding capacities for 3, 7, 12 and 23 months, are shown in Table 26 and Fig. 50. Table 26 and Fig. 49-50 illustrate

the magnitude of hardening that occurred in asphalt materials that were maintained at 60% water holding capacity at the beginning of 12 months. All pucks left in control containers that were maintained at 30%, 60%, and 90% moisture contents and pucks buried in soils that were maintained at 30% water holding capacity developed some degree of "permanent" hardening with the passage of time. Many reports and observations have confirmed that microbes showed degradative activities on asphaltic bitumens and asphalt products such as roofing, roadmats and pipeline coatings et al. Their findings indicated that either a softening or hardening of asphalt resulted from microbial action. It must be realized that bacteria will act on asphalt materials only when certain environmental conditions are favorable to their growth and activity. Mostly because conditions are unfavorable to bacterial activity such as a lack of moisture at the interface of asphalt pavement. This occurred when Marshall pucks were buried in soils maintained at 30% water holding capacity or kept in control containers with the 30% moisture content.

Asphalt materials are more susceptible to hardening, by exposure to air (control containers) and oxidation in the absence of light (asphalt materials buried in soils). The tested asphalt is dull, rough, crumbly materials and has lost its usual rheological characteristics as compared with fresh pucks (Fig. 51 and Fig. 52) due to oxidation of certain asphalt compounds by microbial action. This also caused hardening in asphalt materials. Some low molecular weight oil may be removed from the asphalt at the surface of a piece of pavement aggregate due to microbial stripping action, as a result a slight hardening of the film occurred. This happened in Marshall pucks buried in soils that were maintained at 30% water holding capacity or kept in the control containers. This phenomenon was also observed in Marshall pucks buried in soils that were maintained at 60% water holding capacity during the first year experiment. After the utilization of high molecular weight components of the bitumen by significant microbial biochemical activity as shown on Fig. 48, a softening of the asphalt occurred when Marshall pucks had been buried in soils that were maintained at 60% water holding capacity for 12 to 23 months (Fig. 49 and Fig. 50). The higher visual stripping percentage and higher broken aggregate percentage appeared on Marshall pucks that had been buried in soils for 23 months and maintained at 60% water holding capacity (Table 26, Fig. 53 and Fig. 54).

It is further confirmed that under favorable conditions such as when asphalt materials were buried in soils that were maintained at 60% water holding capacity, microorganisms caused damage to asphalt pavement and bacterial infestations are difficult to suppress.

Soil moisture content is conceded to have an important effect on the deterioration of bituminous roads by reducing adhesive bond between the asphalt and aggregate solid surface. However, it must be recognized that effects of water on bituminous pavements cannot be separated from microbial effect. Water creates a suitable environment for bacterial spores to germinate and become active vegetative cells which act on asphalt pavement, microbes create pinholes in the asphalt film on aggregates and provide access channels for more water and microbes to the asphalt-aggregate interface, these two factors work synergistically. This phenomenon appeared in Marshall pucks buried in soils that were maintained at 60% and 90% water holding capacities (Table 26 and Fig. 49 and Fig. 50).

It should be emphasized that such a research project on "asphalt deterioration and stripping by microbial activity" is both resources and time consuming. A more detailed and longer term study should be carried out to elucidate the mechanism of stripping under all environmental conditions, microbial and water penetration into the asphalt materials, and water and microbial action in different soils adjacent to asphalt materials. Besides, a variety of methods to prevent microbial attacks on asphalt should be investigated. With better understanding of stripping mechanism and preventive measures, methods of improving the longevity of asphalt pavement could be recommended, such as condition control and addition of

anti-microbial agent to minimize microbial activities.

VI. Evaluation:

The results obtained from this study will be evaluated by research engineers and research scientists of Minnesota Department of Transportation and by experts of he U.S. Department of Transportation Research Center in Cleveland, Ohio. If results show a significant degradation/deterioration of asphalt pavement caused by microbial actions under various environmental conditions and if we can establish a correlation of strength loss vs. time in pavement samples exposed to cultures of asphalt-degrading bacteria using standard engineering testing procedures, the next phase of research would be to evaluate asphalt additives as inhibitors of bacterial degradative activity. At that time, the effectiveness of biocidic asphalt additives in halting stripping process will be evaluated thoroughly and optimum dose rates will be recommended to the MNDOT for further field testing. Identifying and categorizing specific causes for stripping could lead to solutions that could save the State of Minnesota considerable amounts of money in maintenance, rehabilitation, and reconstruction costs.

VII. Context within field:

This proposed project is intended to provide a better understanding of microbial deterioration (stripping) of asphalt pavement materials and environmental factors that mediate asphalt stripping activities. We have already isolated a number of microorganisms from deteriorated asphalt samples that may have key roles in asphalt stripping, which will give us a good start to the project. This project will provide useful information on the role of microbial action in asphalt pavement deterioration caused by microbial actions under various environmental conditions. This project would bridge the gap between destruction of asphalt pavement and hydrocarbon degrading microbial activities in the soil underneath the pavement. It would also make a connection between field experience with stripping processes, and substantial research conducted in other fields.

VIII. Budget context:

Currently, MNDOT has allocated $10,000 to support our preliminary study on microbial stripping of asphalt material during the 1994-95 fiscal year (ending June 30, 1995). There is no overlap between this proposed project and the currently funded projects in the investigators' laboratories during the two year period beginning July 1, 1995. Our proposed matching is an in-kind match, we will use faculty time (the project manger is under a 50/50 teaching and research contract) and existing equipments as part of the match. Other funding sources will be sought for additional or matching support to allow for full funding of this important research. The requested LCMR budget of $60,000 will be used solely by Bemidji State University to execute the project.

IX. Dissemination:

The research results will be disseminated at national conferences sponsored by the Department of Transportation and academic organizations. A written summary of the research results and applications will be submitted for publication in relevant scholarly journals. Due to the importance of this research project we have included funding request in this proposal for the project manager to travel to conferences to formally present the results of their study. We feel that the widest possible dissemination of this important information is needed due to its immediate, widespread, and practical remediation possibilities.

X. Time:

This project will last for two years. The first year will concentrate on microbial

enumeration and characterization of asphalt-degrading microorganisms in asphalt pavement and soil samples of different geological regions and on biodeterioration/biodegradation of asphalt materials by asphalt-degrading isolates indigenous in various geological regions. Asphalt strength testing will be initiated in the later half of the first year and finish up in the second year.

XI. Cooperation

This proposed project is a join effort between the Center for Environmental Studies of Bemidji State University and the Minnesota Department of Transportation . The project will be managed by Dr. Fu-Hsian Chang who has been working on biotechnology R&D for 15 years. He will be spending two months (17.5%) of his time on the project. Mr. Olson and Mr. Eastlund are both engineers with the Minnesota Department of Transportation working with the Physical Research Office and the Minnesota Road Research Project respectively. Mr. Olson has been the Research Operation Engineer for 15 years. Mr. Eastlund has been the Assistant Project Manager of MN/Road and previously worked under Mr. Olson for 1.5 years. Dan Wegman is the MnDOT Bituminous Engineer. A position he has held for the past two years. As Bituminous Engineer Dan is responsible for overseeing the quality control and construction of the MnDOT Bituminous paving program. Engineers of MnDOT will assist Chang and his research team with sample collecting and asphalt strength testing. Time spent by MnDOT personnel on the project and equipment use at MnDOT will be considered as a contribution by MnDOT.

XII. Reporting Requirements:

Semiannual six-month workprogram update reports will be submitted no later than January 1, 1996, July 1, 1996, January 1, 1997, and a final six-month workprogram update and final report by June 30, 1997.

LITERATURE CITED

1. Atlas, R.M. 1981 Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiol. Rev. 45:180-209.

2. Cerniglia, C.E. 1984. Microbial metabolism of polycyclic aromatic hydrocarbons. Adv. Appl. Microbiol. 30:31-71.

3. Fedorak, P.M., Semple, K.M., and Westlake, D.W.S. 1984. Oil degrading capabilities of yeasts and fungi isolated from coastal marine environments. Can. J. Microbiol. 30:565-571.

4. Gibson, D.T. 1982. Microbial degradation of hydrocarbons. Environ. Toxicol. Chem. 5:237-250.

5. Texas Research Institute. 1982. Enhancing the microbial degradation of underground gasoline by increasing available oxygen. Report to the American Petroleum Institute, Washington, D.C.

6. Blakebrough, N. 1978. In Charter, K.W.A. and Somerville, H.J. (Eds.), The Oil Industry and Microbial Ecosystems. Institute of Petroleum. London. p. 28.

7. Dietz, D.N. 1980. The intrusion of polluted water into a groundwater body and the biodegradation of a pollutant. Proc. 1980 Conf. Hazardous Material Spills, sponsored by U.S. EPA, et al. pp. 236-244.

8. Nicholas, R.B. 1987. Biotechnology in hazardous-waste disposal: an unfulfilled promise. Amer. Soc. Microbiol. News. 53:138-142.

9. Pettyjohn, W.A. and Hounslow, A.W. 1983. Organic compounds and ground-water pollution. Ground Water Monitoring Rev. 3:41-47.

10. Amdurer, M., Fellman, R., and Abdelhamid, S. 1985 In situ treatment technologies and superfund. In Proc. Intl. Conf. on New Frontiers for Hazardous Waste Management, Sept. 1985. EPA Report No. EPA-600/9-85/025. Hazardous Waste Eng. Res. Lab., Cincinnati, OH.

11. Kobayashi, H. and Rittmann, B.F. 1982. Microbial removal of hazardous organic compounds. Environ. Sci. Technol. 16:170A-183A.

12. Gholson, R.K., Guire, P., and Friede, J. 1972. Assessment of Biodegradation Potential for Controlling Oil Spills. NTIS Report No. AD-759 848. National Technical Information Service, Springfield, VA.

13. Soli, G. 1973. Marine hydrocarbonoclastic bacteria: types and range of oil degradation. In Ahearn, D.G. and Meyers, S.P. (Eds.), The Microbial Degradation of Oil Pollutants, Workshop, Atlanta, GA, Dec. 4-6, 1972. Publ. No. LSU-SG-73-01. Louisiana State Univ., Center for Wetland Resources, Baton Rouge, LA. p. 141.

14. Canter, L.W. and Knox, R.C. 1985. Ground Water Pollution Control, Lewis Publishers, Chelsea, MI. 526 pp.

15. Parr, J.F., Sikora, L.J., and Burge, W.D. 1983. Factors affecting the degradation and inactivation of waste constituents in soils. In Parr, J.E., Marsh, P.B., and Kla, J.M. (Eds.), Land Treatment of Hazardous Wastes. Noyes Publ, Park Ridge, NJ. pp. 20-49, 321-337.

16. Wilson, J.T., Leach, L.E., Henson, M. and Jones, J.N. 1986. In situ biorestoration as a ground water remediation technique. Ground Water Mon. Rev. 56-64.

17. Federle, T.W., Dobbins, D.C., Thornton-Manning, J.R., and Jones, D.D. 1986. Microbial biomass, activity, and community structure in subsurface soils. Ground Water. 24:365-374.

18. Jones, J.G. and Eddington, M.A. 1968. An ecological survey of hydrocarbon-oxidizing microorganisms. J. Gen. Microbiol. 52:381-390.

19. Fewson, C.A. 1981. Biodegradation of aromatics with industrial relevance. In Leisinger, T., Hutter, R., Cook, A.M., and Nuesch, J. (Eds.), FEMS Symp. No. 12. Microbial Degradation of Xenobiotics and Recalcitrant Compounds. 12:141-179.

20. Dagley, S. 1981. New perspectives in aromatic catabolism. In Leisinger, T., Hutter, R., Cook, A.M., and Nuesch, J. (Eds.), FEMS Symposium No. 12. Microbial degradation of Xenobiotics and Recalcitrant Compounds. 12:141:179.

21. Nyns, E.J., Auquiere, I.P., and Wiaux, A.L. 1968. Taxonomic value of property of fungi to assimilate hydrocarbons. Antonie van Leeuwenhoek J. Microbiol. Serol. 34:441-457.

22. Davies, J.S. and Westlake, D.W.S. 1979. Crude oil utilization by fungi. Can . J. Microbiol. 25:146-156.

23. Komagata, K., Nakase, T., and Katsu, N. 1964. Assimilation of hydrocarbons by yeast. I. Preliminary screening. J. Gen. Appl. Microbiol. 10:313-321.

24. Ahearn, D.G., Meyers, S.P., and Standard, P.G. 1971. The role of yeasts in the decomposition of oils in marine environments. Dev. Ind. Microbiol. 12:126-134.

25. Cook, W.L. Massey, J.K., and Ahearn, D.G. 1973. The degradation of crude oil by yeasts and its effect on Lesbistes reticulatus. In Ahearn, D.G. and Meyers, S.P. (Eds.), The Microbial Degradation of Oil Pollutants, Workshop, Atlanta, GA, Dec. 4-6, 1972. Publ. No LSU-SG-73-01. Center for Wetland Resources, Louisiana State Univ., Baton Rouge, LA. p. 279.

26. Walker, J.D., Cofone, L., Jr., and Cooney, J.J. 1973. Microbial petroleum degradation: the role of Cladosporium resinae. In Proc. Joint Conf. on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C. pp. 821-825.

27. Bergey's Manual of Systematic Bacteriology, Volume 1, 1984. J.G. Holt, Editor-in-Chief. Williams and Wilkins Publisher, Baltimore, MD.

28. Gibson, D.T. 1977. Biodegradation of aromatic petroleum hydrocarbons. In Wolfe. D.A. (Ed.), Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems. Pergamon Press, Oxford. pp. 36-46.

29. Atlas, R.M. 1977. Stimulated petroleum biodegradation. CRC Crit. Rev. Microbiol. 5:371-386.

30. Horowitz, A., Gutnick, D., and Rosenberg, E. 1975a. Sequential growth of bacteria on crude oil. Appl. Microbiol. 30:10-19.

31. Horowitz, A.D., Gutnick, D., and Rosenberg, E. 1975h. Sequential growth of bacteria on crude oil. Appl. Microbiol. 30:10-19.

32. Freeze, R.A. and Cherry, J.A. 1979. Groundwater. Prentice-Hall, Inc., Englewood Cliffs, NJ. 227p.

33. Zitrides, T. 1983. Biodecontamination of spill sites. Poll. Eng. 15:25-27. ZoBell, C.E. 1946. Action of Microorganisms on hydrocarbons. Bacteriol. Rev. 10:1-49.

34. Atlas, R.M. 1978c. Microorganisms and petroleum pollutants. BioScience. 28:387-391.

35. Pirnick, M.P. 1977. Microbial oxidation of methyl branched alkanes. Crit. Rev. Microbiol. 5: 413-422.

36. Markovetz, A.J. 1971. Subterminal oxidation of aliphatic hydrocarbons by microorganisms. Crit. Rev. Microbiol. 1:225-237.

37. Sleat, R. and Robinson, J.P. 1984. The bacteriology of anaerobic degradation of aromatic compounds. J. Appl. Bacteriol. 57:381-394.

38. Young, L.Y. 1984. Anaerobic degradation of aromatic compounds. In Gibson, D.T. (Ed.), Microbial Degradation of Organic Compounds. Marcel Dekker, Inc., New York, NY. pp. 487-523.

39. Berry, D.F., Francis, A.J., and Bollag, J.M. 1987. Microbial metabolism of homocyclic and heterocyclic aromatic compounds under anaerobic conditions. Microbiol. Rev. 51:43-59.

40. Shelton, T.B. and Hunter, J.V. 1975. Anaerobic decomposition of oil in bottom sediments. J. Water Pollut. Control fed. 47:2256-2270.

41. Grbic-Galic, D. and Young, L.Y. 1985. Methane fermentation of ferulate and benzoate: anaerobic degradation pathways. Appl. Environ. Microbiol. 50:292-297.

42. Kuhn, E.P., Colberg, P.J., Schnoor, J.L., Wanner, O., Zehnder, A.J.B. and Schwarzenbach, R.P. 1985. Microbial transformation of substituted benzenes during infiltration of river water to groundwater: laboratory column studies. Environ. Sci. Technol. 19:961-968.

43. Reinhard, M., Goodman, N.L., and Barker, J.F. 1984. Occurrence and distribution of organic chemicals in two landfill leachate plumes. Environ. Sci. Technol. 18:953-961.

44. Schnitzer, M. 1982. Organic matter characterization. In Page, A.L., Miller, R.H., and Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd ed. Agronomy Monograph No. 9(2). American Society of Agronomy, Inc., Madison, WI. pp. 581-594.

45. Ramamurti, K., G.P. Jayaprakash, and C.F. Crumpton. 1984. "Microbial biodegradation of asphalt and related hydrocarbons-a literature review." p. 1-44. In Report No. FHWA-KS 84/1. Interim report, Division of Operations, Bureau of Material and Research, Kansas Department of Transportation. Topeka.

46. Hubbard, P. 1938. "Adhesion of Asphalt to Aggregates in the Presence of Water,": Proceedings, Highway Research Board.

47. Burgess, S.J. 1956. "Action of Microorganisms on petroleum-Asphalt Fractions." Bulletin No. 118, Highway Research Board.

48. Harris, J.O., R.M. Kline, and C.F. Crumpton. 1956. "A Study of the Presence of Hydrocarbon Utilizing Bacteria at the Soil-Asphalt Interface of Kansas Highways." Transactions, Kansas Academy of Sciences, Vol. 59.

49. Harris, J.O., H.E. Worley, G.A. McCaskill and L.V. Baldwin. 1958. "Preliminary Studies of the Effect of Microorganisms on Physical Properties of Asphalt." Transactions, Kansas Academy of Sciences, Vol. 61.

50. Benefield, L. and F. Parker, Jr. 1988. "Microbial Degradation as a Factor Contributing to Stripping of Asphalt Pavements." Completion Report for Highway Research Project, Department of Civil Engineering, Auburn University.

51. Brown, L.R. and T.R. Darnell. 1987. "Blistering of Asphalt Paving Overlays Caused by Microorganisms." Proceedings, Vol. 56 Association of Asphalt Paving Technologist.

52. Pendrys, J.P. 1989. "Biodegradation on Asphalt Cement-20 by Aerobic Bacteria." Applied and Environmental Microbiology, Vol: 55(6).

53. Ramamurit, K. and G.P. Jayaprakish. 1987. "Bacteria and Asphalt Stripping." Kansas Department of Transportation Final Report, No. FHWA-KS-87.

54. Traxler, R.W., P.R. Proteau and R.N. Traxler. 1965. "Action of microorganisms on Bituminous Materials." Applied Microbiology, Vol. 13(6).

55. Hironaka, M.S. and T.J. Holland. 1987. "Pressure Heaving of Asphalt Pavement Overlay," Proceedings, Vol. 56, Association of Asphalt Paving Technologist.

56. Standard Methods for the Examination of Water and Wastewater, 1990. Joint Editorial Board: L.S. Clesceri, WPCF Chairman; A.E. Greenberg, APHA, R.R. Trussell, AWWA. 17th Edition, American Public Health Association, Publisher. Washington, D.C.

57. Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 1965. C .A. Black, Editor-in-Chief. American Society of Agronomy, Inc., Publisher, Madison, Wisconsin.

58. Chang, F.H., N. Noben and J.A. Bullert. March 23-27, 1987. Microbial degradation of petroleum in subsurface environments. Published in U.S. Geological Survey Program on Toxic Waste-Groundwater Contamination: Proceedings of the Third Technical Meeting Pensacola, Florida. PP C-035-C40.

59. Chang, F.H., M.F. Hult, and N. Noben. 1987. Quantitative studies of biodegradation of petroleum and some model hydrocarbons in ground water and sediment environments. Presented at the National Conference on Ground Water Quality and Agricultural Practices, May 1-2, 1986, Norman, Oklahoma. Published in Ground Water Quality and Agricultural Practices, pp. 295-318, Lewis Publishers, Deborah M. Fairchild, Ed.

60. Dawson, Timothy D. and F.H. Chang. 1992. A screening test of biodegradative capability of a new strain of Pseudomonas gladioli (BSU 45124) on some xenobiotic organics. Bulletin of Environmental Contamination and Toxicology. 49:10-17.

61. Brown, L.R. and G.S. Pabst. 1988. The contribution of microorganisms to stripping of asphalt pavements. Final Report. Mississippi State Highway Department. Report Number: MSHD-RD-88-89.

62. Jayaprakash, G.P. and K. Ramamurti. 1987. Bacteria and asphalt stripping. Final Report. Kansas Department of Transportation. Report Number: FHWA-KS-87/1.

63. Badgley, W.A., 1962. Private Communication to C.F. Crumpton.

64. Crumpton, C.F., and Badgley, W.A., 1965. A Study of the Clay Mineralogy of Loess in Kansas in Relation to Its Engineering Properties: State Highway Commission of Kansas and U.S. Bureau of Public Roads.

65. Crumpton, C.F. and Badgley, W.A. 1968. Underclay Development Beneath Asphalt Roads in Kansas: Highway Research Record 215. p. 24-34.

66. Robinson, G.W., 1949. Soils: John Wiley and Sons, Inc. New York, New York.

67. Harris, J.O., 1961. Soil bacteria and Corrosion: American Gas Assoc. Production Conference Philadelphia.

68. R.P. Lottman, R.P. Chen, K.S. Kumar, and L.W. Wolf. Laboratory Test System for Prediction of Asphalt Concrete Moisture Damage. TRB, Transportation Research Record 515. 1974. pp. 18-26.

69. R.P. Lottman. predicting Moisture-induced Damage to Asphalltic Concerete-Progress Report on Field Evaluation Phase of NCHRP Project 4-8(3)/1. AASHTO 66th Annual Meeting, Las Vegas, NV. Nov. 16-19. 1980.

70. R.P. Lottman. Predicting Moisture-induced Damage to Asphaltic Concrete-Field Evaluation Phase. NCHRP, Project 4-8(3)/`. Summary Interim Rept. Feb.. 1979.

Fu-Hsian Chang
Center for Environmental Studies
Bemidji State University
Bemidji, MN 56601-2699
Phone (218) 755-4104
Fax (218) 755-4107

Personal: U.S. Naturalized Citizen

Education:

MBA — Business Administration, Carlson School of Management, University of Minnesota, Minneapolis, MN. 1994

Ph.D. — Environmental Microbiology, Department of Land, Air and Water Resources, University of California, Davis, California, 1979.

M.S. — Biology, University of North Texas, Denton, Texas, 1974.

B.S. — Agricultural Chemistry, National Chung-Hsing University, Taichung, Taiwan, 1970.

Professional Experience:

August 1, 1995-Present — President and CEO - The First Environmental Technology and Management Consulting Co., Ltd. Bemidji, Minnesota.

July 1, 1994-June 30, 1995 — Acting Director - Center for Environmental Studies, Bemidji State University, Bemidji, Minnesota

November 1, 1990-Present — Managing Director - Remington International Consultants Co., Ltd. Keelung Taiwan, R.O.C.

August 1, 1990-Present — Task Force Member - Applied Technology Development Program, Agricultural Utilization Research Institute of Minnesota.

July 1, 1989-Present — Professor - Center for Environmental Studies, Bemidji State University, Bemidji, MN 56601-2699. 50/50 teaching and research. Teaching Wastewater Treatment, Environmental Microbiology, Team teaching Introductory Environmental Sciences and Environmental Chemistry. Research in (1) biodegradation and modeling of microbial fate of organic pollutant in surface and subsurface environment; (2) microbial technological utilization of peat; (3) treatment and recovery of agricultural wastes; and (4) wastewater treatment using soils as media that are enriched with screened microbes or genetically engineered microorganisms and study their ecological impacts and economic significance.

May 31, 1989 - June 8, 1989 — Invited Guest Lecturer at the Beijing Agricultural University and the Institute of Microbiology of Chinese Academy of Sciences, Beijing, People's Republic of China. Lecture subjects are on (1) Biodegradation of toxic organics in the environments and (2) Bioconversion of organic wastes to energy and other useful products using enzymatic or genetically engineered microbial system.

Sept., 1990 - Present — Budget Committee Member, Minnesota State Universities, Inter Faculty Organization

Sept., 1989 - Present — Goverment Relation Committee member, Minnesota State Universities, Inter Faculty Organization.

1987-1988 — President of The Association of Chinese Soil and Plant Scientists in North America.

June 8, 1987-Present — Consultant to the Bureau of Rural Energy and Environmental Protection, Ministry of Agriculture, Animal Husbandry and Fishery, People's Republic of China. This consulting was arranged by the United Nations Development Programme. It was to provide technical assistance to Chinese environmental scientists in dealing with the following areas of interest: 1. Soil utilization and environmental pollution, 2. treatment and recovery of agricultural wastes, 3. microbial remediation of organic pollutants, and 4. wastewater treatment system using soils as treatment media.

May 25-May 29, 1987 — Invited Guest Lecturer at the Institute of Public Health, Medical School, National Taiwan University, Taipei, Taiwan. Lecture subjects are on "Utilization of Pesticides in Agriculture and Their Impacts on Environmental Pollution and Public Health", "Hospital Solid Wastes Management and Treatment", "Microbial Degradation and Bioremediation of Xenobiotic Organics and Their Impacts on Environmental Quality", "Soil Wastes Treatments and Their Relationship to Public Health", and "Toxic Chemical Contamination and Its Impacts on Public Health."

June 2-June 6, 1987 — Consultant to the Taiwan Environmental Protection Agency. To provide technical assistance to their national research programs on solid wastes treatments and bioremediation of surface and subsurface contaminated soil, surface water, and groundwater in Taipei, Taichung, Tainan, and Kaoshung cities of Taiwan, Republic of China.

July 1986-Present — Consultant to the United Nations Development Programme in the fields of 1. soil utilization and environmental pollution; 2. treatment and recovery of agricultural waste; 3. pollution and microbiological degradation of organic contaminants; 4. system of waste water treatment on soils.

1986-1987 — Vice President of The Association of Chinese Soil and Plant Scientists in North America.

1985-1986 — Treasurer of The Association of Chinese Soil and Plant Scientists in North America.

May 21-June 12, 1985 — Invited Guest Lecturer at the Advanced Institute of Environmental Ecology, Commission of Environmental Sciences, Institute of Environmental Chemistry and Institute of Microbiology of Academia Sinica (Chinese Academy of Science) in Beijing, China. Lecture subjects are "Microbial Biodegradation and Bioremediation of Crude Oil in Deep Soil Environments", "Effect of Acid Precipitation on Microbial Activities and Their Biochemical Changes in Soils", Chemical and Microbiological Utilization of Peats, "Pollution and Control of Anthropogenic Chemicals in the Environment" and "Xenobiotic Chemicals Contamination and Their Fate in the Environment".

Jan. 1984-September, 1990 — Coordinator and Principal Investigator of microbiological research group on biodegradation of crude petroleum, U.S. Geological Survey research project "Ground Water Contamination by Crude oil near Pinewood, Minnesota."

July 1985-June 1989 — Associate Professor, Center for Environmental Studies, Bemidji State University, Bemidji, MN 56601-2699. 50/50 teaching and research. Teaching Environmental Microbiology, Environmental Chemistry, Soil Microbiology and Wastewater Treatment. Research in (1) biodegradation and modeling of microbial fate of organic pollutant in surface and subsurface environment; (2) microbial technological utilization of peat; (3) treatment

and recovery of agricultural wastes; and (4) wastewater treatment using soils as media that are enriched with screened microbes or genetically engineered microorganisms and study their ecological impacts and economic significance.

Oct. 1981-
June 1985

Assistant Professor, Center for Environmental Studies, Bemidji State University, Bemidji, MN 56601-2699, 50/50 teaching and research, teaching Environmental Microbiology, Waste Water Treatment, Environmental Chemistry and Soil Microbiology. Research areas are in: (1) chemical and microbiological utilization of peat; (2) biodegradation of toxic organic pollutants in unsaturated and saturated environments; (3) microbial purification of waste products and (4) effect of inorganic and organic wastes on nutrient transformations mediated by microorganisms on agriculture or wet land soils.

Dec. 1979-
Sept. 1981

Postdoctoral Associate, Laboratory of Soil Microbiology, Cornell University, Ithaca, NY. Worked on effects of acid precipitation on microbial activities and their biochemical changes in soil. Research includes microbially mediated decomposition and microbial adaptation. Worked with Dr. Martin Alexander.

Sept. 1976-
Nov. 1979

Research Assistant, Department of Land, Air and Water Resources at University of California, Davis, Calif. Worked on effect of nitrification on trace metals mobility in the soil profile, effects of heavy metals (Cd, Cr, Cu, Mn, Pb, Zn) on nitrogen mineralization of the sludge amended soils and the relationship between microbial activity and the concentration of trace metals of the sludge treated soils. Worked for Dr. Francis E. Broadbent.

Sept. 1978 -
Dec 1978

Teaching Assistant, Geomicrobiology in Department of Land, Air and Water Resources at the University of California. Worked under the supervision of Dr. F.E. Broadbent. Repsonsible for geomicrobiology laboratory teaching and laboratory report grading.

Sept. 1974 -
July 1976

Demonstrator, Department of Microbiology, University of Guelph, Guelph, Ontario. Responsible for media and culture preparation for student laboratories, taught in general microbiology laboratories.

May 1975 -
August 1975

Technician, Department of Microbiology, University of Guelph, Guelph, Ontario. In charge of the examination of pathogenic microorganisms inhabiting water runoff and liquid sludge samples sent from Ontario Ministry of Health. Worked with Professor Johnston.

May 1976 -
August 1976

Technician, Department of Microbiology, University of Guelph, Guelph, Ontario. Responsible for 78 stock cultures, purification and identification. Worked with Professor Smith.

Sept. 1972 -
July 1974

Teaching Assistant, Department of Biological Sciences, North Texas State University, Denton, Texas. Responsible for physiology and anatomy laboratory teaching, worked for Dr. James R. Lott.

Sept. 1971 -
Aug. 1972

Research Assistant, Department of Chemistry, North Texas State University, Denton, Texas. Worked on bioorganic anticancer compounds synthesis research under the supervision of Dr. C.G. Skinner.

Memberships:

ACS (American Chemical Society)
ASM (American Society for Microbiology)
ASA (American Society of Agronomy)
SSSA (Soil Science Society of America)
APHA (American Public Health Association)
International Peat Society
Society for Environmental Geochemistry and Health

Special Recognition:

Kearney Foundation Research Assistantship, Davis, CA, 1976-1979.
Welch Research Fellowship, Denton, Texas, 1971-1972.
Who's Who in Technology Today, 1984,1986 and 1988. (Name listed under Environmental Science and Technology).
Merit Award Recipient, Bemidji State University, 1987/88.

Research Grants/Activities:

1995-1997 Microbial Deterioration of Asphalt Materials and Its Prevention. U.S. Department of Energy, Minnesota Department of Administration and Legislative Commission on Minnesota Resources. $60,000

1995-1997 Biochemical Changes of Asphalt Materials During Biodeterioration. Minnesota Department of Transportation. $18,000

1994-1995 Production of Horticultural Products from Aquaculture Wastes. Minnesota Aquafarms Inc. and Minnesota Department of Agriculture. $27,208

1994-1995 Microbial Deterioration of Asphalt Materials and Its Prevention - A Preliminary Investigation. Minnesota Department of Transportation. $10,000

1993-1996 The Use of Innovative Biotechnology in Composting Fish and Other Carbonaceous Wastes. Red Lake Band of Chippewa Indians and U.S. Environmental Protection Agency. $150,000

1993-1994 Treatability and Degradation studies of Pentachlorophenol (PCP) and Petroleum Hydrocarbon Contaminated Soil and Groundwater from Marvin Windows and Doors site in Warroad, Minnesota. Marvin Windows and Doors. $67,556

1992-1994 Bioremediation Study of Leachate Contaminated Soil and Aquifer Material from Kummer Landfill Site. MN Pollution Control Agency and U.S. Env. Protection Agency. $160,000

1992-1995 The Use of Gene Technology to Enhance Cellulase Production for Utilization in Agricultural Biomass Conversion. Minnesota Agricultural Utilization Research Institute. $308,663

1991-1993 Cellulose Rayons for Biodegradable Packaging. U.S. Department of Energy, Minnesota Department of Administration and Legislative Commission on Minnesota Resources. $150,000.

1989-1992 Biotechnological Conversion of Peat to Energy and Other Useful Products. U.S. Department of Energy, Minnesota Department of Administration and Legislative Commission on Minnesota Resources. $173,000

1989-1991	Microbiological Fate of Crude Oil and Hydrocarbons in Subsurface Environment. U.S. Geological Survey. $37,000
1989-1990	Microbiological Fate of Crude Oil and Hydrocarbons. USGS $22,000.
1988-1989	Microbial Degradation of Oil and Hydrocarbons. USGS, $15,000.
1987-1989	Non-energy peat development in Minnesota. Legislative Commission on Minnesota Resources. $98,000.
1986-1988	Biodegradation of crude oil of Pinewood oil spill. U.S. Geological Survey. $40,000.
1985	Bioconversion of peat to useful polysaccharides. MN Dept. Energy and Economic Development and Natural Resources Research Institute. $6,000.
1984-1986	Microbiology of Pinewood oil spill. US Geological Survey. $45,000.
1983-1985	Peat Chemical Utilization. MN Dept. Natural Res. $85,000.
1983	International symposium on peat utilization. MN Dept. Nat. Res. $8,000; Blandin Foundation $10,000; BSU $6,000.
1982	Waxes and chemicals in Minnesota peats. MN Dept. Nat. Res. $2,250.

Publications and Presentations:

Wang, J.W., W.J. Shi and F.H. Chang. 1995. Development of some novel biodegradable cellulosic products. Proceedings of Packaging Polymer Chemistry. American Chemical Society. In Press.

Wang, J.W. and F.H. Chang. 1995. Change of microbial indicator characteristics during composting process of fish wastes. American Society for Microbiology 95th Annual Meeting. May 21-25, 1995. Washington, D.C. Manuscript will be submitted for publication in Biocycle, Journal of Composting and Recycling.

Gu, Z.Y. P. Guilfoile and F.H. Chang. 1995. Isolation of a cellulase gene from Trichoderma harzianum FP108. American Society for Microbiology 95th Annual Meeting. May 21-25, 1995. Washington, D.C. Manuscript in preparation and will be published in Applied and Environmental Microbiology.

Wang, P. T. Y. Xue, Z.Z. Cheng, Y.F. Li, Y.F. Lu, F. Bei and F.H. Chang. 1995. Microorganisms in hydrothermal spring and its biomineralization of gold. American Society for Microbiology 95th Annual Meeting. May 21-25, 1995. Washington, D.C. Published in ASM Meeting Abstract, 1995.

Thomas, S. and F.H. Chang. 1995. Monitoring of carbon mineralization of microbial inoculated fish waste composting process with various C/N ratios. American Society for Microbiology 95th Annual Meeting. May 21-25, 1995. Washington, D.C. Published in ASM Meeting Abstract, 1995.

He, S. and F.H. Chang. 1995. Model of Biodegradation of Trichloroethylene by methanotrophic bacteria in microcosm. American Society for Microbiology Annual Meeting. May 21-25, 1995. Washington, D.C. Manuscript in preparation and will be published in Applied and Environmental Microbiology.

Wang, J.W., W.J. Shi and F.H. Chang. 1995. Some novel biodegradable cellulosic products. American Chemical Society 209th Meeting, Anaheim, CA. April 2-6, 1995. Published in ASM Meeting Abstract, 1995.

A Brief Biography of Mr. Dan Wegman, Bituminous Engineer

EDUCATION:	B.S.C.E., University of Minnesota. March 1984.
WORK EXPERIENCE:	
May 1992 to present	Bituminous Engineer, Matl's Res. and Engineering
Oct. 1991 to Feb. 1992	Acting Resident Engineer, Oakdale Construction
May 1988 to May 1992	Project Engineer, Oakdale Construction
May 1987 to May 1988	Research Project Engineer, Matl's Res. and Eng.
August 1986 to May 1987	MN/DOT Training Program, Grad. Engineer
Feb. 1985 to July 1986	Application Engineer. Osmonics Inc. Mtka, MN.
May 1984 o Feb. 1985	Process Engineer, Basic Technologies Inc., Florida
HOBBIES:	Sports, Hunting and Fishing
STATUS:	Married, two daughters

Resume of CURTIS O. EASTLUND

1712 Barclay St.
Maplewood, MN. 55109

Home (612) 779-8454
Work (612) 779-5514

EXPERIENCE:

MINNESOTA DEPARTMENT OF TRANSPORTATION
June 1992 to present.
Assistant to Project Manager at the Minnesota Road Research Project (Mn/ROAD). Directly responsible for the development of maintenance and rehabilitation plans for a 20 million dollar pavement research facility. Other duties include the administration of consultant contracts worth over 1 million dollars, forensic examination and reporting on the performance of full depth bituminous pavements in Minnesota, and rotations in the offices of Physical Research and Pavement Management.

STRGAR-ROSCOE-FAUSCH, INC. CONSULTING ENGINEERS, Minneapolis., MN.
June 1989 to June 1992.
Project Engineer in Municipal and Highway construction. Duties included preliminary and final design, hydrologic and hydraulic structure design, writing feasibility reports, plan preparation and construction project management.

UNITED STATES FOREST SERVICE, Isabella, MN.
September 1988 to July 1989.
Project Coordinator of a volunteer project to design and build two wooden foot bridges within the Boundary Waters Canoe Area. Project was organized by the Chi Epsilon Chapter at the U of MN.

RAMSEY COUNTY DEPARTMENT OF PUBLIC WORKS, Roseville, MN.
June 1988 to September 1988.
Engineering Assistant during summer Internship. Experience gained included drafting, survey crew member, and construction inspection.

ST ANTHONY FALLS HYDRAULIC LABORATORY, Minneapolis, MN.
September 1987 to June 1988.
Research Assistant in PH.D. research on gas transfer. Performed stereographic photography for topographic mapping of water surfaces.

EDUCATION:

Bachelor of Civil Engineering Degree, 1990.
UNIVERSITY OF MINNESOTA, Minneapolis, MN.

Professional Engineers Exam
Passed Wisconsin Exam, Spring 1991

ORGANIZATIONS:

CHI EPSILON, National Civil Engineering Honor Society.
TAU BETA PI, National Engineering Honor Society
ASCE, American Society of Civil Engineers.
Toastmasters International

Resume of ROGER C. OLSON

8634 Fifth Ave. S.
Bloomington, MN 55420
(612) 881-4086

Materials Research & Engineering
1400 Gervais Avenue
Maplewood, MN 55109
(612) 779-5517

EDUCATION: BSCE North Dakota State University 1968

REGISTRATION: Registered Professional Engineer, Civil, Minnesota

11-78 to Present — Research Operations Engineer, Office of Materials Research & Engineering, MN/DOT. Duties include directing research projects conducted by the Materials Research Unit of the Physical Research Section. Projects are in the areas of pavement design, construction, maintenance and rehabilitation, and such studies as the use of anti-corrosive deicers, waste products, preventative maintenance and new product evaluation.

5-78 to 11-78 — Assistant Bituminous Engineer, Office of Materials, MN/DOT. Duties included supervising trial mix laboratory and reviewing field projects.

3-73 to 5-78 — Assistant County Engineer, Scott County, Minnesota. Involved in design, pre-design, construction, traffic and maintenance.

6-70 to 3-73 — Research Project Engineer, Minnesota Highway Department. Duties included designing, conducting and reporting research projects.

6-68 to 6-70 — Engineer-in-training, Minnesota Highway Department. Rotations in Traffic, Pre-Design, and Materials in Duluth and Foundations in St. Paul.

PROFESSIONAL MEMBERSHIPS:
Association of Asphalt Paving Technologists

Transportation Research Board Committees:
A2DO2 Characteristics of Nonbituminous Components of Bituminous Paving Mixtures
A3C13 Sealants and Fillers for Joints and Cracks
A2BO4 Secretary, Committee on Pavement Rehabilitation
A3CO5 Committee on Pavement Maintenance
MGEC, MSES, Mn AAPT

PERSONAL: Married, Verla, School Secretary; Son, Mitchell Age 16
Member, Bloomington Traffic and Transit Advisory Commission; Officer Bloomington Kennedy Quarterback Club; Chair, Special Gifts Committee, Christ The King Lutheran Church; Coach/Volunteer Bloomington Athletic Association

Proposed Budget (Project Number ZZ19, Fu-Hsian Chang)

Year 1. July 1, 1995 - June 30, 1996

	LCMR	BSU (matching)	Total
I. Personnel			
A. Salaries			
1. Research Associate (1/3 time)	$8,000	- 0 -	$8,000
2. Contracted BSU Faculty	- 0 -	5,740	5,740
3. Non-Contracted BSU Faculty	7,000	- 0 -	7,000
4. Graduate Assistant (1 half-time)	7,000	- 0 -	7,000
5. Lab Helper (1 part-time)	- 0 -	1,500	1,500
B. Fringe Benefit (26% of Staff and Faculty Salaries)	3,900	1,492	5,392
Total Salaries (A+B)	$25,900	$8,732	$34,632
II. Non-Personal			
A. Supplies and Chemicals	$3,000	$500	$3,500
B. Equipment			
1. Water Bath Shaker	- 0 -	1,000	1,000
2. Ultracentrifuge	- 0 -	4,000	4,000
3. Bioreactor	- 0 -	2,000	2,000
4. Concentrater Freeze Dryer	- 0 -	2,000	2,000
5. Spectrophotometer	- 0 -	2,000	2,000
C. Travel			
1. To research sites and MnDOT Lab	1,000	- 0 -	1,000
2. To national meeting	- 0 -	- 0 -	- 0 -
D. Library and Communication Services	100	- 0 -	100
E. Indirect Costs	- 0 -	- 0 -	- 0 -
Total Budget (I+II)	$30,000	$20,232	$50,232

Proposed Budget (Project Number ZZ19, Fu-Hsian Chang)

Year 2. July 1, 1996 - June 30, 1997

	LCMR	BSU (matching)	Total
I. Personnel			
A. Salaries			
1. Research Associate (1/3 time)	$8,000	- 0 -	$8,000
2. Contracted BSU Faculty	- 0 -	5,740	5,740
3. Non-Contracted BSU Faculty	7,000	- 0 -	7,000
4. Graduate Assistant (1 half-time)	7,000	- 0 -	7,000
5. Lab Helper (1 part-time)	- 0 -	1,500	1,500
B. Fringe Benefit (26% of Staff and Faculty Salaries)	3,900	1,492	5,392
Total Salaries (A+B)	$25,900	$8,732	$34,632
II. Non-Personal			
A. Supplies and Chemicals	$2,000	- 0 -	$2,000
B. Equipment			
1. Water Bath Shaker	- 0 -	1,000	1,000
2. Ultracentrifuge	- 0 -	4,000	4,000
3. Bioreactor	- 0 -	2,000	2,000
4. Concentrater Freeze Dryer	- 0 -	2,000	2,000
5. Spectrophotometer	- 0 -	2,000	2,000
C. Travel			
1. To research sites and MnDOT Lab	1,000	- 0 -	1,000
2. To two national meetings	1,000	500	1,500
D. Library and Communication Services	100	- 0 -	100
E. Indirect Costs	- 0 -	- 0 -	- 0 -
Total Budget (I+II)	$30,000	$20,232	$50,232

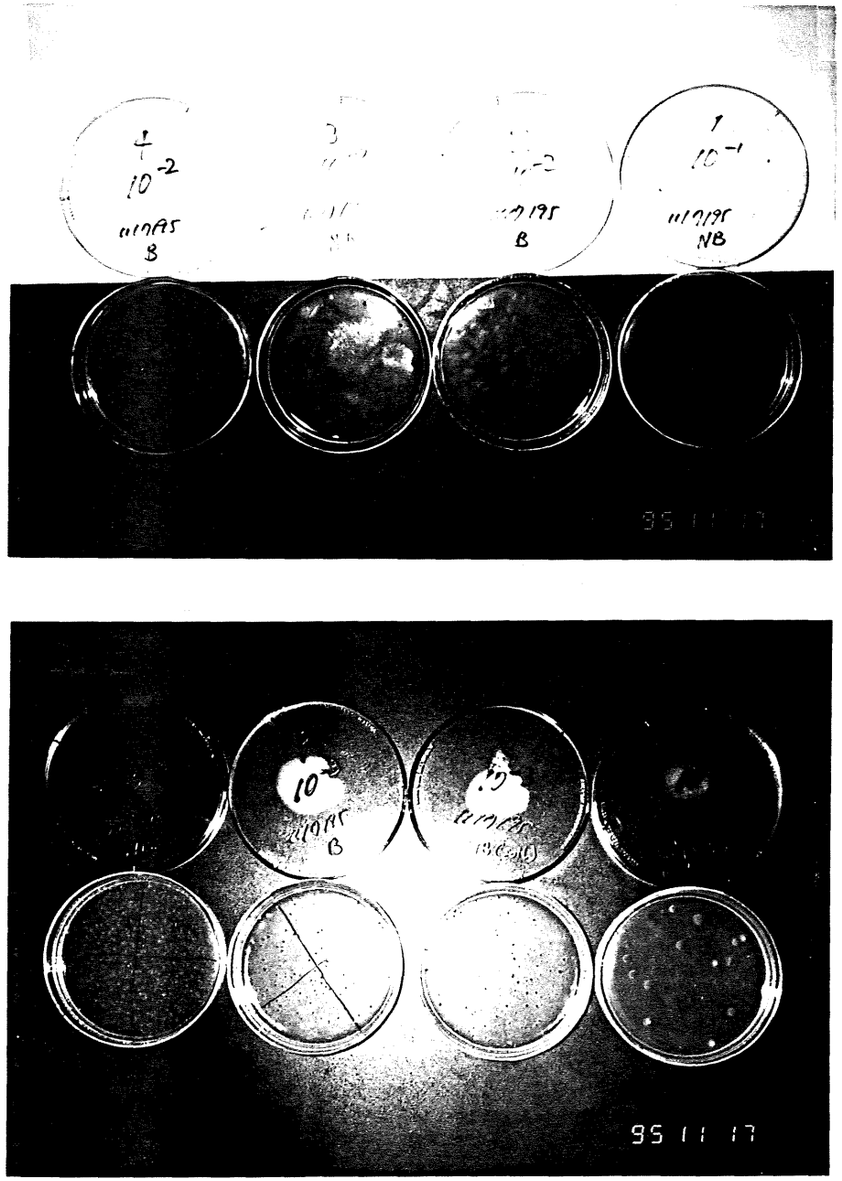


Fig. 1. The appearance of asphalt-degrading microorganisms on mineral agar with asphalt extract as sole carbon source. Top: fungal colonies appear on plates of sample 2 and 4; Bottom: Large Actinomyces and tiny bacteria colonies exhibit on plate of sample 9 and 10.

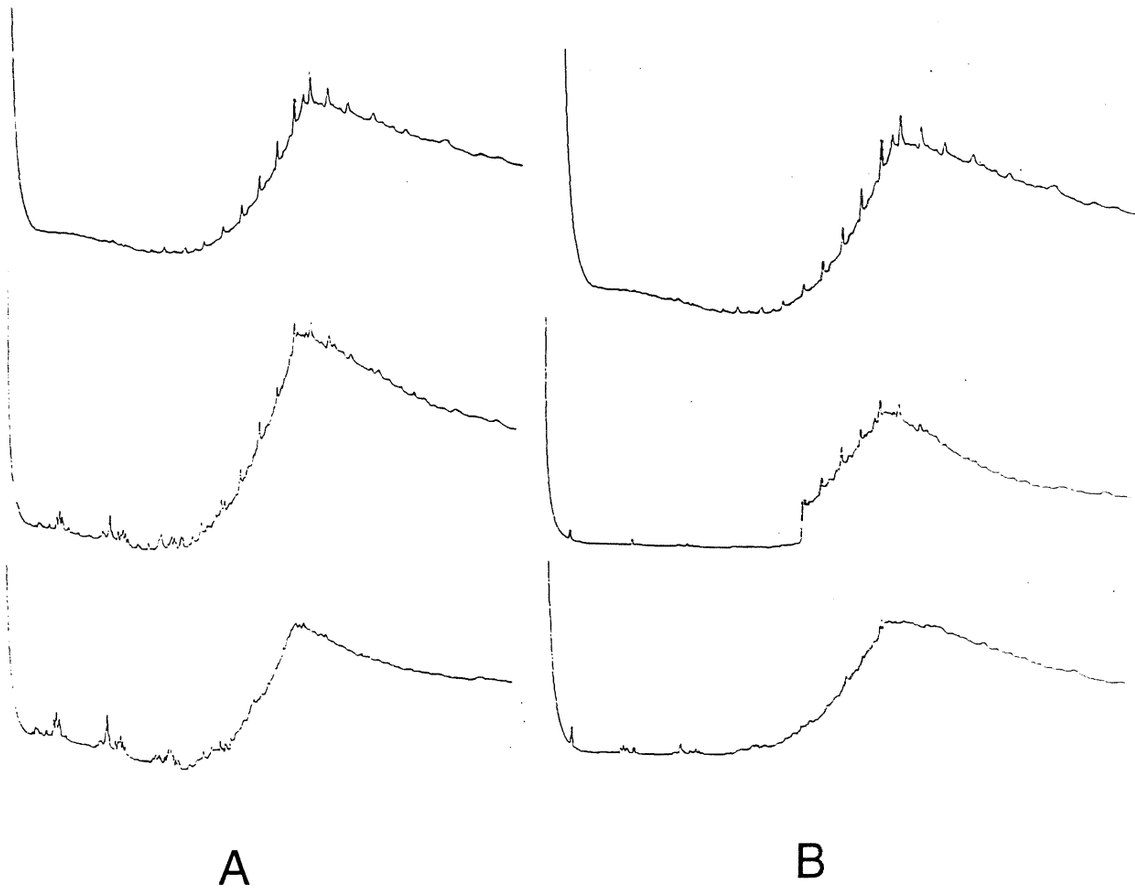


Fig. 2. The chromatograms of GC analysis in asphalt pavement samples.
A. from top to bottom: fresh ashpalt pavement, Sample 3: non-broken asphalt pavement, Sample 2: broken asphalt pavement; B. from top to bottom: fresh asphalt pavement, Sample 1: nonbroken asphalt pavement, Sample 4: broken asphalt pavement.

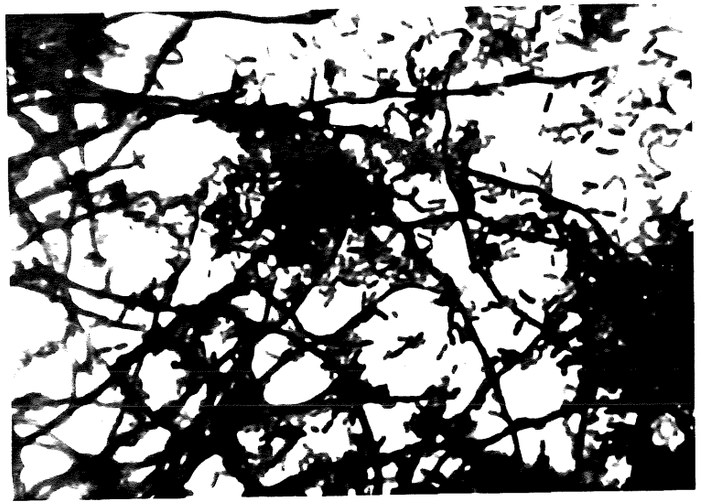


Fig. 3. Mixed asphalt degraders, isolated from 31 asphalt and soil samples (100 X Magnification)

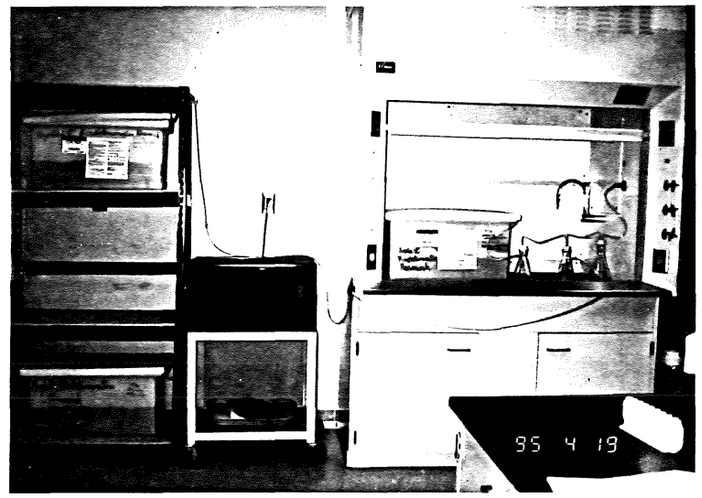
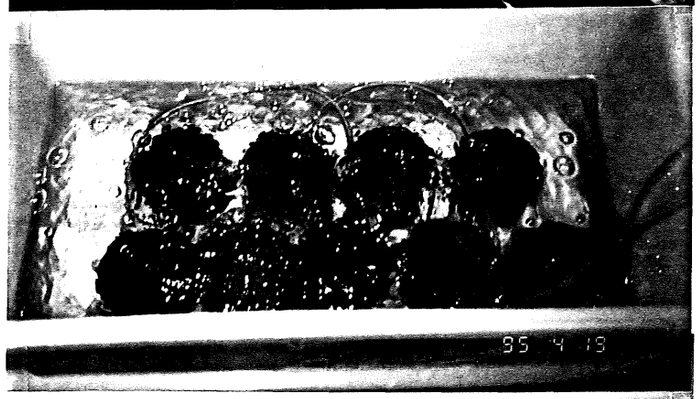
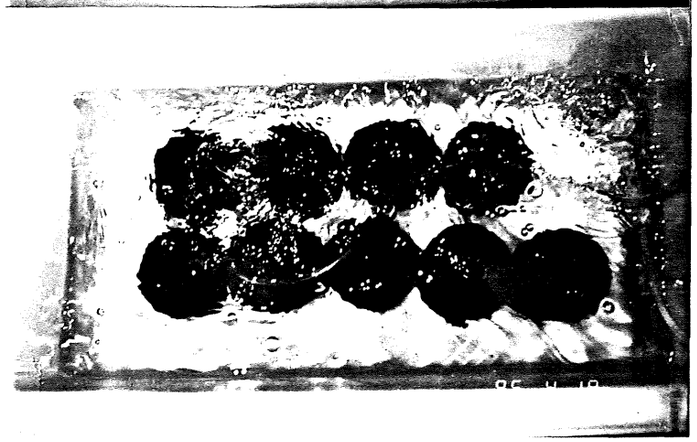


Fig. 4. Schematics side view of apparatus for asphalt biodeterioration/biodegradation studies (Marshall pucks were soaked in mixed culture and lake water)

Soaked in
Asphalt-degrading
microbial culture



Soaked in straight
lake water



Soaked in autoclaved
lake water

Fig. 5. Schematics (top view) illustration of Marshall pucks soaked in three different broth media

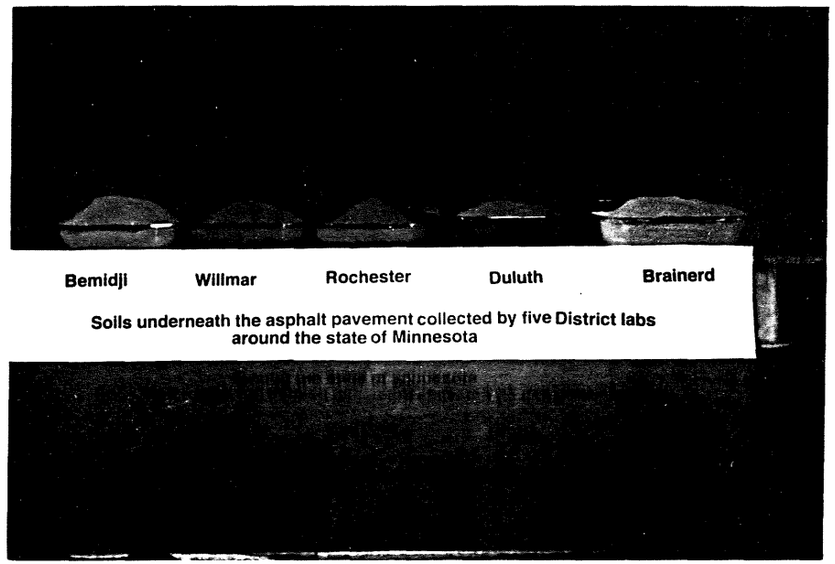


Fig. 6. Soils underneath the asphalt pavement collected by five MN/DOT District Labs around the state of Minnesota

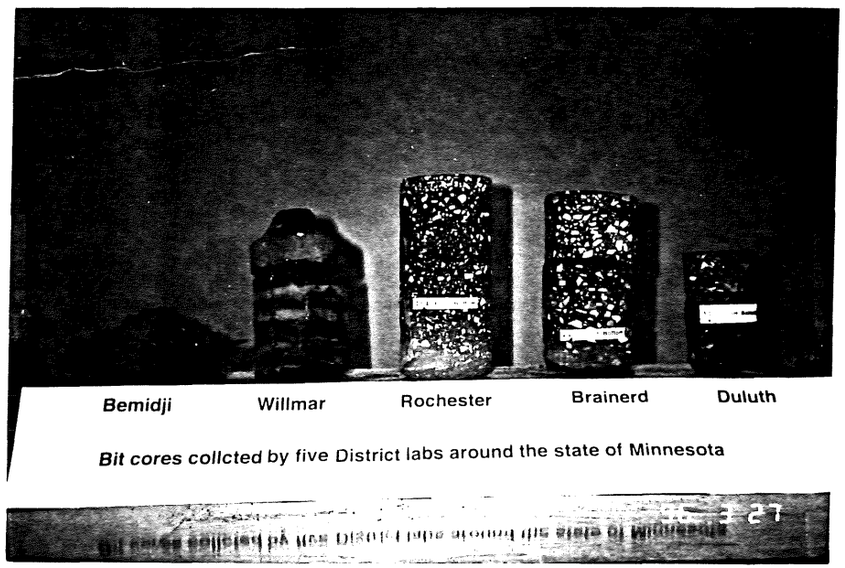


Fig. 7. Bit cores collected by MN/DOT five District Labs around the State of Minnesota

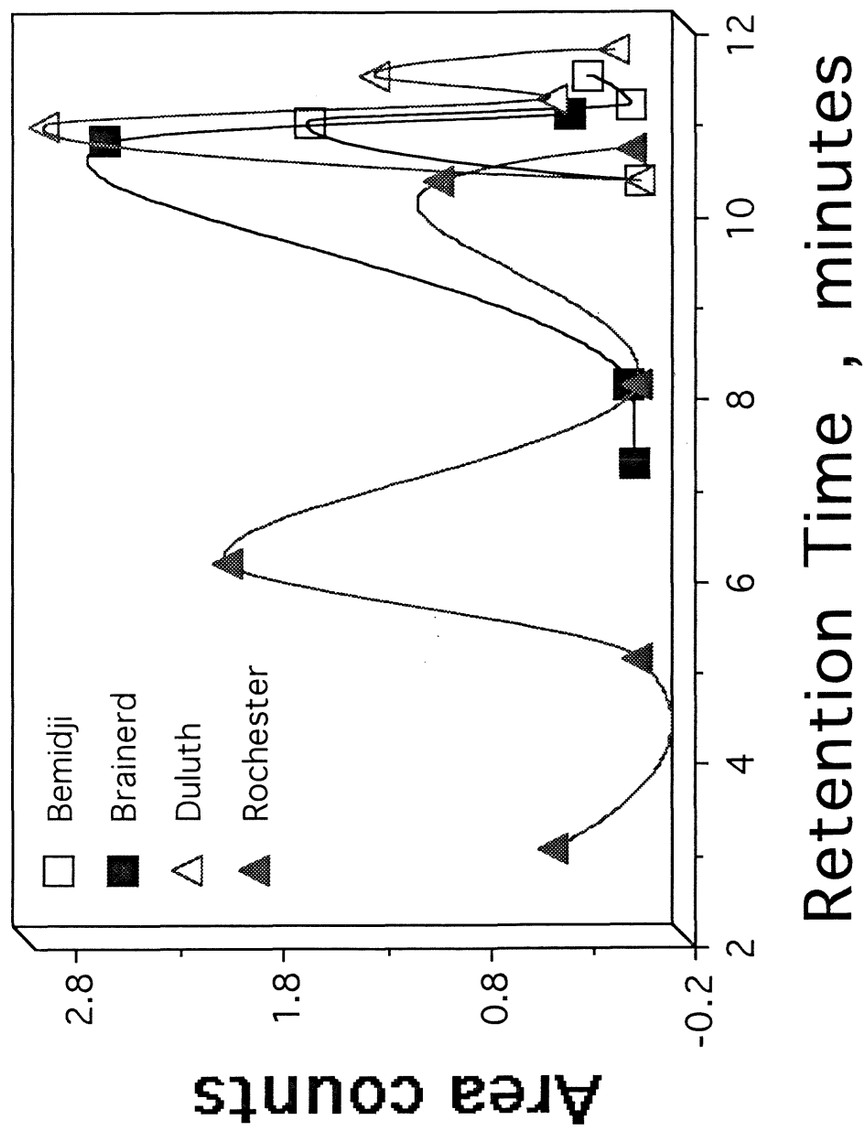


Fig. 8. HPLC analysis of soil samples from different District Labs.

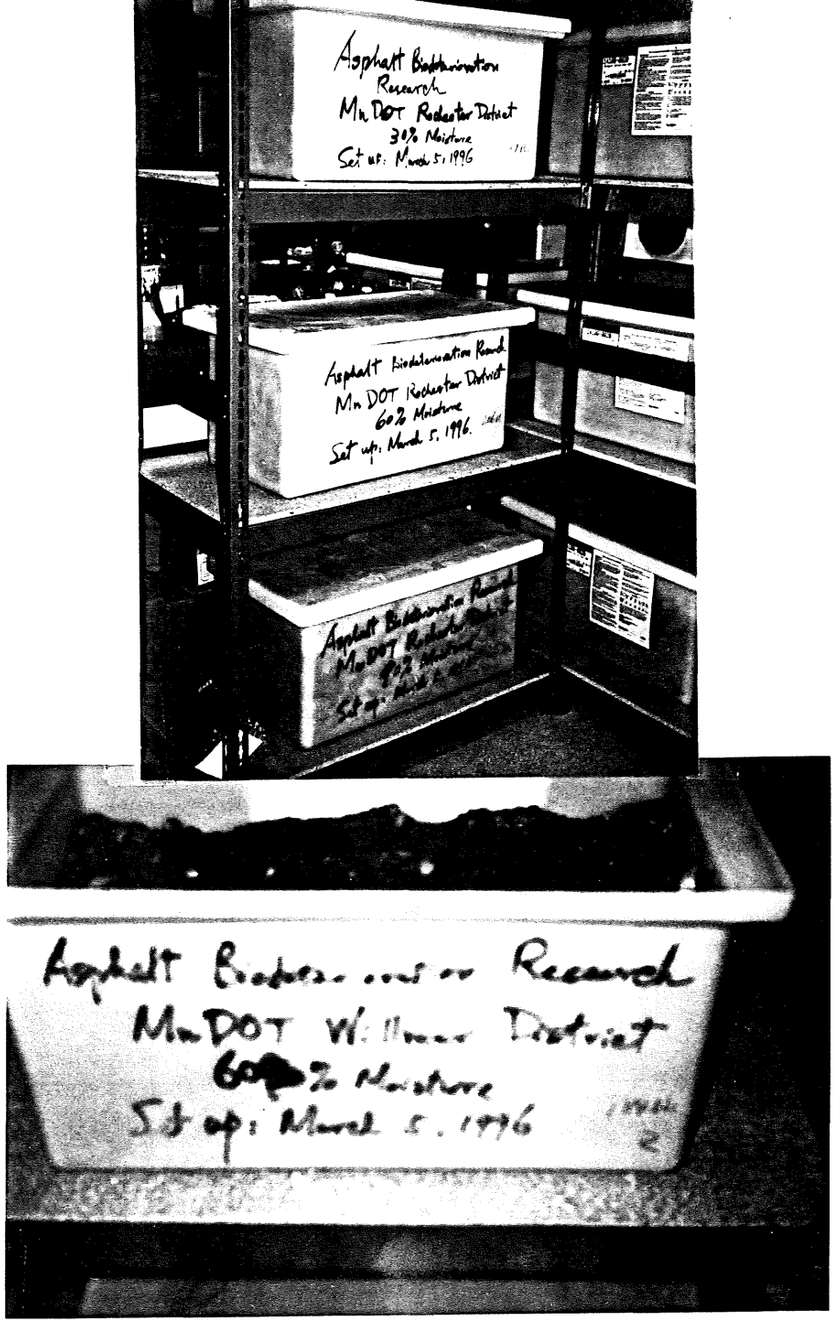


Fig. 9. Schematics (side and top view) of apparatus for asphalt biodeterioration/biodegradation studies buried in soils

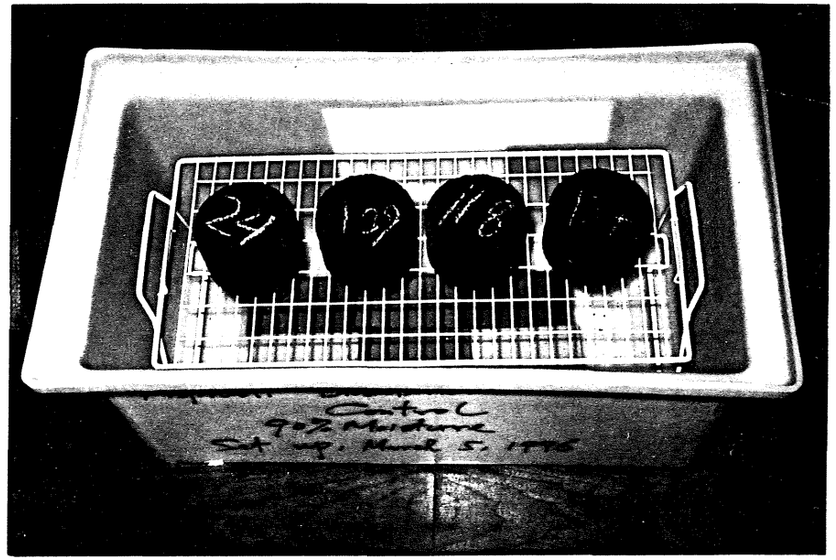


Fig. 10. Schematics (top view) illustrating the control experiment of Marshall pucks buried in soils test.

Fig. 11. Appearance of microbial attachment on Marshall pucks after soaked for 4.5 months in mixed culture and lake waters.

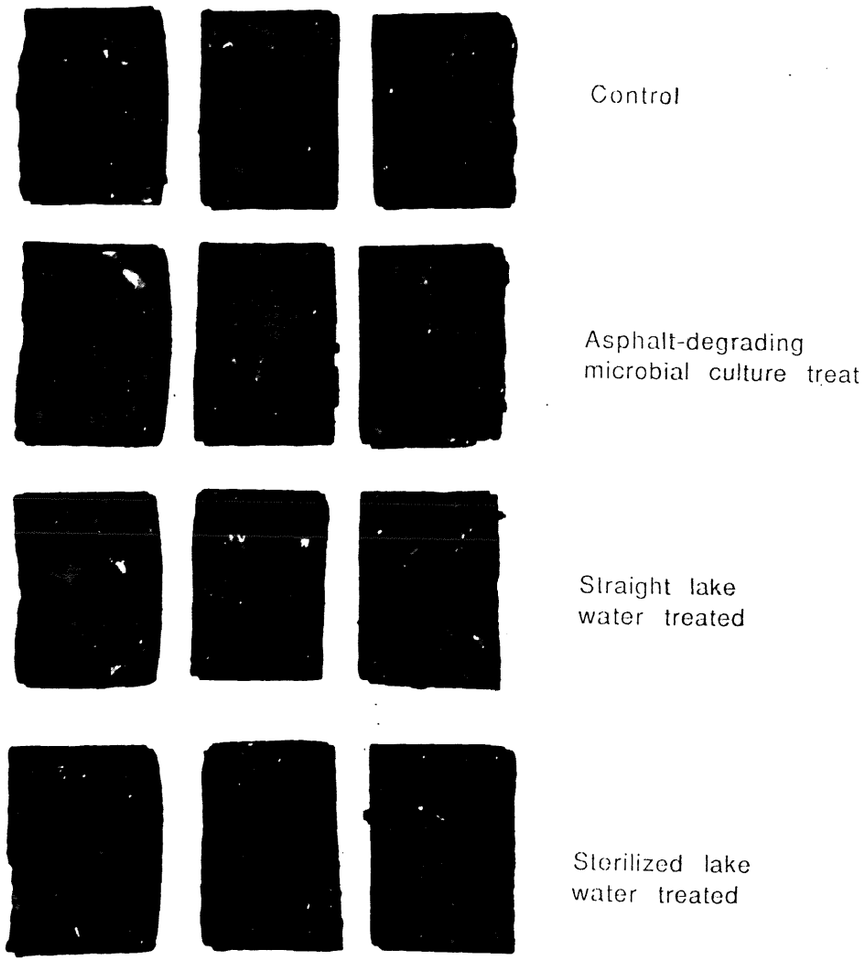
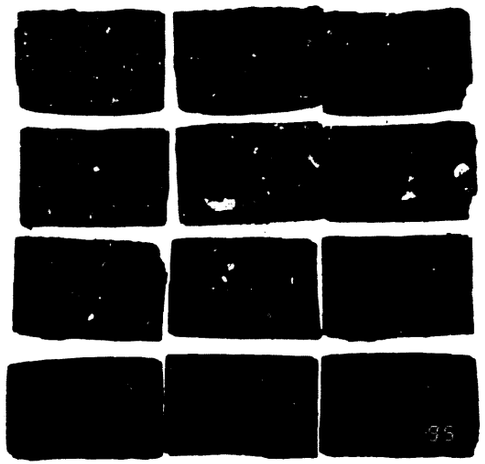


Fig. 12. Cracked Marshall pucks showing broken aggregate after soaked for 1.5 months in different treatements.



Asphalt-degrading microbial culture treated

Straight lake water treated

Sterilized lake water treated

Control

Fig. 13. Broken aggregate shown in cracked Marshall pucks after soaked for 4.5 months in different treatments.

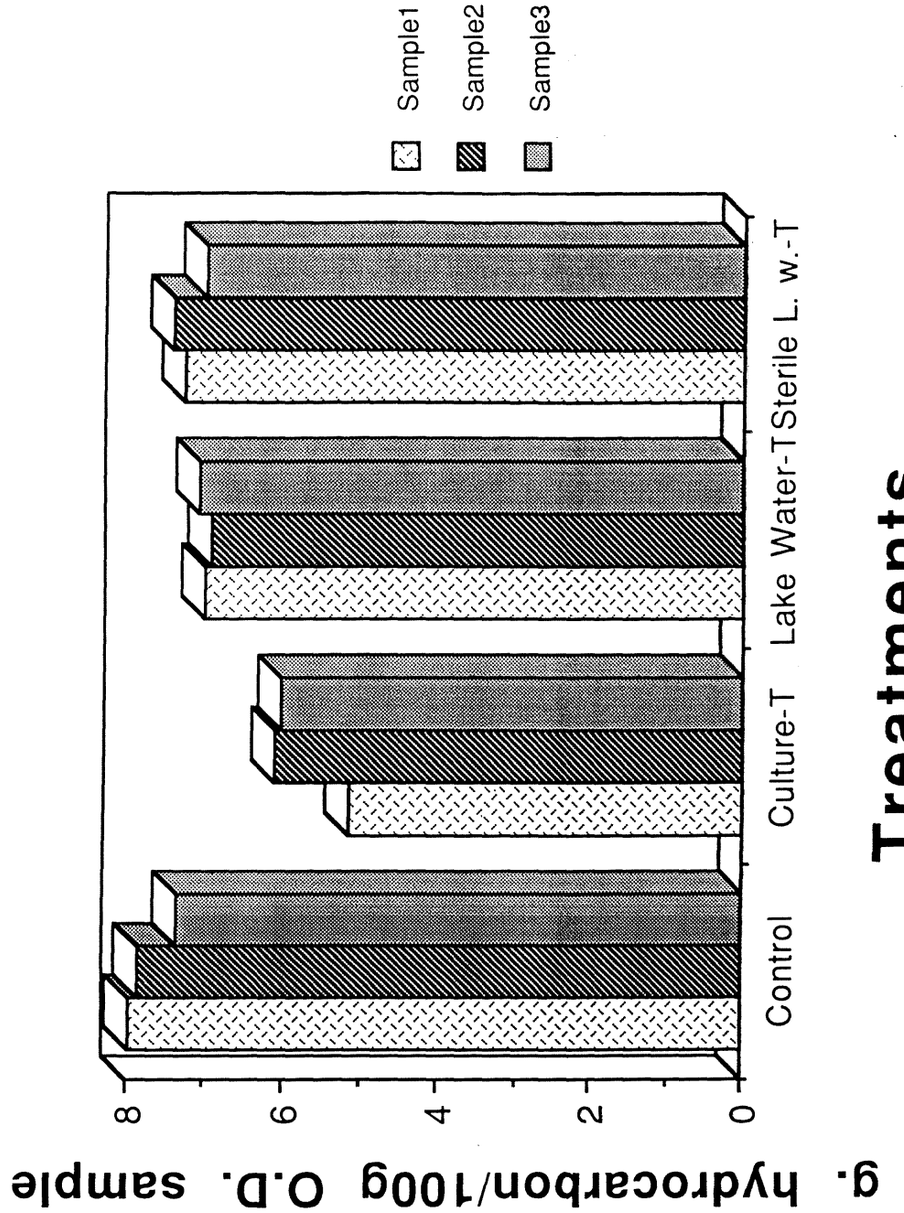


Fig. 14. Total hydrocarbon (%) affected by microbial degradation in Marshall puck samples post-10 months treatments

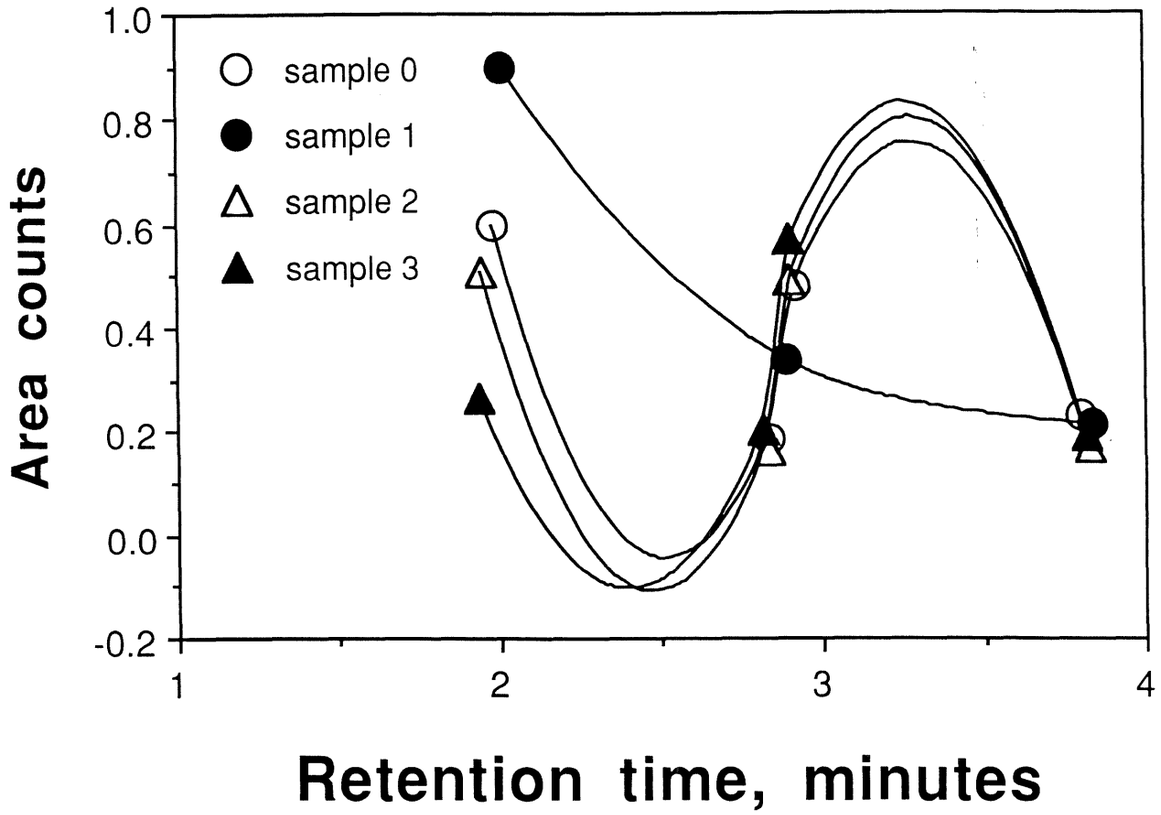


Fig. 15. HPLC analysis of Marshall puck samples post-10 months treatments

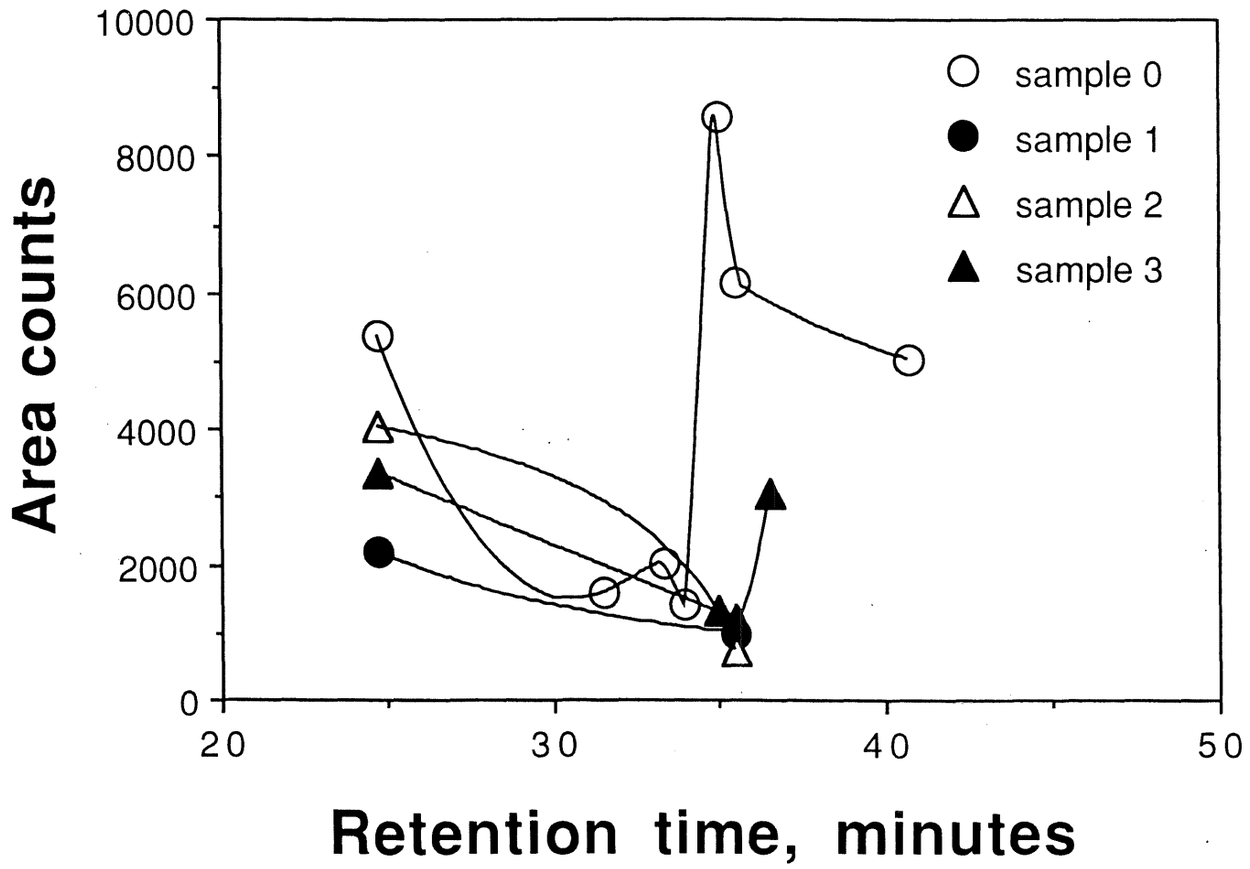


Fig. 16. GC analysis of Marshall puck samples post-10 months treatments

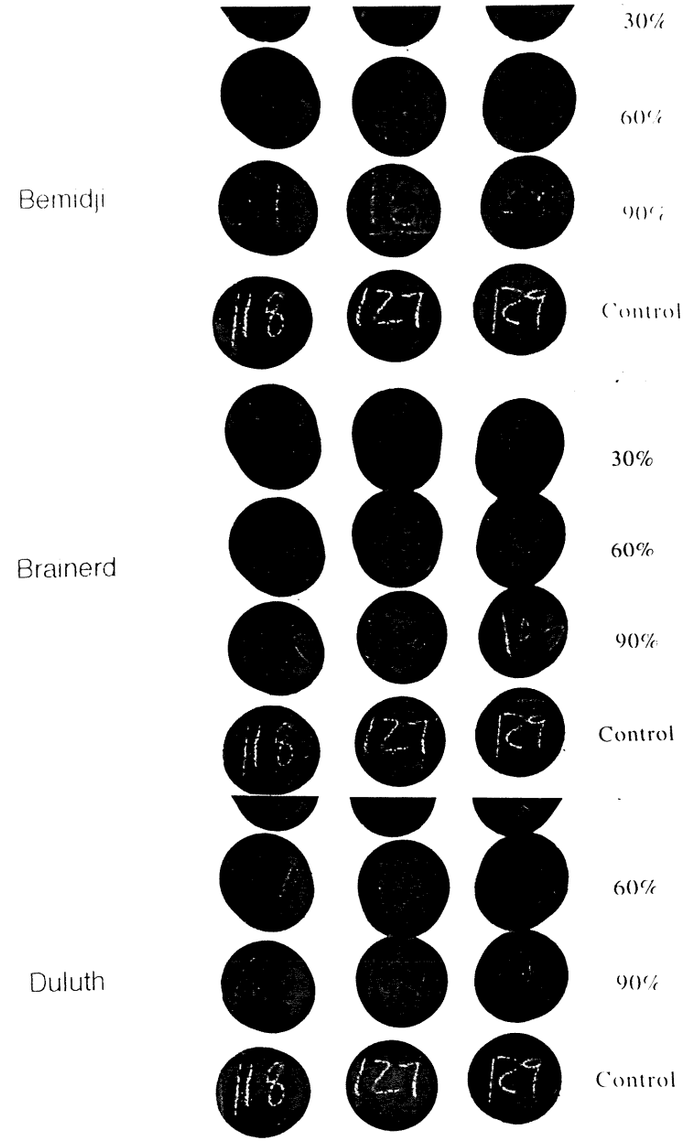


Fig. 17. Appearance of microbial attachment on Marshall pucks after had been buried in soils, collected by Bemidji (top), Brainerd (middle) and Duluth (bottom) Districts, for three months at three water holding capacities.

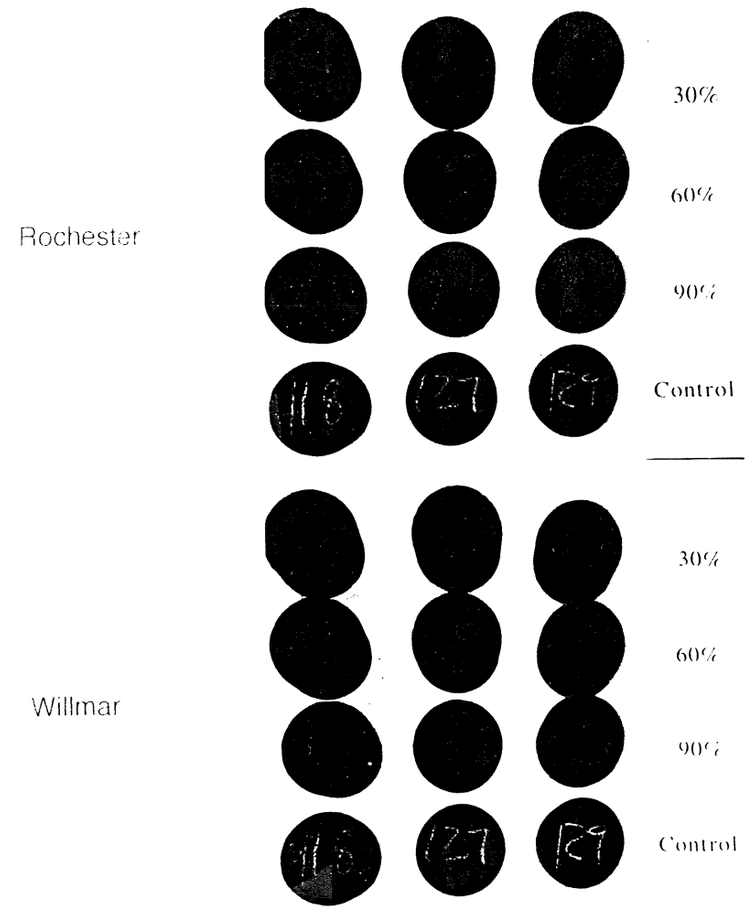


Fig. 18. Appearance of microbial attachment on Marshall pucks after had been buried in soils, collected by Rochester (top) and Willmar (bottom) Districts, for three months at three water holding capacities.

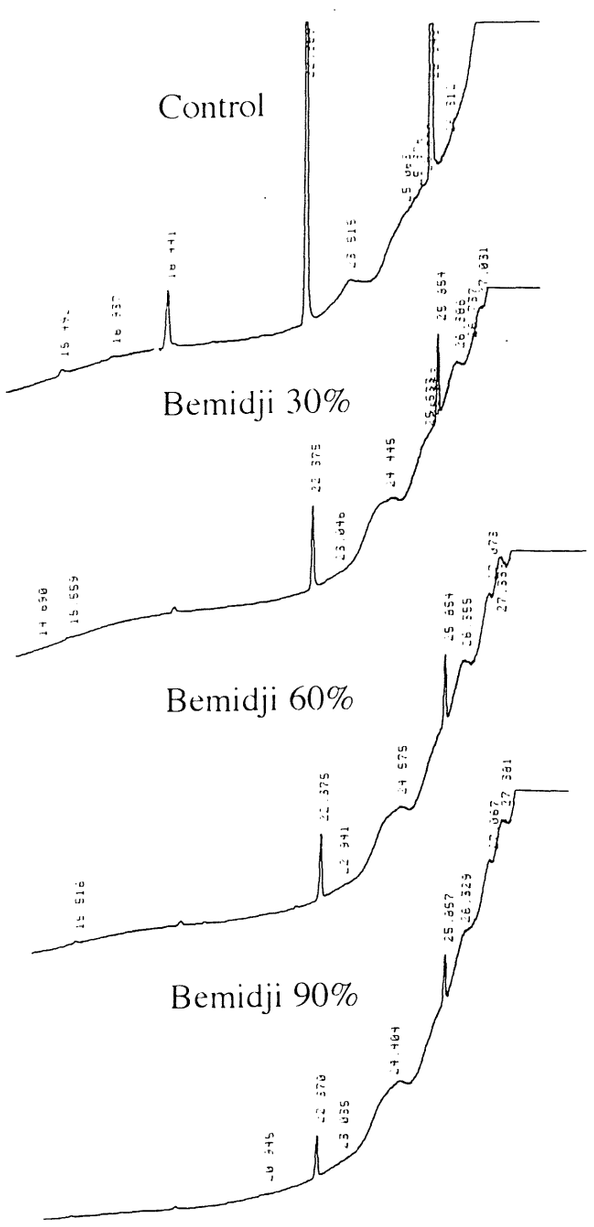


Fig. 19. The Chromatograms of GC analysis in Marshall pucks after buried in control container and soil, collected by Bemidji District, for three months at three water holding capacities.

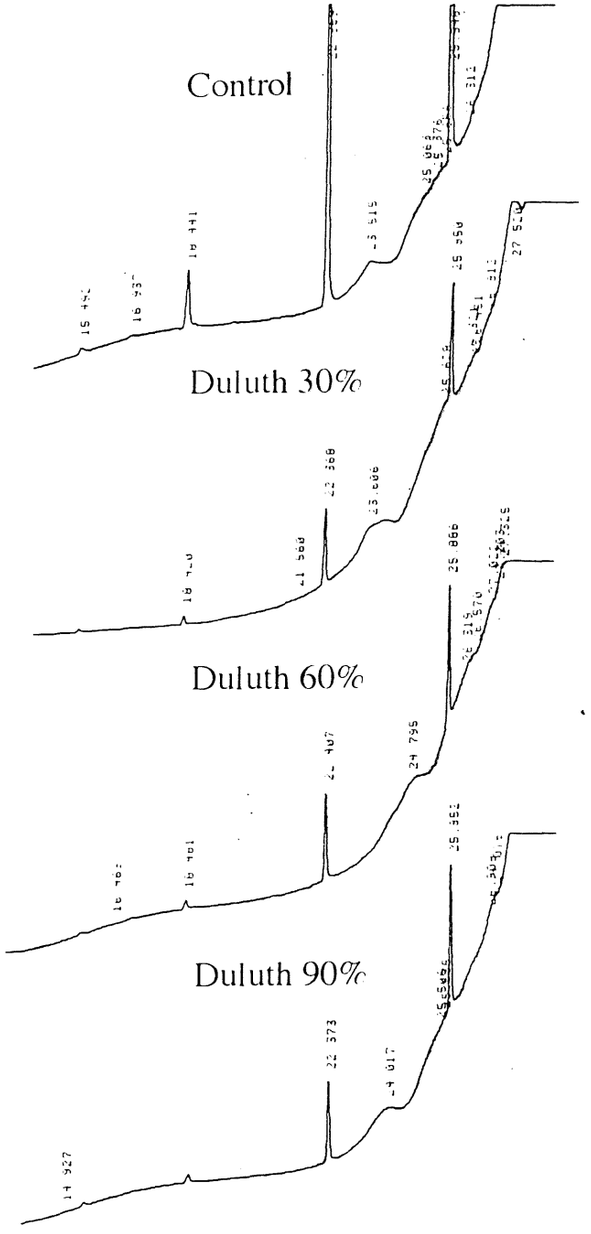


Fig. 20. The Chromatograms of GC analysis in Marshall pucks after buried in control container and soil, collected by Duluth District, for three months at three water holding capacities.

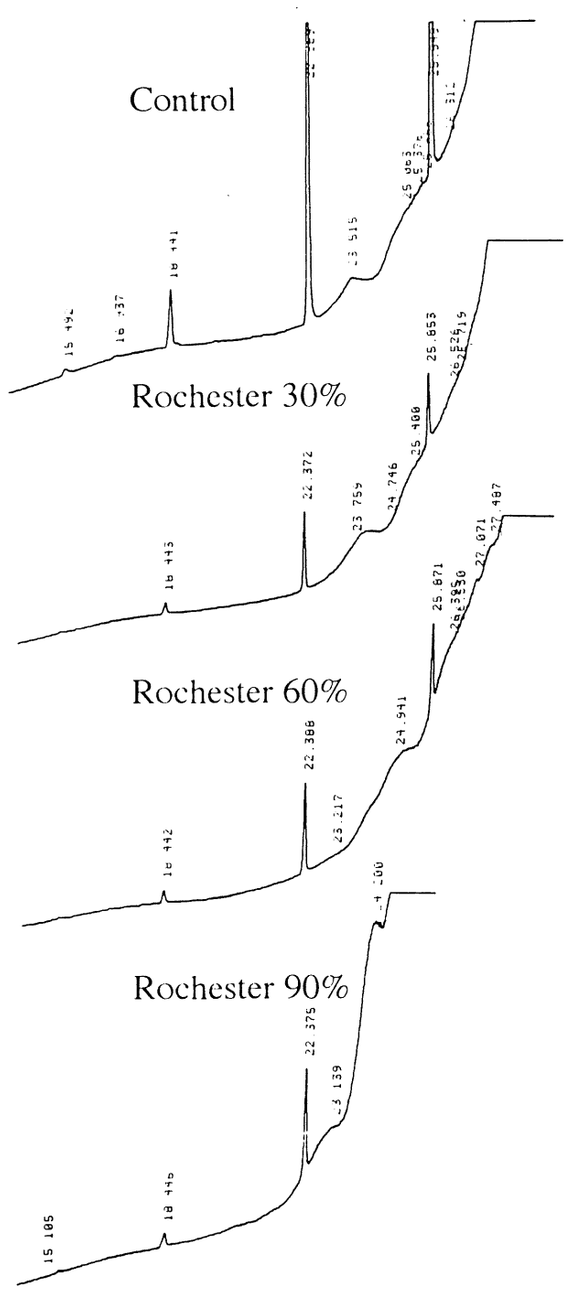


Fig. 21. The Chromatograms of GC analysis in Marshall pucks after buried in control container and soil, collected by Rochester District, for three months at three water holding capacities.

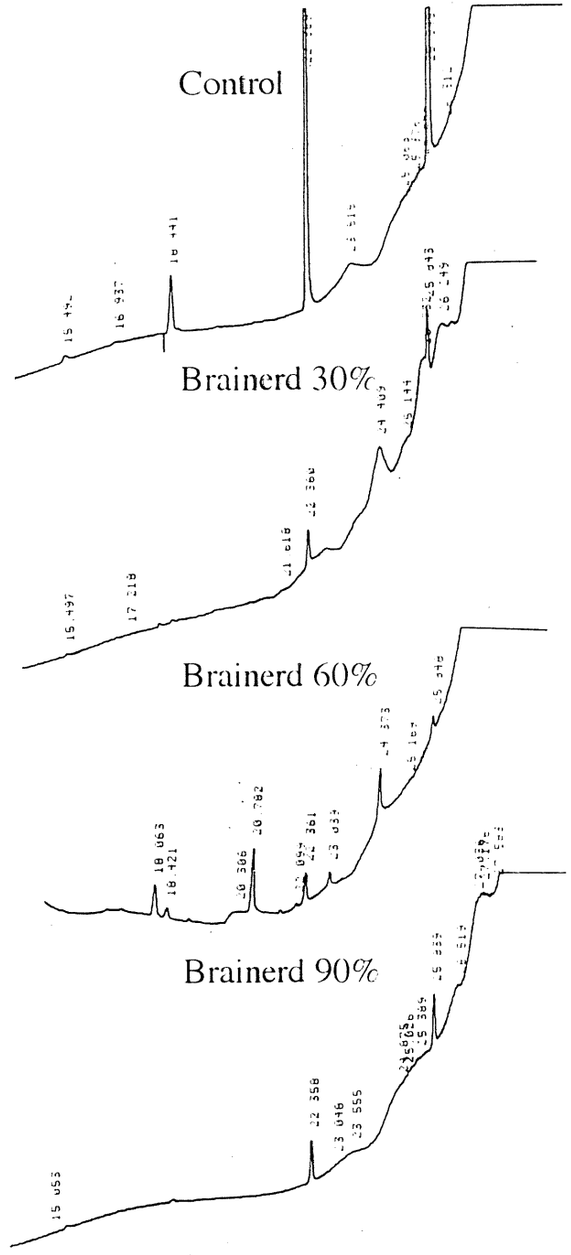


Fig. 22. The Chromatograms of GC analysis in Marshall pucks after buried in control container and soil, collected by Brainerd District, for three months at three water holding capacities.

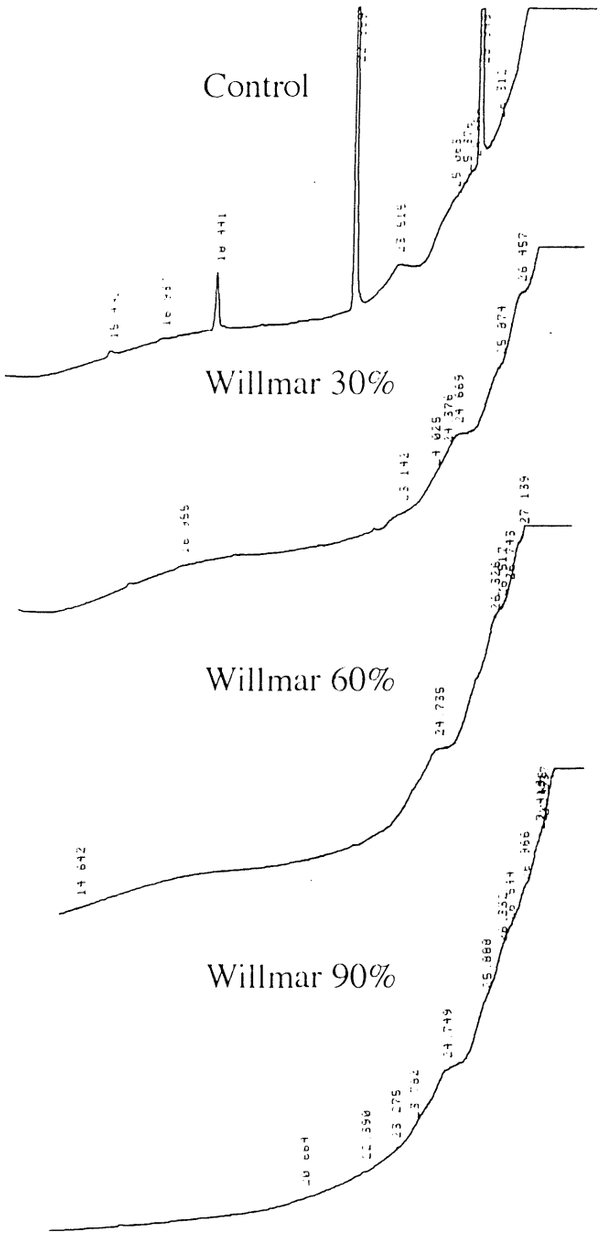
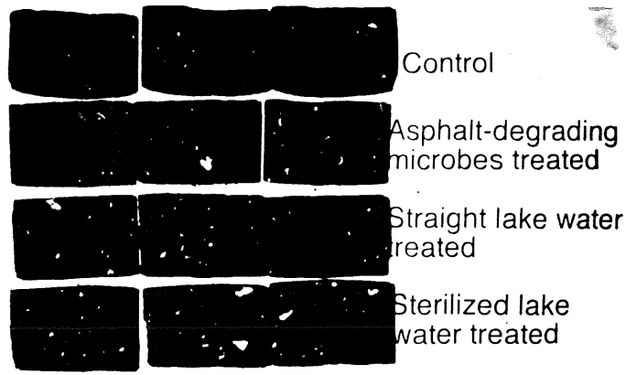


Fig. 23. The Chromatograms of GC analysis in Marshall pucks after buried in control container and soil, collected by Willmar District, for three months at three water holding capacities.



Cracked Marshall pucks with different treatments for 10 months

Fig. 24. Cracked Marshall pucks showing visual stripping and broken aggregate post-10 months treatments.

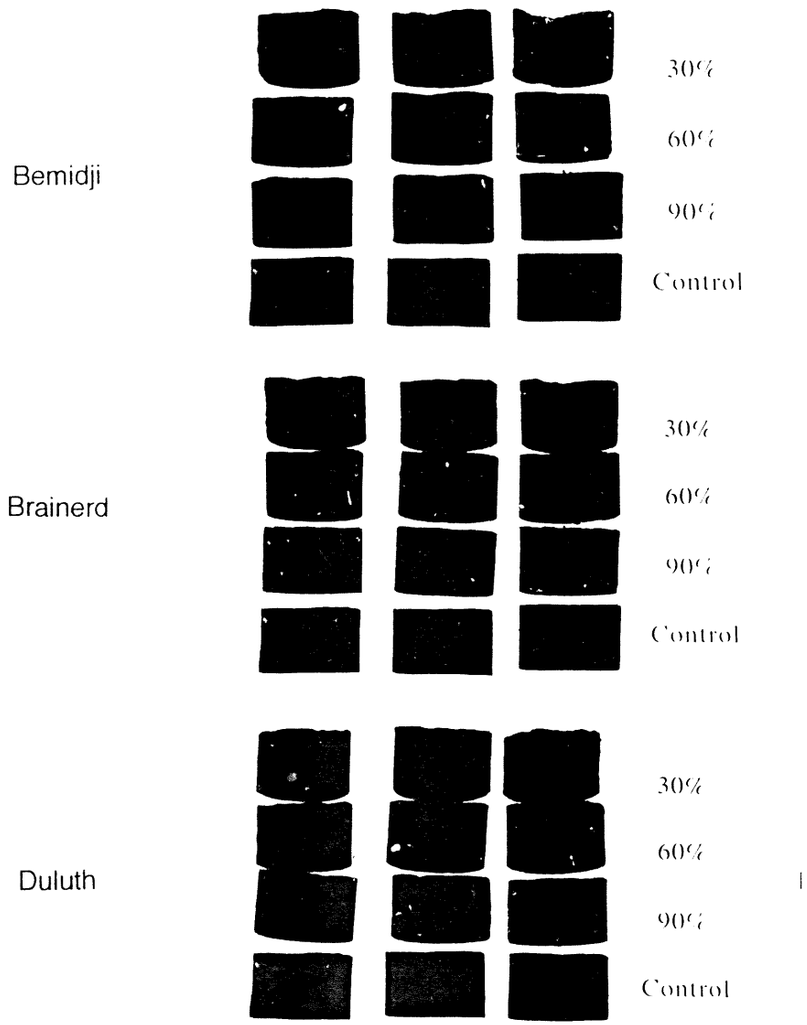


Fig. 25. Broken aggregate shown in cracked Marshall pucks after had been buried in soils, collected by Bemidji (top), Brainerd (middle) and Duluth (bottom) District Labs, for three months with different water holding capacities.

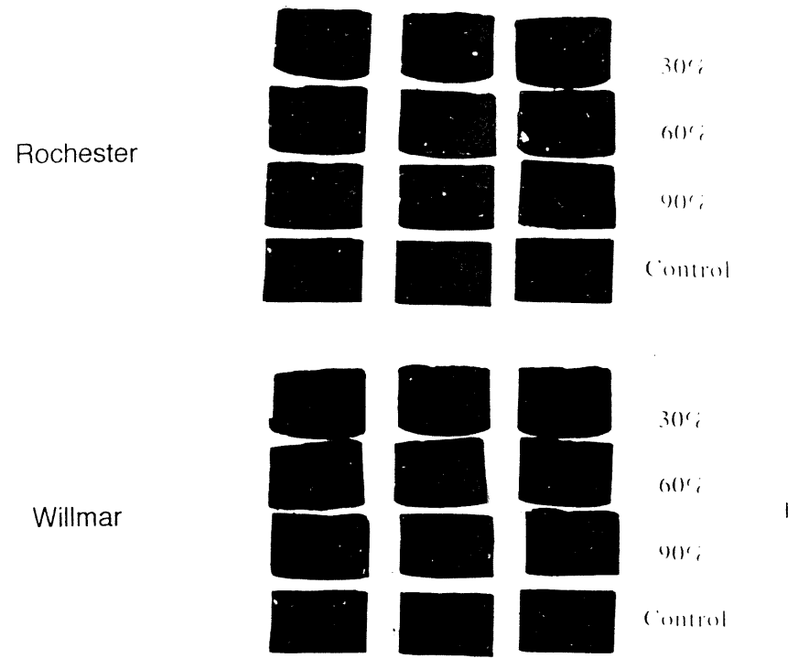


Fig. 26. Broken aggregate shown in cracked Marshall pucks after had been buried in soils collected by Rochester (top) and Willmar (bottom) District Labs, for three months with different water holding capacities.

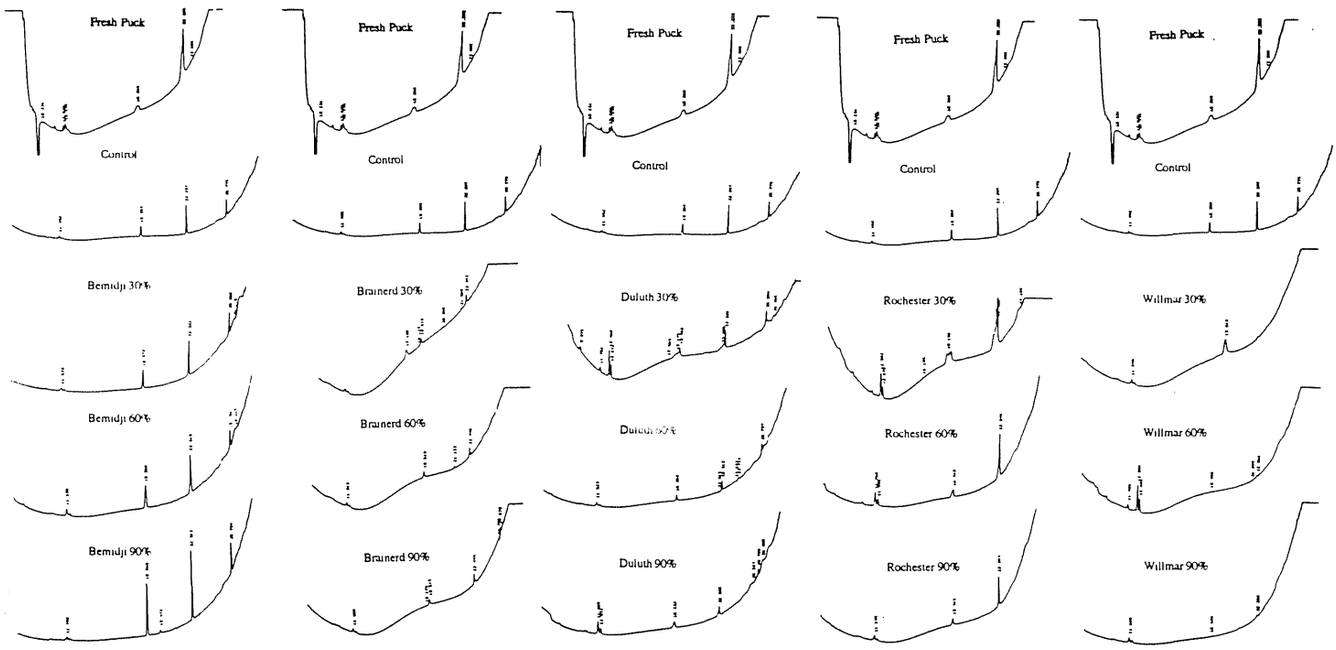


Fig. 27. Chromatograms of GC analysis of Marshall Pucks after buried in soils, collected from five District Labs, or left in containers for control, for seven months at three different water holding capacities and compared with fresh puck extract.

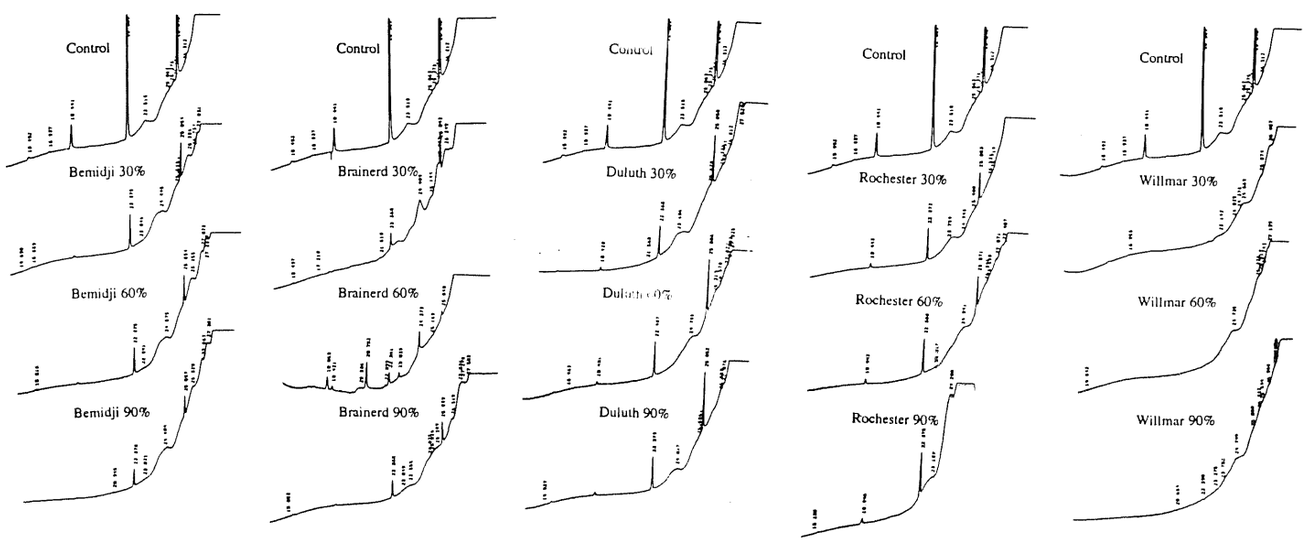


Fig. 28. The chromatograms of GC analysis in Marshall Pucks after buried in soils, collected from five District Labs or left in containers for the control, for three months at three water holding capacities.

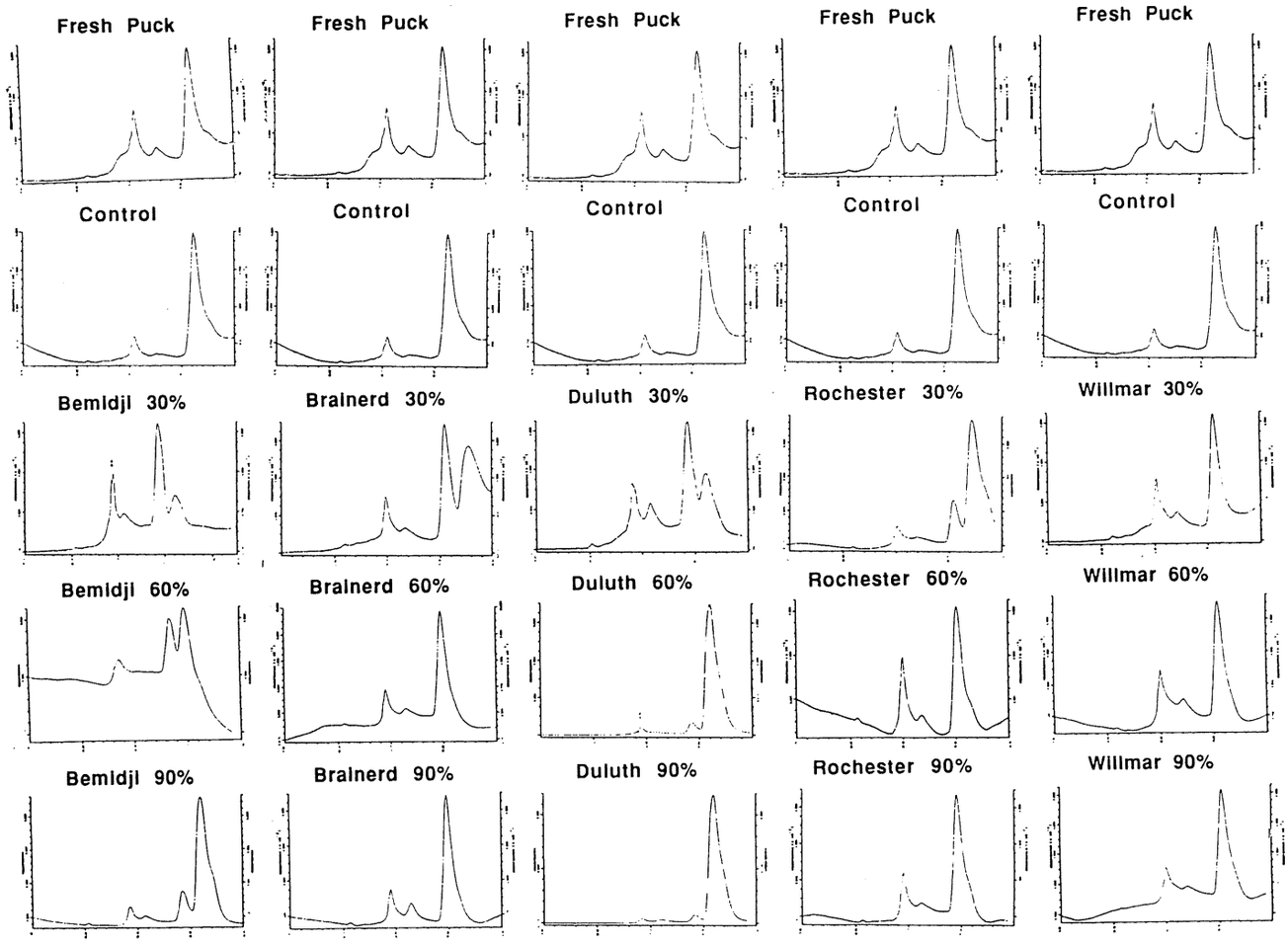


Fig. 29. The chromatograms of HPLC analysis in Marshall pucks after buried in soils, collected from five District labs or left in containers for the control, for seven months at three water holding capacities and compared with fresh puck extract.

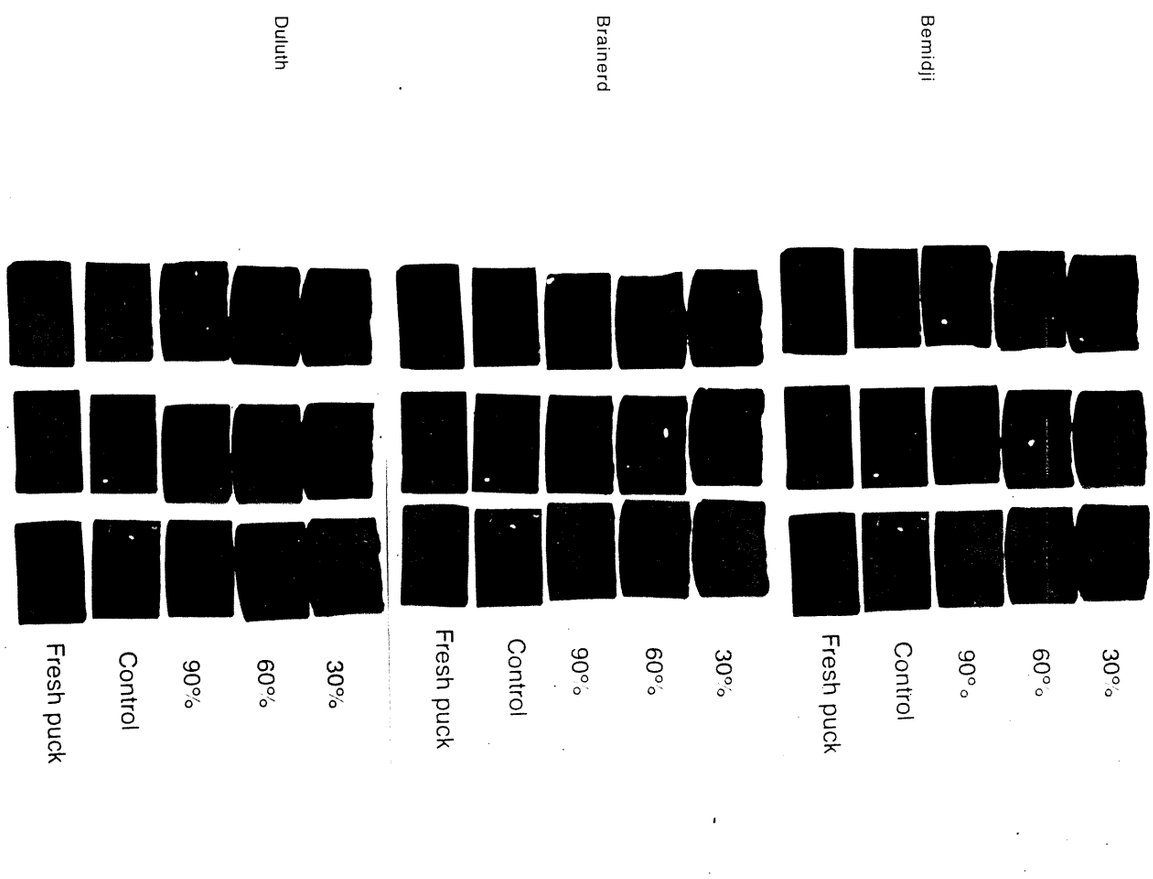


Fig. 30. Broken aggregate shown in cracked Marshall pucks and white aggregate showing on edge of the broken surface after pucks had been buried in soils, collected from Bemidji (top), Brainerd (middle), and Duluth (bottom) Districts, for seven months with different water holding capacities, compared with control samples and fresh puck.

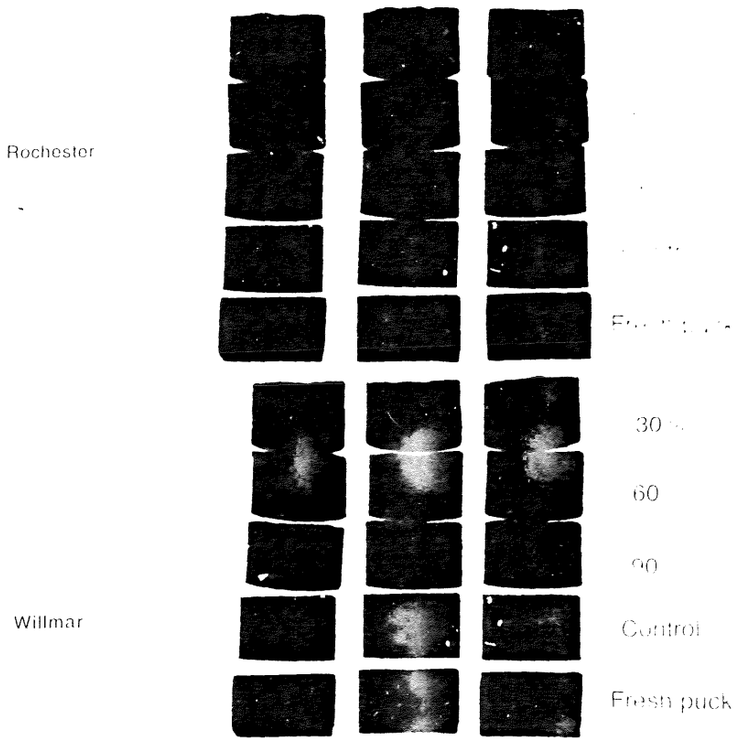


Fig. 31. Broken aggregate shown in cracked Marshall pucks and white aggregate showing on edge of the broken surface after had been buried in soils, collected from Rochester (top) and Willmar (bottom) Districts for seven months with different water holding capacities, compared with control samples and fresh puck.

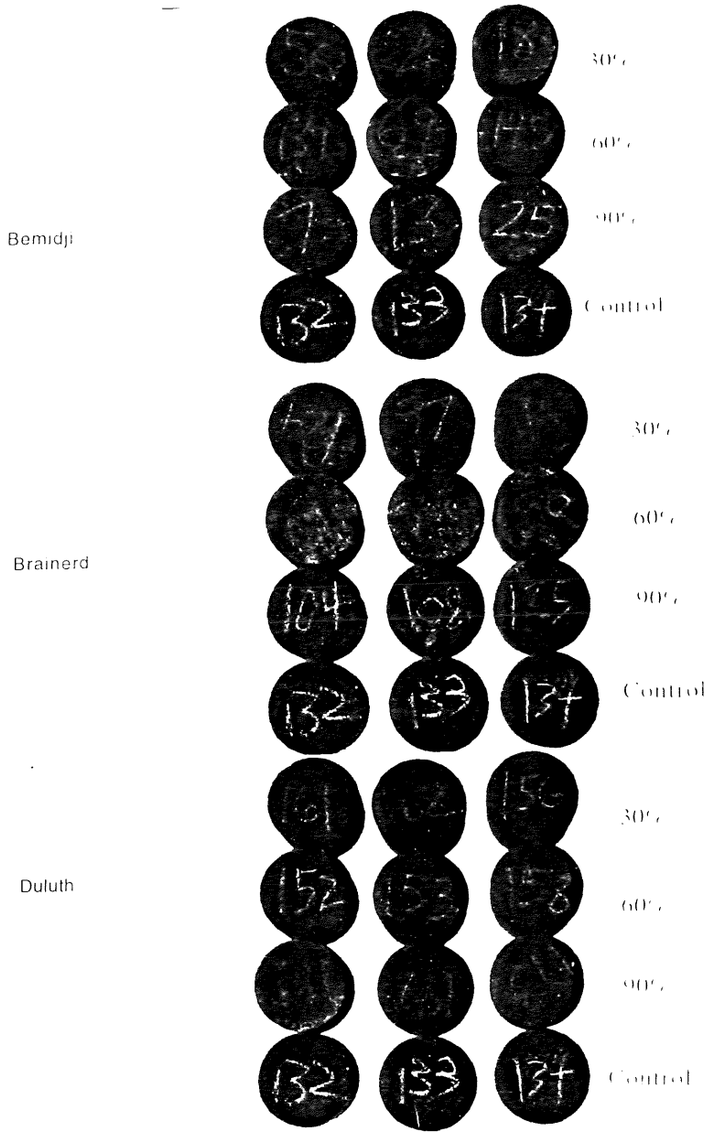


Fig. 32. Appearance of soil microbial attachment on Marshall pucks after had been buried in soils, collected from Bemidji (top), Brainerd (middle), and Duluth (bottom) Districts, for seven months at three different water holding capacities.

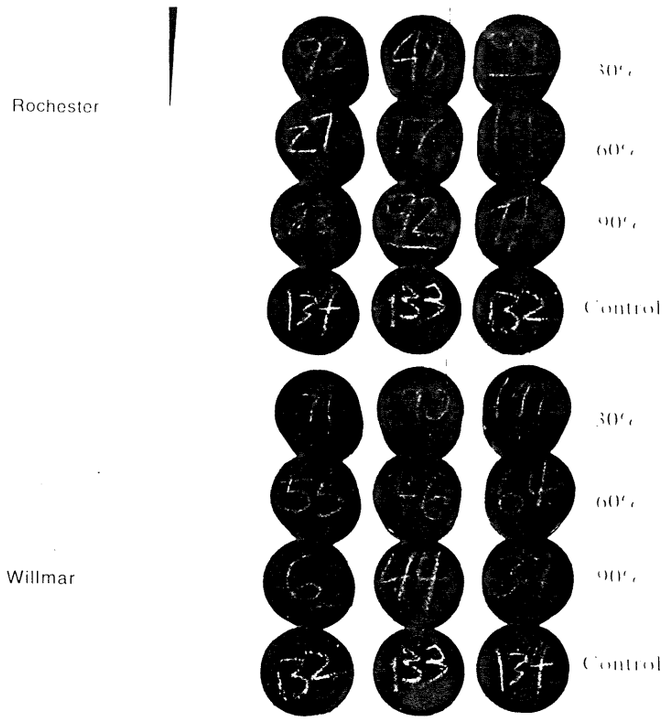


Fig. 33. Appearance of soil microbial attachment on Marshall pucks after had been buried in soils, collected from Rochester (top) and Willmar (bottom) districts, for seven months at three different water holding capacities.

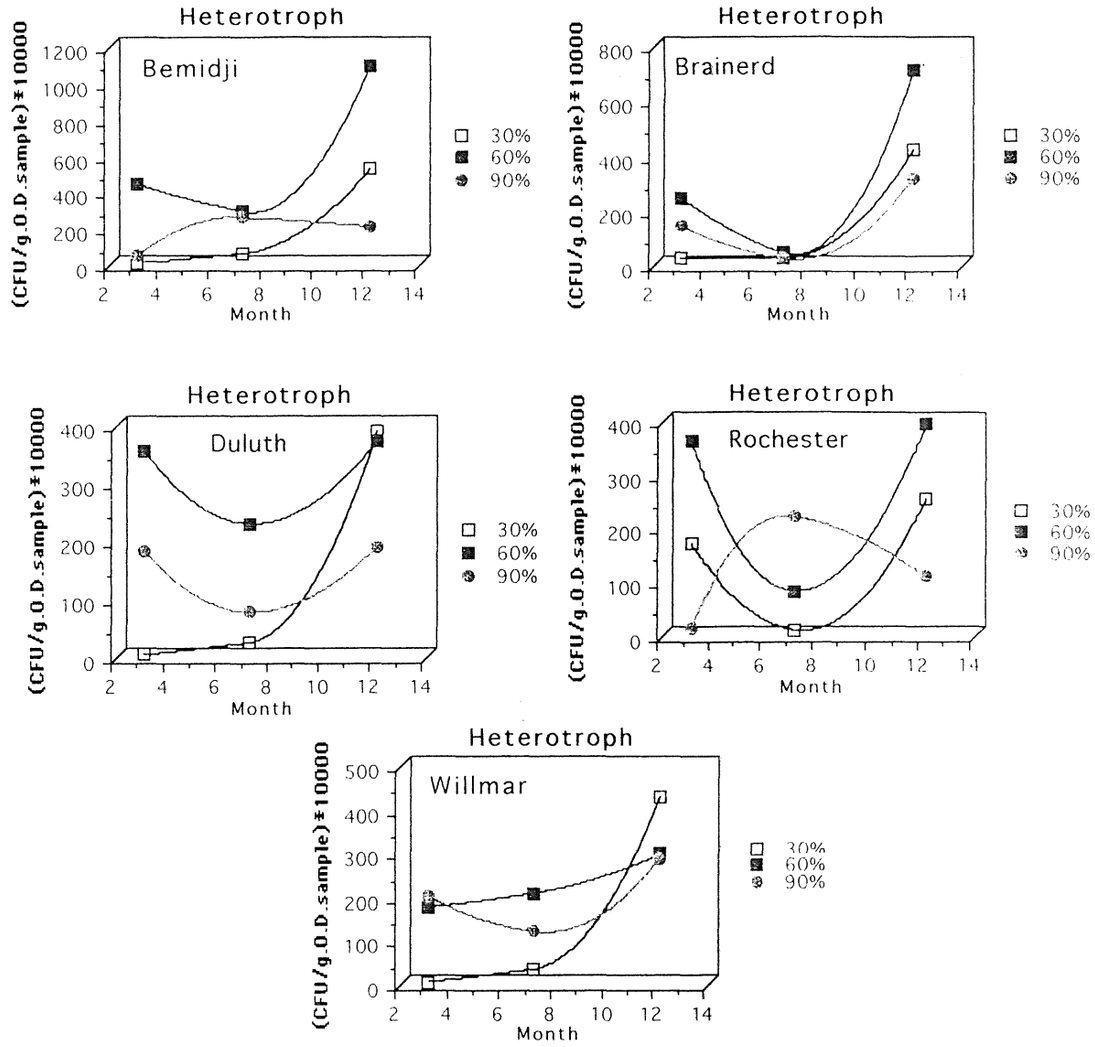


Fig. 34 Viability testing curve for heterotrophs in Marshall pucks after buried for 3, 7, and 12 months in soils collected from five District Labs with different water holding capacities. The number of heterotrophs in control pucks varying from 0.07×10^4 to 1.05×10^4 CFU/g.O.D. sample.

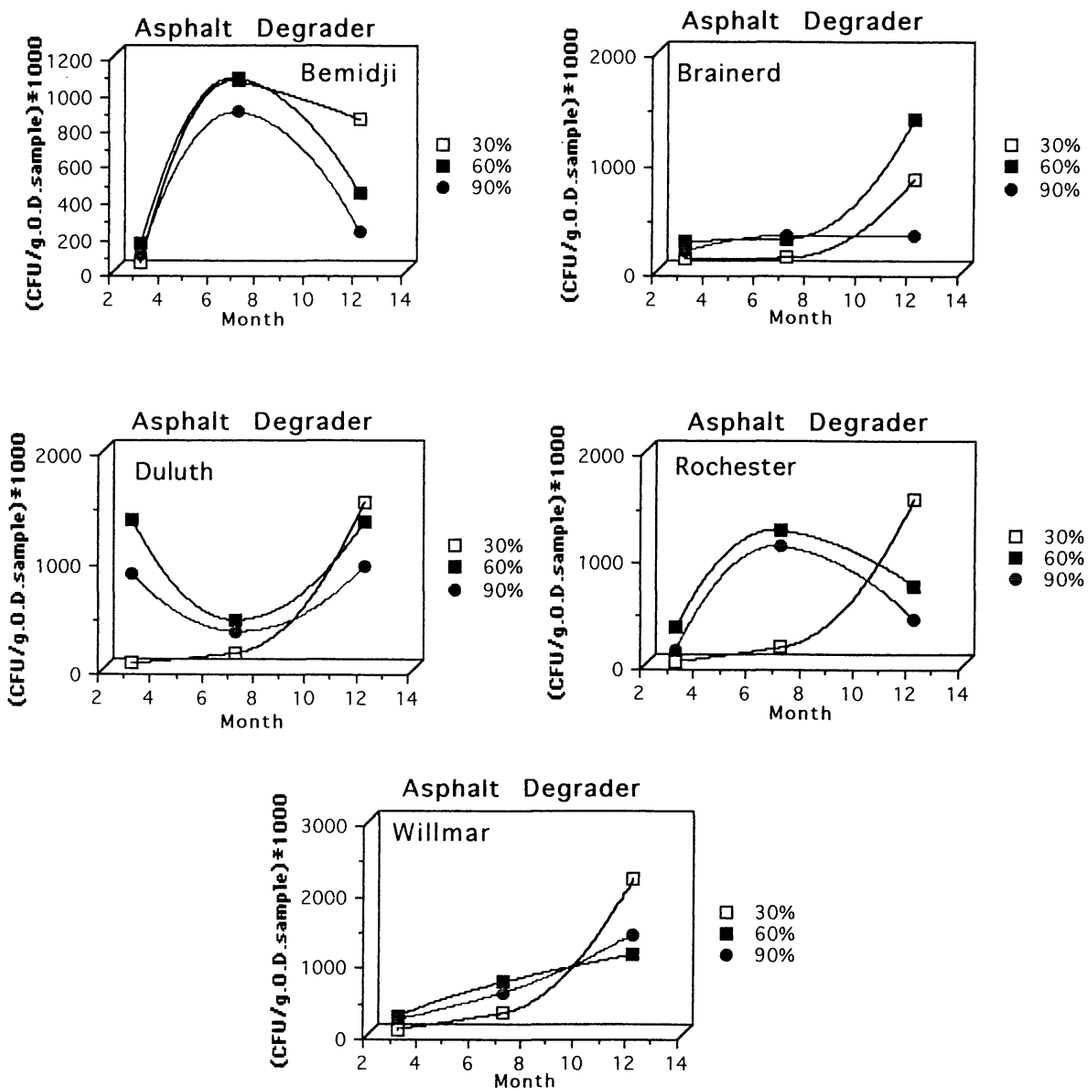


Fig. 35 Viability testing curve for asphalt degraders in Marshall pucks after buried for 3, 7, and 12 months in soils collected from five District Labs with different water holding capacities. The number of asphalt degraders in control pucks varying from 2.00×10^3 to 2.93×10^3 CFU/g.O.D. sample.

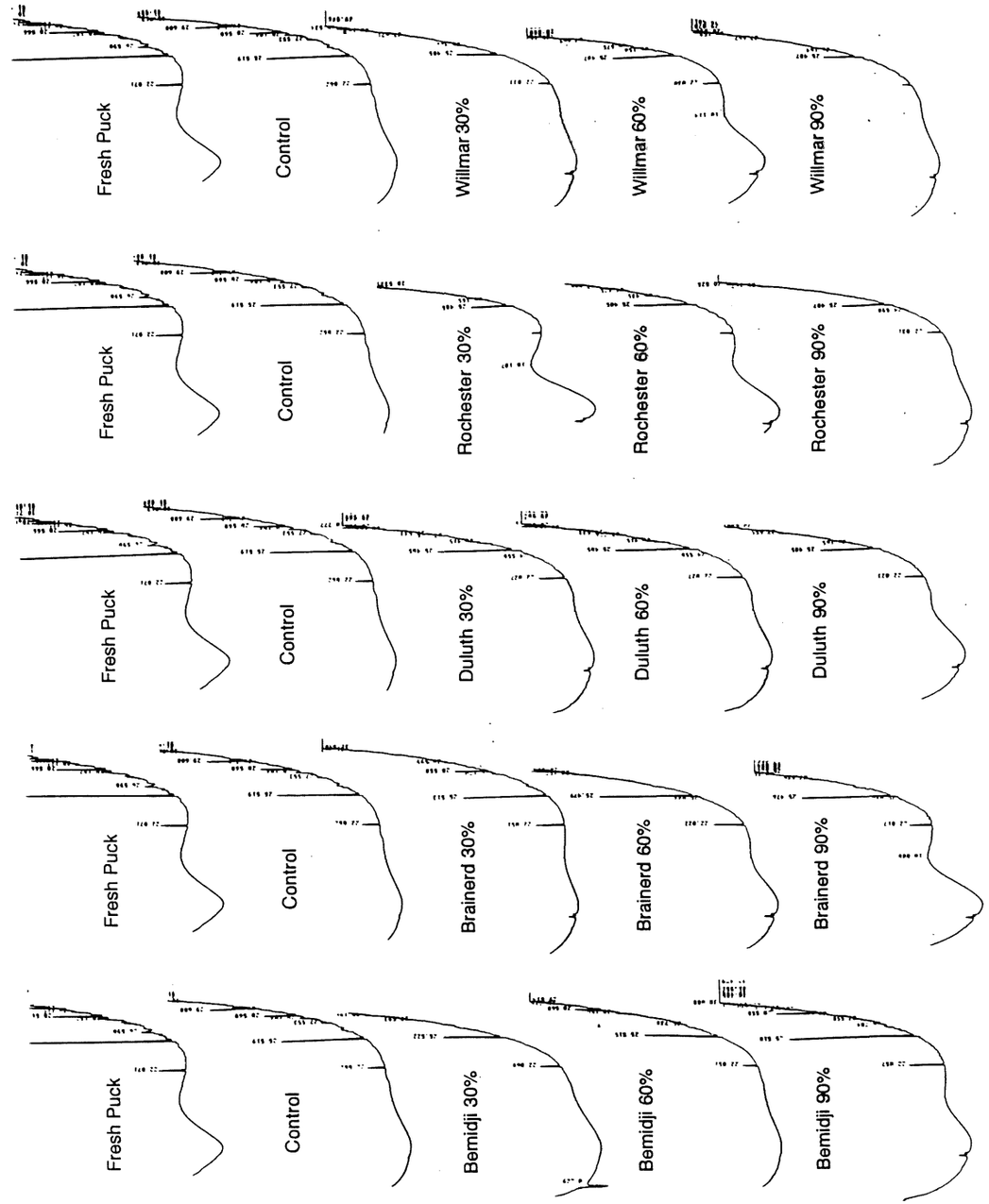


Fig. 36. Chromatograms of GC analysis of Marshall Pucks after buried in soils, collected from five District Labs, or left in containers for control, for twelve months at three different water holding capacities and compared with fresh puck extract.

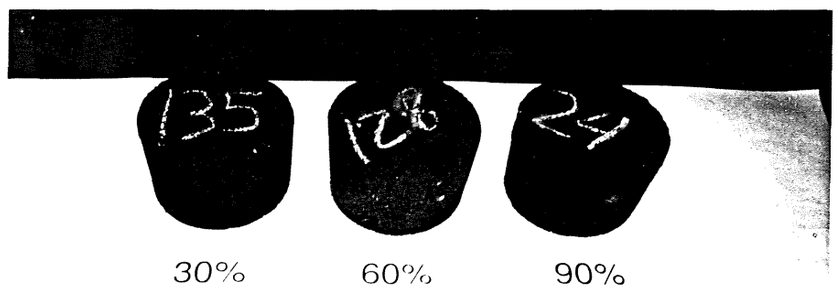


Fig. 37 Molds and bacteria adhering to the surface of the Marshall pucks after left in control containers for 12 months that were maintained at various humidity (30%, 60%, and 90%).

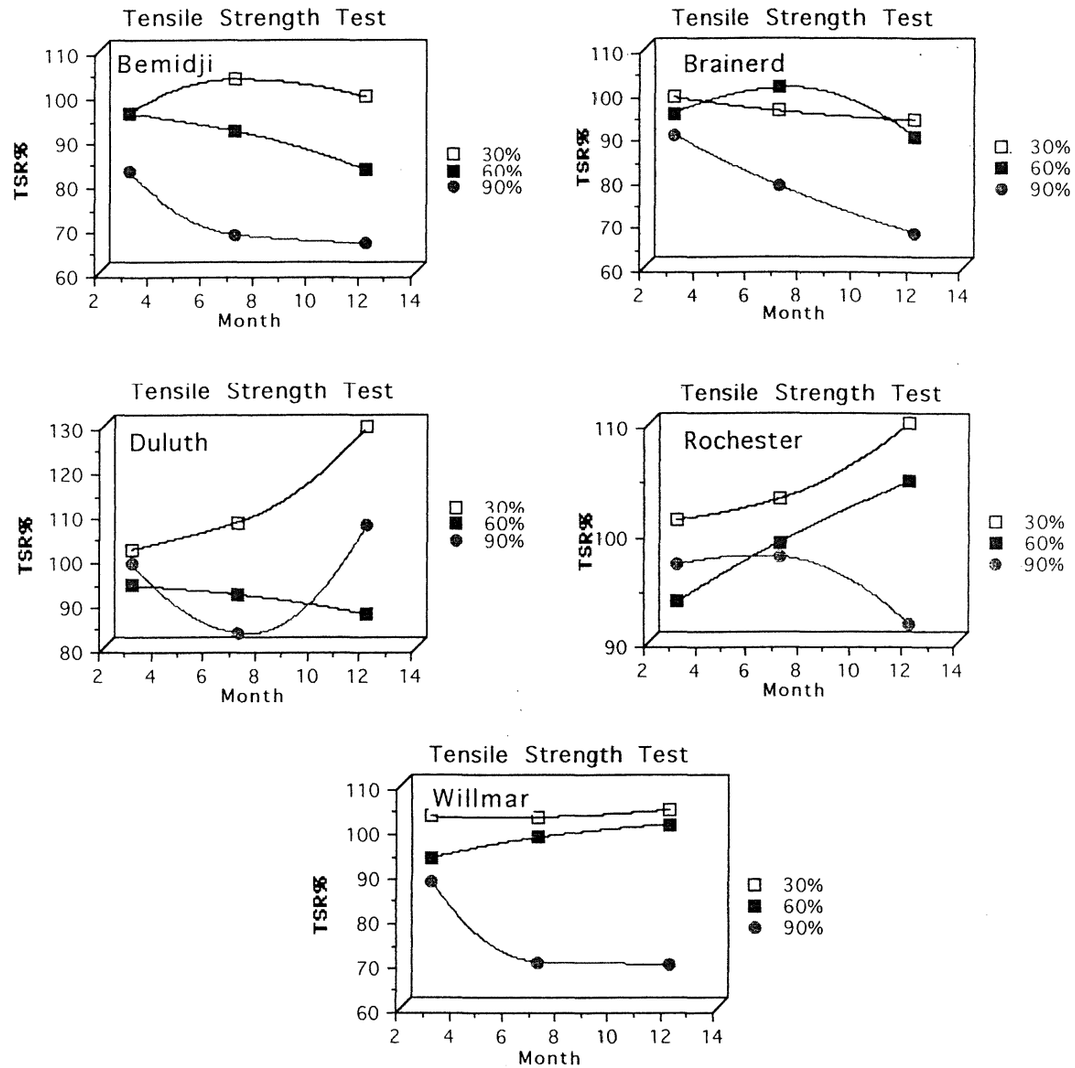


Fig. 38 Tensile strength test of Marshall pucks buried in soils collected from five Minnesota Districts for 3, 7, and 12 months with different water holding capacities. Control of TSR% was 100%.

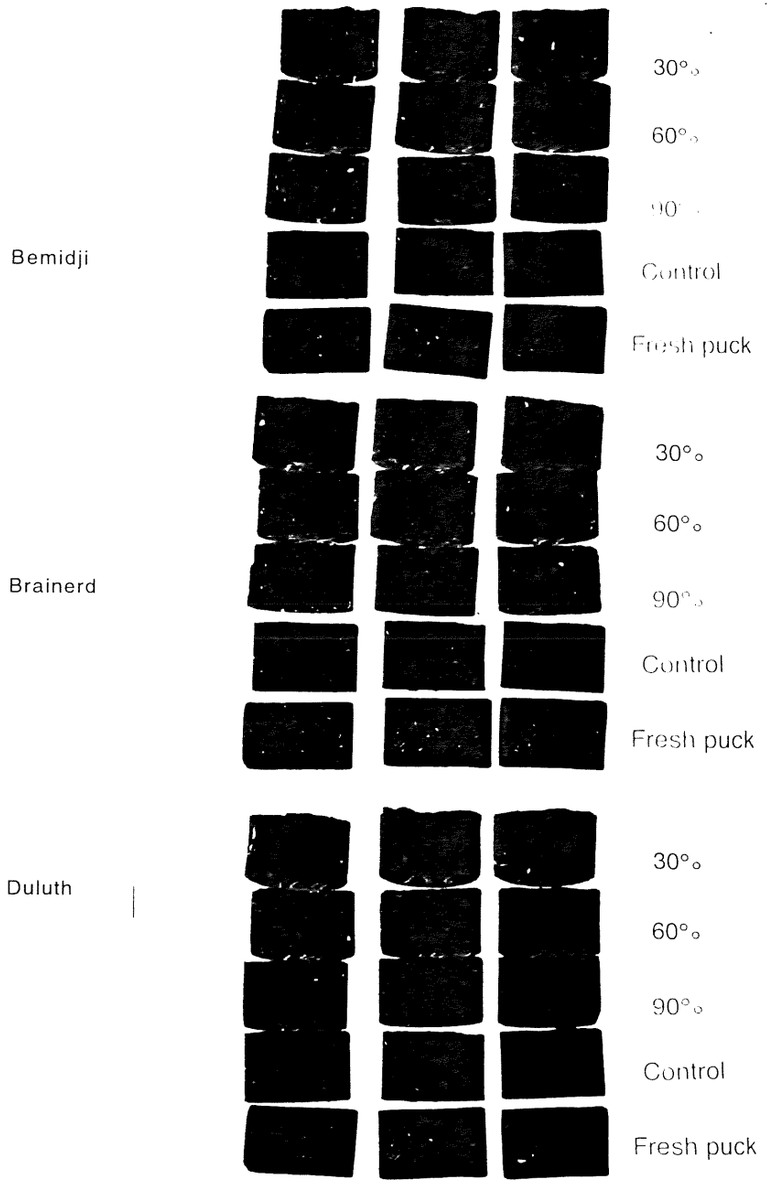


Fig. 39 Broken aggregate shown in cracked Marshall pucks and white aggregate showing on edge of the broken surface after pucks had been buried in soils, collected from Bemidji (top), Brainerd (middle), and Duluth (bottom) Districts, for twelve months with different water holding capacities, compared with control samples and fresh puck.

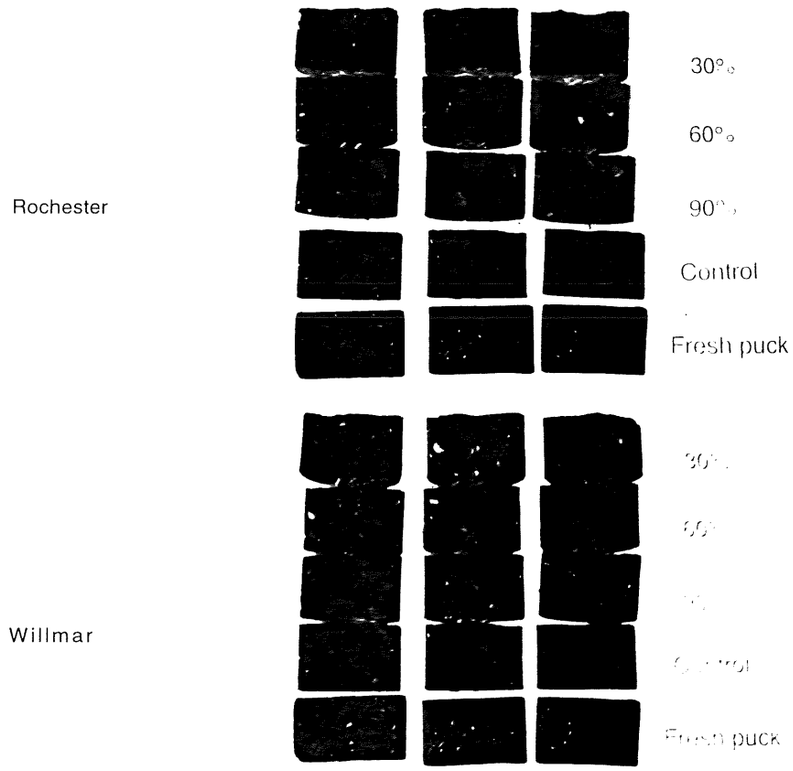


Fig. 40 Broken aggregate shown in cracked Marshall pucks and white aggregate showing on edge of the broken surface after had been buried in soils, collected from Rochester (top) and Willmar (bottom) Districts, for twelve months with different water holding capacities, compared with control samples and fresh puck.

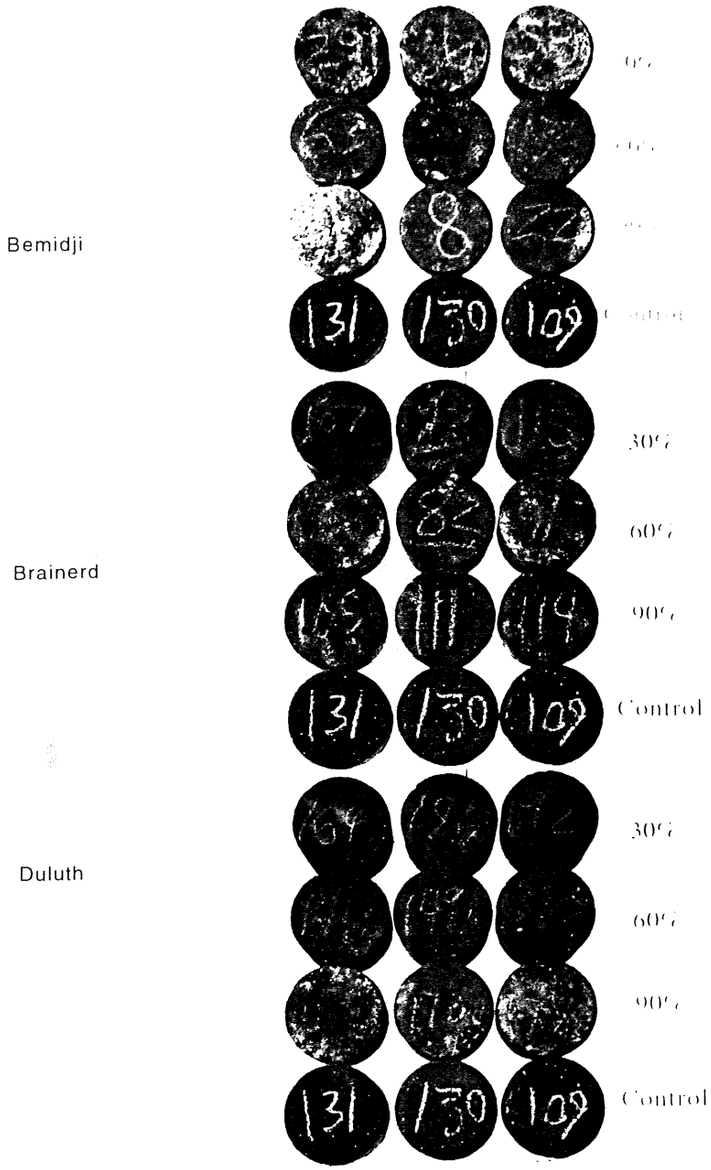


Fig. 41 Appearance of soil microbial attachment on Marshall pucks after had been buried in soils, collected from Bemidji (top), Brainerd (middle), and Duluth (bottom) Districts, for twelve months at three different water holding capacities.

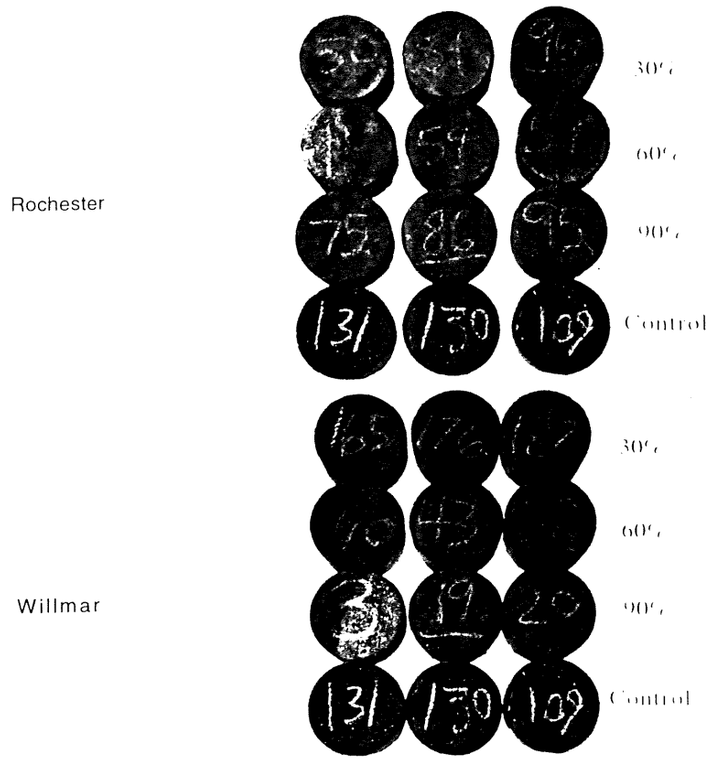


Fig. 42 Appearance of soil microbial attachment on Marshall pucks after had been buried in soils, collected from Rochester (top) and Willmar (bottom) Districts, for twelve months at three different water holding capacities.

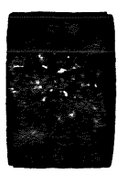


Fig. 43 The crack formation on the Marshall puck buried in Bemidji soil for 12 months with 90% water holding capacity.

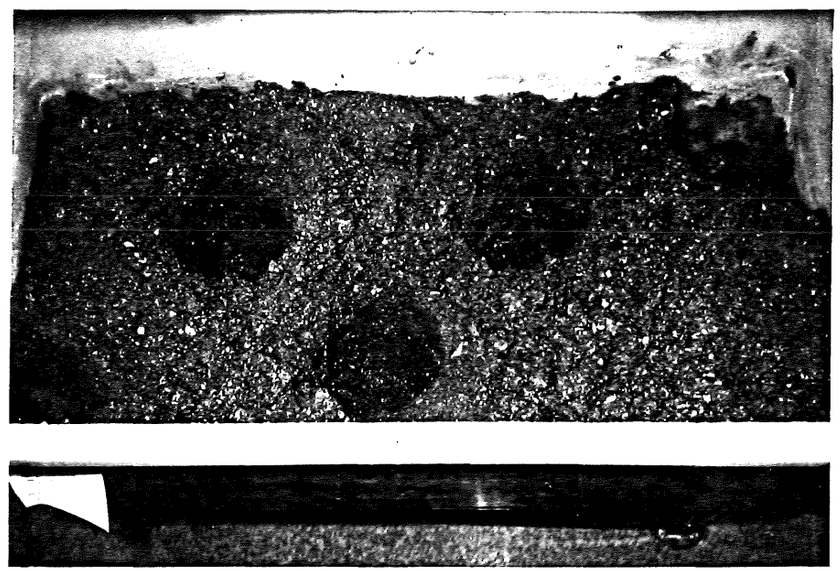


Fig. 44 Soil sampling locations from tanks of Marshall pucks buried - in - soil test.

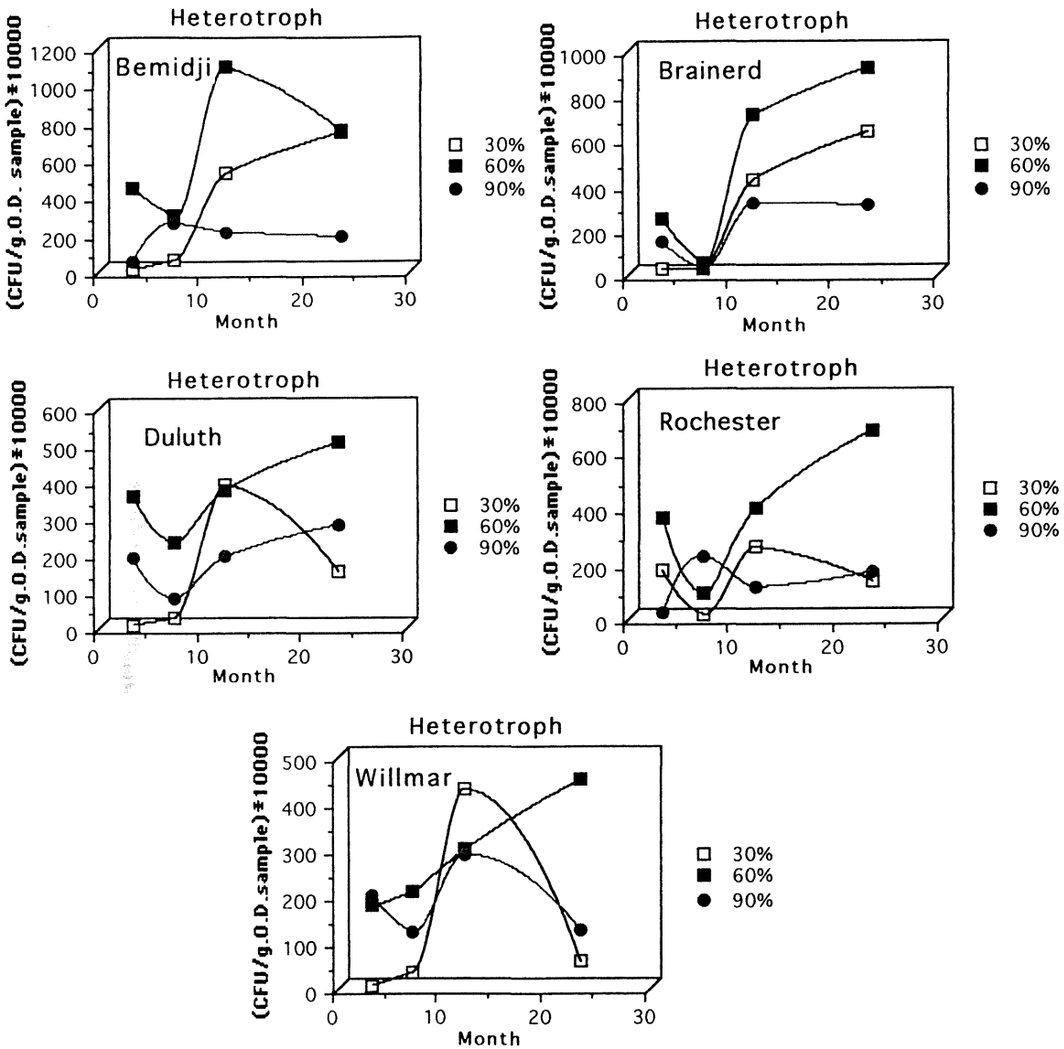


Fig. 45 Viability testing curve for heterotrophs in Marshall pucks after buried for 3, 7, 12 and 23 months in soils collected from five District Labs with different water holding capacities. The number of heterotrophs in control puck varying from 90 to 120 CFU/g.O.D. sample.

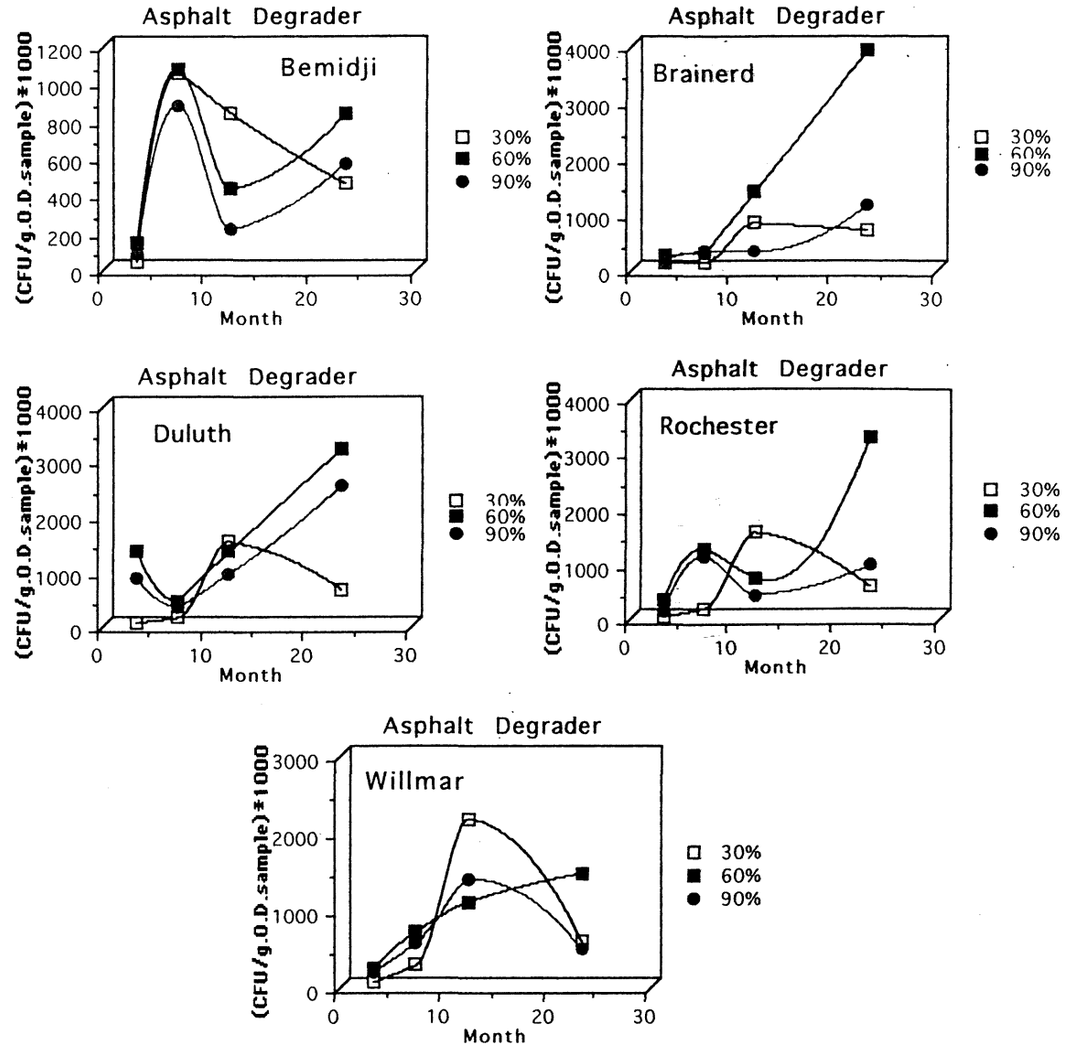


Fig. 46 Viability testing curve for asphalt degraders in Marshall pucks after buried for 3, 7, 12 and 23 months in soils collected from five District labs with different water holding capacities. The number of asphalt degraders in control pucks varying from 4,000 to 7,000 CFU/g.O.D. sample.

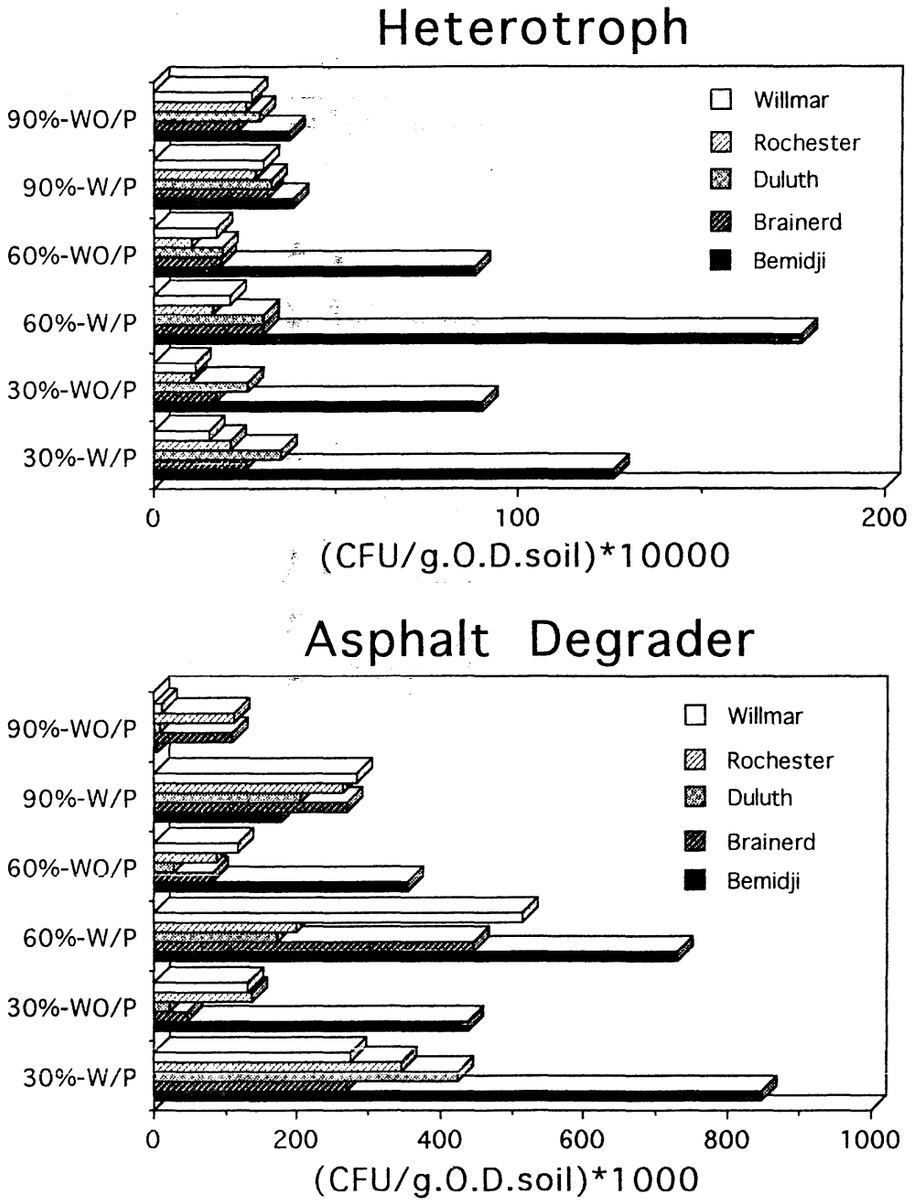


Fig. 47 Comparative numbers of heterotrophs and asphalt degraders in soils adjacent to the buried Marshall pucks with an undistured soil after Marshall pucks had been buried in soils for 23 months with different water hol[illegible]pacities.

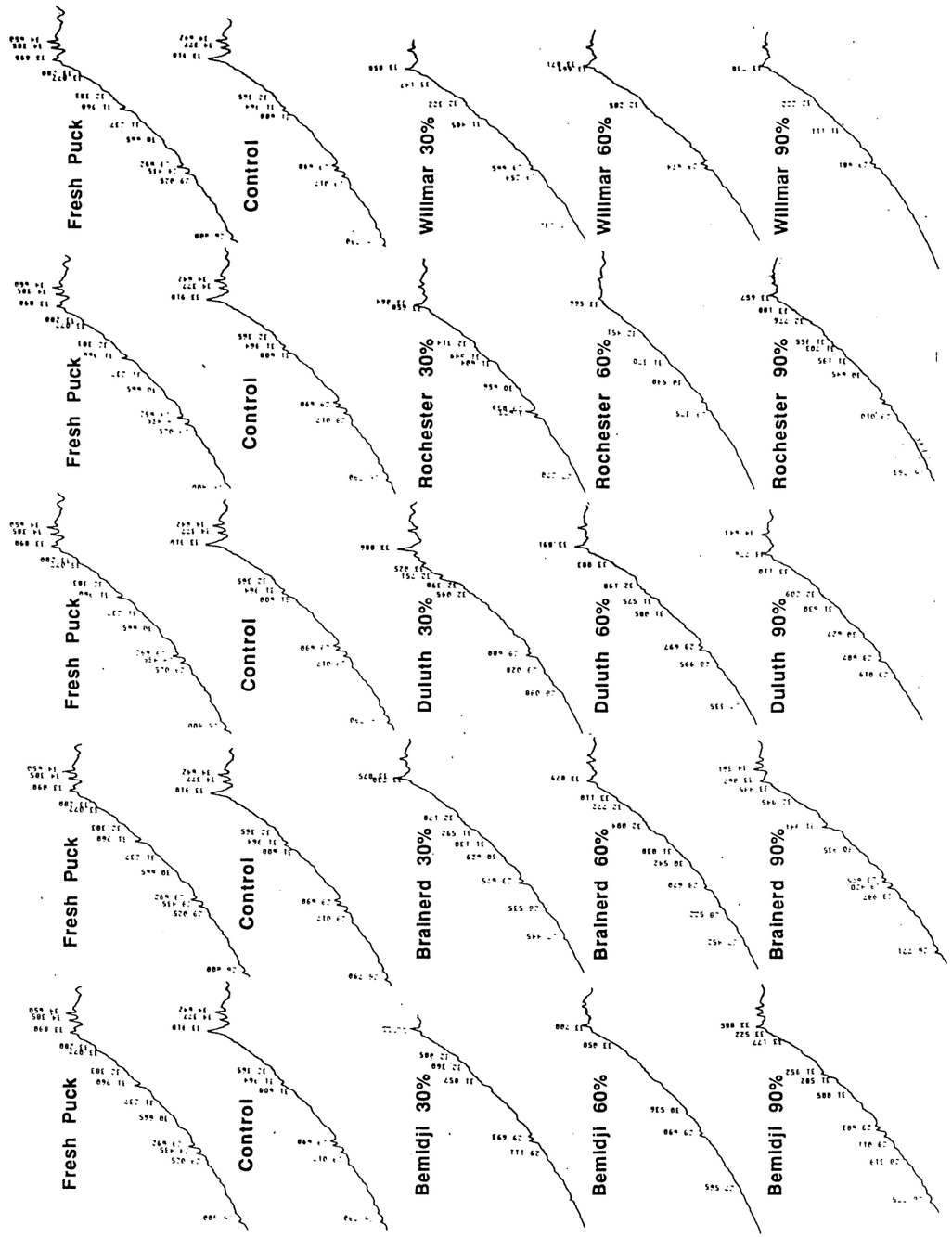


Fig. 48 Chromatograms of GC analysis of Marshall Pucks after buried in soils, collected from five District Labs, or left in containers for control, for 23 months at three different water holding capacities and compared with fresh puck extract.

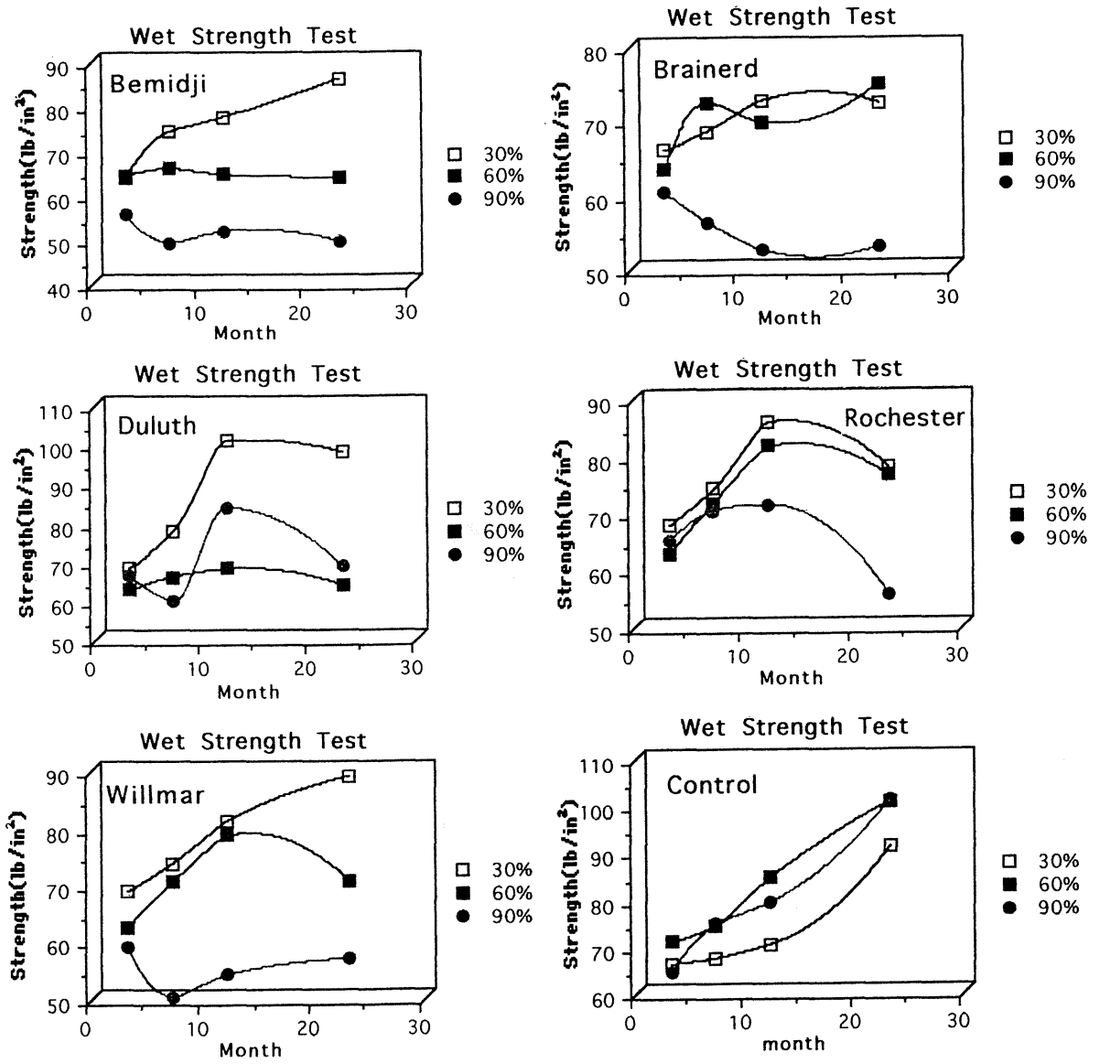


Fig. 49 Physical strength test of Marshall pucks buried in soils, collected from five Minnesota Districts, for 3, 7, 12 and 23 months with different water holding capacities.

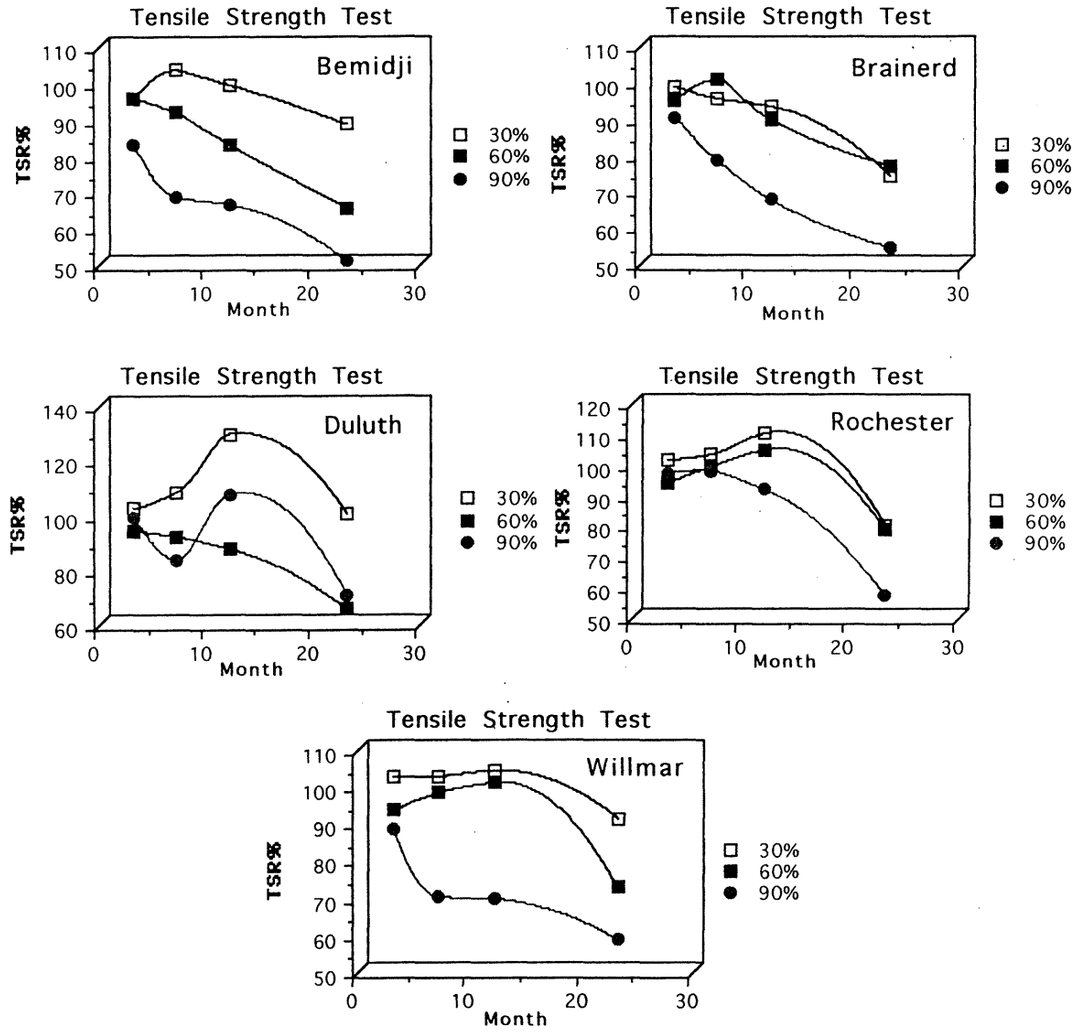


Fig. 50 Tensile strength of Marshall pucks buried in soils, collected from five Minnesota Districts, for 3, 7, 12 and 23 months with different water holding capacities. Control of TSR% was 100%.

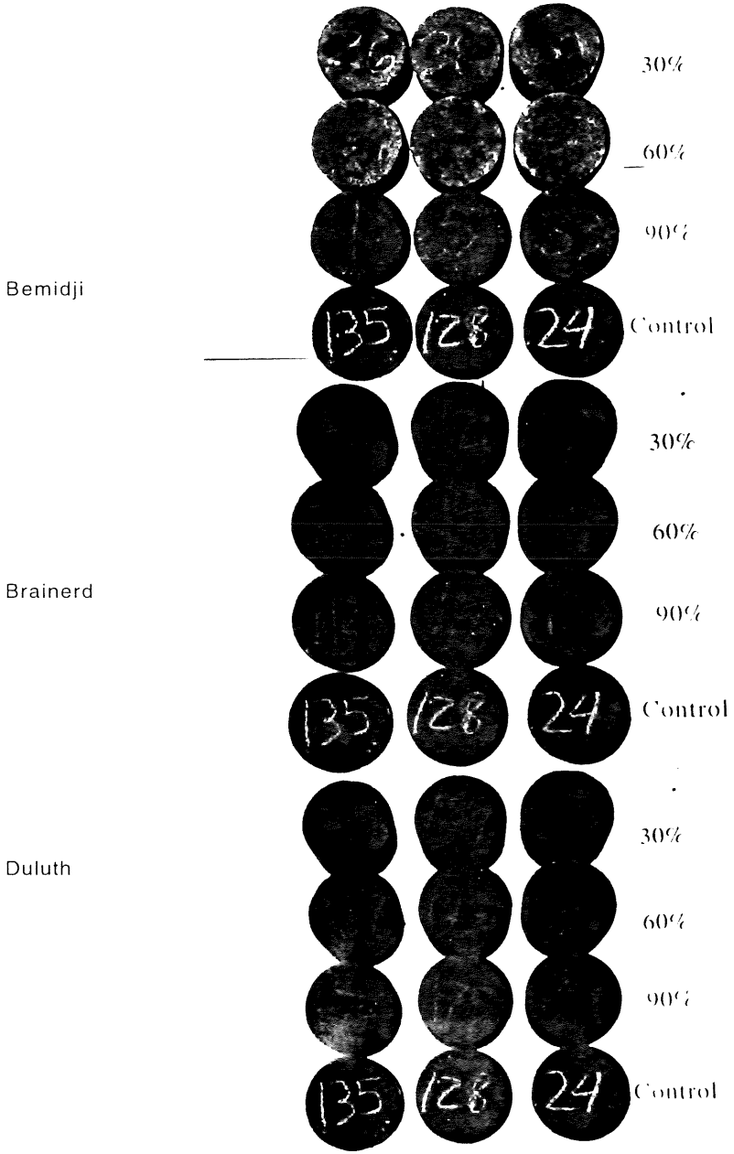


Fig. 51 Appearance of soil microbial attachment on Marshall pucks after had been buried in soils for 23 months at three different water holding capacities.

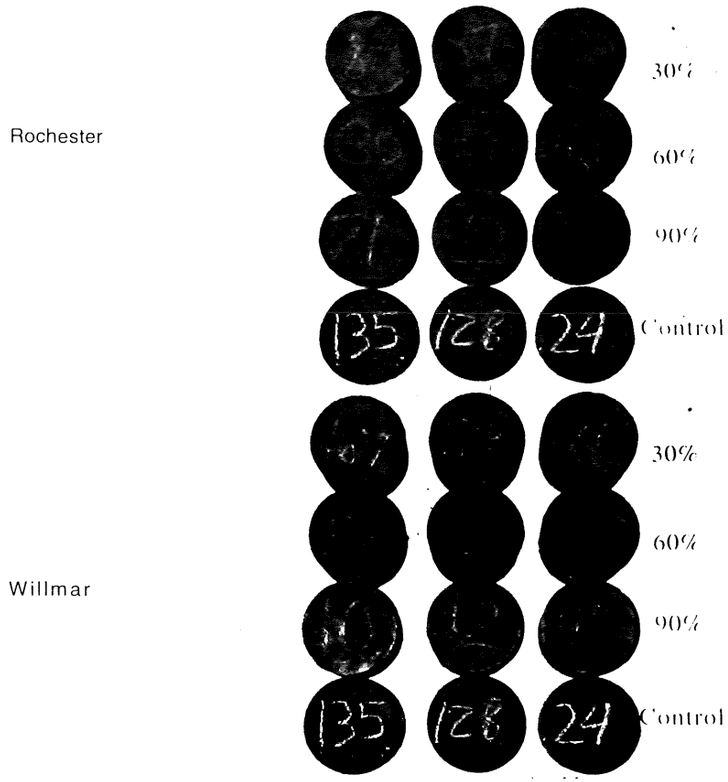


Fig. 52 Appearance of soil microbial attachment on Marshall pucks after had been buried in soils for 23 months at three different water holding capacities.

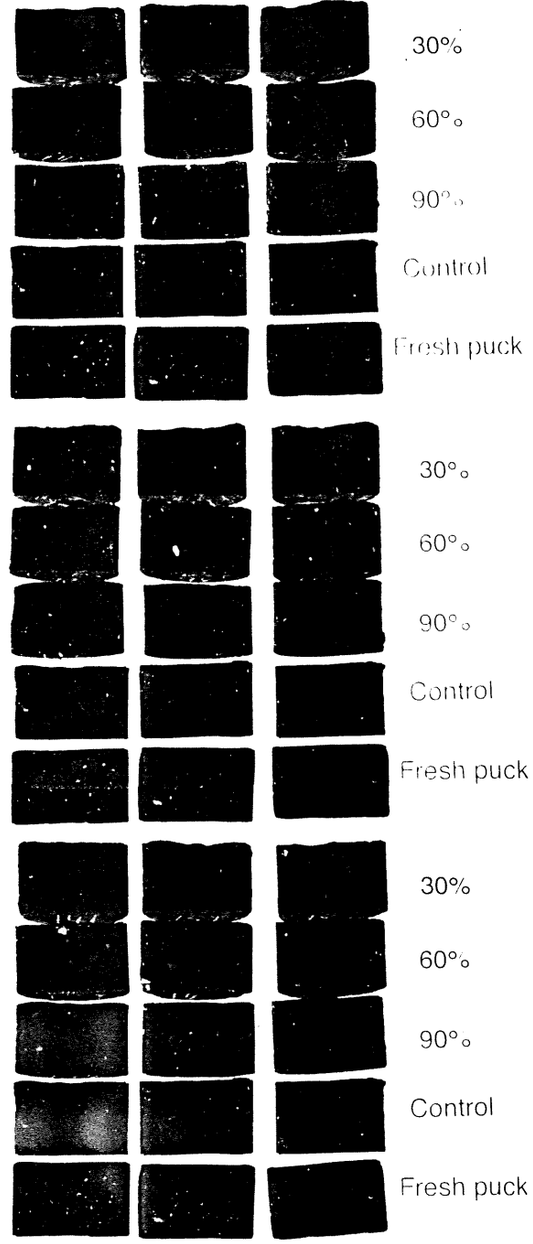


Fig. 53 Broken aggregate and visual stripping shown in cracked Marshall pucks surface after pucks had been buried in soil for 23 months with different water holding capacities. Compared with control samples and fresh puck.

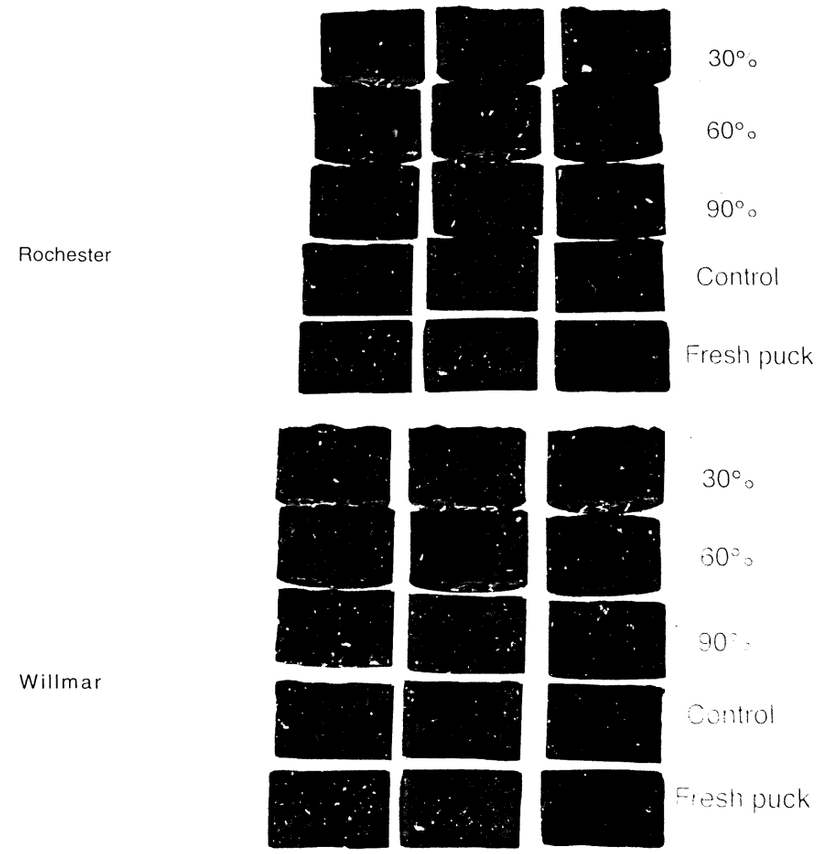


Fig. 54 Broken aggregate and visual stripping shown in cracked Marshall pucks surface after had been buried in soils for 23 months with different water holding capacities. Compared with control samples and fresh puck.

Table 1. Locations and description of collected asphalt and soil samples

Samples No.	Locations	Description
1	Mankato District TH 15. South bound lane 6' right of center line M.P. 31.3. middle of panel sta 1735±	Non Broken Top.
2	Mankato District TH 15. South bound lane 6' right of center line M.P. 31.5. next to joint sta 1745±	Broken Top.
3	Mankato District TH 15. South bound lane 6' right of center line M.P. 31.5. middle of panel sta 1745±	Non Broken Top.
4	Mankato District TH 15. South bound lane 6' right of center line M.P. 31.3. next to joint sta 1735±	Broken Top.
5	Mankato District TH 15. South bound lane 6' right of center line M.P. 31.5. next of joint sta 1735± in place embankment	Broken Top (soil).
6	Mankato District TH 15. South bound lane 6' right of center line M.P. 31.3. middle of panel sta 1735± in place embankment	Non Broken Top (soil).
7	Mankato District TH 15. South bound lane 6' right of center line M.P. 31.5. middle of panel sta 1745 ± in place embankment	Non Broken Top (soil)
8	Mankato District TH 15. South bound lane 6' right of center line M.P. 31.3. next to joint sta 1735±	Broken Top. (soil).
9	Bemidji District TH 71. Tenstrike M.P. 329 + 1710' 7.5' left of center line 0.0-1.0' (approximate) Bitumen in patch area	Broken Top.
10	Bemidji District TH 71. Tenstrike M.P. 329 ± 2710' 7.5' left of center line in patch area grading material 1.0 - 2.0	Broken Top (soil).
11	Bemidji District TH 71. Tenstrike M.P. 329+2143' 7.5' left of center line Grading material (soil). 1.0-2.0'	Non Broken Top (soil).
12	Bemidji District TH 71. Tenstrike M.P. 329+2143' 0.0-1.0' (approximate) Bitumen outside of patch area	Non Broken Top.
13	Detroit Lakes District TH #10. West bound Right lane M.P. 85-between wheel path solid area-15' from #14 1. Bitumen 2. Soil	Non Broken Top.
14	Detroit Lakes District TH #10. West bound Right lane M.P. 85-between wheel path taken over transverse crack 1. Bitumen 2. Soil	Broken Top.
15	Willmar District TH 23. By pass by crack M.P. 144.	Broken Top

16	Willmar District TH 23 M.P. 144. Bitumen, by crack	Broken Top (soil).
17	Willmar District TH 23 By pass M.P. 144. 4 of soil	Non Broken Top (soil).
18	Willmar District TH 23. By pass good area Bitumen M.P. 144.	Non Broken Top.
19	Willmar District TH 23. M.P. 144. Between crack	Broken Top.
20	Rochester District TH 60. CS 2511 East bound R.P. 165.626 6' right of INP. center line Aggregate base Goodhue county Rochester Field 1.0	Broken Top (soil).
21	Rochester District TH 60. CS 2511 East bound lane R.P. 165.626. 6' right of INP center line Bitumen	Broken Top.
22	Rochester District TH 60. CS 2511 East bound lane. R.P. 165.624 6' right of INP center line Bitumen	Non Broken Top.
23	Rochester District TH 60. CS 2511. East bound lane. R.P. 165.635. 5' right of INP center line Aggregate. base	Broken Top (soil)
24	Rochester District TH 60. CS 2511 East bound lane R.P. 165.624 6' right of INP center line. Aggregate. base	Non Broken Top (soil).
25	Rochester District TH 60. CS 2511 East bound lane R.P. 165.634 5' right of INP center line. Bitumen. Transverse crack	Broken Top
26	Rochester District TH 60. CS 2511 East bound lane R.P. 165.634 5' right of INP center line. Bitumen Transverse Crack	Broken Top.
27	Willmar District East bound internal traffic lane (Station 650 + 00) on CR 15.	Broken Top

Table 2. The chemical-physical properties of asphalt pavement and soil sample underneath the asphalt pavement

Sample No.			PH	Total Alkalinity mg $CaCO_3$ /L	Total hydrocarbon (%) g.hydrocarbon/ 100 g.O.D.sample	Total N ngNH_3-N/ g.O.D. sample	NH_3^-	NO_2^- PPM	NO_3^-	Total P ngPO_4^-P/ g.O.D. sample
1	Mankato	N	10.1	105.92±0	9.07±0	400±51.2	0	0	0	40.59±3.18
2	Mankato	B	9.8	101.31±0.28	6.71±0.04	400±28.3	0	0	0	26.62±0.67
3	Mankato	N	10.1	151.96±0.25	8.45±0.38	500±23.5	<1	0	<1	25.28±1.41
4	Mankato	B	9.0	89.79±0.11	7.33±0.86	500±28.1	0	0	0	18.70±0.27
5	Mankato	BS	8.4	75.98±0.05	0.58±0.15	600±39.2	<1.2	0.1	0	19.65±0.28
6	Mankato	NS	7.8	64.47±0.04	0.33±0	1100±19.2	<1.2	0.1	0	31.71±4.06
7	Mankato	NS	7.8	57.56±0.02	0.05±0.04	600±23.4	<1	<0.1	0	5.43±0.09
8	Mankato	BS	8.2	55.26±0.05	0.63±0.5	1300±27.6	<1.2	0.1	<1	6.59±1.73
9	Bemidji	B	8.6	62.16±0.16	6.71±0.35	400±10.7	0	0	0	6.76±1.69
10	Bemidji	BS	8.6	73.68±0.16	1.35±0.17	200±10.7	<1	0	0	18.61±1.78
1	emidji	NS	9.0	73.68±0.12	2.7±0.8	200±14.3	<1	0	0	19.44±3.57
12	Bemidji	N	8.2	62.16±0.04	8.03±0.64	300±21.7	0	0	0	17.12±3.05
13_1	Det. Lakes	N	7.7	46.05±0.14	6.14±0.43	400±25.6	0	0	0	2.69±0.38
13_2	Det. Lakes	NS	7.6	55.26 ±0.04	0.88± 0.15	100±2.27	0	0	0	23.81±0.07
14_1	Det. Lakes	B	8.2	46.05±0.02	3.22±0.39	400±39.2	0	0	0	17.82±1.55
14_2	Det. Lakes	BS	8.5	43.74±0.02	4.80±0.17	100±3.62	0	0	0	28.15±6.21

Sample No.			PH	Total Alkalinity mg $CaCO_3$ /L	Total hydrocarbon (%) g.hydrocarbon/ 100 g.O.D.sample	Total N ngNH_3-N/ g.O.D. sample	NH_3^-	NO_2^- PPM	NO_3^-	Total P ngPO_4^-P/ g.O.D. sample
15	Willmar	B	8.4	46.05±0.02	6.14±0.11	400±27.3	0	0	0	13.61±1.43
1	Willmar	BS	8.3	43.74±0.02	6.70±1.88	400±9.16	0	0	0	18.63±0.26
18	Willmar	N	8.1	43.74±0.03	6.22±0.13	400±2.58	0	0	0	46.07±7.19
19	Willmar	B	8.2	36.84±0.03	6.31±0.02	400±3.03	0	0	0	26.07±2.18
20	Rochester	BS	7.8	46.05±0.02	0.19±0.10	200±16.7	0	0	0	15.55±0.05
21	Rochester	B	8.2	41.44±0	7.08±0.14	400±1.74	0	0	0	15.85±1.30
22	Rochester	N	7.9	41.44±0	5.53±0.26	400±28.5	0	0	0	10.36±3.75
23	Rochester	BS	8.2	46.05± 0.05	2.80±0:49	400±2.09	0	0	0	27.37±6.28
24	Rochester	NS	8.0	55.26±0.04	0.91±0.31	200±8.62	0	0	0	12.68±0.35
25	Rochester	B	8.0	46.05±0	5.19±0.08	500±2.02	0	0	0	13.93±1.66
26	Rochester	B	8.2	46.05±0.14	5.60±0.09	400±23.1	0	0	0	22.39±3.05
27	Willmar	B	10.1	82.84±0	8.84±0.41	500±24.2	0	0	0	9.91±0.07
0	Fresh pavement (control)		8.0	36.84±0	8.17±0.46	500±5.34	0	0	0	16.87±3.33

Table 3. Comparable microbial enumeration of asphalt pavement and soil sample underneath the asphalt pavement.

Sample No.			Heterotrophs		Asphalt degraders	
			MPN (CFU/ml)*	Plate Count (CFU/g.O.D. sample)**x10^5	MPN (CFU/ml)	Plate Count (CFU/g.O D. sample) x10^4
1	Mankato	N	1,500	0.35 ± 0.03	90	1.06±0.08
4	Mankato	B	25,000	1.12±0.09	250	87.3±6.66
3	Mankato	N	400	0.19±0.05	90	4.79±0.81
2	Mankato	B	25,000	0.92±0.10	250	8.06±0.22
7	Mankato	NS	150,000	151±16.8	250	642±90.3
5	Mankato	BS	1400,000	414±45.4	250	977±77.0
6	Mankato	NS	95,000	220±61.4	250	696±62.0
8	Mankato	BS	450,000	351±31.6	250	897±121
12	Bemidji	N	4,500	123±19.3	90	120±15.7
9	Bemidji	B	140,000	187±20.5	250	191±15.8
11	Bemidji	NS	110,000	52.7±3.29	950	54.6±3.16
10	Bemidji	BS	45,000	11.6±1.50	2500	110.5±7.37
13_1	Det. Lakes	N	2,500	0. 49±0.10	40	26.2±9.61
14_1	Det. Lakes	B	110,000	10.8±0.79	250	253±18.4
13_2	Det. Lakes	NS	45,000	10.6±0.40	450	23.8±3.75
14_2	Det. Lakes	BS	25,000	7.52±1.69	90	7.17±1.33
18	Willmar	N	9,500	4.79±0.40	90	22.1±1.22
19	Willmar	B	20,000	79.8±7.56	250	123.0±22.5
22	Rochester	N	25,000	63.2±0.45	250	72.6±3.74
21	Rochester	B	4,500	1.50±0.26	250	92.0±8.65
24	Rochester	NS	2,500	3.51±0.02	250	13.0±1.12
23	Rochester	BS	45,000	5.84±1.52	950	642±45.1

* CFU/ml: colony forming unit per ml
** CFU/g.O.D. sample: colony forming unit per grams sample of oven dry weight.

Table 4. Plate Count and most probably number of heterotrophs and asphalt-degraders in Marshall puck samples post 1.5 and 4.5 months treatments.

		Heterotrophs		Asphalt degraders	
Treatment	Treatment Period (months)	MPN (CFU/ml)*	Plate Count (CFU/g.O.D. sample)**x10^5	MPN (CFU/ml)	Plate Count (CFU/g.O.D. sample) x 10^4
Control	1.5	119	0.0468±0.002	1.5	0.0007±0
	4.5				
Mixed Culture treated	1.5	132,000	623±36.9	883	775±12.8
	4.5	32,600	77.3±44.5	107	19.7±8.4
lake water treated	1.5	35,000	54.8±23.3	100	41.5±8.21
	4.5	18,500	18.2±1.80	67	4.37±3.81
Autoclaved lake water treated	1.5	12,300	39.6±0.35	37	29.7±7.89
	4.5	9,400	22.4±16.0	8.3	1.5±0.5

*CFU/ml: colony forming unit per ml
**CFU/g.O.D. sample: colony forming unit per gram of oven dry weight

Table 5. Locations and description of soils and asphalt samples.

Sampling District	Date of collection	Locations	Description of core	Description of soils
Bemidji	11/20/95	TH 71	323.5 35' RT of E 323.63 40' LT of E 327.7 50' RT of E 329.36 45' LT of E	sandy Loam/ clay Loam/clay (all probably silty)
Brainerd	11/29/95	TH 6 R.P. 77	shoulder edge of bit mat stripping: 2-2.5 in. from bottom	granular base plastic base
Duluth	11/22/95	TH 73 R.P. 24.447	4.5 M W of E shoulder edge of mat stripping: 3-3.5 in. from bottom	S-1: Aggregate base/granular S-2: Native soil= Fine sandy Loam, SLPL.
Willmar	11/3/95	TH 19/15 585+00 LT	Bit core (s) stripping	Two types: 1. Gravel + Granular (sand) 2. 6 in. gravel base on granular
Rochester	11/3/95	TH 56 CS 2006	12'-18' RT of E Bit core (s) stripping: 3.5-4 in. from bottom	shoulder subgrade soil sample taken from 0.5'-1.5'

Table 6. Chemical-physical properties of asphalt pavement and soil sample underneath the asphalt pavement

..pling District	PH	Granulation size (in.) 1"	3/4	5/8	1/2	3/8	4	10	40	100	200	silt	clay	Type	Total Alkalinity (mg $CaCO_3$/L)	Total hydrocarbon (%) g.hydrocarbon/ 100 g.O.D. sample	Total N ng NH4-N/ g.O.D. sample	NH_4^+	NO_2^- ppM	NO_3^-	Total P ng PO_4-P/ g.O.D. sample
soils																					
Bemidji	8.6	100	100	100	100	99	97.3	95.2	89.3	74.9	62.9	41.2	21.7	CL*	70.56	0.24	152.51±19.5	1.0	0	0	165.73±9.45
Brainerd	8.2	100	100	100	100	99.1	98.4	96.5	83.7	56.0	47.9	27.2	20.2	SaCL**	60.48	0.21	85.36±13.38	0	0	0	91.78±9.25
Duluth	7.7	100	99.3	96.5	95.4	90.5	82.3	74.0	52.7	26.6	15.7			CLSa	20.16	0.21	324.8±53.1	0	0	0	187.60±10.8
Willmar	8.8	100	99.6	99.5	98.8	97.3	92.2	79.0	43.8	15.8	10.7			Fine Gravel	60.48	0.22	63.13±14.4	0	0	0	49.72±8.15
Rochester	7.8	100	100	100	100	97.9	96.1	92.9	78.2	58.2	50.1	32.1	18.0	L	75.60	0.24	370.07±34.1	1.2	0	0	199.28±11.8
asphalt																					
Bemidji (s)	8.7														20.16	4.81	241.93±60.4	0	0	0	49.21±7.54
Brainerd (s)	7.9														25.20	5.25	462.82±11.4	0.1	0	0	325.31±3.54
Duluth (s)	8.0														20.16	5.60	432.76±21.5	0.1	0	0	162.39±11.92
Willmar (s)	9.2														40.36	6.13	398.6±35.2	0	0	0	22.73±1.95
Rochester(s)	8.3														45.36	6.46	675.38±77.2	0.1	0	0	207.19±6.95
...ster(NS)	8.4														65.52	7.46	441.73±10.7	0.1	0	0	49.16±5.11

* CL: Coarse Loam
**SaCL: Sandy Coarse Loam

Table 7. Microbial enumeration of asphalt pavement and soil samples underneath the asphalt pavement

District of Sample	Heterotrophs MPN (CFU/ml)*	Heterotrophs Plate Count (CFU/g.O.D. sample)** x 10^4	Aspahlt Degraders MPN (CFU/ml)	Aspahlt Degraders Plate Count (CFU/g.O.D. sample) x 10^3
soils				
Bemidji	1,400,000	48.07±4.17	250,000	18.64±3.25
Brainerd	95,000	7.86±0.83	25,000	1.05±0.17
Duluth	250,000	16.30±5.01	45,000	7.16±1.06
Willmar	95,000	11.99±2.79	25,000	4.05±0.80
Rochester	75,000	3.30±0.40	25,000	2.77±0.39
Asphalt				
Bemidji (s)	7,500	4.76±0.62	450	112.68±6.24
Brainerd (s)	4,500	1.88±0.28	90	2.79±0.12
Duluth (s)	4,500	0.25±0.01	25	3.53±0.21
Willmar (s)	2,500	1.66±0.28	250	29.45±3.27
Rochester (s)	4,500	2.92±0.42	250	65.82±1.03
Rochester (Ns)	900	1.10±0.07	40	4.15±0.43

*CFU/ml: colony forming unit per ml
**CFU/g.O.D. sample: colony forming unit per gram of oven dry weight

Table 8. Trial Mix Verification Worksheet.

%AC 5.5						
			MN/DOT		Contractor	
Marshall ID	A	B	C	D	E	F
Height	2.62	2.59	2.62	2.62	2.61	2.61
Dry Weight	1202.6	1200.5	1202.0	1204.5	1201.9	1203.4
SSD Weight	1206.1	1203.2	1205.7	1208.1	1204.8	1207.0
Imm. Weight	670.6	672.6	666.4	669.9	667.9	671.9
Volume	535.5	530.6	539.3	538.2	536.9	535.1
Bulk Sp. G.	2.246	2.263	2.229	2.238	2.239	2.249
Ave Blk Sp G		2.246			2.242	
DENSITY		139.9			139.7	
Flow	4	5	5	6	6	6
Ave. Flow			4.67			6.00
Stab. Rdg.	42	50	40	49	46	50
Ave. Cor. Stab. Rdg.	297	359		348	326	355
Ave. Stability			313			343
Cont. ID	A	B				
Cont.+SA Dry	2031.4	2029.3				
Cont. Dry						
Sa Dry	2031.4	2029.3				
Cont.+Sa Imm.	1393.5	1395.6				
Cont. Imm.	189.2	192.8				
Sa Imm.	1204.3	1202.8				
Sa Volume	827.1	826.5				
Max. Sp. G.	2.456	2.455				
Ave Max Sp G			2.456			*
Rice Voids			8.55			8.7075

* MN/DOT's Ave Max Sp G used.

Remarks: 12 F[illegible]

Table 9. Test Report on Aggregates
Bituminous-Quality

	% COMPONENT USED				TOTAL	SPEC
	31	25	13	31	100	CONT/REQD
BA#	BA128	BA129	BA130	BA120		FIELD 3139 RESULT
Sieves	Bin1	Bin 2	Bin 3	Bin 4	COMP.	
1						
3/4	100.0	100.0	100.0	100.0	100.0	
5/8	99.0	98.0	86.0	98.0	96.8	
1/2	99.0	94.0	48.0	93.0	89.3	
3/8	95.0	88.0	19.0	88.0	81.2	
# 4	83.0	79.0	2.2	87.0	72.7	
#10	71.0	69.0		66.0	59.7	
#20						
#40	36.0	40.0		21.0	27.7	
#80						
#100						
#200	5.0	10.1		2.3	4.8	
% Passing 1"	100.00	100.00	100.00	100.00		
% Passing 1/2"	99.00	94.00	48.00	93.00		
% Passing #4	83.00	79.00	2.20	87.00		
Wt Sample -#4	270.00	263.10	100.00	100.00		
Wt of Shale -#4	0.54	0.39	0.00	0.00		
Wt Shale + 1/2"	0.00	0.00	0.00	0.00		
Wt Shale Ret #4	0.42	0.00	0.00	0.00		
Wt Spall + 1/2" *	0.00	0.00	3.60	0.00		
Wt Spall Ret #4 *	1.32	0.81	0.00	0.00		
Wt Other + 1/2"	100.00	100.00	945.60	0.00		
Wt Other Rock +#4	477.70	441.20	100.00	0.00		
					COMP.	
% Shale -#4	0.20	0.15	0.00	0.00	0.11	
% Shale Ret #4	0.08	0.00	0.00		0.02	
% Total Shale	0.18	0.12	0.00	0.00	0.09	
% Spall Ret #4	0.34	0.13	0.20		0.19	
% Total Spall	0.22	0.14	0.20	0.00	0.13	
% Other Rock	99.66	99.87	99.80	100.00	99.81	

* DOES NOT INCLUDE SHALE

% CRUSHING (PARTICLE COUNT)

Wt Sample +1/2"	
Wt Crush +1/2"	
Wt Sample 1/2"-#4	
Wt Crush 1/2"-#4	
% Crushed Part.	
TOTAL CRUSHED +4 MATERIAL:	0

Table 10. Tensile strength testing of submersed Marshall puck in different treatements

Type of treatments	Treatment period (months)	Specimens no.		Diameter in.		Thickness in.		Dial Reading		Load		Dry strength lb/in2		Wet strength lb/in2		TSR %		Visual stripping %		Broken aggregate %	
		1.5	4.5	1.5	4.5	1.5	4.5	1.5	4.5	1.5	4.5	1.5	4.5	1.5	4.5	1.5	4.5	1.5	4.5	1.5	4.5
Non-treated		28	28	4.0	4.0	2.56	2.56	155	155	1199	1199	72.54	74.54					0	0	0	0
		29	29	4.0	4.0	2.58	2.58	153	153	1183	1183	72.98	72.98			100	100	0	0	0	0
		30	30	4.0	4.0	2.57	2.57	140	140	1076	1076	71.38	71.38					0	0	0	0
Mixed Culture treated		22	21	4.0	4.02	2.56	2.57	148	92	1142	702			71.00	43.26			0	0	2	2
		25	24	4.0	4.02	2.57	2.57	148	101	1142	781			70.72	48.13	93.12	65.0	0	0	1	1
		20	26	4.0	4.02	2.58	2.59	122	101	935	781			57.68	47.76			0	0	2	3
Lake water treated		10	11	4.0	4.02	2.59	2.58	140	91	1076	702			66.38	43.11			0	0	2	2
		16	12	4.0	4.02	2.58	2.60	125	87	959	670			59.16	40.38	86.35	59.56	0	0	0	3
		18	13	4.0	4.02	2.59	2.58	126	92	966	710			59.36	43.60			0	0	1	2
Autoclaved lake water treated		1	4	4.0	4.04	2.59	2.58	116	75	891	577			54.75	35.26			0	0	0	5
		2	7	4.0	4.03	2.58	2.59	130	84	997	647			61.50	39.48	80.69	52.36	0	0	1	5
		3	9	4.0	4.03	2.57	2.60	119	80	913	615			56.54	37.39			0	0	1	3

Table 11. Comparative results of plate count and most probable number of heterotrophs and asphalt-degraders in Marshall puck samples post 1.5, 4.5 and 10 months treatments.

		Heterotrophs		Asphalt degraders	
Treatment	Treatment Period (months)	MPN (CFU/ml)*	Plate Count (CFU/g.O.D. sample)**x 10^5	MPN (CFU/ml)	Plate Count (CFU/g.O.D. sample) x 10^4
Control	1.5	119	0.0468±0.002	1.5	0.0007±0
	4.5				
	10.0	817	0.15±0.03	20.0	0.75±0.35
Mixed Culture treated	1.5	132,000	623±36.9	883	775±12.8
	4.5	32,600	77.3±44.5	107	19.7±8.4
	10.0	61,667	82.8±8.8	533	367.5±88.2
Lake water treated	1.5	35,000	54.8±23.3	100	41.5±8.21
	4.5	18,500	18.2±1.80	67	4.37±3.81
	10.0	6,167	4.7±2.0	198	6.66±1.42
Autoclaved lake water treated	1.5	12,300	39.6±0.35	37	29.7±7.89
	4.5	9,400	22.4±16.0	8.3	1.5±0.5
	10.0	3,833	4.5±0.83	198	7.9±5.2

*CFU/ml: colony forming unit per ml
**CFU/g.O.D. sample: colony forming unit per gram of oven dry weight

Table 12. Chemical properties of submersed Marshall puck post-10 months treatment.

Treatments	pH	Total Alkalinity mg$CaCO_3$/L	Total hydrocarbon (%) g.hydrocarbon/ 100g.O.D.sample	Total N ngNH_4-N/ g.O.D.sample	NH_4^+	NO_2^- PPM	NO_3^-	Total-P ngPO_4^-P/ g.O.D. sample
Control	8.4	41.44±7.97	7.72±0.32	302.26±13.43	0	0	0	45.91±9.36
Mixed Culture treated	8.1	36.84±4.61	5.77±0.53	417.67±16.80	1.06	0.8	0	24.94±10.06
Lake Water treated	8.3	39.91±5.32	7.00±0.08	366.41±10.07	0.36	0	0	27.86±13.70
Sterile lake Water treated	8.5	44.52±2.66	7.25±0.22	260.64±42.99	0.1	0	0	35.96±2.53

Table 13. Chemical properties of Marshall pucks after buried in soils for three months with different water holding capacities.

District and water holding capacity of sample		PH	Total Alkalinity mgCaCO$_3$/L	Total hydrocarbon* (%) g hydrocarbon/ 100 g.O.D.sample	Total N ngNH$_3$-N/ g.O.D. sample	NH_4^+	NO_2^- PPM	NO_3^-	Total P ngPO$_4$-P/ g.O.D. sample
Bemidji	30%	7.99±0.06	38.08±6.30	6.73±0.13	358.08±32.25	1.02	1	0	103.52±31.43
	60%	8.03±0.08	40.22±7.59	6.28±0.68	344.62±28.88	1.22	1	0	74.88±9.48
	90%	8.10±0.08	47.40±7.84	5.19±0.79	322.12±4.75	0.81	1	0	88.32±36.78
Brainerd	30%	8.12±0.10	42.57±7.85	6.54±0.24	366.40±64.76	0.41	1.22	0	103.98±12.73
	60%	8.32±0.01	45.62±12.5	6.51±0.09	346.45±58.74	0.54	1.22	0	61.69±17.04
	90%	8.42±0.04	57.52±15.03	6.26±0.38	320.76±21.62	0.61	1.22	0	96.09±31.36
Duluth	30%	8.37±0.10	55.63±2.81	6.55±0.25	352.98±13.54	0	1.22	0	164.66±20.02
	60%	8.41±0.04	49.03±10.72	5.92±0.60	355.70±31.47	0	1.22	0	162.91±27.93
	90%	8.37±0.03	52.66±13.69	6.28±0.62	313.07±46.91	0	1.22	0	185.42±9.30
Rochester	30%	8.15±0.14	17.26±4.21	6.60±0.12	308.03±49.85	0.41	1.22	0	202.84±8.12
	60%	8.00±0.18	60.93±4.94	6.37±0.07	328.95±9.68	0	1.22	0	190.84±21.46
	90%	8.24±0.07	60.39±11.80	6.35±0.01	348.45±9.48	0	4.01	0	185.43±50.25
Willmar	30%	8.12±0.15	48.91±12.67	6.75±0.08	322.92±22.37	0.81	1	0	151.68±9.34
	60%	8.11±0.13	46.07±13.62	6.67±0.01	339.63±14.08	0.81	1	0	169.09±17.79
	90%	8.17±0.13	70.29±12.36	6.49±0.34	323.69±14.49	1.22	1	0	172.52±28.77
Control		8.01±0.03	57.91±5.99	6.75±0.05	439.91±34.03	0	0	0	197.14±12.45

*extracted with ethyl ether and dry to constant weight at 105°C

Table 14. Plate count and most probable number of heterotrphs and asphalt-degraders in Marshall pucks after buried for 3 months in soils with different water holding capacities.

District and water holding capacity sample) x 10^3 of sample		Heterotrophs MPN (CFU/ml)*	Heterotrophs Plate Count (CFU/g.O.D. sample)**x10^{-1}	Asphalt degraders MPN (CFU/ml)	Asphalt degraders Plate Count (CFU/g.O.D.
Bemidji	30%	5,167	1.41±0	25	33.93±6
	60%	100,000	435.05±665	32	137.95±105
	90%	93,000	39.42±53	25	77.43±68
Brainerd	30%	25,000	18.69±3	9	89.64±6
	60%	198,333	242.26±209	25	252.07±158
	90%	71,667	138.22±104	20	154.14±118
Duluth	30%	26,333	2.28±1	40	26.66±6
	60%	1,300,000	354.68±264	90	1349.53±539
	90%	600,000	181.62±145	73	846.68±579
Rochester	30%	25,000	169.13±54	147	3.89±2
	60%	45,000	359.02±153	883	318.95±226
	90%	10,000	11.07±5	32	107.16±55
Willmar	30%	19,000	2.03±0	78	39.20±16
	60%	916,667	173.19±122	367	230.04±75
	90%	666,667	197.55±60	198	177.30±38
Control		123	1.05±0	1	2.35±1

*CFU/ml: colony forming unit per ml
**CFU/g.O.D. sample: colony forming unit pr grams sample of oven dry weight

Table 15. Tensile strength of submersed Marshal puck post-10 months treatments*

Type of treatments	Diameter in.	Thickness in.	Dial Reading	Load	Dry strength lb/in^2	Wet strength lb/in^2	TSR % lb/in^2	Visual stripping %	Broken aggregate %
Control	4.0	2.57	149±8	1152±67	72.97±1.58		100	0	0
Mixed Culture treated	4.03	2.58	129±10	990±76		60.51±4.66	84.8	6	2.3
Lake water treated	4.05	2.58	127±12	973±95		59.39±5.69	83.2	5	1.0
Sterile lake water treated	4.04	2.59	119±9	911±69		55.54±4.26	77.8	5	2.6

*Tensile strength in this batch were tested at sub zero temperatures due to harsh weather conditions.

Table 16. Tensile strength test of Marshall puck buried in soils for three months with different water holding capacities.

District and Water holding capacity of sample		Diameter in.	Thickness in.	Dial Reading	Load	Wet strength lb/in^2	TSR %	Visual stripping %	Broken aggregate %
Control		4.0	2.54	139±6	1069±53	66.89±3.28	100		
Bemidji	30%	4.0	2.55	133±9	1019±71	63.65±4.21	95.16	white aggregate on broken surface at edge (fig. 25, top)	
	60%	4.0	2.55	134±4	1026±30	64.03±1.62	95.72	big white aggregate on broken surface at edge (fig. 25, top)	
	90%	4.0	2.56	115±4	884±31	55.03±1.96	82.27	white aggregate on broken surface at edge (fig. 25, top)	
Brainerd	30%	4.0	2.53	136±6	1047±52	65.79±3.03	98.35	no visual stripping	
	60%	4.0	2.56	133±3	1018±30	63.37±2.00	94.73	small amount of white aggregate showing on edges of the broken surface (Fig. 25, middle)	
	90%	4.0	2.54	124±2	953±18	60.08±1.27	89.82	Same as 60% (Fig. 25, middle)	
Duluth	30%	4.0	2.56	142±4	1090±34	67.88±2.15	101.48	1 broken Iron Oxide (Fig. 25, bottom)	
	60%	4.0	2.54	130±10	995±82	62.40±5.60	93.29	2 broken Iron Oxide (Fig. 25, bottom)	
	90%	4.0	2.54	137±4	1047±28	65.62±2.11	98.10	Small white aggregate on broken surface (Fig. 25, bottom)	
Rochester	30%	4.0	2.55	141±4	1082±39	67.54±2.68	100.97	White aggregate showing on edges of the broken surface (fig. 26, top)	
	60%	4.0	2.55	131±10	1003±78	62.51±4.79	93.46	Big white aggregate showing on edges of the broken surface (Fig. 26, top)	
	90%	4.0	2.55	135±3	1039±20	64.78±1.00	96.85	One small white aggregate on the broken surface	
Willmar	30%	4.0	2.54	142±7	1093±62	68.52±4.23	102.43	No visual stripping	
	60%	4.0	2.55	130±4	997±28	62.25±1.86	93.06	Small amount of white aggregate showing on the broken surface (Fig. 26, bottom)	
	90%	4.0	2.56	123±4	946±32	58.88±3.26	88.03	No visual stripping	

Table 17. Chemical properties of Marshall pucks after buried in soils for seven months with different water holding capacities.

District and Water holding capacity of soil sample	pH	Total Alkalinity mg $CaCO_3$/L	Total hydrocarbon (%) g hydrocarbon/ 100g.O.D. sample 60°C	105°C	Total N ngNH_3-N/ g.O.D. sample	NH_4^+	NO_2^- PPM	NO_3^-	Total P ng PO_4-P/ g.O.D. sample
Bemidji 30%	8.23±0.05	43.46±6.88	6.73±0.09	6.67±0.07	336.78±32.89	0	1.0	0	235.77±42.06
60%	8.28±0.04	48.24±1.23	6.69±0.05	6.65±0.05	336.64±25.16	0	1.0	0	261.66±4.71
90%	8.48±0.12	45.76±8.54	6.61±0.00	6.59±0.00	294.24±75.55	0	1.0	0	299.46±48.49
Brainerd 30%	8.17±0.08	34.19±7.84	6.71±0.17	6.61±0.15	296.63±45.42	1.22	1.0	0	246.30±54.76
60%	8.16±0.05	33.79±4.68	6.65±0.00	6.55±0.00	203.01±7.14	1.22	1.0	0	314.70±67.30
90%	8.28±0.04	22.66±4.61	6.30±0.21	6.29±0.21	233.39±66.99	1.22	0.33	0	292.33±21.07
Duluth 30%	7.47±0.05	34.10±4.32	6.63±0.11	6.62±0.13	377.27±28.02	1.22	1.0	0	220.00±35.35
60%	7.76±0.10	39.96±15.69	6.60±0.14	6.58±0.15	377.89±8.54	1.22	0.67	0	289.26±32.89
90%	7.63±0.07	48.36±9.32	6.62±0.11	6.61±0.05	352.43±9.12	1.22	0.67	0	283.91±17.01
Rochester 30%	7.96±0.06	46.35±9.32	6.58±0.05	6.57±0.05	368.73±6.51	0.41	0.67	0	312.68±93.24
60%	8.06±0.01	43.43±12.94	6.25±0.34	6.22±0.32	332.30±21.61	0	1.0	0	275.73±9.17
90%	8.02±0.06	35.00±2.71	6.55±0.24	6.53±0.24	337.68±51.42	0	0.67	0	270.09±18.38
Willmar 30%	8.38±0.12	56.17±5.37	6.66±0.39	6.64±0.39	319.10±26.27	1.22	1.00	0	271.67±24.21
60%	8.44±0.03	85.90±19.93	6.58±0.33	6.55±0.32	341.62±20.23	1.22	1.00	0	304.97±46.37
90%	8.56±0.08	90.86±14.50	6.54±0.31	6.52±0.30	339.35±29.62	1.22	1.00	0	282.22±12.55
Control	8.28±0.15	39.53±2.60	6.51±0.00	6.49±0.00	395.67±8.28	0	0	0	236.38±17.95
Fresh Puck	7.31±0.00	65.29±5.52	6.70±0.00	6.65±0.00	388.36±6.16	0	0	0	181.29±15.75

Table 18. Comparative results of plate count and most probable number of heterotrophs and asphalt-degraders in Marshall pucks after buried for 3 and 7 months in soils with different water holding capacities.

District and water holding capacity of soil sample	Treatment Period (months)	Heterotrophs MPN (CFU/ml)*	Heterotrophs Plate Count (CFU/g.O.D. sample)** x 10^4	Asphalt degraders MPN (CFU/ml)	Asphalt degraders Plare Count (CFU/g.O.D. sample)x10^3
Bemidji 30%	3	5,167	1.41±0.00	25	33.93±6
	7	36,667	52.48±42.04	32	1043.92±860
60%	3	100,000	435.05±665	32	137.95±105
	7	316,667	284.70±215	78	1061.28±169
90%	3	93,000	39.42±53	25	77.43±68
	7	146,667	252.03±88	45	870.81±395
Brainerd 30%	3	25,000	18.69±3	9	89.64±6
	7	148,333	20.05±4	9	98.02±6
60%	3	198,333	242.26±209	25	252.07±158
	7	383,333	41.36±13	62	269.41±61
90%	3	71,667	138.22±104	20	154.14±118
	7	331,667	19.55±10	25	302.56±461
Duluth 30%	3	26,333	2.28±1	40	26.66±6
	7	55,000	22.85±26	20	125.30±96
60%	3	1,300,000	354.68±264	90	1349.53±539
	7	600,000	227.61±172	55	428.42±236
90%	3	600,000	181.62±145	73	846.68±579
	7	85,000	75.31±32	45	325.64±134
Rochester 30%	3	25,000	169.13±54	147	3.89±2
	7	26,667	8.80±2	9	139.34±39
60%	3	45,000	359.02±153	883	318.95±226
	7	131,667	81.07±90	25	1237.35±795
90%	3	10,000	11.07±5	32	107.16±55
	7	248,333	219.90±65	20	1086.27±689
Wilmar 30%	3	19,000	2.03±0	78	39.20±16
	7	51,667	31.06+13	14	262.76±58
60%	3	916,667	173.19±122	367	230.04±75
	7	816,667	204.61±118	38	696.14±374
90%	3	666,667	197.55±60	198	177.30±38
	7	816,667	117.49±44	25	549.16±38
Control	3	123	1.05±0	1	2.35±1
	7	482	0.34±0.4	7	2.93±4
Fresh Puck	3	-	-	-	-
	7	250	0.10±0.00	4	0.04±0

*CFU/ml: colony forming unit per ml. **CFU/g.O.D. sample: colony forming unit per grams sample of oven dry weight.

Table 19. Comparative results of tensile strength test of Marshall puck buried in soils for 3 and 7 months with different water holding capacities.

District and water holding capacity of soil sample		Treatment period (months)	Diameter in.	Thickness in.	Dial Reading	Load	Wet strength lb/in^2	TSR %
Control		3	4.0	2.54	139±6	1069±53	66.89±3.28	100
		7	4.0	2.55	149±8	1153±66	71.84±4.06	100
Bemidji	30%	3	4.0	2.55	133±9	1019±71	63.65±4.21	95.16
		7	4.0	2.55	153±3	1183±21	73.93±1.38	102.91
	60%	3	4.0	2.55	134±4	1026±30	64.03±1.62	95.72
		7	4.0	2.53	136±3	1045±21	65.63±1.57	91.35
	90%	3	4.0	2.56	115±4	884±31	55.03±1.96	82.27
		7	4.0	2.54	101±5	781±34	48.86±2.37	68.01
Brainerd	30%	3	4.0	2.53	136±6	1047±52	65.79±3.03	98.35
		7	4.0	2.53	141±16	1087±129	68.39±8.52	95.19
	60%	3	4.0	2.56	133±3	1018±30	63.37±2.00	94.73
		7	4.0	2.53	149±5	1150±41	72.25±2.90	100.57
	90%	3	4.0	2.54	124±2	953±18	60.08±1.27	89.82
		7	4.0	2.54	116±9	894±69	56.11±4.61	78.10
Duluth	30%	3	4.0	2.56	142±4	1090±34	67.88±2.15	101.48
		7	4.0	2.54	159±9	1231±66	77.27±4.39	107.55
	60%	3	4.0	2.54	130±10	995±82	62.40±5.60	93.29
		7	4.0	2.53	136±5	1043±37	65.61±2.31	91.33
	90%	3	4.0	2.54	137±4	1047±28	65.62±2.11	98.10
		7	4.0	2.56	124±8	954±62	59.39±4.00	82.67
Rochester	30%	3	4.0	2.55	141±4	1082±39	67.54±2.68	100.97
		7	4.0	2.55	153±6	1183±53	73.91±3.44	102.88
	60%	3	4.0	2.55	131±10	1003±78	62.51±4.79	93.46
		7	4.0	2.54	148±3	1140±21	71.51±1.33	98.90
	90%	3	4.0	2.55	135±3	1039±20	64.78±1.00	96.85
		7	4.0	2.54	145±3	1120±27	70.12±2.05	97.61
Willmar	30%	3	4.0	2.54	142±7	1093±62	68.52±4.23	102.43
		7	4.0	2.54	152±7	1174±59	73.48±3.64	102.28
	60%	3	4.0	2.55	130±4	997±28	62.25±1.86	93.06
		7	4.0	2.54	145±10	1121±81	70.22±5.08	97.74
	90%	3	4.0	2.56	123±4	946±32	58.88±3.26	88.03
		7	4.0	2.54	104±2	801±15	50.13±3.26	69.78

Table 20. Chemical properties of Marshall pucks after buried in soils for twelve months with different water holding capacities.

District and Water holding capacity of soil sample	pH	Total Alkalinity mg $CaCO_3$/L	Total hydrocarbon (%) g hydrocarbon/ 100g.O.D. sample	Total N ngNH_3-N/ g.O.D. sample	NH_4^+	NO_2^-	NO_3^-	Total P ng PO_4-P/ g.O.D. sample
					PPM			
Bemidji 30%	8.30 0.04	47.42±14.21	6.48±0.16	360.71±52.59	1.22	1.0	0	283.04±23.26
60%	8.37±0.13	43.87±3.99	6.42±0.03	435.75±68.51	1.22	0.33	0	310.09±34.82
90%	8.61±0.06	43.17±12.28	6.65±0.09	396.19±28.35	1.22	0	0	260.65±30.68
Brainerd 30%	8.01±0.05	25.63±2.78	6.69±0.20	421.52±41.86	0	1.0	0	244.11±24.71
60%	8.02±0.06	36.77±14.09	6.57±0.08	373.24±42.50	0	1.0	0	240.12±17.98
90%	8.12±0.14	34.56±5.66	6.49±0.18	356.35±26.10	0	1.0	0	257.38±16.66
Duluth 30%	7.99±0.08	42.24±2.93	6.54±0.07	371.48±27.82	1.22	0	0	270.63±81.74
60%	7.94±0.05	45.86±13.87	6.65±0.16	440.38±38.86	1.0	0	0	272.49±32.13
90%	7.87±0.05	41.22±7.52	6.55±0.06	379.34±46.14	1.0	0	0	226.99±10.34
Rochester 30%	7.92±0.03	25.86±6.42	6.52±0.19	3.94.13±28.71	0.33	0.33	0	275.73±38.88
60%	7.95±0.04	31.39±10.73	6.62±0.09	340.60±46.97	0.33	0.67	0	251.53±44.66
90%	8.05±0.05	31.36±8.59	6.58±0.24	341.47±9.82	0.33	0.67	0	237.34±14.03
Willmar 30%	7.99±0.06	45.22±5.90	6.65±0.01	486.30±7.33	1.0	0.33	0	268.49±8.08
60%	7.98±0.04	32.45±4.05	6.50±0.01	410.73±59.02	1.0	0	0	259.46±4.81
90%	8.06±0.05	51.71±9.16	6.64±0.02	408.02±79.07	1.0	0	0	284.61±17.33
Control	7.88±0.10	25.97±7.14	6.28±0.06	308.39±58.04	0	0	0	232.26±27.23
Fresh Puck	7.68±0.00	67.39±5.70	6.70±0.00	388.36±6.16	0	0	0	247.78±10.62

Table 21. Comparative results of plate count and most probable number of heterotrophs and asphalt-degraders in Marshall pucks after buried for 3,7,and 12 months in soils with different water holding capacities.

District and water holding capacity of soil sample	Treatment Period (months)	Heterotrophs MPN (CFU/ml)*	Heterotrophs Plate Count (CFU/g.O.D. sample)** x 10^4	Asphalt degraders MPN (CFU/ml)	Asphalt degraders Plate Count (CFU/g.O.D. sample)x10^3
Bemidji	3	5,167	1.41±0.00	25	33.93±6
30%	7	36,667	52.48±42.04	32	1043.92±860
	12	383,333	516.37±93.43	45	830.00±108
60%	3	100,000	435.05±665	32	137.95±105
	7	316,667	284.70±215	78	1061.28±169
	12	1,200,000	1084.19±340	62	420.00±215
90%	3	93,000	39.42±53	25	77.43±68
	7	146,667	252.03±88	45	870.81±395
	12	250,000	201.60±35	32	205.00±149
Brainerd	3	25,000	18.69±3	9	89.64±6
30%	7	148,333	20.05±4	9	98.02±6
	12	666,667	417.84±112	38	817.00±128
60%	3	198,333	242.26±209	25	252.07±158
	7	383,333	41.36±13	62	269.41±61
	12	1,100,000	705.54±275	95	1363.00±372
90%	3	71,667	138.22±104	20	154.14±118
	7	331,667	19.55±10	25	302.56±461
	12	666,667	313.42±8	32	306.00±87
Duluth	3	26,333	2.28±1	40	26.66±6
30%	7	55,000	22.85±26	20	125.30±96
	12	1,200,000	385.40±140	45	1498.00±100
60%	3	1,300,000	354.68±264	90	1349.53±539
	7	600,000	227.61±172	55	428.42±236
	12	1,050,000	370.41±135	62	1320.00±103
90%	3	600,000	181.62±145	73	846.68±579
	7	85,000	75.31±32	45	325.64±134
	12	133,333	188.21±34	55	918.00±101
Rochester	3	25,000	169.13±54	147	3.89±2
30%	7	26,667	8.80±2	9	139.34±39
	12	533,333	252.22±92	32	1530.00±586
60%	3	45,000	359.02±153	883	318.95±226
	7	131,667	81.07±90	25	1237.35±795
	12	350,000	393.36±386	55	717.00±124
90%	3	10,000	11.07±5	32	107.16±55
	7	248,333	219.90±65	20	1086.27±689
	12	448,333	106.33±64	38	403.00±131
Willmar	3	19,000	2.03±0	78	39.20±16
30%	7	51,667	31.06+13	14	262.76±58
	12	600,000	425.52±247	62	2157.00±312
60%	3	916,667	173.19±122	367	230.04±75
	7	816,667	204.61±118	38	696.14±374
	12	883,333	294.40±28	38	1083.00±241
90%	3	666,667	197.55±60	198	177.30±38
	7	816,667	117.49±44	25	549.16±38
	12	883,333	285.33±89	38	1374.00±143
Control	3	123	1.05±0	1	2.35±1
	7	482	0.34±0.4	7	2.93±4
	12	97	0.07±0.02	11	2.00±2
Fresh Puck	3	-	-	-	-
	7	250	0.10±0.00	4	0.04±0
	12	250	0.26±0.00	25	4.00±0

.nl : colony forming unit per ml. **CFU/g.O.D. sample: colony forming unit per gran .e of oven dry weight.

Table 22. Comparative results of tensile strength test of Marshall puck buried in soils for 3,7, and 12 months with different water holding capacities.

District and water holding capacity of soil sample		Treatment period (months)	Diameter in.	Thickness in.	Dial Reading	Load	Wet strength lb/in	TSR %	Visual Stripping %
Control		3	4.0	2.54	139±6	1069±53	66.89±3.28	100	-
		7	4.0	2.55	149±8	1153±66	71.84±4.06	100	-
		12	4.0	2.55	163±16	1258±116	77.90±7.22	100	0
Bemidji	30%	3	4.0	2.55	133±9	1019±71	63.65±4.21	95.16	-
		7	4.0	2.55	153±3	1183±21	73.93±1.38	102.91	-
		12	4.0	2.55	160±5	1237±34	77.08±1.89	98.94	2
	60%	3	4.0	2.55	134±4	1026±30	64.03±1.62	95.72	-
		7	4.0	2.53	136±3	1045±21	65.63±1.57	91.35	-
		12	4.0	2.54	133±4	1025±28	64.28±1.60	82.52	3
	90%	3	4.0	2.56	115±4	884±31	55.03±1.96	82.27	-
		7	4.0	2.54	101±5	781±34	48.86±2.37	68.01	-
		12	4.0	2.53	106±8	818±63	51.40±4.22	65.99	3-5
Brainerd	30%	3	4.0	2.53	136±6	1047±52	65.79±3.03	98.35	-
		7	4.0	2.53	141±16	1087±129	68.39±8.52	95.19	-
		12	4.0	2.53	149±5	1152±36	72.47±2.24	93.03	1
	60%	3	4.0	2.56	133±3	1018±30	63.37±2.00	94.73	-
		7	4.0	2.53	149±5	1150±41	72.25±2.90	100.57	-
		12	4.0	2.56	145±10	1118±74	69.62±4.62	89.37	3
	90%	3	4.0	2.54	124±2	953±18	60.08±1.27	89.82	-
		7	4.0	2.54	116±9	894±69	56.11±4.61	78.10	-
		12	4.0	2.52	107±13	828±98	52.41±6.71	67.27	2-3
Duluth	30%	3	4.0	2.56	142±4	1090±34	67.88±2.15	101.48	-
		7	4.0	2.54	159±9	1231±66	77.27±4.39	107.55	-
		12	4.0	2.53	210±2	1600±15	100.49±0.34	129.00	1
	60%	3	4.0	2.54	130±10	995±82	62.40±5.60	93.29	-
		7	4.0	2.53	136±5	1043±37	65.61±2.31	91.33	-
		12	4.0	2.52	139±2	1072±18	67.68±0.91	86.88	5
	90%	3	4.0	2.54	137±4	1047±28	65.62±2.11	98.10	-
		7	4.0	2.56	124±8	954±62	59.39±4.00	82.67	-
		12	4.0	2.54	173±4	1328±29	83.34±2.21	106.99	3
Rochester	30%	3	4.0	2.55	141±4	1082±39	67.54±2.68	100.97	-
		7	4.0	2.55	153±6	1183±53	73.91±3.44	102.88	-
		12	4.0	2.55	179±6	1371±42	85.57±2.89	109.85	1
	60%	3	4.0	2.55	131±10	1003±78	62.51±4.79	93.46	-
		7	4.0	2.54	148±3	1140±21	71.51±1.33	98.90	-
		12	4.0	2.55	169±5	1302±36	81.37±2.26	104.45	3
	90%	3	4.0	2.55	135±3	1039±20	64.78±1.00	96.85	-
		7	4.0	2.54	145±3	1120±27	70.12±2.05	97.61	-
		12	4.0	2.54	147±3	1136±20	71.18±1.01	91.37	2
Willmar	30%	3	4.0	2.54	142±7	1093±62	68.52±4.23	102.43	-
		7	4.0	2.54	152±7	1174±59	73.48±3.64	102.28	-
		12	4.0	2.54	168±12	1292±84	80.95±5.27	103.92	1
	60%	3	4.0	2.55	130±4	997±28	62.25±1.86	93.06	-
		7	4.0	2.54	145±10	1121±81	70.22±5.08	97.74	-
		12	4.0	2.53	162±12	1247±85	78.38±5.78	100.62	3
	90%	3	4.0	2.56	123±4	946±32	58.88±3.26	88.03	-
		7	4.0	2.54	104±2	801±15	50.13±3.26	69.78	-
		12	4.0	2.55	112±0	864±78	53.98±3.26	69.29	2-3

Table 23. Chemical properties of Marshall pucks after buried in soils for 23 months with different water holding capacities.

District and Water holding capacity of soil sample	pH	Total Alkalinity mg $CaCO_3$/L	Total hydrocarbon (%) g hydrocarbon/ 100g.O.D. sample	Total N $ngNH_3$-N/ g.O.D. sample	NH_4^+	NO_2^-	NO_3^-	Total P ng PO_4-P/ PPM g.O.D. sample
Bemidji 30%	7.26±0.12	37.24±1.33	6.57±0.10	338.69±10.43	0	0	0	246.20±18.98
60%	7.54±0.03	39.90±1.33	6.55±0.03	353.83±20.23	0	0	0	263.77±13.86
90%	7.65±0.08	38.13±1.54	6.59±0.01	355.07±23.62	0	0	0	287.39±18.06
Brainerd 30%	7.52±0.12	33.25±2.66	6.60±0.03	345.37±17.21	0	0	0	254.91±4.47
60%	7.64±0.02	32.81±0.77	6.44±0.09	354.93±25.55	0	0	0	263.20±8.20
90%	7.66±0.02	33.25± 0.00	6.68±0.08	359.38±11.52	0	0	0	259.07±14.04
Duluth 30%	7.65±0.07	33.70±4.06	6.63±0.30	334.71±22.31	0	0	0	233.63±15.71
60%	7.96±0.06	38.13±2.03	6.61±0.03	333.84±24.85	0	0	0	271.07±5.39
90%	7.84±0.05	37.68±2.77	6.52±0.80	350.46±21.05	0	0	0	275.29±5.39
Rochester 30%	7.41±0.04	37.24±3.52	6.47±0.10	413.06±24.11	0	0	0	224.75±73.13
60%	7.58±0.12	35.47±2.03	6.54±0.08	385.71±55.66	0	0	0	251.41±29.06
90%	7.73±0.13	34.58±2.66	6.51±0.07	391.59±19.39	0	0	0	309.12±10.93
Willmar 30%	7.88±0.03	32.36±1.54	6.49±0.30	357.30±39.68	0	0	0	212.18±13.26
60%	7.93±0.05	33.69±0.77	6.46±0.05	209.36±32.02	0	0	0	263.37±14.57
90%	7.98±0.05	34.58±0.00	6.50±0.06	324.59±50.49	0	0	0	290.04±5.81
Control	7.59±0.05	31.92±1.33	6.92±0.11	380.64±50.49	0	0	0	316.12±33.24
Fresh Puck	7.66±0.00	-	7.02±0.01	-	0	0	0	-

Table 24. Comparative results of plate count and most probable number of heterotrophs and asphalt-degraders in Marshall pucks after buried for 3, 7, 12, and 23 months in soils with different water holding capacities.

District and water holding capacity of soil sample		Treatment period (months)	Heterotrophs MPN (CFU/ml)*	Heterotrophs Plate Count (CFU/g. O.D. sample)** x 10^4	Asphalt Degraders MPN O.D. (CFU/ml)	Asphalt Degraders Plate Count (CFU/g.O.D.Sample) x10^3
Bemidji	30%	3	5,167	1.41±0.00	25	33.39±6
		7	36,667	52.48±42.04	32	1043.92±860
		12	383,333	516.37±93.43	45	830.00±1.08
		23	600,000	733±168	25	454±108
	60%	3	100,000	435.05±665	32	137.95±105
		7	316,667	284.70±215	78	1061.28±169
		12	1,200,000	1084.19±340	62	420.00±215
		23	916,667	748±418	55	823±298
	90%	3	93,000	39.42±5.3	25	77.43±68
		7	146,667	252.02±88	45	870.81±395
		12	250,000	201.60±35	32	205.00±149
		23	548,333	177±67	45	558±33
Brainerd	30%	3	25,000	18.69±3	9	89.64±6
		7	148,333	20.05±4	9	98.02±6
		12	666,667	417.84±112	38	817.00±128
		23	816,667	630±42	32	683±166
	60%	3	198,333	242.26±209	25	252.07±158
		7	383,333	41.36±13	62	269.41±61
		12	1,100,000	705.54±275	95	1363.00±372
		23	983,333	978±340	62	3885±1508
	90%	3	71,667	138.22±104	20	154.14±118
		7	331,667	19.55±10	25	302.56±461
		12	666,667	313.42±8	32	306.00±87
		23	200,000	303±21	32	1131±695
Duluth	30%	3	26,333	2.28±1	40	26.66±6
		7	55,000	22.85±26	20	125.30±96
		12	1,200,000	385.40±140	45	1498.00±100
		23	16000	146±75	20	627±178
	60%	3	1,300,000	352.68±264	90	1349.53±539
		7	600,000	227.61±172	55	428.42±236
		12	1,050,000	370.41±135	62	1320.00±103
		23	641,667	500±216	423	3190±1650
	90%	3	600,000	181.62±145	73	846.68±579
		7	85,000	75.31±32	45	325.64±134
		12	133,333	188.21±34	55	918.00±101
		23	165.000	276±194	78	2532±1703

Table 24 Continued

Rochester	30%	3	25,000	169.13±54	147	3.89±2
		7	26,667	8.80±2	9	139.34±39
		12	533,333	252.22±92	32	1530.00±586
		23	156,000	129±44	18	544±368
	60%	3	45,000	359.02±153	883	318.95±226
		7	131,667	81.07±90	25	1237.35±795
		12	350,000	393.36±386	55	717.00±124
		23	548,333	673±44	38	3254±1132
	90%	3	10,000	11.07±5	32	107.14±55
		7	248,333	219.90±65	20	1086.27±689
		12	448,333	106.33±64	38	403.00±131
		23	401,667	164±91	25	932±464
Willmar	30%	3	19,000	2.03±0	78	39.20±16
		7	51,667	31.06±13	14	262.76±58
		12	600,000	425.52±247	62	2157.00±312
		23	10,333	56±14	20	57±80
	60%	3	916.667	173.19±122	367	230.04±75
		7	816,667	204.61±118	38	696.14±374
		12	883,333	294.40±28	38	1083.00±241
		23	883,333	445±100	38	1455±725
	90%	3	666,667	197.55±60	198	177.30±38
		7	816,667	117.49±44	25	549.16±38
		12	883,333	285.33±89	38	1374.00±143
		23	40,000	122+100	32	463±69
Control		3	123	1.05±0	1	2.35±1
		7	482	0.34±0.4	7	2.93±4
		12	97	0.07±0.02	11	2.00±2
		23	25	0.01±0.00	3	4.00±3
Fresh Puck		3	-	-	-	-
		7	250	0.10±0.00	4	0.04±0
		12	250	0.26±0.00	25	4.00±0
		23	45	0.02±0.00	3	0.01±0

*CFU/ml: colony forming unit per ml. **CFU/g O.D. sample: colony forming unit per gram of given dry weight

Table 25. Comparative results of plate count and most probable number of heterotrophs and asphalt-degraders in soils adjacent to or far away from the buried Marshall pucks that were buried in soil for 23 months with different water holding capacities.

		Heterotrophs		Asphalt degraders	
District and water holding capacity of	Soils adjacent or far away from pucks (Adj or FAF)	MPN (CFU/ml)*	Plate Count (CFU/g.OD. sample)** x 10^4	MPN (CFU/ml)	Plate Count (CFU/g.O.D. sample x 10^3
Bemidji					
30%	Adj	555,000	126±12	1,300	847±29
	FAF	60,000	90±18	170	440±69
60%	Adj	766,667	177±17	383	729±145
	FAF	272,500	88±21	250	354±10
90%	Adj	95,000	38±6	143	176±9
	FAF	70,000	37±8	90	5±1
Brainerd					
30%	Adj	26,333	25±9	143	271±76
	FAF	25,000	17±4	90	47±6
60%	Adj	481,667	30±15	250	447±65
	FAF	95,000	18±8	90	82±88
90%	Adj	53,333	31±6	142	270±62
	FAF	45,000	23±10	90	109±81
Duluth					
30%	Adj	425,000	35±3	250	426±83
	FAF	242,500	26±6	170	21±0
60%	Adj	481,667	30±2	417	171±32
	FAF	70,000	19±1	250	26±1
90%	Adj	516,667	32±2	300	203±9
	FAF	272,500	29±4	120	8±4
Rochester					
30%	Adj	9,000	21±7	317	345±30
	FAF	9,000	10±2	250	137±8
60%	Adj	9,000	16±4	250	200±12
	FAF	6,500	10±0	170	86±9
90%	Adj	9,000	28±9	250	265±72
	FAF	6,500	25±6	40	111±11
Willmar					
30%	Adj	13,000	15±2	110	275±70
	FAF	11,500	11±4	65	130±6
60%	Adj	131,667	21±5	533	516±80
	FAF	60,000	17±8	170	117±28
90%	Adj	38,333	30±14	197	284±118
	FAF	35,000	27±2	90	12±3

*CFU/ml: colony forming unit per ml. **CFU/g.O.D. sample: colony forming unit per gram sample of oven dry weight

Table 26. Comparative results of tensile strength test of Marshall puck buried in soils for 3,7, 12 and 23 months with different water holding capacities.

District and water holding capacity of soil sample		Treatment period (months)	Diameter in.	Thickness in.	Dial Reading	Load	Wet strength lb/in^2	TSR %	Visual Stripping %	Broken Aggregate %
Control		3	4.0	2.54	139±6	1069±53	66.89±3.28	100	-	-
		7	4.0	2.55	149±8	1153±66	71.84±4.06	100	-	-
		12	4.0	2.55	163±16	1258±116	77.90±7.22	100	0	-
		23	4.01	2.55	208+13	1567+93	97.58+5.73	100	Edges	1.0
Bemidji	30%	3	4.0	2.55	133±9	1019±71	63.65±4.21	95.16	-	-
		7	4.0	2.55	153±3	1183±21	73.93±1.38	102.91	-	-
		12	4.0	2.55	160±5	1237±34	77.08±1.89	98.94	2.0	-
		23	4.0	2.55	182+5	1377+39	85.85+2.07	87.98	1.7	1.3
	60%	3	4.0	2.55	134±4	1026±30	64.03±1.62	95.72	-	-
		7	4.0	2.53	136±3	1045±21	65.63±1.57	91.35	-	-
		12	4.0	2.54	133±4	1025±28	64.28±1.60	82.52	3.0	-
		23	4.0	2.54	132+3	1013+20	63.56+1.37	65.13	2.7	1.3
	90%	3	4.0	2.56	115±4	884±31	55.03±1.96	82.27	-	-
		7	4.0	2.54	101±5	781±34	48.86±2.37	68.01	-	-
		12	4.0	2.53	106±8	818±63	51.40±4.22	65.99	3-5	-
		23	4.0	2.57	103±1	803±4	49.23±0.41	50.50	1.7	1.7
Brainerd	30%	3	4.0	2.53	136±6	1047+52	65.79+3.03	98.35	-	-
		7	4.0	2.53	141±16	1087±129	68.39±8.52	95.19	-	-
		12	4.0	2.53	149±5	1152±36	72.47±.2.24	93.03	1.0	-
		23	3.93	2.54	148+7	1130+50	72.22+2.82	74.01	1.0	0.3
	60%	3	4.0	2.56	133±3	1018±30	63.37±2.00	94.73	-	-
		7	4.0	2.53	149±5	1150±41	72.25±2.90	100.57	-	-
		12	4.0	2.56	145±10	1118±74	69.62±.4.62	89.37	3.0	-
		23	3.95	2.49	152+5	1156+35	74.88+4.01	76.74	1.7	1.3
	90%	3	4.0	2.54	124+2	953+18	60.08+1.27	89.82	-	-
		7	4.0	2.54	116±9	894±69	56.11±4.61	78.10	-	-
		12	4.0	2.54	107±13	828±98	52.41±6.71	67.27	2.3	-
		23	3.90	2.50	104+10	810+74	52.96+3.14	54.28	0.7	0.0
Duluth	30%	3	4.0	2.56	142+4	1090+34	67.88+2.15	101.48	-	-
		7	4.0	2.54	159±9	1231±66	77.27±4.39	107.55	-	-
		12	4.0	2.53	210±2	1600±15	100.49±0.34	129.00	1.0	-
		23	3.90	2.56	208+10	1569+74	97.56+5.09	99.98	1.0	1.3
	60%	3	4.0	2.54	130±10	995±82	62.40±5.60	93.29	-	-
		7	4.0	2.53	136±5	1043±37	65.61±2.31	91.33	-	-
		12	4.0	2.52	139±2	1072±18	67.68±0.91	86.88	5.0	-
		23	4.01	2.56	133+8	1021+59	63.40+3.54	64.97	3.0	2.3
	90%	3	4.0	2.54	137±4	1047±28	65.62±2.11	98.10	-	-
		7	4.0	2.56	124±8	954±62	59.39±4.00	82.67	-	-
		12	4.0	2.54	173±4	1328±29	83.34±2.21	106.99	3.0	-
		23	3.9	2.56	144+5	1102+38	68.14+2.01	69.83	1.3	1.3
Rochester	30%	3	4.0	2.55	141+4	1082+39	67.54+2.68	100.97	-	-
		7	4.0	2.55	153±6	1183±53	73.91±3.44	102.88	-	-
		12	4.0	2.55	179±6	1371±42	85.57±2.89	109.85	1.0	-
		23	4.0	2.56	166+3	1256+18	77.88+1.45	79.81	2.0	1.7
	60%	3	4.0	2.55	131+10	1003+78	62.51+4.79	93.46	-	-
		7	4.0	2.54	148±3	1140±21	71.51±1.33	98.90	-	-
		12	4.0	2.55	169±5	1302±36	81.37±2.26	104.45	3.0	-
		23	4.02	2.57	163+8	1235+55	76.37+3.86	78.26	3.3	3.0
	90%	3	4.0	2.55	135±3	1039±20	64.78±1.00	96.85	-	-
		7	4.0	2.54	145±3	1120±27	70.12±2.05	97.61	-	-
		12	4.0	2.54	147±3	1136±20	71.18±1.01	91.37	2.0	-
		23	4.03	2.56	117+2	898+14	55.48+1.16	56.85	3.0	2.3
Willmar	30%	3	4.0	2.54	142±7	1093±62	68.52±4.23	102.43	-	-
		7	4.0	2.54	152±7	1174±59	73.48±3.64	102.28	-	-
		12	4.0	2.54	168±12	1292±84	80.95±5.27	103.92	1.0	-
		23	4.02	2.56	190+20	1463+147	88.75+8.88	90.95	2.3	1.0
	60%	3	4.0	2.55	130+4	997+28	62.25+1.86	93.06	-	-
		7	4.0	2.54	145±10	1121±81	70.22±5.08	97.74	-	-
		12	4.0	2.53	162±12	1247±85	78.38±5.78	100.62	3.0	-
		23	4.02	2.54	148±4	1131±31	70.43±1.73	72.18	4.0	2.0
	90%	3	4.0	2.56	123±4	946±32	58.88±3.26	88.03	-	-
		7	4.0	2.54	104±2	801±15	50.13±3.26	69.78	-	-
		12	4.0	2.55	112±0	864±78	53.98±3.26	69.29	2-3	-
		23	4.02	2.55	120±2	917±16	56.87±.95	58.28	4.3	1.0