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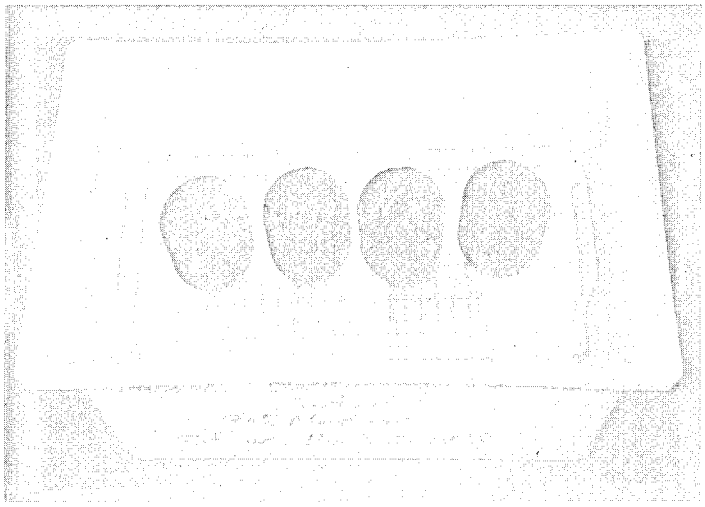

Microbial Deterioration of Asphalt Materials and Their Biochemical Changes During Stripping Process

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Chemical analysis, enumeration of heterotrophic microorganisms and asphalt degraders, metabolites produced by asphalt degraders, and tensile strength were assayed after Marshall pucks had been buried for 3, 7, 12, and 23 months in soils that were held in 20-gallon aquarium tanks and maintained at 30, 60, and 90 percent water holding capacities.

Good correlation was found between loss of hydrocarbons in asphalt materials and reduction of tensile strength of Marshall pucks. Total hydrocarbon (percentage) in Marshall pucks that were buried in soils was less than those in control and fresh puck samples. The study confirmed that asphalt samples buried in soils that were maintained at 60 percent water holding capacity for 23 months had significantly more asphalt degraders and had undergone most severe biodegradation. GC, HPLC, MS, and IR chromatographs show that major peaks of biochemicals and meabolites almost disappeared in most asphalt samples. The higher visual stripping and broken aggregate percentages also were found in Marshall pucks that had been treated under the same environmental conditions.

The study also confirmed that under optimum environmental conditions, microorganisms caused significantly more damage to asphalt pavement in all instances. Therefore, microbial degradative activities are considered major mechanisms to stripping process of asphalt pavement.

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Microbial Deterioration Of Asphalt Materials And Their Biochemical Changes During Stripping Process

Final Report

Prepared by:
Fu-Hsian Chang, Ph.D.
Professor of Environmental Studies
Bemidji State University
1500 Birchmont Drive N.E.
Bemidji, MN 56601-2699

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EXECUTIVE SUMMARY

Relationships were found between biochemical changes caused by asphalt degrades and physical failure of asphalt pavement using Marshall pucks buried in soil tests.

Chemical analysis, enumerations of heterotrophic microorganisms and asphalt degraders, metabolites produced by asphalt-degraders were assayed using GC, HPLC, MS and IR. chromatographic standard methods and tensile strength test were conducted in Marshall pucks after having been buried for 3, 7, 12 and 23 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 were found alkaline, pHs were between 7.26-8.44. 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.

Total hydrocarbon (%) in Marshall pucks that were buried in soils was less than those in control and fresh puck samples. 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.

Growth of heterotrophs and asphalt-degraders was observed when Marshall pucks were 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 remained at optimum for about seven months in most samples that were maintained at 30% water holding capacity. 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. 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.

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. 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. Also, significant number of heterotrophs and asphalt degraders were found in soil adjacent to the asphalt materials.

GC, HPLC, MS and IR. chromatograms for biochemical/metabolites changes and biodegradation of asphalt materials buried in various soils for 3, 7, 12 and 23 months show that major peaks almost disappeared in most asphalt samples buried in soils that were maintained at 60% water holding capacities. 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 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. 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.

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CHAPTER 1

INTRODUCTION

Recently, more evidence concerning the involvement of microbes in the degradation process of asphalt concrete has been reported [1-6]. Numerous studies [7-9] 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 concern to asphalt-aggregate mixture performance. The effect on asphalt-aggregate bond is particularly acute since microbial activities are concentrated on mineral aggregate surfaces where the primary source of nutrients for microorganisms are present.

During our previous study on "Microbial Deterioration of Asphalt Materials and its Prevention" funded by the Minnesota Department of Transportation (Mn/DOT), we have isolated 74 asphalt-degrading microbial genera and species. The study revealed that physical strength was significantly weakened after pucks had been soaked in mixed asphalt-degrading microbial culture or in lake waters, and a large number of microorganisms were found bound to aggregates after soaked for 1.5 and 4.5 months. A result obtained from the soaking test showed 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. However, the higher broken aggregate percent and visual stripping percent the greater loss of hydrocarbons (%) occurred in Marshall pucks submersed in enriched asphalt-degrading cultures for 10 months than those submersed for 1.5 and 4.5 months. Biochemical/Metabolites produced by asphalt-degraders revealed that significant mineralization or bioconversion of asphalt also occurred in the samples that have been soaked in enrichment culture for 10 months.

Our previous year study has revealed that degradation/deterioration of asphalt materials by microorganisms is not a simple process and microbial activity have contributed to a

significant cause of failure of asphalt pavements in which environmental conditions are optimal for microbial degradative activities which usually would take longer terms otherwise. It also has revealed that biodegradation of asphalt materials by microorganisms may involve a variety of different biochemical mechanisms. Therefore, this project is intended to provide enumeration of heterotrophic microorganisms and asphalt degraders and biochemical changes information that leads to physical failure of asphalt pavement under various environmental conditions of buried-in-soil tests.

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[10]. Five soils with different physical-chemical properties were collected from the road side of the stripped asphalt pavements around the State of Minnesota. More than two hundred pounds of soil from each site were put in separate 20-liter containers and adjusted to 30%, 60% and 90% water holding capacities. Fresh Marshall pucks were buried in soils and incubated for certain periods. At the end of each incubation period triplicated pucks were removed from each container and tested for chemical-physical properties, microbial enumerations, biochemicals/metabolites changes and physical strength. The purpose of this study is to evaluate the extent of destruction deterioration of asphalt materials by microorganisms. This project of study has emphasized the nature and properties of microorganisms that attack asphalt materials, the nature of the microbial actions on asphalt materials, and their biochemical/metabolites changes during asphalt stripping.

CHAPTER 2

MATERIALS AND METHODS

I. Procedures of pucks-buried-in-soil tests.

1. Collection of pavement and soil samples.

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. 1,2). The locations and description of those samples were described in Table 1. Fresh Marshall pucks were prepared by the Mn/DOT Bemidji District Lab and buried in the soils.

2. The experiment of Marshall puck-buried-in-soil.

The experiments of Marshall puck buried-in-soil were set up using 120-140 lbs. of each soil put in 20-liter Nalgene rectangular tank and adjusted to 30%, 60%, 90% water holding capacity (Fig. 3). Triplicated Marshall pucks were buried in the soil of each container and incubated for four time period (3, 7, 12, 23 months). The control Marshall pucks were kept in the containers that were maintained at the same humidity (Fig. 4). At the end of each incubation period three pucks from each soil and control container were removed and tested for chemical-physical properties, microbial enumerations, biochemical/metabolic products produced by asphalt-degraders and physical strength.

Fragments were taken from the fracture planes produced during the tensile-strength test. These fragments were homogenized by blender and hammer and passed through a 0.0469 in. or 0.0787 in. sieve. Samples were then stored in a 4°C walk-in refrigerator for future analysis.

II. 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 analyzed according to the methods described in "Methods of soil analysis, Part II: Chemical and Microbiological Properties" [11] and "Standard Methods for the Examination of Water and Wastewater"[12].

1. pH

PH determination was measured by 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 the determination of alkalinity, exchangeable ammonium, nitrate, and nitrite in asphalt and soil samples, the asphalt or soil sample was shaken with 2N NCl for 1 hour using 10 ml of this reagent per gram of soil. The KCl was filtered and filtrate analyzed for alkalinity, NH4+, 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 KCl titrant (M ml). Total titration (ml) of 0.1 N HCl solution is equal to Pml + Mml. Calculate alkalinity according to the following formula.

$$\text{Total alkalinity, mg } CaCO_3/L = \frac{\text{Total (P \& M) ml HCl x normality HCl x 50000}}{\text{ml sample}}$$

4. Total hydrocarbon

Hydrocarbon in asphalt materials was extracted in a Soxhlet apparatus. Fifty to 100 g of finely ground sample were 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.

$N_{H_2SO_4}$ = concentration of H_2SO_4 solution (normality).

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 wavelength of 425 nm. The nitrite is analyzed by a modification of the Griess-Ilosvay (the diazotization and coupling reactions) at wavelength of 520 nm. The colorimetric method for nitrate is the phenoldisulfonic acid technique and measurements were made at wavelength of 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 wavelength of 690 nm.

III. Enumeration of heterotrophic and asphalt-degrading microorganisms.

Microbial numbers in asphalt and soil samples taken from five District Labs were enumerated using plate count method and most probable number (MPN) techniques for total heterotrophic microorganisms and asphalt-degrading microorganisms. The medium for the enumeration of heterotrophic microorganisms is nutrient agar or nutrient broth (Difco products). The medium for the enumeration of asphalt degraders was basal medium that contained 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% K_2HPO_4 per liter of distilled water, concentrated PO_4^{-3} salt solution (pH 7.0) was autoclaved separately and added to the autoclaved basal broth. Asphalt extract (10%) was extracted using 500 g asphalt materials in 1 liter distilled water, autoclaved and filtered. The extract was added to basal medium as sole carbon source for asphalt degraders. For a solid medium, 2% agar (Difco Laboratories, Detroit, Mich.) was added to basal medium. Growth in broth medium incubated at 27°C incubator for 3 to 7 days. Turbidity of growth medium indicates growth of asphalt-degrading microorganisms in the broth medium.

IV. Extraction and analysis of biochemicals and metabolites.

1. Extraction of Metabolites

Two different solvents, methanol and toluene, were used to extract the asphalt samples. Twenty-four grams of each asphalt sample (8 g portion of sample from three replicates of each treatment were mixed uniformly) were extracted in a 125-ml flask overnight with solvent in a Soxhlet apparatus. Two milliliters of liquid from methanol extract were centrifuged at 15,000 rpm for 20 minutes. The liquid was kept in a vial (12 mm x 32 mm) with polypropylene screw caps at 4°C. Liquid extracted by toluene was introduced to the top of a liquid column (25 ml) packed by Sephadex LH-20 (Supelco Inc., PA 16823) with toluene and rinsed with toluene. Eluent was collected and kept in a vial (12 mm x 32 mm with polypropylene screw caps) at 4°C.

2. GC Analysis

The methanol extract of each sample was analyzed with a HP 5890 Series II gas chromatograph equipped with a flame ionization detector (Hewlett-Packard, Avondale, PA). An Ov-1 fused silica open tabular column (50 m x 0.32 mm) with 0.3µm film coating was used (Alltech, Deerfield, IL). Nitrogen was used as a carrier and makeup gas for analyzing the samples after 3 and 7 months of treatment. Helium was used instead of nitrogen for analyzing the samples after 12 months of treatment. The analysis was conducted using a temperature program. The oven temperature was initially set at 55°C for 1 minute and increased at 8°C per minute to 280°C and maintained at 280°C for 10 minutes. During the analysis, the injection port and detector were maintained at 220°C and 250°C, respectively.

3. HPLC analysis

High performance liquid chromatography (HPLC) analysis was conducted using

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 µl of above methanol 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 wavelength of 254 nm. Chromatograms were recorded and integrated using Beckman's System Gold V510 software.

4. MS analysis

Selected samples of both methanol and toluene extracts were analyzed at the Department of Biochemistry of the University of Minnesota. A Carlo Erba/Kratos MS 25 model mass spectrometer was used.

5. IR analysis

The methanol extract was scanned in A 1600 Series FTIR (PERKIN-ELMER) IR spectrometer to determine the functional groups of the metabolites. Several drops of liquid sample with a volume of approximate 15µl was used. Pure methanol was scanned prior to analysis of the samples and excluded as background from the spectrum of each sample. The spectra of Transmittance (T%) and wavenumber (cm^{-1}) were plotted and the peak height (T%) were measured by the IR spectrometer.

6. Statistical analysis

All determinations were made in triplicates and the relative standard deviation of the data were less than 5% (95% significant level) significant differences between treatments (means of three replicates) were differentiated using multiple range test.

V. Physical/Tensile strength test

Physical/Tensile strength was tested at Mn/DOT's Bemidji District Lab using Standard Lotmann Test Procedures [13-15], soaking the samples in 77°F water bath

and then load tested to determine the tensile strength.

CHAPTER 3

RESULTS AND DISCUSSIONS

I. Properties of pavement and soil samples.

Most of the soil samples collected from underneath the damaged asphalt pavement by five Mn/DOT District Labs belonged to coarse loamy sand or clay or silty clay according to granulation tests. Granulation of soil samples was analyzed at the Mn/DOT Bemidji District Lab. (Tables 1 and 2). Table 2 shows the stripped asphalt pavement and soil samples underneath the asphalt pavement were both alkaline, 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 from Brainerd and Willmar District. It might have influenced 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 have resulted from worse biodeterioration by microbial degradative activity.

The hydrocarbon components of soil samples underneath the asphalt pavement were fractionated by chromatographic techniques using HPLC. The chromatograms for soil samples underneath asphalt pavements of Bemidji, Brainerd, Duluth and Rochester Districts are shown in Fig. 5. 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. The results of heterotrophs and asphalt degraders enumeration in soils underneath asphalt pavements and samples of asphalt pavements are shown in Table 3. Heterotrophs were significantly more abundant than asphalt-degrading microbial populations in all soil samples. However, the stripped pavement had 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 had more asphalt-degrading microbes and heterotrophs than non-stripped pavement samples. The greatest asphalt-degrading-microbial count and heterotrophs count were found in soil samples underneath asphalt pavement and the sample of asphalt pavement from Bemidji District. In general, the higher the asphalt degrading microbial counts were the worse the biodeterioration of asphalt pavement.

II. Biodeterioration/Biodegradation of asphalt (bituminous) materials buried in soils with different water holding capacities.

Marshall pucks were removed from each treated or control containers after having been buried for 3, 7, 12 and 23 months in soils or left in the control containers that were maintained at 30%, 60%, and 90% water holding capacities. Enumeration of heterotrophic and asphalt degrading microorganisms were assayed using plate counts and most probably number (MPN) techniques and chemical characteristics and metabolites produced by asphalt-degraders were analyzed using GC, HPLC, and MS chromatographic and IR spectrometric techniques. Physical/tensile strength test was done at Mn/DOT's Bemidji District Lab. An additional experiment was conducted to compare populations of heterotrophs 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. 6). Data reported here deals with the numbers and types of asphalt-utilizing microorganisms in Marshall pucks and soils adjacent to the Marshall pucks, and their biochemical/metabolites changes as a result of asphalt-degrading microbial activity relative to physical strength and degree of stripping.

1. Chemical and physical properties:

Chemical and physical properties of Marshall pucks after having been buried in soils or placed in control containers for 3, 7, 12 and 23 months are shown in Table 4. All pucks had pHs between 7.26-8.44 and bicarbonate alkalinity was predominant in most asphalt samples. Most pucks buried (treated) in soils had less nitrogen and phosphorus than those pucks kept in control containers. It might be due to microbial assimilation of nitrogen and phosphorus during degradation of asphalt materials. Denitrification might have occurred to form NH_4^+ and NO_2^- in most samples due to an insufficient dissolved oxygen present in the containers when Marshall pucks were buried for 3, 7, 12 months in soils that contained 30%, 60% and 90% water holding capacities. However, denitrification did not occur in any asphalt samples when Marshall pucks were buried in soils for 23 months (Table 4). In most cases, total hydrocarbon (%) in Marshall pucks that had been buried in soils that were maintained at different water holding capacities was less than that of the control pucks sample. The Marshall pucks buried in soils with 60% and 90% water holding capacities had less hydrocarbons (%) than those samples with 30% water holding capacity (Table 4).

2. Enumeration of heterotrophic and asphalt-degrading microorganisms:

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 5, Fig. 7 and Fig. 8. Show 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. 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. 7 and Fig. 8). 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 etc. 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. 7). Fig. 7 also shows that heterotrophs proliferated with a second exponential phase after Marshall pucks had 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. 8 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. 8).

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. 7 and Fig. 8). The second logarithmic phase of heterotrophs and asphalt degraders (Fig. 7 and Fig. 8) 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 phase. Fig. 7 and Fig. 8 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 etc. are most suitable for microbial growth.

Table 6 and Fig. 9 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 stripping asphalt road and have been given extensive data reporting high microbial counts in soils near asphalt materials.

3. Biochemical/metabolic products produced by asphalt-degraders.

a. GC analysis:

The chromatograms for biodegradation of asphalt materials buried in various soils for 3 months are shown in Fig. 10. 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 10 shows 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. 10) 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 had 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.

Gas chromatograms for biochemical/metabolites changes and biodegradation of asphalt materials buried in various soils for seven months are shown in Fig. 11. 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. 11). The biochemical/metabolites changes and biodegradation of asphalt material are also noticed in control Marshall puck samples (Fig. 11) 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. 11 shows relatively small or almost absence of four major peaks 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 (Fig. 10). 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 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.

Gas chromatograms for biochemical/metabolites changes and biodegradation of asphalt materials buried in various soils for 12 months are shown in Fig. 12. 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. 12) 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 of 29.600, 28.560, 25.519, 22.062 minutes were present in the control pucks extract.

Fig. 1 2 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. 12). 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 5, even if the samples buried in soils with 30% water-holding capacity) are responsible for the biodeterioration of asphalt materials. It was noticed (Fig. 12) that even though the Marshall 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 Marshall puck sample. Molds and bacteria (Fig. 13) 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 5). Those organisms present in the control container only rely on asphalt as the sole source of carbon and energy because there was only Marshall 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.

Gas chromatograms for biochemical/metabolite changes and biodegradation of asphalt materials buried in various soils for 23 months are shown in Fig. 14. It shows that 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 (control pucks extract). Major peaks almost

disappeared in most asphalt samples buried in soils that were maintained at 60% water holding capacities (Fig. 14). 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. 14), especially, the asphalt sample buried in soils with 60% water holding capacity had undergone most severe biodegradation.

2. HPLC analysis

The HPLC chromatograms for biochemical/metabolites changes and biodegradation of asphalt materials after having been buried in soils for seven months are depicted in Fig. 15. 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 area 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. 15).

3. MS Analysis

Methanol Extraction

Extracts from Control and the samples buried in Bemidji soil with 60% water holding capacity were analyzed using GC-MS technique. MS spectra are shown in Fig. 16 (1), (2) and Fig. 17 (1) (2). Here the tested samples are referred as MeOH control and MeOH treated. Two high molecular weight compounds were found in both the control and treated sample.

(1) Octadecanoic acid, methyl ester

- Formula: $C_{19}H_{38}O_2$
- Molecular Weight: 298.50
- CAS Registry Number: 112-61-8
- Chemical Structure

(2) Heptadecanoic acid

- Formula: $C_{17}H_{34}O_2$
- Molecular Weight: 270.45
- CAS Registry Number: 506-12-7
- Chemical Structure:

As we can see from the MS spectra, the concentration of these two compounds was much lower in the treated sample than in the control one. This might be because in the treated sample high molecular weight compounds have been transformed to lower molecular weight compounds or even inorganic CO_2 and H_2O by microbial activity. Comparison of the MS spectra between the control and the treated sample also shows that treated sample had lower molecular weight compounds than the control. Asphalt degraders actually used those high molecular weight compounds as an energy source and transformed them to lower molecular weight compounds or even inorganic end products.

Toluene Extraction

Extracts from the controls and the samples buried in Bemidji soil with 60% water holding capacity were analyzed using GC-MS method. Here tested samples are referred to as toluene control and toluene treated. Each sample had eight 2-ml bottles of elution collected from the liquid column. The second and third bottles of elution of each sample were analyzed using GC-MS method. Here tested samples are referred to as toluene control and toluene treated. Each sample had eight 2-ml bottles of elution collected from the liquid column. The second and third bottles of elution of each sample were analyzed using GC-MS and EI-MS (Electronic Impact Mass Spectrometry). it turns out that GC-MS wasn't effective enough to separate the individual compounds, because toluene extract contained a lot of high molecular weight compounds in asphalt material, and it is very hard to separate them through GC, so electron impact mass spectrometer (EI-MS) was used instead to see what exactly were consisted in the elution. The MS spectra of the control and treated samples are shown in Fig. 16-17. The spectra indicate that there were a lot of different molecular weights of compounds in

the samples. It's actually difficult to separate the MS spectrum of a single compound from the others, and the spectra showed several large peaks instead of some separate single peaks. However, it's quite obvious that in the control, there were more compounds with a molecular weight between 301 and 500 than those in the treated sample (peak intensity in the control was 7229440, peak intensity in the treated sample was 3627072). There were also less compounds with molecular weight between 201 and 300 than those in the treated sample (peak intensity in the control was 426976, peak intensity in the treated sample was 875216). So in the treated sample, some high molecular weight of hydrocarbons have been transformed to lower molecular weight hydrocarbons or turned into inorganic end products. This result was in accordance with the microbial activity found in the treated sample.

IR Analysis

The results of IR analysis are shown in Fig. 18-22. The relative absorbance of each peak is shown in Table 7.

Due to the difference among concentrations of liquid samples scanned through IR spectrometer, the absorbance of each band was adjusted in order to compare the relative absorbance. Considering the larger amount of solvent than analyses, it is assumed that the absorbance of the 3336-3595 cm^{-1} band, which is mainly contributed by -OH functional group in alcohol, remained the same from sample to sample and served as an internal standard. The relative absorbency of the other bands was calculated as the percentage absorbency of the main band between 3336-3595 cm^{-1}.

Controls without soil treatment showed great changes in the IR. spectra after each treatment period (Fig. 18). The band at 1650 cm^{-1} was caused by C=O stretching in aldehyde, ketone, and carboxylic compounds. The band at 1450 cm^{-1} was due to $-CH_2$

scissoring. In IR. spectrum of control after 3 months of treatment, there was a big peak at 1450 cm-1, and no peak was observed at 1650 cm-1. The original asphalt sample extracted by Methanol seems to be comprised of mainly long chain alkanes. After 7 months of treatment, the IR. spectrum of the control showed a very broad peak ranging from 1650 cm-1 to 1450 cm-1. After 12 months of treatment, the IR spectrum of control had a big peak at 1650 cm-1 with absorbency of 0.669. It can be concluded from the comparison of IR spectra of controls after each treatment period that oxidized hydrocarbons, such as aldehydes, ketones, and carboxylic hydrocarbons, were formed after 12 months. Since there was no soil used in the control treatment, evidence was obvious that the indigenous microorganisms in asphalt samples and water slowly degraded control asphalt under favorable condition even without soil.

The samples buried in Bemidji soil with 60% waster holding capacity after each treatment period had similar differences in their IR spectra to those of controls as discussed above (Fig. 19). The IR spectra of samples after 12 months of treatment had a big peak at 1650 cm-1, indicating the formation of compounds with C=O functional group. The intense (A=0.849) of this C=O stretching was much higher than that of control's (A=0.669). it was the microbes in soil that aggravated the degradation of long-chain alkane to its oxidizing forms.

Different soil types play different roles in he biodegradation of asphalt samples. Spectra of control and five samples buried in Bemidji, Brainerd, Duluth, Rochester, and Willmar soil with 60% water holding capacity after 12 months of treatment are compared as shown in Fig. 20 and Fig. 21. All of the spectra showed peaks at 1650 cm^{-1} with various intensity. The order of Absorbance of the C=O stretching peak were: Bemidji>Control>Willmar>Duluth>Brainerd>Rochester. The soil type affected the degree

of degradation of asphalt samples.

It is also interesting to compare the IR spectra of sample in one soil type with different water holding capacities. IR spectra of the samples buried in Bemidji soil after 12 months of treatment are shown in Fig. 22. All of the spectra showed high to medium peaks at 1650 cm-1. The order of Absorbance of the C=O stretching peak are: 30%>60%>90%. The samples with 30% and 60% water holding capacity had almost the same intense peak of C=O stretching. Since the soil type was fixed, the results showed that the soil water holding capacity also affected the degradation of asphalt samples.

III. Physical Strength, visual stripping and broken aggregate percentage examination and their correlation to biochemical/metabolites changes.

Changes of tensile strength, broken aggregate percent and visual stripping percent of Marshall pucks after buried in soils for 3, 7, 12 and 23 months in soils that were maintained at 30%, 60% and 90% water holding capacities are summarized in Table 8 and fig. 23-28. Fig. 23 shows wet strength (lb/in2) 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, as shown in Table 8 and Fig. 24. Table 8 and Fig. 23-24 illustrate the magnitude of hardening that occurred in asphalt materials that were maintained at 60% water holding capacity for12 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 etc. 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 maintained at 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 has changed to dull, rough, crumbly materials and has lost its usual rheological characteristics as compared with fresh Marshall pucks (Fig. 25 and Fig. 26) 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. 14, 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. 23 and Fig. 24). 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 8, Fig. 27 and Fig. 28).

CHAPTER 4

CONCLUSION

It is concluded from this research 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 might be difficult to suppress if no effective inhibiting chemical is applied.

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. 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.

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 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.

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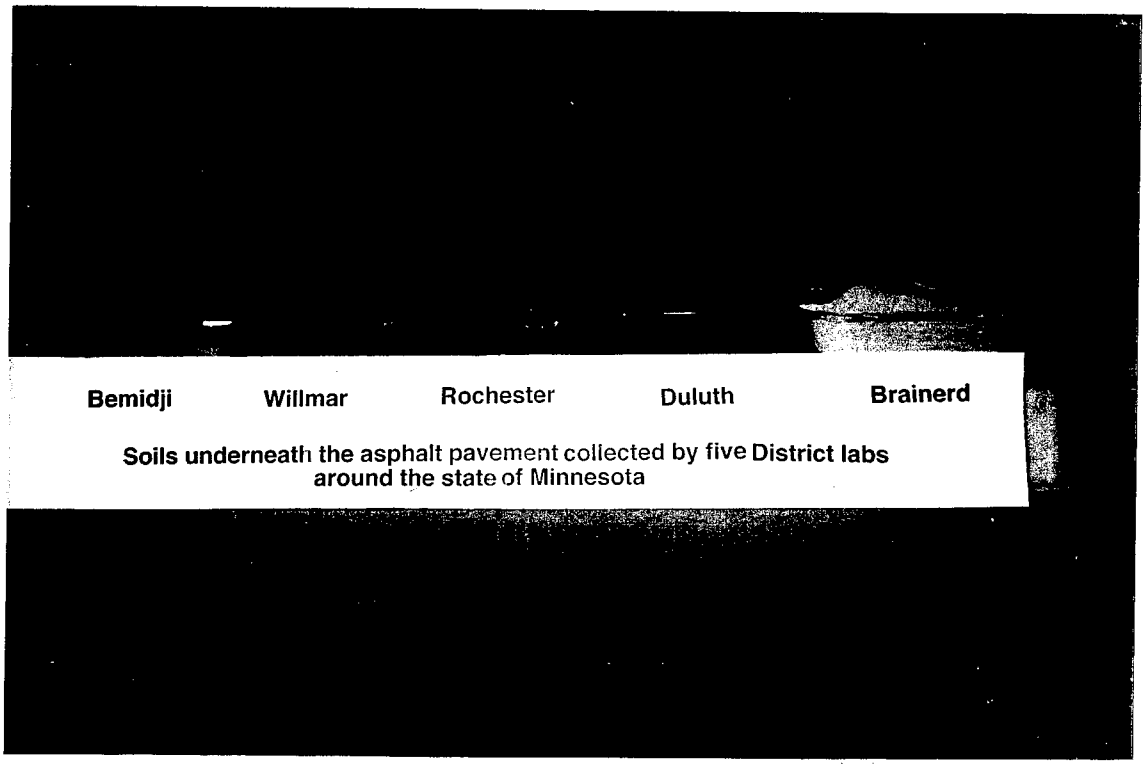


Fig. 1. Soils underneath the asphalt pavement collected by five Mn/DOT District Labs around the State of Minnesota.

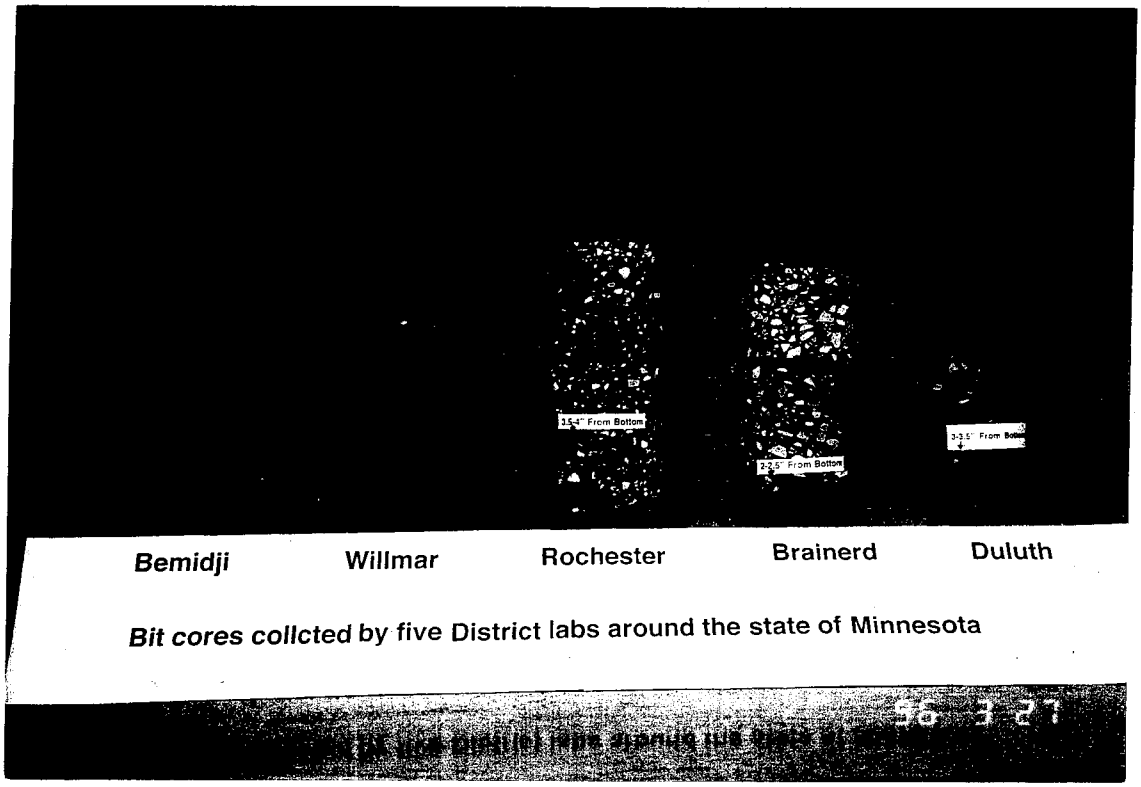


Fig. 2. Bit cores collected by Mn/DOT five District Labs around the State of Minnesota

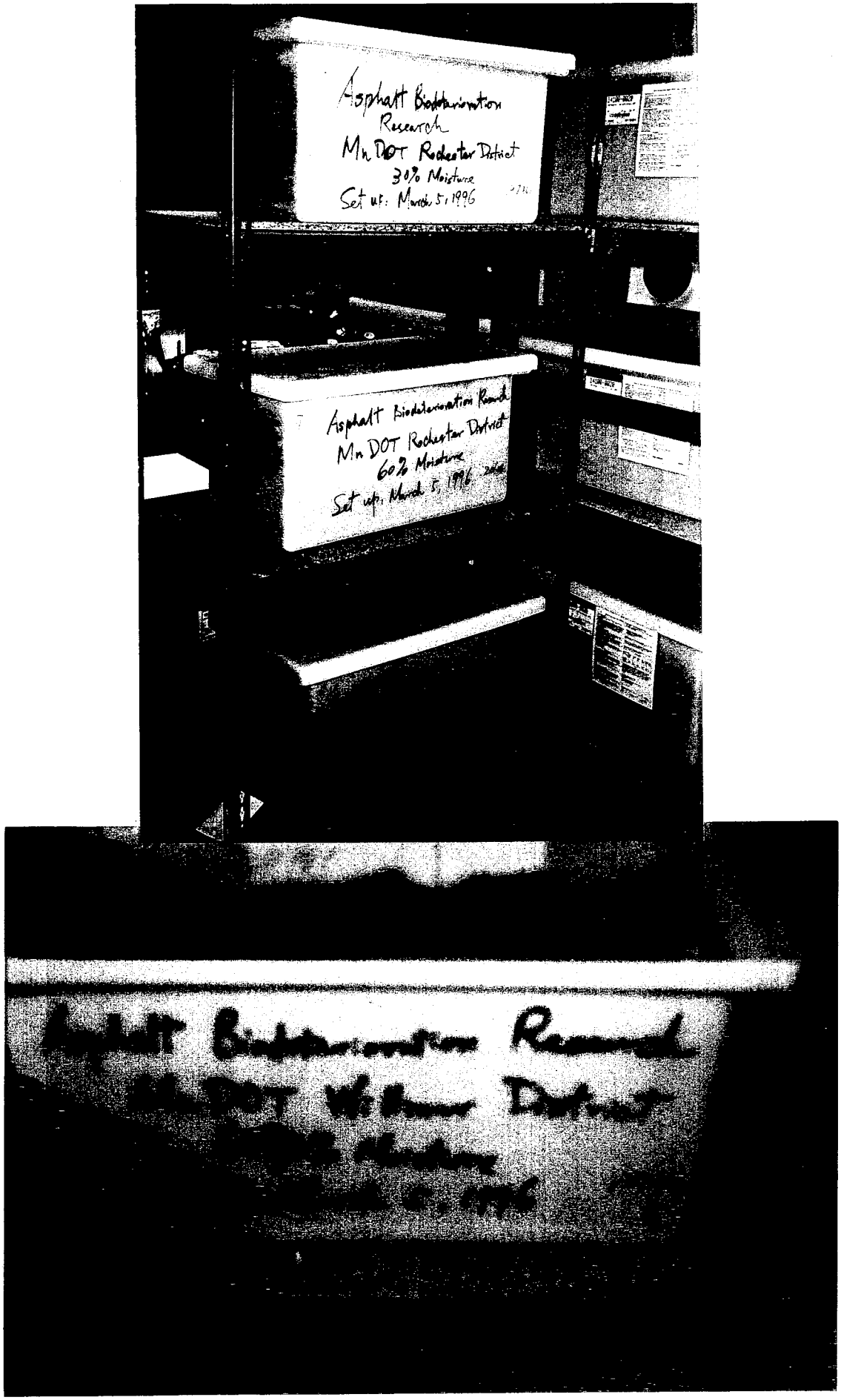


Fig. 3. Schematics (side and top view) of apparatus for asphalt biodeteroration/ biodegradation studies buried in soils.

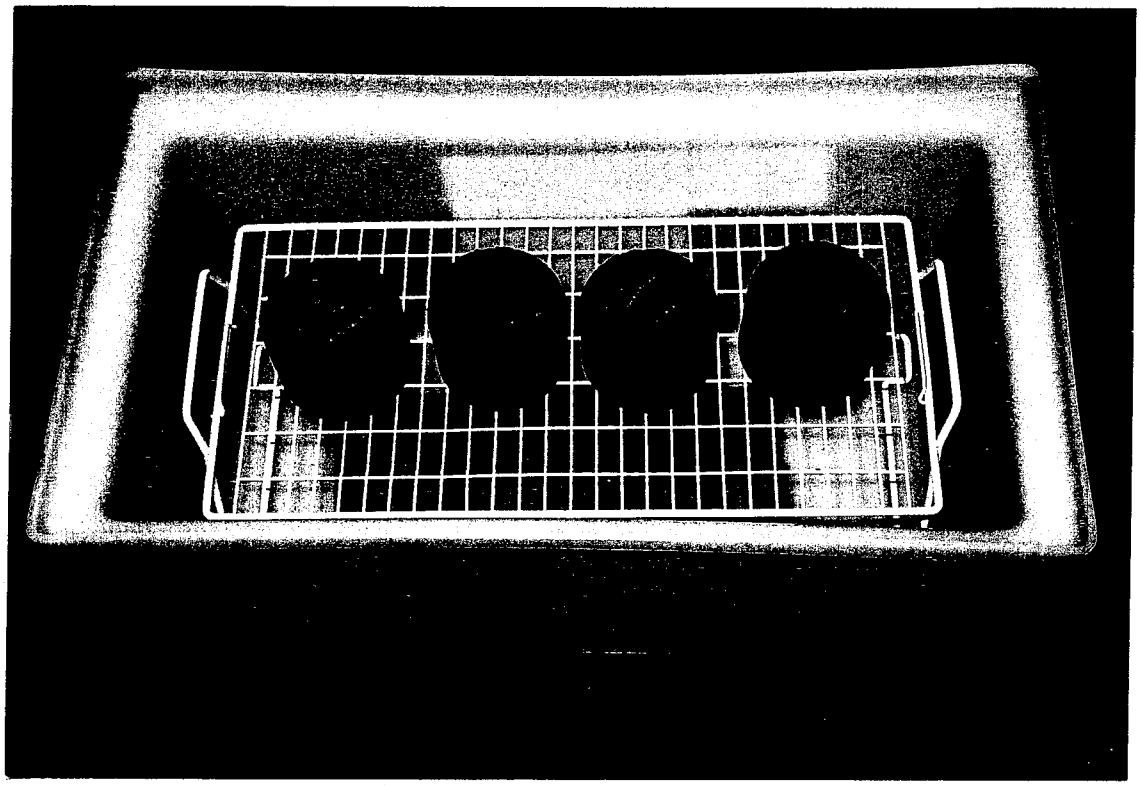


Fig. 4. Schematics (top view) illustrating the control experiment of Marshall pucks buried in soils test.

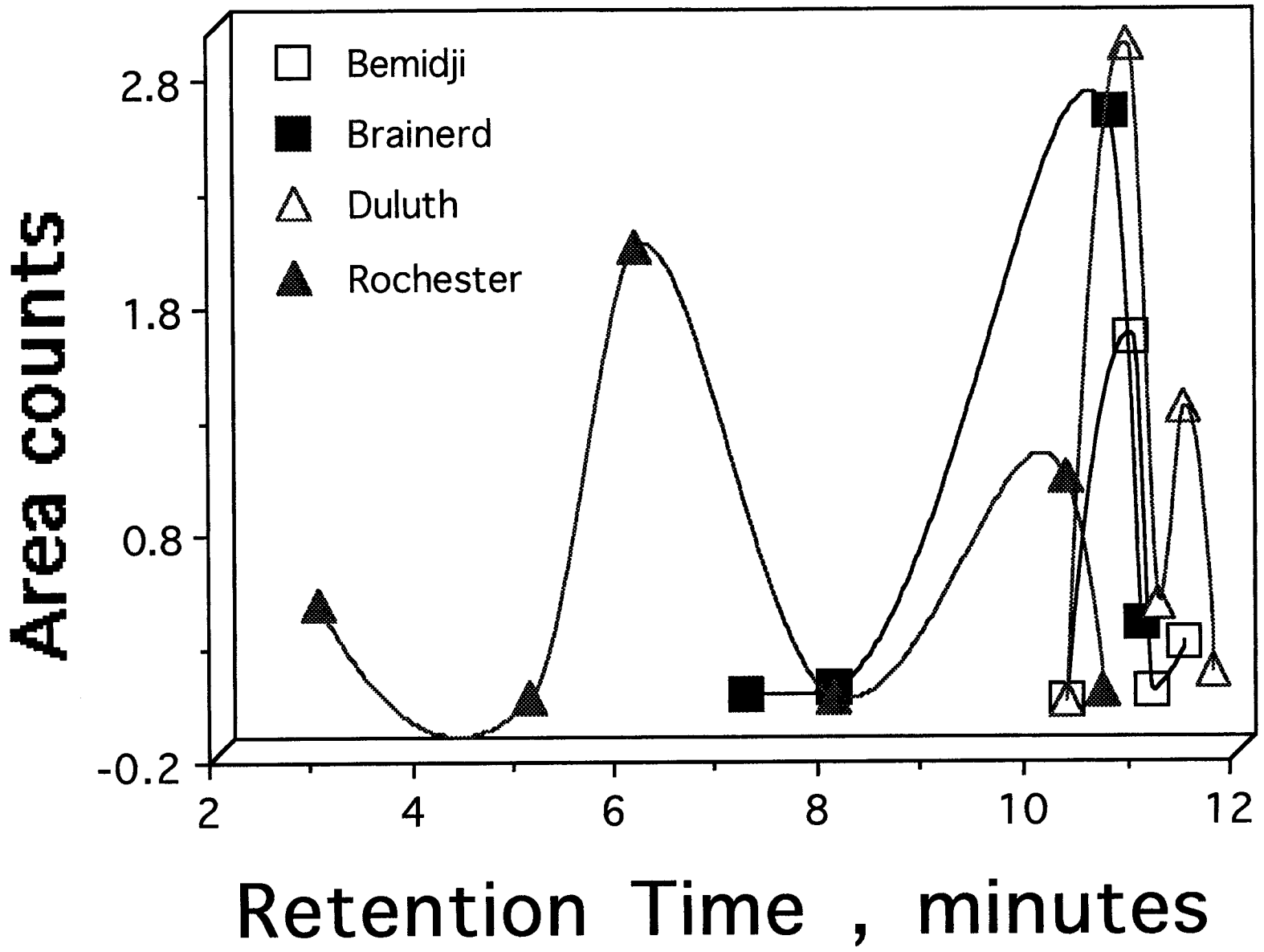


Fig. 5. HPLC analysis of soil samples from different District Labs.

Fig. 6. Soil sampling locations from tanks of Marshall pucks buried-in-soil test

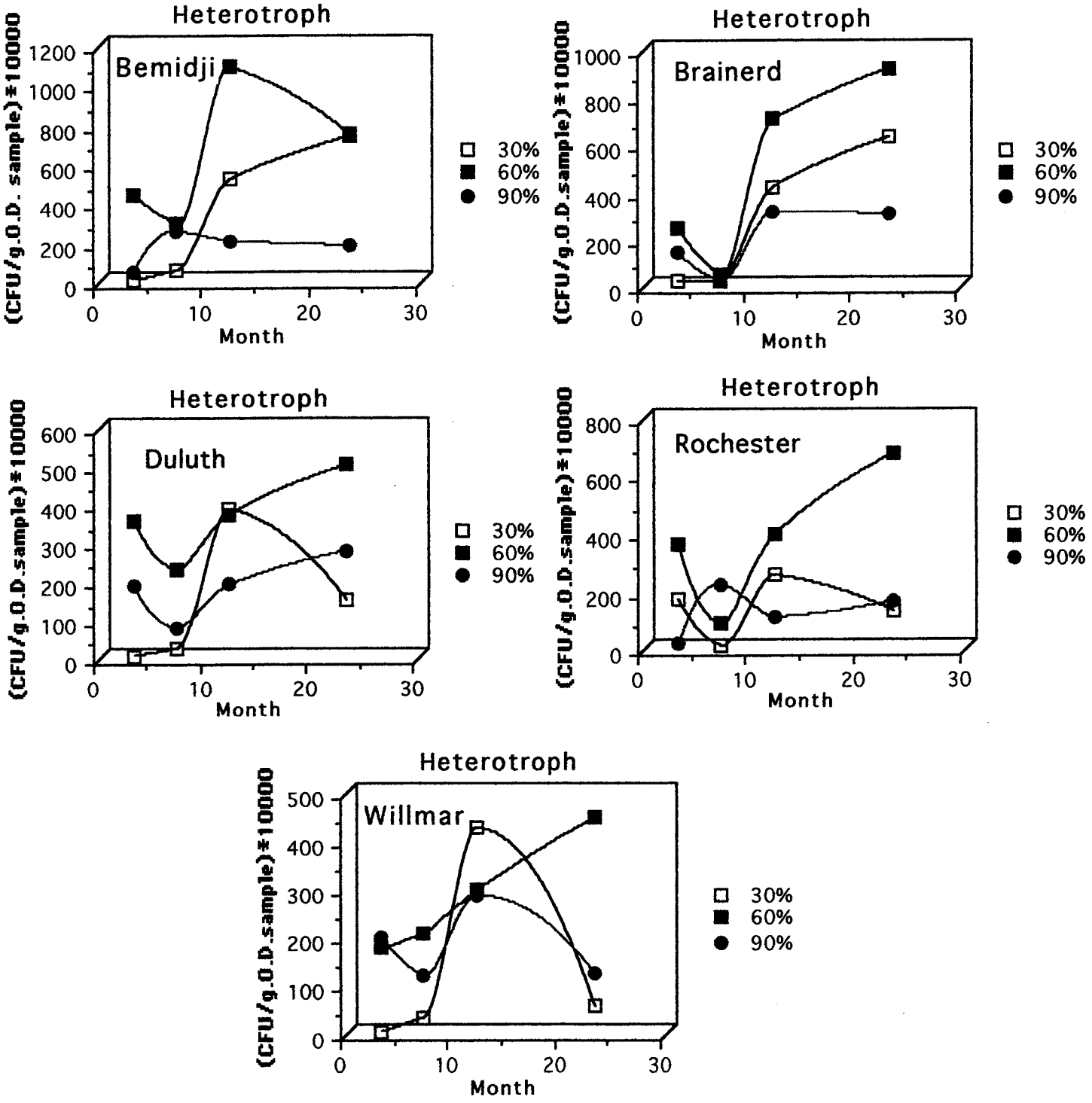


Fig. 7. Viability testing curve for heterotrophs in Marshall pucks after buried for 3, 7, 12 and 23 months in soils collected from five District Labs with different water holding capacities. The number of heterotrophs in control puck varying from 90 to 120 CFU/g.O.D. sample.

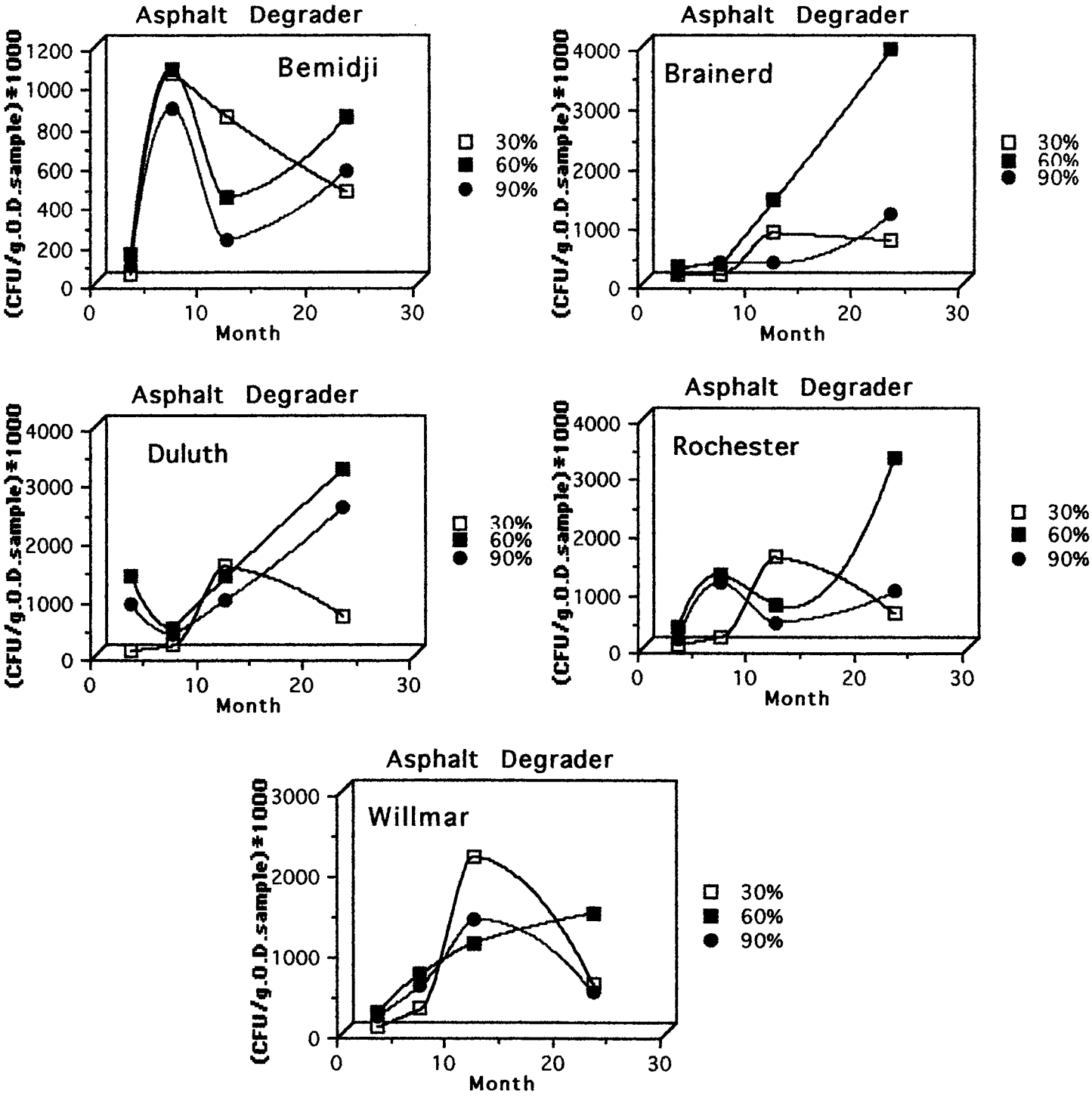


Fig. 8. Viability testing curve for asphalt degraders in Marshall pucks after buried for 3, 7, 12 and 23 months in soils collected from five District labs with different water holding capacities. The number of asphalt degraders in control pucks varying from 4,000 to 7,000 CFU/g.O.D. sample.

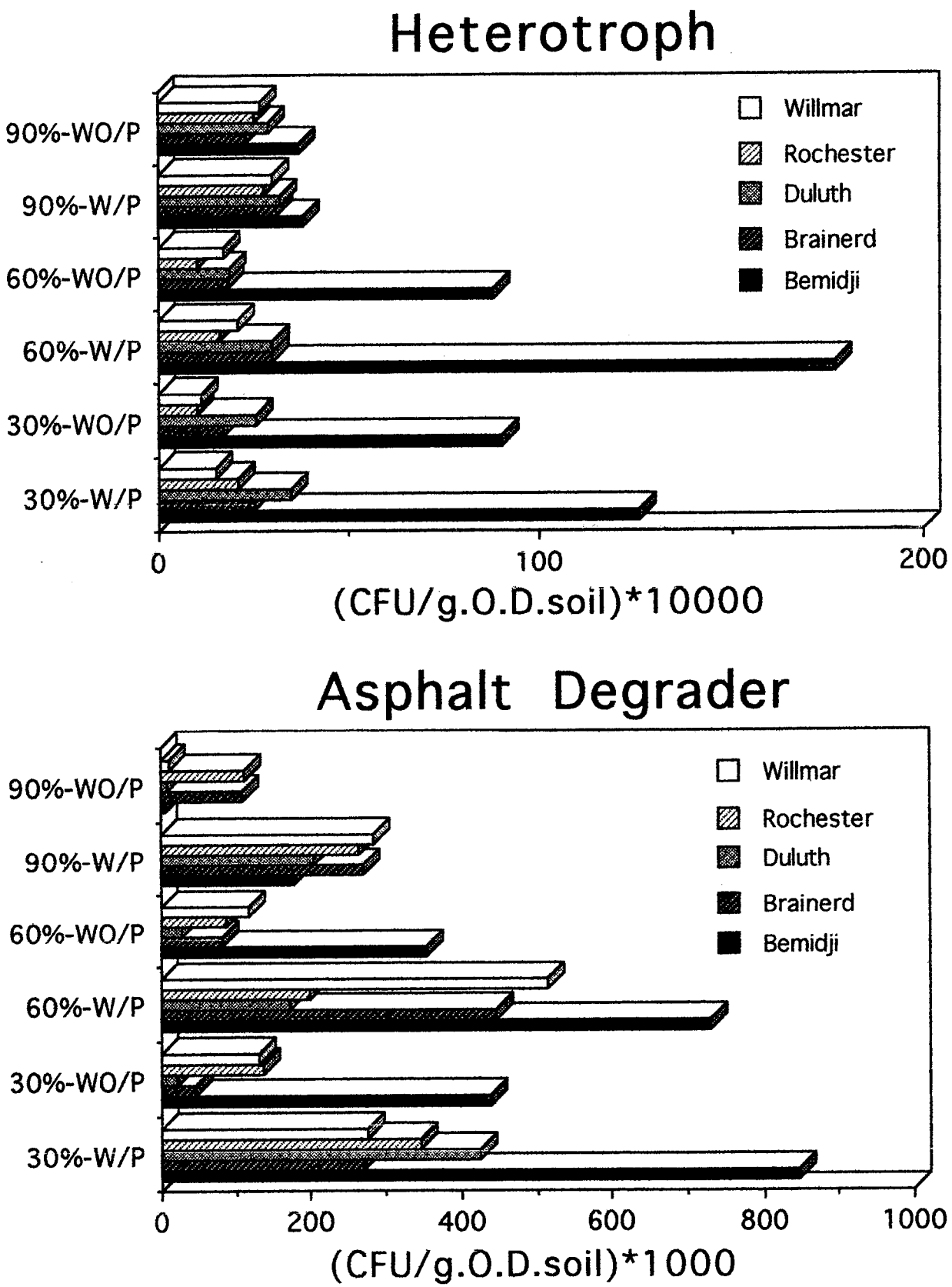


Fig. 9. Comparative numbers of heterotrophs and asphalt degraders in soils adjacent to the buried Marshall pucks with an undisturbed soil after Marshall pucks had been buried in soils for 23 months with different water holding capacities.

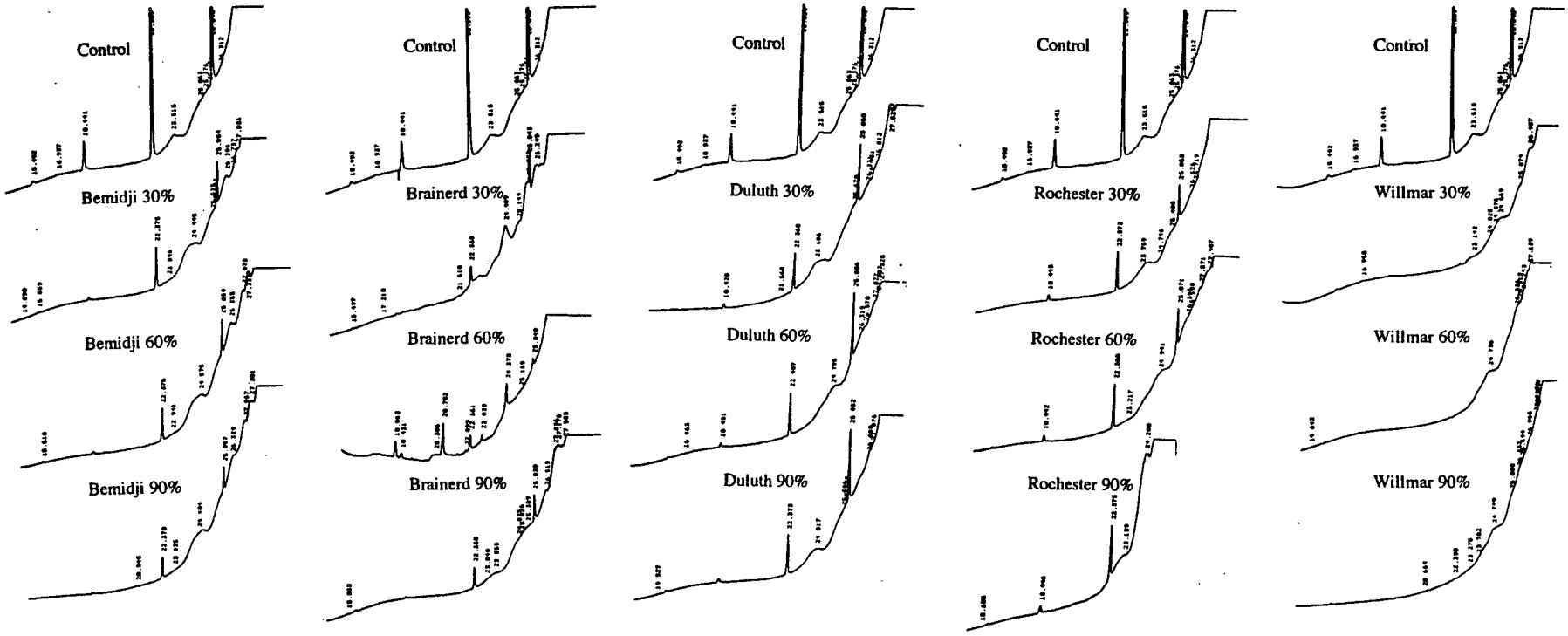


Fig. 10. The chromatograms of GC analysis in Marshall Pucks after buried in soils, collected from five District Labs or left in containers for the control, for three months at three water holding capacities.

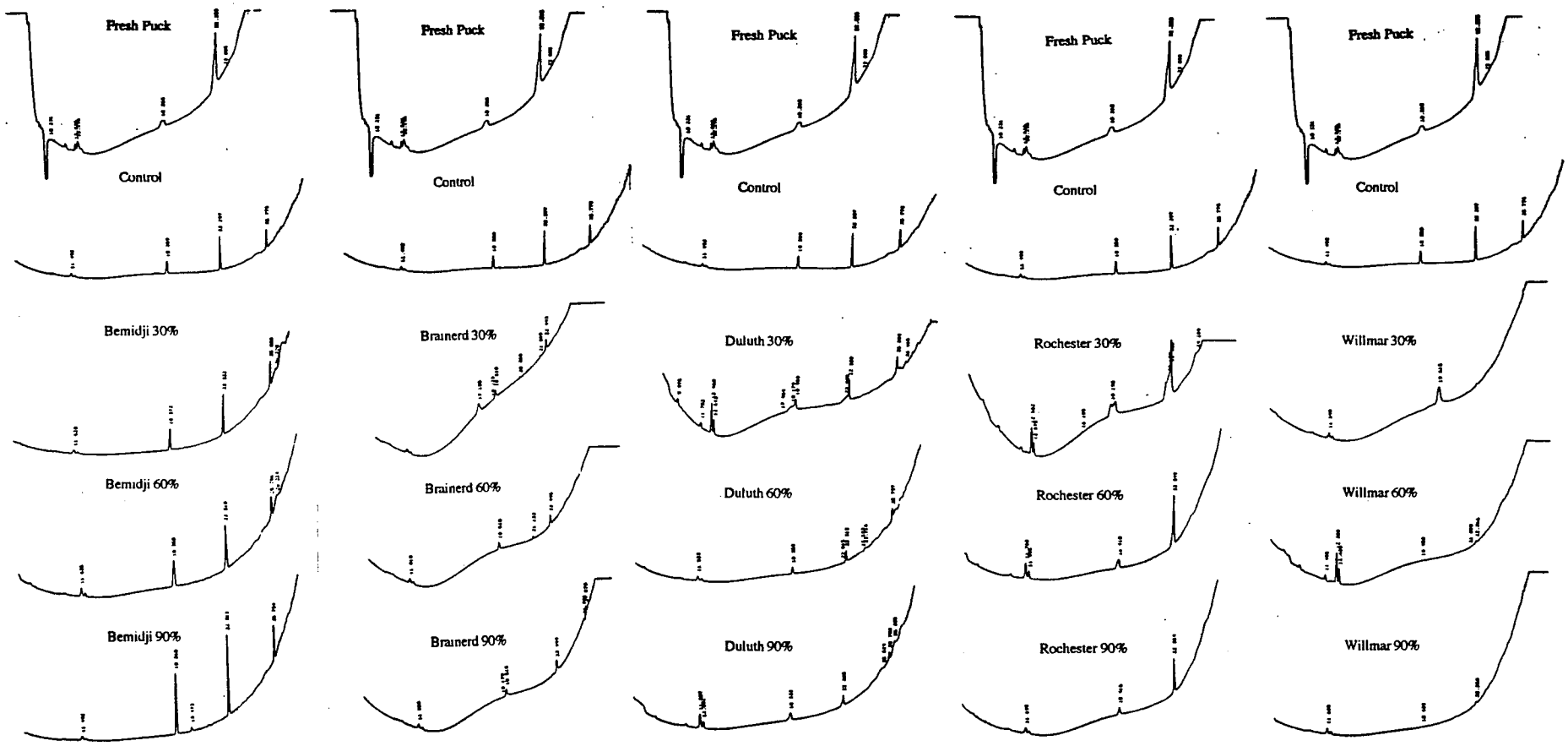


Fig. 11. Chromatograms of GC analysis of Marshall Pucks after buried in soils, collected from five District Labs, or left in containers from control, for seven months at three different water holding capacities and compared with fresh puck extract.

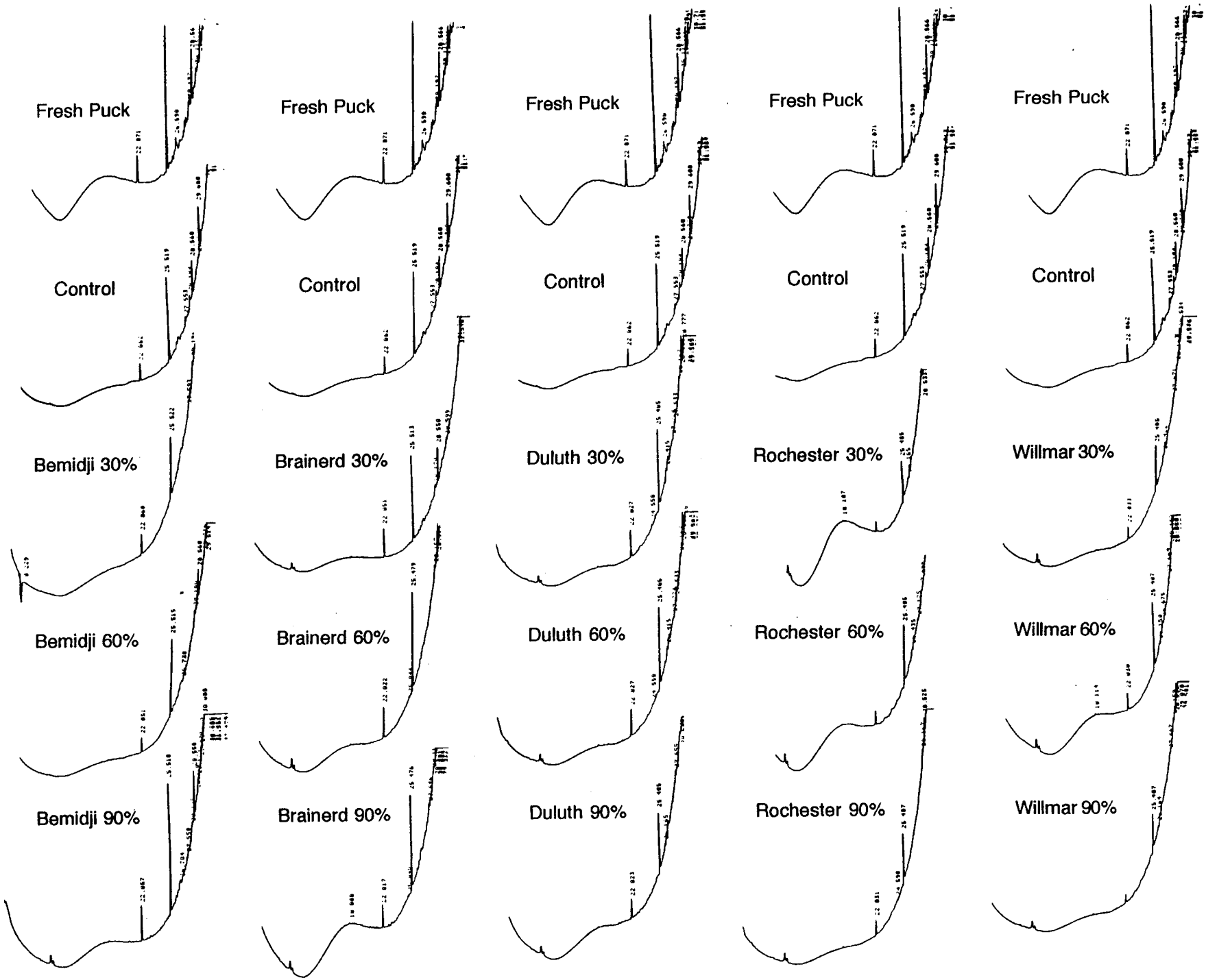


Fig. 12. Chromatograms of GC analysis of Marshall Pucks after buried in soils, collected from five District Labs, or left in containers for control, for twelve months at three different water holding capacities and compared with fresh puck extract.

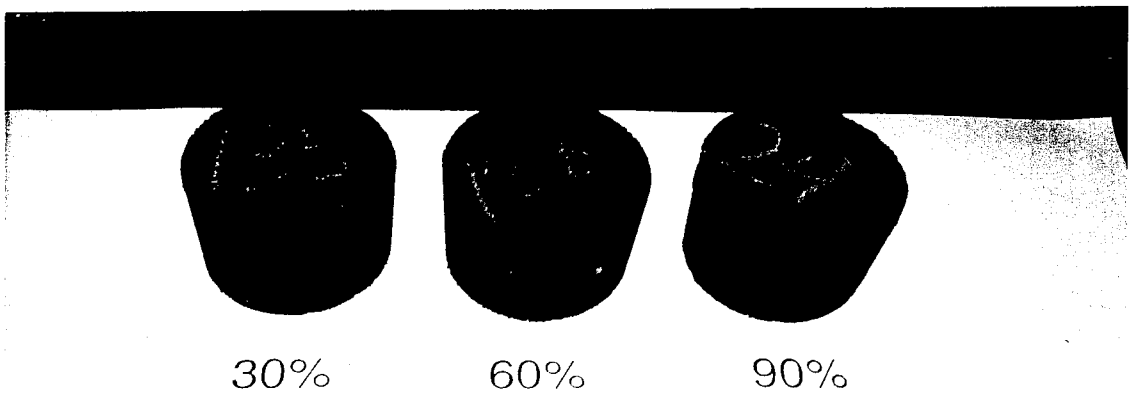


Fig. 13. Molds and bacteria adhering to the surface of the Marshall pucks after left in control containers for 12 months that were maintained at various humidity (30%, 60%, and 90%).

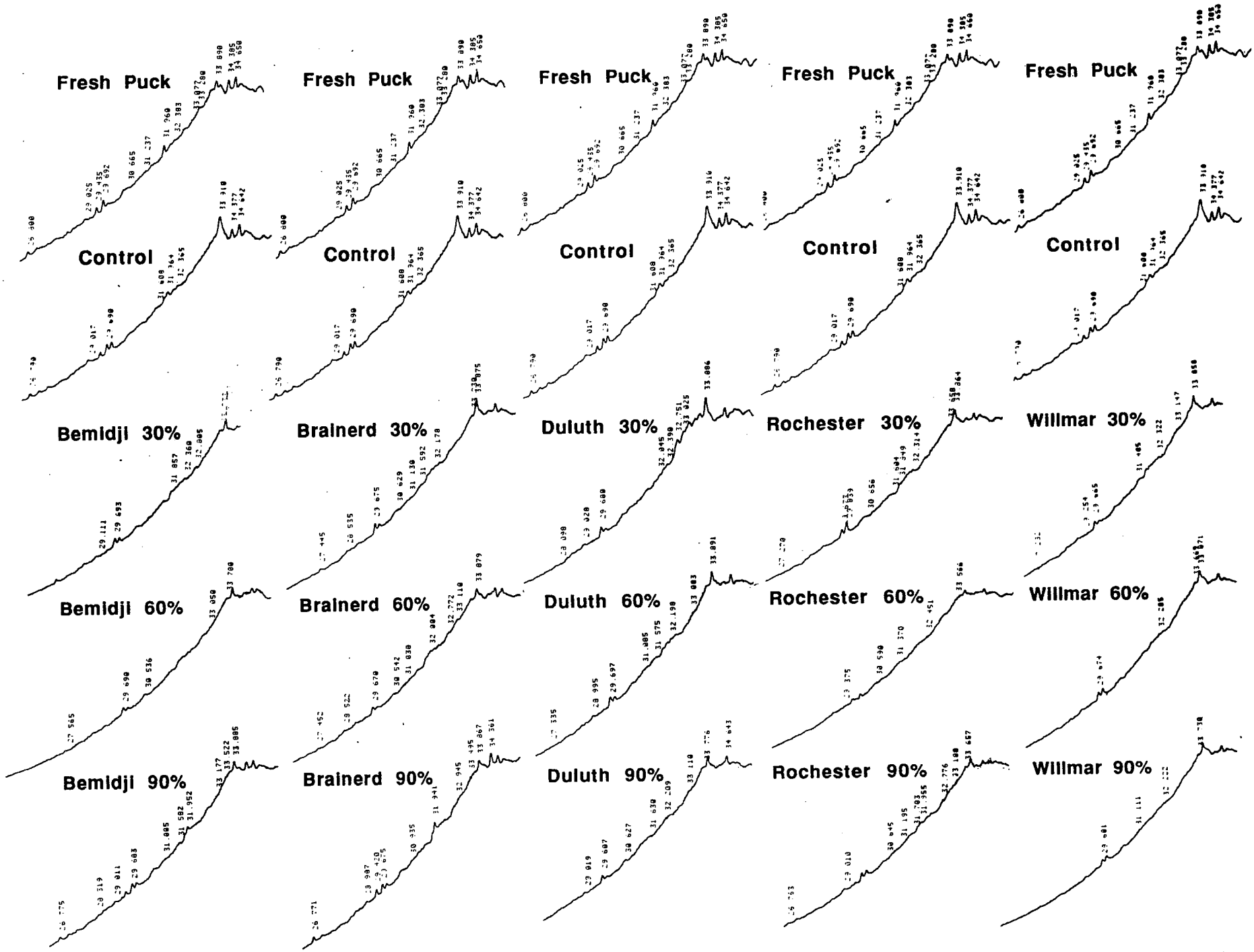


Fig. 14. Chromatograms of GC analysis of Marshall Pucks after buried in soils, collected from five District Labs, or left in containers for control, for 23 months at three different water holding capacities and compared with fresh puck extract

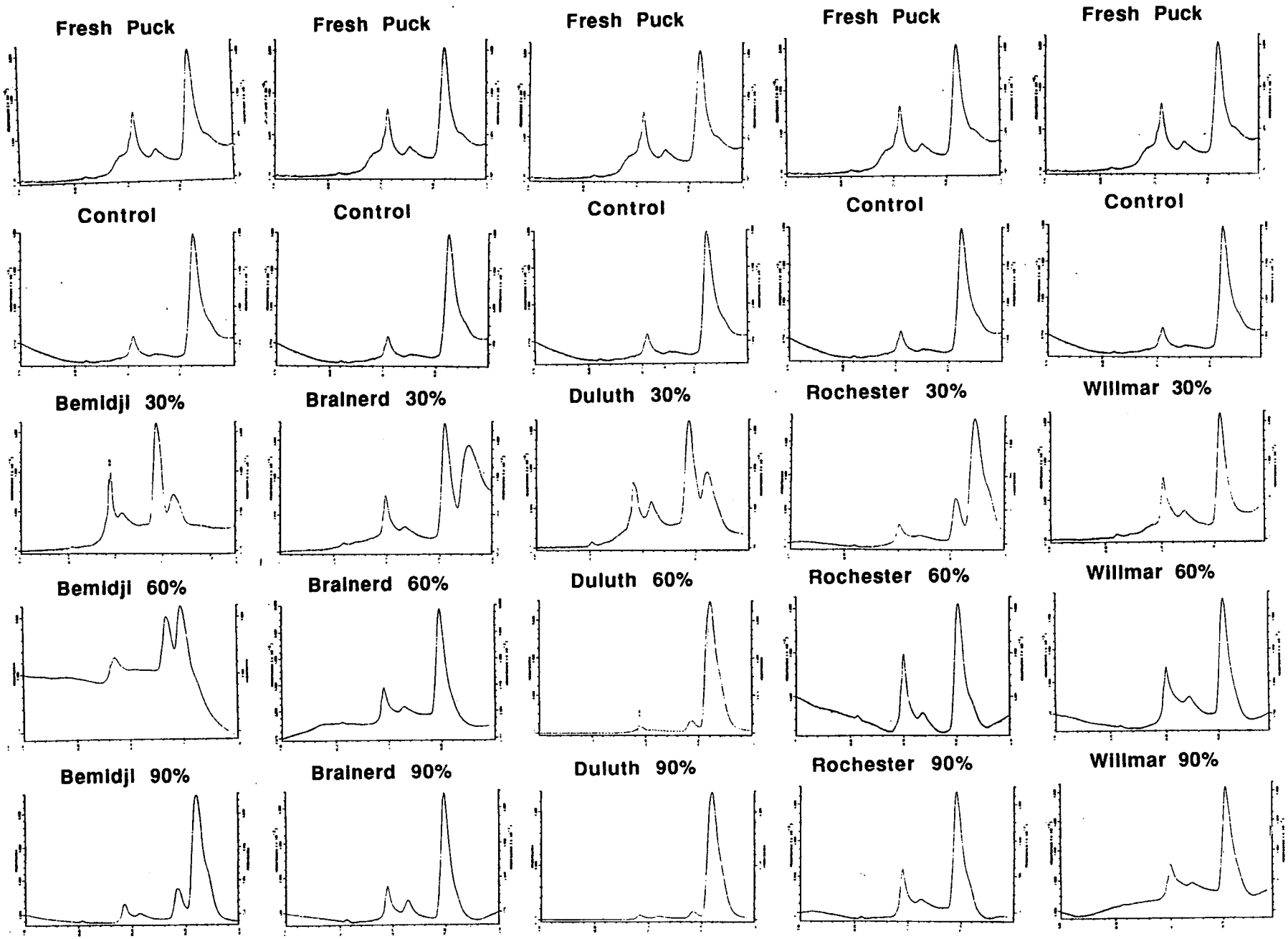
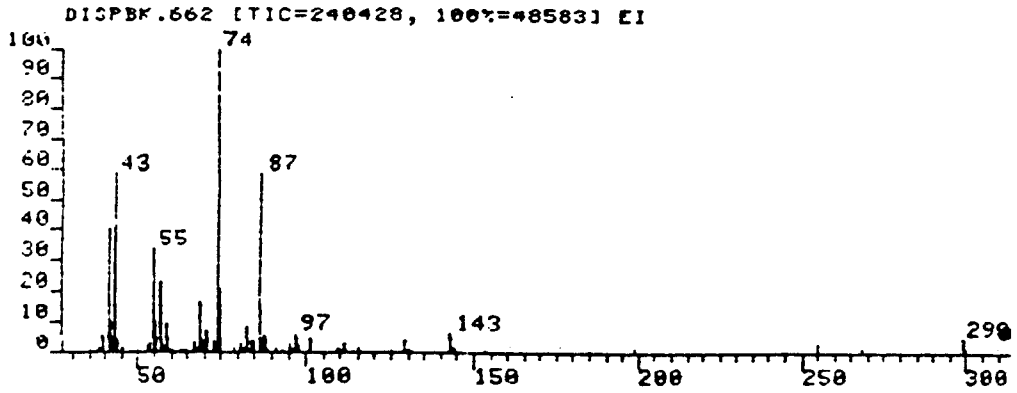
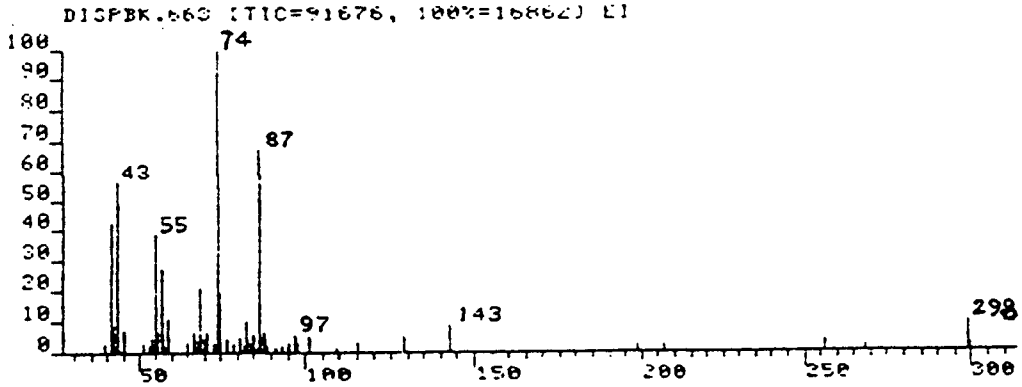


Fig. 15. The chromatograms of HPLC analysis in Marshall pucks after buried in soils, collected from five District labs or left in containers for the control, for seven months at three water holding capacities and compared with fresh puck extract.

(1) Methanol Control



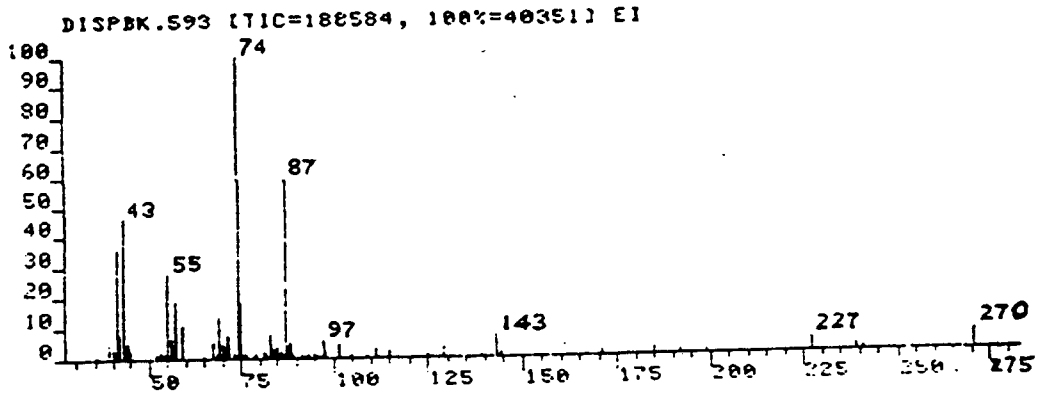
(2) Methanol Treated



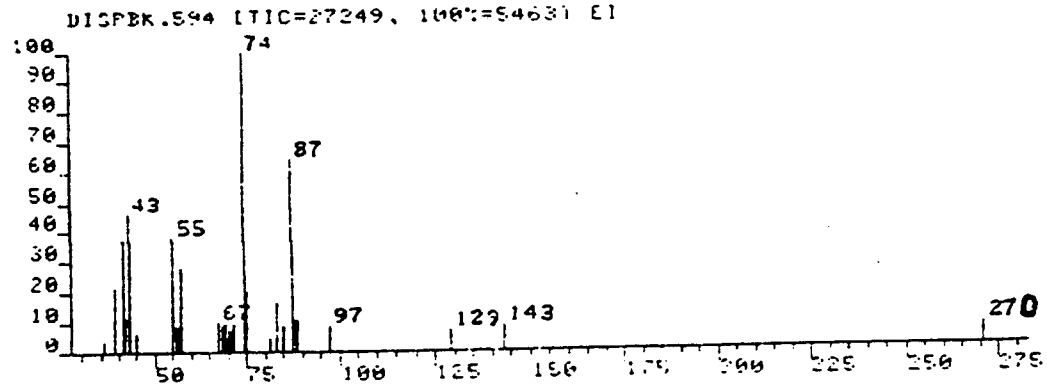
Methyl Sterate $C_{19}H_{38}O_2$ **MW: 298**

Fig. 16 (1).Mass Spectrometry MS Analysis (1) Methyl Sterate

(3) Methanol Control



(4) Methanol Treated



Hexadecanoil acid $C_{17}H_{34}O_2$ MW: 270

Fig. 16 (2).MS Analysis (2) Hexadecanoic acid

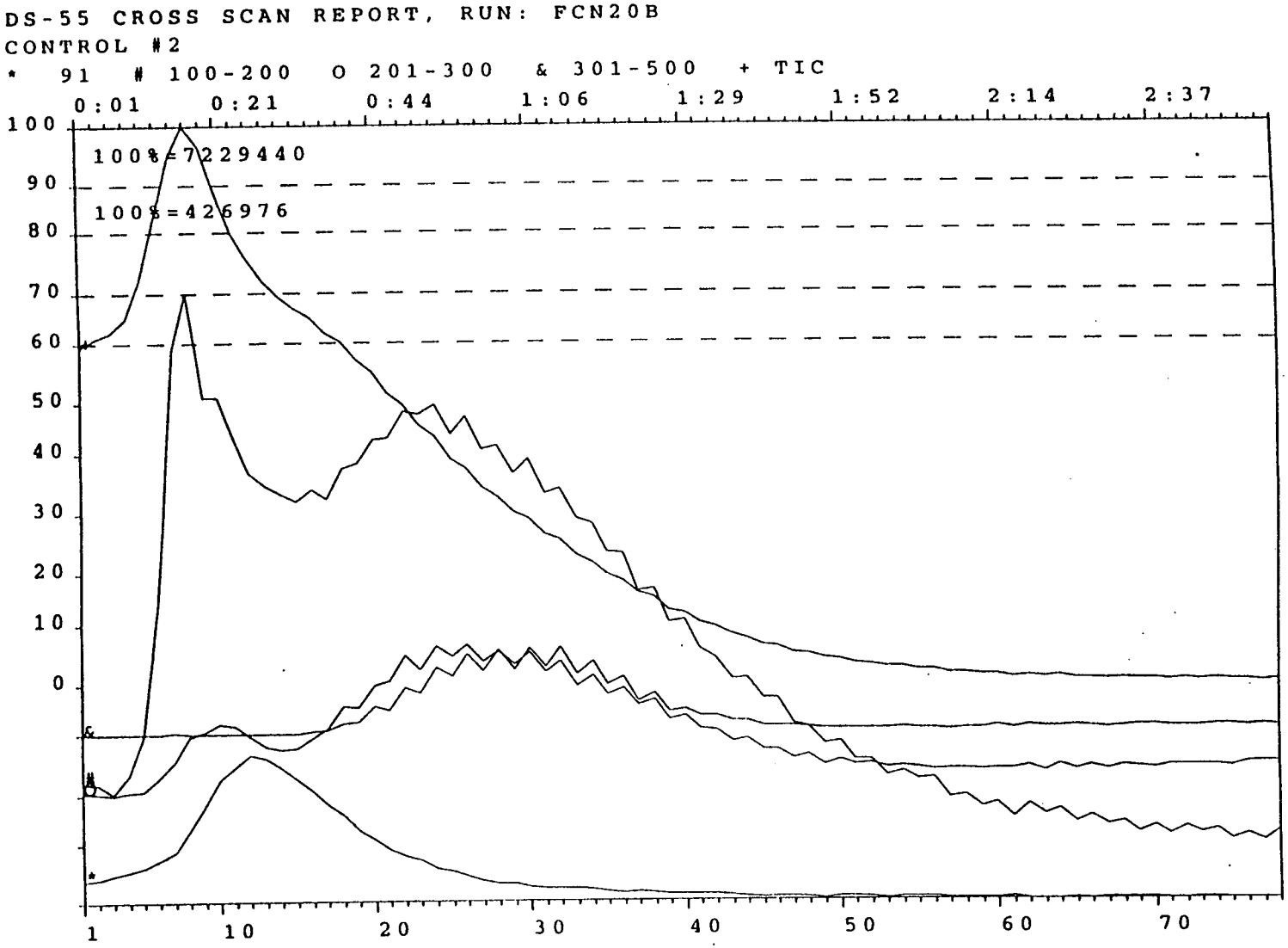


Fig. 17 (1).MS Analysis (1) Toluene Control

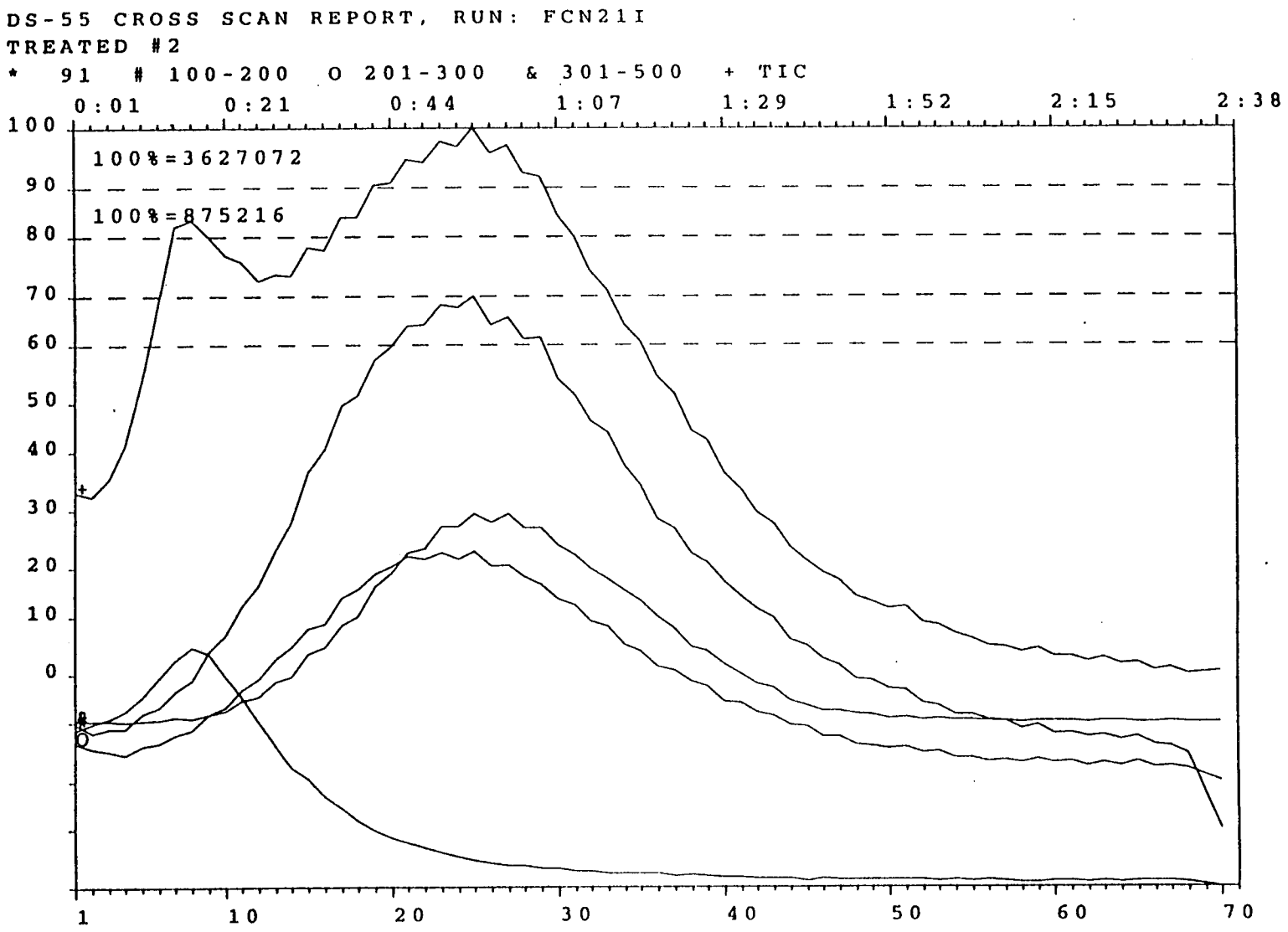


Fig. 17 (2).MS Analysis (2) Toluene Treated

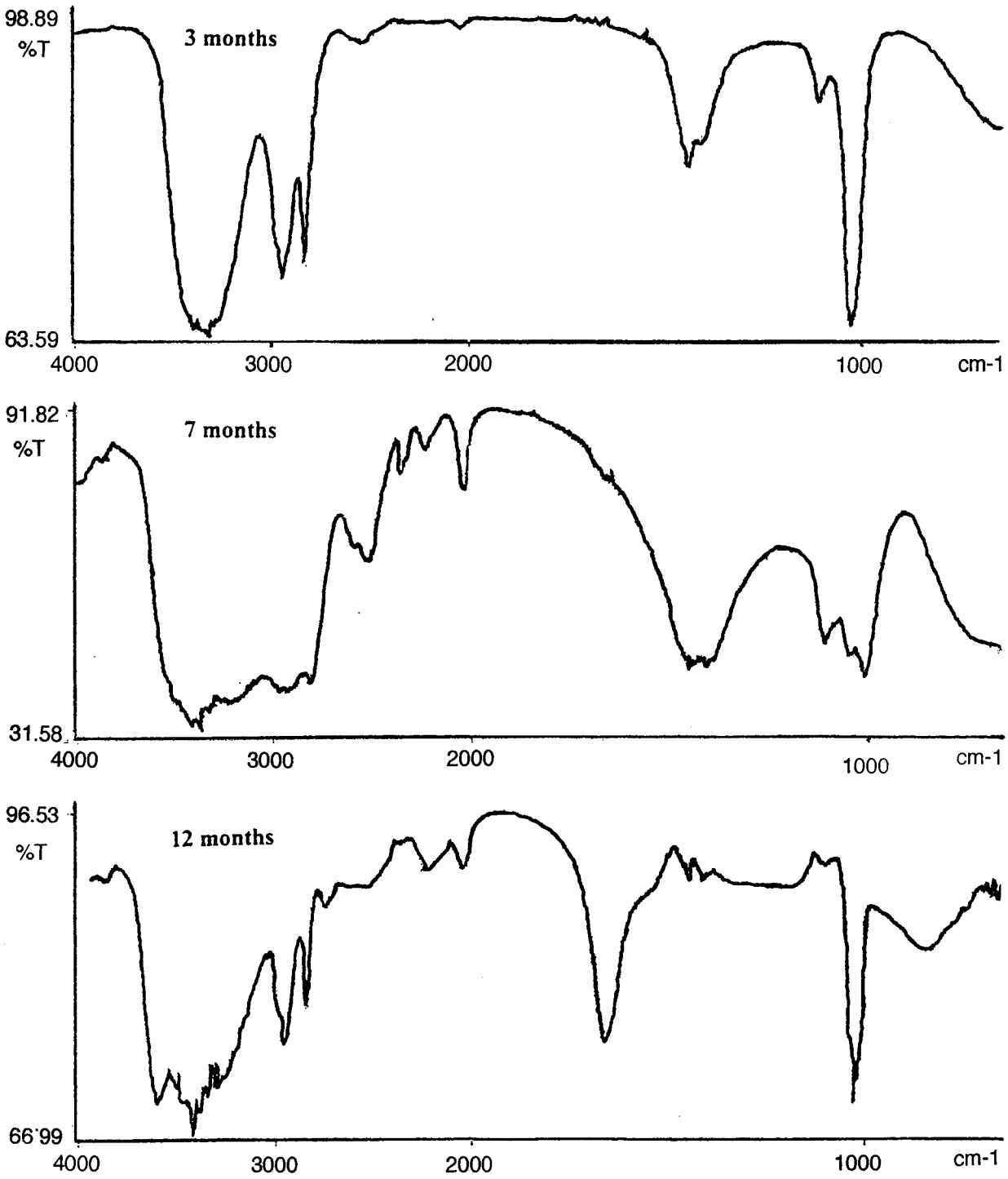


Fig. 18. IR Analysis (1) Control.

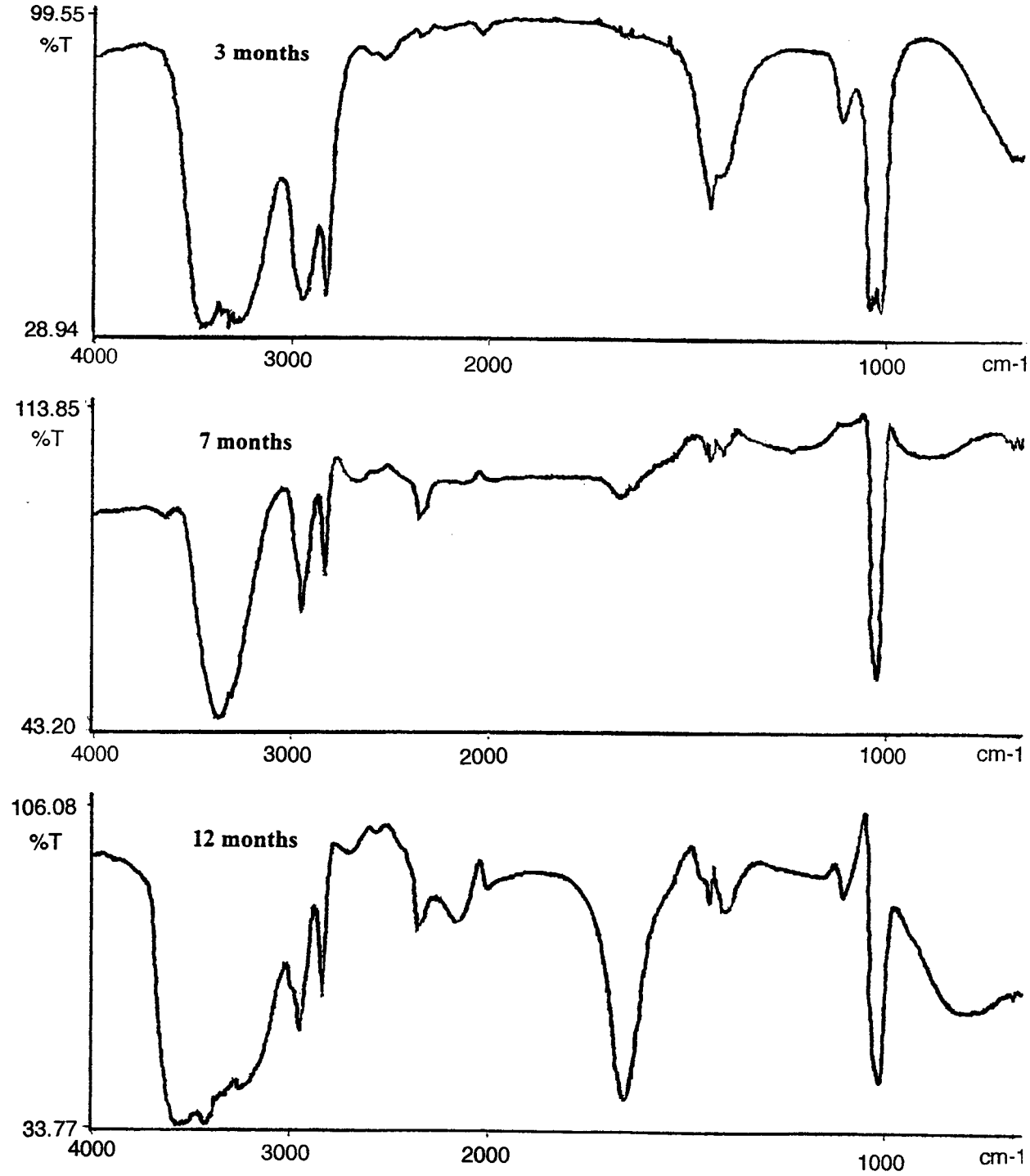


Fig. 19. IR Analysis (2) Bemidji 60%

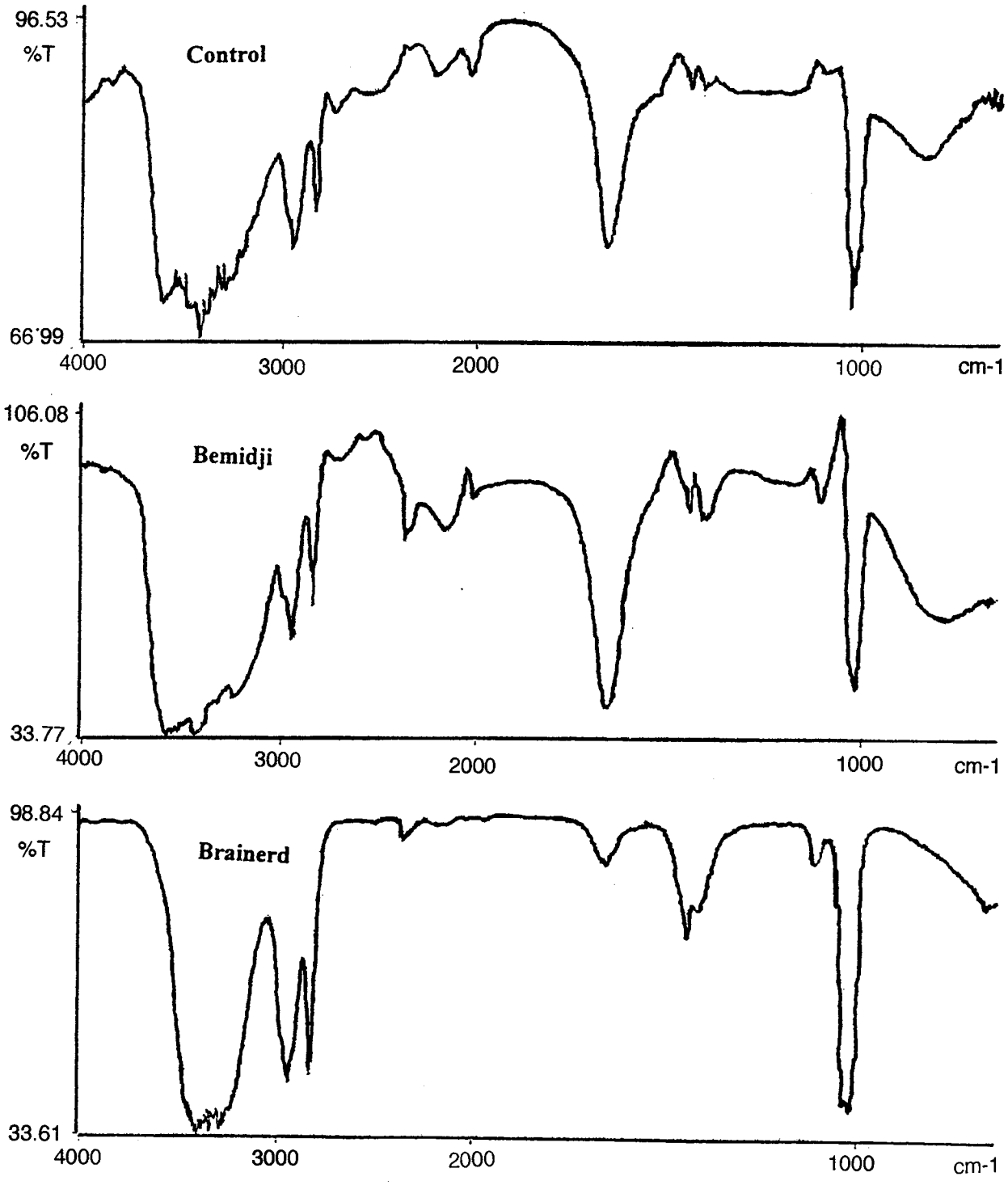


Fig. 20. IR Analysis (3) buried in soil for 12 months at 60% water holding capacity

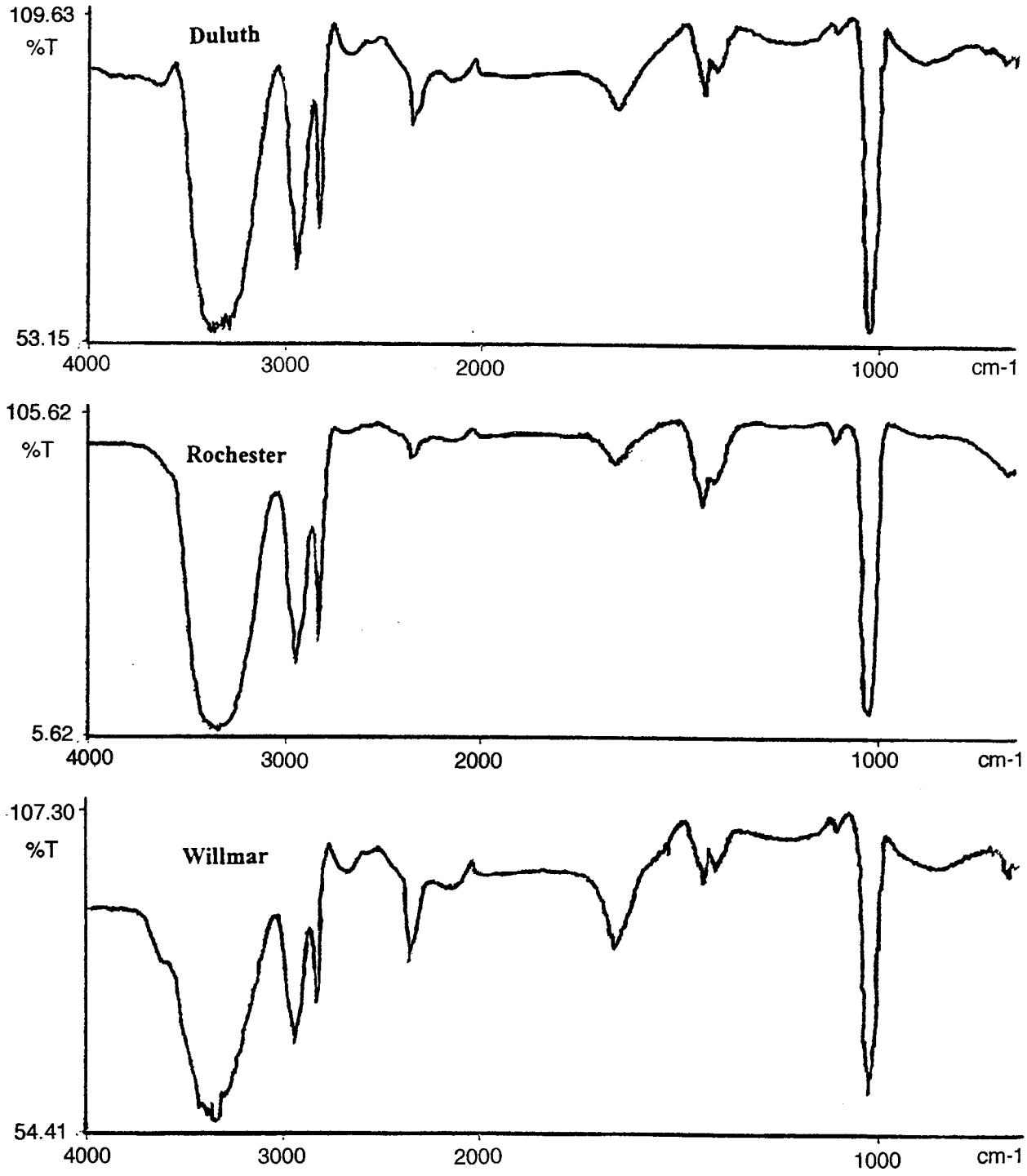


Fig. 21. IR Analysis buried in soils of Duluth, Rochester and Willmar at 60% water holding capacity

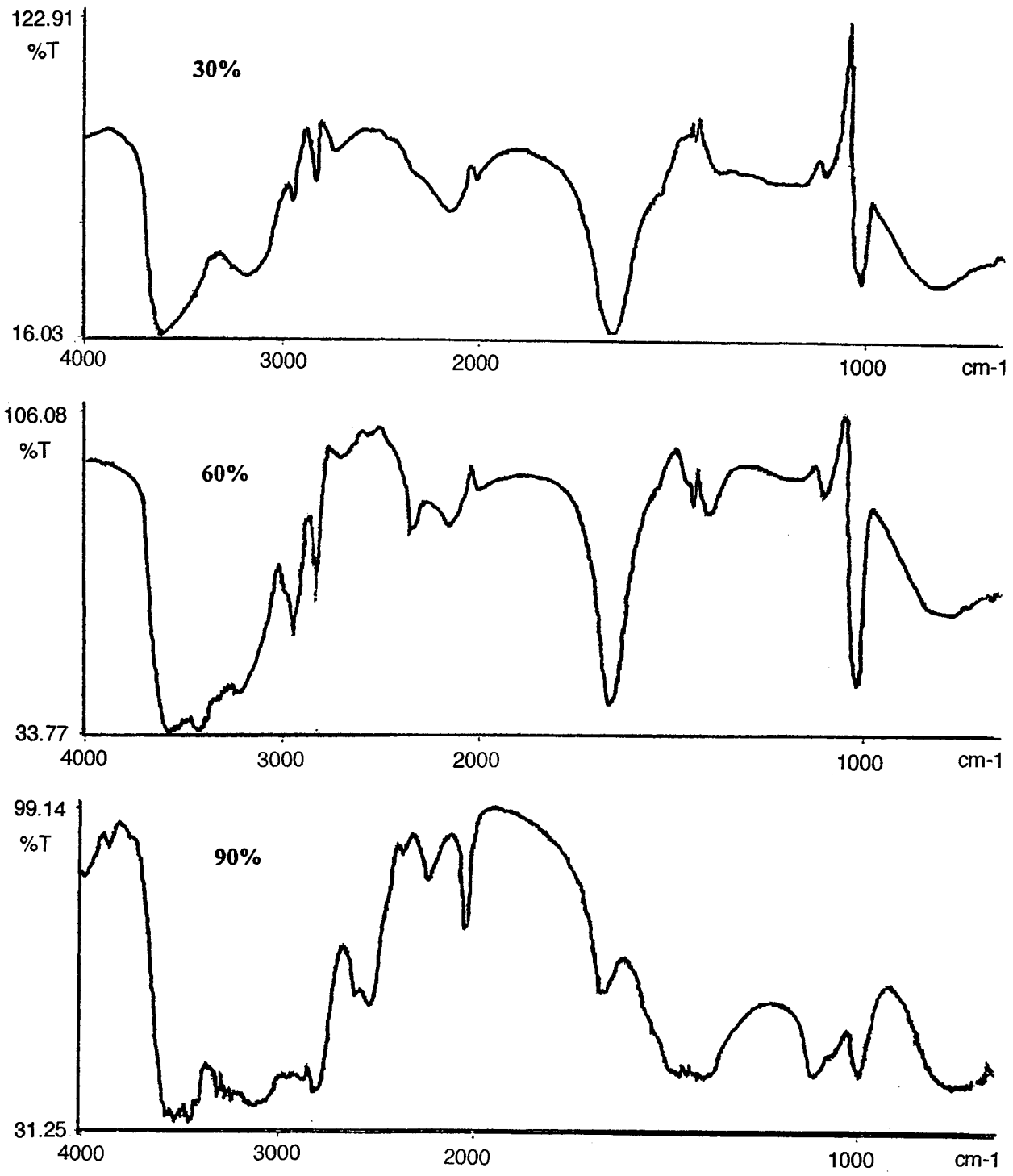


Fig. 22. IR Analysis buried in Bemidji soil for 12 months at 30%, 60% and 90% water holding capacity

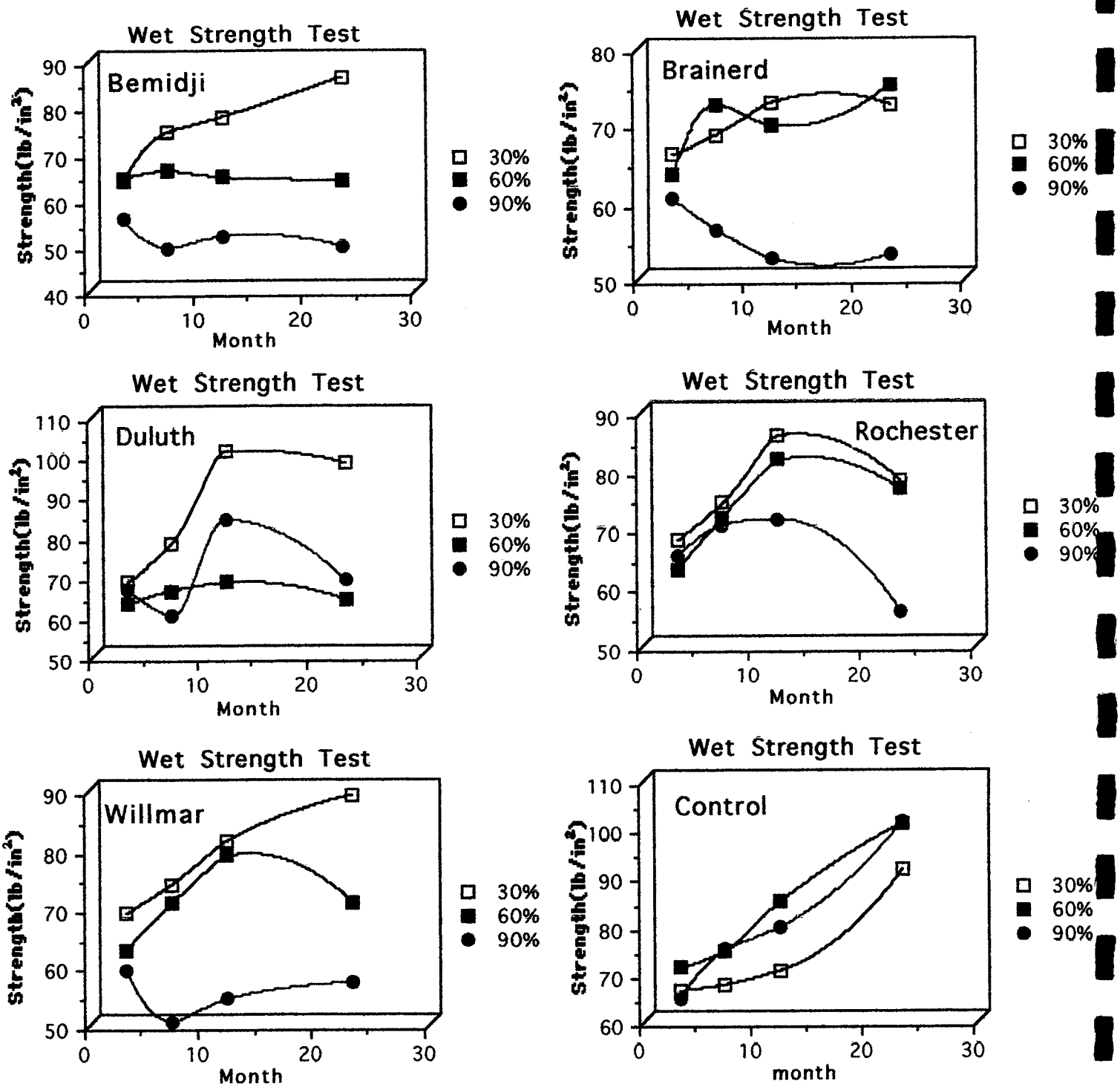


Fig. 23. Physical strength test of Marshall pucks buried in soils, collected from five Minnesota Districts, for 3, 7, 12 and 23 months with different water holding capacities.

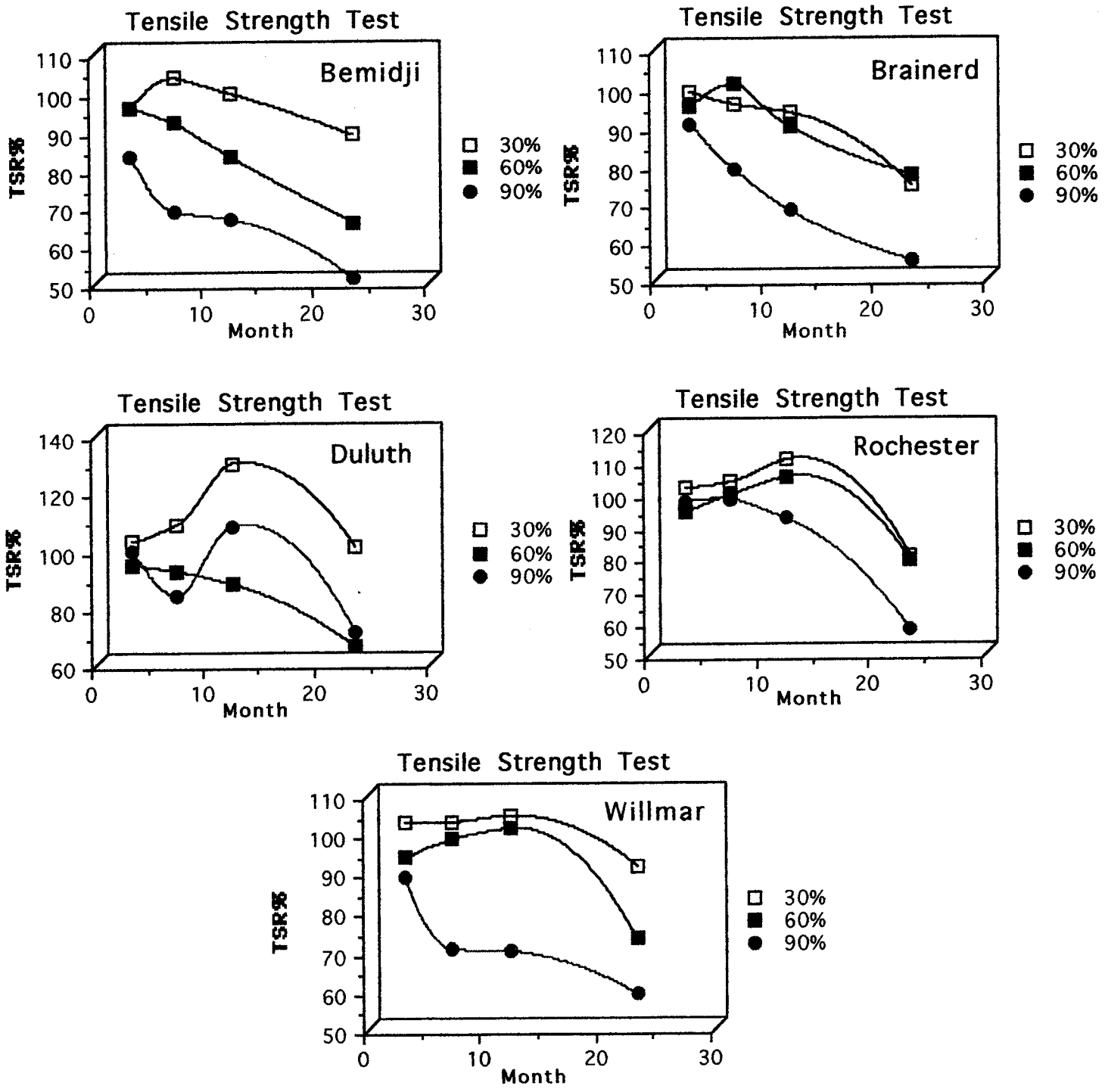


Fig. 24. Tensile strength of Marshall pucks buried in soils, collected from five Minnesota Districts, for 3, 7, 12 and 23 months with different water holding capacities. Control of TSR% was 100%.

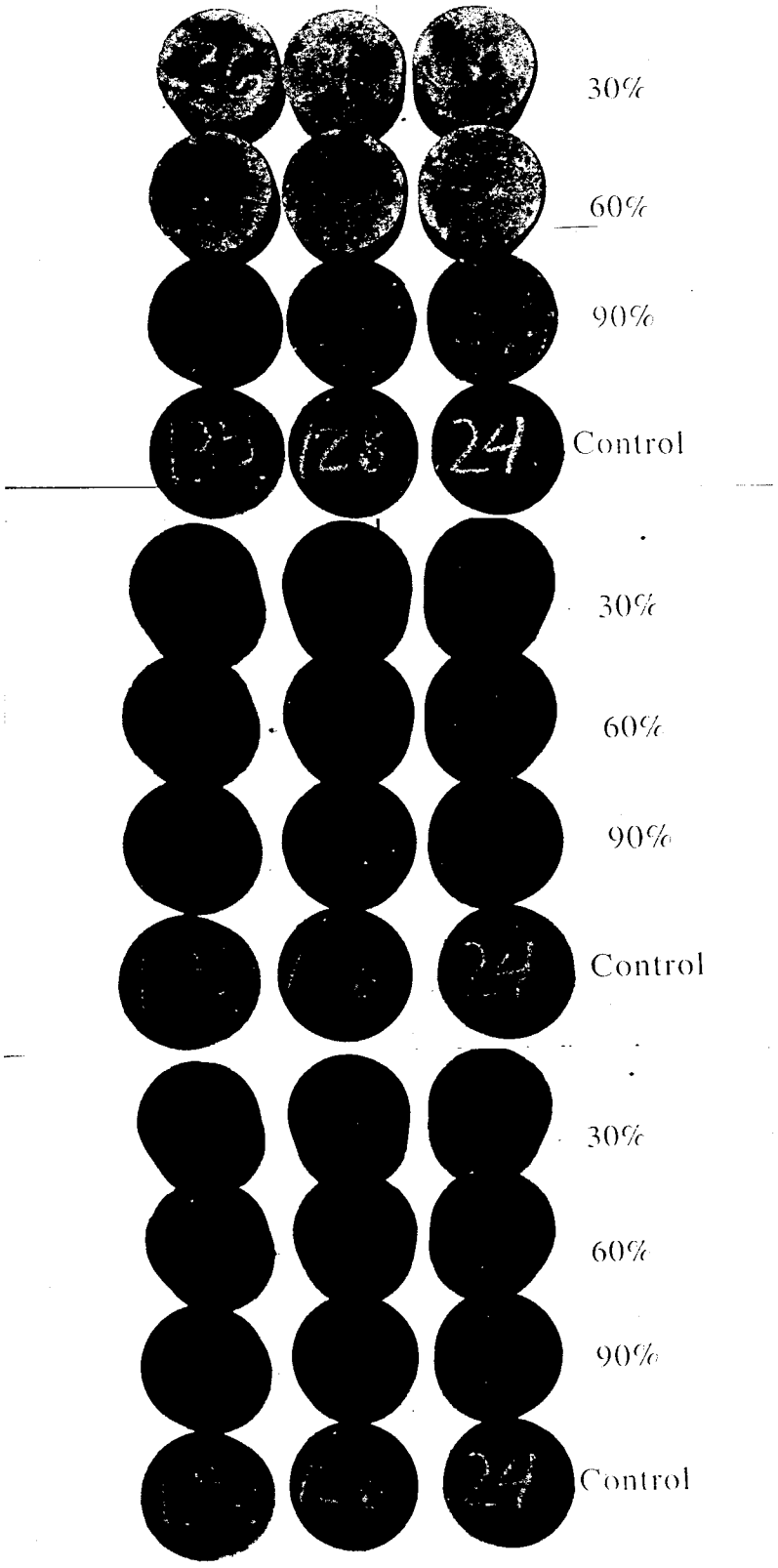


Fig. 25. Appearance of soil microbial attachment on Marshall pucks after had been buried in soils for 23 months at three different water holding capacities.

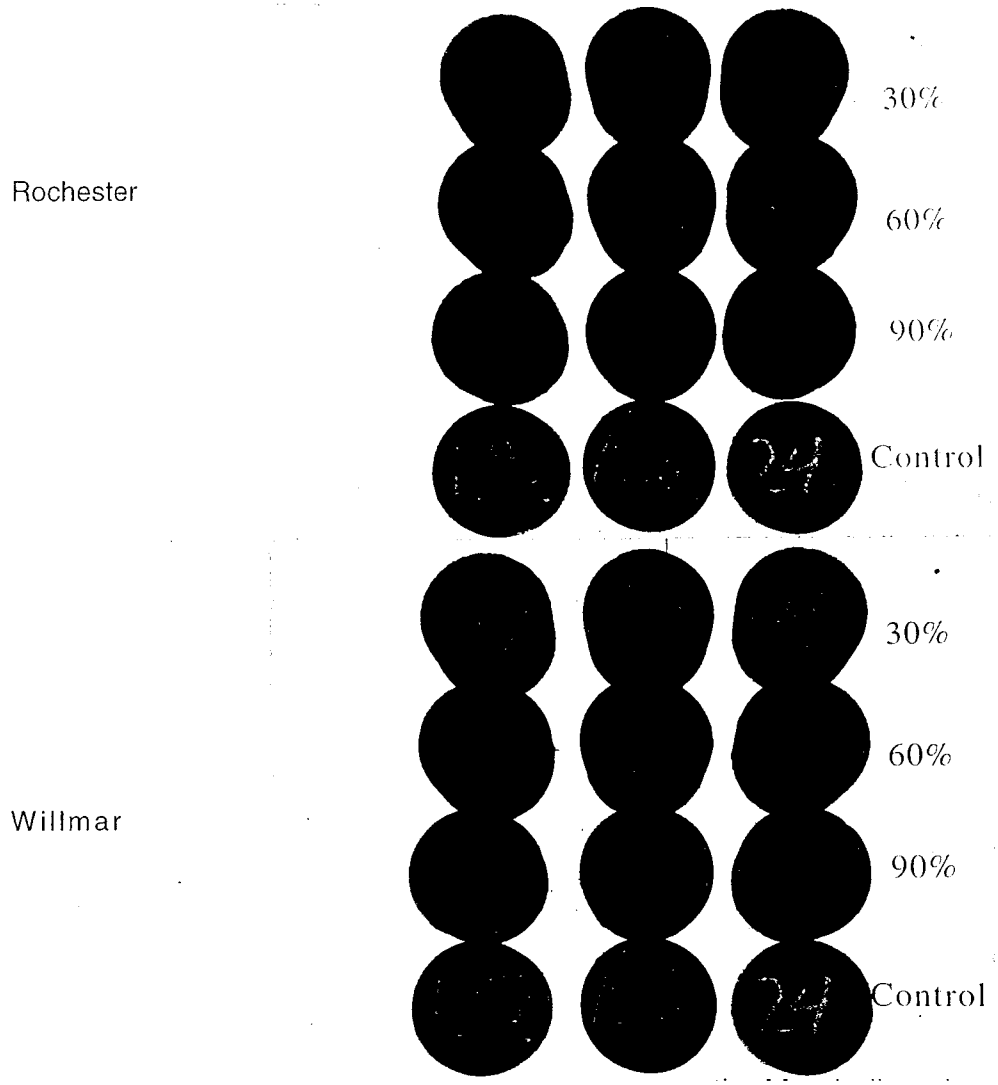


Fig. 26. Appearance of soil microbial attachment on Marshall pucks after had been buried in soils for 23 months at three different water holding capacities.

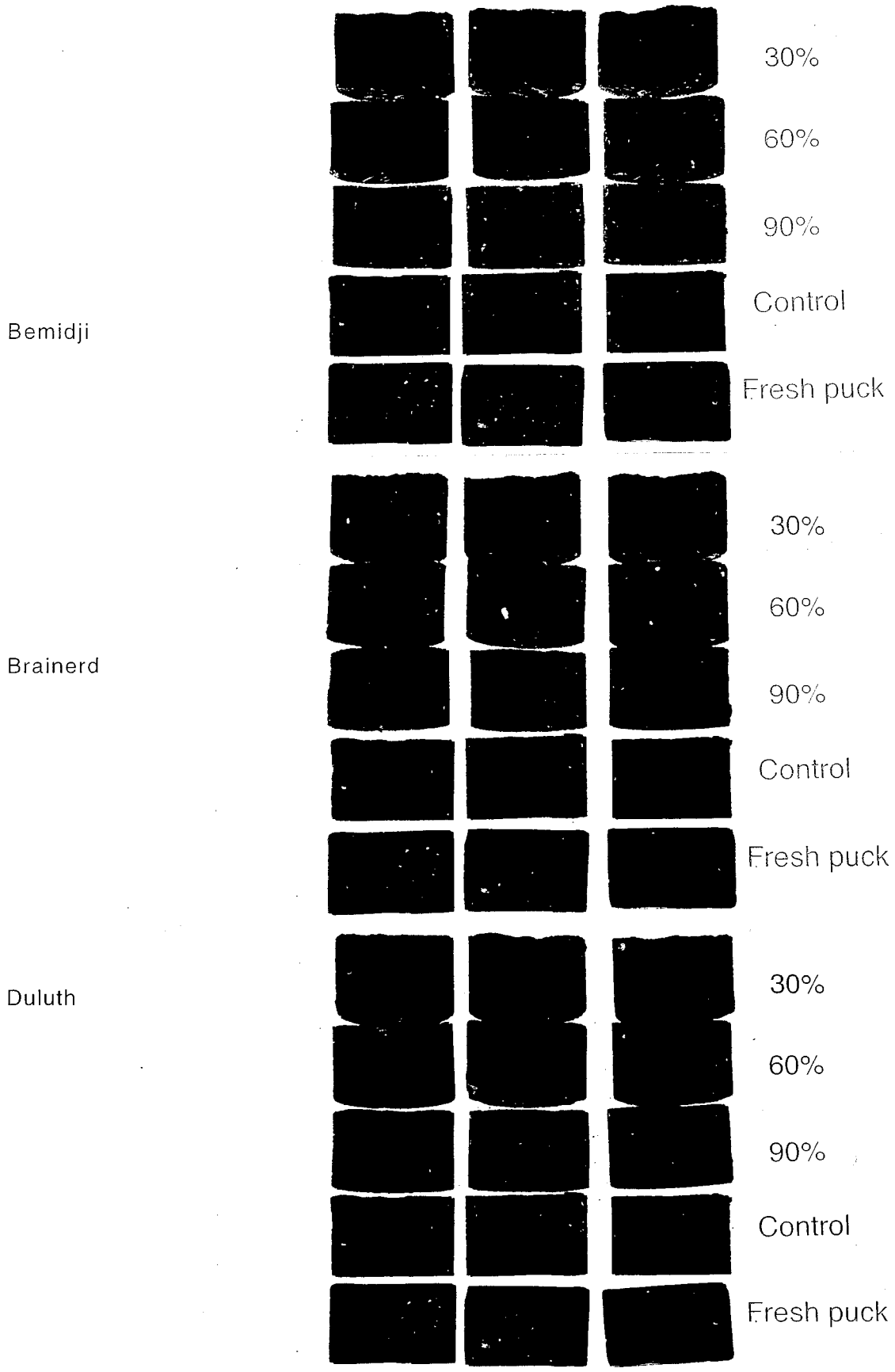


Fig. 27. Broken aggregate and visual stripping shown in cracked Marshall pucks surface after pucks had been buried in soil for 23 months with different water holding capacities. Compared with control samples and fresh puck.

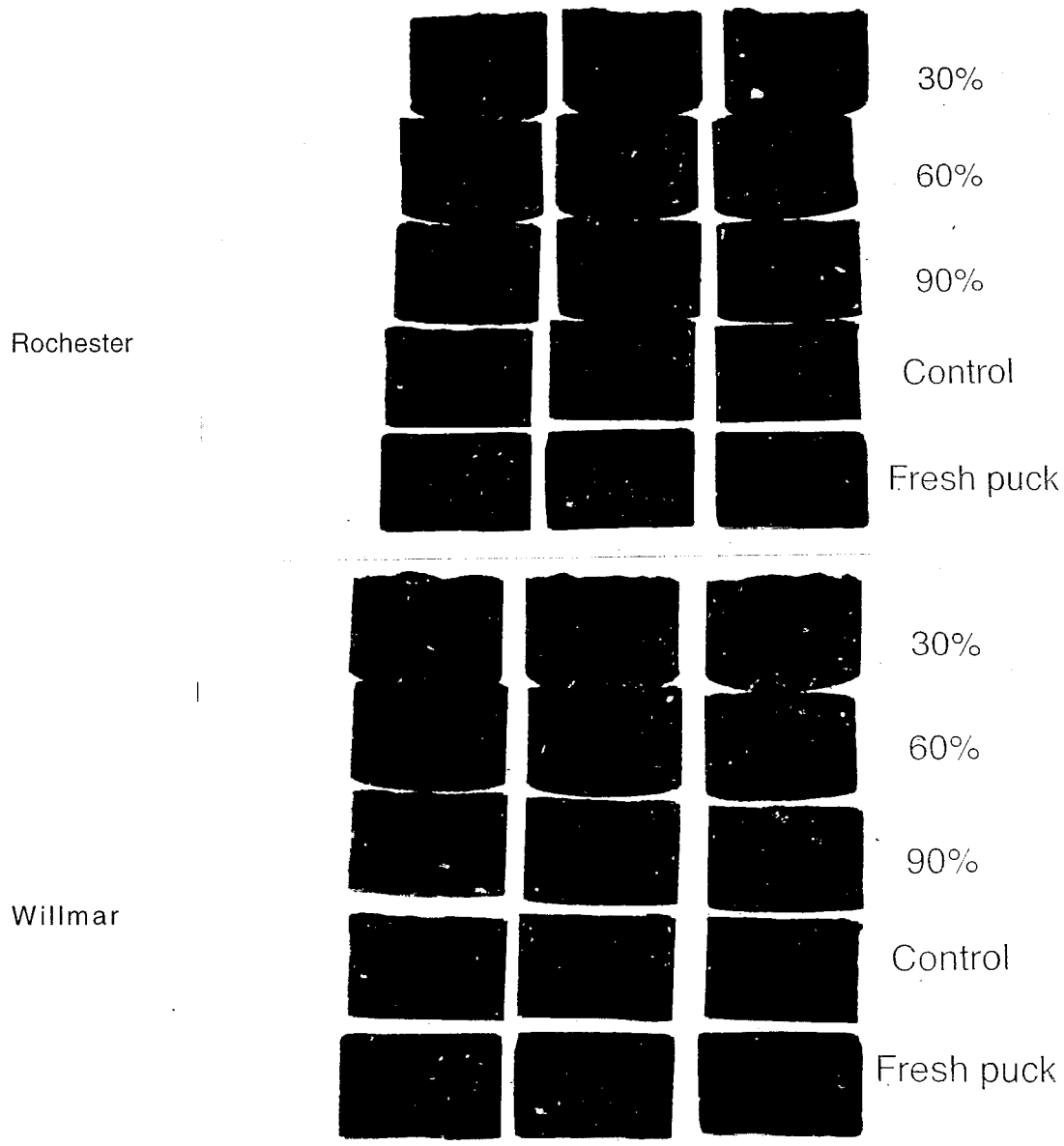


Fig. 28. Broken aggregate and visual stripping shown in cracked Marshall pucks surface after had been buried in soils for 23 months with different water holding capacities. Compared with control samples and fresh puck.

Table 1. Locations and description of 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 2. Chemical-physical properties of asphalt pavement and soil sample underneath the asphalt pavement

Sampling 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 NH_4-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	98.5	95.4	90.5	82.3	74.0	52.7	26.6	19.7			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	432.78±21.5	0.1	0	0	182.39±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
Rochester(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 3. Microbial enumeration of asphalt pavement and soil samples underneath the asphalt pavement

District of Sample	Heterotrophs		Aspahlt Degraders	
	MPN (CFU/ml)*	Plate Count (CFU/g.O.D. sample)** x 10^4	MPN (CFU/ml)	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 4. Chemical Properties of Marshall pucks after buried in soils for 3, 7, 12, 23 months with different water holding capacities.

District and water holding capacity of soil sample		Treatment period (months)	PH	Total Alkalinity mg $CaCO_3$/L 100 g.O.D. sample	Total hydrocarbon (%) g. hydrocarbon/ g.O.D. Sample	Total N ngNH_3-N/	NH_4^+	NO_2^- Sample	NO_3^-	Total P mg PO_4-P/ g.O.D.
Bemidji	30%	3	7.99±0.06	38.08±6.30	6.73±0.13	358.08±32.25	1.02	1	0	103.52±31.43
		7	8.23±0.05	43.46±6.88	6.67±0.07	336.78±32.89	0	1	0	235.77±42.06
		12	8.30±0.04	47.42±14.21	6.48±0.16	360.71±52.59	1.22	1	0	283.04±23.26
		23	7.26±0.12	37.24±1.33	6.57±0.10	338.69±10.43	0	0	0	246.20±18.98
	60%	3	8.03±0.08	40.22±7.59	6.28±0.68	344.62±28.88	1.22	1	0	75.88±9.48
		7	8.28±0.04	48.24±1.23	6.65±0.05	336.64±25.16	0	1	0	261.66±4.71
		12	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
		23	7.54±0.03	39.90±1.33	6.55±0.03	353.83±20.23	0	0	0	264.77±13.86
	90%	3	8.10±0.08	47.40±7.84	5.19±0.79	322.12±4.75	0.81	1	0	88.32±36.78
		7	8.48±0.12	45.76±8.54	6.59±0.00	294.24±75.55	0	1	0	299.46±48.49
		12	8.16±0.06	43.17±12.28	6.65±0.09	396.19±28.35	1.22	0	0	260.65±30.68
		23	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%	3	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
		7	8.17±0.08	34.19±7.84	6.61±0.15	296.63±45.42	1.22	1	0	246.30±54.76
		12	8.01±0.05	25.63±2.78	6.69±0.20	421.52±41.86	0	1	0	244.41±24.71
		23	7.52±0.12	33.25±2.66	6.60±0.03	345.37±17.21	0	0	0	254.91±4.47
	60%	3	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
		7	8.16±0.05	33.79±4.68	6.55±0.00	203.01±7.14	1.22	1	0	314.70±67.30
		12	8.02±0.06	36.77±14.09	6.57±0.08	373.24±42.50	0	1	0	240.12±17.98
		23	7.64±0.02	32.81±0.77	6.44±0.09	354.93±25.55	0	0	0	263.20±8.20
	90%	3	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
		7	8.28±0.04	22.66±4.61	6.29±0.00	233.39±66.99	1.22	0.33	0	292.33±21.07
		12	8.12±0.14	34.56±5.66	6.49±0.18	356.56±26.10	0	1	0	257.38±16.66
		23	7.66±0.08	33.25±0.00	6.68±0.08	359.38±11.52	0	0	0	259.07±14.00

Table 4 cont'd. Chemical Properties of Marshall pucks after buried in soils for 3, 7, 12, 23 months with different water holding capacities.

District and water holding capacity of soil sample		Treatment period (months)	PH	Total Alkalinity mg $CaCO_3$/L 100 g.O.D. sample	Total hydrocarbon (%) g. hydrocarbon/ g.O.D. Sample	Total N ngNH_3-N/	NH_4^+	NO_2^- Sample	NO_3^-	Total P mg PO_4-P/ g.O.D.
Duluth	30%	3	8.37±0.10	55.63±2.81	6.55±0.25	352.98±13.54	0	1.22	0	164.66±20.02
		7	7.47±0.05	34.10±4.32	6.62±0.13	377.27±28.02	1.22	1	0	220.00±35.35
		12	7.99±0.08	42.24±2.93	6.54±0.07	371.48±27.82	1.22	0	0	270.63±81.74
		23	7.65±0.07	33.70±4.06	6.63±0.30	334.71±22.31	0	0	0	233.63±15.71
	60%	3	8.41±0.04	49.03±10.72	5.92±0.60	355.70±31.47	0	1.22	0	162.91±27.93
		7	7.76±0.10	39.96±15.69	6.58±0.15	377.89±8.54	1.22	0.67	0	289.26±32.89
		12	7.94±0.05	45.86±13.87	6.65±0.16	440.38±38.86	1	0	0	272.49±32.13
		23	7.96±0.06	38.13±2.03	6.61±0.03	333.84±24.85	0	0	0	271.07±5.39
	90%	3	8.37±0.03	52.66±13.69	6.28±0.62	313.07±46.91	0	1.22	0	185.41±9.30
		7	7.63±0.07	48.36±9.32	6.61±0.05	352.43±9.12	1.22	0.67	0	283.91±17.01
		12	7.87±0.05	41.22±7.52	6.55±0.06	379.32±46.14	1	0	0	226.99±10.34
		23	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%	3	8.15±0.14	17.26±4.21	6.60±0.12	208.03±49.85	0.41	1.22	0	202.84±8.12
		7	7.96±0.06	46.35±9.32	6.57±0.05	368.73±6.51	0.41	0.67	0	312.68±93.14
		12	7.92±0.03	25.86±6.42	6.52±0.19	394.13±28.71	0.33	0.33	0	275.73±38.88
		23	7.41±0.04	37.24±3.52	6.47±0.10	413.06±24.11	0	0	0	224.75±73.13
	60%	3	8.00±0.18	60.93±4.94	6.37±0.07	328.95±9.68	0	1.22	0	190.84±21.46
		7	8.06±0.01	43.43±12.94	6.22±0.32	332.30±21.61	0	1.0	0	275.73±9.17
		12	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
		23	7.58±0.12	35.47±2.03	6.54±0.08	385.71±55.66	0	0	0	251.41±29.06
	90%	3	8.24±0.07	60.39±11.80	6.35±0.01	348.45±9.48	0	4.01	0	185.43±50.25
		7	8.02±0.06	35.00±2.71	6.53±0.24	337.68±51.42	0	0.67	0	270.09±18.38
		12	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
		23	7.73±0.13	34.58±2.66	6.51±0.07	391.59±19.38	0	0	0	309.12±10.93

Table 4 cont'd. Chemical Properties of Marshall pucks after buried in soils for 3, 7, 12, 23 months with different water holding capacities.

District and water holding capacity of soil sample		Treatment period (months)	PH	Total Alkalinity mg $CaCO_3$/L 100 g.O.D. sample	Total hydrocarbon (%) g. hydrocarbon/ g.O.D. Sample	Total N ngNH_3-N/	NH_4^+	NO_2^- Sample	NO_3^-	Total P mg PO_4-P/ g.O.D.
Willmar	30%	3	8.12±0.15	48.91±12.67	6.75±0.08	322.92±22.37	0.81	1	0	151.68±9.34
		7	8.38±0.12	56.17±5.37	6.64±0.39	319.10±26.27	1.22	1	0	271.67±24.21
		12	7.99±0.06	45.22+5.90	6.65±0.01	486.30±7.33	1	0.33	0	268.49±8.08
		23	7.88±0.03	32.36±1.54	6.49±0.30	357.30±39.68	0	0	0	212.18±13.26
	60%	3	8.11±0.13	46.07±13.62	6.67±0.01	339.63±14.08	0.81	1	0	169.09±17.79
		7	8.44±0.03	85.09±19.93	6.55±0.32	341.62±20.23	1.22	1	0	304.97±46.37
		12	7.98±0.04	32.45±4.05	6.50±0.01	410.73±59.02	1	0	0	259.46±4.81
		23	7.93±0.05	33.69±0.77	6.46±0.05	309.36±32.02	0	0	0	263.37±14.57
	90%	3	8.17±0.13	70.29±12.36	6.49±0.34	323.69±14.49	1.22	1	0	172.52±28.77
		7	8.56±0.08	90.86±14.50	6.52±0.30	339.35±29.62	1.22	1	0	282.22±12.55
		12	8.06±0.05	51.71±9.16	6.64±0.02	408.02±79.07	1	0	0	284.11±17.33
		23	7.98±0.05	34.58±0.00	6.50±0.06	324.59±50.49	0	0	0	290.04±5.81
Control		3	8.0±0.03	57.91±5.99	6.75±0.05	439.91±34.03	0	0	0	197.14±12.45
		7	8.28±0.15	39.53±2.60	6.49±0.00	395.67±8.28	1.22	0	0	236.38±17.95
		12	7.88±0.10	25.97±7.14	6.28±0.06	308.39±58.04	0	0	0	232.26±27.23
		23	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		3	-	-	-	-				-
		7	7.31±0.00	65.29±5.52	6.65±0.00	388.36±6.16	0	0	0	181.29±15.75
		12	7.68±0.00	67.39±5.70	6.70±0.00	388.36±6.16	0	0	0	247.78±10.62
		23	7.66±0.00	-	7.02±0.01	-	0	0	0	-

Table 5. 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 5 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 6. 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.

District and water holding capacity of	Soils adjacent or far away from pucks (Adj or FAF)	Heterotrophs MPN (CFU/ml)*	Heterotrophs Plate Count (CFU/g.OD. sample)** x 10^4	Asphalt degraders MPN (CFU/ml)	Asphalt degraders 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 7. IR Analysis

#	Sample D+	T*	WHC%	Relative Absorbance 3336 - 3595 cm-1	2940 - 2980 cm-1	2837 - 2839 cm-1	2160 - 2360 cm-1	2020 - 2048 cm-1	1620 - 1670 cm-1	1400 - 1460 cm-1	1015 - 1040 cm-1
1	Control	3	60	1.000	0.472	0.422	0.145	0.180	0.017	0.186	0.797
		7		1.000	0.821	0.778	0.145	0.180	0.177	0.753	0.735
		12		1.000	0.704	0.568	0.155	0.158	0.669	0.217	0.956
2	Bemidji	3	60	1.000	0.817	0.778	0.043	0.037	0.040	0.439	0.836
		7		1.000	0.521	0.160	0.266		0.205	0.159	0.750
		12		1.000	0.574	0.454	0.276	0.243	0.849	0.218	0.769
3	Control	12	60	1.000	0.704	0.568	0.155	0.158	0.669	0.217	0.956
	Bemidji			1.000	0.574	0.454	0.276	0.243	0.849	0.218	0.769
	Brainerd			1.000	0.762	0.714	0.057		0.097	0.273	0.854
	Duluth			1.000	0.718	0.582	0.268		0.216	0.185	0.962
4	Rochester			1.000	0.455	0.387	0.052		0.056	0.111	0.720
	Willmar			1.000	0.657	0.555	0.400		0.348	0.205	0.971
5	Bemidji	12	30	1.000	0.346	0.310	0.372	0.288	0.959	0.203	0.637
			60	1.000	0.574	0.454	0.276	0.243	0.849	0.218	0.769
			90	1.000	0.761	0.807	0.150	0.268	0.460		0.582

\+ D=District from where soil samples were taken
* T = Treatment period (months)
% WHC = Water holding capacity (%)

Table 8. 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