Dale C. Mann,[1] *B.S. and William R. Gresham,*[2] *Ph.D.*

Microbial Degradation of Gasoline in Soil

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ABSTRACT: Forensic science analysis of soil samples for the presence of flammable liquids occasionally results in the detection of volatile mixtures that lack some of the diagnostic features of common petroleum products. The presence of these mixtures is not consistent with evaporation or with a chemical or physical process, but is reported in microbiological literature that addresses bacterial degradation.

Microbiological research has shown that crude oil spilled in the environment is sometimes degraded by bacteria. A study was conducted to demonstrate how automotive gasoline is degraded. Gasoline was spiked into 36 containers of soil (12 were stored at −5°C; 12 were stored at room temperature; and 12 of the soil samples were sterilized prior to the addition of the gasoline and were then stored at room temperature). These samples were monitored, and the results were compared using static heated headspace sampling and capillary gas chromatography.

The gasoline in the unsterilized samples stored at room temperature degraded rapidly, while the gasoline in the other two sets of samples was unaffected. This degradation followed trends that can be recognized in casework and can assist in the identification of affected petroleum product residues in soil.

KEYWORDS: forensic science, gasoline, soil, bacteria

The detection of petroleum product residues in fire debris is dependent on several factors, which include the quantity and type of accelerant used to initiate the fire, the intensity and duration of the fire, the length of time between suppression of the fire and collection of the sample, and the sensitivity and selectivity of the analytical methods. Certain physical characteristics of the substrate to which the accelerant is added (that is, porosity, flammability, and ability to insulate) will also play major roles in the survival of the accelerant. For example, one study has shown that soil retains an accelerant more efficiently than wood or carpeting during and after burn tests [*1*].

Arson casework experience in the Washington State Patrol Seattle Crime Laboratory supports the study just mentioned [*1*]. However, analysis of some soil samples has resulted in the detection of a volatile mixture that lacks some (and, in some cases, most) of the diagnostic features associated with common petroleum products [*2–5*], while other debris types in these same cases contained easily recognized petroleum products. This degradation was independent of the vapor pressure range of the suspected accelerant, and it occurred in samples less than a week old, as well as in old samples. The alteration was generally restricted to a loss of aromatic and normal alkanes; other hydrocarbons were less affected. This alteration was first noticed in soil samples, although occasionally it occurred in samples containing vegetation or rotting wood.

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[1]Supervising forensic scientist, Washington State Patrol Crime Laboratory, Tacoma, WA.

[2]Supervising forensic scientist, Washington State Patrol Crime Laboratory, Seattle, WA.

A review of the environmental microbiology literature showed that crude oil is degraded in the environment by available bacteria [6–9]. The rate of consumption was demonstrated to be dependent on the species of bacteria present, the temperature, and the nutrient concentration. The cited microbial degradation studies produced results consistent with results obtained in some of our casework in which volatile mixtures were recovered from soils.

Experimental Design

An experiment was designed to test (1) if the alteration of petroleum products sometimes seen in soil is due to microbial degradation (and is, therefore, not a physical phenomenon), (2) how fast this alteration may occur, (3) if there is a practical way to recognize the degradation, (4) how to recognize the original product after alteration, and (5) if low-temperature storage of samples eliminates or retards the degradation.

Soil from an organically fertilized garden was sieved through a 2.36-mm sieve. Samples of this soil, 250 g each, were weighed out and sealed into 36 separate 1-pt (0.5-L) cans. Twelve of these samples were autoclaved for 60 min on three successive days in order to sterilize the soil completely.[3] All 36 soil samples were then spiked with 200 μL of a Chevron unleaded regular gasoline. After thorough mixing, 12 of the nonautoclaved samples were stored at −5°C. The remaining 24 samples (12 autoclaved and 12 nonautoclaved) were stored at room temperature, which is the normal storage practice for arson samples.

The volatile contents of these samples were analyzed, using an 85°C static-heated headspace recovery method and capillary gas chromatography. Samples were analyzed on Days 0, 1, 2, 4, 6, 11, 20, and 60. Samples analyzed on one day were not reanalyzed at a later date, since they had been heated for headspace sampling.

To help answer questions raised by test results in a case, a preliminary experiment was also conducted a few months prior to the above study. Soil from the same source as that in the formal study was spiked with gasoline and the samples were analyzed in the same manner as in the follow-up formal study. No controls, sterilization procedures, or alternative storage methods were used in this preliminary experiment. The results of this preliminary experiment are discussed at the end of the Results section.

All analyses were performed on a Hewlett-Packard Model 5840A gas chromatograph equipped with a 60-m by 0.25-mm inside diameter DB-1 (methyl silicone) column with a film thickness of 1.0 μm. The temperature program increased from 35°C (for 2 min) to 260°C at 8°C/min. Hydrogen was used as the carrier at a linear velocity of 45 cm/s at 200°C. All component identification was based on flame ionization detection (FID), using retention time correspondence to gas chromatography (GC) of known compounds and to prior gas chromatography/mass spectrometry (GC/MS) analyses.

Results

In the formalized experiment, the gasoline spiked into unsterilized soil stored at room temperature showed a dramatic decrease in the concentrations of numerous aromatic and all *n*-paraffinic compounds within a few days. Figure 1 shows the decrease in Peaks 5 (benzene), 10 (toluene), 13 (ethylbenzene and *p*- and *m*-xylenes), and 15 (isopropylbenzene, *n*-propylbenzene, 1-methyl-3-ethylbenzene, and 1,2,4-trimethylbenzene) by Day 2. On Day 4, Peaks 1, 3, 8, and 12 (*n*-paraffins), 13 (*o*-xylene), and 15 (1-methyl-4-ethylbenzene, 1-methyl-2-ethylbenzene, and 1,2,3-trimethylbenzene) had degraded. Peaks 15 (1,3,5-trimethylbenzene) and 2,4,6,7,9, and 11 (isoparaffins) did not degrade

[3]Rodgers, J., U.S. Environmental Protection Agency, personal communication, 1985.

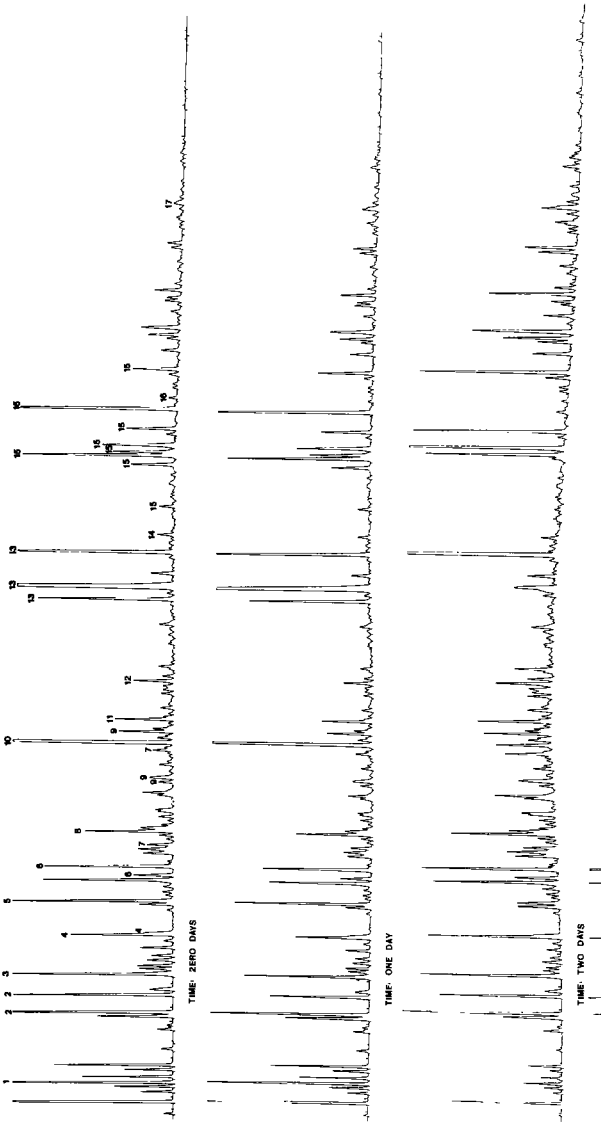

FIG. 1—*Static headspace/capillary gas chromatography comparison of microbial degradation effects on gasoline in nonsterilized soil stored at room temperature. The component identification is as follows:* (1) n-*pentane*, (2) *methylpentanes*, (3) n-*hexane*, (4) *dimethylpentane*, (5) *benzene*, (6) *methylhexanes*, (7) *trimethylpentanes*, (8) n-*heptane*, (9) *dimethylhexanes*, (10) *toluene*, (11) *trimethylhexane*, (12) n-*octane*, (13) C_3-*benzenes*, (14) n-*nonane*, (15) C_4-*benzenes*, (16) n-*decane*, *and* (17) *naphthalene. Note the different rates of degradation for different classes of compounds (aromatics versus aliphatics), for compounds of different molecular weight (benzene versus* C_3-*benzenes), and for isomers of the same molecular weight* (C_3-*benzenes).*

through Day 60. It is not known if all the degraded compounds were completely depleted from the samples or not. For some of these compounds, small peaks remained stable at the appropriate retention time; however, mass spectrometry was not available to confirm the identities of these persistent components. It should be noted again that peak identification during this study was based on previous GC/MS and GC analyses of standard compounds; the lack of a GC/MS in the Seattle laboratory during this study precluded the conclusive identification of the small persistent compounds.

The reproducibility of the rate or order of degradation was not examined closely, although a duplicate sample of a unsterilized soil stored at room temperature was analyzed on Days 2 and 11, and the results corresponded closely to those illustrated in Fig. 1, Day 2, and Fig. 2, Day 11.

The gasoline alteration at Day 11 in the unsterilized room temperature sample is compared in Fig. 2 with the sample stored at −5°C and with the sterilized soil. The results for gasoline spiked into the sterilized soil and the gasoline in soil stored at −5°C compare favorably with those for the original gasoline. (Compare these results with those for the Day 0 gasoline in Fig. 1.) Analysis of the gasoline in the sterilized soil and that stored at −5°C on Days 20 and 60 also showed little perceptible change relative to the original gasoline. The gasoline spiked into the unsterilized soil, which was stored at room temperature, showed little additional change at Days 20 and 60.

The initial informal study conducted with soil from the same source showed a similar trend of degradation of certain classes, but not necessarily the same exact chemical species. The *n*-alkanes, benzene, toluene, ethylbenzene, and propylbenzene all show a dramatic decrease by Day 6 (Fig. 3). Numerous other substituted benzenes, which were degraded in the more formal study, persisted in this initial experiment through Day 22 (the last day samples were analyzed). The isoparaffins also persisted to the end of the experiment, as they did in the formal experiment conducted later.

Discussion

From the results depicted in Fig. 2, in which the hydrocarbon component losses are retarded by sterilization and storage at −5°C, it is evident that these losses are due to consumption by bacteria and not to some irreversible chemical or physical adsorption process. The consumption process was specific, with a decided preference toward normal alkanes and substituted benzene compounds with the least substitution. (Compare the results in Fig. 2 with those for Days 1, 2, and 4 in Fig. 1). Note that, within a certain class of compounds (such as the C_3-benzenes), there is not a uniform degradative loss among all the compounds. In the initial study, using soil from the same source but at a different time of year, there was generally less degradation of the aromatics, although benzene and toluene were still degraded completely (Fig. 3). This indicates that degradative loss rates of specific compounds are dependent on various factors, including the presence of specific species of bacteria [9]. There is no reason to believe that any hydrocarbon profile generated in such a study would be accurately reproduced using a different soil, bacteria, temperatures, moisture level, and so forth; however, the general trends of degradation do seem to be predictable.

The reason for the lack of additional degradation after the initial attack by available bacteria (after Day 11 in the formal study) is not addressed in these experiments. It is not known if the remaining chemical species are inert to the bacteria present in this soil or if the environment in the sample containers was altered in some way to hinder further bacterial action, such as by reduced oxygen, changing moisture content, or production of toxic materials. The cited microbiological literature indicates that the remaining chemical species are inert to degradation.

The effect of increasing the hydrocarbon concentration was not studied in detail.

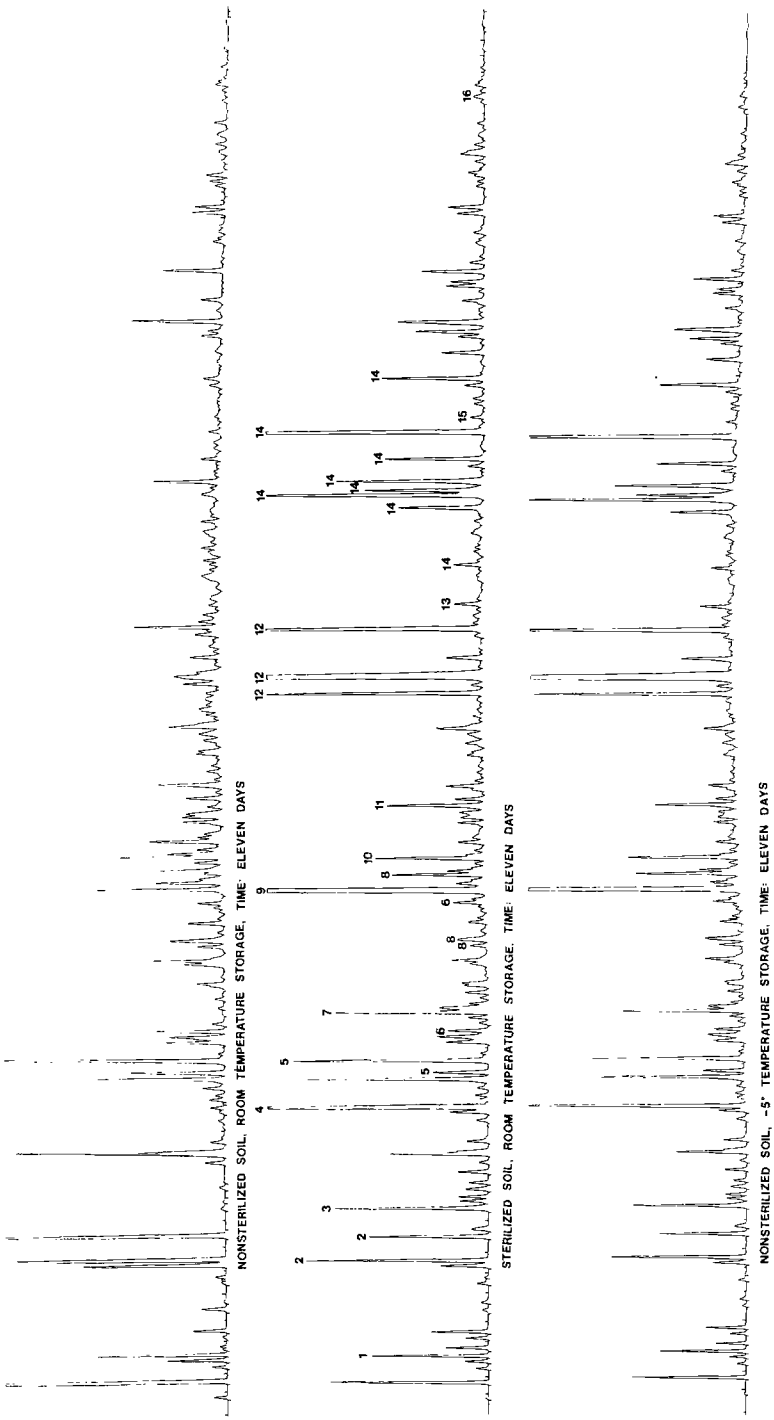

FIG. 2—*Static headspace/capillary gas chromatography comparison of microbial degradation effects on gasoline in soil. The component identification is as follows:* (1) n-*pentane*, (2) *methylpentanes*, (3) n-*hexane*, (4) *benzene*, (5) *methylhexanes*, (6) *trimethylpentanes*, (7) n-*heptane*, (8) *dimethylhexanes*, (9) *toluene*, (10) *trimethylhexane*, (11) n-*octane*, (12) C_9-*benzenes*, (13) n-*nonane*, (14) C_{10}-*benzenes*, (15) n-*decane*, *and* (16) *naphthalene. The gasoline used in these samples is the same as that in Fig. 1.*

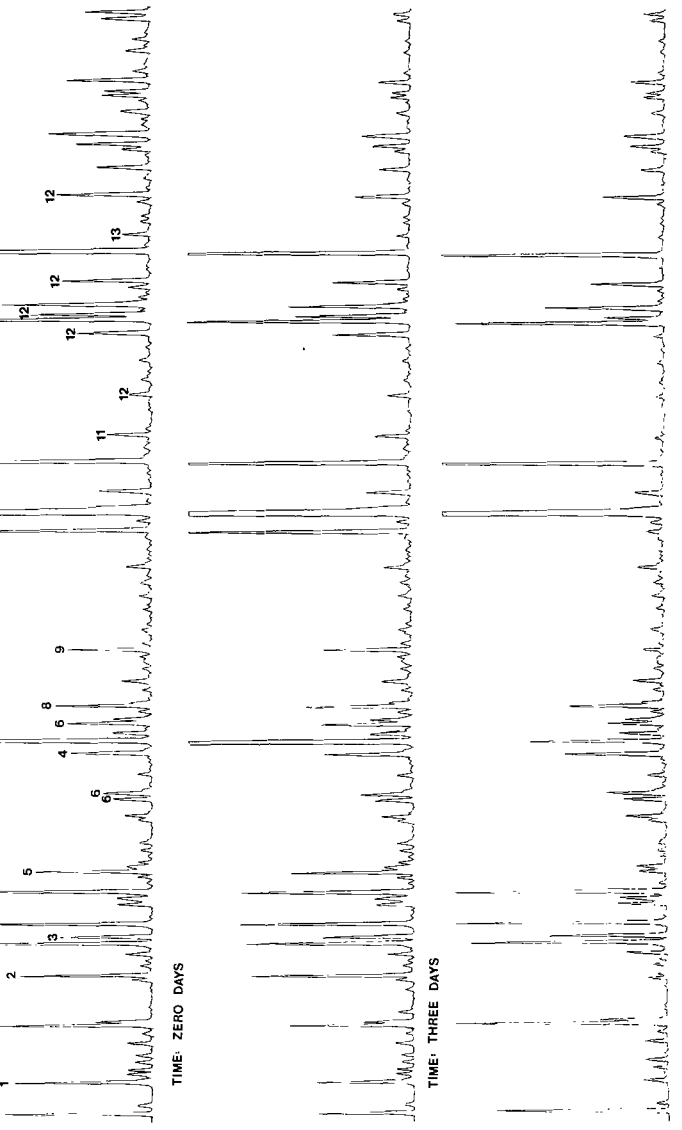

FIG. 3—*Static headspace/capillary gas chromatography analysis of gasoline spiked into soil. The component identification is as follows:* (1) n-*hexane*, (2) *benzene*, (3) *methylhexanes*, (4) *trimethylpentanes*, (5) n-*heptane*, (6) *dimethylhexanes*, (7) *toluene*, (8) *trimethylhexane*, (9) n-*octane*, (10) C_9-*benzenes*, (11) n-*nonane*, (12) C_{10}-*benzenes*, *and* (13) n-*decane*.

However, when a few millilitres of gasoline were added to a unsterilized, room-temperature storage sample (run in conjunction with the formal study), no change in the hydrocarbon profile was noted after three weeks. This lack of alteration may be due to several factors, such as a low relative concentration of bacteria, lack of sufficient available oxygen, or toxic effects of some components in gasoline.

Many soil samples received in casework show little or no apparent degradation of the absorbed petroleum product. Those soil samples, which appear to be primarily clay (containing little organic material), and those samples from next to foundations or under crawl spaces (which may normally be very dry or possibly alkaline) are less likely to contain bacteria capable of degrading petroleum products.

Three of the major causes of alteration of a flammable liquid in arson casework are evaporation (due to exposure to the environment or fire), pyrolytic addition (from thermal degradation products from materials in the fire), and bacterial degradation. Evaporative changes in a flammable liquid profile are predictable; deviation from available standards can be explained with some confidence. The chromatographic profiles of pyrolysis products from materials in the sample are not reproducible and can sometimes be difficult to distinguish from actual refined petroleum products because of the variety of aromatic compounds commonly produced during the pyrolysis of many types of synthetic materials. Bacterial degradation of gasoline can also result in an array of results which would not be specifically predictable, based on information at hand at the time of analysis. Deviation of test results from available petroleum-based liquid standards is not as readily or easily explained as the effects of evaporation. Differences between the chromatographic pattern obtained from the evidence and that diagnostic of a petroleum-based liquid should not be dismissed as due to weathering or bacterial degradation without critical assessment and knowledge of the sample history and the degradation process.

Identification of a petroleum product affected by bacterial degradation may not be possible when using the classical diagnostic hydrocarbon features, such as normal alkanes and pseudo-cumenes, or by a comparison of the results with available liquid or evaporated standards [*2–5*]. Identification of a refined petroleum product in such cases may have to be based on the presence of more stable chemical species, such as branched alkanes, indanes, and possibly substituted naphthalenes, which are present in low concentrations in the original petroleum products. FID analysis in the above studies was not adequate to evaluate the degradative effect on some of these species. Mass spectral analysis of cases involving degradation may be desirable in order to identify the compounds which are resistant to this degradative process. It would also be advantageous to compare samples containing suspected microbial degradation with prepared reference samples of degraded liquid standards. Beyond the cited caveats, we recommend a conservative approach to attempted identifications of degraded products.

As a result of these studies, the Washington State Patrol Crime Laboratory now freezes all fire debris samples containing soil or vegetation until analysis can be completed. Fire investigators are also encouraged to store these types of samples in a similar manner.

Case Examples

Case 1

A fire was discovered in a vacant house and quickly extinguished. Fire investigators determined the point of origin of the fire and collected partially burned carpet samples from that location. Investigation outside yielded a gas can and an adjacent "trailer" leading from the back door into the yard. The can, soil from the trailer, and the carpet samples were submitted to the laboratory for analysis. Figure 4 illustrates the results of the static headspace analysis. The gasoline from the can shows little sign of evaporation.

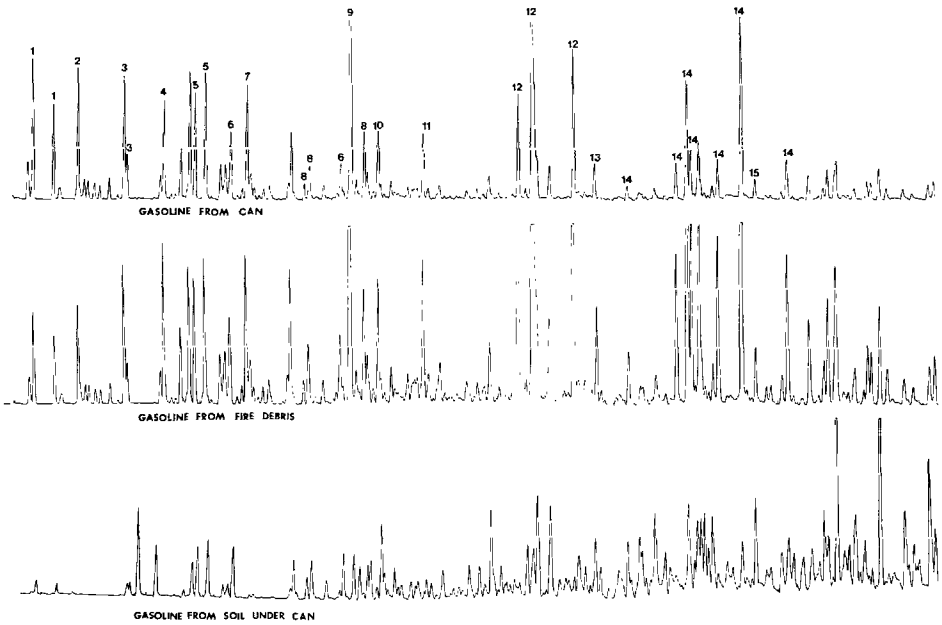

FIG. 4—*Static headspace/capillary gas chromatography comparison of gasoline recovered from soil with gasoline found in a gasoline can and with gasoline recovered from the fire scene. The gasoline can was found adjacent to the soil sampled. The component identification is as follows:* (1) *methylpentanes,* (2) n-*hexane,* (3) *dimethylpentanes,* (4) *benzene,* (5) *methylhexanes,* (6) *trimethylpentanes,* (7) n-*heptane,* (8) *dimethylhexanes,* (9) *toluene,* (10) *trimethylhexane,* (11) n-*octane,* (12) C_3-*benzenes,* (13) n-*nonane,* (14) C_3-*benzenes, and* (15) n-*decane.*

The gasoline from the burned carpet shows some signs of evaporation, as would be expected from a fire debris sample. The product recovered from the trailer in the yard is presumably gasoline; however, this sample shows evidence of evaporation and of extensive microbial degradation (similar to that in Fig. 1, Day 4). For the soil sample, identification of a residue of automotive gasoline would have been difficult without knowledge of the microbial degradation process and knowledge of the relationship of the soil sample to the other items.

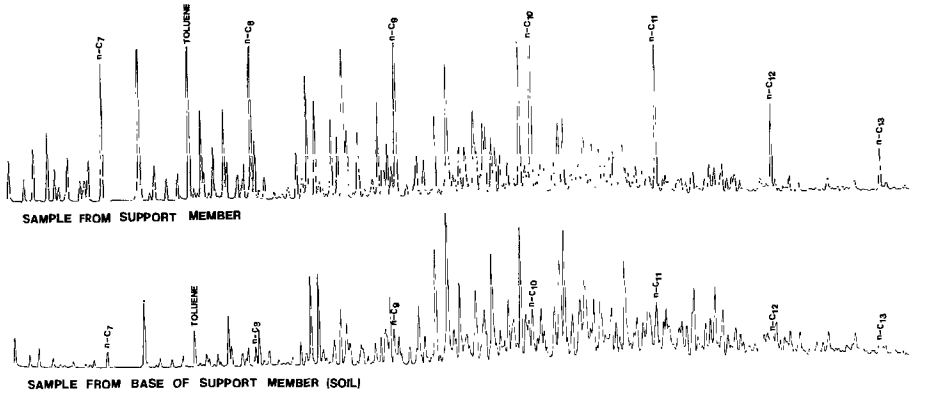

FIG. 5—*Static headspace/capillary gas chromatography analyses of a mid-range petroleum distillate product recovered from two samples collected adjacent to each other at the fire scene.*

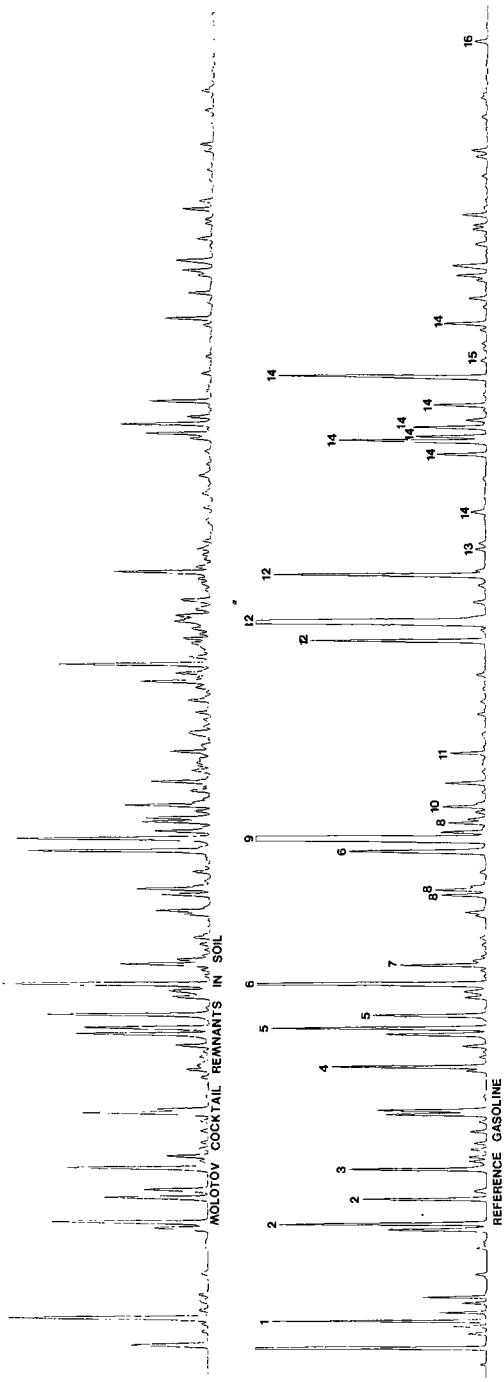

FIG. 6—*Static headspace/capillary gas chromatography analysis of a gasoline recovered from soil. The results are compared with an arbitrary reference gasoline taken from the laboratory's flammable liquid library. The component identification is as follows:* (1) n-*pentane,* (2) *methylpentanes,* (3) n-*hexane,* (4) *benzene,* (5) *methylhexanes,* (6) *trimethylpentanes,* (7) n-*heptane,* (8) *dimethylhexanes,* (9) *toluene,* (10) *trimethylhexane,* (11) n-*octane,* (12) C_3-*benzenes,* (13) n-*nonane,* (14) C_3-*benzenes,* (15) n-*decane, and* (16) *naphthalene.*

Case 2

A fire was determined to have originated in an open carport attached to a single-family residence. Samples from a wood support pillar and from soil at the base of the pillar were submitted to the laboratory for analysis. The static headspace test results are presented in Fig. 5. A typical mid-range petroleum distillate product was recovered from the wood pillar. The soil sample contained a volatile mixture with a boiling range similar to that found for the mid-range product in the wood pillar; however, the traditional landmarks associated with a distillate product (the *n*-alkanes) were not present. The concentrations of many of the unidentified trace components were also altered. Identification of the presence of a mid-range petroleum product was made possible by combining the known history of the sample and knowledge of the microbial degradation process.

Case 3

The remnants of a Molotov cocktail, asphalt, and soil (from under the device) were submitted to the laboratory for confirmation of an incendiary device. The static headspace results are presented in Fig. 6. This sample contains an automotive gasoline which exhibits little evaporation but shows the "typical" loss of the aromatic and normal hydrocarbons as a result of microbial degradation. Data for unaltered reference gasoline (not associated with the case) is included in Fig. 6 for comparison.

Summary

Microbial degradation of liquid petroleum products collected in soil may occur occasionally unless steps are taken to prevent it. Freezing those samples containing soil or vegetation has been shown to be an effective way to prevent this degradation. Refrigeration would most likely slow this process. Despite these measures, however, test results will occasionally indicate that degradation has already taken place prior to submission of the sample. Reference chromatograms of bacteria-degraded accelerants may aid in the interpretation in such cases. Such chromatograms have the same usefulness as those produced from evaporated petroleum products in the flammable liquid libraries of most arson analysis laboratories. Identification of significantly degraded flammable liquids or residues should be undertaken very conservatively.

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Address requests for reprints or additional information to
Dale C. Mann
Washington State Patrol Crime Laboratory
County City Building, B-70
930 Tacoma Ave.
Tacoma, WA 98402-2102