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The Effect of Microbial Degradation on the Gasoline Residues Identification in Fire Debris

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Short report

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Abstract

The identification of ignitable liquid residues in fire debris is a key finding for determining the cause of the fire and indicate that it was intentional fire. However, ignitable liquids in the samples are subject to evaporation as well as microbial degradation. The aim of this work is to investigate accelerators and their changes due to microbial degradation in fire debris. Gasoline changes in different soil types and different time intervals were compared. Based on several authors, mainly alkanes decompose and thus change the chromatogram, from which ignitable liquids are subsequently incorrectly identified. The results indicate the need for rapid and timely analysis of fire debris in laboratories or their storage under specified conditions, therefore freezing to the specified temperature.

Keywords: Gas chromatography – mass spectrometry, gasoline, ignitable liquids, microbial degradation, soil

1 Introduction

Detection of ignitable liquids is crucial in determining cases of arson. In many cases, an arson uses an ignitable liquid to accelerate a fire. Most ignitable liquids are hydrocarbon-based fuels. Gasoline is the most used ignitable liquid because it is easily accessible, inexpensive, and easily ignited. Gasoline and other ignitable liquids are classified according to the American Society for Testing and Materials (ASTM) guidelines according to their boiling point range and chemical composition. Other common consumer products, which are classified according to chemical composition and boiling point, may also be used. Other product classes include petroleum distillates (e.g., diesel), isoparaffins (e.g., paint thinner), aromatics (e.g., degreasers), naphthenic paraffinic substances (e.g., lamp oil), n-alkanes (e.g., candle oil), de-aromatized distillates (e.g., camping fuel), oxidized solvents (e.g., ketones) and various products such as turpentine. Identification of ignitable liquids is a challenging task that can be affected by several factors. Microbial degradation is one of the three main processes that can change the composition of ignitable liquid residues. Because biodegradation is a time-related aspect, it should be studied at different stages of its development. Although the debate on microbial degradation is relatively new to investigators of the causes of fires, it has long been of interest to environmental researchers and the oil industry. As early as 1946, Claude ZoBell documented that hydrocarbon could be used by microorganisms as their only source of energy (Atlas, 1981; Hybská et al., 2018, Stauffer et al., 2008; ASTM E1618-19; ASTM E1618-06).

The role of the investigator of the causes of the fire in these cases is twofold: to remove the remnants of ignitable liquid from the matrix and to determine the type of ignitable liquid present. While the analysis of volatile compounds in solid matrices is an active area of research, forensic scientists have adopted only a few selected methods for the analysis of fire samples. The use of standardized methods ensures consistency between laboratories and confidence in the results after they are brought to court. Methods for which American Society for Testing and Materials (ASTM) standards have been established include steam distillation, solvent extraction, headspace sampling, passive and dynamic headspace method, and solid phase microextraction (SPME) (Turner, Goodpaster, 2009).

Each of these methods has its strengths and weaknesses. For example, steam distillation is useful only if there is a large amount of ignitable liquid in the sample. Solvent extraction is also an approved ASTM method and is a useful method when the samples are very small, when the ignitable liquid contains compounds with very high boiling points, or when the matrix is unsuitable for extraction of the ignitable liquid by other methods. However, steam distillation and solvent extraction are time and labour intensive compared to their efficiency, and key compounds may be lost in the process where background interference may occur. Modern extraction methods use dynamic and passive headspace methods. These methods allow ignitable liquids to escape and concentrate on the adsorption medium, which can then be desorbed for analysis. The dynamic headspace method involves the use of an inert gas to continuously clean the headspace of the sample, allowing complete extraction of the ignitable liquid from the matrix. Thermal desorption is then used to release the ignitable liquid from the sorption material. The dynamic headspace method is not as often used as passive due to the work involved and can thus allow contamination of the ignitable liquid (Dolan, 2003; Sandercock, 2008).

The method used in forensic laboratories is primarily a passive headspace method. Its advantage is a much simpler method for sample extraction and is also non-destructive, which makes it the preferred method for sample extraction. As with the dynamic method, the ignitable liquid is evaporated and collected on an adsorbent material, usually a porous polymer or carbon. The ignitable liquid can then be thermally desorbed from the strip or extracted with a solvent. The difference between the passive and dynamic methods is that for the passive, the inert gas is not used to expel the ignitable liquid from the headspace to the adsorbent material. Instead, the adsorbent material is suspended in the headspace, during heating of the sample. This method also uses a closed system, while the dynamic method does not. Another advantage of the passive method is that it allows the sample to be stored for re-analysis if necessary. Suitable solvents for the passive method should be able to displace the compounds in the ignitable liquid from the adsorbent material and should have a high solubility for these compounds. Typical solvents include carbon disulfide or pentane. Solid phase microextraction (SPME) is a relatively new technique for analyzing fire samples that uses silica fiber instead of a carbon strip. The fiber is enclosed in a hypodermic syringe so that the fiber can be exposed to the sample and then withdrawn for analysis. SPME is a versatile method that can be used in the headspace method, the direct method, or the partial headspace method (Turner, Goodpaster, 2009).

Regardless of the extraction method, the method of identifying samples of ignitable liquids in laboratories is universal gas chromatography, usually using silicone columns and either by flame ionization or mass spectrometry. In contrast, the aspect by which microorganisms metabolize oil components is not as well known among forensic scientists, although it has been well studied over the last few decades. For example, the original microbial communities found in oil fields are very diverse and include many species of bacteria. Numerous trends and observations that have been reported in the literature regarding the degradation of certain classes of hydrocarbons in oil can be summarized as follows (Hybská et al., 2018, Magot, 2005; Turner, Goodpaster, 2009):

- C_6 to C_{15} n-alkanes are the most easily degradable components of petroleum.
- A typical first sign of biodegradation is the loss of n-alkanes ranging from C_{10} to C_{13}.
- Aromatic hydrocarbons are more resistant to degradation than aliphatic hydrocarbons.
- Cyclic and branched alkanes are more resistant than direct hydrocarbons.
- Resistance to degradation increases with the degree of substitution in isoalkanes, alkylcyclohexanes, alkylcyclopentanes and alkylbenzenes.
- Resistance to degradation also depends on substitution effects (e.g., 3-methylalkanes> 4-methylalkanes> 2-methylalkanes).

- Adjacent methyl groups (e.g., 1,1-dimethylcyclohexane and 1,2,3-trimethylbenzene) also increase resistance to biodegradation.
- Degradation in heavier aliphatic hydrocarbons tends to occur in the order in which n-alkanes are removed first and then acyclic isoprenoid alkanes.

The aim of the paper is to point out the microbial degradation of ignitable liquids in the soil. Whereas ignitable liquids in fire samples may, due to microbial degradation, change their formula, according to which they can be distinguished and determined in ion chromatograms. In microbial degradation, the diagnostic formulas used to identify ignitable liquids change more unpredictably than in weathering. Instead of gradually losing entire groups based on volatility, losses are limited to individual compounds in the groups based on the preferences of the microbes present in each sample.

2 Material and Methods

The data and results of two studies of microbial degradation of gasoline in different soil types and at different time intervals were compared. Based on their research and results, the decomposition of ignitable liquids due to microbial degradation was evaluated.

Bacteria tend to consume petroleum products, and this has an adverse effect on the identification of ignitable liquids, especially in highly organic samples such as soils. D.C. Mann and W. R. Gresham (1990) from a laboratory in Washington first examined this phenomenon in the context of the analysis of fire samples. Using gasoline-enriched garden soil, this study showed that degradation occurred rapidly unless the soil was either thoroughly sterilized prior to the introduction of gasoline or gasoline or soil samples were stored at -5 °C. For unsterilized samples stored at room temperature, the degradation process was characterized by the loss of substituted benzenes and all n-paraffin compounds over several days. However, the isoparaffinic compounds were not affected. Based on these findings, the authors stated that all soil to be transferred to the laboratory will henceforth be stored in a freezer until analysis is complete (Dann, Gresham, 1990; Turner, Goodpaster, 2009).

Dinan et al., (1992) isolated two species of bacteria (Pseudomonas putida and Pseudomonas fluorescens biovar III) from soil samples that generated an anomalous chromatographic pattern. The ability of these bacteria to degrade gasoline and petroleum gasoline was evaluated in vitro and the two species were found to complement each other because Pseudomonas putida consumed the aromatic parts of the fuels, while Pseudomonas fluorescens biovar III the aliphatic part. Finally, the authors offered recommendations to prevent microbial degradation, such as storing samples at reduced temperature or adding a non-volatile bactericidal agent to fire residues (Mann, Gresham, 1990; Dinan et al., 1992).

In an article by Turner et al. (2014) focused on microbial changes of ignitable liquids in soil obtained from an agricultural field and was clay, the second was taken from the yard and it was sandy Miami clay, and the last sample was ordinary urban soil from the brownfield area, and it was Wawaka clay. All material was collected from the surface to a depth of 20 cm using a sampling probe, which was then sieved. Bacterial populations were determined for each soil type. For each soil sample, 20 µl of standard unleaded petrol (87 octane) per 100 g of soil was added three times in a clean, unused container. Samples were sealed and stored for 0, 2, 4, 7, 11, 15, 22 and 30 days. On designated days, samples were extracted by the passive headspace method. The activated carbon strip, which hung with a clip on the nylon fiber, was placed in a can in the headspace. The sealed cans were heated at 85 °C for 4 hours. After cooling, the strips were removed and extracted with 400 µl of pentane for approximately 1 minute. Samples were then analysed by GC-MS (Agilent 6890 GC with Agilent 5975 MSD) using a standard method for the analysis of fire residues, which includes 1 µl injection volume, 20: 1 partition ratio, 250 °C inlet temperature, 1 ml / min (helium), DB-5 column 30 mx 0.25 mm x 0.25 µm, initial column temperature 40 °C maintained for 2 minutes, subsequent temperature increase by 20 °C / min, final temperature 280 °C maintained for 3 minutes, MS scanning 40-300 m / z, MS quad temperature 150 °C and MS source temperature 230 °C (Turner et al., 2014).

Turner and Goodpaster (2009) used a thin layer of soil weighing 40-90 g, which was placed on the bottom of the can and formed into a square shape. 20 µl of ignitable liquid was poured onto this layer. Gasoline (87 octane), lighter fluid, charcoal starting fluid, kerosene and heating oil were used as ignitable liquids. The cans were sealed for 2-7 days at room temperature. The passive adsorption technique from the headspace was used to extract residual ignitable liquids. In this procedure, the can

was opened, and the carbon strip was hung on a paper clip. Subsequently, the sealed can was placed in an oven at 85 °C for 4 hours. The can was cooled back to room temperature after 4 hours and the carbon strip was removed. The strip was then placed in a test tube and the ignitable liquid was extracted by adding 300 μl of pentane and stirring for 1 minute (Turner, Goodpaster, 2009).

3 Results and Discussion

Microbial degradation of gasoline in an experiment by Turner et al. (2014) was observed in residential, agricultural and brownfield soils. In all three soil types, n-alkanes degraded in a similar fashion in that degradation is almost complete after 7 days. In fact, no peaks remained in the chromatograms after 15 days except those attributed to the volatile aldehydes that are present in the headspace of all soil samples. In contrast, we noted differences in the ratios of the C3-alkylbenzenes depending upon soil type. For example, all profiles appear nearly identical on day 0, but on day 2 propylbenzene (peak 1 in Fig. 2) is significantly reduced in residential soil compared to 3-ethyltoluene (peak 2 in Fig. 2), while in agricultural soil and brownfield there is only a minimal reduction. By 30 d the gasoline in residential and agricultural soils experienced the greatest microbial degradation while the gasoline in the brownfield soil experienced the least. These trends may be the result of the higher levels of nutrients in the residential and agricultural soils - both soils contained higher NO_3, NH_4 and K concentrations compared with the brownfield soil. Furthermore, the residential soil contained more than twice the extractable P compared with the brownfield soil (154 versus 74 mg/kg, respectively). Another factor may be the Pb concentration in the brownfield soil (497 mg/kg), which may have impaired the activity of heterotrophic bacteria. Furthermore, Pb was found to pose a greater stress to soil microbes than did other heavy metals. Application of Pb at concentrations of >500 $mg.kg^{-1}$ caused an immediate and significant decline in microbial biomass (Turner et al., 2014).

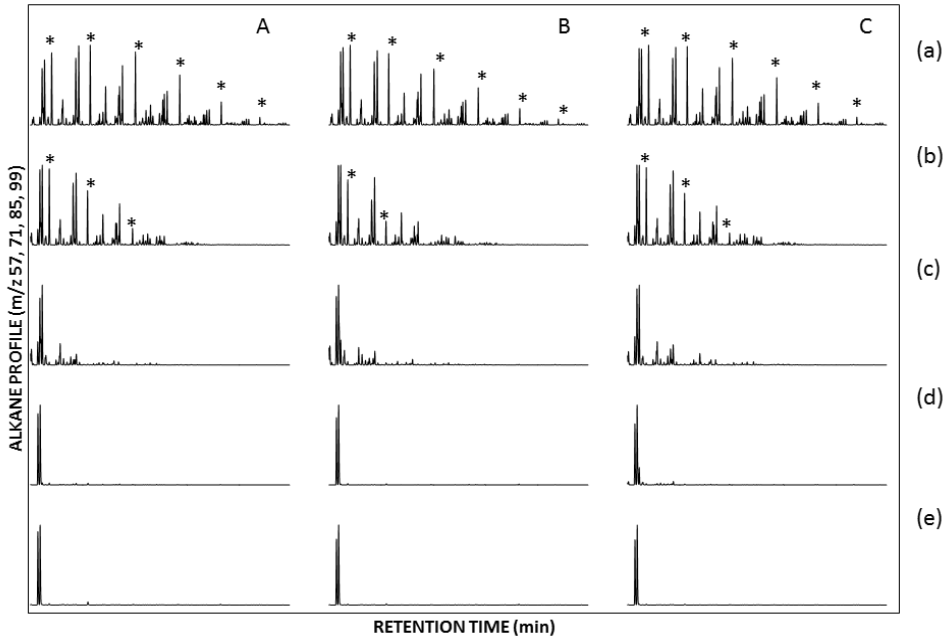

Fig. 1 Alkane profile for the soil type comparison of microbial degradation of gasoline on: (A) agricultural soil, (B) residential soil, and (C) brownfield soil over (a) 0, (b) 2, (c) 7, (d) 15, and (e) 30 days. Peaks from the homologous series of n-alkanes are marked with an asterix.

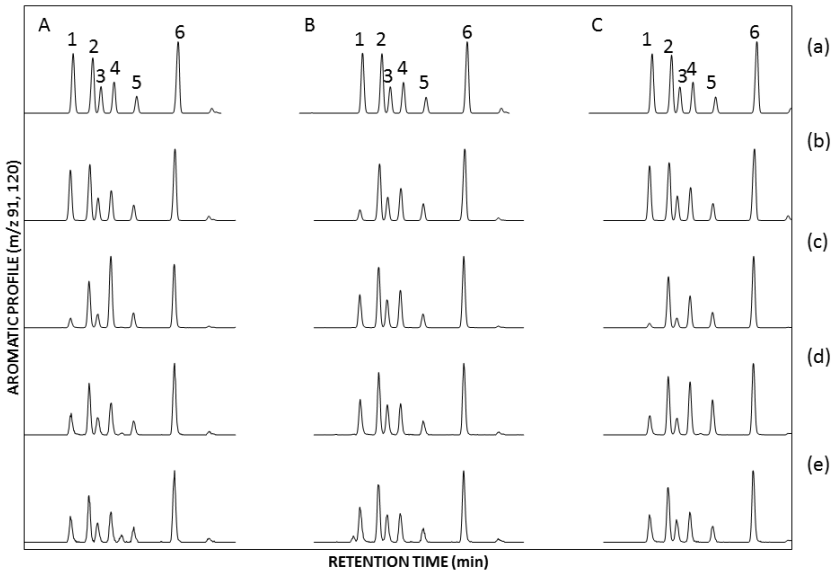

Fig. 2 Aromatic profile for the soil type comparison of microbial degradation of gasoline on: (A) agricultural soil, (B) residential soil, and (C) brownfield soil over (a) 0, (b) 2, (c) 7, (d) 15, and (e) 30 days. Peaks: (1) propylbenzene, (2) 3-ethyltoluene, (3) 4-ethyltoluene, (4) 1,3,5-trimethylbenzene, (5) 2- ethyltoluene, and (6) 1,2,4-trimethylbenzene.

Gasoline contains n-alkane and aromatic compounds, as well as branched and cycloalkane compounds. This complex mixture may be subject to pertubations such as weathering (evaporation) or microbial degradation. Therefore, in a study by Turner and Goodpaster (2009), an initial comparison of fresh gasoline and weathered gasoline was made to contrast the effects of weathering (which should depend largely on boiling point) to that of microbial degradation (which demonstrates compound selectivity) (Turner, Goodpaster, 2009).

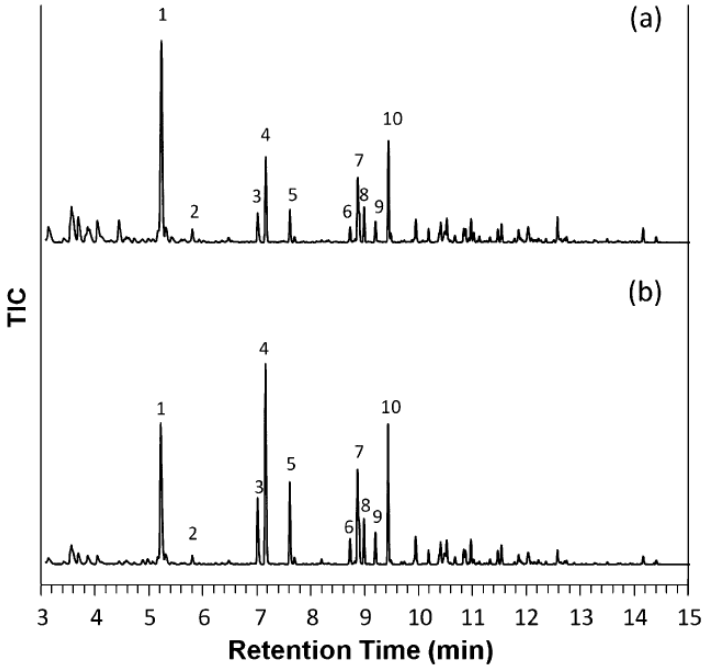

Fig. 3 Total ion chromatograms of a fresh and b slightly weathered gasoline standards diluted 0.67% v/v in pentane. Peaks: *1* toluene, *2* *n*-C_8, *3* ethyl benzene, *4* *m*- and *p*-xylene, *5* *o*-xylene, *6* propylbenzene, *7* 3-ethyl toluene, *8* 1,3,5-trimethylbenzene, *9* 2-ethyl toluene, *10* 1,2,4-trimethylbenzene

Gasoline degradation was studied over time periods extending up to 7 days. The use of a “0 day” time point allows for the effects of soil absorption and other physical/chemical factors affecting recovery of the ignitable liquid to be accounted for. Hence, the effects of microbiological activity after 2 days are readily visible, in that n-alkanes such as octane (peak 2 in Fig. 4) and decane (peak 11 in Fig. 4) largely disappear from the TIC, and the monosubstituted benzenes also show significant decreases. In addition, the peak height ratio of 3-ethyltoluene and 1,2,4-trimethylbenzene reverses. After 7 days, there are no peaks in the TIC that are readily attributable to gasoline. The peaks seen at - 4 minutes correspond to volatile short-chain aldehydes that are detected in the headspace of the soil under normal circumstances (Turner, Goodpaster, 2009).

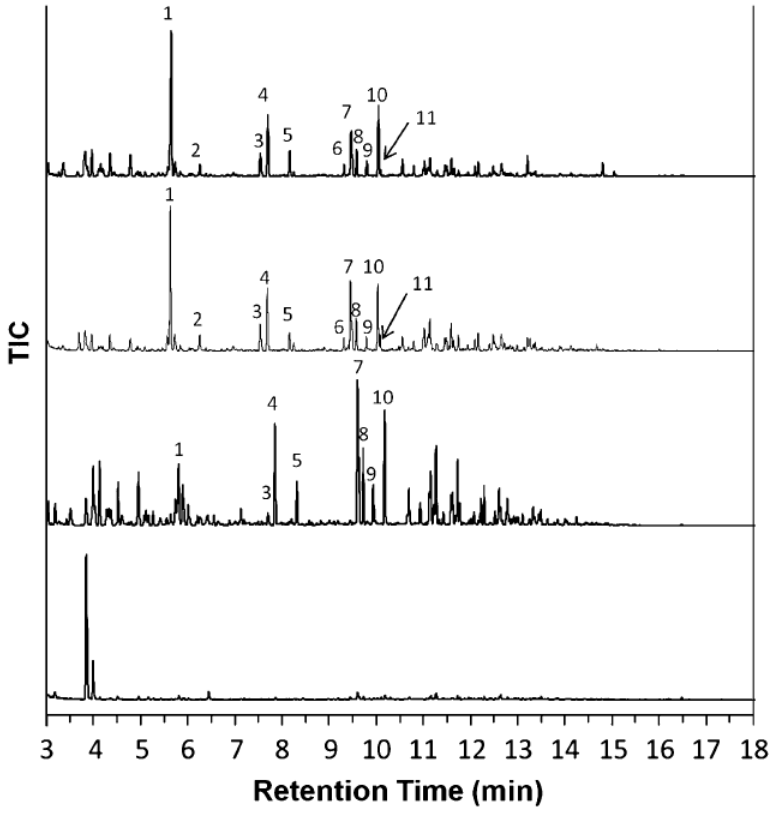

Fig. 4 Total ion chromatogram of gasoline: a standard diluted 0.67% v/v in pentane, b after 0 day on soil, c after 2 days on soil, and d after 7 days on soil. Peaks: *1* toluene, *2* *n*-C_8, *3* ethyl benzene, *4* *m*- and *p*-xylene, *5* *o*-xylene, *6* propyl benzene, *7* 3-ethyl toluene, *8* 1,3,5-trimethyl benzene, *9* 2-ethyl toluene, *10* 1,2,4-trimethylbenzene, *11* *n*-C_{10}

The potential risk of misclassifying an ignitable liquid due to microbial degradation is particularly relevant when dealing with petroleum distillates. This stems from the fact that petroleum distillates comprise branched and n-alkane compounds, the latter of which are more susceptible to microbiological attack. After 7 days, very little of the n-alkanes are present, and the resultant profile resembles that of an isoparrafin. In contrast, odorless lighter fluid comprises branched alkanes solely, which shows little to no degradation even after 7 days on soil (Turner, Goodpaster, 2009).

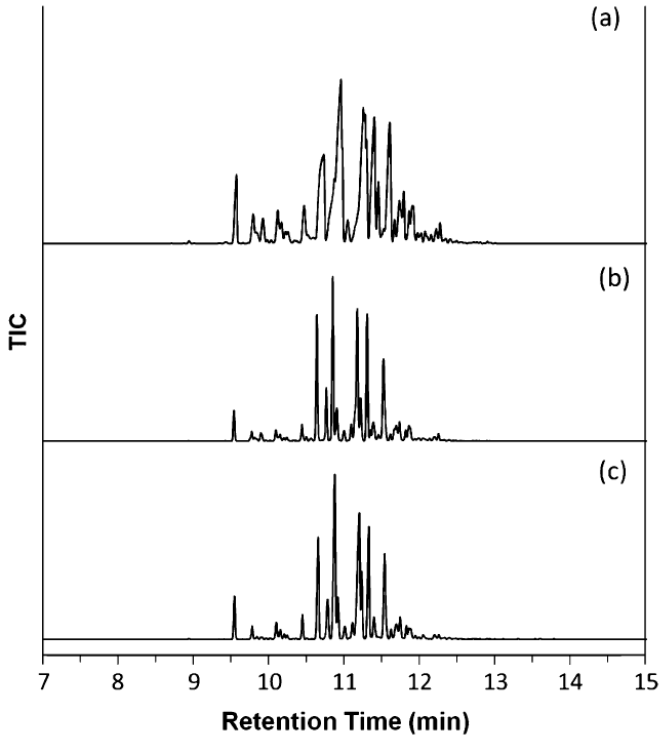

Fig. 5 Total ion chromatogram of an odorless lighter fluid, a medium–heavy isoparaffin: **a** standard diluted 1% v/v in pentane, **b** after 0 day on soil, and **c** after 7 days on soil. Peaks all branched alkanes between C_{11} and C_{15}

4 Conclusions

The ability of microbes to degrade petroleum products is a well-known phenomenon that can be used to remediate the environment. The results clearly show the need for rapid analysis of samples from the fire, for the correct identification of ignitable liquids. Microbial degradation is evident from the results for all soil types. In particular, the loss of straight chain alkanes was evident in all samples, and key compounds in gasoline were also subject to degradation. Of particular interest is the observation that bacterial degradation showed selectivity for n-alkanes composed of an even number of carbons. This represents a new type of susceptibility to ignitable liquid residues that may affect the interpretation of chromatographic data from fire evidence.

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