

PAPER**CRIMINALISTICS**

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Comparing the Effects of Weathering and Microbial Degradation on Gasoline Using Principal Components Analysis*

ABSTRACT: Ignitable liquid residues recovered from a fire scene will often show signs of weathering as a result of exposure to the heat of the fire. In addition, when the substrate is rich in organic matter, both weathering and microbial degradation may be observed. In this study, 20 μ L aliquots of fresh gasoline samples were intentionally weathered and also subjected to microbial degradation in potting soil. These samples were then analyzed using a passive adsorption–elution recovery method and gas chromatography/mass spectrometry. Peak areas from compounds of interest were normalized and autoscaled and then subjected to principal components analysis. This analysis showed that while lower boiling compounds are subject to weathering, a different set of compounds are subject to microbial degradation. Of the compounds studied, heptane, octane, toluene, and ethylbenzene were the most vulnerable to both weathering and microbial degradation. In contrast, 1,3,5-trimethylbenzene and 2-ethyltoluene were the most resistant to both phenomena.

KEYWORDS: forensic science, fire debris, microbial degradation, gasoline, principal components analysis

Identifying ignitable liquid residues (ILR) such as gasoline in fire debris samples using gas chromatography/mass spectrometry (GC/MS) is an important part of an arson investigation. Currently, one of the most popular methods for isolating ILR from fire debris is passive adsorption onto an activated charcoal strip followed by solvent elution of the ILR using carbon disulfide, pentane, or another appropriate solvent. The performance of this technique has been well studied (1–4) and its procedures have been standardized (5).

Following isolation of the ILR, analysis by GC/MS is used almost universally to detect and classify the type of ignitable liquid that may be present. Extracted ion profiles (EIPs) that are characteristic of particular compound classes are generated (i.e., alkanes, aromatics, cycloparaffins, indanes, and polynuclear aromatics) (3,6,7). In addition, specific target compounds are identified by their mass spectra and retention times. In particular, 2-ethyltoluene, 3-ethyltoluene, 4-ethyltoluene, 1,3,5-trimethylbenzene, and 1,2,4-trimethylbenzene must be present according to the American Society for Testing and Materials standard governing the analysis of ILR by GC/MS (8).

There are several challenges to the interpretation of GC/MS results. One of the most common is the presence of a large background of pyrolysis products that can obscure ILR patterns (9,10). EIPs are specifically intended to filter out these signals. Another challenge is weathering, which distorts the ILR chromatographic profile as a result of evaporation or partial burning, which results in the loss of low-boiling compounds. Comparison of a weathered sample with a library of weathered exemplars can assist in the

interpretation of these results. Chemical markers of weathered gasoline have also been reported (11).

Less commonly, microbial degradation can occur in samples rich in organic matter. Microbial degradation of compounds found in petroleum is a well-known phenomenon in environmental science where indigenous bacteria can metabolize petroleum compounds to remediate contaminated areas (12–16). In contrast, microbial degradation of hydrocarbons in fire debris is problematic. In these cases, the identification of ILR can become difficult or even impossible given enough microbial action. The forensic literature contains several examples where microbial degradation of ignitable liquids has been studied in controlled laboratory experiments (17–20). Bacteria from the genus *Pseudomonas*, which are believed to be largely responsible for biodegradation in fire debris, have been cultured and identified by Kirkbride et al. (18). We have previously demonstrated that ignitable liquids are being degraded instead of adsorbed by the organic matter that makes up soil (20). In these studies, gasoline was spiked onto autoclaved (sterilized) soil and a comparison with a gasoline standard and gasoline exposed to “living” soil showed that the losses observed in the live soil were not observed in sterile soil, and hence, they are caused by microbial action, not because of soil adsorption effects.

The complex nature of gasoline and other ignitable liquids makes statistical approaches attractive for the data analysis of fire debris samples. A recent review by Sandercock (21) discusses the many statistical approaches that have been applied to the analysis of various neat and weathered ignitable liquids. One of the most common statistical methods being used is principal components analysis (PCA) (22–25). Overall, these studies have been focused on the chemical fingerprinting of ignitable liquids, with a particular emphasis on discriminating and identifying different ignitable liquids in varying stages of weathering.

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The work described here takes a different approach in that the effects of weathering and microbial degradation on gasoline samples will be compared using PCA to elucidate relationships between variables that may be less obvious. In particular, the effect of the two processes on the chromatographic profile of gasoline will be discussed so that those compounds that are least vulnerable to either weathering or degradation can be identified.

Materials and Methods

Chemicals

Gasoline (87 octane, unleaded) was obtained locally. Pentane was obtained from Fisher Scientific Company LLC (Chicago Branch, Hanover Park, IL). Standards of various alkane and aromatic compounds identified in gasoline were purchased from VWR International (Batavia, IL), Sigma-Aldrich Inc. (St. Louis, MO), and Fisher Scientific. Activated charcoal strips and unlined quart-sized paint cans were obtained from Albrayco Technologies, Inc. (Cromwell, CT) and Lab Safety Supply Inc. (Janesville, WI), respectively. Hyponex[®] brand potting soil was obtained from Wal-Mart (Indianapolis, IN).

Weathering Study

Approximately 2 mL of fresh gasoline was transferred to each of 15 culture tubes. The tubes were then capped and the mass of the gasoline in each tube was determined. The caps were then removed from 12 of the tubes, which were placed under a stream of nitrogen until approximately 25%, 50%, 75%, and 90% of the original volume was evaporated (three replicates per level). The evaporated samples were then recapped and weighed again to obtain the mass of evaporated gasoline. Unweathered samples were allowed to stand during the evaporation of the other samples with the caps remaining tightly in place. 20 μ L of each sample were spiked onto a Kimwipe (Fisher Scientific) in a quart-sized paint can and analyzed by passive headspace adsorption followed by solvent elution.

Degradation Study

Twenty microliter aliquots of gasoline were spiked onto approximately 90 g of potting soil and stored in a sealed quart-sized paint cans for 0, 7, 11, and 22 days. The samples, as well as a soil control and a can control, were then analyzed using passive headspace adsorption followed by solvent elution.

Sample Preparation

The protocol used here is consistent with the analysis of fire debris in the Indiana State Police laboratory. In this procedure, one-third of a charcoal strip (*c.* 7 \times 9 mm²) was suspended on a pre-baked paper clip using a strand of nylon string. The can was sealed and baked in an oven at 85°C for 4 h. After cooling the can to room temperature, the carbon strip was removed. The gasoline was eluted off the strip by adding 400 μ L of pentane to the strip in a small test tube and vortexing for approximately 1 min. The samples were then subjected to GC/MS analysis.

GC/MS Method

All data were acquired using an Agilent 6890 gas chromatograph with an Agilent 5975 mass spectrometer (Agilent Technologies

Inc., Wilmington, DE). A Gerstel MPS2 autosampler (Gerstel Inc., Linthicum, MD) was used for liquid injections. The GC was equipped with a DB-5 column (30 m \times 0.25 mm \times 0.25 μ m). The carrier gas was helium with a flow rate of 1 mL/min. The method utilized an inlet temperature of 250°C, 1 μ L injection volume, and a 20:1 split ratio. The default oven temperature program started at 40°C for 3 min, ramped to 280°C at 10°C/min, and held at 280°C for 3 min. The MS parameters included a 3 min solvent delay and a scan range of *m/z* 40–300.

Data Analysis

The compounds of interest to this study are shown in Table 1, along with abbreviations used for variable labels. The software used was XLSTAT (AddinSoft, Paris, France), an add-in for Microsoft Excel. Each component was identified based on the comparison of its retention time and mass spectrum to authentic standards and the National Institute of Standards and Technology mass spectral database. Summed EIPs were generated corresponding to the characteristic fragments of *n*-alkanes (*m/z* 57, 71, 85, and 99) and aromatics (*m/z* 91, 105, and 119). The peak areas in each of the EIPs were generated using the Xcaliber data analysis software (Thermo Scientific, Waltham, MA).

To pretreat the data for statistical analysis by PCA, the peak areas from the EIPs were normalized and then autoscaled. Normalization divides the peak areas for a given sample by a constant and thus places them on the same scale. Each peak area can be divided by the sum of all peak areas (normalizing to unit area) or by the square root of the sum of squares of all peak areas (normalizing to unit length). This form of data pretreatment corrects for variation in peak areas because of sample amount and instrument response. In this study, the data were normalized to unit length (26–29).

The normalized peak areas were then autoscaled. Autoscaling is a form of variable weighting that applies mean centering followed by variance scaling. Mean centering subtracts the mean peak area for a compound from each value in a variable. Variance scaling divides each value in the variable by the standard deviation of that

TABLE 1—List of gasoline compounds included in principal components analysis.

Compounds	Formula	Abbreviation	Boiling Point, °C
Heptane	C ₇ H ₁₆	C7	98
Toluene	C ₇ H ₈	tol	111
Octane	C ₈ H ₁₈	C8	125–127
Ethylbenzene	C ₈ H ₁₀	eb	136
m- and p-xylene	C ₈ H ₁₀	p-xyl	138–139
o-xylene	C ₈ H ₁₀	o- & m-xyl	143–145
Nonane	C ₉ H ₂₀	C9	151
Isopropylbenzene	C ₉ H ₁₂	isoprop b	152–154
Propylbenzene	C ₉ H ₁₂	prop b	158
3- and 4-ethyltoluene	C ₉ H ₁₂	3- & 4-et	158–159
1,3,5-trimethylbenzene	C ₉ H ₁₂	1,3,5-tmb	163–165
2-ethyltoluene	C ₉ H ₁₂	2-et	164–165
1,2,4-trimethylbenzene	C ₉ H ₁₂	1,2,4-tmb	168
Decane	C ₁₀ H ₂₂	C10	172–174
Undecane	C ₁₁ H ₂₄	C11	196
Dodecane	C ₁₂ H ₂₆	C12	216
Tridecane	C ₁₃ H ₂₈	C13	234
Tetradecane	C ₁₄ H ₃₀	C14	252–254
Pentadecane	C ₁₅ H ₃₂	C15	270
Hexadecane	C ₁₆ H ₃₄	C16	287
Heptadecane	C ₁₇ H ₃₆	C17	302
Octadecane	C ₁₈ H ₃₈	C18	317
Nonadecane	C ₁₉ H ₄₀	C19	330
Eicosane	C ₂₀ H ₄₂	C20	343

variable (26,30). Taken together, autoscaling sets the mean of each variable to zero and the variance of each variable to one. This technique is recommended when variables have large differences in variance or are measured in different unit systems. In this study, autoscaling was used to remove variability in peak areas owing to differences in the variance of the ions that are used to construct EIPs (26–29).

The primary objectives of PCA are to effectively reduce the dimensionality of the data as well as to provide insight into any underlying structure. In PCA, a new coordinate system is created; whereby the new variable axes describe the maximum amount of variability in the data set. These new axes are the principal components. Principal components are uncorrelated, orthogonal to one another, and each represents a portion of the variance, the largest residing with the first component and descending with subsequent components. Factor scores for each sample are tabulated that depict the locations of the sample with respect to each of the principal component axes. Factor loadings plots illustrate the contributions of the original variables to each principal component. Examination of the factor loadings plot aids in the discerning chemical trends potentially associated with each component axis. Owing to normalization and autoscaling, the sum of the squares of the loadings for a given variable is equal to unity.

Results and Discussion

The effects of weathering on the total ion chromatogram (TIC) and the extracted ion chromatogram (EIC) of fresh gasoline are shown in Figs 1 and 2, respectively. In this case, the TIC is dominated by the aromatic components of gasoline, whereas the EIC is selective for the alkane fraction based upon the masses that are being monitored. As can be seen in these figures, weathering does not discriminate against specific classes of chemical compounds, such as alkanes or aromatic hydrocarbons; rather it discriminates

based upon boiling point (20). Therefore, a severely weathered gasoline sample (as shown in Figs 1*d* and 2*d*) will have little to none of the lower boiling compounds, such as toluene and *n*-octane, and an increased relative abundance of the higher boiling compounds, such as 1,2,4-trimethylbenzene and undecane.

In contrast, the effects of microbial degradation on the TIC and the EIC of fresh gasoline are shown in Figs 3 and 4, respectively. In microbial degradation, the bacteria selectively utilize the hydrocarbons based upon chemical structure. In particular, mono-substituted aromatic compounds such as toluene, ethylbenzene, and propylbenzene are significantly reduced within 14 days, as shown in Fig. 3*a–d*. The alkane profile is also significantly altered, with drastic reductions in all *n*-alkanes within 7 days, as shown in Fig. 4*a–d*. In fact, the degradation is so severe after 22 days that the only readily detected peaks in the TIC are short-chain aldehydes (appearing at *c.* 2.25 min) that are naturally found in the headspace of all soil samples.

PCA was then performed on the combined data set of both weathered and degraded chromatograms. The scores plot, which shows how the observations are projected into the space defined by the first two principal components, is shown in Fig. 5. In this case, 80.31% of the variance is captured in two dimensions. As expected, the fresh gasoline samples and the Day 0 degraded samples cluster closely together in the upper left quadrant. This indicates that any differences in recovery as a result of the matrix (Kimwipe vs. soil) are relatively minor compared with the overall changes owing to the processes of weathering or degradation. However, as the processes of weathering and degradation continue, the observations follow two completely different trajectories. While the observations from the weathered gasoline samples are separated horizontally along the first principal component, the observations from the degraded gasoline samples are largely separated vertically along the second principal component. The data labels in Fig. 5 have also been coded according to the sign of the factor score along the third

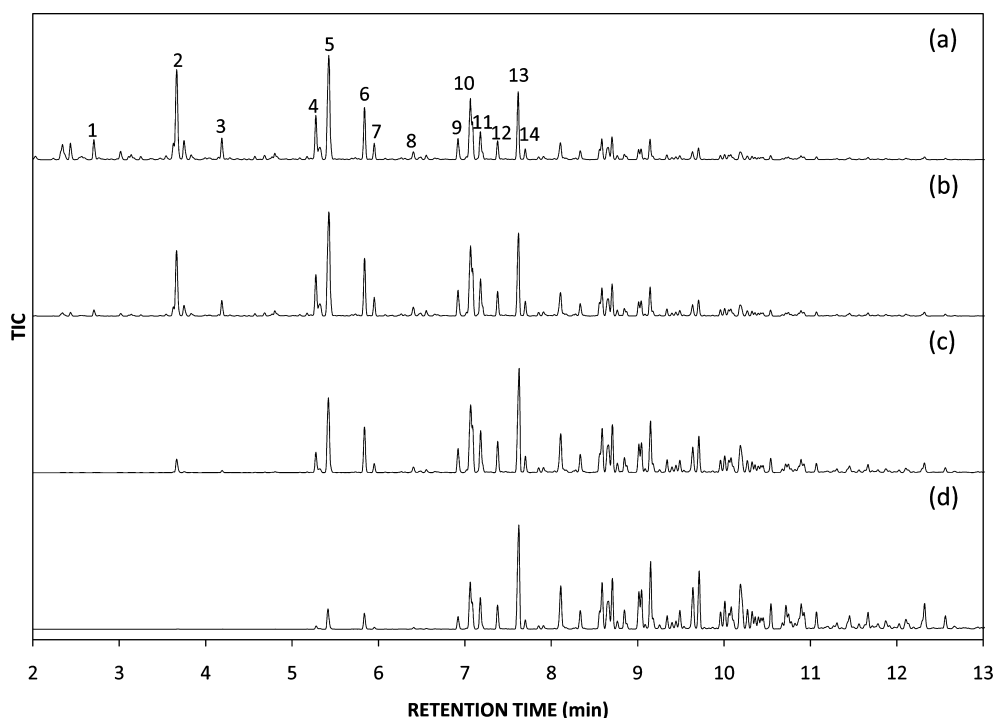


FIG. 1—Total ion chromatogram (TIC) of gasoline after weathering (a) 0%, (b) 50%, (c) 75%, and (d) 89%. Peaks: (1) *n*-C₇, (2) toluene, (3) *n*-C₈, (4) ethylbenzene, (5) *m*- and *p*-xylene, (6) *o*-xylene, (7) *n*-C₉, (8) isopropylbenzene, (9) propylbenzene, (10) 3- and 4-ethyltoluene, (11) 1,3,5-trimethylbenzene, (12) 2-ethyltoluene, (13) 1,2,4-trimethylbenzene, and (14) *n*-C₁₀.

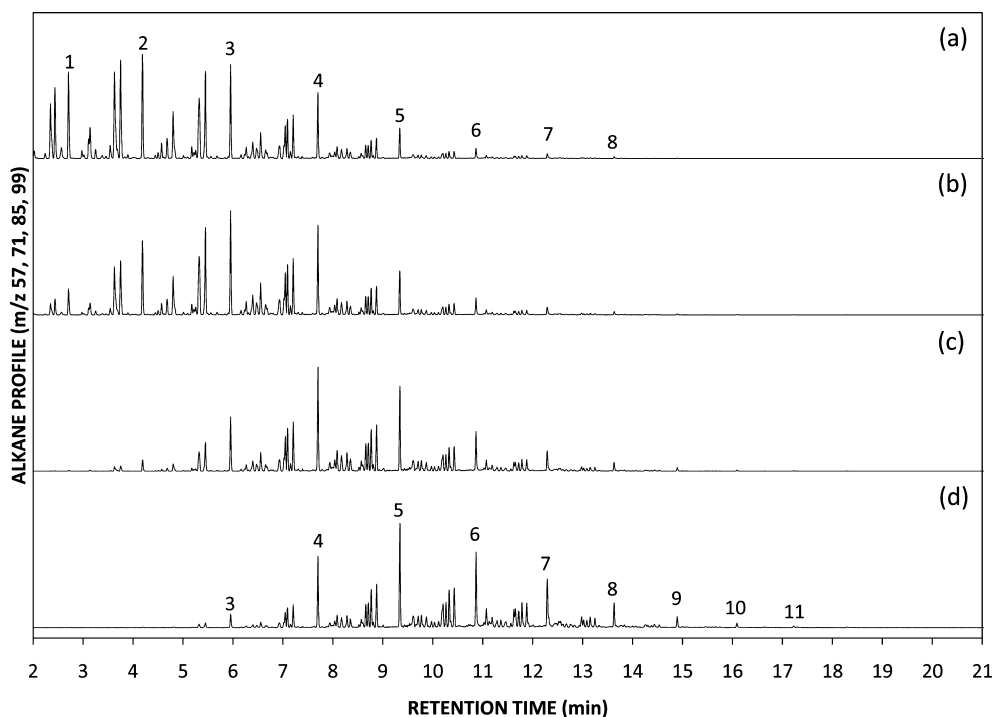


FIG. 2—Extracted ion chromatogram of gasoline after weathering (a) 0%, (b) 50%, (c) 75%, and (d) 89%. Peaks: (1) *n*-C₇, (2) *n*-C₈, (3) *n*-C₉, (4) *n*-C₁₀, (5) *n*-C₁₁, (6) *n*-C₁₂, (7) *n*-C₁₃, (8) *n*-C₁₄, (9) *n*-C₁₅, (10) *n*-C₁₆, and (11) *n*-C₁₇.

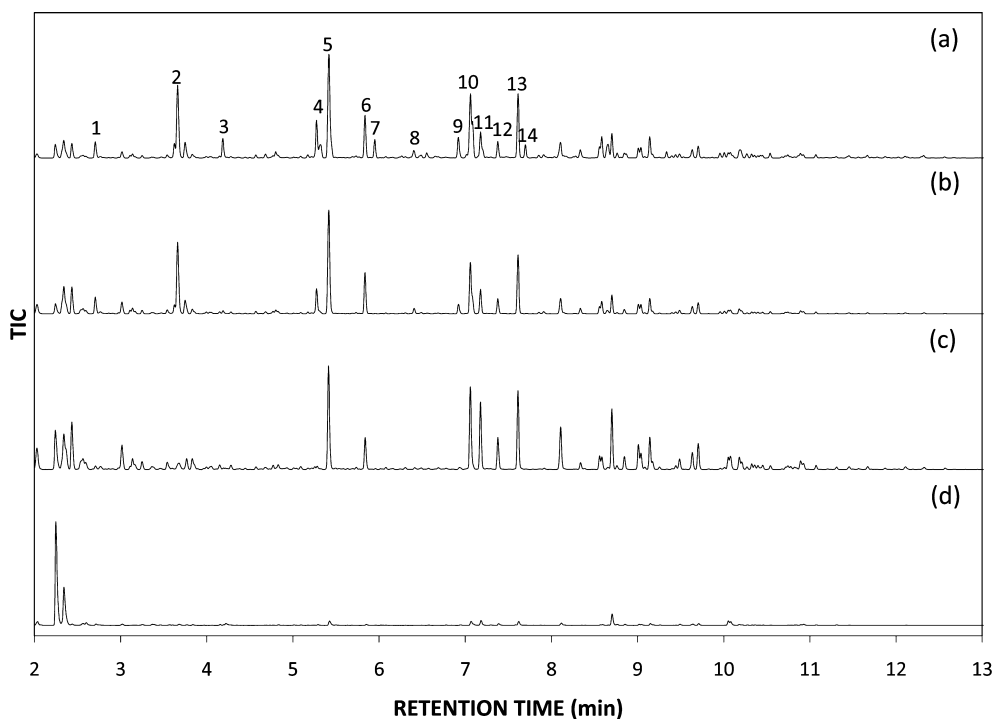


FIG. 3—Total ion chromatogram (TIC) of fresh gasoline after exposure to bacteria in potting soil (a) 0 days, (b) 7 days, (c) 11 days, and (d) 22 days. Peaks: (1) *n*-C₇, (2) toluene, (3) *n*-C₈, (4) ethylbenzene, (5) *m*- and *p*-xylene, (6) *o*-xylene, (7) *n*-C₉, (8) isopropylbenzene, (9) propylbenzene, (10) 3- and 4-ethyltoluene, (11) 1,3,5-trimethylbenzene, (12) 2-ethyltoluene, (13) 1,2,4-trimethylbenzene, and (14) *n*-C₁₀.

principal component. In this case, both the weathered and degraded samples appear to have some curvature in this dimension (i.e., alternating from positive to negative and then positive scores).

Inspection of a scores plot including the third principal component shows that these variations are neither large nor especially informative.

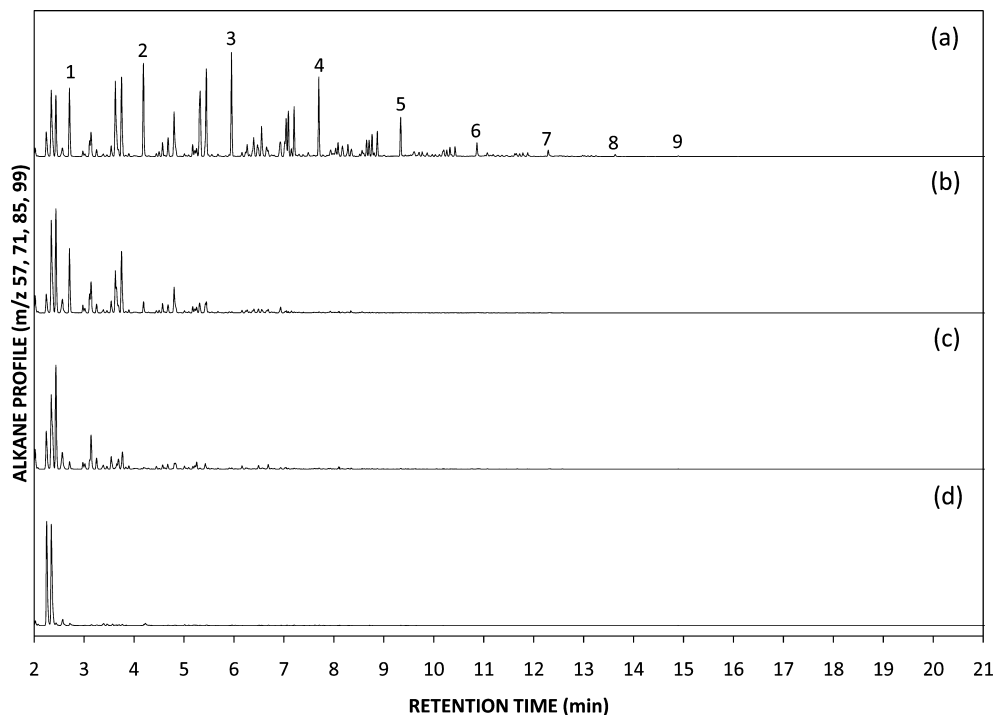


FIG. 4—Extracted ion chromatogram of fresh gasoline after exposure to bacteria in potting soil (a) 0 days, (b) 7 days, (c) 11 days, and (d) 22 days. Peaks: (1) *n*-C₇, (2) *n*-C₈, (3) *n*-C₉, (4) *n*-C₁₀, (5) *n*-C₁₁, (6) *n*-C₁₂, (7) *n*-C₁₃, (8) *n*-C₁₄, and (9) *n*-C₁₅.

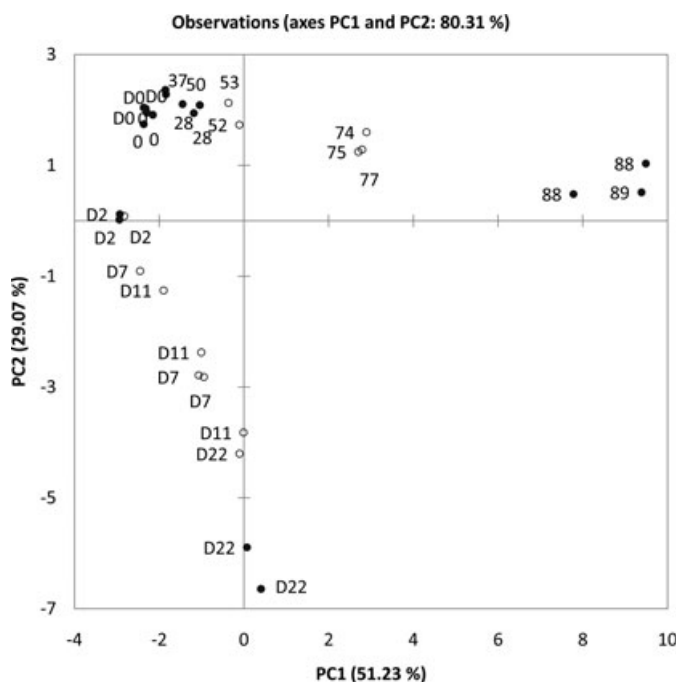


FIG. 5—Scores plot for all chromatograms as projected into the space defined by the first two principal components. Open circles denote observations that have positive scores along the third principal component. Filled circles denote observations that have negative scores along the third principal component.

Insight into the chemical differences between the two processes is gained by inspecting the factor loadings of the variables (the peak areas for the alkane and aromatic compounds), as shown in Fig. 6. In this plot, a group of compounds that have relatively

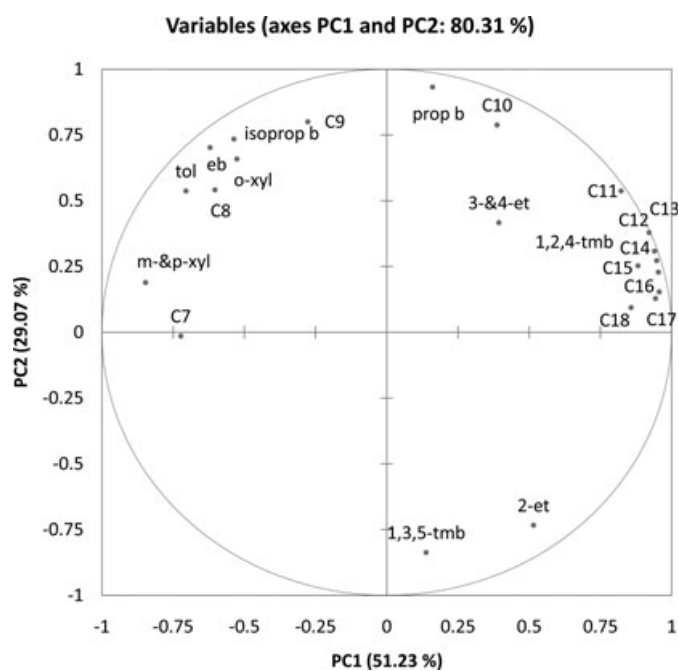


FIG. 6—Plot of the factor loadings for a data set that includes all chromatograms.

low-boiling points (i.e., <155°C) appear in the upper left quadrant. This explains the placement of gasoline samples that are <75% weathered in the upper left quadrant of Fig. 5, as their factor scores have a large contribution from low-boiling *n*-alkanes and aromatics. Compounds in the upper right quadrant of the factor loadings plot have higher boiling points (i.e., >155°C). As a result, highly weathered samples appear in the upper right quadrant of Fig. 5, as their

factor scores have a large contribution from high-boiling n-alkanes and aromatics. Overall, the factor scores of samples undergoing weathering systematically increase along the first principal component as the samples decrease in the relative concentration of low-boiling compounds and increase in the relative concentration of high-boiling compounds.

In the case of degradation, compounds that undergo rapid degradation (i.e., monosubstituted alkylbenzenes and small n-alkanes) appear in the upper left quadrant of Fig. 6. As a result, gasoline samples that have been exposed to soil for <2 days appear in the upper left quadrant of Fig. 5, as their factor scores have large contributions from these compounds. Two compounds in particular, 1,3,5-trimethylbenzene and 2-ethyltoluene, appear in the lower right quadrant of Fig. 6. As a result, gasoline samples that have been exposed to soil for >2 days appear in the lower quadrants of Fig. 5, as their factor scores have large contributions from these compounds. This agrees with the observation that highly substituted aromatics are the most resistant to microbial degradation (17–20). These two compounds also have relatively high-boiling points (165°C) and therefore would not be readily lost owing to weathering.

Conclusions

Weathering and microbial degradation of ILR are two separate processes that can be readily distinguished by PCA. Inspection of the factor loadings for the various compounds in gasoline indicate that compounds with boiling points <155°C are the most susceptible to weathering. In contrast, long-chain alkanes and lower substituted aromatics are susceptible to microbial degradation, regardless of boiling point. In particular, PCA revealed that 1,3,5-trimethylbenzene and 2-ethyltoluene were not only resistant to weathering, but they were the least susceptible to microbial degradation.

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