



### A189 The Effect of Season and Soil Type on the Microbial Degradation of Gasoline

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After attending this presentation, attendees will understand the concept of microbial degradation of gasoline and the effects of soil type and season on this process.

This presentation will impact the forensic science community and the justice system by highlighting seasonal and soil type differences in the microbial degradation of gasoline.

Substrates rich in organic matter such as soil provide an excellent source of carbon and therefore contain a high bacterial load. Since ignitable liquids are comprised of various hydrocarbons, the bacteria can utilize these fuels as a carbon source. In particular, common soil bacteria have been shown to selectively metabolize lower substituted alkylbenzenes (e.g., toluene, ethylbenzene, and propylbenzene) and normal alkanes (e.g., n-octane, n-nonane and n-decane). Previous work completed on various ignitable liquids (e.g., petroleum distillates, gasoline, isoparaffinic, and naphthenic-paraffinic products) has shown a significant loss of normal alkanes in the range of C<sub>9</sub> to C<sub>16</sub>. Branched alkanes appear to be more resistant to degradation than normal alkanes. In addition the degree of degradation was positively correlated to the length of the alkyl chain on the mono-substituted alkylbenzenes. Also, the position of the alkyl branches plays a significant role in the ability for the bacteria to metabolize the alkylbenzenes. This is problematic for fire debris analysis as samples often sit for many weeks before they are analyzed due to case backlog. As a result, selective loss of key components due to bacterial metabolism can make identifying and classifying ignitable liquid residues by their chemical composition and boiling point range very difficult.

Of interest to this study are the effects of soil type (e.g., residential, industrial or agricultural) as well as the physical and chemical characteristics of soil on bacterial populations. These characteristics include the pH, nitrogen and phosphorus content, total organic carbon (TOC), and the soil composition (e.g., silt, clay, or sand). In addition, the season (e.g., fall, winter, spring, or summer) can strongly influence the nature and activity of some bacteria. Overall, these variables (soil type and season) could impact the degree of microbial degradation observed in fire debris samples containing soil substrates. Therefore, the microbial degradation of gasoline in agricultural, industrial, and residential soils was monitored for up to 30 days over four different seasons. The top two inches of these soils were collected from specific areas in Northern Indiana each season. Degradation studies were carried out by spiking these soils in quart-sized paint cans with 20µL gasoline and stored for analysis after 0, 2, 4, 7, 11, 15, 22, and 30 days. These samples were then subjected to passive headspace concentration and GC-MS analysis.

The chromatographic profiles showed that residential soil was the most active and the industrial soil was the least active in terms of the microbial degradation of gasoline. Furthermore, a positive correlation was noted between the outdoor temperature when the soil was collected and the degree of degradation. It was noted that storage at ambient conditions upon collection prior to degradation studies had no apparent effect on the rate of degradation of the gasoline.

Principal Components Analysis was also utilized to elucidate trends of microbial degradation among the different soil types and seasons. These trends showed clear differences in the overall rate of degradation of the ignitable liquid in the different types of soil as well as over the various seasons. In particular, the industrial soil lagged significantly behind the agricultural and residential soils. Even though gasoline was degraded more slowly, by 30 days the chromatographic profile was significantly altered. The aromatic profile became very important for PCA, particularly for the C<sub>3</sub>-alkylbenzenes. It was noted that as degradation progressed, the chromatographic resolution was lost between 3-ethyltoluene and 4-ethyltoluene. This was due to benzaldehyde (a possible hydrocarbon metabolite) becoming more abundant relative to the other components being degraded. Benzaldehyde has major ions of 77 and 105 in its mass spectrum. Therefore, the previously used aromatic profile which included m/z 91, 105, and 119 was adjusted to include only m/z 91 and 120. This allowed for the exclusion of benzaldehyde while still allowing toluene, the C<sub>2</sub>- and the C<sub>3</sub>-alkylbenzenes to be captured.

#### **Fire Debris, Ignitable Liquid, Principal Components Analysis**