DEVELOPMENT OF A SAMPLING SYSTEM TO STABILIZE IGNITABLE LIQUID RESIDUES IN FIRE DEBRIS

Award Number: 2010-DN-BX-K036

FINAL TECHNICAL REPORT

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ABSTRACT

Ignitable liquid residues that are found in soils or similar materials are susceptible to microbiological attack following sample collection at a fire scene. The fact that microorganisms can metabolize components of petroleum is a well-known phenomenon to chemists responsible for analyzing fire debris. Unfortunately, the turnaround time for a fire debris case is typically more than sufficient for significant and irreversible microbial decomposition of ignitable liquid residues to occur under the right conditions. If allowed to progress unchecked, microbial degradation can eliminate the vast majority of compounds that constitute an ignitable liquid residue and ultimately lead to incorrect, inconclusive or false negative findings in fire debris cases that involve soil samples.

In this work, two approaches were pursued to avoid microbial degradation of ignitable liquid residues in soil. The first was to inhibit or eliminate the microbes naturally present in soil. While some laboratories refrigerate or freeze fire debris to minimize bacterial attack, this technique is expensive, space consuming and of mixed effectiveness. We have developed a practical and effective preservative that can be used by investigators in the field to stabilize fire debris evidence. This solution consists of the anti-microbial agent triclosan, which is non-volatile and could be safely deployable by investigators. The effectiveness of triclosan has been evaluated using microbiological techniques and testing of realistic soil samples using ASTM methods verified that ignitable liquid residues can be effectively preserved for periods of up to 30 days.

The second approach involved a sampling container that could immediately partition ignitable liquid residues from fire debris. This container utilized an activated charcoal strip that is isolated from the fire debris, thereby adapting a passive adsorption method that can immediately begin to extract ignitable liquid residues from fire debris upon collection at a fire scene. It was hoped that such a container would increase the recovery of ignitable liquids from any matrix and also help to avoid any degradation that may occur within the soils. However, the influence of discrimination effects and a lack of improved recovery as compared to traditional methods made this approach unattractive.

Overall, we have sought to monitor, characterize and prevent microbial degradation of ignitable liquid residues in soil. In particular, it is anticipated that the antimicrobial solution developed here will result in increased sample integrity and a vastly improved ability to accurately identify an ignitable liquid in a fire debris sample that contains soil.

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EXECUTIVE SUMMARY

Purpose, Goals and Objectives

This project was carried out in response to the NIJ solicitation "Research and Development on Forensic Crime Scene and Medicolegal Death Investigations" and it specifically addressed the need for "Improved means to locate, identify, capture, and stabilize samples (kit development), which are applicable to trace particulate, liquid, chemical, and biological evidence, and which provide immediate partitioning of samples for secondary testing". The work described below was a collaborative effort involving personnel in the Forensic and Investigative Sciences Program at Indiana University Purdue University Indianapolis (IUPUI) and the Departments of Biology and Natural Resources and Environmental Management at Ball State University (BSU) in Muncie, IN.

At its outset, the research had two primary goals:

- Devise a preservative for ignitable liquid residues in fire debris that is effective, non-volatile, non-corrosive, chemically inert towards ignitable liquid residues and has low toxicity to humans.
- Design a sample container that is airtight, but allows for an adsorbent material to be exposed to the container headspace and thus passively adsorb any ignitable liquid vapors that may be present.

In order to accomplish these goals, the following tasks were accomplished:

1) Several soil types were collected over different seasons and fully characterized for relevant physical, chemical and biological characteristics. Bacterial species isolated from the soils and known to degrade petroleum hydrocarbons were identified using genetic profiling.
2) Controlled decomposition experiments were conducted where native soil microorganisms were allowed to degrade ignitable liquids. The extent of degradation was monitored using passive adsorption-elution followed by gas chromatography-mass spectrometry (GC-MS). The extent and speed of decomposition was compared as a function of soil type and season.
3) The effect of various chemical preservatives was evaluated. The ability of the treatments to maintain the integrity of the ignitable liquid residue was evaluated using the techniques described in (2) above.
4) The performance of a sample container that begins the process of passive adsorption immediately upon collecting the fire debris was evaluated.
5) A series of practical tests were conducted whereby ignitable liquids were burned over a soil substrate and the two countermeasures described in (3) and (4) above were evaluated.

RESEARCH DESIGN AND METHODS

Phase I: Physical, Chemical and Biological Characterization of Soils

Several soil samples were gathered over the course of one year at various locations, including agricultural, residential and brownfield sites. These soils were packaged and delivered to Dr. John Pichtel of the Department of Natural Resources and Environmental Management at Ball State University (BSU) in Muncie, IN. Dr. Pichtel's laboratory then determined various chemical and physical properties of the soils including particle size distribution, pH, total organic carbon, and concentrations of ammonium, nitrate, P, K, Cd, Cr, Fe, Zn, and Pb. The same soil samples were also examined by Dr. John McKillip in the Department of Biology at BSU. Dr. McKillip's laboratory determined the populations of total bacteria, fungi and actinomycetes in the study soils. In addition, bacteria in the samples were identified using genetic (DNA) techniques; strains of interest were *Acinetobacter*, *Pseudomonas*, *Alcaligenes*, *Burkholderia*, *Arthrobacter*, *Flavobacterium* and *Bacillus.*

Phase II: Microbial Degradation Experiments

For controlled degradation experiments, procedures developed in the Goodpaster lab were employed. [1] In these tests, 20µL of an ignitable liquid was spiked onto approximately 100 grams of soil and stored in a sealed quart-sized paint can for periods of up to 30 days. The samples, as well as an unspiked soil control and an empty can control, were then extracted using passive headspace adsorption onto a charcoal strip (cut into thirds). The samples were heated at 85°C for 4 hours and the strip eluted with 300µL of pentane, in accordance with Indiana State Police Laboratory protocols. All extracts were analyzed using an Agilent GC/MS. This procedure was also carried out on all soil samples treated with anti-microbial agents.

Phase III: Development of Antimicrobial Treatments

The following criteria were established for a successful fire debris preservative:

1. Can be safely and immediately deployed by an on-scene investigator at the time of evidence collection.
2. Suppresses or eliminates the activity of microbes responsible for degradation of ignitable liquids in soil for periods of up to 30 days;
3. Does not degrade ignitable liquids, damage evidence containers, nor interfere with standard methods for ignitable liquid concentration and identification

Testing of the anti-microbials was carried out in one-quart paint cans. The cans contained a thin layer of soil (100 grams) that was spiked with 20µL of ignitable liquid, followed by either an anti-microbial agent or deionized water. The cans were allowed to stand for periods of up to 30 days and then processed using the passive adsorption-elution protocol discussed in Phase II. The practicality of these agents was also evaluated (e.g., degree of potential toxicity, shelf life, and simplicity of storage and use).

Chemical agents that were evaluated in this work included cationic surfactants, bleach, iodine, colloidal silver, broad spectrum antibiotics and triclosan. Each of these agents was tested for their efficacy at sterilizing soil samples. Five grams of soil was placed in a test tube and mixed with either 10 mL of a chemical agent solution or 10 mL of deionized water to produce a slurry. The treated soil samples were allowed to stand for periods of up to 30 days. The supernatant was sampled periodically by removing 10µL of the liquid and inoculating agar plates. The plates were incubated at room temperature for 48 hours and visually inspected to determine the efficacy of the treatment in eliminating microbes.

Phase IV: Development of a Sample Container that Incorporates Passive Adsorption Sampling

The following criteria were established for a successful sampling container:

1) Immediately isolates the ignitable liquid residue from the fire debris onto an adsorbent material and preserves the sample against biodegradation;
2) Is easy to use and can be provided at relatively low cost to field investigators;
3) The adsorbent material remains free of contaminants until properly deployed.

The eventual container design utilized a small metal tin with a perforated lid that was secured to the underside of a paint can lid. An activated charcoal strip was then placed in the tin so that as soon as fire debris was sealed within the paint can, any ILR that were present began to be collected immediately. Following exposure to the headspace and subsequent heating/cooling of the container, the strip was eluted with a solvent and the extract injected into the GC/MS.

FINDINGS

Phase I: Physical, Chemical and Biological Characterization of Soils

The findings for the physical/chemical analysis of the soil samples were:

- All soil samples contained high percentages of clay. Soil textures ranged from sandy clay loam to clay.
- Over the study period soil pH declined by 0.6 pH units in both the urban and the agricultural soil
- Total soil NO_3 varied as a function of both soil type and sampling period
- Soil NH_4 levels were low and relatively stable for most soil types
- Levels of extractable Cd, Cr, Fe and Zn were all within range for non-contaminated soils

We have also completed both traditional microbiological culture-based and real-time PCR standard curve-based quantification of the following soil borne bacterial genera in agricultural, brownfield and residential soil samples:

1. *Acinetobacter* spp. (*A. calcoaceticus* semi-conserved 16S rDNA primers for PCR)

2. *Alcaligenes* spp. (*A. faecalis* semi-conserved 16S rDNA primers)
3. *Arthrobacter* spp. (*A. globiformis* semi-conserved 16S rDNA primers)
4. *Bacillus cereus* (conserved species-specific 16S rDNA primers)
5. *Flavobacterium* spp. (*F. capsulatum* semi-conserved 16S rDNA primers)
6. Pseudomonas spp (conserved 16S rDNA primers (Widmer *et al*. 1998))

Phase II: Microbial Degradation Experiments

After completing degradation experiments for gasoline on all of the soil types across all seasons, the following observations were made:

- Alkanes are degraded more quickly than aromatics in all soil types.
- The overall rate of degradation decreases from Agricultural > Residential > Brownfield
- In agricultural soil, the overall rate of degradation decreases from Fall > Spring > Winter > Summer
- The compound benzaldehyde was identified in several degraded gasoline samples. This is thought to be the degradation product of toluene.

Phase III: Development of Antimicrobial Treatments

After completing culturing experiments, the following observations were made:

- Most of the household/commercial products tested were unable to sterilize soil samples in that they showed bacterial growth within 24h. Even commonly used antibiotics were not effective in killing all bacteria in soil samples.
- Of all the agents tested, only bleach and triclosan were effective in killing bacteria in soil samples.
- However, bleach signficantly corroded the metal cans and even undiluted household bleach was unable to preserve ignitable liquid residues for longer than three months.
- By far, the most successful anti-microbial agent used was triclosan and it was adopted for all future studies.

Phase IV: Development of a Sample Container that Incorporates Passive Adsorption Sampling

A comparison of the recovery of ignitable liquid residues from soil using the sampling container and/or triclosan resulted in the following observations:

- Only triclosan treated samples were preserved over the length of the study, while untreated samples were significantly degraded.
- None of the samples stored in the specialized sampling container showed any signs of evaporative loss (i.e., “weathering”) but there was evidence of significant discrimination effects whereby recovery of the more volatile components was reduced over time.

CONCLUSIONS

The overall conclusions of this study are as follows:

- Microbial degradation of ignitable liquid residues in soil is a rapid, irreversible, but preventable phenomenon.
- Through the use of well-characterized soils with known physical, chemical and biological properties, we have demonstrated that biodegradation impacts straight-chain alkanes and mono-substituted benzenes more severely than branched alkanes and highly-substituted benzenes.
- A thorough investigation of chemical agents that could be used to sterilize soil samples revealed that only bleach and triclosan exhibited any promise, with the latter being more effective than the former.
- Triclosan-treated soils were effectively preserved for extended periods
- A sampling container was designed to begin recovery of ignitable liquid residues immediately upon collection but it was ultimately unsuccessful.

IMPLICATIONS FOR CRIMINAL JUSTICE POLICY AND PRACTICE

Fire debris evidence that contains soil and subsequently stored until personnel are available to analyze it is indeed “perishable”. If allowed to progress unchecked, microbial degradation can eliminate the vast majority of compounds that constitute an ignitable liquid residue. This can lead to high rates of false negative or inconclusive findings in fire debris cases. This effect is particularly important given that laboratory confirmation is required for a canine alert or observation of a “pour pattern” by a fire investigator. This project has developed a practical and effective chemical preservative that can be utilized by investigators in the field. This will result in increased sample integrity and a vastly improved ability to accurately identify an ignitable liquid in fire debris. Inconclusive or false negative results will be reduced and the overall reliability of the analysis will be increased.

MAIN BODY

INTRODUCTION

Statement of the problem

The primary task of a fire debris chemist is to determine if there is an ignitable liquid present in a fire debris sample and if so, to classify it according to its boiling point and carbon number range. However, in organic-rich substrates such as soil, the ignitable liquid residue is exposed to native microorganisms. Normally, soil bacteria will actively utilize the carbon-rich compounds present in the soil for their metabolic needs. However, ignitable liquids that come into contact with soil are subject to microbial degradation due to the ease with which bacteria can metabolize the various hydrocarbons present. In particular, previous studies have shown that normal alkanes and lesser substituted aromatic compounds are most susceptible to degradation while more highly substituted aromatics and branched alkanes are less susceptible to degradation. This can be a rapid process which can occur in many forensic laboratories as fire debris is often stored for extended periods of time due to case backlog. The detrimental effect of this phenomenon in a forensic context is widely recognized because the identification and classification of highly biodegraded ignitable liquids can become difficult or even impossible.

Literature Review

Microbial Decomposition of Hydrocarbons

Ignitable liquids found at or near the point of origin of a fire are often partially to severely burned, although it is not unusual to encounter unburned samples. Complicating matters is the nature of fire debris, which consists of an extensive suite of organic and inorganic compounds including the matrix, pyrolysis products, combustion products and unburned fuel. The chemical composition of the residue isolated from fire debris is therefore a function of: (1) initial fuel composition; (2) presence of other combustible fuels (e.g., wood, plastic, paper, etc.); and (3) overall combustion conditions. Forensic scientists are most concerned, however, with the classes of organic compounds that occur in ignitable liquid residues, such as aliphatic hydrocarbons, cyclic alkanes, alkylbenzenes, polynuclear aromatics and indanes .[2]

The aliphatic fraction of hydrocarbons consists of straight chain, branched chain and cyclic chain carbon components. This fraction contains the most readily degraded hydrocarbon compounds [3] and most of the reported hydrocarbon-degrading bacteria have shown relatively good growth on these compounds.[4] Numerous reports exist on the isolation of hydrocarbon-degrading bacteria from petroleum-contaminated sites,[5] and the literature reports on degradation of short carbon chain (C_8–C_{16}) [6] to very long carbon chain (C_{44}) hydrocarbons.[5] The bacterial species *Acinetobacter*, *Pseudomonas*, *Alcaligenes*, *Burkholderia*, *Arthrobacter*, *Flavobacterium* and *Bacillus* are among the most documented degraders of aliphatic fractions of fuel hydrocarbons.[4b, c, 6]

The cycloalkane class of hydrocarbons is generally resistant to microbial degradation, due to the toxic effects on cellular structures.[7] However, research has demonstrated the utilization of cycloalkanes by *Pseudomonas citronellolis, Brevibacterium erythrogenes* and *Saccharomyces cerevisiae* [7b, 8] as well as a novel hydrocarbon degrading and surfactant-producing bacterium, *Alcanivorax borkumensis*.

The aromatic hydrocarbon compounds contain a benzene ring in their structure and are ubiquitous in the biosphere.[9] Aromatic hydrocarbons tend to be quite stable which leads to the relative recalcitrance of these molecules to numerous chemical and biological processes.[10] Many bacterial species have, nonetheless, evolved to use aromatics as a source of energy.[11] The microbial degradation of the aromatic fraction of petroleum hydrocarbons was reviewed by Kerr and Capone.[12] It was determined that the low-molecular weight aromatics were most susceptible to microbial attack. Higher aromatic hydrocarbons are less available to biological uptake due to their hydrophobicity.[13]

Many bacterial, fungal and algal strains have been shown to degrade a wide variety of low molecular weight polycyclic aromatic hydrocarbons (PAHs). The most commonly reported bacterial species include *Acinetobacter calcoaceticus, Alcaligens denitrificans, Mycobacterium* sp., *Pseudomonas putida, Pseudomonas fluorescens, Pseudomonas vesicularis, Pseudomonas cepacia, Pseudomonas paucimobilis, Rhodococcus* sp., *Corynebacterium renale, Moraxella* sp., *Bacillus cereus, Beijerinckia* sp., *Micrococcus* sp., and *Sphingomonas* sp..[14] A *Pseudomonas putida* strain isolated from oil fields of Gujarat, India used naphthalene as sole source of carbon and energy and mineralized naphthalene via salicylate. *Brevibacterium* HL4 and *Pseudomonas* DLC-P11 could also utilize naphthalene as an energy source.[15]

In most cases, biological decomposition of a hydrocarbon molecule is carried out by communities of microbial species rather than one or a few species. This is because most media are already enriched with stable and complex microbial communities which have become adapted to the physical and chemical milieu specific to a site. The degradation caused by microbial communities is also more efficient because different populations of organisms within a community will survive under different conditions (*i.e.*, dry vs. wet, cool vs. warm soils, etc.). Finally, no single group will be capable of acting upon all contaminant types; a range of organisms, and hence a range of physiological processes, will provide for more complete hydrocarbon decomposition.[16]

<u>Practical Implications of Microbial Degradation of Ignitable Liquid Residues</u>

The detrimental effect of microbial action on the identification of ignitable liquids in evidentiary samples that contain soil, decomposing wood or other organic matter is a well-known phenomenon in fire debris analysis. For example, the Indiana State Police Laboratory estimates that as many as 10% of their fire debris cases exhibit some degree of bacterial degradation (Mark Ahonen, Personal Communication). Microbial degradation in fire debris was first studied by Mann and Gresham of the Washington State Highway Patrol Crime Laboratory.[17] Using garden soil spiked with gasoline, they demonstrated that degradation occurred rapidly unless when the soil was thoroughly sterilized prior to introduction of gasoline or the gasoline/soil samples were stored at -5 ^{o}C. For unsterilized samples stored at room

temperature, the degradation process was characterized by a loss of substituted benzenes and all *n*-paraffinic compounds within days. The isoparaffinic compounds, however, were not affected. As a result of these findings, the authors stated that all soil samples submitted to their laboratory would henceforth be stored in a freezer until analysis is completed.

Kirkbride and co-workers isolated two species of bacteria (*Pseudomonas putida* and *Pseudomonas fluorescens biovarIII*) from fire debris samples that had generated an anomalous chromatographic pattern.[18] The ability of these bacteria to degrade gasoline and petroleum naphtha was evaluated *in vitro* and the two species were found to be complementary in their action, in that *P. putida* decomposed aromatic portions of the fuels while *P. fluorescens biovarIII* decomposed the aliphatic portion. The authors offered recommendations for avoiding microbial degradation such as storing samples at reduced temperature (as per [17]) or adding a non-volatile bactericide to the fire debris (although this approach was not subsequently explored). Alternatively, if microbial degradation is detected, the authors recommended demonstrating the presence of bacteria by culturing the fire debris samples and screening for species that are known to degrade petroleum and generate anomalous chromatographic profiles.

Cherry and co-workers analyzed gasoline and a medium and a heavy petroleum distillate on three different types of soil using a dynamic heated headspace technique and gas chromatography.[19] In this case, significant degradation in the samples was not detected until after two weeks. Degradation was prominent in the soil that was darker (i.e., more enriched in organic matter) and more moist than the other two soil types. It was determined that microbial degradation occurred among the *n*-alkanes in both of the petroleum distillates. Microbial degradation occurred among the aromatics in gasoline, but to a lesser extent than the degradation of the *n*-alkanes in the petroleum distillates.

Chalmers and co-workers repeated the work by Mann and Gresham using GC/MS technology as well as evaluating the effect of microbes on gasoline, a medium petroleum distillate (MPD) and a heavy petroleum distillate (HPD) [20]. Both rural and garden soils were used as substrates and significant degradation of *n*-alkanes and mono-substituted aromatics was noted in all samples after 7 - 14 days.

Recent work by Turner and Goodpaster has focused on characterizing the effect of microbial degradation on ignitable liquids that have not been previously studied.[1] The degradation of gasoline, odorless lighter fluid, charcoal starter fluid, kerosene and fuel oil have been evaluated in potting soil as a function of time. Key findings include the rapid loss of *n*-alkanes in all samples. For example, concentrations of nonane, decane and undecane in gasoline decreased dramatically over 7 days. Generally, branched alkanes remained unchanged. Mono-substituted benzenes such as toluene, ethylbenzene and propylbenzene also experienced dramatic losses. Decreases in higher molecular weight alkanes (C_{10}-C_{16}) were detected in the medium and heavy petroleum distillates.

Ongoing research in the Goodpaster laboratory has studied the effects of microbial degradation on Tiki torch fuel, lamp oil, and turpentine. A loss of the *n*-alkanes was observed in the Tiki torch fuel, making it difficult to distinguish from an isoparaffinic product. However,

branched alkanes were also subject to microbial degradation, which was demonstrated by the loss of 2-methylundecane and 3-methylundecane in the lamp oil. Terpenes also provided a carbon source for microbial degradation, particularly limonene, o-cymene, and β-pinene, which occur in turpentine. Branched alkanes that are more highly substituted such as 2,6-dimethylundecane and those with methyl groups at a higher position on the alkyl chain such as 7-methyltridecane were more resistant to degradation.

Potential Counter-Measures for Microbial Degradation in Soil

In general, microbial growth and activity can be controlled by physical and/or chemical methods.[21] Physical methods include applying extreme heat (e.g., autoclave) or cold (freezing), filtration, or radiation (UV or gamma). Known chemical agents that can be used as germicides include phenolics, alcohols, halogens, heavy metals, quaternary ammonium compounds, aldehydes and sterilizing gases.[21] Hewitt describes the use of chemical agents as a counter-measure for microbial degradation of volatile organic compounds (VOCs) in environmental soil analysis.[22] The author notes that aromatic hydrocarbons can be degraded even when samples are refrigerated at 4 ^{o}C. Hence, small soil samples were treated with relatively large quantities of sodium bisulfate and water (approx. 20% and 24% w/w respective to the sample), generating an acidic slurry. Levels of VOCs were stable for up to 28 days.

There are several practical problems with adapting any of the methods discussed above to a forensic laboratory. For example, although immediately freezing samples has been suggested, samples are typically not collected by laboratory personnel nor delivered promptly for proper storage. In addition, laboratories generally do not possess the resources or adequate space to maintain samples at low temperature. The remaining physical methods are also impractical at fire scenes. Sodium bisulfate appears to be effective, but the quantity required to produce an acidic slurry makes it unattractive for treating relatively large amounts of soil [22].

Techniques for Isolating Ignitable Liquid Residues from Fire Debris

One of the primary tasks of a fire debris examiner is to remove any ignitable liquid residue from the matrix of fire debris. A number of validated methods exist for concentration and/or isolation of liquid residues from fire debris. These include steam distillation[23], solvent extraction [24], headspace sampling [25], dynamic headspace concentration [26], and passive headspace concentration. The latter technique can utilize either activated charcoal strips [27] or solid phase microextraction (SPME).[28] All of these techniques were conceived and designed for use in the laboratory after fire debris has been gathered and stored until time of analysis.

In the last decade, SPME has been demonstrated to be a valuable sample preparation technique for analysis of chemical traces from fire and explosion debris [29]. SPME provided improved sensitivity for the recovery of light, medium and heavy petroleum distillates with significantly reduced analysis times and elimination of toxic solvents, when compared to the established activated charcoal strip-solvent elution method [30]. The SPME method has been optimized in recent years for a range of ignitable liquids and combustion conditions [31]. The value of SPME, including lack of interference problems, was confirmed in a study of gasoline in the presence of wood or plastic pyrolysis products. SPME was able to provide reproducible multiple analyses from a single sample [32]. The recovery of ignitable liquids (e.g., diesel fuel)

from aqueous solvents using has been demonstrated, with SPME proving to be more than an order of magnitude more sensitive than conventional solvent extraction methods.[33]

To date, there has been only one example of a product that attempts to isolate liquid residues from fire debris immediately upon collection. Diffusive Flammable Liquid Extraction (DFLEX®) charcoal strips, which are no longer commercially available, were added to debris when the sample container is sealed. Ignitable liquid residues would then adsorb onto the charcoal strip until the evidence container was opened, at which point the strip was extracted with a suitable solvent. This technique raises the possibility that ignitable liquid residues could be protected from microbial action, however, use of DFLEX strips in a soil degradation study by Chalmers showed that degradation still occurred after 7 – 14 days.[20]

Statement of Hypothesis or Rationale for the Research

The ultimate rationale for this research was to monitor and mitigate the microbial degradation of ignitable liquids on soil given its deleterious effect on fire debris analysis. It was anticipated that microbial degradation may vary depending upon the season and soil type in question. However, it was also expected that if an effective anti-microbial could be found, then all modes of degradation could be stopped. An alternative hypothesis was also formed. This was that ignitable liquid residues could be promptly adsorbed onto a charcoal strip as soon as the fire debris was sealed into its container. As long as the process of degradation was relatively slow as compared to the pre-concentration of ignitable liquid vapors onto the charcoal strip, the ignitable liquid would remain intact.

METHODS

Phase I: Physical, Chemical and Biological Characterization of Soils

Physical and Chemical Analysis

Soil material was obtained from an agricultural field (Pella clay), a residential property (Miamian sandy clay), a brownfield site (Urban land/Wawaka-Miami complex clay), and an urban site (Urban land/Fox complex, sandy clay) in central Indiana.

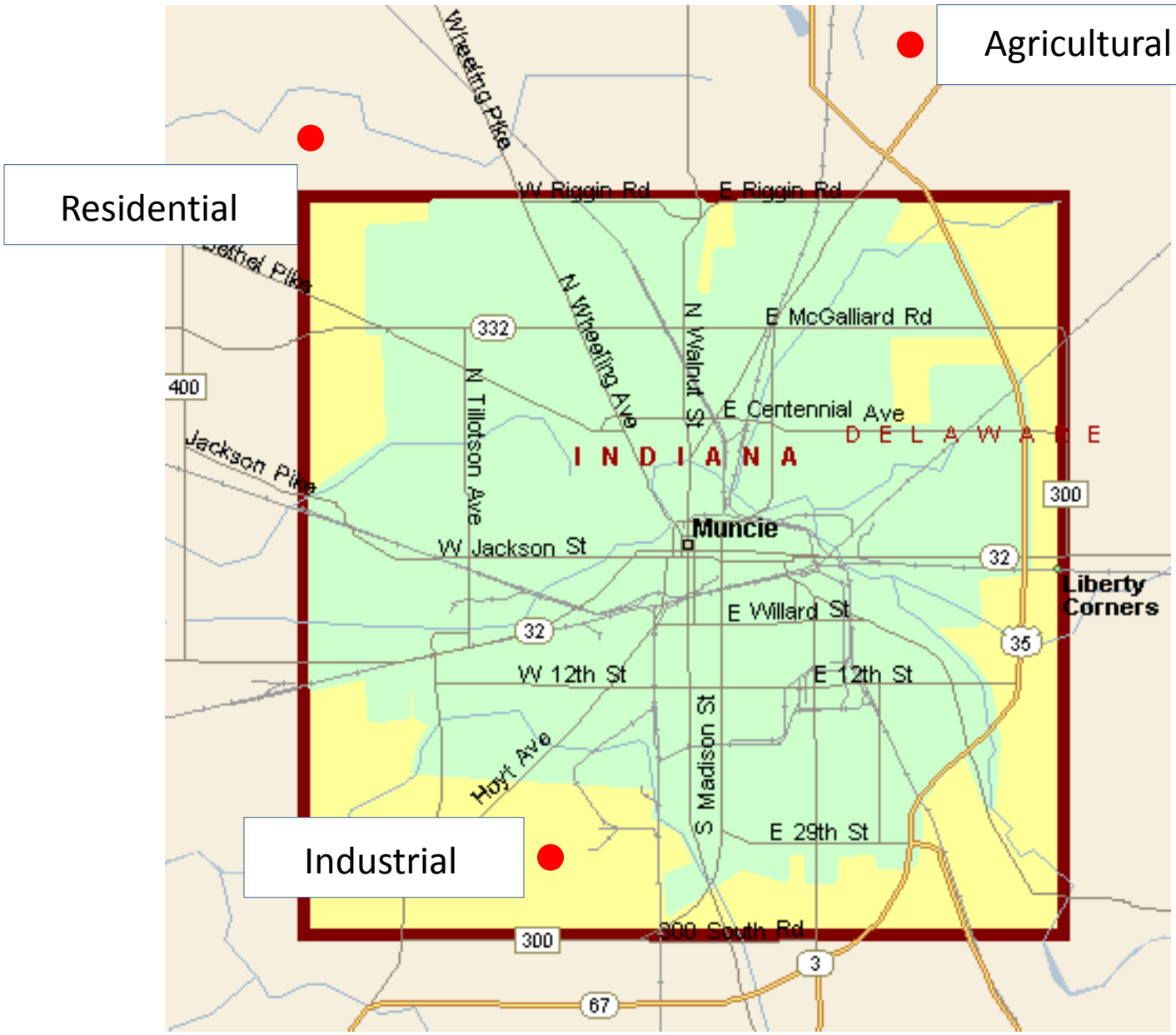

Figure 1: A map of the locations around Muncie, IN from which soils samples were gathered.

In October 2010, and January, April and July 2011, soil material was collected from the surface 0-20 cm of each site using a stainless steel sampling probe. The low/high temperatures for January, April, July, and October (the four months in which sampling occurred) are as follow:

16/33; 39/61; 64/85; and 42/64.[34] The surface was not frozen during the winter soil collection (the air temperature was approximately 38^{o}F). Following collection, the soil was composited, air-dried and sieved (< 2 mm mesh). There is no standardized procedure for air-drying a soil sample. Common practice is to spread soil onto a plastic sheet and allow it to dry over 2-3 days at room temperature.[35]

Particle size distribution of the soils was determined using the hydrometer method.[36] Total organic carbon (TOC) and total nitrogen (N) were analyzed on a Perkin Elmer Series II CHNS/O Analyzer 2400 (Shelton, CT). Acetanilide was the standard used. Soil pH was determined using a 1:2 (w:v) solids:deionized water slurry with an AB15 Accumet® pH meter with a digital readout.

Soil NO_3 concentrations were measured using Szechrome reagents (Polysciences, Inc., Warrington, PA) in a BioteK PowerWave XS2 microassay system. Soil NH_4 concentrations were determined using a modified indophenol blue technique.[37] The method was adapted for the BioteK PowerWave system. Soil extractable P was determined by the Bray-1 method.[38] Soil K was extracted with neutral 1.0 M ammonium acetate and analyzed using atomic emission spectrophotometry (Perkin Elmer AAnalyst 2000). Extractable metal (Cd, Cr, Fe, Zn, Pb) concentrations were determined by extraction with 5 mM DTPA (diethylenetriaminepentaacetic acid) with 10 mM $CaCl_2$, pH adjusted to 7.3. Briefly, the method involved mechanical shaking (120 osc./min. for 2 h) of 5 g soil with 25 ml of 5 mM DTPA in Nalgene® bottles. The suspension was filtered through Whatman No. 2 filter paper and analyzed for Cd, Cr, Fe, Zn and Pb using flame atomic absorption spectrophotometry (Perkin Elmer AAnalyst 2000). For the above analyses, there were four replicates of each sample.

Biological Analysis

Total bacteria, fungi and actinomycetes were counted using the spread plate technique [39]. Plate Count agar was used for bacterial identification, Sabouraud Dextrose agar for fungi, and Actinomycete Isolation agar for actinomycetes. Sterilization of the agar was carried out in a Tomy autoclave SS-325E at 121^{o}C for 15 min. Agar was then poured into sterile polystyrene Petri dishes and stored at 4^{o}C. There were six replicates of all treatments. Soil dilutions (10^{-3} to 10^{-7}) were spread onto Petri dishes with a sterilized L-shaped bent glass rod. Petri dishes were then incubated in the inverted position at room temperature for two days. Microbial enumeration was subsequently carried out in a Darkfield 3330 Quebec® colony counter.

For the genetic identification of bacteria, DNA was obtained from 3-5 g soil samples using a commercial system (MoBIO, Solana Beach, CA) and quantified spectrophotometrically. Real-time PCR was carried out in a Smart Cycler II (Cepheid, Sunnyvale, CA). Extracted DNA (1

µg) was added to 24 µl of master mix; a no-template contamination control was run for each sample/primer set, as well as positive control specimens consisting of ATCC (Manassas, VA) type strains of *Acinetobacter*, *Pseudomonas*, *Alcaligenes*, *Burkholderia*, *Arthrobacter*, *Flavobacterium* and *Bacillus* At least four samples of each soil were subjected to DNA extractions and each quantified extract was tested in triplicate for primer specificity and overall reaction condition optimization.

TaqMan real-time PCR was carried out using a master mix consisting of 0.025 U/µl Hot *Taq* DNA polymerase (BioGene, Kimbolton, UK), 1X PCR buffer, 0.2 mM each deoxynucleoside triphosphate (dNTP), 5.5 mM $MgCl_2$, 5% trehalose (wt/vol), 300 nM of each forward and reverse primers, consisting of 16S rDNA primers for each genus listed above, designed to be specific for key intergenic spacer regions. All primers were designed with the software analyses options available through the National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLAST) (www.ncbi.nlm.nih.gov/BLAST), which allows for sequences to be screened for nonspecific annealing frequencies and non-target homology determination. Internal standard primer targets in each case were the highly conserved prokaryotic gyrase subunit B gene, *gyrB* [4a]. Lastly in each reaction, 100 nM appropriate TaqMan probe was included. Cycling conditions were 10 min at 95°C, followed by 40 two-step cycles of 15 s at 95°C and 1 min at 60°C, with fluorescence acquisition monitored at the end of each cycle.

Standard curves to determine number of copies of target genomes for each bacterial genus were constructed using quantified bacterial templates obtained from each ATCC type strain 1:10 serially diluted in nuclease-free water to 10^{-6} (each diluted in triplicate) and subjected to amplification as described above. Bacterial template concentrations were converted to amplicon (PCR product) copies by multiplying the mean grams of DNA purified for each set of extraction replicates by 6.02 X 10^{23}, and dividing that product by the product of the respective amplicon length in base pairs X 650 Daltons. Plots depicted the number of amplicon copies as a function of respective cycle threshold (Ct) values.

Phase II: Microbial Degradation Experiments

As discussed above, controlled degradation experiments were carried out using procedures developed in the Goodpaster lab [1]. A number of ignitable liquids were degraded and any changes in their chromatographic profile were noted. Test liquids included gasoline (87 octane), light/medium/heavy petroleum distillates, turpentine, an isoparaffinic liquid, a naphthenic paraffinic liquid, a de-aromatized distillate, and an n-alkane liquid. The final group of experiments used 20µL of gasoline (87 octane), which was deposited, using a 100µL gas-tight syringe, on 80-100g of residential, industrial and agricultural soil samples that had been gathered over the course of one year. The same gasoline was used for all soil samples over all seasons and was kept in a flammable-safe refrigerator when not in use. The samples were shaken and stored in air-tight quart-sized unlined paint cans for 0, 2, 4, 7, 11, 15, 22, and 30 days. In the most recent experiments, 20µL of 2.5% (m/v) hexamethylbenzene in pentane was added directly to the soil as a surrogate internal standard and the charcoal strips were eluted with 0.01% tetrachloroethylene in pentane as a volumetric internal standard. On the day of analysis, a third of an 8mm x 20mm charcoal strip (Albrayco Technologies) was suspended on a

pre-baked paper clip using a nylon string. The samples were baked at 85°C for 4h and then extracted after cooling with 400µL of 0.01% tetrachloroethylene in pentane (pentane only for the first soil sampling).

The GC/MS conditions used in this study were standardized and matched the parameters used in many practicing forensic science laboratories:

- Column: DB-5, 30m x 0.25mm x 0.25µm
- Carrier gas: He, 1mL/min
- Injection volume.: 1µL
- Split ratio: 20:1
- Inlet temperature: 250°C
- Oven program: 40°C (3 min), ramp at 10°C/min, 280°C (3 min)
- Solvent delay: 2 minutes
- Scan range: m/z 40-300

Interpretation of the GC/MS results generally consisted of inspecting the entire chromatographic profile as well as identifying and monitoring the relative amounts of several key compounds, as shown below:

Table 1: Key compounds used in the assessment of degradation in ignitable liquids

Compound	bp (°C)
heptane	98
toluene	111
octane	125-127
ethylbenzene	136
p-xylene	138-139
o-xylene	143-145
nonane	151
isopropylbenzene	152-154
propylbenzene	158
3-&4-ethyltoluene	158-159
1,3,5-trimethylbenzene	163-165
2-ethyltoluene	164-165
1,2,4-trimethylbenzene	168
decane	172-174
undecane	196
dodecane	216
tridecane	234
tetradecane	252-254

pentadecane	270
hexadecane	287
heptadecane	302
octadecane	317

Phase III: Development of Antimicrobial Treatments

Initial Culturing Experiments

Preliminary experiments in the Goodpaster laboratory were used to determine the efficacy of various household chemicals in halting microbial growth. Screw-capped culture tubes were filled with 10mL of minimal media. A 10µL loop of a 0.9% saline extract of soil was used to inoculate the media. Varying volumes of agent were added after bacteria were given 72 hours to grow. Sub-cultures were then prepared to ensure that sterilization was complete. Observations of all sub-cultures were made after a minimum of 24 hours. A positive control of inoculated media (i.e., without chemical agent) and a negative control of media (not inoculated) were also prepared.

In addition, soil samples were treated directly with various chemical solutions for 24h at room temperature and then 10µL of the supernatant was transferred to a growth medium (tryptic soy broth (TSB), minimal media (MM) or tryptic soy agar (TSA)). The cultures were inspected daily for bacterial growth. The effective concentration of a chemical agent was determined from the sample that contained the lowest concentration of the chemical and exhibited the longest period of time wihtout bacterial growth.

Evaluation and Validation of Triclosan as an Anti-Microbial Agent

A bacterial growth study was conducted where soil samples were treated with bleach and 1.81% triclosan in 0.1M NaOH (5mL per 2g of soil). Control samples were also prepared using water and 0.1M NaOH. All samples were gently shaken for 60s at room temperature on a shaker table. 10µL of the supernatant was then transferred, using a 10µL loop, to 10mL of TSB in a screw-capped glass culture tube. Growth was then monitored three times daily for up to 4 days via a single beam diode array UV-Vis Spectrophotometer in the 400 to 800nm range. Absorbance was recorded at 600nm.

The ability of triclosan to preserve residues of gasoline on soil was evaluated by a longitudinal study with seven time points (Day 0, 2, 7, 11, 15, 22, and 30). Each time point was run in triplicate. Three soil mixtures were used:

A) Soil + Water (to monitor degradation)
B) Soil + NaOH (materials control)
C) Soil + NaOH + Triclosan (to preserve soil)

All cans received ~100 g Hyponex potting soil, 20 µL of 87 octane gasoline and 60 mL of either A) H_2O, B) 0.2 M NaOH or C) 2 % (w/v) triclosan in 0.1 M NaOH. The volume of liquid used was

based on the amount required to completely cover the soil and ensure that all bacteria in the soil were exposed to the liquid.

Samples were stored at room temperature for the allotted time in airtight quart-sized paint cans. Charcoal strips were then suspended in the cans and the cans were incubated at 85^{o}C for 4 hrs. Pentane was used to elute the gasoline residue from the charcoal strips. The resultant extracts were analyzed via GC/MS

A practical test of the preservation solution was conducted by filling a beer bottle to the bottom of the neck with gasoline. A cloth was inserted into the bottle so that the gasoline wicked up the cloth. The wick was lit and the Molotov cocktail was broken over a brick onto a 3' x 3' patch of lawn soil. Once the fire had self-extinguished the glass fragments were collected and then approximately 3 gallon of soil was collected and homogenized in a 5gal pail. The soil was split into 48 paint cans, filling each can no more than half full. 24 of the cans were then treated with the Triclosan solution while the other 24 remained untreated. The cans were sealed and stored until the specified time point when the cans were opened and one-third of a carbon strip was suspended in each can. The cans were resealed and baked at 85°C for 4hr. Upon cooling, each strip was removed and the gasoline residue was extracted from the strip using 400µL of pentane with vortexing for ~1min. The solution was then transferred to an autosampler vial and analyzed by GC-MS.

Phase IV: Development of a Sample Container that Incorporates Passive Adsorption Sampling

Various means (e.g., adhesives and magnets) were evaluated to attach a screw-capped 2oz "sniffer" tin to the lid of a quart can. None of these methods proved to be effective 100% of the time. Finally, the bottom of the screw-capped sniffer tin was attached to the lid of the quart can using an 8-32 size nut and bolt. A 5/32" drill bit was used to drill a hole in the center of the quart can lid and the bottom of the sniffer tin. Two rubber septa were used (between the bolt head and the inside sniffer tin and between the outside quart can and the nut) to seal any gaps around the bolt. The lid of 2oz sniffer tins were perferated with holes of approximately 1 mm diameter placed in an astrisk pattern. The sniffer tin stayed in place and the samples did not show any signs of evaporation. At this point, a short term study was conducted to test the sampling container design. Soil samples were spiked with gasoline. Half of the samples were treated with 2% triclosan in 0.2M NaOH the other half were left untreated. The samples were allowed to age for 0, 4, and 7 days and then subjected to the passive headspace method. Soil and can controls were also tested to ensure no part of the sampling container would produce volatile compounds that would contaminate a fire debris sample.

Finally, soil samples from a burn study using either gasoline, Tiki gtorch fuel or deisel fuel were placed inside the sample containers. A whole carbon strip was placed inside the perferated sniffer tin and the containers were allowed to stand at room temperature for periods of time up to 154 days. After a set time point, one half of the charcoal strip was removed and eluted with pentane (denoted the "pre-bake" sample) while the other half remained in the sniffer tin and the sample was subjected to the passive headspace method (denoted the "post-bake" sample).

Phase I: Physical, Chemical and Biological Characterization of Soils

Physical/Chemical Characterization

All soil samples contained high percentages of clay (29.8 to 53.9%, Table 2). Soil textures ranged from sandy clay loam to clay. These textures are typical for much of the northern two-thirds of the state of Indiana, which experienced substantial deposits of till from the Wisconsin glacial epoch.[40]

Table 2. Selected chemical and physical properties of the study soils.

	Agricultural	Residential	Brownfield
Total N	0.44	0.23	0.23
TOC*	1.0	1.0	0.9
Bray-1 P	5.3	137.3	5.3
Extractable K	122.8	154.5	74.0
Extractable metals			
Cd	0.32	0.43	0.54
Cd	0.01	0.16	0.11
Fe	48.5	39.5	18.0
Zn	13.5	24.2	22.7
Pb	11.8	12.7	497.0
Particle size analysis			
Sand, %	28.8	46.8	29.6
Silt, %	20.6	12.7	16.5
Clay, %	50.6	40.5	53.9
Texture	clay	sandy clay	clay

*TOC = total organic carbon.

Over the study period soil pH declined by 0.6 pH units in both the urban and the agricultural soil (Fig. 2). This may be partly explained by the oxidation of commercial nitrogen fertilizers, which ultimately result in acid generation.[41] Total soil N ranged from 0.23 mg/kg (residential and brownfield) to 0.44 mg/kg (agricultural) (Table 2). Soil TOC was similar across treatments -- samples ranged from 0.9 to 1.4 % (Table 2). Soil TOC remained relatively constant during the study period (data not shown).

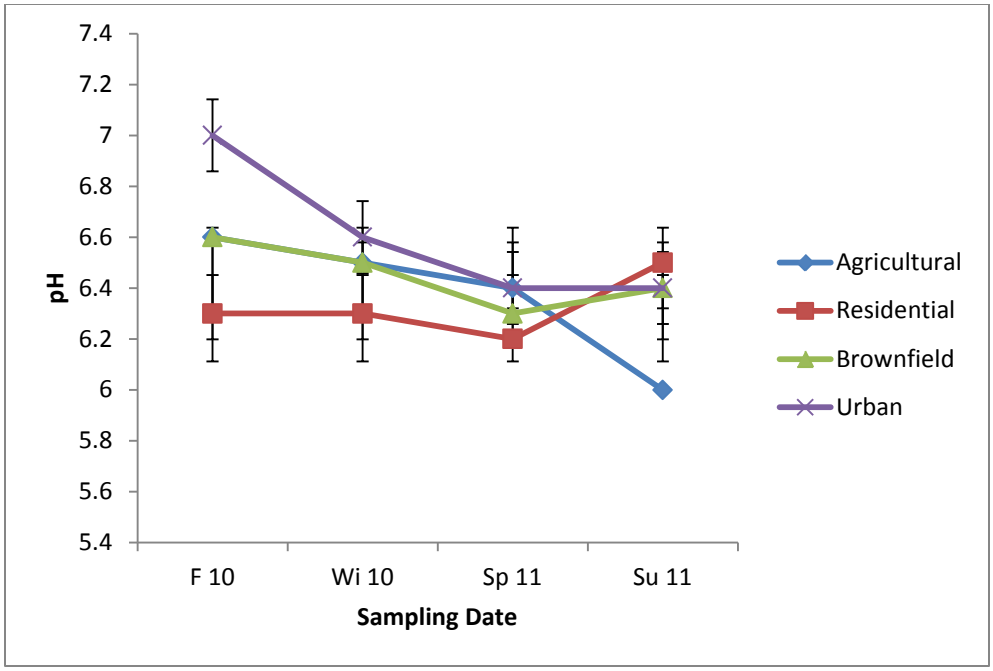

Figure 2. Soil pH levels over four sampling periods.

Total soil NO_3 varied as a function of both soil type and sampling period (Fig. 3). The brownfield soil was consistently lowest in NO_3 levels (range 5.7-26.3 mg/kg), whereas the urban soil had highest levels (221 mg/kg, fall 2010 mg/kg). In the urban soil NO_3 levels decreased significantly ($p < 0.05$) from the fall to winter sampling (Fig. 3), likely due to leaching of late-applied soluble N fertilizer. Several researchers have determined substantial NO_3 leaching from fertilized turfgrass.[42] Single dose, high rate, water-soluble N applications to mature turf grass stands tend to result in excess N losses as NO_3.[42a] The remaining three soil types experienced a modest decline in NO_3 levels during the study period (Fig. 3). From the spring to summer sampling NO_3 levels in the urban soil increased from 13.4 to 114.7 mg/kg. Such fluctuations in soil NO_3 during the growing season have been reported elsewhere.[43]

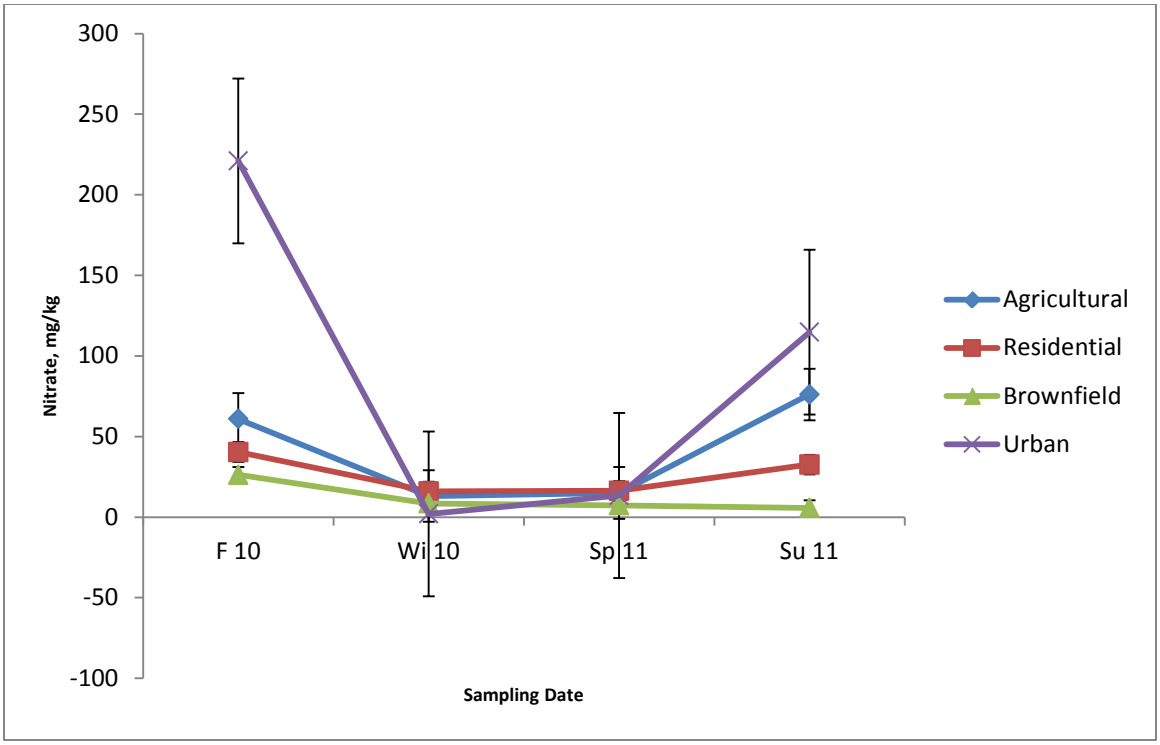

Figure 3. Soil nitrate levels over four sampling periods.

Soil NH_4 levels were low and relatively stable for most soil types, ranging from non-detectable to 12.9 mg/kg (Fig. 4). Brown et al. noted negligible NH_4 losses from golf greens treated with several N fertilizer types.[42d] In the case of the agricultural soil, NH_4 levels increased from 4.1 mg/kg in winter to 12.9 mg/kg in summer (Fig. 4). These fields had recently received fertilizer application as anhydrous ammonia. Some may have also been released from exchangeable (colloid-bound) forms. Mengel and Scherer measured a decline in soil NH_4 during the growing season followed by a subsequent rebound due to release from exchangeable forms.[44]

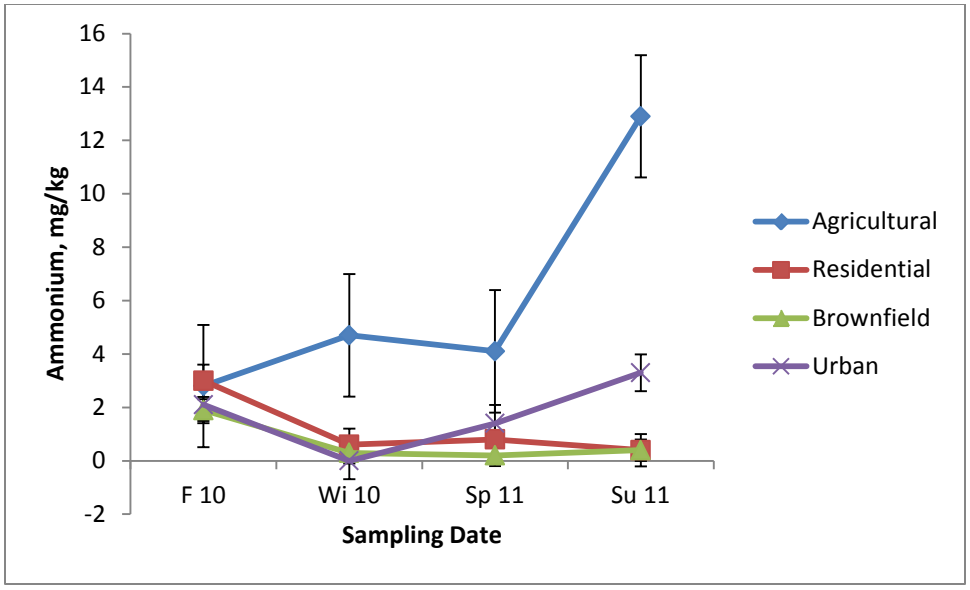

Figure 4. Soil ammonium levels over four sampling periods.

Levels of extractable Cd, Cr, Fe and Zn were all within range for non-contaminated soils (Table 2). In the brownfield soil, extractable Pb levels measured 497 mg/kg. An upper limit for the Pb content of a normal soil is approximately 70 mg/kg.[45] The levels of Pb in soils that are toxic to soil microorganisms and plants are a function of species, Pb concentration and soil factors (e.g., pH, fertility status, presence of other toxins); thus, threshold toxicity levels will vary. Soil Pb levels considered toxic to biota have ranged from 100 to several thousand mg/kg.[45-46].

Biological Characterization

Early efforts in the Goodpaster laboratory to identify the soil bacteria responsible for degradation have entailed inoculating bacteria into minimal media spiked with 0.1% v/v gasoline and where other nutrients were limited. Bacterial colonies were sub-cultured and then spread onto agar plates. A single colony was then isolated and determined to contain gram-positive short rods that also produced a positive presumptive test for *Pseudomonas*, the same genus isolated by Kirkbride.

Enumeration of soilborne bacteria may be completed with the traditional culture-based methods familiar to all microbiologists (standard plate count), using appropriately selective media for the target group(s) of interest. Our results from this approach revealed no significant differences in total bacteria or fungi counts across the soil types by season (Table 3).

Table 3. Microbiological plating-based results. Values shown represent mean colony counts of eight replicates, which were not significantly (p>0.05) different across the sampling times (F10, Wi10, Sp11, & Su11). PCA= Plate count agar for total chemoheterotrophic bacteria; ACT = actinomycete agar, for soilborne actinomycetes; SDA = Sabouraud dextrose agar for total molds and yeasts.

Media	Residential	Brownfield	Agricultural
PCA	2.54×10^5	6.86×10^5	5.02×10^5
ACT	7.1×10^5	1.38×10^6	4.62×10^5
SDA	4.48×10^4	1.24×10^5	2.28×10^5

However, these findings do not necessarily reflect the relative levels of viable target microorganisms in the soils, since recovered densities of each group may not be in proportion to their relative numbers in the natural soil communities. Moreover, only a fraction of the viable microorganisms in an environmental sample are recoverable on artificial media (although all microbiologists still expect these experiments to be performed). Thus, a more sensitive measure of these genera was performed that targets genomic DNA from select bacteria of interest based on a precedent for bioremediation or breakdown of chemical adulterants in soil communities. This DNA-based approach was real-time PCR targeting conserved ribosomal DNA sequences that allow for total genome equivalents (copy number) for each genus to be determined.

Our results, presented in Table 4, reveal an actual density of each genus markedly higher than that seen in the culture-based experiments. Using standard curves constructed by a type strain of each genus, we determined how many genome equivalents were detectable across each soil type. While the average value did not differ significantly ($p > 0.05$) from F10, Wi10, Sp11, or Su11, they did differ by soil type and by genus, as summarized collectively in Fig. X. *Alcaligenes faecalis* genomic DNA copies were consistently the highest in all soil types. This genus is known to be involved in remediation of soils contaminated with polychlorinated biphenyls (PCB) and chlorobiphenyls (Menn et al., 2008). Most other detectable genome copies across soil types were comparable (although at lower levels that that seen for *Alcaligenes*) except for *Flavobacterium* spp. in residential soil (Fig. X), which was found to be 10^{10} genome equivalents. At this time, the reason for this specific finding remains unclear, although the consistency of the result among replicates tends to rule out variables associated with DNA extraction or PCR aspects.

Table 4. Total genome copies per gram of soil as detected by real-time PCR. Values shown are the average of three replicates.

Soil Type	***Acinetobacter***	***Alcaligenes***	***Arthrobacter***	***Bacillus***	***Flavobacterium***	***Pseudomonas***
Residential	$3.12x10^{16}$	$2.37x10^{20}$	$1.636x10^{13}$	$5.12x10^{14}$	$4.53x10^{10}$	$1.682x10^{14}$
Agricultural	$4.32x10^{16}$	$7.29x10^{19}$	$4.135x10^{10}$	$4.06x10^{14}$	$1.8x10^{16}$	$1.624x10^{14}$
Brownfield	$1.31x10^{17}$	$2.26x10^{18}$	$1.396x10^{12}$	$3.36x10^{14}$	$4.21x10^{11}$	$2.56x10^{15}$

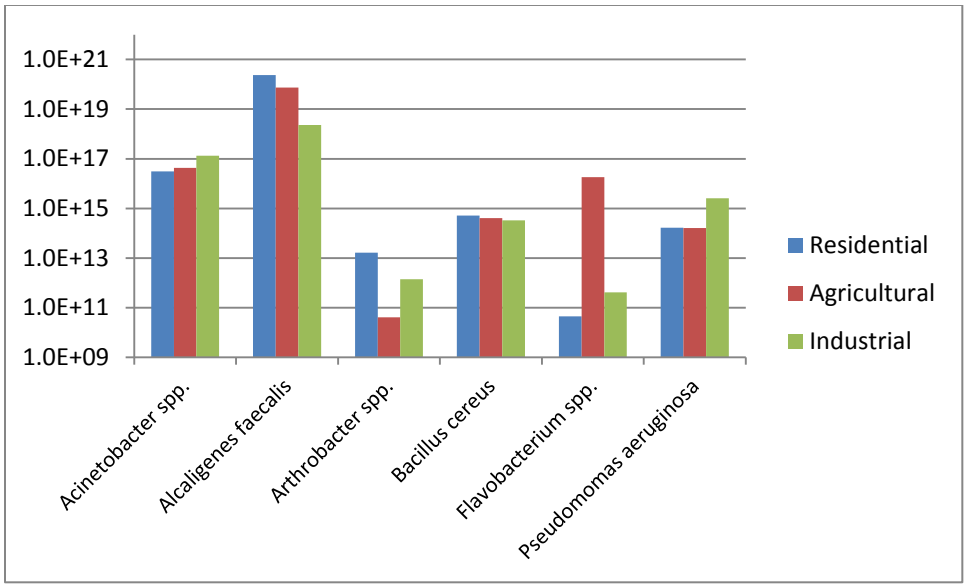

Figure 5: Collective 16S rDNA-based PCR quantification comparisons for all six bacterial genera. Values plotted are averages of triplicate experiments and did not differ significantly ($p > 0.05$) across sampling times.

Phase II: Microbial Degradation Experiments

The following general observations were made based upon the degradation experiments that were conducted using various ignitable liquids:

- n-alkanes from $C_8 - C_{16}$ were significantly degraded

- Significant degradation also occurred in the aromatics, but higher substituted benzenes were not degraded as severely
- Branched alkanes were not significantly degraded
- Of greatest concern is that degradation of n-alkanes caused the chromatographic profile of petroleum distillates to resemble that of isoparaffinics

The effect of season and soil type was also investigated using the soil samples that had been physically, chemically and biologically characterized. As an example, figures 6 and 7 show the alkane and aromatic profiles of gasoline after exposure to a residential soil during four different seasons. Figures 8 and 9 show the alkane and aromatic profiles of gasoline after exposure to an agricultural, residential and brownfield soil during the same season (Fall). Taken together, the data from all soil types across four seasons results in the following general observations:

- The most degraded samples were seen in soil collected in the fall
- The least degraded samples were seen in soil collected in the summer
- Alkanes are degraded more quickly than aromatics in all soil types.
- The overall rate of degradation decreases from Agricultural > Residential > Brownfield.

While it may seem logical that summer samples would exhibit the highest rate of microbial activity and sample degradation, it is important to keep in mind that the temperatures of soil removed for this study during Fall and Winter would likely be more within the range of ideal growth temperatures for most of the bacterial genera included in this present study. ACINETOBACTER, ARTHROBACTER, and most of the others not only tolerate what may be considered as lower temperatures (~20 deg. C), but in fact will remain metabolically active. In contrast, summer temperatures at the soil depths measured in this study in many instances will exceed the optimum growth range for these bacterial genera, with the exception of BURKHOLDERIA spp..[47] Furthermore, temperatures were very high (mid-90s) during summer soil collection and it was an exceptionally dry season. It is well established that soil microbial activity is inhibited during drought and/or high temperatures.[48] Soil drying causes stress to soil microorganisms[49] because they require more energy for water uptake and become substrate-limited as substrate and nutrient diffusion are restricted.[50]

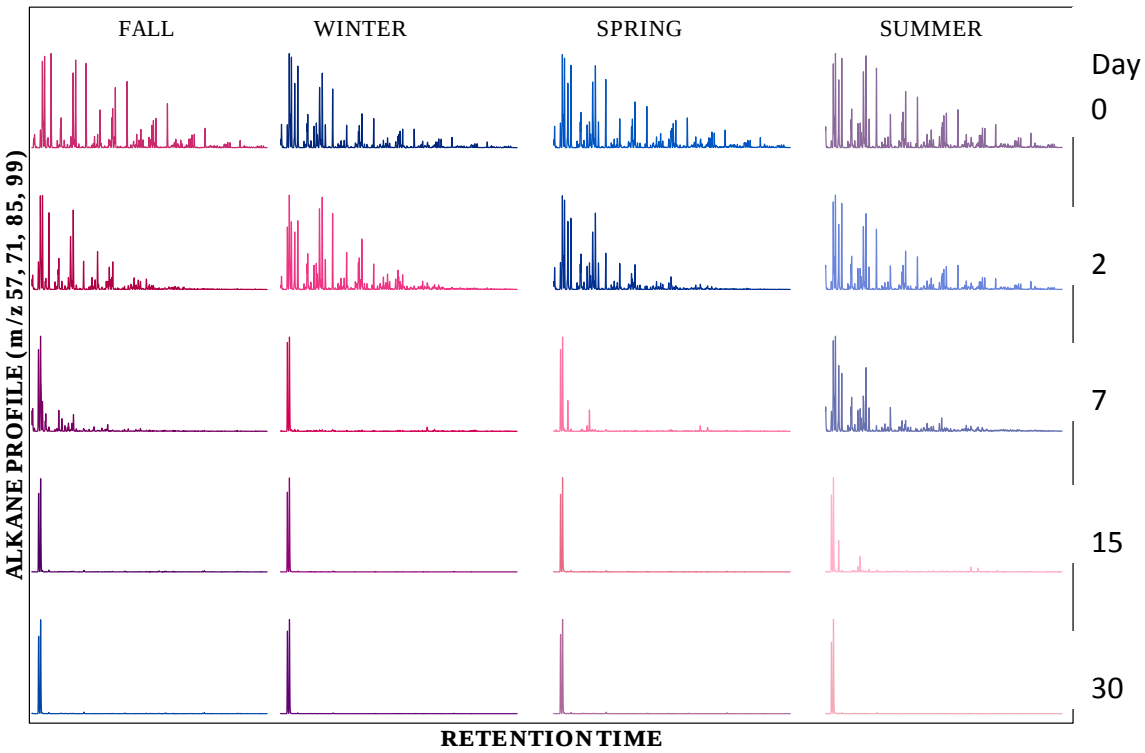

Figure 6: Extracted ion profile for alkanes in gasoline after exposure to a residential soil during four different seasons.

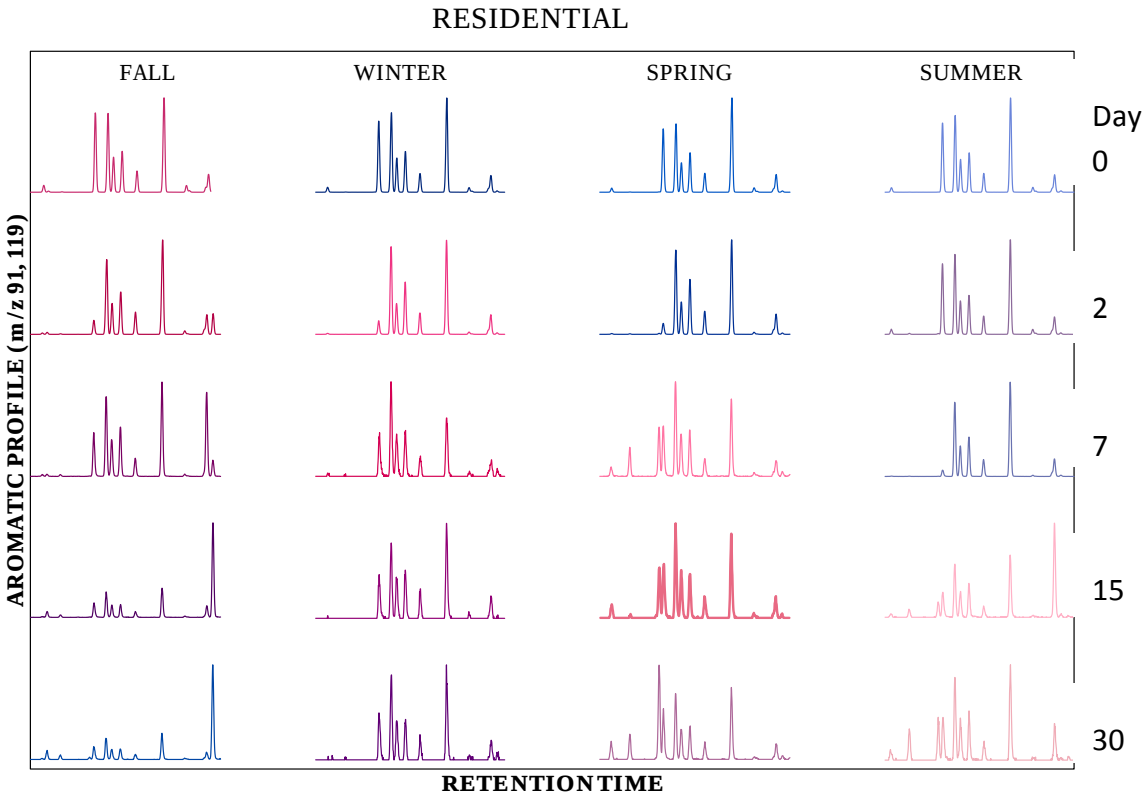

Figure 7: Extracted ion profile for aromatics, showing the trialkylbenzenes, in gasoline after exposure to a residential soil during four different seasons.

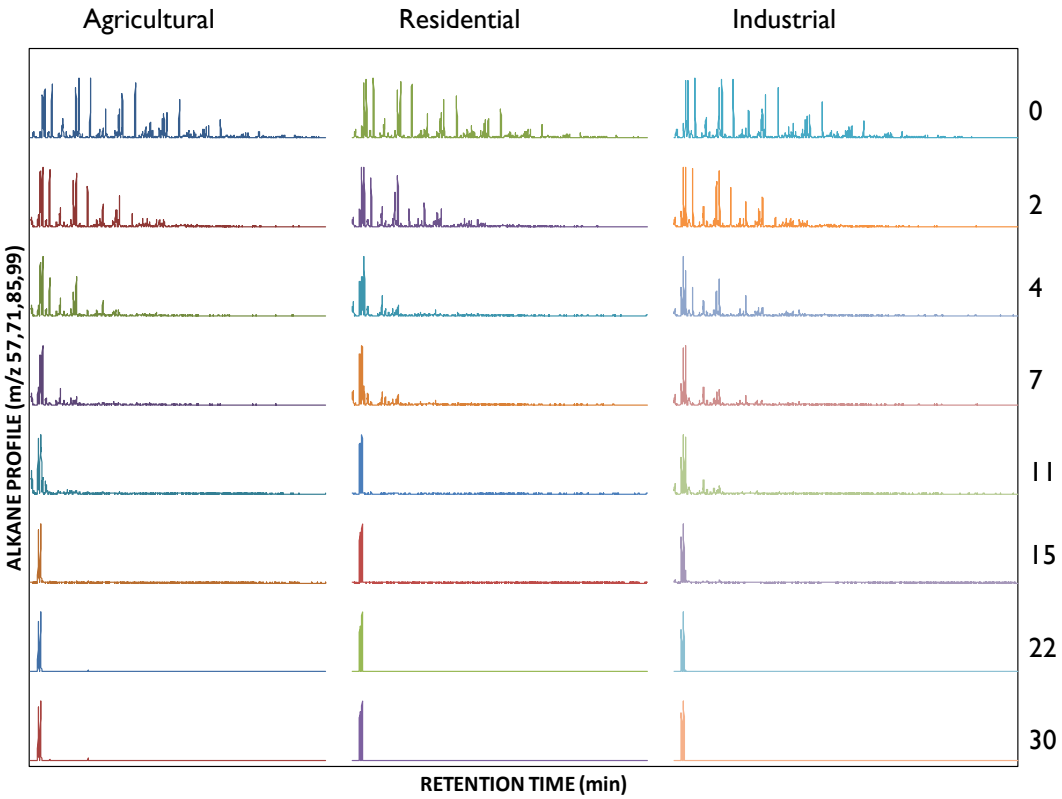

Figure 8: Extracted ion profile for alkanes in gasoline after exposure to an agricultural, residential and brownfield soil during the same season (Fall).

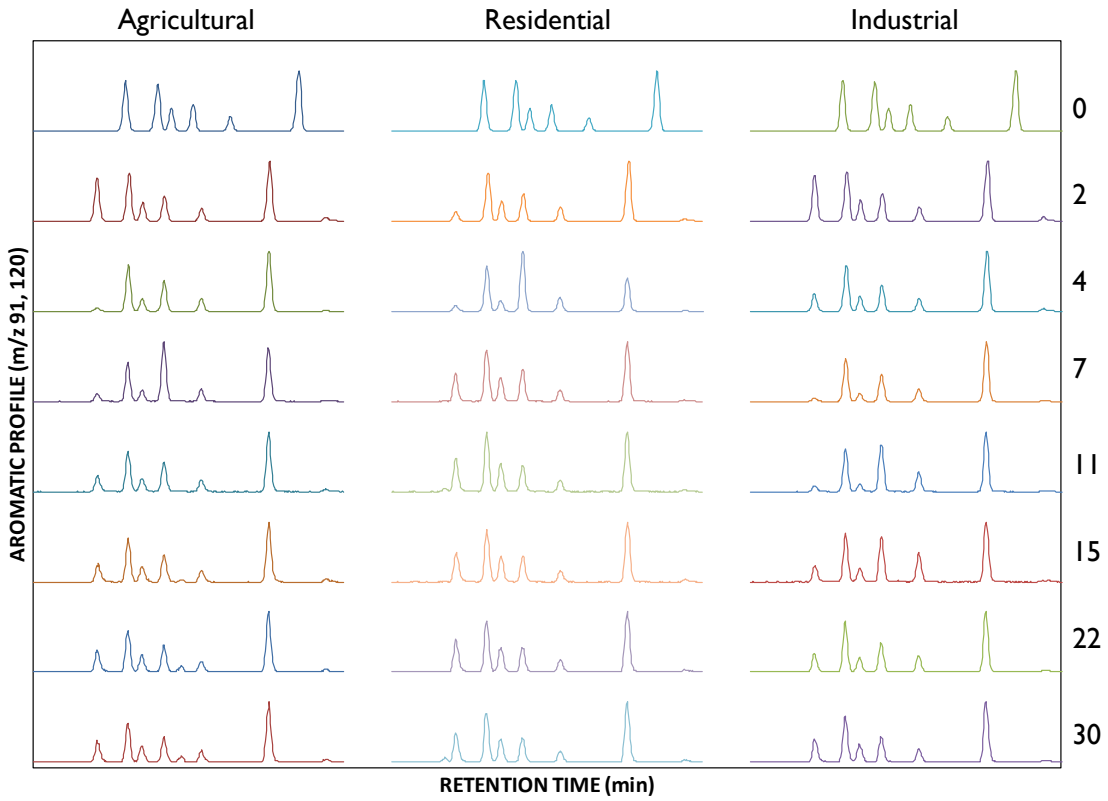

Figure 9: Extracted ion profile for aromatics, showing the trialkylbenzenes, in gasoline after exposure to an agricultural, residential and brownfield soil during the same season (Fall).

Phase III: Development of Antimicrobial Treatments

Initial Culturing Experiments

These experiments served as a rapid means to test the efficacy of varying levels of each chemical agent because visual inspection of the culture broth can determine whether the agent is effective in killing bacteria. Furthermore, it provided a minimum concentration of agent to use for future studies involving actual soil. However, most of the household/commercial products tested showed bacterial growth in culture prepared from treated soil within 24h. Overall, household bleach (sodium hypochlorite) was the most effective chemical agent – 2% v/v was capable of sterilizing an active culture of soil bacteria. However, subsequent attempts to sterilize soil samples with bleach as well as prevent microbial degradation showed that a 10% v/v solution of bleach could prevent degradation for only 7 days. Even undiluted household bleach was not effective at preserving ignitable liquid residues for longer than 3 months. A key disadvantage to using bleach is its tendency to corrode metal cans. These efforts also revealed that regardless of which chemical agent is used, it must be in intimate contact with the sample (i.e., a slurry) in order for effective sterilization to occur. In turn, this means that one cannot avoid using a relatively large amount of chemical solution.

Evaluation and Validation of Triclosan as an Anti-Microbial Agent

By far, the most effective anti-microbial tested was triclosan, an organic compound found in anti-microbial soaps and other cleansers. This compound is widely available, relatively inexpensive, non-volatile and non-corrosive. For example, a growth study was completed on a 2% w/v solution of triclosan in 0.2 M NaOH where a control soil sample is compared to a soil sample treated with both the NaOH solvent and triclosan solution. As shown in the figure below, triclosan maintains a clean culture for up to 75 hours.

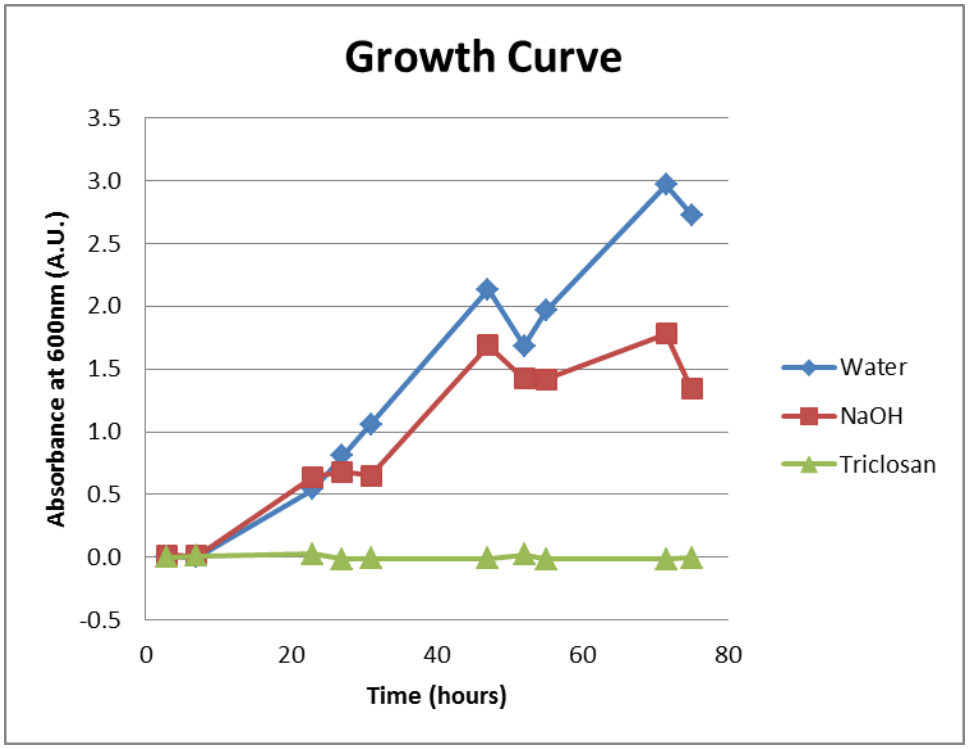

Figure 10: Absorbance of inoculated culture broth samples that were treated with water, 0.2M NaOH or 2% (w/v) triclosan in 0.2M NaOH over the course of 75 hours

In the first validation of triclosan, it was evident from the gasoline chromatograms that gasoline is severely degraded after just 7 days. However, triclosan treated samples remained intact and the resultant chromatograms could be readily identified as gasoline. In fact, the chromatograms were easily identified until day 25. Furthermore, minimal corrosion and rust was seen in the paint cans used to house the soil solutions. Therefore, the antimicrobial solution used in this study will not impact the structural integrity of the evidence container. Also, the basicity of the antimicrobial solution is on par with common household cleaning agents, such as drain openers.

This finding was further validated by monitoring the chromatographic profile of gasoline that was either allowed to degrade, preserved with bleach or preserved with a slightly more concentrated solution of triclosan (Figure 11). The chromatograms from triclosan-treated soil remained unchanged for up to 30 days. A practical burn study was also conducted where a Molotov cocktail was constructed and deployed onto soil. The gasoline residues were then either left untreated, whereupon they became degraded, or treated with triclosan, whereupon they were preserved (Figure 12).

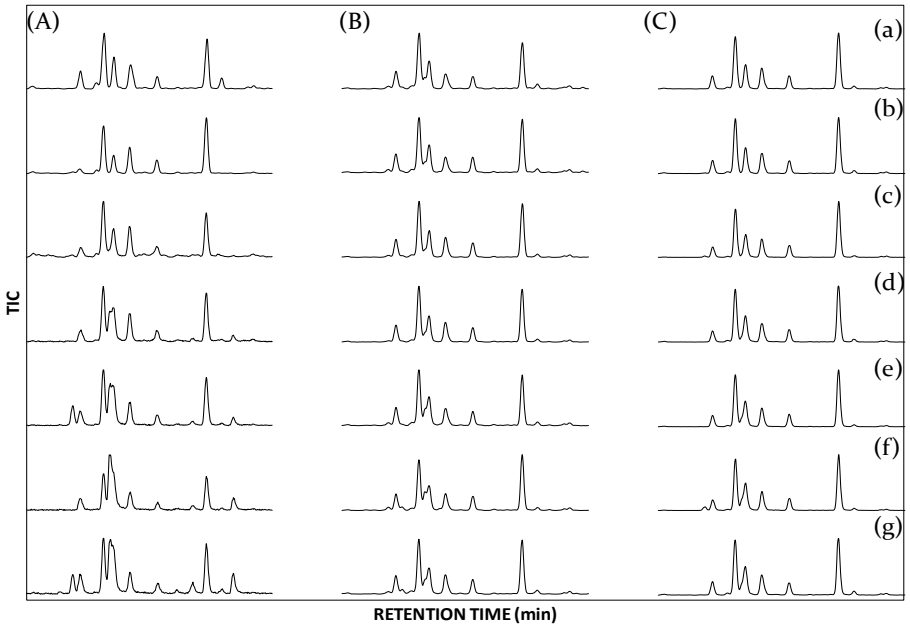

Figure 11: Chromatograms showing the "group of five" trialkylbenzenes under various conditions. A) unpreserved gasoline on soil, B) gasoline preserved with 10% bleach, (C) gasoline preserved with 2% (w/v) triclosan in 0.2M NaOH. Time points: (a) 0, (b) 2, (c) 7, (d) 11, (e) 15, (f) 22, and (g) 30 days.

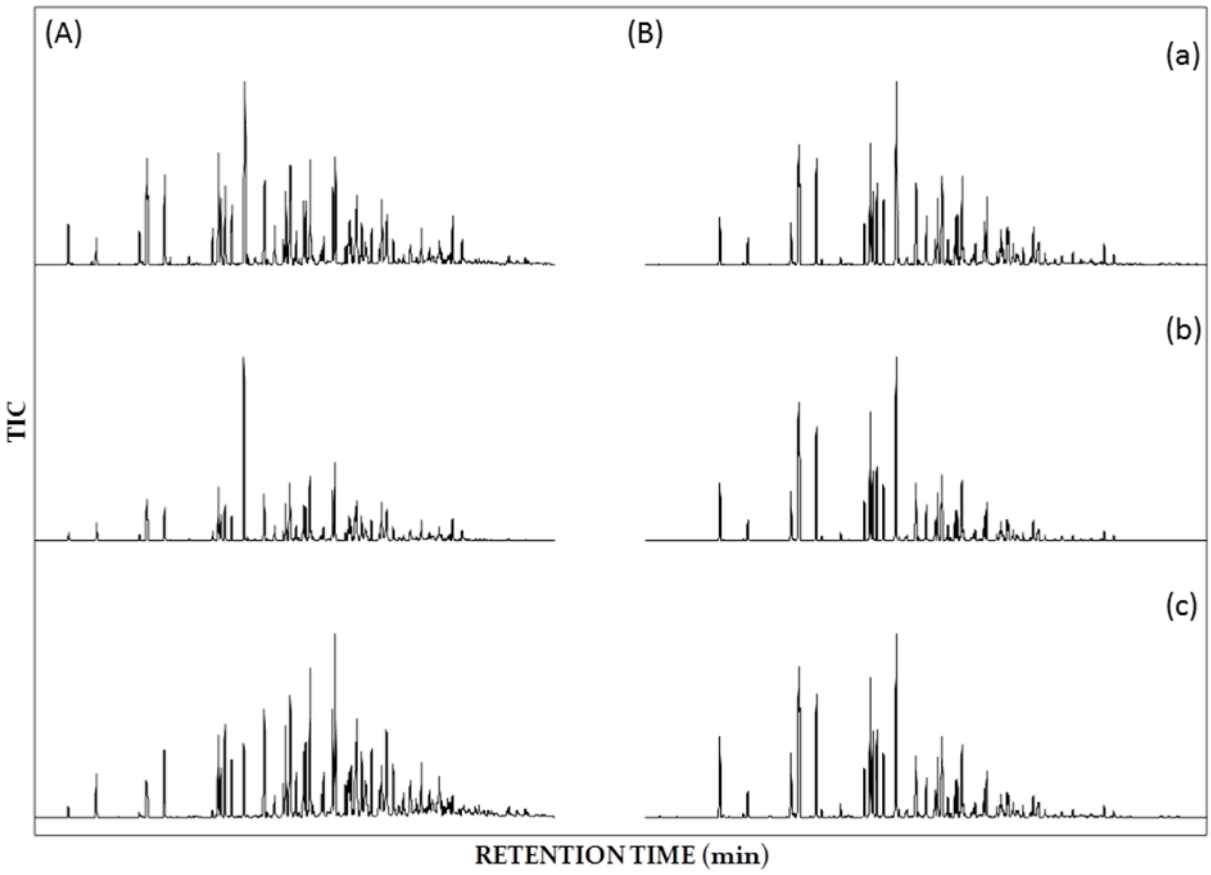

Figure 12: Molotov cocktail study: Degradation of gasoline from an incendiary device with (A) no additional treatment, (B) treatment with 2% Triclosan in 0.2M NaOH after (a) o, (b) 28, and (c) 140 days.

Phase IV: Development of a Sample Container that Incorporates Passive Adsorption Sampling

Our experiments indicate that while immediate sampling of a can did not significantly improve the recovery of ignitable liquid residues. For example, and as shown in Figures 12 and 13, a charcoal strip that is in immediate contact with the sample headspace at room temperature ("pre-bake") yields a chromatogram that is very similar to a chromatogram obtained from a charcoal strip that has been heated in the usual way ("post-bake"). There also appears to be discrimination effects the modified sample container. For example, when "post-bake" chromatograms are compared to those obtained from a fresh charcoal strip inserted into a traditional can (Figure 14), there is a marked loss of less volatile components from the charcoal strip that had been exposed to the sample headspace for extended times. This implies that the less volatile components in the ignitable liquid residue would saturate the charcoal strip and displace any lesser volatiles.

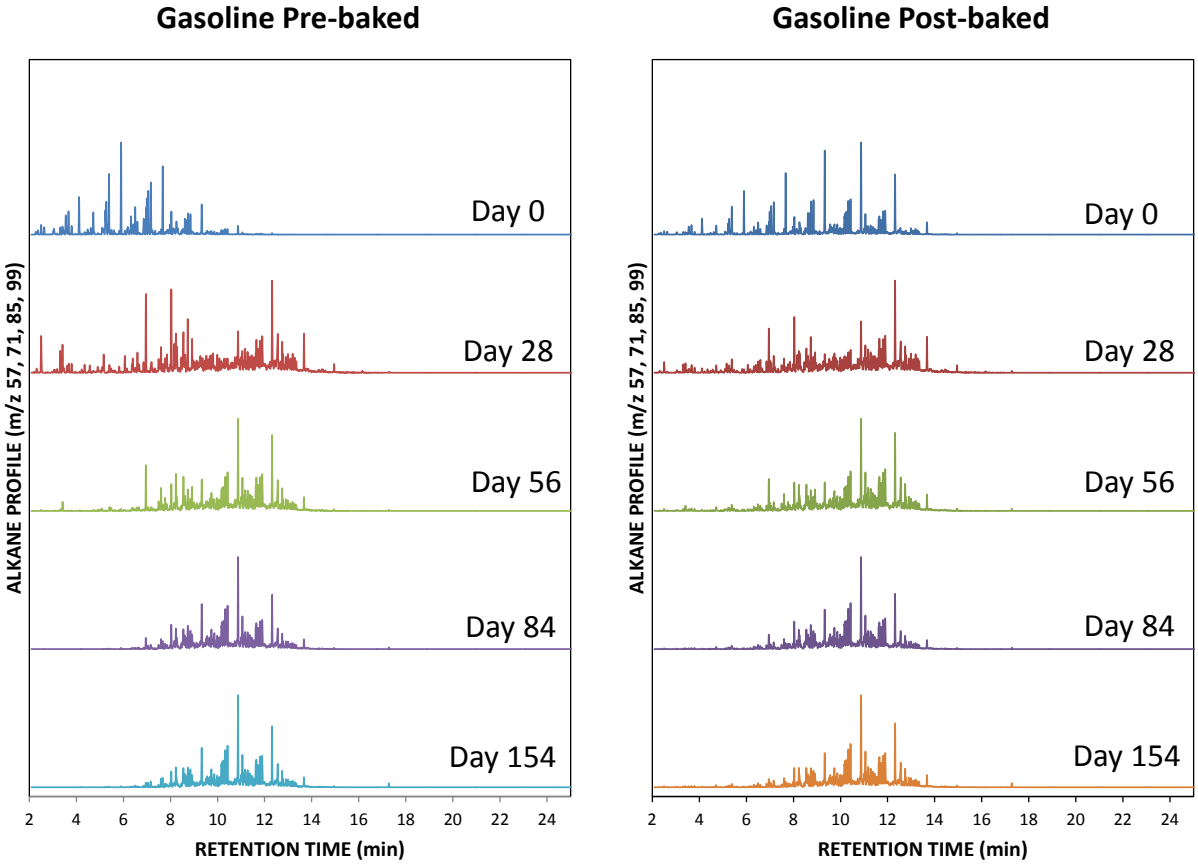

Figure 13: Summed extracted ion profiles for the alkane fraction of gasoline residues from burn study samples. This figure compares the residues recovered from a charcoal strip that was exposed to the sample headspace at room temperature ("pre-bake") to residues recovered from a charcoal strip that was exposed to the sample headspace at room temperature followed by heating in an oven as per a passive headspace elution method.

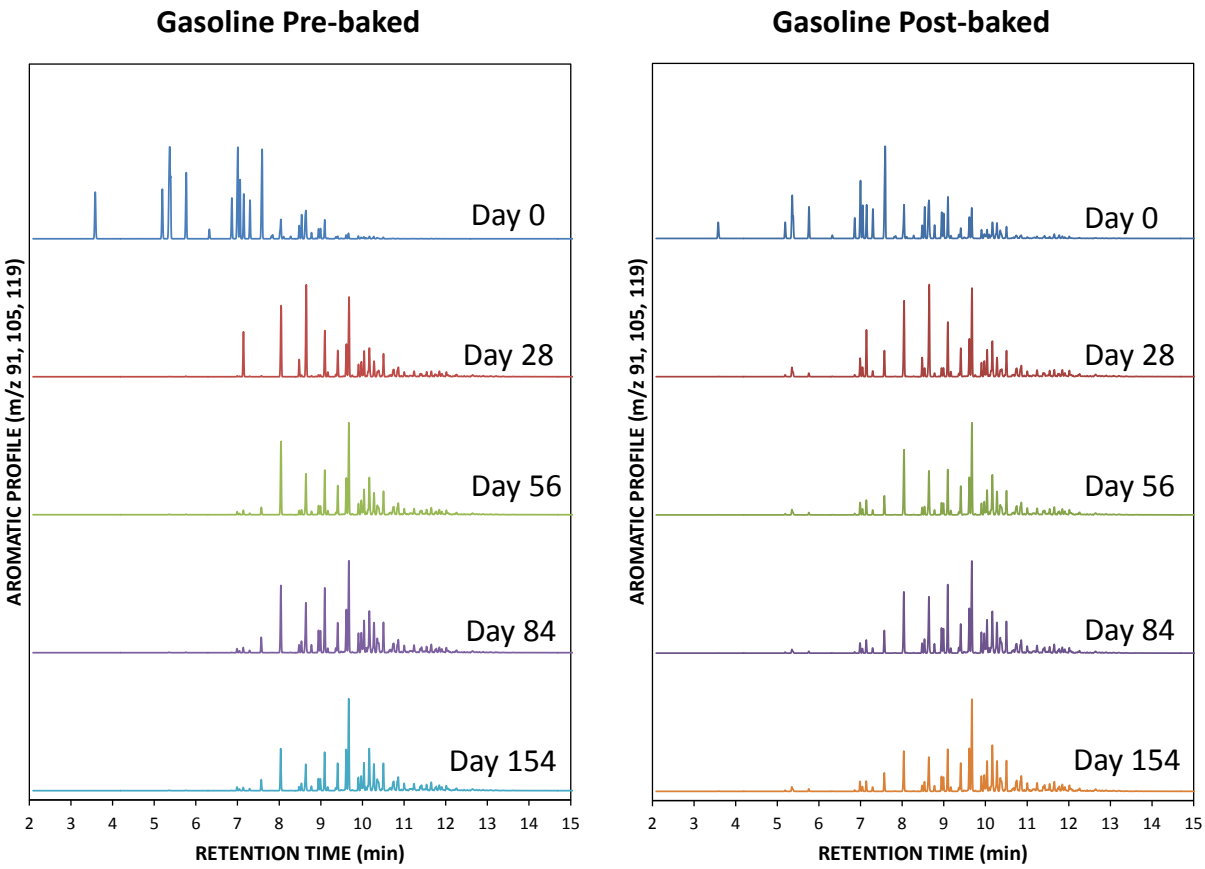

Figure 14: Summed extracted ion profiles for the alkylbenzene fraction of gasoline residues from burn study samples. This figure compares the residues recovered from a charcoal strip that was exposed to the sample headspace at room temperature ("pre-bake") to residues recovered from a charcoal strip that was exposed to the sample headspace at room temperature followed by heating in an oven as per a passive headspace elution method.

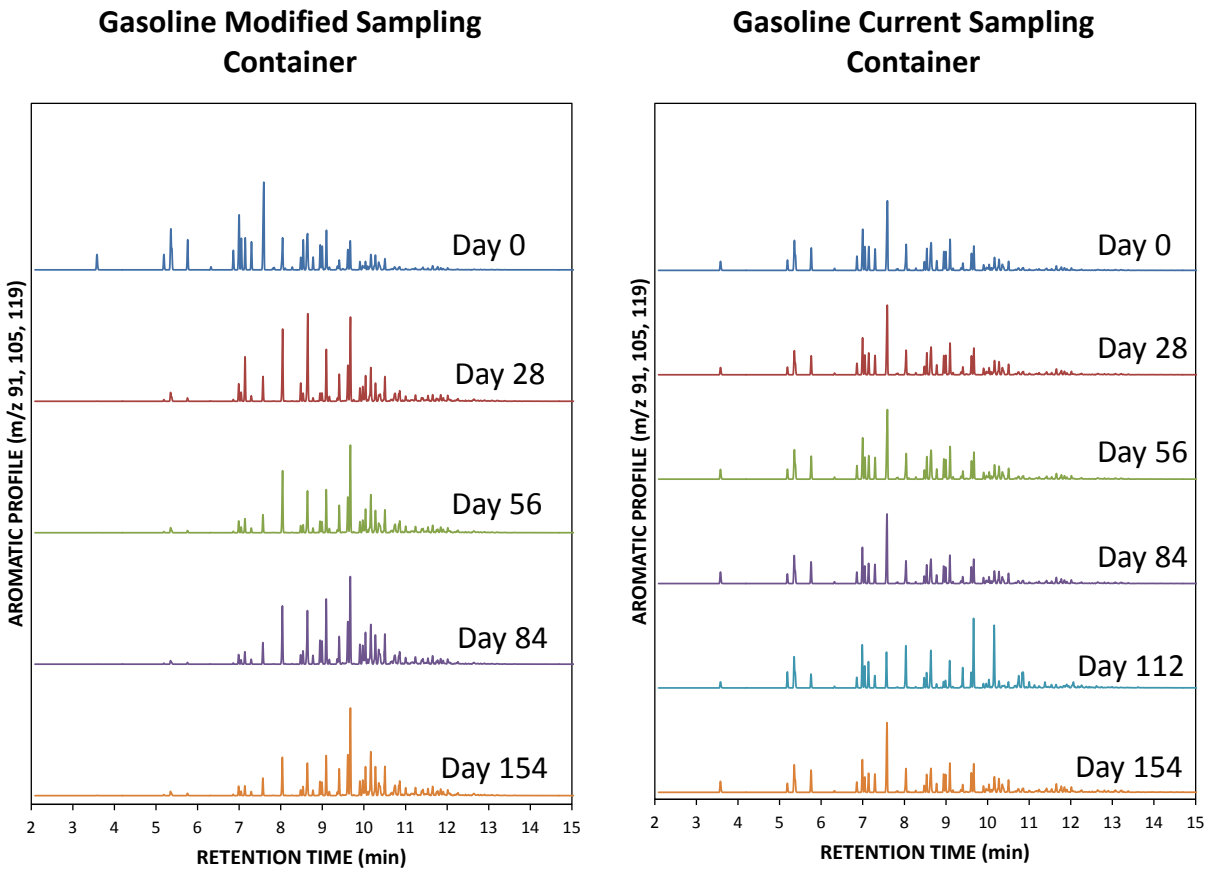

Figure 15: Summed extracted ion profiles for the alkylbenzene fraction of gasoline residues from burn study samples. This figure compares the residues recovered from a charcoal strip that was exposed to the sample headspace at room temperature followed by heating in an oven as per a passive headspace elution method ("Modified Sampling Container") to residues recovered from a fresh charcoal strip that was inserted into the can as per a typical passive headspace method.

CONCLUSIONS

Discussion of Findings

Given that there were two major goals of the study, there were also two major conclusions:

- A concentrated solution of triclosan in sodium hydroxide acts as a preservative for ignitable liquid residues on soil. It is effective up to 30 days, non-volatile, non-corrosive, and does not interfere with standard methods for extracting ignitable liquid residues from fire debris.
- A sample container has been designed that is airtight, but allows for a charcoal strip to be exposed to the container headspace and thus passively adsorb any ignitable liquid vapors that may be present.

Current practice in fire debris analysis does not always include steps to mitigate biodegradation. As a result, degraded samples can yield poor quality results. This, in turn, can contribute to incorrect classification of an ignitable liquid residue or even false negatives. Adopting the chemical preservative developed in this work would immediately impact the issue of biodegradation and improve the results of fire debris analysis.

Implications for Policy and Practice

One implication of these results is that a chemical solution could be made available for purchase by fire investigators, who are primarily responsible for gathering fire debris. In order for this type of product to be successful, both fire investigators and fire debris chemists must be satisfied that it is affordable, safe and validated. Therefore, one aspect of this work that remains undone is allowing investigators and labs to evaluate the chemical solution. This will be necessary so that end users can determine their applicability within the practices and protocols of their agencies.

Implications for Further Research

In order to establish a correlation between observed degradation levels in each soil type and bacterial activity, we would need to investigate two aspects of this work further: a) messenger ribonucleic acid (mRNA), and b) in vitro culture validation of key genera identified in quantitative mRNA analysis. We are currently using real-time reverse transcriptase polymerase chain reaction (RT-PCR) to enumerate each of the six bacterial genera by soil type and season in order to ascertain which of these bacterial groups may be most active in degradation. Once this is determined (end of Dec. 2012), we can use pure cultures of the key genera identified (using type/reference strains already obtained) on some IN VITRO biodegradation studies to confirm ability of each to act on chemical adulterants of interest. This could easily be quantified.

DISSEMINATION OF RESEARCH FINDINGS

PUBLICATIONS, CONFERENCE PAPERS, AND PRESENTATIONS

Publications

D.A. Turner, J.V. Goodpaster; "The effects of season and soil type on microbial degradation of gasoline residues from incendiary devices," *Anal Bioanal Chem* 405, 1593-1599 (2013).

Presentations

D.A.Turner and J.V. Goodpaster. "Die Critters Die: The Challenges of Mitigating Microbial Degradation in Fire Debris Samples," *Young Forensic Scientists Forum Bring Your Own Slides* at the *Annual Meeting of the American Academy of Forensic Sciences,* Chicago, IL 2/23/11.

D.A.Turner, A. Flores, and J.V. Goodpaster. "Monitoring and Characterizing Microbial Degradation of Gasoline on Different Soil Types," *Central Regional Meeting of the American Chemical Society,* Indianapolis, IN 6/10/11.

D.A. Turner, and J.V. Goodpaster. "The Effect of Season and Soil Type on the Microbial Degradation of Gasoline," *Annual Meeting of the American Academy of Forensic Sciences,* Atlanta, GA 02/25/12.

D.A. Turner, and J.V. Goodpaster. "Microbial Degradation of Gasoline Used in Incendiary Devices: Triclosan as a Solution," PITTCON CONFERENCE AND EXPO, Orlando, FL 03/14/12.

D.A. Turner, and J.V. Goodpaster. "Microbial Degradation of Gasoline Used in Incendiary Devices: Triclosan as a Solution," *National Meeting of the American Chemical Society*, Orlando, FL 03/25/12 and 03/26/12.

INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

We have submitted a PCT International Patent Application entitled "Biodegradation Suppression Solution for Forensic Samples" (Attorney Docket: 19202.096128)

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