ANALYSIS OF THE VOLATILE ORGANIC COMPOUNDS IN GRAVE SOIL USING SOLID PHASE MICROEXTRACTION COUPLED TO GAS CHROMATOGRAPHY MASS SPECTROMETRY (SPME-GC-MS)

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ABSTRACT

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Locating and recovering bodies that are buried in clandestine graves is a challenging task and may provide significant information about the deceased in a forensic investigation. Currently, human remains detection (HRD) dogs are the most commonly used method to find human remains. There have been very few human decomposition studies conducted due to the lack of human decomposition research facilities and the ethical and legal restrictions regarding the use of human bodies in human decomposition studies. Many HRD dogs are trained using synthetic training aids commonly known as pseudo scents due to the restrictions and costs surrounding true human remains. The focus of this research is to generate human decomposition odor profiles in Western Carolina University's Forensic Osteology Research Station (FOREST) facility by determining the volatile organic compounds (VOCs) in grave soil, soil surrounding decomposing human bodies. Volatile compounds in grave soil are analyzed at different stages of decomposition and at different weather conditions to determine how the VOC profiles are changed. This data will also be used to assess the chemical composition of training aids and to develop better training aids. This research is conducted using solid phase microextraction (SPME) to pre-concentrate the VOCs before analysis using gas chromatography-mass spectrometry (GC-MS). Soil is collected around decomposing bodies in different stages of

decomposition from WCUs FOREST facility and exposed to a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber to pre-concentrate VOCs. After exposure, the fiber is injected into the GC-MS for analysis. Over 200 compounds were identified during this research and over 90 of those compounds have been reported in previous decomposition studies. The results of this research depend on many factors such as weather, donor, location of the donors in the FOREST facility, and stage of decomposition. Analysis of synthetic training aids showed few compounds were present. More research will be conducted in the future using different fibers and methods to increase the data available.

CHAPTER ONE: INTRODUCTION

1.1 Locating Human Remains

Locating human remains is extremely important and can be very time-consuming. It can allow for many criminal cases to be solved, questions to be answered, and peace and closure be given to families of missing person cases. If detectives were unable to locate human remains, many questions would go unanswered. This research will hopefully allow for current techniques used in locating human remains to be improved and new techniques to be created.

Currently, there are only ten anthropological research facilities in the world that allow for the study of human decomposition. There are so few facilities due to the legal and ethical restrictions surrounding the use of human cadavers in decomposition studies. This is one reason why there is very little known about the volatile organic compounds (VOCs) produced during the decomposition process. The purpose of this research is to provide more data on the VOCs produced during human decomposition and use it to further the knowledge in this area and improve the current methods used in locating human remains.[1]

The methods currently used in locating human remains are manual probing, ground penetrating radar (GPR), and cadaver or human remains detection (HRD) dogs. Manual probing is used to locate regions of disturbed soil. It is an inexpensive method but can only be used in small areas and cannot confirm the presence of a corpse.[2] Ground penetrating radar (GPR) is also used to locate regions of disrupted soil and can sometimes indicate the presence of a corpse if the ground conditions are met. It is favorable compared to manual probing as it does not disrupt the ground or any evidence that may be present.[3] However, GPR is expensive and requires an extensive amount of training to interpret the data as it can give false positives due to objects in the environment showing as a possible corpse.[2] It can also only penetrate to a certain depth

depending on the frequency of the GPR source signal and soil conditions.[2,4] Dogs have been used in law enforcement for many years to detect explosives, narcotics, missing people, and more. They excel in these tasks because of their olfactory cell counts which allow them to have a much better sense of smell than humans do.[2] They are also able to distinguish human remains from animal remains. If well trained, dogs are a good choice to use in helping locate human remains.

1.2 Cadaver Dog Training

There are three main ways that cadaver dogs or human remains detection (HRD) dogs are trained. They can be trained using true material such as tissue, bones, or blood, pseudo-odors or synthetic aids, and non-pseudo alternatives such as diluted, encapsulated, and ab/adsorbed true material.[5] Many cadaver dogs are trained using synthetic training aids commonly known as pseudo-scents due to the restrictions and costs surrounding true human remains.[5]

While there are many different training aids available to cadaver dog trainers, there are different levels of effectiveness for each of these types of training. Each type of training aid produces a different VOC profile. Generally, it is seen as best to train using true material to ensure reliable detection. The one issue with true remains is that training on a single pure odor, while it gives a strong success, is it reduces the tendency to detect variations of the single odor.[5] Combinations and variations of odors are what will typically be present when locating human remains. This is why it is common to use multiple types of training aids to improve the dog's ability to detect complex odor mixtures. There have been studies conducted to determine the effectiveness of using cadaver tissue and decomposition fluid for training.[6] The dogs had an overall high success in locating human remains after training with tissue and decomposition fluid. However, there were some issues when trying to locate dry remains and bones as there are

fewer VOCs present causing lower odor intensity. This suggests that all types of tissues and bones, in varying stages of decomposition, should be used for training.[6]

There have been multiple studies that determined the effectiveness of training dogs with blood. The studies suggested that if a dog was continuously exposed to fresh and degraded blood for training they could out-perform instruments such as gas chromatography-mass spectrometry (GC-MS). However, this is only true in relatively ideal conditions. Depending on the surface interactions and procedures such as washing, when the blood gets diluted, the VOC profile of the blood can be altered which can impact the compounds the dogs respond to. If the dog is trained using all types of conditions, there a is higher probability of being able to locate blood.[6]

Synthetic training aids are the most common when true material is not available or to use alongside true material. These synthetic aids are commonly made by identifying the major chemical compounds present in the decomposition odor and using those compounds to make a mixture to simulate the true odor profile.[5] However, determining what compounds to include in these aids has proven to be difficult due to the limited research on human decomposition odors.[7] Previous studies conducted by Dargan and Forbes using solid phase microextraction (SPME), show that the synthetic aids include mostly alcohols and ketones with some aldehydes, acids and esters, sulfur compounds, nitrogen compounds, and hydrocarbons.[6] Before complex training aids that contain multiple compounds were created, many handlers trained dogs using cadaverine and putrescine, two biogenic amines formed during decomposition. However, due to the toxicity of these compounds to dogs, they are not widely used. Two studies were conducted by Dargan et. al. and Tipple et. al. to determine the effectiveness of three commercial synthetic pseudo scents.[6,7] The pseudo scents used were Sigma pseudo corpse scent (PS) formulation I (PSI), formulation II (PSII), and drowned victim (PSDV). The studies showed that dogs showed no

positive response to any of the formulations and concluded that the synthetic formulations do not accurately represent the human decomposition odor. More studies need to be conducted to determine the suitability and enhancement of these synthetic aids before they can be used efficiently in training dogs.[6,7]

A common non-pseudo alternative used in training is to place clothing made of cotton or other natural fibers near true material to collect the odor or pull the odor through the clothing using a STU-100, a scent collection device, or other similar vacuum device.[5] There have been two studies conducted that showed that dogs can detect textiles, such as cotton blankets and gauze, that are contaminated with decomposition odor.[6] It is unknown how long the odor would remain for a positive detection. Additionally, it was shown that the textiles having direct contact with the remains acquired a better response from the dogs.[6] Overall, it seems that true human remains, and decomposition fluid produce the most VOCs out of the training aids studied.[6]

1.3 Scientific Basis

Dogs are useful to law enforcement when locating human remains. They can detect scents extremely well, even in lower concentrations, due to their high olfactory cell count. Their sense of smell allows them to have the capability to detect human remains with proper training. However, it is still unknown what compounds dogs detect from the VOCs released from decomposing remains as there have been hundreds of specific compounds that have been identified in human decomposition studies.[8]

Scientific basis is important in forensics. It helps ensure that there is a justified explanation for the techniques used and data found. For the first time, during the case of Casey Anthony, it was called into question whether the evidence based on decomposition odor analysis should be admitted into the court of law. They had to verify that the methods used to analyze and

process the evidence were generally accepted in the scientific community.[9] Using the thresholds set by *Frye v. United States* and *Daubert v. Merrell Dow Pharmaceuticals* is important in ensuring that there is a scientific basis in the evidence being admitted and could even be used to justify that research has been properly conducted.[9]

1.4 Previous Studies

There have been very few human decomposition studies conducted due to the lack of human decomposition research facilities and the ethical and legal restrictions regarding the use of human bodies in human decomposition studies. Many of the recent studies focused on decomposition odor profiles have used pigs as human analogs. Pigs are considered to be a suitable analog to human decomposition due to their internal anatomy and gut biota being similar to humans.[10] The VOCs produced from pig decomposition are also similar to those produced in human decomposition. However, the overall odor profiles, based on abundance and variation in ratio, between pig and human decomposition have proven to be different.[10] The VOCs present could also be dependent on the environment where the research is conducted as it has been shown that climate has an effect on decomposition.[11]

One study, conducted by Knobel et. al. in Sydney Australia, compared human and pig decomposition patterns in the summer and winter months. It was concluded that during early decomposition, pigs are not reliable human analogs based on visual decomposition findings. The chemical odor profiles also showed that while both pigs and humans produce many of the same VOCs, the overall odor profiles were different. In the cooler months, human and pig decomposition, both visually and chemically, were more similar than in the warmer months.[10] Another study, conducted in Tennessee, also compared human and pig decomposition. They also concluded that the rate and process of decomposition are different between humans and pigs with

humans having a more variant decomposition.[1,10,12] While pigs may not be the most suitable analogs for human decomposition, they may still be able to provide useful information regarding decomposition research.[1]

Another study, conducted by Perrault et. al. in Sydney Australia, characterized the soil VOC profile throughout the decomposition process of pigs using SPME and sorbent tubes.[13] They found that 47 of the VOCs present were only detected using sorbent tubes, 48 of the VOCs present were only detected using SPME, and 36 of the VOCs present were detected by both. The compounds predominantly identified using sorbent tubes were sulfur and nitrogen-containing compounds throughout the majority of decomposition while short-chain esters, short-chain ketones, short-chain alcohols, and short-chain aldehydes, were identified throughout the full duration of decomposition. The compounds predominately identified using SPME were carboxylic acids, longer chain acid esters, and monoterpenoid ketones. Both techniques collected a range of aldehydes with saturated aldehydes only being identified using SPME.[13]

One human study conducted by Vass et. al., in Tennessee, identified VOCs at the surface of burial sites during the decomposition process of human remains over many years to expand the decomposition odor analysis database. They identified 478 volatile and semi-volatile compounds. They identified 30 of the 478 compounds as key markers of human decomposition detectable at the soil surface of buried remains. Toluene, ethylbenzene, nonanal, hexane, and carbon tetrachloride are just a few of the 30 compounds that were identified.[14]

1.5 Decomposition Process

The decomposition process can be classified into five stages: fresh, bloat, active decay, advanced decay, and dry remains or skeletonization. The body undergoes many different changes throughout these stages. During the fresh stage, there will be fly activity, early putrefaction, and

reduction and liquefaction of tissues and of contents of the intestinal tract. During the bloat stage, there will be maggot activity, skin slippage, decomposition odor, gas by-product of decomposition, and the tissues will begin to liquify. During the decay stages, insect activity will begin to diminish, the body cavities will begin to rupture, the soft tissues will begin to decay, and the skin will start to dry out. Typically, the body will begin to skeletonize by the end of the first year but can be faster depending on the conditions the body is in.[11]

When a body begins to decompose, carbohydrates, lipids, and proteins are broken down by bacteria. These macromolecules then degrade into smaller molecules and gases. Proteins denature into amino acids, which get broken down even further. Amino acids that undergo desulfhydration produce dimethyl disulfide and other sulfide compounds. Decarboxylation of amino acids produces carbon dioxide and amines such as cadaverine and putrescine. The adipose tissue, lipids, breaks down into fatty acids. The fatty acids commonly found during decomposition are oleic, palmitic, myristic, and stearic acids. Depending on the environment, aerobic or anaerobic, the unsaturated fatty acids are saturated or oxidized to aldehydes and ketones. The carbohydrates are broken down into glucose monomers which are then converted into organic acids such as butyric acid and acetic acid, or related alcohols depending on the environment.[15]

It is reported that certain stages of decomposition occur faster in the warmer months suggesting that the climate can influence the decomposition process. Temperature has a major effect on the decomposition process due to its effect on the microbial activities in a corpse.[11] In warm temperatures, decomposition may start in minutes while in colder temperatures it can take days. However, in freezing temperatures, below -5 °C, decomposition will not occur due to the inhibition of enzymatic and microbial activites.[11]

The rate of decomposition also depends on whether the body was buried or placed on the ground surface. It has been shown that the rate of decomposition is faster for a body placed on the ground surface than for a buried body. This is because on the surface, microorganisms and insects have better access to bodies, more oxygen is present, and there is a higher rate of gaseous diffusion compared to being under the soil.[11]

This shows the importance of collecting odor profile data at different environmental conditions to have sufficient data regarding human decomposition to locate human remains and clandestine graves. Western Carolina University's Forensic Osteology Research Station (FOREST) is one of the few decomposition facilities in the USA and around the world. The decomposition odor data collected in the higher elevation and oceanic climate in this region would expand the knowledge on human decomposition.

1.6 Instrumentation

There are various types of instruments used to analyze and identify VOCs while the main instrument used for this task is gas chromatography (GC). Several types of gas chromatographic systems are used with some adaptations such as comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGCxTOFMS). This system is used because of its ability to separate complex mixtures based on volatility and polarity. The sample is injected into the system and mixed with a carrier gas, typically helium, hydrogen, or nitrogen. This gas mixture then enters the column coated with a chemical, the stationary phase, where the compounds are separated based on their chemical properties. The separated compounds are then sent to the detector which gives an electric signal that is proportional to the amount of molecules of the same identity.[16] The mass spectrometer is typically referred to as the mass selective detector when attached to the GC. It consists of an ionization chamber that

typically uses electron ionization (EI) to fragment the analytes and generate the ions that are detected. Chemical ionization (CI) can also be used when no molecular ions are obtained using EI. Once the analytes are fragmented, they go through a mass filter that allows only fragments with a certain mass to charge (m/z) ratio through at a time. The ions will then be measured by the detector.[16,17]

Analysis of VOCs from grave soil with GC typically requires a preconcentration method due to the low concentrations. There are several preconcentration methods employed. SPME is a good method for preconcentrating VOCs prior to GC analysis because it is solvent-free and a very simple extraction technique. A sample is introduced into the injection port of the GC either as a liquid or as molecules adsorbed on a surface, as it is in SPME. When SPME and GC-MS are not used for soil VOC analysis many studies used thermal desorption with GCxGCxTOFMS for air VOC analysis.[18–20] GC×GC provides a greater degree of separation than GC. GC is unable to differentiate some compounds in the complex VOC mixture produced by decomposition whereas GCxGC is.[20] TOFMS has a faster acquisition rate that can accommodate the narrower peaks produced by GC×GC.[10] One soil study, using cryofocusing, collected soil in vials, heated them, then withdrew 2 mL from the headspace of the vial and injected into a cryofocusing GC port and analyzed using GC-MS.[18]

1.7 Preconcentration Methods

Solid phase microextraction (SPME) is commonly used as a preconcentration method due to its simplicity. The SPME method uses a capillary fiber coated with a polymeric adsorption material that can extract target compounds from the samples. This is done by the mass transfer of analytes from the sample, until the chemical potential of each substance is the same, to the coated fiber.[16] This fiber can be exposed to the headspace of a sample, such as in a vial, exposed

to the air near a sample, typically in a chamber with an air pump pulling air through, or directly immersed in the sample.[16] This method is used due to its simple sample preparation and the variety of fibers available to capture different classes of compounds. The fiber chosen for this project was a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber because it is suitable for analysis of both polar and non-polar volatiles and semi-volatiles, which are the compounds commonly found in decomposition odors.[21] There are many other types of fibers with different materials that are used for polar compounds, non-polar compounds, low molecular weight compounds, and a combination of these compounds.

Thermal desorption uses sorbent tubes that contain adsorbent material. Air is pulled through the tube using an air sampling pump. The volatile compounds are trapped onto the adsorbent material then desorbed into the GC using a thermal desorption unit.[10] Sorbent tubes are used because of their suitability for use in field studies.[10]

The focus of this research was to generate human decomposition odor profiles. There are many factors that can affect the decomposition odor profile such as weather, donor, location of the donor in the FOREST, and stage of decomposition of the donor. These factors were monitored to determine their effects on the VOC content in grave soil.

CHAPTER TWO: MATERIALS AND METHOD

2.1 Materials

Soil was collected in two time periods in 2023. It was collected in the spring semester, from January-April, and again in the fall semester, from September-December. The temperature was recorded on each day soil was collected using the weather channel app. It was also noted if it had rained in the days prior to collection and the soil conditions surrounding each donor.

Soil, surrounding decomposing donor bodies, was collected from Western Carolina University's Forensic Osteology Research Station (FOREST). In the FOREST, the donors are placed on top of the soil in various locations under anti-scavenging cages. The size of the enclosure that the donors are placed in is approximately 5,000 square feet and is on a slight incline. Some donors are placed a few feet from each other while others are placed further apart, approximately 10-15 feet. There are also several trees and plants inside the enclosure. Approximately five grams of soil was collected from five to six donors from between the legs of the donors or on the left or right side of the donors each week. Soil from 18 donors was collected and analyzed during the year 2023. From January to April in 2023, 50 total soil samples were collected from donors 23-02, 23-06, 23-07, 23-09, 22-23, 22-29, 22-31, 22-32, and 22-34. From September to December in the year 2023, 51 total soil samples were collected from donors 23-25, 23-26, 23-29, 23-30, 23-32, 23-34, 23-35, 23-36, and 23-37. The donors that provided reasonable data were monitored until they did not provide a detectable amount of VOCs, or the collection period was finished. The soil samples were placed in 15 mL clear glass vials and sealed with a screw cap containing a polytetrafluoroethylene/silicone septum immediately after the collection at the collection site. The vials were stored in the refrigerator, immediately after collection, until the analysis. At the end of October, the GC-MS was out of operation for

maintenance until mid-December. The soil samples collected at that time were stored in the freezer until the analysis in January 2024.

Pseudo-scents from Sigma-Aldrich chemical company (Sigma Pseudo Corpse Scent Formulation I and II) were obtained. To prepare for analysis, 500 microliters of each pseudo-scent were transferred to 15 mL clear glass vials and sealed with a screw cap containing a polytetrafluoroethylene/silicone septum. The pseudo-scents were analyzed immediately after preparation.

2.2 Solid Phase Microextraction

A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) stableflex SPME fiber (50/30 um, 24 Ga, Supelco) was used as it is suitable for analysis of both polar and non-polar volatiles and semi-volatiles. New fibers were conditioned by placing the fiber into the inlet of the GC at 250 °C, for 30 minutes, and then conditioned at the same temperature for five minutes before each blank was run. A blank was run before each sample by desorbing the fiber in the inlet of the GC for five minutes. The fiber was exposed in the headspace vial, above the soil, for 20 minutes at approximately 40 °C to pre-concentrate the fiber with VOCs. The vials were heated in a Fisher Scientific Isotemp dry bath heating block during exposure for uniform heating of the soil. After exposure, the fiber was desorbed for five minutes in the inlet of the gas chromatograph at 250 °C using a 0.75 mm I.D. SPME direct inlet liner in splitless mode. This same method was used to analyze pseudo-scents.

2.3 Gas Chromatography-Mass Spectrometry

For the analysis performed before October 2023, an HP-5MS column was used (30m x 0.250 mm x 0.25 μm) with an Agilent 7890A GC coupled to a 5975C mass selective detector (MSD). A hydrogen carrier gas flow rate of 1.5 mL/min was used. The oven temperature was

held at 35 °C for 3 minutes then increased by 3 °C/min to 80 °C. The temperature was further increased to 120 °C at a rate of 10 °C/min and finally increased to a temperature of 250 °C at 40 °C/min. For the analysis conducted in 2024, an HP-1MS ultra inert column was used (20 m x 0.180 mm x 0.18 μm) with an Agilent 7890A GC coupled to a 5975C MSD. For most samples, a hydrogen carrier gas flow rate of 1.08 mL/min was used. The oven temperature was held at 35 °C for 1.9 minutes then increased by 4.7 °C/min to 80 °C. The temperature was further increased to 120 °C at a rate of 15.6 °C/min and finally increased to a temperature of 250 °C at 62.5 °C/min. For the last 7 samples ran in 2024, a hydrogen carrier gas flow rate of 0.8 mL/min was used. The oven temperature was held at 35 °C for 3 minutes then increased by 3 °C/min to 80 °C. The temperature was further increased to 120 °C at a rate of 10 °C/min and finally increased to a temperature of 250 °C at 40 °C/min. For all samples, the transfer line was held at 310 °C, the source temperature was held at 230 °C, and the MS quadrupole temperature was held at 150 °C. The MSD was operated in full electron ionization (EI) scan mode from 50 to 550 m/z. The column was changed to a shorter and smaller diameter to create a better vacuum in the MS. There was a chance for better sensitivity with the new column, but no other major differences were expected.

2.4 Data Processing

The MSD ChemStation software was used for identifying the compounds present in each soil sample through mass spectral library comparison. Each major peak in the chromatogram was selected and its mass spectrum was searched against the National Institute of Standards and Technology (NIST) library. The matches were visually confirmed by comparing the library or literature mass spectrum of the target compound with the experimental mass spectrum. Many compounds had a NIST library match of <30% however the visual observations showed that the

experimental mass spectra matched relatively well with the NIST library mass spectra of the target compounds. This comparison revealed that extra peaks that are very low in abundance were present in the experimental spectra in the high mass region which likely reduced the matching score. The source of these high mass peaks is likely noise from the instrument or from the material of the fiber itself. The fiber is made up of siloxane compounds that have a high mass which could be the source of the extra peaks that are seen.

When the library match was a lower percentage, the mass spectra were visually compared to confirm that the target compound was correctly identified. This was done by considering the fragmentation of the compound and confirming that the peaks of the mass spectrum matched the fragmentation patterns. There were also some compounds that gave a higher percent match but visually the mass spectra did not match up. This was also considered when determining what compounds could confidently be included (see Figure A1).

Other studies confirmed the presence of compounds by standard comparison using mass spectral library and retention time comparison or mass spectral library searching only if standards were not available.[13,15] Another study identified the peaks by comparing the baseline subtracted spectra with the NIST mass spectral database using a probability-based matching algorithm to give the compounds with the highest match qualities, which were typically >80%.[22]

CHAPTER THREE: RESULTS AND DISCUSSION

3.1 Overview

The compilation and analysis of data have been challenging due to factors such as temperature, donor, placement of the donor, and stage of decomposition. Results show that many compounds found in our soil analysis are consistent with previously published decomposition studies.[14,20,22–48] Tables 1 and 2 contain the most abundant compounds that were present in soil samples collected from FOREST and reported in previous studies. Table 1 shows the most abundant compounds from the 2023 fall semester collection while Table 2 shows the most abundant compounds from the 2023 spring semester collection. A full list of all the compounds that were found in FOREST soil samples that were previously reported is shown in Table A1. Some of these compounds were only reported in animal decomposition studies while others were reported in both human and animal studies. Toluene and ethylbenzene, among others, were reported as key markers of human decomposition detectable at the soil surface of buried remains.[14] While this research analyzed soil surrounding decomposing remains on the surface, these compounds being detected, in this research, indicated that these compounds likely originate from human decomposition. The compounds that were detected the most were slightly different for the spring and fall semesters. However, for both, they were mainly hydrocarbons and aromatics. There were also some differences in the overall compounds detected between the spring and fall semesters. Some compounds, such as toluene, heptane, and 2-pentylfuran, were detected in both semesters while other compounds, such as ethyl ester acetic acid, were only detected in one semester. This is likely due to the factors mentioned above in section 1.5.

Table 1**.** The most abundant compounds found in soil, 2023 fall semester, and training aid samples that have been reported in decomposition studies.

Compounds	Number of times found in soil samples	Number of times found in training aids
3-Carene^	28	0
(-)-beta-Pinene^[a]	25	0
1R/alpha-Pinene*^[b]	21	0
Heptane*^	15	0
Octane*^	14	0
2-Pentylfuran*^	12	0
Copaene^	12	0
Pentadecane*^	11	0
Toluene*^	11	2
o-Cymene^	10	0
L-Calamenene^	8	0
Terpinolene^	8	0
p-Xylene*^	7	0
2-Pentanone*^	6	0
d-Cadinene^	5	0
Acetophenone^	5	0
Ethylbenzene*^	5	0
D-Limonene*	5	0
Heptanoic acid^	4	0
Dodecane*^	4	1
Acetic acid^*	4	1
Tridecane^*	4	0
Acetone*^	4	0
Acetic acid, ethyl ester*^	0	2
Palmitic acid^	0	1
1,4-Dioxane^	0	1
1H-Pyrrole^	0	1

*Indicates reported in human studies. ^Indicates reported in animal studies. [a]Beta-pinene is reported in previous studies but (-)-beta-pinene has a very similar mass spectrum so it is classified as being previously reported. [b]Alpha-pinene is reported in previous studies but 1R-alpha-pinene has a very similar mass spectrum so it is classified as being previously reported.

Table 2. The most abundant compounds found in soil, 2023 spring semester, and training aid samples that have been reported in decomposition studies.

Compounds	Number of times found in soil samples	Number of times found in training aids
Toluene*^	32	2
2-Pentylfuran*^	20	0

Table 2 Cont.

(-)-beta-Pinene^[a]	19	0
Heptane*^	18	0
Octane*^	17	0
3-Octanone^	15	0
Dodecane*^	11	1
Pentadecane *^	11	0
1R-alpha-Pinene*^[b]	10	0
3-Carene^	10	0
Tridecane*^	9	0
1-Hexanol*^	9	0
Hexanal*^	9	0
1-Butanol*^	6	0
2,3-Epoxybutane^	6	0
2-Butanone*^	5	0
Butanoic acid*^	5	0
Hexadecane^*	5	0
Acetic Acid*^	5	1
Butyl ester butanoic acid*^	4	0
Propyl ester butanoic acid^*	4	0
Acetic acid, ethyl ester*^	2	2
1,4-Dioxane^	0	1
1H-Pyrrole^	0	1
Palmitic acid^	0	1

*Indicates reported in human studies. ^Indicates reported in animal studies. [a]Beta-pinene is reported in previous studies but (-)-beta-pinene has a very similar mass spectrum so it is classified as being previously reported. [b]Alpha-pinene is reported in previous studies but 1R-alpha-pinene has a very similar mass spectrum so it is classified as being previously reported.

There were also compounds found that have not been reported in previous studies. The most abundant of these compounds are shown in Tables 3 and 4. A full list of these compounds is shown in Table A2. Some compounds have a role as a human metabolite such as 2,4-dimethylhexane and methylmalonic acid that could indicate human decomposition.[49,50] Another compound digitoxin is a medication typically used to treat heart failure. While medical records are not available for every donor, this medication has been present in the soil of at least one

donor with a known heart condition. Other compounds such as D-camphene, m-cymene, and m-cresol were detected and are closely related to camphene, o-cymene, and p-cresol that have been reported in previous studies. One explanation for this finding is that the library currently used for data analysis matches to the compound closest to the experimental mass spectrum which may be an isomer of the compound reported in previous studies as the mass spectra are almost the same between isomers. While hi-oleic safflower oil was detected in many soil samples, it is believed that hi-oleic safflower oil is not present in the soil. It is believed to be a large lipid as there is no source of safflower around. It is believed that the library matched the closest compound which happened to be safflower oil.

Table 3. The most abundant compounds found in soil, 2023 fall semester, and training aid samples that have not been reported in decomposition studies and are not plant related.

Compounds	Number of times found in soil samples	Number of times found in training aids
D-Camphene	20	0
Doconexent	13	0
m-Cymene	12	0
Hi-Oleic safflower oil[a]	12	1
alpha-Muurolene	9	0
Cadinene	6	0
alpha/beta-Terpinyl acetate	6	0
beta-Guaiene	5	0
2,4-Dimethylhexane	5	0
m-Cresol	4	0
Dotricontane	4	1
Dimethyl silanediol	4	1
Digitoxin	4	0
E-2,3-Epoxycarene	3	0
Spironolactone	3	0
Octadecanoic acid	2	1
2-Pyrrolidinone	0	1

[a]Hi-oleic safflower oil is plant-based but is included in this table because it was found in training aids.

Table 4. The most abundant compounds found in soil, 2023 spring semester, and training aid samples that have not been reported in decomposition studies and are not plant related.

Compounds	Number of times found in soil samples	Number of times found in training aids
Hi-oleic safflower oil[a]	24	1
Dimethyl silanediol	23	1
D-Camphene	9	0
1-Chloro-tetradecane	7	0
Doconexent	7	0
Glutaraldehyde	6	0
Methyl arachidonate	6	0
1-Chloro-octadecane	5	0
1-Tetradecanol	5	0
Calamenene	5	0
1,3-Butanediol	4	0
13-Heptadecyn-1-ol	4	0
gamma-Cadinene	4	0
gamma-Undecalactone	4	0
Octadecanoic acid	4	1
1,2-Diethoxyethane	3	0
2,4-Dimethylhexane	3	0
alpha/beta-Terpinyl acetate	3	0
gamma-Nonalactone	3	0
Iso amyl alcohol	3	0
m-Cymene	3	0
Nonadecane	3	0
Dotriacontane	1	1
2-Pyrrolidinone	0	1

[a]Hi-oleic safflower oil is plant-based but is included in this table because it was found in training aids.

There were compounds found that have not been reported in previous studies but are plant-related (see table A3). Some of the compounds detected are junipene, a pine derivative, (+)-cyclosativene, natural in red fir, and sabinene, a plant metabolite. These compounds may be related to the decomposition process but there is also a likely chance they are present due to the location of the FOREST as there are many trees, plants, and natural vegetation around the

donors. While the control soil, collected directly outside of the FOREST, did not have any compounds detected, there is still a possibility some of the plant-based compounds detected are still from the environment. It is difficult to get a comprehensive control sample while not being in the exact same environment as the donors are placed in.

3.2 Donor-to-Donor Trends

Some compounds detected seem to be donor-specific, such as phenol and o-xylene, while other compounds, such as beta-pinene and 3-carene, are detected regardless of the donor. There have been donors that have similarities in compounds present and others that have virtually nothing in common. Two donors in the spring semester, 23-07 and 23-09, compared at five weeks since placement, had similar chromatograms and compounds present (see Figure 1). There were five compounds in common between them at this collection time. Donor 23-07 was placed at the top left of the FOREST while donor 23-09 was placed at the bottom right of the FOREST. Another two donors in the spring semester, 23-06 and 23-07, also compared at five weeks since placement, had different chromatograms and compounds present (see Figure 2). There were two compounds in common between them at this collection time. Donor 23-06 was placed at the middle bottom of the FOREST.

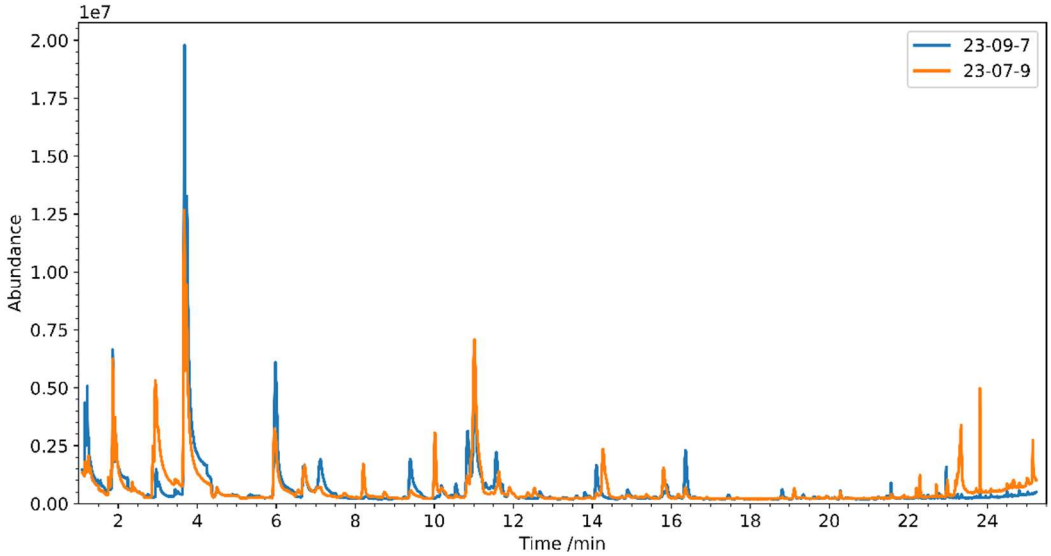

Figure 1. Overlayed chromatogram comparing donors 2023-09 and 2023-07 five weeks since placement. There were five compounds in common between these donors at this collection time.

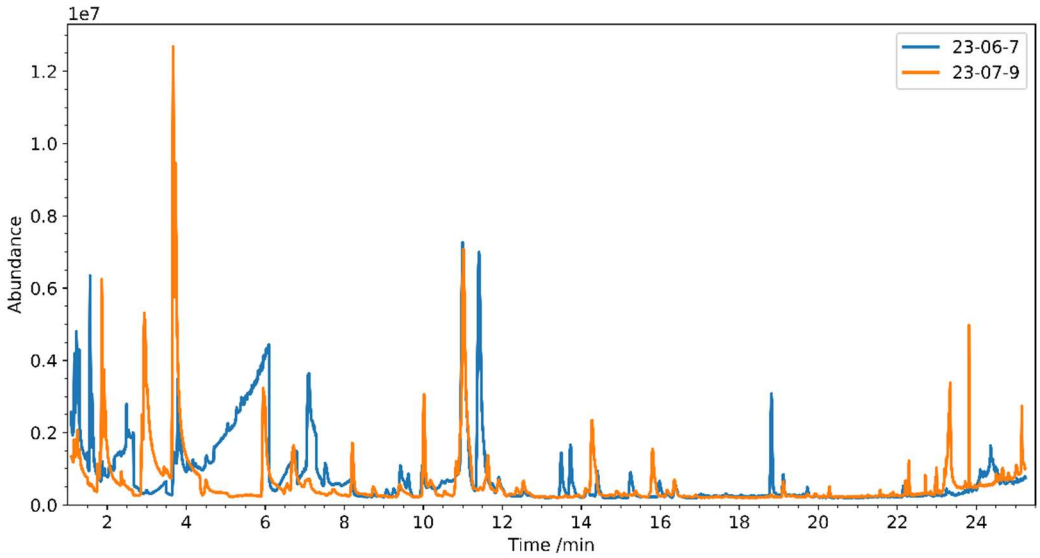

Figure 2. Overlayed chromatogram comparing donors 2023-06 and 2023-07 five weeks since placement. There were two compounds in common between these donors at this collection time.

Two donors in the fall semester, 23-34 and 23-36, compared at three weeks since placement, had similar chromatograms and compounds present as seen in Figure 3. There were

six compounds in common between them at this collection time. Donor 23-34 was placed at the bottom middle left of the FOREST while donor 23-36 was placed at the very bottom in the middle of the FOREST. Another two donors in the fall semester, 23-30 and 23-29, compared at five weeks since decomposition, had different chromatograms and compounds present as seen in Figure 4. There was one compound in common between them at this collection time. Donor 23-30 was placed at the top right of the FOREST while donor 23-29 was placed at the middle bottom of the FOREST.

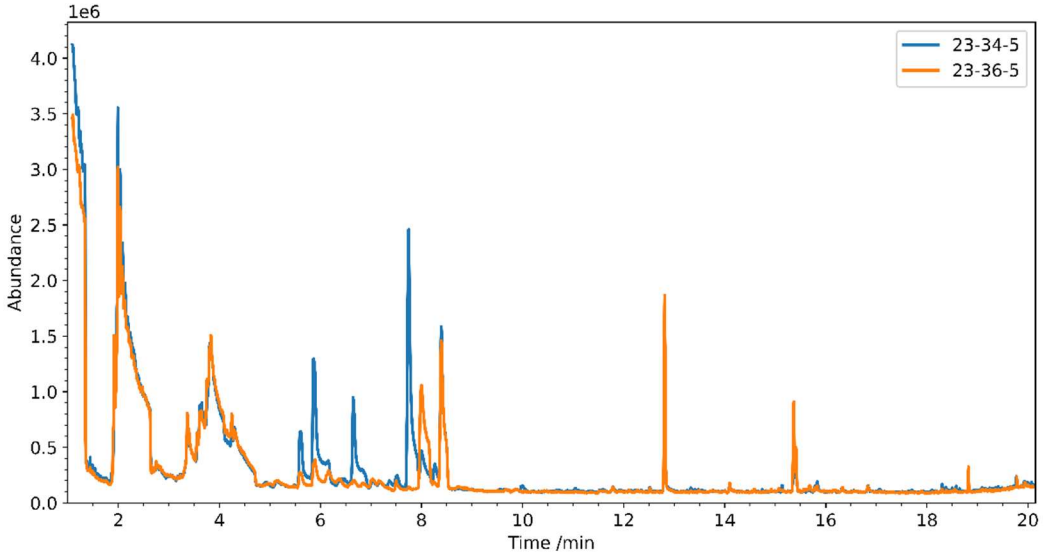

Figure 3. Overlayed chromatogram comparing donors 2023-34 and 2023-36 three weeks since placement. There were six compounds in common between these donors at this collection time.

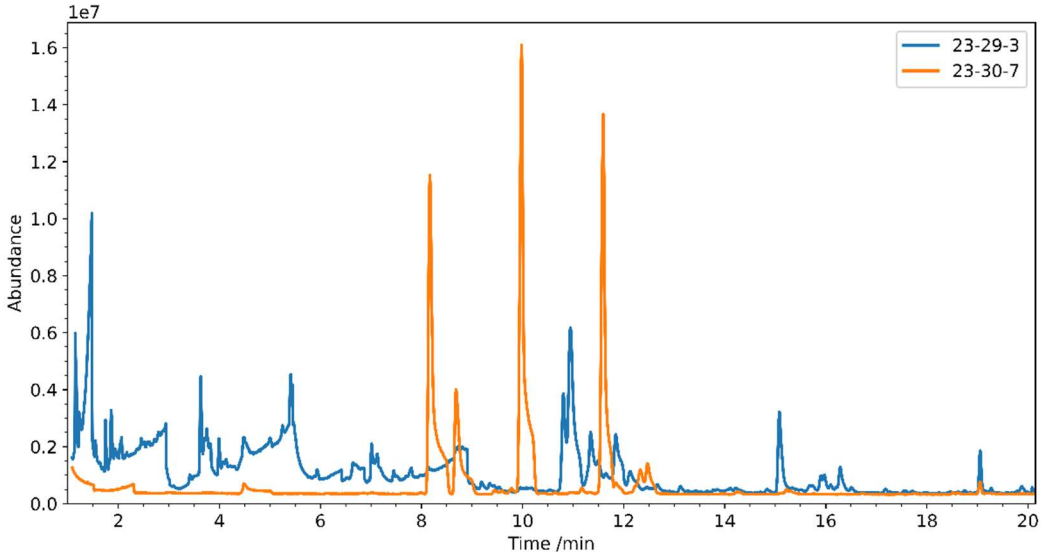

Figure 4. Overlayed chromatogram comparing donors 2023-29 and 2023-30 five weeks since placement. There was one compound in common between these donors at this collection time.

This suggests that the compounds that are present in the soil depend on the donor. It is still not known whether this factor dictates most of the compounds present, but it is a possibility that the donor impacts the compounds that are detected. It is also possible that the location where the donors are placed in the FOREST has an impact on the compounds detected. There were two donors, one in the spring semester, 23-06, and one in the fall semester, 23-29, that were placed around the same location. Donor 23-06, a diabetic person, was recovered around the time that 23-29, a prediabetic person, was placed. Both donors gave similar compounds which led to the question of if the location had a role in what compounds were present in the soil or if it was due to the medical condition. Additionally, the FOREST staff confirmed that donor 23-06 underwent decomposition in a very short period compared to other donor bodies. There have also been times when donors at the bottom of the FOREST have more compounds or fewer compounds

than donors at the top of the FOREST in the same stage of decomposition. However, Figure 1 shows two donors with similar compounds that were placed in different places in the FOREST. This makes it difficult to confirm if it's the placement that produces these compounds or the donors themselves.

3.3 Stage of Decomposition

There were no apparent trends based on the compounds detected during different stages of this project. However, when classifying the compounds detected it was shown that esters, alcohols, acids, and ketones were not detected until week three or four after being placed and were not regularly detected after week ten. For the spring semester, alkanes were the most abundant three weeks after placement while esters were the most abundant six weeks after placement. For the fall semester, acids and ketones were the most abundant seven weeks after placement while aromatics were the most abundant three and eight weeks after placement. It was also noted, in the spring semester, that when toluene was in high abundance, octane and heptane were in low abundance and the reverse is true when toluene was present in low abundance, as shown in Figure 5.

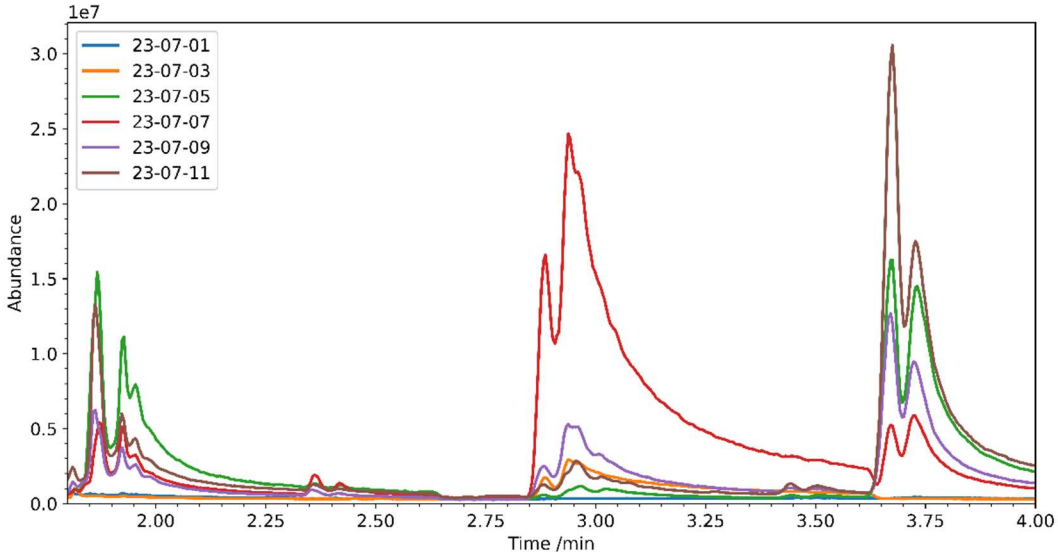

Figure 5. Overlayed chromatogram of soil from donor 2023-07 for 6 weeks of collection. Heptane is shown from 1.8-1.9 minutes, toluene is shown from 2.8-2.9 minutes and octane is shown from 3.7-3.9 minutes.

Data was compiled for four donors, 23-06, 23-02, 23-32, and 23-30, that showed the compounds detected and the week since placement they were detected in. This is shown in Tables A4-A7. Depending on the donor, some compounds, such as toluene and junipene, showed up in only one week or a few weeks out of the collection period while other compounds, such as 3-carene, beta-pinene, and (+)-cyclosativene, showed up almost every week in the collection period. These trends seemed to change depending on the donor which could mean that the stage of decomposition does not have a major effect on when these compounds are detected. However, other studies were able to classify compounds based on the stage of decomposition meaning there is a possibility that there was not enough data to accurately show how the compounds change from week to week.[42] While soil was collected for some donors for 3 months, others were collected for a shorter amount of time, meaning many of the donors are still in an early stage of

decomposition. Not having more data following the decomposition process longer makes it harder to get overall trends.

3.4 Temperature

There was not a very clear trend when looking at the temperature on the day of collection. For the fall semester, on the days when the temperature was between 60-69 °F, there were a lot of compounds present in many different donors. This was also true when the temperature was between 50-59 °F in the spring semester. However, much of the data was from the same donors at different stages of decomposition which could explain why there were many compounds in common. The highest toluene count was when the temperature was between 40-49 °F, for both the fall semester and spring semester, when considering the temperature overnight and in the morning of collection and not just the temperature at the time of collection.

3.5 Other Factors

There were also other variable factors such as insect activity, scavenging activity, rainfall, and intrinsic, cadaver-related factors. While anti-scavenging cages were placed over most donors some did not have the cages on all the time or were only used weeks after the donors were placed. This meant that vultures and other animals had access to the donors during this time. There were also times when there was a lot of insect activity and times when there was very little activity. These factors likely influenced the decomposition process but there is no way to know exactly how it may have affected the results from this research. The rainfall was also a variable that may have influenced the results. Rainfall varied from time to time, and this may have affected the moisture of the soil that was collected as well as possibly affecting results from donors at the bottom of the FOREST as there is an incline that may have caused a runoff from the top to the bottom.

Another factor that likely affects the decomposition process is the factors specific to the donors. Donors that were collected around in the FOREST had differences in body composition, sex, and medical conditions. It has been shown that differences in body mass index affected the pH response of the soil during decomposition. If the individual had more fat tissue present, more acidic products would be expected during decomposition.[51] In this same study, it was shown that diseases and medication may also influence the decomposition process. The soil surrounding donors that had cancer at the time of death had an altered microbial pattern compared to the soil around donors without cancer. This could have been due to medications, differences in body tissue, or an altered microbiome due to the disease.[51] One donor that was collected from during the spring semester, 2023-06, had diabetes. The overall decomposition process for this donor was different than that of other donors placed around the same time. One difference was that the entire decomposition process only took four months which is significantly shorter than other donors. The compounds that were produced were also different than most of the other donors collected from at that time. Around five weeks after placement, many ester and acid compounds were produced. This could be due to the heath conditions and further studies should be conducted to investigate the connection between heath conditions and decomposition.

3.6 Peak Splitting

In the spring semester 2023, there were issues with peak splitting in the chromatograms that we obtained for soil samples. Many of the peaks between 1-4 minutes split into two or more peaks. While mass spectrum library searching indicates that the splitting has the same identity, the mass spectra differ slightly for the splitting peaks (Figures 6 and 7). In the lower m/z regions, the mass spectra were identical while there were some differences observed in the higher m/z ratios. This is likely an indication of isomers or other low-volatile compounds coeluting. Initial

investigation of this problem by injecting neat toluene, heptane, and isooctane resulted in a similar splitting pattern. This issue was not able to be explored further due to time constraints, however, more research will be conducted in the future regarding this issue.

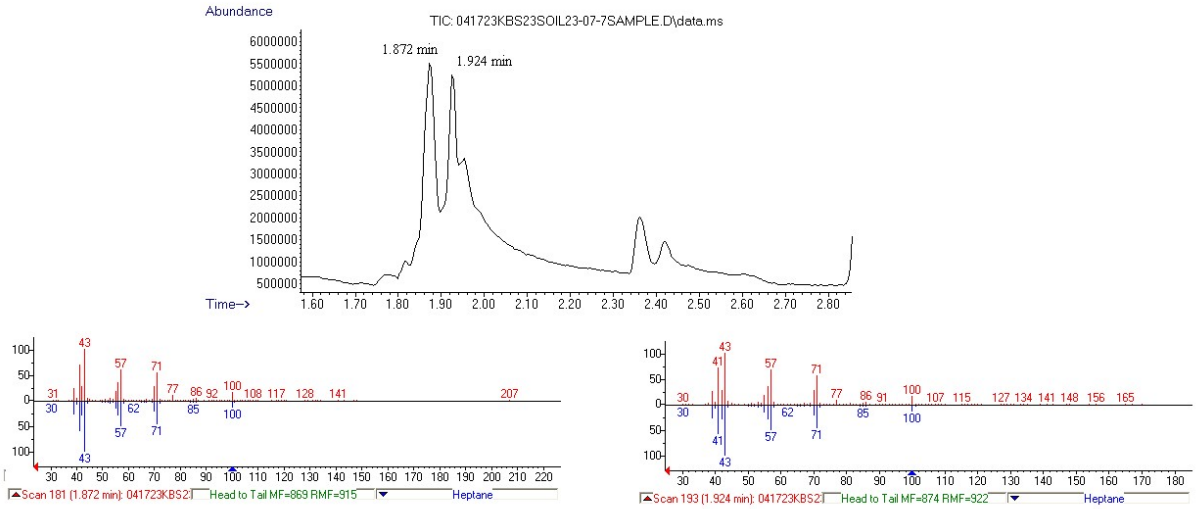

Figure 6. Chromatogram and mass spectra of soil sample 23-07-7 from donor 2023-07 showing heptane.

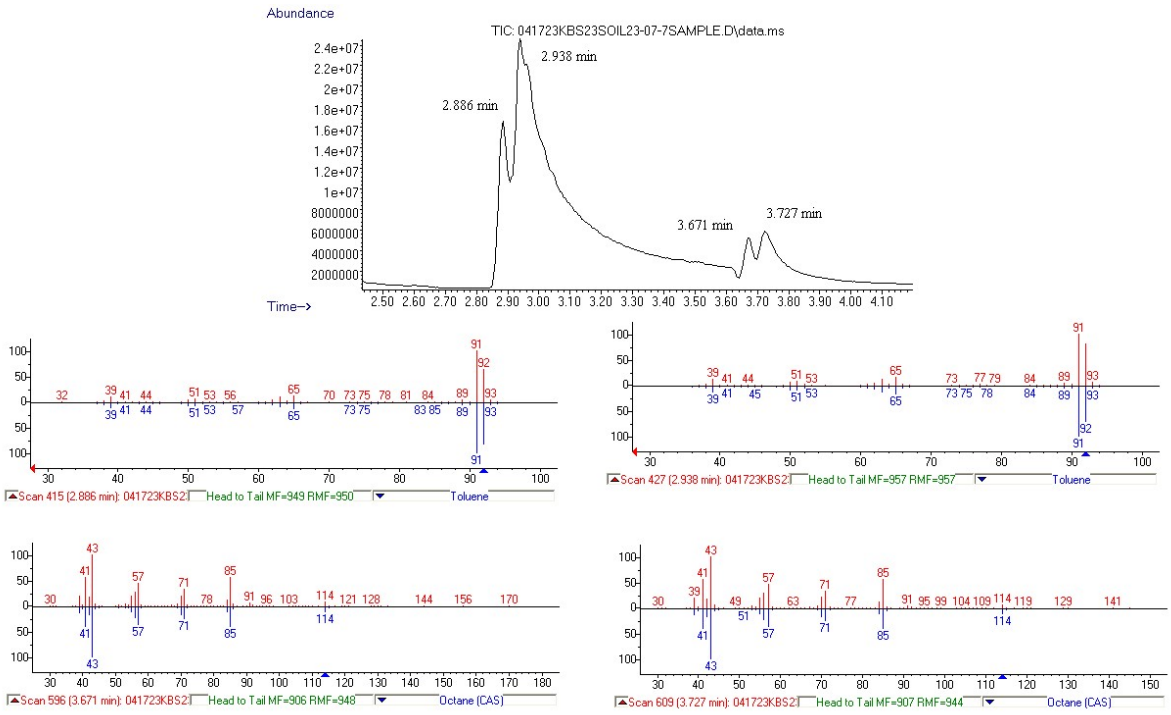

Figure 7. Chromatogram and mass spectra of soil sample 23-07-7 from donor 2023-07 showing toluene (left and top) and octane (right and bottom).

CHAPTER FOUR: CONCLUSION

Many compounds were present in the soil samples collected over 2023. Over 90 compounds present in the soil samples have been reported in previous decomposition studies indicating these compounds are from the decomposition process. There were also many compounds present in the soil samples that have not been reported in previous decomposition studies. Some of these compounds were determined to have likely come from the soil itself and be environmentally related. However, other compounds present could be an indication of human decomposition.

The effect the donors and placement of the donors had on the decomposition process and compounds that were present was considered. It was determined that some donors produced more compounds while others produced fewer. It was also noted that some compounds were only present around certain donors while other compounds were present around almost every donor. It could not be determined if the placement influenced the compounds present or if it was simply donor related.

The stage of decomposition seemed to influence the compounds present as well. Esters, alcohols, acids, and ketones were not present in the soil until three weeks after the placement of the donor. Some compounds were only present in certain weeks after placement while other compounds were present almost every week of the collection period. The abundance of certain compounds also changed week to week. However, this was slightly different depending on the donor, so it is unsure if this was due to the stage of decomposition or the donor.

The temperature during collection was taken into consideration. While it is known that the temperature has an effect on the decomposition process no trends were observed in the

compounds present in the soil samples. The only thing to note was that toluene was present in soil samples mostly when the temperature was in the range of 40-49 °F.

There were additional factors such as rain, insect activity, scavenging activity, and donor-specific factors that may have also affected the decomposition process and compounds present. The insect activity and scavenging activity could have influenced the rate of the decomposition but there is no way to definitively know what effect these factors had. The rain also changed the moisture content of the soil, but it is unknown if this had an impact on the compounds present in the soil. There were also donors with varying fat content and conditions such as diabetes that could have influenced the compounds present in the soil.

Future studies will be conducted to obtain more information on the compounds produced during human decomposition. As more data is collected, it may be possible to make further conclusions on how certain factors influence both the decomposition process and the compounds produced during this process. There will also be different collection and analysis methods tested to determine if more compounds can be discovered.

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APPENDIX A

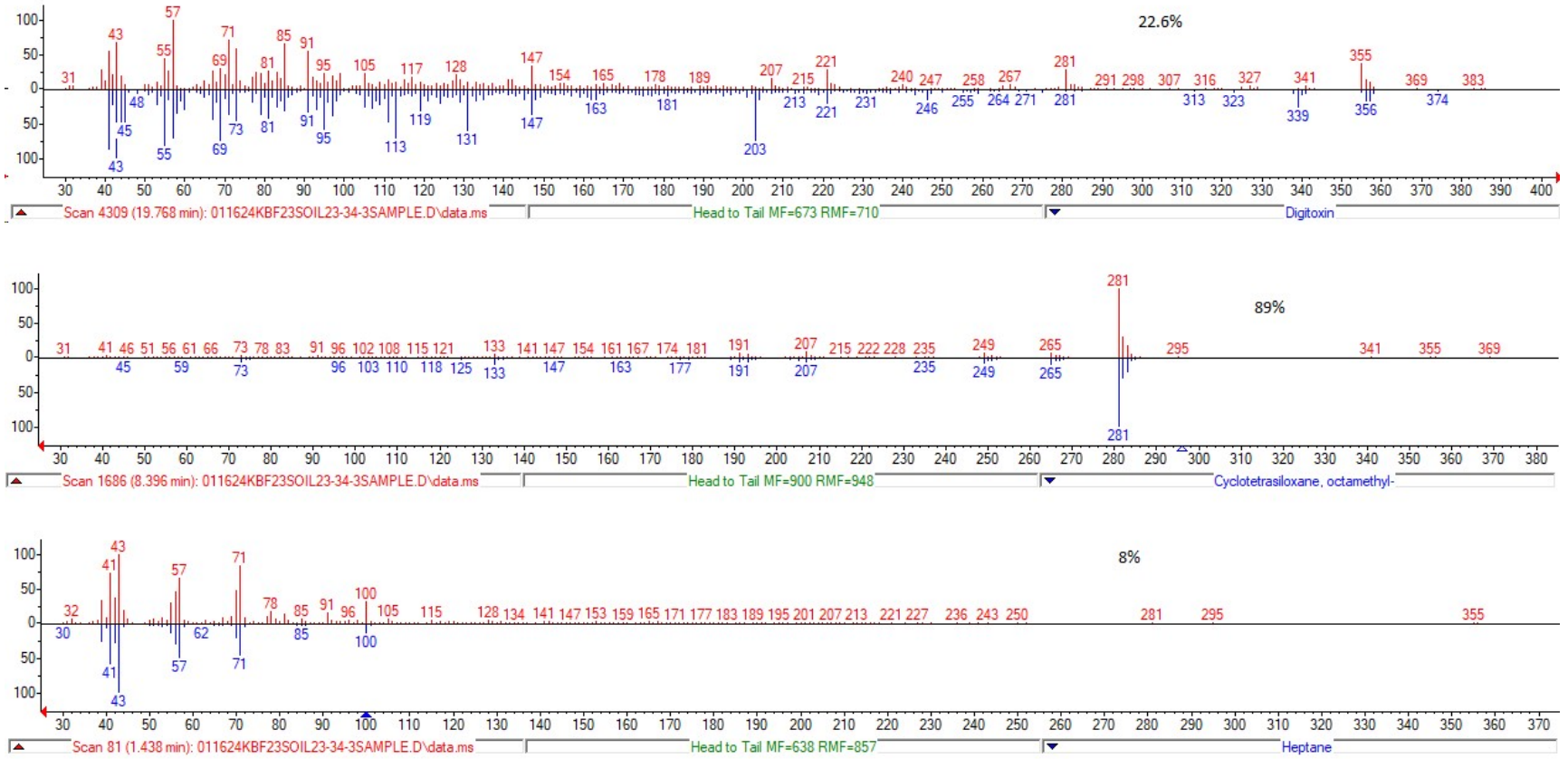

Figure A1. Comparison of experimental and library mass spectra of varying percent matches.

Table A1. All compounds found in soil, from all donors, that have been reported in previous studies.

Compound	Times present	23-25	23-26	23-29	23-30	23-32	23-34	23-35	23-36	23-37	23-02	23-06	23-07	23-09	22-31	22-34	23-23	22-29	22-32
Acids																			
2-Methylbutanoic acid	2			X								X							
3-Methylbutanoic acid	1											X							
Acetic acid	9			X							X	X							
Butanoic acid	5											X		X					
Heptanoic acid	6			X								X							
Octanoic acid	2			X															
Oleic acid	4			X							X								
Pentanoic acid	4			X								X							
Propanoic acid	6			X								X							
Alcohols																			
1-Butanol	6										X	X	X	X					
1-Heptanol	1									X									
1-Hexanol	11									X	X		X	X		X			
1-Pentanol	3									X				X					
1-Propanol	1										X								
2-Pentanol	1			X															
p-Cresol	4			X								X							
Aldehydes																			
2-n-Butylacrolein	1						X												
2-Octenal	2											X		X					

Table A1 Cont.

Decanal	2												X						
Heptanal	4									X			X	X					
Hexanal	11			X						X	X	X	X	X		X			
Nonanal	4									X			X	X					
Octanal	4											X	X	X					
Alkanes																			
2,3-Epoxybutane	6										X		X	X		X			
2,4-Dithiapentane	1																		X
2-Methyl pentane	1			X															
3-Methylhexane	1													X					
Dodecane	18			X		X	X				X	X		X			X		
Heptane	36	X		X		X	X			X	X	X	X	X	X	X	X		
Hexadecane	5											X		X					
Hexane	3										X		X						
Nonane	2										X								
Octane	34	X		X		X	X			X	X	X	X	X	X	X	X		
Pentadecane	22			X		X	X			X	X	X	X						
Tetradecane	5						X				X	X	X						
Tridecane	14			X			X				X		X				X		
Undecane	3												X				X		
Alkenes																			
(-)-beta-Pinene[a]	45	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	
1-Dodecene	2						X				X								
1-Hexene	2													X					
1R/alpha-Pinene[b]	31	X	X		X	X	X	X		X	X	X	X			X	X	X	
2-beta-Pinene	2					X											X		
8-Heptadecene	1																		
D-Limonene	5		X		X		X												

Table A1 Cont.

Limonene	1				X														
Aromatics																			
2-Heptylfuran	2						X												
2-Pentylfuran	36	X		X		X	X	X		X	X	X	X	X	X	X	X		
3-Methyl-1H-Indole	2			X															
Benzene	1																		
Ethylbenzene	5			X			X		X										
m-Xylene	1								X										
o-Cymene	11	X	X		X	X			X	X							X		
o-Xylene	1						X												
Phenol	6			X							X	X							
p-Xylene	7					X	X	X	X										
Toluene	44				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Esters																			
Acetic acid, ethyl ester	2																	X	
Butyl 2-methylbutanoate	2											X							
Butyl 3-methylbutanoate	3											X							
Butyl ester butanoic acid	4											X		X					
Butyl ester hexanoic acid	3											X							
Butyl ester propanoic acid	3											X		X					
Butyl pentanoate	3											X							
Ethyl 4-methylpentanoate	2											X							

Table A1 Cont.

Ethyl ester butanoic acid	3											X		X					
Ethyl pentanonate	1			X															
Propyl ester butanoic acid	6			X							X	X							
Propyl hexanoate	5			X								X							
Propyl pentanoate	3			X															
Propyl propionate	3			X															
Ether																			
2,3-Dimethyloxirane	2			X															
Ketones																			
2-Butanone	7			X							X	X		X					
2-Heptanone	2			X										X					
2-Hexanone	1																		
2-Nonanone	5			X									X						
2-Octanone	3			X															
2-Pentanone	9			X						X			X						
3-Hydroxy-2-Butanone	1										X								
3-Octanone	18			X							X	X	X	X			X		
Acetone	4					X	X	X	X										
Acetophenone	8			X								X	X						
Other																			
L-Calamenene	8	X			X	X		X		X									
1,3,5-Cycloheptatriene	4							X			X					X			
3-Carene	37	X			X	X	X	X	X	X	X	X	X			X			X
Camphene	6		X			X					X	X							X

Table A1 Cont.

Copaene	14				X	X	X	X		X	X						X		
d-Cadinene	5		X		X			X		X									
Methoxy-phenyl oxime	1										X								
Terpinolene	9				X	X		X		X						X			
Sulfur-containing																			
Dimethyl disulfide	2											X							X

[a]Beta-pinene is reported in previous studies but (-)-beta-pinene has a very similar mass spectrum so it is classified as being previously reported. [b]Alpha-pinene is reported in previous studies but 1R-alpha-pinene has a very similar mass spectrum so it is also classified as being previously reported.

Table A2. All compounds found in soil, from all donors, that have not been reported in previous studies and are not plant based.

Compound	Number of times present
D-Camphene	29
Dimethyl silanediol	26
Doconexent	20
m-Cymene	15
alpha-Muurolene	10
alpha/beta Terpinyl acetate	9
1-Chloro-tetradecane	8
2,4-Dimethylhexane	8
Glutaraldehyde	8
Methyl arachidonate	7
beta-Guaiene	7
1-Tetradecanol	6
Cadinene	6
gamma-Cadinene	6
Octadecanoic/stearic acid	6
1,3-Butanediol	5
1-Chloro-octadecane	5
Calamenene	5
Ditgitoxin	5
Dotricontane	5
m-Cresol	4
gamma-Nonalactone	4
Valencene	4
13-Heptadecyn-1-ol	4
gamma-Undecalactone	4
2,4-Pentadienal	3
3-Methylhexane	3
E-2,3-epoxycarane	3
Methylmalonic acid	3
Nonadecane	3
Retinol	3
Spironolactone	3
1,2-Diethoxyethane	3
Iso amyl alcohol	3
1,2,4-Butanetriol	2
2,5-Dimethoxytoluene	2
4-Methyl anisole	2
5-Methyl-3-heptanone	2

Table A2 Cont.

d-Verbenone	2
Ethyl stearate	2
Gitoxigenin	2
Heptyl benzene	2
Pentyl ester butanoic acid	2
Thymol methyl ether	2
2-Ethoxyethanol	2
2-Ethylbutanal	2
Cis-2-nonen-1-ol	2
Tropacocaine	2
(+)-3-carene	1
(+)-Sativene	1
(+/-)-Dihydrocarveol	1
(Z)-5-Undecene	1
1,3-Dimethyl cyclohexane	1
1,4-Dichlorobenzene	1
11-Benzylheneicosane	1
17-Chloro-7-heptadecane	1
1-Chloro-hexane	1
1-Heptatracontanol	1
1-Heptene	1
2-Carene	1
2-Decyn-1-ol	1
2-Methylbutyl hexanoate	1
2-Octyn-1-ol	1
3-Deoxyestradiol	1
3-Ethylhexane	1
3-Methyl anisole	1
4-Ethylcyclohexanol	1
4-Isopropenyltoluene	1
4-Methyl-2-hexanone	1
5-Methyl-2-hexanone	1
Allyl 2-ethylbutyrate	1
alpha,p-Dimethylstyrene	1
alpha-Copaene	1
Aspartame	1
Benzyl bromide	1
Benzyl palmitate	1
beta-Neoclovene	1
beta-Phellandrene	1
Canrenone	1

Table A2 Cont.

Carvacrol methyl ether	1
Cathine	1
Chlorozotocin	1
E-2-Octenal	1
Ethyl iso-allocholate	1
Fenretinide	1
Geranyl bromide	1
Lactaropallidin	1
Norethynodrel	1
Ocimene	1
o-Cresol	1
o-Methylthymol	1
p-Cymene	1
Pentadecanoic acid	1
p-Xylenol	1
Sclarene	1
Tetradecanoic acid	1
Trimethyltetrahydropyran	1
1,2,6-Hexanetriol	1
1-Chloro-2-propanol	1
2-Methyl-4-penten-2-ol	1
2-Methyltetrahydrofuran-3-one	1
3-Methyl-2-pentanone	1
9-Hexadecanoic acid	1
alpha-Curcumene	1
Androstanolone acetate	1
Caryophyllene	1
Chavicol	1
Cholic acid	1
Cis-ocimene	1
Cuparene	1
Diethyl ether	1
Guaiazulene	1
Verbenone	1

Table A3. All compounds found in soil, from all donors, that have not been reported in previous studies and are plant based.

Compound	Number of times present
Hi-oleic safflower oil	36
Junipene	32
(+)-Cyclosativene	30
Sabinene	16
alpha-Guaiene	14
beta-Elemene	10
1-Monopalmitin	9
2-Dodecanone	6
1-Phenyl-1-butene	5
alpha-Cubebene	5
Curzerene	5
2,4,6-Trichloroanisole	4
alpha-Fenchene	4
Falcarinol	3
gamma-Muurolene	3
Cedrene	3
beta-Cedrene	2
beta-Cubebene	2
Fenchone	2
1,7-Dimethyl naphthalene	1
1,8-Cineole	1
2,4-Dimethylanisole	1
Aromadendrene	1
Docosane	1
Isopinocarveol	1
Ipsdienol	1
1-Nonadecane	1
Isolongifolene	1
delta-Guaiene	1
Neoclovene	1
Camphor	1
Cuparene	1

Table A4. Compounds present for donor 2023-06 for all weeks of collection.

Compound	Week 1	Week 2	Week 3	Week 5	Week 6	Week 7
(-)-beta-Pinene*	X	X				
1,2,4-Butanetriol					X	
1,8-Cieneole	X					
1-Butanol*				X	X	X
1-Chlorotetradecane	X					X
2,4-Dimethylhexane		X		X	X	
2-Butanone*				X		
2-Dodecanone						X
2-Methylbutanoic acid*					X	
2-Methyltetrahydrofuran-3-one				X		
2-Octenal*						X
2-Pentanone*						X
2-Pentylfuran*	X	X	X			X
3-Carene*			X			
3-Methyl butanoic acid				X		
3-Octanone*						X
4-Methyl phenol*				X	X	
Acetic acid*				X	X	X
Acetophenone*						X
alpha-Pinene*		X				
Butanoic acid*				X	X	X
Butyl 2-methylbutanoate*					X	X
Butyl 3-methylbutanoate*				X	X	X
Butyl ester butanoic acid*				X	X	X
Butyl ester hexanoic acid*				X	X	X
Butyl ester propanoic acid*					X	X
Butyl pentanoate*				X	X	X
Camphene*	X					
Cis-2-nonen-1-ol					X	X
Cis-ocimene	X					
Dimethyl disulfide*				X		
Dimethyl silanediol		X				
Dodecane*	X			X	X	X
Ethyl 4-methylpentanoate*				X	X	
Ethyl ester butanoic acid*				X	X	
gamma-Undecalactone					X	
Glutaraldehyde		X				
Heptane*		X	X			X
Heptanoic acid*					X	X
Hexadecane*	X	X	X			

Table A4 Cont.

Hexanal*	X	X				X
Hi-oliec safflower oil	X	X	X			X
Junipene		X	X			
Methyl arachidonate		X				
Octanal*						X
Octane*		X		X		
Phenol*				X	X	
Pentadecane*				X	X	X
Pentanoic acid*				X	X	
Pentyl ester butanoic acid*						X
Propanoic acid*				X	X	X
Propyl ester butanoic acid*				X	X	
Propyl hexanoate*				X	X	X
Sabinene	X					
Tetradecane*					X	
Toluene*			X			

*Indicates reported in previous studies

Table A5. Compounds present for donor 2023-02 for all weeks of collection.

Compound	Week 3	Week 4	Week 5	Week 7	Week 9	Week 10	Week 11	Week 13	Week 14	Week 15
beta-Elemene	X									
(-)-beta-Pinene*	X			X	X	X		X	X	X
(+)-Cyclosativene	X									
1,3,5-Cycloheptatriene*					X					
1,3-Butanediol				X	X	X				
13-Heptadecyn-1-ol		X								
1-Butanol*					X					
1-Chloro-tetradecane						X		X	X	X
1-Dodecene*								X		
1-Hexanol*					X	X	X			
1-Monopalmitin		X		X			X			
1-Propanol*				X						
1R-Alpha pinene*	X			X						
1-Tetradecanol							X		X	
2,3-Epoxybutane*					X		X			
2-Butanone*				X	X			X		
2-Dodecanone					X			X		
2-Octanone*				X						
2-Pentylfuran*					X	X	X	X	X	X
3-Carene*	X									X
3-Hydroxy-2-butanone*				X						
3-Methyl-2-pentanone								X		
3-Methylhexane						X	X			
3-Octanone*					X	X	X	X	X	X
9-Hexadecenoic acid										X
Acetic acid*				X			X			
alpha-Fenchene			X							
alpha-Guaiene	X									
alpha-Terpinyl acetate	X									

Table A5 Cont.

beta-Cubebene	X									
beta-Pinene*							X			
Butanoic acid*				X						
Butanoic acid, propyl ester*				X						
Camphene*								X		
Copaene*	X									
D-Camphene	X									X
Dimethyl-silanediol	X	X	X							X
Doconexent	X	X			X					
Dodecane*				X		X				X
Falcarinol					X					
gamma-Nonalactone					X			X		
gamma-Undecalactone						X	X			
Glutaraldehyde							X			X
Heptane*				X	X	X	X	X	X	
Hexanal*									X	
Hexane*						X				
Hi-oliec safflower oil		X		X	X	X	X	X	X	X
Iso amyl alcohol						X				
Junipene	X					X				X
m-Cymene	X									
Methoxy-phenyl oxime*	X									
Methyl arachidonate					X				X	X
Nonadecane					X					
Nonane*							X	X		
Octadecanoic/Stearic acid				X						
Octane*				X	X	X	X	X	X	
Oleic acid*			X					X		X
Pentadecane*						X	X	X	X	

Table A5 Cont.

Pentadecanoic acid								X		
Phenol*				X						
Sabinene			X							
Tetradecane*								X		
Tetradecanoic acid								X		
Toluene*	X	X	X	X						X
Tridecane*					X		X	X	X	
Tropacocaine										X

*Indicates reported in previous studies

Table A6. Compounds present for donor 2023-32 for all weeks of collection.

Compound	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 9	Week 11	Week 12
beta-Elemene		X	X		X					
(-)-beta-Pinene*		X	X	X	X		X	X	X	
(+)-Cyclosativene		X	X	X	X	X	X	X		X
1-Tetradecanol				X						
2,4-Dimethylhexane									X	
2,5-Dimethoxy toluene								X		
2-beta-Pinene					X					
2-Pentylfuran										X
3-Carene*	X	X	X	X	X	X	X	X	X	
3-Ethylhexane						X				
4-Isopropenyltoluene					X					
Acetone*									X	
alpha-Pinene*		X	X	X	X			X	X	
alpha,p-Dimethylstyrene								X		
alpha-Cubebene			X		X					
alpha-Guaiene		X	X	X	X			X		
alpha-Muurolene			X		X			X		
alpha-Terpinyl acetate				X						
Benzyl bromide					X					
beta-Guaiene		X						X		
beta-Phellandrene			X							
beta-Terpinyl acetate					X					
Cadinene			X		X			X		
Camphene*									X	
Carvacrol methyl ether					X					
Cathine					X					
Copaene*			X	X	X			X		
Curzerene	X									

Table A6 Cont.

D-Camphene		X	X	X	X	X	X	X		
delta-Guaiene					X					
Dimethyl silanediol	X	X								
Doconexent	X	X	X	X						
Dodecane*	X									
Dotriacontane		X								
Ethyl iso-allocholate						X				
Fenchone								X		
Geranyl bromide								X		
Heptane*	X					X		X	X	X
Heptylbenzene							X			
Hi-oleic safflower oil	X		X							
Junipene		X	X							
L-Calamenene								X	X	
m-Cymene				X		X	X		X	X
Methyl arachidonate		X								
Norethynodrel		X								
Octane*										X
o-Cymene*			X		X					
o-Methylthymol			X							
p-Cymene								X		
Pentadecane*	X									
p-Xylene*									X	
Retinol			X							
Sabinene			X	X	X					
Spironolactone						X				
Terpinolene*			X		X			X		
Thymol methyl ether								X		
Toluene*								X	X	

*Indicates reported in previous studies

Table A7. Compounds present for donor 2023-30 for all weeks of collection.

Compound	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 10	Week 13
(-)-beta-Pinene*	X	X	X	X		X	X	X	
(-)-alpha-Pinene					X				
(+)- 3-Carene	X								
(+)-Cyclosativene	X	X	X	X	X	X	X	X	X
(+)-Sativene									X
1R-alpha-Pinene*	X	X	X	X			X	X	
3-Carene*	X	X	X	X		X	X	X	X
alpha-Guaiene	X	X	X						
alpha-Muurolene	X		X						
alpha-Cubebene			X						
alpha-Fenchene		X							
beta-Elemene	X		X						
beta-Cubebene			X						
beta-Guaiene								X	
beta-Terpinyl acetate	X								
Cadinene	X		X						
Copaene*	X		X					X	
d-Cadinene*	X								
D-Camphene	X		X	X		X	X	X	X
Digitoxin						X	X		
Dimethyl silanediol	X								
Divinyl sulphone					X				
d-Limonene				X			X		
Doconexent		X	X	X					
Docosane		X							
d-Verbenone								X	
E-2,3-Epoxycarene	X								
Fenretinide				X					

Table A7 Cont.

Hi-oliec safflower oil	X			X					
Junipene		X	X	X					
Lacteropallidin				X					
L-Calamenene*		X							X
Limonene*					X				
m-Cymene								X	
Ocimene					X				
Octadecanoic/Stearic acid									X
o-Cymene*	X						X		
Sabinene	X								
Terpinolene*	X								
Toluene*							X	X	

*Indicates reported in previous studies