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ORIGINAL ARTICLE

Detection of decomposition volatile organic compounds in soil following removal of remains from a surface deposition site

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Abstract
Purpose Cadaver-detection dogs use volatile organic compounds (VOCs) to search for human remains including those deposited on or beneath soil. Soil can act as a sink for VOCs, causing loading of decomposition VOCs in the soil following soft tissue decomposition. The objective of this study was to chemically profile decomposition VOCs from surface decomposition sites after remains were removed from their primary location.
Methods Pig carcasses were used as human analogues and were deposited on a soil surface to decompose for 3 months. The remains were then removed from each site and VOCs were collected from the soil for 7 months thereafter and analyzed by comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–TOFMS).
Results Decomposition VOCs diminished within 6 weeks and hydrocarbons were the most persistent compound class. Decomposition VOCs could still be detected in the soil after 7 months using Principal Component Analysis.
Conclusions This study demonstrated that the decomposition VOC profile, while detectable by GC×GC–TOFMS in the soil, was considerably reduced and altered in composition upon removal of remains. Chemical reference data is provided by this study for future investigations of canine alert behavior in scenarios involving scattered or scavenged remains.

Keywords Forensic Science · Decomposition chemistry · Carrion scavenging · Comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–TOFMS) · Residual odor

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Introduction

Scent-detection canines (*Canis lupus* var. *familiaris*) are often used to locate evidence of forensic significance. Specifically, cadaver-detection dogs are commonly used by police agencies to search for and locate deceased victims. Their ability to search large areas rapidly and under adverse conditions is beneficial, especially in critical situations where agility is required (i.e., mass disasters, urban search and rescue, missing persons cases). As such, they are regularly used in outdoor scenarios.

Although the exact suite of compounds involved in eliciting a positive alert is still unknown, alert behavior is a response of the canine olfactory system to volatile organic

compounds (VOCs) [1–4]. Under ideal circumstances where the remains are present and discovered soon after death, a strong odor is released from the remains and the cadaver-detection dog will alert with high efficacy to the detectable decomposition VOCs. During extended post-mortem intervals (PMIs) the remains may be severely decomposed or scavenged, leaving behind minimal soft tissue with a reduced presence of detectable decomposition VOCs. Cadaver-detection dogs can still be effective in these situations, yet often require additional training to maintain their efficacy [5].

Due to the sorptive capacity of soil [6], VOCs remaining in soil at the deposition site may contribute largely to the persisting decomposition odor that is available for cadaver-detection dogs. The term "residual odor" has recently been used to describe VOCs that adhere to soil particles after remains are removed from the soil surface [7]. A recent study highlighted the high efficacy with which cadaver-detection canines are able to alert on residual decomposition odor as a result of persisting decomposition VOCs [7]. However, to date there have been no published studies that chemically profile the decomposition VOCs that remain in residual decomposition odor, which has made it difficult to interpret the chemical foundation for this behavior [7].

Scavenging of remains associated with outdoor scenes can impact the success of search and recovery teams [8]. Cadaver-detection dogs can be tasked with locating clandestine graves or scavenged body parts which can be valuable for assembling remains for burial purposes [5]. They can also be used to identify the original deposition site of scavenged remains. Animal scavengers can cause extensive soft tissue or bone removal from the primary deposition site, reducing the VOCs available to cadaver-detection dogs and/or rendering the original location absent of visible remains [2]. Locating a primary deposition site can provide valuable investigative information for substantiating suspect or witness testimony. In speculative searches with a large area of interest, identification of the primary deposition site can narrow the search area and assist in locating scattered remains. However, the reduced presence of decomposition VOCs can challenge cadaver-detection dog odor recognition and the handler's ability to assess canine efficacy. The handler may mistake a true positive alert to the deposition site as being a false positive alert due to the lack of confirmation of visual remains. There is currently no published research characterizing the decomposition VOC profile where decomposed remains have been removed from deposition sites. Lack of scientific substantiation may lead to court challenges of cadaver-detection dog use in complex scenarios [9]. The need for improved chemical characterization of the VOCs associated with residual decomposition odor has recently been highlighted [7].

Analysis of the decomposition VOC profile has been under investigation for many years using gas chromatography–mass spectrometry (GC–MS) [3, 10–15]. The complex reactions occurring during decomposition produce a dynamic VOC profile which varies over time. Post-mortem processes produce numerous compounds from a wide range of chemical classes causing difficulty when targeting all classes in a single analysis. These challenges have led to the use of comprehensive two-dimensional gas chromatography (GC×GC) coupled with time-of-flight mass spectrometry (TOFMS) [4, 16–20]. The GC×GC–TOFMS system reduces compound co-elution that often occurs in complex mixtures by subjecting the sample to two independent separations, thereby increasing the number of VOCs that can be accurately detected in a single sample. Higher sensitivity of this instrumentation is especially beneficial for trace mixture analysis. These features improve the overall reliability in decomposition VOC profiling and can therefore aid in the characterization of residual decomposition odor.

The objective of this study was to investigate the persistence of decomposition VOCs in soil using GC×GC–TOFMS following simulated scavenging involving removal of the remains from the deposition site. Characterization of the changes in the VOC profile following the removal of remains was desired, in addition to determining those compounds that persist for a longer period. Considering soil as a VOC sink, this provides chemical data regarding the VOCs remaining in soil that are detectable following long term PMIs and when remains have been scavenged. High peak capacity and improved sensitivity were necessary for this study due to the complexity of the matrix and the trace levels of compounds expected as time proceeded; thus, GC×GC–TOFMS provided a means of achieving both of these goals.

Experimental

Decomposition trial

A decomposition trial was performed using four 70 kg pig (*Sus scrofa domesticus*) carcasses and four control sites containing no remains. Pigs are often used in decomposition research as odor analogues due to anatomical similarities to humans [21]. Although there may be differences between pigs and humans in the minor VOCs released during decomposition [12, 22], the major compounds in the profile have been shown to be comparable and therefore pigs provided acceptable odor analogues for this study [12, 16, 22–24]. The pig carcasses were purchased post-mortem from a licensed abattoir and therefore did not require

animal ethics approval since the subjects were not killed specifically for the purposes of this study.

An open eucalypt woodland with sandy clay topsoil was used as the study site, located outside of Sydney, Australia. Carcasses were deposited on the soil surface approximately 4 m apart, and were allowed to decompose from January to April 2013, representing the transition from summer to autumn in Australia. Control and experimental sites were separated by a minimum distance of 20 m. Hot and humid conditions were typical during this time with average daily temperatures of 16–31 °C and relative humidity averaging 80 %. Accumulated degree days (ADD) is used to represent the duration of stages in many taphonomic studies and is a measure of time that accounts for the effects of temperature on the decomposition rate [25]. ADD is represented by a cumulative total of the average daily temperature (in °C) for each day [25]. Decomposition was rapid in this environment and the carcasses reached skeletonization with extensive mummification by the 17th day post-mortem (413 ADD). During the decomposition period, each set of remains was covered by a large stainless steel anti-scavenging cage with 1 cm wire mesh to prevent the premature loss of biomass prior to the removal of remains. Cages were removed periodically in order to sample the soil VOCs during the decomposition period. The VOC profile detected in soil during the decomposition period (prior to the removal of the remains for persistence sampling) has been previously published and will not be repeated herein [23, 26].

Persistence sampling

Artificial scavenging was performed after 3 months of decomposition (94 days, 1986 ADD) by removing the remains manually from each deposition site. Mummified soft tissue and large bones were removed from each experimental site while small bones were left on the soil surface. A 30 cm VOC-Mole™ Soil Probe (Markes International Ltd., UK) was inserted in the ground at each experimental site ($n = 4$) and control site ($n = 4$) in order to collect VOC samples from the soil and is described in further detail in previous publications [23, 26]. Pumped sampling was performed (100 mL min^{-1} for 15 min) from each probe onto a Tenax TA/Carbograph 5TD dual sorbent tube (Markes International Ltd., UK) using an ACTI-VOC low flow sampling pump (Markes International Ltd., UK). The sorbent tube was connected to the external side of the probe cap using a ¼″ union containing a polytetrafluoroethylene (PTFE) ferrule. VOC samples were collected from the four experimental and four control sites on each sampling day. Sorbent tubes were sealed with brass storage caps, wrapped in aluminum foil, and transported to the laboratory in an air-tight glass jar to prevent contamination or sample loss. Field blank samples were collected on each sampling day in order to account for analyte artefacts, as outlined in the EPA Method (TO-17). Determination of volatile organic compounds in ambient air using active sampling onto sorbent tubes. These field blank samples bracketed the sample collection period by exposing a blank sorbent tube to the atmosphere for 10 s between the control and experimental sites. Sample collection was performed immediately following scavenging (day 0) and on days 14 (2 weeks), 28 (1 month), 55 (2 months), 94 (3 months), 125 (4 months), and 214 (7 months) post-scavenging. Each site was covered with a layer of wire mesh affixed with steel pegs between sampling periods to prevent animal activity at the sites (e.g., burrowing, defecation, etc.). Weather variables were recorded hourly during the study using a HOBO® U30 No Remote Communication (NRC) data logger and sensors (OneTemp, Marleston) for ambient temperature (°C), relative humidity (%), solar radiation (Wm^{-2}), wind speed (m s^{-1}), wind direction (ø), and rainfall (mm). Soil pH was measured using a direct soil pH measurement kit (Hanna Instruments, Australia). A soil moisture sensor with LabQuest 2 Interface (Vernier, Australia) was used to measure volumetric water content of the soil at each site as an estimate of soil moisture. Before analysis of each sorbent tube, 2 µL of 150 ppm bromobenzene (GC grade, Sigma Aldrich, Australia) in methanol (HPLC grade, Sigma Aldrich, Australia) was injected onto the tube using an eVol® XR hand-held automated analytical syringe (SGE Analytical Science, Australia) to allow for internal standard normalization of analytes.

GC×GC–TOFMS analysis

VOCs were thermally desorbed from sorbent tubes using a Series 2 Unity Thermal Desorber and ULTRA™ multi-tube autosampler (Markes International Ltd.). Sorbent tubes were desorbed for 4 min at 300 °C onto a general purpose cold trap (Tenax TA/Carbograph 1TD) at −10 °C followed by trap desorption for 3 min at 300 °C under a 20 mL min^{-1} split flow. The Unity 2™ was connected to a Pegasus® 4D GC×GC–TOFMS (LECO, Australia). An Rxi®-624Sil MS primary column (30 m × 0.250 mm ID, 1.40 µm film thickness, Restek Corporation, Australia) was used and attached to a Stabilwax® secondary column (2 m × 0.250 mm ID, 0.50 µm film thickness, Restek Corporation) using a SilTite™ µ-Union (SGE Analytical Science). Helium was used as the carrier gas in constant flow at 1.00 mL min^{-1}. The primary GC oven was held initially at 35 °C for 5 min, followed by an increase to 240 °C at a rate of 5 °C min^{-1}, and was then held for 5 min. The modulator offset was 5 °C and the secondary oven temperature offset was 15 °C. A 5 s modulation

period was used with a hot pulse time of 1 s. The MS transfer line was held at 250 °C and the acquisition rate was 100 Hz from 29 to 450 amu. The electron ionization energy was −70 eV and the source temperature was 200 °C. The detector voltage was optimized prior to each sample run and used with a 200 V offset above the optimized detector voltage.

Data processing and analysis

ChromaTOF® (version 4.50.8.0, LECO) was used to process data using baseline tracking with an 80 % offset and automatic baseline smoothing. A 30 s peak width in ^{1}D and 0.15 s peak width in ^{2}D was used. A signal-to-noise ratio (S/N) of 150 was used with a minimum similarity match >700 to the NIST (2011) mass spectral library database. Peak identification was also confirmed by retention indices and using a range of 84 chemical standards (alkanes, alkylbenzenes, aromatic hydrocarbons, heterocyclic aromatics, chlorinated hydrocarbons, ketones, aldehydes, sulfur-containing compounds, phthalates, primary alcohols, secondary alcohols, fatty acid methyl esters, phthalates, and Grob test mix compounds). Unique mass was used for peak area calculation and alignment was performed using Statistical Compare in ChromaTOF® and normalized using the internal standard. A 50 % filter was applied to the data and peaks >20 S/N were included for analytes not identified by initial peak finding (method and data validation have been reported elsewhere [27]). The 50 % filter increased the reliability in the results because compounds reported in fewer than 50 % of the samples in a class (i.e., control or experimental) were not reported.

Output from Statistical Compare was exported into Microsoft Excel. Logarithmic transformation of raw data was performed and compounds were identified that were exclusive to controls or significantly higher than controls using an independent Student's t test ($n = 4$, $p < 0.05$). These data handling processes have been previously published and incorporate the use of replicate measurements, control data, and field blanks [27]. Normalized peak areas were summed between chemical classes, mean-centered and then analyzed using principal component analysis (PCA) in The Unscrambler® X (version 10.3, CAMO Software, Norway). This allowed for reduced data dimensionality, increasing the ability to visualize the data on PCA plots of scores and loadings. In this manner, the presence of a decomposition VOC signature in experimental sites could be assessed in comparison to control soils. Chemical classes used for classification were: sulfur-containing, nitrogen-containing, aromatic, carboxylic acid, ester, aldehyde, ketone, alcohol, ether, halogenated, hydrocarbon, or other. The normalized peak area for each compound of interest was averaged between sites and summed for each class.

Results and discussion

VOCs of interest and compound class trends

The VOC profile was most abundant and complex immediately following simulated scavenging and was similar in composition and abundance to the VOC profile documented for the skeletonized stage with mummification during the decomposition period [23, 26]. This was anticipated because the residual odor left by the remains should have been highest at this point, as it was the closest measurement to the point at which the remains were present. A list of significant compounds can be found in Table 1. Figure 1 displays the relative amount of each compound summed by their compound class. This figure demonstrates general trends that were observed throughout the trial for each compound class. Only significant compounds have been included and their abundance was summed to produce the plots in Fig. 1. The control data is also shown, as there were many compounds which were found in common between the control and the experimental samples (i.e., the compounds where t testing needed to be applied). This demonstrates that the background VOC profile was low after compound selection by t testing.

The relative amounts of each compound class were high during initial measurements (i.e., immediately following simulated scavenging as this represented the closest time point at which the remains were present) but decreased thereafter. The majority of compound classes were greatly reduced 6 weeks after the removal of remains from the deposition site. An elevated number of aromatics, ketones, and alcohols were detected after 94 days (3 months, Table 1). Environmental conditions on this day may have been favorable for the release of these VOCs from the soil. Above-average rainfall was experienced leading up to and during this time, allowing more effective volatilization of remaining polar VOCs in moist soil [6, 11, 28]. Cadaver-detection dog use is often more effective in moist soil or humid conditions [29] due to the improved VOC release from soil particles. However, increased soil moisture at the surface level may have caused non-polar hydrocarbons to recede into the soil where water was not accumulated. This is further supported by the level of hydrocarbons detected in the soil at the end of the trial (Day 214, Table 1).

Hydrocarbons represented the largest class of compounds detected as shown in Table 1. Hydrocarbons remained elevated (in comparison to control soil) over the majority of the trial (Fig. 1) and constituted a major

Table 1 Volatile organic compounds of interest identified throughout the study

	Day 0	Day 14	Day 28	Day 55	Day 94	Day 125	Day 214
Sulfur-containing							
Carbon disulfide [12, 39]	✔				✔	✔	
Dimethyl sulfide [3, 11–15, 17, 23, 39]	✔						
Dimethyl disulfide [3, 11, 13–15, 18, 23, 39, 40]	✔	✔	✔	✔			✔
Dimethyl trisulfide [11, 13–15, 17, 18, 23, 30]	✔	✔					
Methanesulfonic anhydride						✔	✔
Methanesulfonyl chloride					✔		
Aromatic							
Benzene [11, 13, 22, 23, 39]	✔	✔					
Benzene, 1,3-dimethyl- [13, 14]	✔	✔			✔		
Benzene, 1-methyl-3-(1-methylethyl)-	✔				✔		
Benzene, ethyl- [11, 13, 39]	✔	✔			✔		
Benzene, propyl- [10, 13]					✔		
Benzaldehyde [3, 10, 15, 16, 18, 22, 23, 30, 39, 41]					✔		
Furan, 2-methyl- [12]	✔						✔
Furan, 2-ethyl- [13, 23]		✔					
Furan, 2-propyl		✔					
Furan, 2-butyl- [23]		✔					
Furan, 2-pentyl- [3, 22, 23, 40]	✔	✔			✔		
Furan, tetrahydro-2-methyl- [23]		✔					
Xylene [3, 11, 13, 14, 39]				✔	✔		
Styrene [11, 12, 14, 23, 30, 39]					✔		
Toluene [3, 11, 13, 30, 39]					✔		
4-isopropyltoluene					✔		
Nitrogen-containing							
Acetamide, 2-cyano-			✔				
Benzonitrile [15, 16, 18, 23, 39]	✔		✔				
Butanenitrile,3-methyl- [18]		✔					
Formamide, N,N-dimethyl- [10]						✔	
Methenamine [11, 12, 23, 39]							✔
Esters							
Acetic acid, methyl ester [23]		✔					
Propanoic acid, methyl ester	✔						
Benzoic acid, methyl ester	✔				✔		
Butanoic acid, 2-methyl-, methyl ester		✔					
Butanoic acid, methyl ester [23]	✔						
Tetradecanoic acid, 1-methylethyl ester	✔						
Tetradecanoic acid, methyl ester							✔
Thiocyanic acid, methyl ester		✔					
Ketone							
2,4-Hexanedione, 5,5-dimethyl-							✔
2-Butanone [10, 12–14, 16, 23]	✔				✔		
2-Butanone, 3-methyl- [10, 16, 23]			✔		✔		
2-Pentanone [10, 13, 16, 23]			✔		✔		
3-Pentanone [10, 13]					✔		
1-phenylethanone [10, 18, 19, 23]			✔		✔		
Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-	✔			✔			

Table 1 continued

	Day 0	Day 14	Day 28	Day 55	Day 94	Day 125	Day 214
Alcohol							
Ethanol [11–15, 23]					✔		
2-Butanol [10, 15, 16, 23]	✔						
1-Pentanol [3, 10, 13, 15, 16, 22, 23, 40]		✔					
2-Pentanol [10, 13, 23]	✔						
2-Penten-1-ol, 2-methyl-	✔				✔		
1-Hexanol [3, 10, 11, 13, 22, 41]		✔					
1-Hexanol, 2-ethyl- [10, 13, 14, 22, 30, 39]			✔		✔		
Phenol [10, 14–16, 18, 19, 23, 30]						✔	✔
Ether							
Propane, 1,1-dimethoxy-	✔						
Propane, 1,1-dimethoxy-2-methyl-	✔						
3-methoxybut-1-ene	✔				✔		
1-methoxy-3-methylbutane	✔		✔				
1-methoxy-3-methylbut-1-ene	✔						
2-Methoxy-1-hexene	✔						
Methoxybenzene [18]	✔						
1,3-Dioxolane			✔				
2-methyl-1,3-Dioxolane		✔					
1,4-Dioxane		✔	✔				
Hydrocarbon							
Butane, 2-methyl- [12]					✔		✔
1-Butene, 3-methyl- [15]			✔				
Pentane [10, 12, 23]	✔	✔	✔				✔
Pentane, 2-methyl- [10, 12, 39]		✔					✔
Pentane, 3-methyl- [10, 12, 13]		✔					
2-Pentene [12]	✔	✔					
Hexane [10–14, 22, 23]		✔	✔		✔		✔
1-Hexene [10, 13, 23]	✔	✔					
Heptane [10, 12–14, 23, 39]	✔	✔		✔	✔		✔
Heptane, 3-methylene-					✔		
1-Heptene [10, 13, 23]	✔	✔		✔	✔		✔
3-Heptene, 3-methyl-				✔	✔		
Octane [12–14, 16, 23]	✔	✔		✔	✔	✔	
Nonane [10, 12, 16]					✔		
Undecane [10–12, 22, 23, 30, 39]					✔		
Tetradecane [10, 30]					✔		
Eicosane [15, 23]						✔	
Pinene [10, 13, 23]				✔	✔		
Cyclopropane, 1,2-dimethyl-			✔				✔
Cyclopropane, 1-ethyl-2-methyl-			✔				
Cyclopropane, ethyl-		✔	✔		✔	✔	✔
Cyclopropane, ethylidene-	✔	✔	✔				✔
1,3,5,7-Cyclooctatetraene	✔						
1,3,5-Cycloheptatriene	✔		✔				
Bicyclo[4.2.0]octa-1,3,5-triene		✔			✔		
Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-		✔					

Presence was determined by a *t* test ($p < 0.05$) comparing each compound in control and experimental samples. References are given where compounds have been previously reported in decomposition odor research

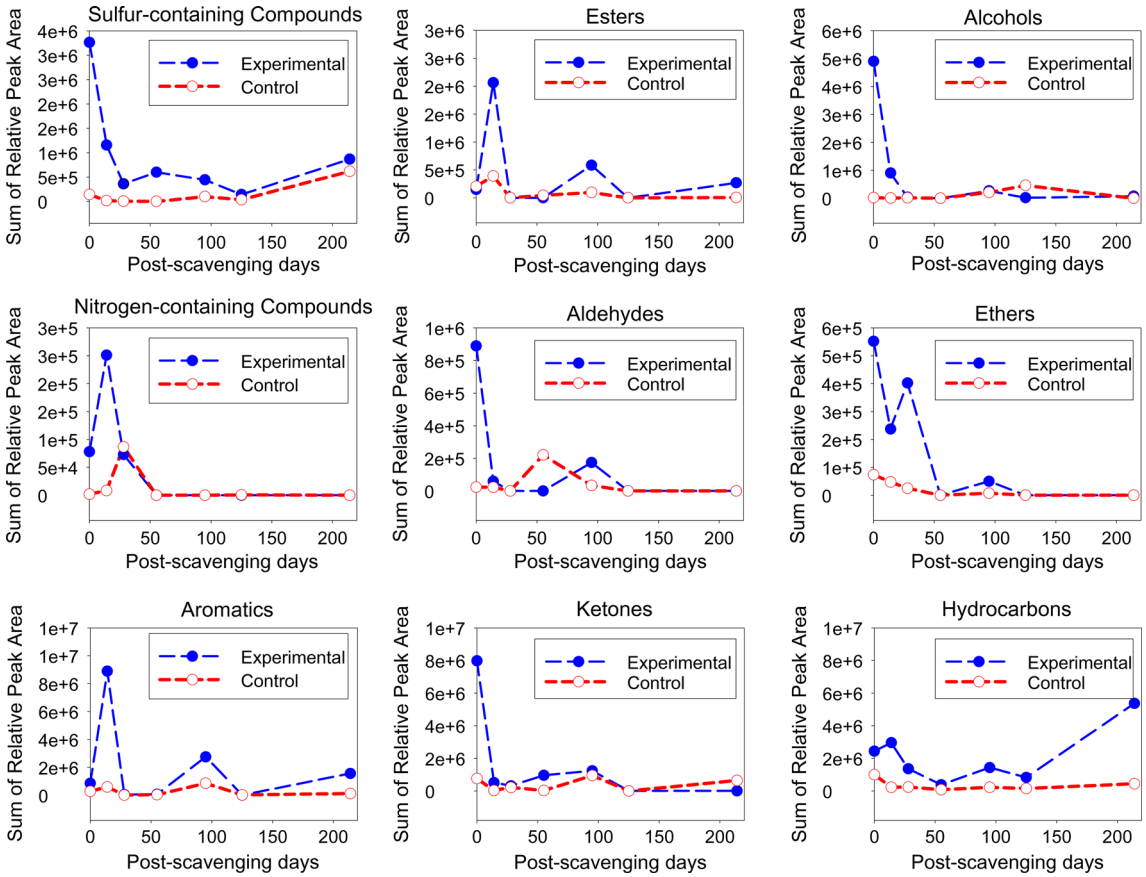

Fig. 1 Volatile organic compound (VOC) class trends in soil after artificial scavenging for 214 days (7 months)

proportion of the VOCs identified at the end of the study. Hydrocarbons have been shown to be a dominant compound class in soils collected from burials [18]. The persistence of these hydrocarbons in soil indicates that they may possibly be involved in cadaver-detection dog alerts in extended post-mortem intervals where other highly volatile VOCs no longer persist. However, there is also the potential that interactive effects of hydrocarbons with other compounds may be responsible for the specific odor to which the cadaver-detection dogs respond. Decomposition VOCs from other compound classes cited in previous literature were identified at the completion of the trial (Table 1, i.e., dimethyl disulfide, 2-methylfuran, methenamine, phenol) yet the relative amount of these compounds constituted a minor component of the overall profile.

A number of esters and ethers were detected in the decomposition VOC signature throughout the study (Table 1). Although many of the esters from this study have not been previously reported, many closely related esters have been previously identified in decomposition soil [23, 26]. It has been hypothesized that the most evident source of esters results from microbial processing of decomposition VOCs in the soil [26]; however, it is also possible that these compounds could be produced during lipid oxidation or other biochemical processes occurring in this soil. It is possible that the longer evaluation period in this study (allowing a longer period of microbial action in soil) produced these structurally-related esters. Ethers are a decomposition by-product of carbohydrate metabolism and although various ethers have been reported in decomposition studies [12, 18, 19, 30], consistent detection of these compounds in literature does not occur. A large number of ethers (structurally related to those identified in Table 1) have been detected in a previous study using GC×GC–TOFMS [19]. It is possible that the use of this instrument provided improved identification of ethers thereby improving the comprehensive profiling of decomposition VOCs. Ethers have also been reported as by-products of bacterial and fungal metabolism [31], soil litter decomposition [32] and from gram-negative soil bacteria [33]. Metabolic pathways resulting in the presence of ethers in decomposition soil are likely a complex interaction of many factors, leading to the inconsistency of identification of these compounds in previous research. Notable peaks in ether detection were observed in Fig. 1 at the beginning of the study (day 0), on day 28 (1 month), and on day 94

(3 months), which further supports this complex biological activity that could potentially be taking place in the soil. There is also the possibility that some of these compounds may be associated with the use of pig remains, as the only studies to identify ethers have not involved the use of human remains in the experimental design.

Carboxylic acids are a decomposition by-product first produced during putrefaction of soft tissues and were not detected in the VOC profile in this study. Other researchers have also experienced difficulty detecting such carboxylic acids in decomposition soil [12, 18]. GC–MS analysis of the carcasses during decomposition showed that carboxylic acids detected in the headspace above a carcass were not detected in soil [23]. These compounds may not exhibit a tendency to sorb to the soil type, or they potentially undergo microbial processing in soil resulting in related compounds such as esters. Carboxylic acids are challenging to detect using gas chromatography, often requiring derivatization prior to their analysis. Hence, carboxylic acids may have been present but not detectable using the GC×GC–TOFMS method.

Aldehydes were also not detected following scavenging, with the exception of benzaldehyde (Table 1). Although some aldehydes were identified in initial data processing (3-methylbutanal, propanal, 2-propenal, and hexanal), they were not found to be significantly higher than corresponding amounts in control soil. Similar aldehydes were present in the decomposition VOC profile prior to scavenging [23], and thus the absence of aldehydes is likely attributed to the scavenging process.

Day 0 marks the point at which the odor source was removed, and therefore this day should have theoretically yielded the highest amount of decomposition VOCs throughout the entire study. This was true for sulfur-containing compounds, aldehydes, ketones, alcohols, and ethers. These classes exhibit secondary smaller peaks in VOC detection but they did not surpass the value for the measured relative peak area on day 0 of the study. However, for nitrogen-containing compounds, aromatics, esters, and hydrocarbons, an increase in relative peak area was measured on day 14 of the trial compared with day 0 (Fig. 1). This finding demonstrates that the persisting VOCs constituting the decomposition odor signature does not simply decrease beyond its fixed initial point over time. The VOCs in the environment must be interacting with the soil (e.g., through biological activity or due to physicochemical properties of the soil). These interactions are further developed in the sections below.

Principal component analysis

PCA allowed visualization of distinctions between the experimental soils and control soils. Within the first 2 weeks following removal of the remains, the experimental soil could only be partially distinguished from control soil on the axis of the first principal component (PC-1) (Fig. 2a). Due to the high axis loading on PC-1 (73 %), this separation of day 0 and day 14 from the control soils indicates that there were only minor differences in the VOC profile of experimental and control soils on these days. By day 125 (4 months) and day 214 (7 months) clear discrimination was demonstrated between experimental and control soil samples (Fig. 2a). Day 125 was clearly separated from control soil samples based on the axis of the second principal component (PC-2) and day 214 was clearly separated from control soil samples based on the axis of the first principal component (PC-1). The loadings plot (Fig. 2b) suggests that aromatics were the most discriminatory class for day 125 followed by a transition to aromatics and hydrocarbons as the most discriminatory classes for day 214. The higher amount of aromatics and hydrocarbons exhibited on days 125 and 214 provides support that these two compound classes may be relevant for cadaver-detection dogs in extended post-mortem interval cases where scavenging has occurred. However, in order to generate further suppositions for the correlation of statistical results with cadaver-detection dog responses, additional studies should confirm these trends by monitoring samples more frequently and for an extended duration. This should also be monitored in combination

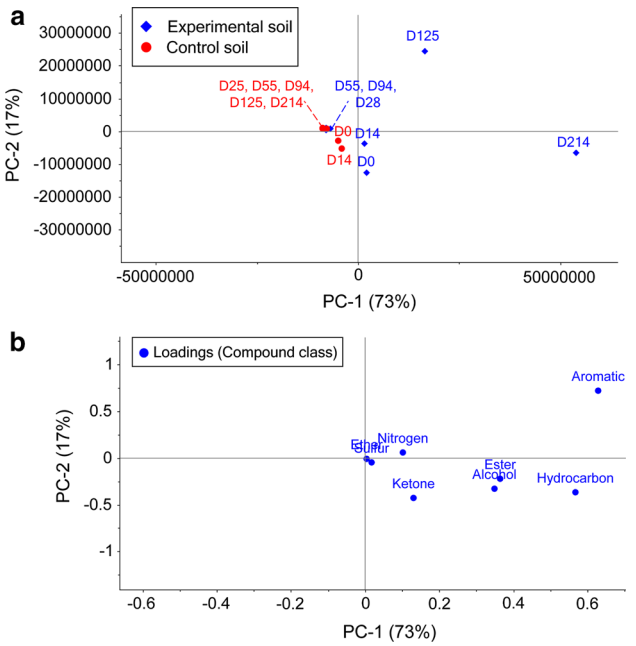

Fig. 2 **a** PCA score plot of average control soil and average experimental soil replicates ($n = 4$) for each day (e.g. D14 = day 14) based on compound class sums. **b** PCA loadings plot of compound classes responsible for discriminating control soil and experimental soil scores

with real-time cadaver-detection dog responses to samples of interest to provide information about the profile's physiological relevance. Esters, alcohols, ketones, and nitrogen-containing compounds were also important for the discrimination of days 125 and 214 but to a lesser extent (Fig. 2b). For soil samples collected at 28 days (1 month), 55 days (2 months), and 94 days (3 months), a decomposition VOC signature could not be differentiated from the control soil samples (as evidenced by clustering of these points in Fig. 2a). A cycling of nutrients in the experimental soil may have caused certain characteristic decomposition VOCs to reduce to concentrations below the instrumental limits of detection.

Cadaver-detection dogs must be capable of distinguishing background odors and distractor odors from their target odor in order to effectively produce positive alerts [1]. PCA allowed the input of a suite of compounds and provided a visual representation of the chemical differentiation between experimental soil and the natural background VOCs associated with the control soil. Whether statistical discrimination correlates with canine alert behavior and whether the persisting compounds are odor-active to canines is still currently unknown. For example, although aromatics and hydrocarbons appeared to be the most persistent and discriminatory decomposition VOC class, it is possible that cadaver-detection dogs may be alerting on a number of trace decomposition VOCs at specific ratios in the residual odor, which may not have been reflected in the statistical analysis performed herein. Cadaver-detection dog trials using experimental soil from this study would provide further information. Comparison could then be made between persisting VOCs and alert behavior.

Persistence of VOCs in soil

Given the fact that cadaver-detection dogs are often effective in extended post-mortem scenarios or where scavenging has occurred, it was anticipated that the decomposition VOC signature would persist in soil for an extended period. The focus of this study was to determine the variation in the VOC profile in soil during the transition from carcass presence to carcass absence. During this transition, there are many soil interactions that contribute to resulting VOC persistence.

Soil physicochemical characteristics impact the concentration, mobility, and retention of VOCs in soil [28]. Particle size and texture of the soil will affect how VOCs penetrate the soil and sorb to soil particles [34–36]. For example, soils with high clay content will have a higher adsorptive capacity than those with a high content of sand or limestone [37]. Larger particles exhibit more surface area resulting in more sites for sorption from soil gas [36]. Soil porosity will affect the movement of gaseous VOCs between particles [37], allowing for VOCs in the vapor phase to diffuse through the soil more easily in soils with high porosity. Soil physicochemical properties were not investigated, yet these characteristics are nonetheless important when relating trends to soils at other geographical locations. Since these characteristics were relatively consistent between the experimental and control sites, their impact was considered to be minimal for establishing longitudinal trends within the study.

Soil moisture affects the dissolution, mobility, and sorption of VOCs differently depending on whether analytes are hydrophilic or hydrophobic. Hydrophilic molecules such as polar analytes will be more effectively dissolved in moist soils thus increasing mobility, while hydrophobic molecules such as hydrocarbons will have reduced mobility through moist soils [28]. An anomaly in the decreasing trends of aromatics, esters, aldehydes, ethers, and hydrocarbons was observed on day 94 (Fig. 1). High rainfall leading up to day 94 resulted in moist soils at the research location which may explain the high number of compounds collected on this day but not on earlier days (i.e., 28 and 55). Soil pH can also affect the retention of VOCs by soil particles, whereby alkaline soils retain VOCs better than acidic soils [38]. Average soil pH throughout the study was measured in the range of 6.00–7.00, however on day 94 the average soil pH at experimental sites was measured at 5.60 compared to the average pH of control soil measured at 7.00. The soil acidity and increased moisture content on this day likely affected the VOC retention causing an increase in the number of compounds detected on this day. This is supported by the corresponding elevation of control VOCs on day 94 in these compound classes (Fig. 1).

The decomposition process is extremely complex and many interacting factors can influence the resulting VOC profile. The persistence of compounds in the soil will be dependent on the initial pulse of decomposition material into the soil which is affected by variables such as carcass mass, initial composition, and decomposition rate. VOC production and retention is also affected by the soil microbial community [28]. Bacterial and fungal metabolism produces volatile by-products and can also transform existing soil VOCs [28]. Nutrient loading in the soil due to decomposition will subsequently have an impact on bacterial and fungal metabolism. It is possible that scavenging may occur prior to sufficient decomposition, resulting in reduced influx of decomposition by-products in soil. Although these factors have an impact on obtained results, the pre-scavenging interval and site setup were chosen to represent a situation that may be encountered in a forensic investigation.

Considerations for future work

The decision to remove the remains after 3 months of decomposition at the deposition site was based on anecdotal information from local police agencies. The study aimed to target scavenging that occurred during late-stage decomposition which often occurs in the study environment, promoting the early onset of mummification. Once the remains were skeletonized, it was expected that the profile complexity would not change considerably as the major decomposition VOCs had already been produced during soft tissue decomposition. A decision to mimic scavenging earlier in the study (i.e., during the bloat or active decay stages) may have prevented the formation of certain key decomposition VOCs, thus changing the profile reported. These circumstances are important to study as it is plausible that animal scavengers will locate the remains during early-stage decomposition in some environments. In such studies, it would be valuable to implement both a positive and negative control which would allow for the comparison of the VOC profile from a scavenged site to both control soil and from a site that had not been scavenged. Further investigation of the persistence of decomposition VOCs in other soil types will also provide more information about the nature of residual decomposition odor.

Due to the large number of trace compounds present in this study, comparison to a single standard may need to be reconsidered in future work. The choice of bromobenzene as the internal standard for this study was based on its use in previous work and therefore facilitated comparison between studies. However, in the future, using several deuterated internal standards from a variety of compound classes would provide an improved representation of VOC behavior in soil. In addition, this also further ensures that the internal standard is not present from surrounding environmental sources.

It is also important to note that this study was limited strictly to the chemical profiling of residual odor. This provides a general basis for residual decomposition VOC profiling, as there are no existing studies that provide this analytical data. Further studies should investigate the physiological implications of the presence of the decomposition VOCs in residual odor for cadaver-detection dog olfaction. The mechanisms behind canine alert behavior are still poorly understood and linking the chemical characteristics of the target odor to the physiological and neurological pathways producing an alert is extremely complex. The ratio of decomposition VOCs in relation to each other and their interactive effects is an important consideration for future work as it can impact the perceived odor available to the canine. Therefore, it is challenging to provide strong conclusions on the implications of residual decomposition VOCs to the canine olfactory process solely based on the chemical data because it is still unknown whether the remaining decomposition VOCs provide a similar scent picture compared to the full decomposition VOC profile originally present. This study aimed to provide valuable chemical reference data for residual decomposition odor that will serve as a foundation for other research areas (e.g., animal physiologists) to interpret the complex mechanisms underlying canine olfaction.

Conclusions

An accurate representation of the decomposition VOC profile from decomposed remains in soil has only recently been investigated. At present, there is a lack of information regarding how this profile can change under complex situations such as scavenging and/or extensive post-mortem intervals. This study investigated the persistence of decomposition VOCs in soil over the duration of 7 months following the removal of the remains from the deposition site in order to identify changes in the VOC profile during this period. The presence of most compound classes was high immediately following removal and became considerably reduced after 6 weeks, with the exception of hydrocarbons which remained elevated throughout the study period. A peak in the number of compounds identified occurred on day 94 which may have been attributed to an increase in soil moisture and decrease in soil pH. PCA identified hydrocarbons and aromatics as the two major compound classes responsible for the lasting decomposition VOC signature at the end of the study. These results provide information on the decomposition VOC signature that remains at a deposition site which may be useful for understanding the need for specialized training of cadaver-detection dogs when locating scavenged remains.

Key points

1. Residual odor profiling was performed on soil from surface decomposition after the removal of remains.
2. Analysis of soil VOC samples from pig decomposition was performed using GC×GC–TOFMS.
3. Residual odor changed in concentration and composition after remains were removed.
4. The chemistry of VOCs in residual decomposition odor provides reference data for animal behavior studies.

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