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Odor mortis☆

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ABSTRACT

This study, the third of a series on the odor signature of human decomposition, reports on the intermittent nature of chemical evolution from decomposing human remains, and focuses primarily on headspace analysis from soil associated with older human remains (10–60+ years) from different environments around the globe. Fifty grams of soil were collected in 40 mL glass vials with polypropylene sealed lids from soil above known or suspected graves and from subsurface chemical plumes associated with human decompositional events. One hundred eighty six separate samples were analyzed using gas chromatography–mass spectrometry (GC–MS). After comparison to relevant soil controls, approximately fifty volatile chemical compounds were identified as being associated with human remains. This manuscript reports these findings and identifies when and where they are most likely to be detected showing an overall decrease in cyclic and halogenated compounds and an increase in aldehydes and alkanes as time progresses. This research identifies the "odor signatures" unique to the decomposition of human remains with projected ramifications on cadaver dog training procedures and in the development of field portable analytical instruments which can be used to locate human remains in shallow burial sites.

1. Introduction

This paper is the third of a multi-part research series which is attempting to identify the volatile chemical components of human decomposition. In Part I [1], the Decompositional Odor Analysis (DOA) Database was established. Four burial sites were established at the University of Tennessee's Anthropological Research Facility with a sampling strategy that allowed for the collection of volatile organic compounds (VOCs) below and above the body. Air samples were also collected at the surface of these sites, which had different burial depths and where burials occurred at different times of the year. Chemical classes were identified, separated into individual compounds, and correlated to the time of their evolution in relation to environmental factors, which illustrated the complexity of human burial decomposition.

In Part II [2], the DOA Database was expanded to define the chemical fingerprint at the surface of burial sites produced by volatile compounds during the decomposition process of human remains over a span of approximately 4 years. This research, which included surface decompositional events of both human and select animal remains, identified over 478 separate compounds, identified which chemicals are primarily associated with aerobic and anaerobic processes, established significant chemical differences between human and animal remains, provided approximations of expected concentrations and time periods of maximum production, and ranked the top thirty most significant chemicals produced during human decomposition.

The primary goal of the current phase of this study was to collect and analyze soil samples (in addition to other matrices) associated with significantly older human decompositional events (10–60+ years) from a variety of different environmental sources to document the cyclic chemical progression that occurs with residual decompositional material (fluids and matrix-adsorbed chemical components of soft tissue decomposition) and bone diagenesis. This work differs from other studies using volatile organic compounds [3–7] by using the entire body (not individual organs which can bias the results), using human remains (pigs, cows, chicken, etc. have significantly different odor signatures), and by studying remains that have been decomposing for a minimum of several years.

In Latin, *mortis* means "of death". When describing a recent death, evaluating the early post-mortem interval or relating the "fresh" stage of decomposition to the corpse [8], oftentimes the *mortis* triad is invoked. This triad is concerned with the onset and eventual dissipation of three aspects of the death process: *rigor mortis* (a temporary stiffening of the musculature primarily due to the failure of sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA)

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umps) [9–11], *algor mortis* (the cooling of the body to ambient temperatures since the metabolic regulation of body core temperature has failed), and *livor mortis* (the settling of blood in dependent areas of the body) [12,13]. What has historically been omitted from death investigations has been the value of *odor mortis* – or the "smell of death". The advantage to the death investigator of expanding the triad into a tetrad is that the 'smell of death' lingers well past the fresh stage of decomposition (>100 years as discussed in this manuscript) and, as the odor changes and migrates, can be a valuable aid to investigators in the areas of post-mortem interval (PMI) determinations [14–33], location of clandestine graves, and verification of decompositional events [34].

The production of chemicals liberated during decompositional events depends on numerous taphonomic factors, many of which are poorly understood [2]. For this reason, it is currently not possible to say with 100% certainty that a particular chemical will be present at a particular time during the decompositional process. The research over the last decade has provided a guideline for establishing which chemicals are produced during human decomposition, their concentration range and when they should be at their maximum concentration. What is not currently known are the physical and biochemical mechanisms (microbial fermentation pathways, catalytic reactions, necessary enzymes, mitigating circumstances, oxygen concentrations, drug interactions, which chemicals adhere or adsorb to what types of matrices, microbial transformation of base compounds, plant uptake of specific chemicals, etc.) that produce them. Without this basic knowledge, statistics cannot currently be applied to these data as a predictor of what chemicals will be present at any given time and involving any given circumstance since every crime scene is unique. In a perfect world, the evolution of the chemicals associated with human decomposition should follow a Gaussian distribution. While it is not known whether these chemicals would, in fact, follow this pattern, the reality of human decomposition odor studies shows us that numerous taphonomic factors alter this pattern. One such factor is barometric pressure – a critical modulator of chemicals detected at the soil surface. When determining what chemicals are liberated at the surface of known human graves, barometric pressure is a key player due to the dynamic nature of soil. High atmospheric pressure will push chemicals down into the soil column (suppressing or eliminating surface detection) whereas low pressure environments will tend to draw these chemicals up to the surface making their detection at the surface possible [34]. This trend could be reversed depending on the soil moisture content. Certain hydrophobic compounds will be pushed downward in the soil column during rain events, potentially reducing their detection at the soil surface (depending on the amount of moisture, their solubility, and their density), regardless of the rise or fall of barometric pressure.

While quite complex, this does not mean that all hope is lost when it comes to documenting, predicting, and establishing the presence of decompositional events, or using odor as a PMI determinant. There are some commonalities to every human decompositional event. The first is that the corpse (along with its resident bacterial population) either is or was there at one time. Also, the peri-mortem chemical composition of soft tissue and bone will be relatively stable. It is possible to get a rough approximation of accumulated degree day (ADD) or burial accumulated degree day (BADD) [2,14,35] values and the environmental conditions (aerobic, anaerobic, temperature, humidity, barometric pressure, soil texture, etc.) can be documented [15,36] regardless of the death scene circumstances. It is also possible to identify and document lateral plume flows in the subsurface. It is known which of these chemicals are water soluble or water insoluble, their density (as a predictor of how these chemicals migrate through the soil column), which compounds adhere to silicates or clay soil particles, and what chemicals are in control samples in the vicinity of the site in question. Some of these parameters have even been included in recent computer modeling of hazardous chemicals in the subsurface as a way of predicting their movement in the soil column [37–41].

While still a challenging endeavor, understanding the chemistry of human decay processes, identification of odor compounds, and knowledge of the required range of sensitivity in shallow burial sites or during surface decomposition, can provide specific data vital for the development of reliable, cost-effective, portable analytical field detection instrumentation capable of locating clandestine burial sites. Additionally, determining the volatile chemical signature emanating from a burial site has projected ramifications on cadaver dog training procedures allowing human remains detection (HRD) canine handlers to begin standardizing and optimizing their training procedures and perhaps even as a foundation for understanding entomological scent attractants.

2. Materials and methods

2.1. Volatile organic chemical (VOC) vials

VOC vials (Precleaned/quality certified), 40 mL borosilicate glass vials with 0.125″ septa were purchased from Environmental Sampling Supply (Houston, TX, USA). Vial diameter is approximately 2.5 cm, vial length is approximately 9.5 cm.

2.2. Triple sorbent traps (TSTs) used to collect VOC samples

A description of TSTs, their use in sampling and analytical procedures, calibration and quantitation are described in great detail in previous manuscripts [1,2]. When TSTs are used, it is advisable to collect a minimum of 20 L of air sample at a relatively slow sampling rate (~100–300 mL/min – ADM 3000 Flowmeter from J & W Scientific®) to avoid degassing a small confined area, but this rate can be increased to ~500 mL/min when sampling surface decompositional events or large areas. If a TST is used to collect an air sample from a hole dug in the soil, it is advisable to place a small air filter (pre-TST) to avoid dust or soil particles from being drawn into the trap. This filter should only be used as a particulate filter (filter paper material) and not as any type of sorbent trap.

2.3. Chemicals

Analytical grade chemicals as standards were purchased from Supelco (Bellefonte, PA, USA). EPA 524 Rev 4 Ketones Mix (47573-4); carbon disulfide (4-8361); KDWR VOC Mix A (5-06575).

2.4. Samples

For this manuscript, 186 soil samples were analyzed over the course of 9 years using soil headspace analysis. These samples (primarily collected from known or suspected gravesites) ranged in age from <1 month to 119 years. Suspected gravesite locations were subsequently confirmed using canines or geophysical instrumentation such as ground-penetrating radar (GPR) or magnetometers. Samples were obtained from forensic cases, cemeteries, and military conflict areas in Europe, the Pacific rim, Canada, Africa, and 14 states in the United States representing desert, deciduous, tropical, marsh/swamp and grassland biomes. Most of the soils collected were associated with burials (1.5–8.1 ft. {0.48–2.47 m} in depth), but also included soils associated with surface decompositional events as well as simple air samples (using TSTs) taken close to corpses (<0.5 m) found decomposing on the surface (with or without clothing or loosely wrapped). While not the specific focus of this manuscript, data encompassing odor obtained from clothing or various other fabrics stained with decompositional fluids were also evaluated and showed results quite similar to what is seen when using TST samples collected above aerobic decompositional events.

2.5. Soil collection procedure

Soil intended for the purpose of locating, identifying or verifying clandestine graves is best placed in 40 mL pre-cleaned glass volatile organic analysis (VOC) vials fitted with caps and a polypropylene-lined septum (see Fig. 1). The polypropylene-lined septum provides a convenient means of using a syringe to withdraw a few milliliters (mLs) of headspace from the vial for analysis without removing the cap. Through the use of cryofocusing techniques it is possible to extract and concentrate the chemical vapor components from the headspace before injection onto the column of the gas chromatograph. The gas chromatograph then separates the individual constituents in a complex mixture and they are identified one at a time using the mass spectrometer [1,2]. This process can also be used for quantification if required, but for clandestine grave detection, these analyses are usually only used

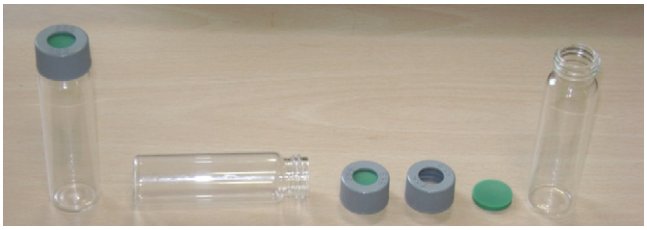

Fig. 1. Typical volatile organic compound (VOC) sampling vials with caps which allow for a gas sample to be withdrawn from the headspace of the vial without removing the cap. These vials should be filled approximately ¾ full with soil, mud, water, vegetation, etc.
Courtesy: Dr. Laurance Donnelly, Forensic Geologist, Wardell Armstrong LLP, England.

to determine the presence or absence of specific chemicals and their relative abundance.

Soil collection depends on the type of environment that is being sampled, but generally consists of utilizing either soil cores or probes (e.g. 21″ stainless steel soil probe with soil ejector, Forestry Suppliers Inc., Jackson, MS, USA), or manually scooping the soil into the VOC vials. When using soil cores, soil is collected with a pre-cleaned soil coring device which should be rinsed with clean distilled water after every sampling event (fabrics or other non-soil matrices should be placed directly into the sampling vials). Since every sampling area is different, it is difficult to stipulate the depth at which to collect the samples, but typically the corer is pushed into the soil to the greatest possible depth (which varies with the soil texture), withdrawn, and then rapidly segregated after the core is removed from the ground (while still intact in the coring devise) into top, middle, and bottom sections which correspond roughly to between 2–6 in. (5.1–15.2 cm), 8–12 in. (20.3–30.5 cm) and 14–18 in. (35.6–45.7 cm), respectively. Historically, the best results are obtained if the middle sample is collected at a depth of approximately 8–12 in. (20.3–30.5 cm). Soil obtained from each selected section is then placed directly into VOC vials so that they are approximately ¾ full and quickly sealed with the vial caps and properly labeled with the time, date, segment, location, name, etc.

Depending on the type of soil, the moisture content, associated debris, and degree of compaction, this is roughly 50 g of collected soil material. Speed is critical since as soon as the core samples are withdrawn from the soil, they begin to de-gas and vital chemicals signatures could be lost if they remain in the open air for lengthy periods of time. If coring is not possible, small shovels, trowels, or similar digging tools can be used to dig down approximately 8 in. (20.3 cm) into the soil column. If the soil is loose enough, the VOC vial itself can be used to directly scoop the soil into the tube, or the trowel can be used to fill the vial up to ¾ full.

This must be done rapidly to minimize degassing of the soil sample since very light VOCs can be lost using this procedure. If this procedure is used, it is important to be sure that the lip of the vial is free of debris (so the cap seals tightly) and also that the soil sample has not come in contact with skin (it is advisable to wear gloves during this procedure), sweat, or any other external contaminants. For this manuscript, both techniques (segregated soil cores and manual collection methods) were utilized. For most of the 186 samples collected for this study, law enforcement agencies, crime scene investigators, or other researchers performed the collection and sent us the collected soil samples.

It is also critical to collect control samples from the area for comparative purposes (at the same depth as the test samples). These should be collected from areas devoid of obvious surface contamination, but in areas with similar soil composition as the suspect area and, if possible, parallel to established groundwater flows.

2.6. Transport of collected soils

During transport of the vials back to the laboratory it is recommended that the samples be cooled (placed on ice or refrigerated), if possible, to minimize changes that may occur due to bacteria, fungi, or other microorganisms in the samples. If this is not possible, the samples should be refrigerated or frozen once they arrive at the laboratory if not analyzed immediately upon receipt (which is recommended).

2.7. VOC vial heating and sampling

Soil samples (if stored refrigerated or frozen) were brought to room temperature and then the 40 mL VOC vials were placed in a close-fitting aluminum sleeve equipped with a Chromel–Alumel thermocouple and a 120 V 25 W cartridge heater. The sleevecovers about ¾ of the vial. If the experiment was particularly temperature sensitive, the remaining portion of the vial was wrapped in an insulating material such as fiberglass for better temperature homogeneity. The temperature was regulated by a 1/32 DIN CAL 3300 heater controller manufactured by CAL Controls, using a PID control algorithm. The temperature was maintained at 60 °C for routine soil headspace analysis, but should be set at 35 °C for samples which have a very high water content so that expanding water vapor does not rupture the vials. Samples should be heated for at least 30 min. After this time period, 2.0 mL of headspace was withdrawn from the vial by piercing the vial septum (the cap is not removed) using a glass syringe (while the vial is still in the heating block), and this volume of headspace was directly injected through the septum into the injection port of the GC for analysis using cryofocusing techniques, described below, with the helium flow and the GC oven turned off and the cryoloop already immersed in liquid nitrogen.

2.8. Instrumentation

Sample analyses during this study were conducted on a Hewlett-Packard (HP – Agilent) 5890/5972 GC–MS (Table 1). The GC was equipped with a Restek Crossbond® Rtx®-1PONA column (100 m, 0.25 mm ID, 0.5 μm d_f) for the analytical separations.

During cryofocusing, the column flow and oven heater were turned off. These were reestablished after the sample had been injected. The cryofocusing technique used a short loop (between 5 and 6 in long [12.7 and 15.24 cm]) of 1/16 in (1.6 mm) OD stainless steel tubing (0.030 in ID [0.76 mm]) connected to a low dead volume 1/16 in (1.6 mm) stainless steel "tee" connector. The "tee" outlet was either to an atmospheric vent (during injection) or when this was closed, to the analytical column. The cryofocusing loop was immersed in liquid nitrogen prior to injection and during flow stabilization.

2.9. Data reduction

GC–MS data were transferred to a satellite PC for processing. The HPCHEM software (Hewlett-Packard G1701BA Version B.01.00) was used to calculate the peaks and areas using the quantitation database generated from aggregate data reviews. After identification and quantitation was complete, these data were reviewed manually using the QEdit Quant Result feature of the HPCHEM software. In this review, individual compounds were identified and the total ion chromatogram (TIC) quantitated manually. Unknowns with a peak height of greater than 5000 were also reviewed and listed as unidentified if no identification was possible from the software database. When the QEdit review was complete, the results were saved, and both a file and screen summary report were generated. The screen report was saved as a text file, which was then imported into Microsoft Excel®.

3. Results and discussion

The previous two papers in this series concentrated primarily on VOC analysis of human remains, buried and on the surface [1,2], using triple sorbent traps (TSTs) during the first 4–5 years of decomposition. This manuscript differs from those studies by extending the time period of the decompositional event to many decades and also by using soil headspace analysis in addition to TSTs. Soil headspace analysis has distinct advantages over TST analyses when dealing with the identification and location of unknown gravesites and mapping subsurface chemical plumes. Soil collection and analysis is much faster, easier and simpler than dealing with TSTs. Mapping the collected soil sample is much more accurate and allows presentation of the collected sample to cadaver locating canines and field instrumentation. Negative aspects to the collected soil samples include possible shipping (United States Department of Agriculture permits) and disposal

Table 1
Operating conditions for the GC/MS used for *odor mortis* analyses.

Initial temperature	30 °C
Initial hold time	7.0 min
Initial rate	10 °C/min
Second temperature	45 °C
Second hold time	5.0 min
Second rate	1.5 °C/min
Third temperature	70 °C
Third hold time	5 min
Third rate	3 °C/min
Final temperature	250 °C
Final hold time	5 min
Injector temperature	250 °C
Detector temperature	280 °C
Scanned from	35–550 *m*/*z*
Helium flow	1 mL/min

Table 2
List of *odor mortis* compounds and pertinent taphonomic variables deemed important in the location and interpretation of human remains.

Compounds	Most common location of compound in soils	Approximate time interval in years when compound is most commonl y detected	Decomposition phase when compound is most likely to be present	Most favorable s oil conditions for compound detection[e]	Most favorable soil texture for compound detection	Most favorable formation environment	Compound is a component of identified skeletal material	Comments/ob servations
1,1,2-Trichloro-1,2,2-trifluoroethane[a]	Corpse/plume	0–1.5	Early	Dry or moist	Clay/loamy	Aerobic/ anaerobic	Human/dog	Primarily corpse associated as gravesite ages
1,1-Dichloro-1-fluoroethane[b]	Corpse	0–0.8	Early	Dry or moist	Clay/loamy	Anaerobic		
1,2 Benzenedicarboxylic acid, diethyl ester	Corpse/plume	0–2.5	Early	Dry or moist	Clay/loamy	Aerobic/ anaerobic	Human/deer	
1,2-Dichloroethene[a]	Plume	10–20+	Late	Dry or moist	Sand/silt/loamy	Aerobic/ anaerobic		
1,2-Dimethyl benzene	Corpse/plume	0–10 (60+)	Anytime	Dry	Sand/clay/loamy	Aerobic/ anaerobic		If dry, usually seen late in decomp
1,4-Dimethyl benzene	Corpse/plume	0–8 (60+)	Anytime	Dry	Sand/clay/loamy	Aerobic/ anaerobic	Human/dog	If dry, usually seen late in decomp
1-Ethyl, 2-methyl benzene	Plume	0–2.5	Early	Dry or moist	Clay/loamy	Aerobic/ anaerobic	Deer	
1-Methoxypropyl benzene	Plume	0–1	Early	Dry or moist	Clay/Loamy	Anaerobic		
2-Butanone (methyl ethyl ketone – MEK)	Corpse	0–4 (20+)	Anytime	Dry	Sand/loamy	Anaerobic	Human/deer	Also detected in cemetery settings
2-Methyl butanal	Corpse/plume	10–40+	Late	Dry or moist	Sand/silt/peat	Anaerobic	Human/dog/deer	
2-Methyl butane	Corpse/plume	2–3 (20+)	Late	Dry or moist	Sand	Aerobic/ anaerobic		
2-Methyl furan[b,c]	Corpse	0–2 (10+)	Late	Dry or moist	Sand/silt	Anaerobic	Deer	
2-Methyl pentane/3-methyl pentane	Corpse/plume	1–4 (20+)	Late	Dry or moist	Sand/peat	Aerobic/ anaerobic		
2-Methyl propanal	Corpse	10–40+	Late	Dry or moist	Sand/silt/peat	Anaerobic		
2-Methyl propene	Plume	20+	Late	Moist	Silt/peat	Anaerobic		
2-Pentene	Plume	30+	Late	Dry or moist	Sand	Aerobic/ anaerobic		
3-Methyl butanal	Corpse/plume	10–40+	Late	Dry or moist	Sand/silt/peat	Anaerobic	Human/deer	Higher than 2-Me butanal in human remains
Acetone (2-propanone)	Corpse/plume	0.5–40+	Late	Dry or moist	Sand/silt/peat	Aerobic/ anaerobic	Human/pig/sheep	
Benzene[c]	Corpse/plume	0–4 (10+)	Anytime	Dry or moist	Clay/loamy	Aerobic/ anaerobic	Human/dog/deer	Primarily corpse associated as gravesite ages
Benzenemethanol-alpha, alpha, dimethyl	Corpse/plume	0–2	Early	Dry or moist	Clay/loamy	Aerobic /anaerobic	Human/dog	
Butanal	Plume	10+	Late	Dry	Sand/clay	Aerobic	Human/pig/deer	
Carbon disulfide[a]	Corpse/plume	0–3 (10+)	Anytime	Dry or moist	Sand/clay/loamy	Aerobic /anaerobic		
Carbon oxide sulfide (COS)[a]	Corpse/plume	0–2 (10+)	Late	Dry or moist	Sand	Anaerobic		
Carbon tetrachloride[a]	Plume	0–3	Early	Dry or moist	Clay/loamy	Aerobic/ anaerobic	Human	
Chloroform[a]	Corpse/plume	0–4 (10+)	Early	Dry or moist	Sand/clay/loamy	Anaerobic	Dog	If dry, usually seen late in decomp
Decanal	Corpse/plume	0–15+	Anytime	Dry or moist	Clay/loamy	Aerobic/ anaerobic	Human/pig/deer/dog	Primarily corpse associated as gravesite ages
Decane	Corpse	0–1 (20+)	Late	Dry or moist	Peat/loamy	Anaerobic	Human	

Table 2 (*Continued*)

Compounds	Most common location of compound in soils	Approximate time interval in years when compound is most commonl y detected	Decomposition phase when compound is most likely to be present	Most favorable s oil conditions for compound detection[e]	Most favorable soil texture for compound detection	Most favorable formation environment	Compound is a component of identified skeletal material	Comments/ob servations
Dichlorodifluoromethane[b]	Corpse/plume	0–1 (10+)	Mid	Dry or moist	Clay/loamy	Aerobic/ anaerobic	Deer/dog	Primarily corpse associated as gravesite ages
Dichlorotetrafluoroethane[b]	Corpse/plume	0–1.5	Early	Dry or moist	Clay/loamy	Anaerobic		
Dimethyl disulfide[a]	Corpse	0–3 (10+)	Mid	Dry or moist	Sand/ clay/loamy	Aerobic/anaerobic		Primarily corpse associated as gravesite ages
Dimethyl sulfide	Corpse/plume	20+	Late	Dry or moist	Sand	Aerobic/anaerobic		
Dimethyl trisulfide[a,d]	Corpse/plume	0–1.5	Early	Dry or moist	Clay/loamy	Aerobic/anaerobic		Primarily corpse associated as gravesite ages
Ethanal (acetaldehyde)	Plume	7–20+	Late	Dry or moist	Sand	Anaerobic	Pig	
Ethanol	Corpse/plume	10–40+	Late	Dry or moist	Sand/peat	Anaerobic	Pig	
Ethyl benzene	Corpse/plume	0–2 (10+)	Mid	Dry or moist	Clay/loamy	Aerobic/anaerobic	Human/dog	Primarily corpse associated as gravesite ages
Heptanal	Corpse/plume	20–40+	Late	Moist	Peat	Aerobic/anaerobic	Human/pig/deer/dog	
Heptane	Corpse/plume	1–4 (20+)	Late	Dry or moist	Sand/peat	Aerobic/anaerobic	Human/dog/deer	
Hexadecanoic acid, methyl ester	Corpse/plume	0–2	Early	Dry or moist	Clay/loamy	Anaerobic		
Hexanal	Plume	2–10+	Anytime	Dry or moist	Sand/clay	Aerobic/anaerobic	Human/pig/deer/dog	
Hexane	Corpse/plume	0–40+	Anytime	Dry or moist	Sand/peat/ clay/loamy	Aerobic/anaerobic	Human/deer	
Methane, thiobis[a]	Plume	10–40+	Late	Dry or moist	Sand/peat	Aerobic/anaerobic		
Methenamine[a]	Corpse/plume	0–1.5	Early	Dry or moist	Clay/loamy	Aerobic/anaerobic	Dog	
Naphthalene[a]	Plume	0–2	Early	Dry or moist	Clay/loamy	Anaerobic	Dog	
Nonanal	Corpse/plume	0–20+	Anytime	Dry or moist	Clay/loamy	Aerobic/anaerobic	Human/pig/deer/dog	Primarily corpse associated as gravesite ages
Nonane	Corpse	1–4 (20+)	Late	Moist	Peat	Anaerobic	Human/deer	
Octanal	Plume	15+	Late	Dry	Sand/clay	Aerobic/anaerobic	Human/pig/deer	
Octane	Corpse	1–40+	Late	Dry or moist	Sand/peat	Anaerobic	Human/dog/deer	If dry, usually seen late in decomp
Pentanal	Plume	2–40+	Anytime	Moist	Peat	Aerobic/anaerobic	Human/pig/deer/dog	
Pentane	Corpse	1–40+	Late	Dry or moist	Silt/peat	Anaerobic	Human	If dry, usually seen late in decomp
Styrene	Corpse/plume	0–2	Early	Dry or moist	Clay/ loamy	Anaerobic		
Sulfur dioxide[a]	Corpse/plume	0–3	Early	Dry or moist	Clay /loamy	Anaerobic		
Tetrachloroethene[a]	Corpse/plume	0–5 (~10)	Early	Dry or moist	Sand/clay/loamy	Anaerobic	Deer/dog	
Toluene (methyl benzene)[b]	Corpse/plume	0–100+	Late	Dry or moist	Sand/silt/clay/loamy	Aerobic/anaerobic	Human/dog/deer	Identified in cremain samples
Trichloroethylene[a]	Plume	0–1	Early	Dry or moist	Clay/loamy	Anaerobic		
Trichloromonofluoromethane[b]	Corpse/plume	0–12+	Mid	Dry or moist	Sand/clay/loamy	Anaerobic	Deer/dog	
Undecane	Corpse/plume	0–4	Early	Dry or moist	Clay/loamy	Aerobic/anaerobic	Human	

[a] Density > 1.0.
[b] Not seen in children <4 years old ($n = 3$).
[c] Additional substituted forms also seen (e.g. 1,4-dichlorobenzene).
[d] Dimethyl tetrasulfide also seen early during human decomposition.
[e] Dry soil is defined as soil with <50% moisture content (by weight), moist soil is soil with >50% moisture content.

issues as well as the fact that only a fraction of headspace is analyzed (making the detection of compounds at trace levels difficult) compared to a usually large volume of air (liters) collected with TSTs.

The results of VOC analysis of soil headspace associated with human decompositional events are presented in Table 2. During the course of this research, 56 chemicals have been shown to be consistently associated with decompositional events. Interestingly the appearance or detection of these chemicals is heavily dependent on the environment, or rather the taphonomy, associated with the human remains. It is also critical to note that the characteristics of the compound (solubility, density, molecular weight, etc.) have a profound impact on whether the compound is detected in the chemical plume (surface or subsurface) or only in close association with the corpse. Some of the taphonomic variables studied include the moisture content of the soil, the type of soil (soil texture) and whether or not the decompositional event was anaerobic (typically a deeper burial) or aerobic (typically found on the surface, a very shallow burial or loosely wrapped). For the purposes of this manuscript, as determined by the predominant Genera of microorganisms associated with a decompositional event (manuscript in preparation) and the hydrological component of these soil textures [36], an anaerobic environment is created during a decompositional event if: (1) the remains are buried a minimum of 2 ft. (0.61 m) deep in predominately clay textured soils, 2.5 ft. (0.76 m) in peat or silty textured soil, and 3.5 ft. (1.07 m) deep in sand or loamy soils; or (2) the remains are wrapped or placed in an airtight container or matrix. Aerobic is defined as a surface or near-surface decompositional event – loosely wrapped in clothing above ground, in a non-airtight container above ground or loosely covered (not compacted) with any matrix less than 1 ft. (0.3 m) deep. Other scenarios not mentioned are, for the purposes of this manuscript, considered between an aerobic and anaerobic environment. Submerged remains are not discussed in this manuscript.

Soil was segregated into broad categories for ease of identification for the layman into clay, sand, peat, silt and loamy soil textures. For soil to be considered one of these textures, more than half of the composition of the soil sample must fall into that category. Soils, for the purposes of this manuscript, were considered 'moist' if the moisture content was greater than 50% of the total weight. This determination is easily accomplished by weighing a small amount of the soil sample before and after placement in a drying oven (100 °C) for 24 h [2]. Additionally, Table 2 also provides the readers with an indication of when the compounds are most commonly detected (years since death and phase of decomposition). Note that stages are not used in the description of *odor mortis* evolution, but phases. The early phase encompasses approximately 0–3 years, mid 5–20 and late 40+.

During analysis of the data, samples were grouped together according to whether they were from plumes or directly over a corpse, sample depth, the age of the source material, soil moisture, and soil texture (columns in Table 2). Compounds detected in each grouping were then compared. This allowed us to formulate the most favorable perceived environment allowing for the detection of a particular compound.

As previously reported in this series, decomposition is cyclic (with an intermittent release of VOCs) and rarely does one see a constant evolution of odor chemicals being produced. For this reason, time frames listed in Table 2 are not always continuous, but represent the periods during decomposition when the chemicals are most apparent given the additional listed favorable circumstances. Even though environmental conditions can change on an hourly basis, there are conditions that affect the chances of detecting trace quantities of chemical vapors in soil found significant in human decompositional events. These include: barometric pressure, temperature, soil texture, soil moisture, air humidity, rainfall, and wind speed [34].

Table 2 also indicates which of the 56 compounds are found in skeletal material of humans and select animals. This does not mean that ethanol, for example, is only detected during the decomposition of pigs. This simply indicates that if a bone, devoid of tissue, is found and analyzed as previously described [2], then ethanol has only been detected in pig bone and not in deer bone or dog bone, etc. Ethanol, while not detected in human skeletal material, is still an important compound produced by microorganisms during the decomposition of human soft tissue and can be found in soil and other matrices and persists for many decades. These data were included in Table 2 as a possible means of identifying skeletal elements (in the absence of tissue) using the concept of *odor mortis*.

Several important observations become apparent when studying Table 2. Early decomposition appears to liberate many of the fluorinated halogen compounds, the substituted benzene compounds and large amounts of very specific sulfur compounds (e.g. sulfur dioxide). These will either stay associated with the corpse (or in the general vicinity) or migrate away from the corpse in a plume depending on a host of factors which, at present, are poorly understood. As time progresses, the substituted benzene compounds become simpler in composition, more complex sulfur compounds are detected and one begins to see the appearance of aldehydes and ketones. Interestingly, as one approaches the late phase, a noticeable increase in aldehydes becomes apparent with significant increases in compounds such as butanal, decanal, heptanal, nonanal and octanal (among others). Also straight chain hydrocarbons (alkanes) become more prevalent (hexane, decane, nonane, octane, etc.) as do the appearance of furans. Depending on the taphonomy (and negative control comparisons), there is a subset of compounds that appear to be consistent and commonly associated with most decompositional events. These include xylenes, 2-butanone, acetone, dimethyl disulfide, hexane, pentane, toluene, chloroform and carbon disulfide. When comparing human decompositional events with animal subsets (pig, deer, dog, cat, squirrel and sheep), carbon tetrachloride, pentane, decane and undecane appear to be human specific, the latter two only when evaluating skeletonized remains. Additionally, it was noted that when animal carcass VOCs are analyzed, 2-methyl butanal is always greater than 3-methyl butanal (this trend is also seen in animal fecal samples). This is reversed (or equal – within 10% in one instance) in human remains and is potentially a key marker to determine if the remains are human or not, especially in older gravesites. Some compounds, such as benzene, some fluorinated halogens, important sulfur compounds (dimethyl disulfide, dimethyl trisulfide), and a few aldehydes, tend to concentrate near the corpse rather than in the plume as the gravesite ages. Others, such as the xylenes (e.g. 1, 2 dimethyl benzene), chloroform and straight chain compounds (alkanes) such as pentane and octane, tend to predominate late in decomposition, but only when the conditions are predominately dry. Fig. 2 illustrates these concepts and represents the soil headspace analysis of an 11 year old shallow grave containing skeletonized human remains. The grave was 1.8 ft. (0.55 m) deep in loose (not compacted) sand. This represents an aerobic/anaerobic environment (since the remains were discovered between a depth of 1–4 ft. in sand); a mid-phase decompositional event since it was 11 years old (mid phase has a range of 5–20 years); and is considered dry since the sand was well drained and had much less than 50% moisture content. While not all peaks are labeled for visual clarity, note that the decomposition compounds xylene, 2-butanone (MEK), acetone, dimethyl disulfide, hexane, pentane, toluene, chloroform, and carbon disulfide are indeed present. Also note that 3-methyl butanal is much greater than 2-methyl butanal indicating (confirming) the presence of human remains. Carbon tetrachloride, undecane,

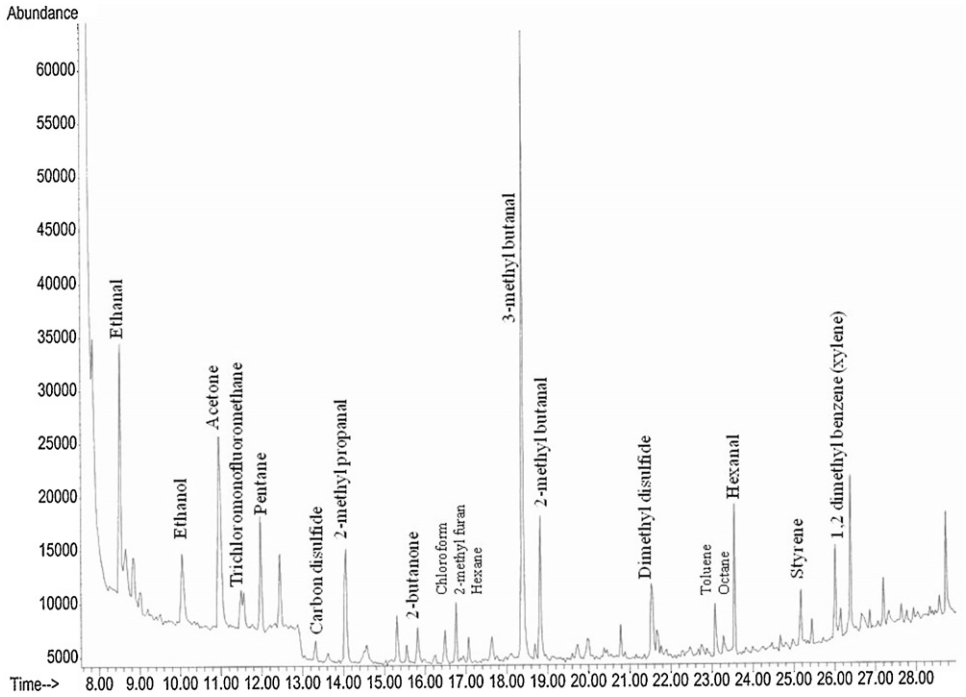

Fig. 2. GC/MS chromatogram showing the soil headspace analysis collected from an 11 year-old shallow grave in sandy soil indicating relevant compounds commonly seen using *odor mortis* as a means of locating human clandestine graves.

and decane were not identified in this chromatogram. This potentially indicates that the 11 year time period is not favorable for detection of these compounds and neither is the soil texture. It is interesting to note that styrene was detected in this sample even though the conditions are not considered favorable for its detection (since favorable conditions for styrene include early decomposition, clay/loamy soils and anaerobic conditions), but does illustrate the need for additional research in this area attempting to understand all the taphonomic variables important in chemical liberation.

4. Conclusions

Odor mortis is an important component of the death process, acting both as an attractant (insects, rodents, reptiles, mammals) and as a repulsant (meat spoilage). The study of *odor mortis* has just begun, but it has many potential benefits, especially in the forensic community. These include:

- Confirmation of *decompositional events*.
- Potential use for *post-mortem interval estimates*.
- Potentially confirming the *presence of human remains*.
- Potentially *distinguish human from animal remains* and other environmental sources.
- Identification of the shape, location, and *point source of odor plumes*.
- Aiding in cadaver dog training/verification.
- Development of detection instrumentation.

Significant additional research must be performed in order to fully understand the source of these compounds and how they are modified (or created) by bacteria, fungi and other microorganisms, especially in the soil column as they migrate not only upwards to the soil surface, but also with the lateral plume flow.

Currently it is not yet possible to accurately predict which compounds will be present at any given decompositional event since the mechanisms of compound formation and the taphonomic influences are not yet fully understood. In terms of searching for clandestine graves, one must consider the compounds that appear predominantly near/at the corpse as opposed to those present in the plume. It is highly advisable to consult with a forensic geologist when sampling soils from a suspect area to determine groundwater flow and land slope effects. Rarely do the compounds identified in Table 2 migrate uphill from a gravesite. They can be drawn short distances contrary to groundwater movement by the root systems of plants, but it is always advisable to collect sufficient soil samples to properly map the area under investigation. Once the overall environment, soil texture, moisture content, control sample composition, and groundwater flow have been established, a determination of whether the *odor mortis* signature is human or not should be undertaken using the guidelines previously described. One must also determine, based on the environment and control samples, which compounds could or should be present (Table 2). Once this list has been established, it is our current 'rule of thumb' that if the list of remaining compounds match at a level of approximately 50% or higher, then there is a strong indication of a decompositional event in the vicinity of the sampling. We have seen plumes in the subsurface from a human decompositional event migrate over 800 m (2625 ft.), so a well thought-out collection/mapping strategy is crucial to the identification of possible clandestine gravesites or confirmation of decompositional events.

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