Forensic Chemistry 37 (2024) 100542

Contents lists available at ScienceDirect

Forensic Chemistry

journal homepage: www.sciencedirect.com/journal/forensic-chemistry

On the styx bank - characterization of the headspace cadaveric volatiles released by submerged decaying rats

Clément Martin [1], Marta Malevic [1], François Verheggen [*]

TERRA, Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés 2, 5030 Gembloux, Belgium

ARTICLE INFO

Keywords:
Cadaveric volatilome
Submerged remains
Headspace sampling
Forensic science

ABSTRACT

The cadaveric volatilome of terrestrial decomposition, including buried corpses, has been extensively studied in recent taphonomic research. However, there has been comparatively less attention given to the volatile organic compounds associated with submerged vertebrate remains. This decaying process is distinct, as evidenced by the succession of decay stages that significantly differ from terrestrial decomposition. Indeed, five stages can be delineated: fresh, early floating, floating decay, deterioration, and sunken remains. Due to the unique nature of underwater decomposition, we anticipate the release of different cadaveric volatiles from submerged remains. In this study, we characterize the volatile compounds emitted during underwater decomposition and that reach the surface. Rat cadavers were placed individually in glass chambers filled with water. The volatiles released at the surface were subsequently collected three times per week over the course of a month. Two types of water, fresh and marine, were used to assess the potential influence of the salinity level on the cadaveric volatilome. A total of 33 volatile compounds were identified, with the majority having previously been reported in the headspace of cadavers undergoing decomposition in a terrestrial environment. Among these compounds, those containing sulfur were the most abundant, with dimethyl disulfide being the major one. Our findings did not reveal any discernible impact of salinity levels on the volatile profile, which was, however, affected by the specific decaying stage. Notably, 3-methyl-indole emerged as a promising candidate for distinguishing between the first two stages of decomposition and the subsequent third stage.

Introduction

For years, the characterization of volatile cadaveric compounds has been performed on both human and surrogate models (*e.g., pig*). Despite this extensive interest, research has primarily concentrated on terrestrial decomposition, encompassing both above or below-ground conditions [1–5]. Comparatively less attention has been directed towards the volatiles associated with submerged vertebrate remains [6,7]. The mechanisms governing the decomposition of submerged remains are unique to this environment [6–8]. The succession of stages (as illustrated in Table 1) differs from terrestrial decomposition, as do the associated necrophagous insects that hasten the decomposition process in terrestrial environments [7,9–12]. Furthermore, the absence of oxygen and the consistent water temperature induce a slower decomposition process, favoring anaerobic decomposition [6,13,14]. Among the many other factors that can impact the decomposition of immerged bodies: flora and fauna, temperature, water chemical composition, water flow and the presence of clothes [12].

To date, the consensus has been that the cadaveric volatilome consists of a multitude of molecules spanning nearly all chemical classes [1,16]. These compounds belong to alkanes, alkenes, sulfur compounds, nitrogen compounds, aromatic compounds, aldehydes and ketones [2,16–18]. Among the more frequently identified compounds released by decaying remains are dimethyl-disulfide and dimethyl-trisulfide [18]. These compounds are associated with the decomposition of sulfur containing amino acids (methionine and cysteine) [19], and mediate the host finding behavior of necrophagous insects [20]. In terrestrial environments, the different decaying stages are known to release specific compounds [17]. For instance, the bloat stage is associated with a stronger odor, compared to fresh stage [1,18,21]. The bloat stage is typically made of sulfur compounds, methane and ammonia. In addition, the cadaveric volatilome differs depending on the species of the carrion [22]. This high variability in addition to the high number of VOCs associated with the decomposition make the characterization of a

* Corresponding author.
E-mail address: fverheggen@uliege.be (F. Verheggen).
[1] Authors contributed equally to the work.

https://doi.org/10.1016/j.forc.2023.100542
Received 1 September 2023; Received in revised form 6 November 2023; Accepted 2 December 2023
Available online 6 December 2023

Table 1
Differences between decomposition stages of surface, buried and immerged corpses [15].

Decaying stages	Above/below ground decaying	Under water decaying
Fresh	No evident signs of decomposition	
Bloat / Early float	Inflation of carcass	Breaching water-line
Active / floating decay	Deflation of carcass	Inflation of carcass
Advanced decay / deterioration	Drying of soft tissue	Deflation of carcass
Skeletonisation / Sunken remains	Dried tissue	Sinking

cadaveric VOCs profile difficult to perform.

Despite an extensive literature on airborne cadaveric volatile organic compounds (VOCs), VOCs released by submerged bodies have been subject to minimal investigation. A better understanding of (i) the composition a the cadaveric profile of submerged bodies, and (ii) the characterization of the partition of the VOCs between the solution and the gas phase, would help to improve detection dogs' performance. The later are known to be able to detected cadaveric VOCs above the water surface [23]. Only two other publications were found in the literature [23,24]. Both studies explored the cadaveric volatilome of submerged and non-submerged remains, revealing twice the number of VOCs in open-air decomposition [23]. Among these, dimethyl disulfide contributes significantly to the overall profile abundance, whereas the profile exhibits greater diversity during terrestrial decomposition [24]. In both studies, solid-phase-microextraction (SPME) was used to characterize the cadaveric VOCs released during the decomposition. Although SPME is an economical and fast sampling technique, it does not facilitate quantitative analyses of the outcomes, and neither of these SPME studies performed quantitative analyses of the identified compounds.

The objective of the present work was to characterize the cadaveric VOCs released during the decomposition of submerged rat remains by using dynamic headspace sampling coupled with thermal desorption and gas chromatography. Moreover, we assessed the impact of water salinity on the release of cadaveric VOCs.

Material and methods

Biological material

We were provided with 8 dead laboratory male rats (*Rattus Norvegicus*, 3–5 months old, 266.5 ± 10.4 g) by the faculty of veterinary medicine of the University of Liège (Belgium). None were killed for this specific experiment (Ethics agreement n°18-2021); they had been used in a previous assay (which could not be communicated to the authors of the present research). Rats remains did not have any apparent damages. They all came from the same experiment batch, had been euthanized and kept frozen for about ten weeks prior to our experiment.

Rat decomposition setup

Three 15 L open glass tanks were filled to the top with soft water (collected in a water source in Sugny, Belgium); and three other tanks were filled with sea water (collected at Middelkerke beach, Belgium). Four rats were defrosted in a hot water bath set at 40 °C. Two of them were individually introduced into two tanks containing soft water, the other two were individually introduced inside two tanks containing sea water. Two additional control tanks were filled with soft and sea water, respectively. All glass tanks were placed in a room set at 14 °C, and the rats were left undisturbed to decompose for one month. A net was placed on the top of the glass tanks to prevent insect presence on the remains. This experiment was then replicated one month later using the remaining four rats (n = 4 per water salinity). The duration of each stage of decomposition was recorded during the month of experiment. Stages were assigned based on Payne and King's (1972) [15].

Odor collection

One hour prior to sampling, a glass lid was positioned to seal the tanks, allowing for equilibration of the VOCs present in the headspace. This lid featured multiple openings, which remained closed throughout this equilibration period. Then, one of the openings was connected to the hydrophobic inert-coated dual sorbent thermal desorption tube containing a Tenax® TA/Carbograph^{TM} B (Markes International Ltd, Llantristant, UK), connected to an air pump (GilAir1 plus pump, Sensidyne1, St. Petersburg, Florida, USA). Another opening was utilized to house a charcoal tube, serving to filter incoming air. All other openings were securely sealed. The pump was adjusted to operate at 200 ± 3 mL/min and the sampling lasted for one hour. After sampling, the tube was sealed and stored at 4 °C prior to analysis. Seven microliters of a 5 ng/µl solution of chlorobenzene (Sigma Aldrich®) were spiked in the sampling tubes to serve as internal standard (35 ng).

Gas chromatography analyses

The separation of the sampled compounds was performed on a gas chromatograph (QP 2020 NX, Shimadzu, Kyoto, Japan). Prior the separation, the compounds were desorbed at 280 °C for eight minutes with a helium flow set at 20 mL/min in a TD-30R Thermal desorption unit (Shimadzu, Kyoto, Japan). Subsequently, these compounds were cryofocused in a cooling trap (d = 3.2 mm, id = 2 mm, L = 102 mm) filled with Tenax TA (60 mg, 60–80 mesh) set at −20 °C by Peltier effect. The trap was then fast heated to 280 °C to inject the compounds through the transfer line in the GC column at a linear velocity mode at 45.7 kPa. The carrier gas was helium (purity 99.999 %, AirLiquide, Namur, Belgium). The flow rate was set at 0.94 mL/min. Employing a split ratio of 20, the compounds were carried out on a HP-5MS column (30 m x 0.25 mm x 0.5 µm; Agilent technology, Santa Clara, California, USA). The temperature program started at 40 °C, a temperature held for two minutes. The temperature was then incrementally raised to 90 °C at a rate of 2 °C/min, and then to 300 °C at 10 °C/min. This final temperature was held for three minutes. Chemicals were detected on a quadrupole mass spectrometer (QP 2020 NX, Shimadzu, Kyoto, Japan) set at 1.1 kV. The ion source temperature was set at 200 °C. All *m/z* ratios ranging from 30 to 500 were acquired each 5 scans/s. The detected compounds were identified based on the interpretation of their mass spectra and by comparison with spectral libraries (NIST version 2017 and FFNSC version 3). Identification was confirmed by calculating the retention index. The compounds detected in the control were removed from the data as well as the compounds which are not considered as VOCs (as defined by EU directive 2004/42/CE). While the mean profile was generated, a single ion monitoring method (SIM) was applied for each individual compound within each chromatogram, enabling their quantification at trace levels.

Statistics

Statistical analyses were performed using R version 3.4.2. (R Foundation for statistical computing). To compare the duration of each decaying stage under the different water conditions, a generalized linear mixed model (GLMM) was performed. The comparison of the volatile profiles was conducted with a permutational multivariate analyses of variance (i.e., perMANOVA) using a Euclidean distance matrix and 999 permutations ("adonis" command, R-package vegan, (Oksanen et al., 2020)). This allowed to assess the potential impact of the decaying stage, the type of water and the interaction of these factors. As the experiment was split into two blocks (March and April), the month of the experiment was placed in stratum using the ''strata'' function in adonis. It allowed to perform intra-month permutation in the data frame instead of inter-

month permutations. Additionally, this inclusion was accounted for in the model, thereby enabling the incorporation of the random factor during residue calculation. In order to mitigate the potential inflation of Type I errors due to multiple testing, p-values were adjusted using Bonferroni's correction. When a *p*-value was significant, pairwise comparisons were performed also with a Bonferroni's adjustment. Differences are illustrated with a linear discriminant analysis (LDA) used for pattern recognition between groups. This method allowed to determine which of the independent variables contributed the most to the differences in the average score profiles of the different conditions of immerged decaying rats. A generalized linear mixed model (GLMM) was used on variables to highlight candidate VOCs driving the difference when observed.

Results

Decomposition

In both sea and soft water, all rat cadavers went through 3 stages of decomposition, except for two rats from the first batch which did not reach the floating stage (Fig. 1). Consequently, the floating stage duration was excluded from comparison with the first two stages. The water salinity level had no impact on the decaying process duration: sea water = 9.7 ± 0.6 days; soft water = 9.7 ± 2.0 days (Z = -0.96, *p*-value = 0.34). However, the salinity level impacted the duration of the first stage (*p*-value = 0.026) but not the second one (*p*-value = 0.77). Indeed, rats remained in the fresh stage for a longer period when placed in soft water as opposed to sea water (Fig. 1).

Volatile collection

In the present study, a total of 33 cadaveric VOCs were identified in the headspace of both soft and sea water. Eight chemical classes were represented (Table 2). The class most prominently represented is that of sulfur-containing compounds. Remarkably, three compounds accounted for half of the entire cadaveric volatilome released throughout the entire process in both water types: dimethyl disulfide (DMDS), nonane and toluene. DMDS alone constituted over 30 % of the cadaveric VOC profile. The majority of these compounds have been previously associated with the decomposition of vertebrate remains (Table 2). Specifically, only two compounds were exclusively released in sea water conditions: dimethyl sulfoxide and 2,4-diathiapentane. All other compounds were released in at least one of the three investigated decomposition stages for each water type.

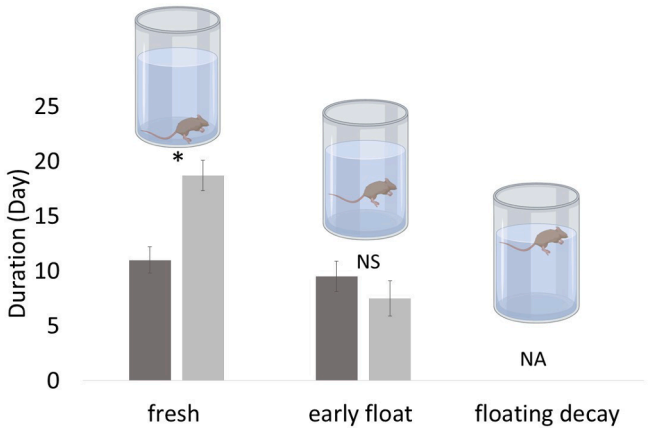

Fig. 1. Stage duration in days in two water salinity conditions (dark grey: sea water; light grey: soft water) for each stage of decomposition, except for the floating decay stage, as we stopped the experiment at this last stage. (NS: no significant; NA: not applicable).

Decomposition stages and water salinity

Water salinity did not impact the cadaveric volatilome released at the water surface ($F_{1,95} = 2.39$; *p*-value = 0.124). However, a pattern of VOCs emerged, corresponding to each stage of decomposition ($F_{2,95} = 13.65$; *p*-value = 0.001). No differences were identified when investigating the interactions between water salinity and decaying stage ($F_{2,95} = 0.39$; *p*-value = 0.717). The LDA was therefore only performed by considering the decaying stage (Fig. 2). Regarding the influence of the decomposition stage, differences were observed between the fresh and the floating decay stages (*p*-value = 0.003) and between the early floating and the floating decay stages (*p*-value = 0.012). However, no differences could be demonstrated between the fresh and early floating stages (*p*-value = 0.129).

Starting by the effect of the stage, the first two discriminant functions accounted for 100 % of the observed variability (LD1: 73.6 %, LD2: 26.4 %). LD1 was mainly correlated with terphenyl-2-ol, tetramethylbutyl disulfide, 6,10-dimethylundecadien-2-one, skatole, phenylmaleic anhydride (supp Table S1). Upon conducting a GLMM on these factors to compare the decaying stages, all highlighted significative differences (p-value $_{\text{6,10-dimethyl-5,9-undecadien-2-one}} = 0.036$, p-value $_{\text{terphenyl-2-ol}} = 0.048$, p-value $_{\text{tetramethylbutyl disulfide}} = 0.162$, p-value $_{\text{skatole}} = 0.002$, *p*-value $_{\text{phenylmaleic anhydride}} < 0.001$). LD2 was correlated with tetramethylbutyl disulfide, 2,4-dithiapentane, dimethyl sulfoxide, 6,10-dimethyl-5,9-undecadien-2-one, 2-hexanone. Upon performing a GLMM concerning the stage of decomposition, only tetramethylbutyl disulfide and dimethyl sulfoxide were not significantly different across them (p-value $_{\text{tetramethylbutyl disulfide}} = 0.162$, p-value $_{\text{2,4-dithiapentane}} = 0.007$, p-value $_{\text{dimethyl sulfoxide}} = 0.058$, p-value $_{\text{6,10-dimethyl-5,9-undecadien-2-one}} = 0.036$, *p*-value $_{\text{2-hexanone}} = 0.01$).

Discussion

Our findings confirm previous observations of a deceleration in the decaying process when a corpse is submerged, as compared to the more conventional terrestrial decomposition [7]. Intriguingly, during our experiment, the fresh stage duration extended even beyond that which has already been documented [7]. The presence of a net prevented the colonization of insects, known to hasten the decay process [33]. While the carcass is fully submerged during the fresh stage, aquatic insect larvae typically feed on the remains [7]. The duration of the early floating stage was close to the one already reported in the literature during the same year period. Notably, salinity levels did not yield differences in terms of decomposition duration. While data on marine submerged cadavers remain limited, it is evident that the decomposition follows distinct patterns in fresh and marine waters [34]. These differences appear unrelated to water salinity levels, but rather arise from variations in environmental conditions inherent to freshwater and marine settings (*e.g.*, sediment, scavengers, depth, water movement) [34]. Finally, rats remains used for the present study were frozen after death and kept for 10 weeks which could have an impact on the decaying process [3,35]. Indeed, frozen remains are known to produce a more complex cadaveric volatilome than fresh remains [3]. Moreover, freezing process could impact the fatty acid analytes presence in the VOCs profile [35].

More than 30 volatile compounds were identified in the cadaveric decay of submerged rats under the two salinity conditions. Two-third of these compounds had been previously documented in both surface and subterranean decomposition processes [2,5,10,17,25,26,28,29,31]. Of particular note, sulfur-containing compounds were the most abundant, contributing to one-third of the total VOC profile. These compounds play a significant role in decomposition, with dimethyl disulfide (DMDS) being one of the most frequently cited among them [1,17,36,37]. They are by-products of sulfur-containing amino acids (*e.g.*, cysteine, methionine) undergoing desulfhydralation [38]. This high diversity of sulfur containing compounds was already reported in a study dedicated to

Table 2
List of volatile organic compounds released by rat remains decaying either in soft or sea water, according to the three decomposition stages.

	Soft water			Sea water			References related to terrestrial decomposition odour	References related to aquatic decomposition odour
	fresh	early floating	floating decay	fresh	early floating	floating decay		
sulfur compounds								
dimethyl disulfide	X	X	X	X	X	X	[2,5,10,17,25–29]	[23,30]
dimethyl trisulfide	X	X	X	X	X	X	[2,5,10,17,25,26,28,29,31]	[23,30]
dimethyl tetrasulfide	X			X	X	X	[10,31]	[23]
dimethyl sulfoxide				X	X	X	[10,28]	
methyl ethyl disulfide		X	X	X	X	X	[10,28]	
methyl butanethioate	X		X	X	X	X		
methyl (methylthio)methyl disulfide			X	X		X	[28]	
bis(1,1,3,3-tetramethylbutyl) disulfide	X	X	X	X	X	X	[31]	
dihydro-2(3H)-thiophenone	X	X	X	X	X	X		
2,4-dithiapentane				X	X	X	[5,25,28]	
1,1-bis(methylthio)ethane	X	X	X	X	X	X		
hexathiane			X	X	X	X		
aldehydes								
decanal	X	X	X	X	X	X	[10,26,27,29,32]	
benzaldehyde	X	X	X	X	X	X	[5,10,17,27,29]	[23]
cyclic compounds								
toluene	X	X	X	X	X	X	[3,10,27,29]	[23]
styrene	X	X	X	X	X	X	[3,29]	
3-carene		X	X	X	X	X		
7-methylbicyclo[4.4.1] undeca-2.4.8-triene	X	X	X	X	X	X		
phenylmaleic anhydride	X	X	X	X	X			
ketones								
3-hexanone	X	X	X	X	X	X	[3]	
2-hexanone	X	X	X	X	X	X	[5]	
acetophenone	X	X	X	X	X	X	[10,29]	[23]
6.10-dimethyl-5.9-undecadien-2-one	X	X	X	X	X	X		
2-butyl-1.1.3-trimethylcyclohexane	X	X	X	X	X	X		
alkanes								
nonane	X	X	X	X	X	X	[10,26,32]	
dodecane	X	X	X	X	X		[3,27,32]	
tridecane	X	X	X	X	X	X	[3,10,25]	
tetradecane	X	X	X	X	X	X	[10]	[23]
carboxilic acids								
benzoic acid	X	X	X	X	X	X	[17,25,29]	
pentanoic acid	X	X	X	X	X	X	[17,25,27–29]	[23]
alcohols								
2-ethyl-1-hexanol	X	X	X	X	X		[3,27]	
[1.1′:3′.1′′-Terphenyl]-2′-ol	X	X		X	X			
nitrogen compounds								
3-methyl-indole		X	X		X	X	[32]	[23]

adipocere [13], a substance known to be formed under moist conditions [39]. A similar array of compounds was highlighted by Irish et al. (2019) albeit with a greater diversity of nitrogen containing compounds and a lesser diversity of sulfur containing ones [23]. They also identified more molecules probably because they reached the advanced deterioration stage.

As during terrestrial decomposition, the cadaveric volatilome of submerged remains varies across stages of decay [17]. Notably, the floating decay stage was the only one that exhibited a significant difference. However, a trend towards differentiation can be discerned in the LDA concerning the distinction between fresh and early floating stages. Detecting differences might be more challenging as the remains stay submerged during these stages [7]. Clear differentiation becomes evident when the remains fully breach the water's surface. These disparities are shaped by the compounds that compose the primary axes of the LDA. Statistical analyses focused on compounds most correlated with these axes highlighted the role of skatol, phenylmaleic anhydride and 6,10-dimethyl-5,9-undecadien-2-one. While the latter two have not previously been reported as cadaveric VOCs, they could potentially be contaminants. Yet, 3-methyl-indole is known to originate from the breakdown of amino acids, such as phenylalanine, tryptophan and tyrozine [40].

Conclusion

Future research endeavours should delve into the realm of cadaveric VOCs released during aquatic decomposition, thus paving the way for the creation of an olfactory library specific to this distinct decay process. Although our study successfully prevented insect colonization of the corpses, investigating their impact remains a valuable avenue, given their pivotal role in terrestrial decomposition [33]. Additionally, we recommend investigating the volatile compounds that remain dissolved in the water, rather than being released at the water surface. Finally, we propose that future researchers work on human surrogates to bring more information about aquatic decomposition and associated volatilome, which could directly be applied on specific field (*e.g.*, human remains detection dogs training).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

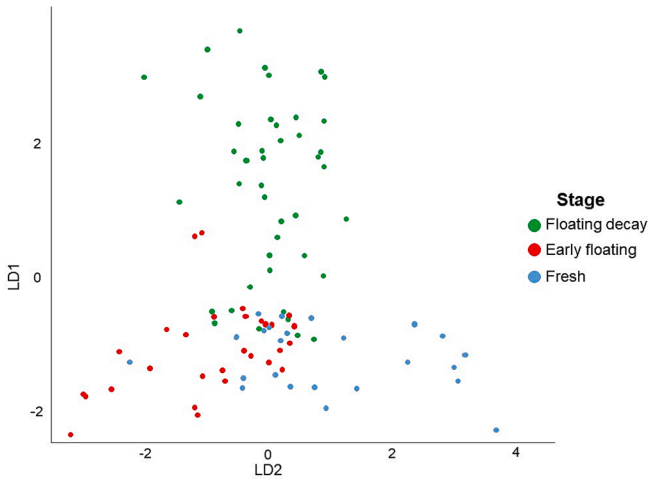

Fig. 2. Linear discriminative analyses (LDA). Visualization of the VOCs profile during the decaying process of immerged rats remains, depending on the decomposition stage. Each sampling is represented in a two-dimensional plan constituted by the two first axes of the LDA.

the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.forc.2023.100542.

References

[1] F.J. Verheggen, K.A. Perrault, R. Caparros Megido, L. Dubois, F. Francis, E. Haubruge, S.L. Forbes, J.-F. Focant, P.-P.-H. Stefanuto, The odor of death : an overview of current knowledge on characterization and applications, Bioscience. 67 (2017) 600–613, https://doi.org/10.1093/biosci/bix046.

[2] E. Rosier, S. Loix, W. Develter, W. Van de Voorde, J. Tytgat, E. Cuypers, Time-dependent VOC-profile of decomposed human and animal remains in laboratory environment, Forensic Sci. Int. 266 (2016) 164–169, https://doi.org/10.1016/j.forsciint.2016.05.035.

[3] S.L. Forbes, L.T. Rust, K. Trebilcock, K.A. Perrault, L.T. McGrath, Effect of age and storage conditions on the volatile organic compound profile of blood, Forensic Sci. Med. Pathol. 10 (2014) 570–582, https://doi.org/10.1007/s12024-014-9610-3.

[4] L. Dubois, P.H. Stefanuto, K.A. Perrault, G. Delporte, P. Delvenne, J.-F. Focant, Comprehensive Approach for Monitoring Human Tissue Degradation, Chromatographia. (2019), https://doi.org/10.1007/s10337-019-03710-3.

[5] K.A. Perrault, B.H. Stuart, S.L. Forbes, A Longitudinal Study of Decomposition Odour in Soil Using Sorbent Tubes and Solid Phase Microextraction, Chromatography. 1 (2014) 120–140, https://doi.org/10.3390/chromatography1030120.

[6] J. Haefner, J. Wallace, R. Merritt, Pig Decomposition in Lotic Aquatic Systems: The Potential Use of Algal Growth in Establishing a Postmortem Submersion Interval (PMSI), J. Forensic Sci. 49 (2004) 1–7, https://doi.org/10.1520/JFS2003283.

[7] J. Dalal, S. Sharma, T. Bhardwaj, S.K. Dhattarwal, K. Verma, Seasonal study of the decomposition pattern and insects on a submerged pig cadaver, J. Forensic Leg. Med. 74 (2020), 102023, https://doi.org/10.1016/j.jflm.2020.102023.

[8] V. Heaton, A. Lagden, C. Moffatt, T. Simmons, Predicting the postmortem submersion interval for human remains recovered from U.K. waterways, J. Forensic Sci. 55 (2010) 302–307, https://doi.org/10.1111/j.1556-4029.2009.01291.x.

[9] J.K. Tomberlin, H.P. Adler, Seasonal Colonization and Decomposition of Rat Carrion in Water and on Land in an Open Field in South Carolina, J. Med. Entomol. 5 (1998) 704–709.

[10] C. Martin, M. Vanderplanck, A. Boullis, E. Haubruge, F. Verheggen, Impact of necrophagous insects on the emission of volatile organic compounds released during the decaying process, Entomol. Gen. 39 (2019) 19–31, https://doi.org/10.1127/entomologia/2019/0663.

[11] J. Amendt, R. Krettek, R. Zehner, Forensic entomology, Naturwissenschaften. 91 (2004) 51–65, https://doi.org/10.1007/s00114-003-0493-5.

[12] B.H. Stuart, M. Ueland, Decomposition in Aquatic Environments, Taphon, Hum. Remain. Forensic Anal. Dead Depos. Environ. (2017) 235–250, https://doi.org/10.1002/9781118953358.ch17.

[13] L. Dubois, P.-H.-P. Stefanuto, L. Heudt, J.-F.-J. Focant, K.A. Perrault, Characterizing decomposition odor from soil and adipocere samples at a death scene using HS-SPME-GC×GC-HRTOFMS, Forensic Chem. 8 (2018) 11–20, https://doi.org/10.1016/j.forc.2018.01.001.

[14] J.B. Keiper, D.A. Casamatta, Benthic organisms as forensic indicators, 10.2307/1468325. 20 (2015) 311–324. 10.2307/1468325.

[15] J. Payne, E. King, Insect succession and decomposition of pig carcasses in water, J. Georg. Etomol. Soc. (1972) 153–162.

[16] J. Dekeirsschieter, P.H. Stefanuto, C. Brasseur, E. Haubruge, J.-F. Focant, Enhanced characterization of the smell of death by comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GCxGC-TOFMS), PLoS One. 7 (2012), https://doi.org/10.1371/journal.pone.0039005.

[17] J. Dekeirsschieter, F.J. Verheggen, M. Gohy, F. Hubrecht, L. Bourguignon, G. Lognay, E. Haubruge, Cadaveric volatile organic compounds released by decaying pig carcasses (Sus domesticus L.) in different biotopes, Forensic Sci. Int. 189 (2009) 46–53, https://doi.org/10.1016/j.forsciint.2009.03.034.

[18] C. Martin, F. Verheggen, Odour profile of human corpses: A review, Forensic Chem. 10 (2018) 27–36, https://doi.org/10.1016/j.forc.2018.07.002.

[19] M. Statheropoulos, A. Agapiou, E. Zorba, K. Mikedi, S. Karma, G.C. Pallis, C. Eliopoulos, C. Spiliopoulou, Combined chemical and optical methods for monitoring the early decay stages of surrogate human models, Forensic Sci. Int. 210 (2011) 154–163, https://doi.org/10.1016/j.forsciint.2011.02.023.

[20] W. Liu, M. Longnecker, A.M. Tarone, J.K. Tomberlin, Responses of Lucilia sericata (Diptera: Calliphoridae) to compounds from microbial decomposition of larval resources, Anim. Behav. 115 (2016) 217–225, https://doi.org/10.1016/j.anbehav.2016.03.022.

[21] C. Martin, F. Verheggen, All equal in the face of death! – Characterization of the volatile cadaveric compounds of fresh stage human corpses, Forensic Chem. 35 (2023), https://doi.org/10.1016/j.forc.2023.100516.

[22] E. Rosier, S. Loix, W. Develter, W. Van De Voorde, J. Tytgat, E. Cuypers, The search for a volatile human specific marker in the decomposition process, PLoS One. 10 (2015) 1–15, https://doi.org/10.1371/journal.pone.0137341.

[23] L. Irish, S.R. Rennie, G.M.B. Parkes, A. Williams, Identification of decomposition volatile organic compounds from surface-deposited and submerged porcine remains, Sci. Justice. 59 (2019) 503–515, https://doi.org/10.1016/j.scijus.2019.03.007.

[24] N.I. Caraballo, B. Sc, K.G. Furton, D. Ph, An Investigation into the Volatile Organic Compounds Released from Submerged Remains, (2016).

[25] S.L. Forbes, K.A. Perrault, Decomposition odour profiling in the air and soil surrounding vertebrate carrion, PLoS One. 9 (2014) 21–22, https://doi.org/10.1371/journal.pone.0095107.

[26] A.A. Vass, Odor mortis, Forensic Sci. Int. 222 (2012) 234–241, https://doi.org/10.1016/j.forsciint.2012.06.006.

[27] M.E. Cablk, E.E. Szelagowski, J.C. Sagebiel, Characterization of the volatile organic compounds present in the headspace of decomposing animal remains, and compared with human remains, Forensic Sci. Int. 220 (2012) 118–125, https://doi.org/10.1016/j.forsciint.2012.02.007.

[28] P. Armstrong, K.D. Nizio, K.A. Perrault, S.L. Forbes, Establishing the volatile profile of pig carcasses as analogues for human decomposition during the early postmortem period, Heliyon. 2 (2016) 1–24. 10.1016/j.heliyon.2016.e00070.

[29] K.A. Perrault, P.-H. Stefanuto, B.H. Stuart, T. Rai, J.-F. Focant, S.L. Forbes, Reducing variation in decomposition odour profiling using comprehensive two-dimensional gas chromatography, J. Sep. Sci. 38 (2015) 73–80, https://doi.org/10.1002/jssc.201400935.

[30] M. Malevic, F. Verheggen, C. Martin, Potential of direct immersion solid-phase microextraction to characterize dissolved volatile organic compounds released by submerged decaying rat cadavers, Forensic Chem. 34 (2023) 8–11, https://doi.org/10.1016/j.forc.2023.100488.

[31] P.-H. Stefanuto, K.A. Perrault, S. Grabherr, V. Varlet, J.-F. Focant, Postmortem internal gas reservoir monitoring using GC×GC-HRTOF-MS, Separations. 3 (2016) 24–37, https://doi.org/10.3390/separations3030024.

[32] K.D. Nizio, M. Ueland, B.H. Stuart, S.L. Forbes, The analysis of textiles associated with decomposing remains as a natural training aid for cadaver-detection dogs, Forensic Chem. 5 (2017) 33–45, https://doi.org/10.1016/j.forc.2017.06.002.

[33] V. Bugelli, M. Gherardi, M. Focardi, V. Pinchi, S. Vanin, C. Pietro Campobasso, Decomposition pattern and insect colonization in two cases of suicide by hanging, Forensic Sci. Res. 3 (2018) 94–102, https://doi.org/10.1080/20961790.2017.1418622.

[34] G.S. Anderson, N.R. Hobischak, Decomposition of carrion in the marine environment in British Columbia, Canada, Int. J. Legal Med. 118 (2004) 206–209, https://doi.org/10.1007/s00414-004-0447-2.

[35] M. Ueland, S. Collins, L. Maestrini, S.L. Forbes, S. Luong, Fresh vs. frozen human decomposition – a preliminary investigation of lipid degradation products as biomarkers of post-mortem interval, Forensic Chem. 24 (2021), 100335, https://doi.org/10.1016/j.forc.2021.100335.

[36] A.A. Vass, R.R. Smith, C. V. Thompson, M.N. Burnett, D.A. Wolf, J.A. Synstelien, N. Dulgerian, B.A. Eckenrode, N. Dulgerian, Decompositional odor analysis database, J. Forensic Sci. 49 (2004) 760–769. JFS2003434.

[37] B.B. Dent, S.L. Forbes, B.H. Stuart, Review of human decomposition processes in soil, Environ. Geol. 45 (2004) 576–585, https://doi.org/10.1007/s00254-003-0913-z.

[38] R.C. Janaway, S.L.S. Percival, A.S.A.S. Wilson, icrobiology and Aging, Helicobacter. (2009) 275–289, https://doi.org/10.1007/978-1-59745-327-1.
[39] R.J. Moses, Experimental adipocere formation: implications for adipocere formation on buried bone, J. Forensic Sci. 57 (2012) 589–595, https://doi.org/10.1111/j.1556-4029.2011.02032.x.
[40] S. Paczkowski, S. Schutz, Post-mortem volatiles of vertebrate tissue, Appl. Microbiol. Biotechnol. 91 (2011) 917–935, https://doi.org/10.1007/s00253-011-3417-x.