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Manuscript Details

Manuscript number	FORC_2017_31
Title	The analysis of textiles associated with decomposing remains as a natural training aid for cadaver-detection dogs
Short title	Analysis of textiles associated with decomposing remains
Article type	Full Length Article

Abstract

Cadaver-detection dogs are employed by law enforcement agencies to locate human remains. The ability of cadaver-detection dogs to locate human remains relies heavily on the use of effective training aids. Cadaver-detection dogs may be trained using a variety of materials ranging from natural scent sources to synthetic materials. Natural scent sources are typically considered to be the most effective training aids; however, there is concern that using individual tissue types as natural training aids may not be indicative of the scent of an intact human cadaver. The objective of this work was to determine how well textiles associated with decomposing remains retain and mimic the odour of natural training aids. To test this, the chemical odour profile of textile samples collected from decomposing porcine remains that were buried clothed in 100% cotton t-shirts was examined. Throughout various stages of decomposition, the pig carcasses were exhumed and cotton samples were obtained. The volatile organic compound (VOC) profile of the textiles was collected using headspace solid phase microextraction (HS-SPME) and analysed using comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS). This study provides evidence that textiles associated with decomposing remains may represent a useful natural training aid with a VOC profile reflective of a large subset of cadaveric decomposition odour. The odour profile is dynamic and changes over time suggesting that obtaining textiles from different postmortem intervals would be useful for providing training aids that represent the full spectrum of decomposition odour that cadaver-detection dogs may encounter during a search.

Keywords	Forensic taphonomy; Buried remains; Textiles; Cadaver-detection dogs; GC×GC-TOFMS
Manuscript category	Technology Readiness Level 1
Corresponding Author	Katie Nizio
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Suggested reviewers	Chris Tipple, Robert Janaway, Laurny DeGreeff, Mary Cablk

Submission Files Included in this PDF

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Cover Letter.pdf [Cover Letter]

Conflict of Interest Declaration.pdf [Conflict of Interest]

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Manuscript.docx [Manuscript File]

Fig1.tiff [Figure]

Fig 2.tiff [Figure]

Fig 3.tiff [Figure]

Fig 4.tiff [Figure]

Fig 5.tiff [Figure]

Highlights.docx [Highlights]

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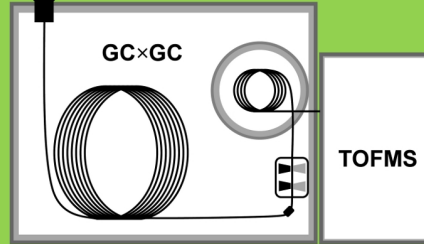


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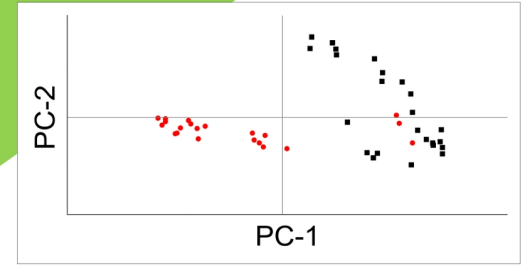
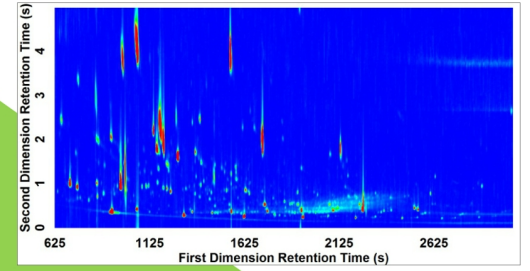
SPME



SPME



Cotton Sample



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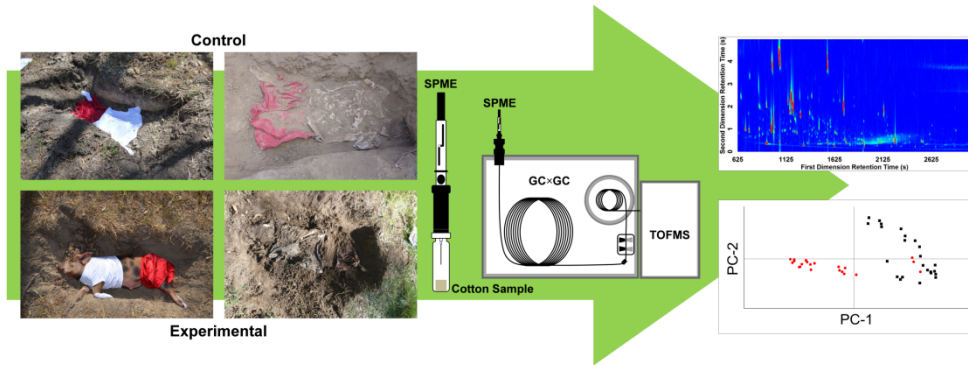
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62 **Abstract**
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65 Cadaver-detection dogs are employed by law enforcement agencies to locate human remains in cases
66 of missing persons, suspected homicides and following natural or man-made disasters. The ability of
67 cadaver-detection dogs to locate human remains relies heavily on the use of effective and reliable
68 training aids. Cadaver-detection dogs may be trained using a variety of materials ranging from natural
69 scent sources (e.g. flesh, bone, blood or decomposition soil) to synthetic materials (e.g. Pseudo™
70 Scents). Commercially available synthetic scents often have an overly simplistic chemical
71 composition that is inconsistent with decomposition odour. Therefore, natural scent sources are
72 typically considered to be the most effective training aids; however, there is concern that using
73 individual tissue types as natural training aids may not be indicative of the scent of an intact human
74 cadaver. The objective of this work was to determine how well textiles associated with decomposing
75 remains retain and mimic the odour of natural training aids. To test this, the chemical odour profile of
76 textile samples collected from decomposing porcine remains that were buried clothed in 100% cotton
77 t-shirts was examined. Throughout various stages of decomposition, the pig carcasses were exhumed
78 and cotton samples were obtained. The volatile organic compound (VOC) profile of the textiles was
79 collected using headspace solid phase microextraction (HS-SPME) and analysed using comprehensive
80 two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS). This
81 study provides evidence that textiles associated with decomposing remains may represent a useful
82 natural training aid with a VOC profile reflective of a large subset of cadaveric decomposition odour.
83 The odour profile is dynamic and changes over time suggesting that obtaining textiles from different
84 postmortem intervals would be useful for providing training aids that represent the full spectrum of
85 decomposition odour that cadaver-detection dogs may encounter during a search. This information is
86 particularly beneficial for law enforcement agencies searching for effective and reliable cadaver-
87 detection dog training aids.
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100 **Keywords:** *Forensic taphonomy; Buried remains; Textiles; Cadaver-detection dogs; Natural training*
101 *aids; GC×GC-TOFMS*
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106 **Abbreviations:** ¹D, first dimension; ²D, second dimension; DVB/CAR/PDMS, divinylbenzene/
107 carboxen/polydimethylsiloxane; F_{crit} , critical value; GC-MS, gas chromatography – mass
108 spectrometry; GC×GC-TOFMS, comprehensive two-dimensional gas chromatography – time-of-
109 flight mass spectrometry; HS-SPME, headspace solid phase microextraction; NIST, National Institute
110 of Standards and Technology; PC-1, first principal component; PC-2, second principal component;
111 PCA, principal component analysis; PDMS/DVB, polydimethylsiloxane/divinylbenzene; *S/N*, signal-
112 to-noise ratio; TIC, total ion current; TVOCs, total volatile organic compounds; VOCs, volatile
113 organic compounds
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121 **Graphical Abstract**
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180 **1. Introduction**
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182 The innate ability of canines to locate and hunt prey makes them an ideal candidate for use as a scent-
183 detection tool. Both wild and domesticated canines have a natural ability to detect the scent of their
184 prey [1], an ability that can be used to train canines towards almost any desired scent. The use of
185 canines in a forensic investigation dates to the 1800s, when bloodhounds were used in an attempt to
186 locate Jack the Ripper in England [2]. Currently canines are used for the detection of drugs,
187 explosives, agricultural products, accelerants, currency, missing persons, human remains and certain
188 diseases. Cadaver-detection dogs are specially trained canines employed by law enforcement agencies
189 to locate human remains in cases of missing persons believed dead, suspected homicides and
190 following natural or man-made disasters. These canines evolved when handlers observed that the
191 search and rescue dogs, trained to locate living humans, would lose their tracking ability once the
192 individual was no longer alive, causing their scent to change [3]. Cadaver-detection dogs are still
193 currently one of the preferred search methods for the localisation of human remains as they can cover
194 large areas rapidly and can work both day and night [4,5].
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202 The ability of cadaver-detection dogs to detect human remains relies heavily on the use of effective
203 aids during training. Several materials, both natural and man-made can be used when training these
204 dogs on a specific target odour. Natural training aids include biological tissues such as blood, bone or
205 flesh, decomposition fluid or soil that has been in contact with decomposing remains [1]. Although
206 ideal, the use of whole human cadavers for training is uncommon due to the ethical and legal
207 restrictions associated with acquiring bodies. In addition to natural training aids, synthetic scents have
208 also been developed such as Pseudo™ Scents. These man-made scents are easier to obtain as there are
209 fewer ethical restrictions than those associated with natural training aids, and the synthetic scents are
210 easier to store. Despite these advantages, commercially available synthetic scents often have an overly
211 simplistic chemical composition that is not representative of decomposition odour [6,7], and in some
212 cases can also comprise hazardous chemicals. Cadaverine and putrescine, two very odorous
213 compounds often associated with decomposition, are commonly found in synthetic scents [8].
214 Although these scents are associated with human decomposition, they also result from the
215 decomposition of any organic matter and are thus not human specific [9].
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223 Due to the current inconsistencies associated with synthetic scents, natural training aids are
224 considered more reliable and provide a better representation of the scent of human remains. However,
225 Hoffman et al. [10] demonstrated that the odour produced from individual tissue samples (e.g. blood,
226 muscle, skin, adipocere, fat, bone, teeth, etc.) shared similarities, but varied enough in their odour
227 profile that care should be taken when using a specific individual tissue type as a cadaver-detection
228 dog training aid. The use of individual bones, flesh and blood samples as training aids may not
229 provide an adequate odour representation of a cadaver or intact human remains. An alternative
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239 training aid that may provide a more comprehensive profile than using bone, blood or specific tissue
240 samples is the use of textile samples that have been in contact with decomposing remains. The use of
241 textiles as training aids is beneficial over the use of bones or blood as they can trap scent molecules
242 from the whole body rather than specific tissues types [11]. Textiles are commonly found in
243 association with decomposing remains [10,11], either fully or partially clothed. As the remains
244 decompose the resulting fluid released will cause the clothing to become stained. It has already been
245 established that decomposition fluid will be absorbed into textiles and the fluid composition will
246 change over time [12]. Additionally, fluid generated by decomposing remains can become embedded
247 in-between the fibres of certain textile types, and might thus remain trapped as decomposition odour
248 for longer than the remains. Cadaver-detection dogs are often trained on multiple training aids to
249 ensure that the full range of decomposition odour is accounted for and that the remains can be
250 successfully located.
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258 The decomposition process is initiated the moment the heart stops beating. The process of breaking
259 down the soft tissue to completion may take as little as days or progress slowly for years. Initially the
260 pH of blood will decrease and the skin colour will fade in a process known as pallor [13]. During this
261 process the early insect colonisers such as blow flies (*Calliphoridae*) and flesh flies (*Sarcophagidae*)
262 arrive at the corpse [13]. The arrival of insects to the remains demonstrates that a scent is being
263 emitted from the body. This scent will change and amplify as internal bacteria break down the
264 macromolecules in the body. The breakdown causes the release of gases (i.e. volatile organic
265 compounds; VOCs) such as methane and hydrogen sulfide. This large gas accumulation inside the
266 remains will eventually cause the skin to rupture, effectively releasing the gases. During this period
267 the strong odour anecdotally associated with decomposing remains is detected.
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274 The location of human remains greatly impacts the decomposition process. Whether the remains are
275 deposited on the surface or buried changes the immediate surrounding environment and can alter
276 access by insects and other scavengers. Temperature aboveground is generally higher than below the
277 surface [14,15]. Burial environments tend to result in decomposition over a longer period of time due
278 to this temperature difference as well as due to the protection of the remains from scavenging, large
279 and small [13] and general weathering activities [15]. As the process of decomposition in a burial
280 environment is generally slower than a body decomposing on the surface, it is hypothesised that a
281 burial environment might allow the analysis of the VOC profile from textiles for a longer time period.
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286 Headspace solid phase microextraction (HS-SPME) combined with gas chromatography – mass
287 spectrometry (GC-MS) has been used to identify trace amounts of volatile compounds emitted from a
288 variety of forensic specimens [3,16]. A previous study by Zhu et al. [17] resulted in the development
289 of a method used to detect six target VOCs along with the total volatile organic compounds (TVOCs)
290 from textile samples using HS-SPME and GC-MS. The one-dimensional targeted GC-MS analysis
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298 was deemed successful; however, would be unlikely to provide sufficient resolution for the analysis of
299 VOCs from textiles associated with decomposed remains. Decomposition odour has previously been
300 reported to consist of a complex mixture of VOCs [18–24] and it is still not known which VOCs are
301 responsible for the detection and localisation of remains by cadaver-detection dogs. The initial
302 decomposition odour studies also used GC-MS for VOC analysis, however, due to the complexity of
303 the resulting profiles, comprehensive two-dimensional gas chromatography – time-of-flight mass
304 spectrometry (GC×GC-TOFMS) instrumentation was introduced. GC×GC employs a second
305 dimension GC column for further separation of the eluent from the first dimension column, and has
306 been shown to provide increased peak capacity, higher resolution separations and improved
307 sensitivity, providing a more comprehensive VOC profile [25]. The current study used HS-SPME-
308 GC×GC-TOFMS to investigate the VOC profile emitted from textiles buried with decomposing
309 remains over a two year period. The objective was to investigate how well the odour retained in
310 clothing associated with these remains reflected the VOC profile of decomposition typically reported
311 in the literature for humans and human analogues. This information will provide further insight into
312 the value of textiles as a training aid for cadaver-detection dogs.
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323 **2. Materials and Methods**

324 *2.1. Experimental Design*

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326 Field experiments consisted of burying and subsequently exhuming a total of seven pig carcasses (*Sus*
327 *scrofa domesticus L.*) at various intervals over a 24 month period (January 2013 – January 2015). Pigs
328 were chosen for this study as they are widely accepted as human decomposition analogues in
329 taphonomic studies due to their similarity in internal anatomy, fat distribution, size of chest cavity,
330 skin, gut flora and lack of heavy fur [26]. Pigs were purchased postmortem as excess stock from
331 Hawkesbury Valley Meat Processors, a licenced abattoir in Wilberforce, NSW, Australia confirmed to
332 follow established animal welfare guidelines. All pigs were killed using captive-headbolt, the standard
333 procedure employed in Australian abattoirs. The carcasses were wrapped in a large polyethylene
334 tarpaulin and transported to the field site within an hour of death. Following the guidelines of the
335 *Australian Code for the Care and Use of Animals for Scientific Purposes* (8th ed. 2013)
336 (<http://www.nhmrc.gov.au/guidelines-publications/ea28>), animal ethics approval was not required for
337 this study because the experimental subjects were: 1) purchased postmortem; and 2) not killed
338 specifically for the purposes of this research.
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346 The field site is an open eucalypt woodland located on the Cumberland Plain in Western Sydney,
347 NSW, Australia (33° 38S, 150° 39E). The land is privately owned by the University of Technology
348 Sydney and has been approved for research and educational purposes. The soil consists of layers of
349 sandy clay topsoil to a depth of approximately 0.70 – 1.00 m, shale clays to a depth of approximately
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357 1.50 – 1.80 m and yellow and grey sandstone bedrock beyond 1.50 – 1.80 m. The topsoil is mostly
358 acidic, typically ranging between pH 4 – 5 throughout the year.
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361 The seven pig carcasses, weighing approximately 70 kg each, were clothed in 100% cotton t-shirts
362 (Alpha Brand, Kmart, Broadway, NSW, Australia) prior to burial in individual experimental graves.
363 Cotton was chosen for this study as it represents a textile that is commonly worn and often associated
364 with human remains. Seven cotton t-shirts were also buried in individual graves in the absence of
365 remains to serve as control samples. All graves were dug a minimum of 3 m apart (with a minimum of
366 5 m between the control and experimental graves) to a depth of 50 cm using an excavator and were
367 backfilled using an excavator. The clothed pig carcasses and control textiles were exhumed after 1, 3,
368 6, 12, 18 and 24 months post-burial.
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373 After exhumation, the textile samples were packaged into small paper envelopes, placed into
374 individually labelled paper bags and stored in a cooler for transportation to the laboratory. Paper was
375 selected as the packaging material in order to prevent the textile samples from storing moisture and
376 degrading during transport and storage. To prevent bacterial and fungal growth the textile samples
377 were air-dried under ambient temperature by hanging vertically inside a laboratory fume cupboard,
378 and any adhering tissue, soil or hair was removed after drying. Using sterilised scissors, triplicate
379 1 × 1 cm cotton samples were collected from each experimental and control textile. Triplicate
380 1 × 1 cm cotton samples were also collected from a t-shirt prior to burial (referred to as day 0 control
381 textiles) for the purpose of determining background VOCs associated with the cotton textiles. The
382 scissors were washed with acetone between each replicate and between experimental and control
383 samples. The 1 × 1 cm cotton samples were placed into individual 20 mL headspace vials, sealed
384 airtight with a screw cap containing a 1.3 mm thick polytetrafluoroethylene/ silicone septum (Sigma-
385 Aldrich, Castle Hill, NSW, Australia) and stored at -18 °C prior to analysis. VOC profiles of the
386 cotton samples were collected using HS-SPME and analysed using GC×GC-TOFMS.
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395 2.2. HS-SPME Sample Collection

396 Based on previous literature examples that used SPME for the headspace collection of VOCs
397 produced from textiles [27] and decomposing remains [3,10,28–30], two different SPME fibres were
398 chosen for testing using the experimental textile samples collected after 6 months post-burial:
399 namely a 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibre and a 50/30 µm
400 divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre. Following optimisation of
401 other parameters, the DVB/CAR/PDMS fibre (50/30 µm, 24 Ga Stableflex; Supelco, Bellefonte, PA,
402 USA) collected the widest possible range of compounds in the test samples and was therefore chosen
403 for the headspace collection of VOCs in this study. Before first use, the fibre was conditioned for 60
404 min at 270 °C in the GC×GC sample inlet, according to the manufacturer's recommendations. Fibre
405 reconditioning was performed for 5 min at 250 °C at the beginning of each sampling day. A fibre
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414 blank was completed before sampling and after every 3 sample collections. Prior to sample collection,
415 the SPME fibre was pre-loaded with an internal standard [31] by exposing the fibre to the headspace
416 of a 200 μ L solution of 100 ppm bromobenzene (GC grade, Sigma-Aldrich), prepared in methanol
417 (HPLC grade, Sigma-Aldrich), inside a sealed headspace vial for 15 s at room temperature. After
418 incubating the sample vial for 10 min in a dry bath heating block (Thermoline Scientific, Wetherill
419 Park, NSW, Australia) at a constant temperature of 40 $^{\circ}$ C, sample extraction was performed by
420 exposing the SPME fibre to the headspace within the sample vial for 10 min while the sample vial
421 was continuously maintained at 40 $^{\circ}$ C.
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429 2.3. GC \times GC-TOFMS Sample Analysis

430 A Pegasus[®] 4D GC \times GC-TOFMS system (LECO, Castle Hill, NSW, Australia) equipped with a liquid
431 nitrogen cryogenic quad jet modulator was used for sample analysis. The column configuration
432 consisted of a mid-polar Rxi[®]-624Sil MS column (30 m \times 0.250 mm inner diameter, 1.40 μ m film
433 thickness; Restek Corporation, Bellefonte, PA, USA) in the first dimension (¹D) and a polar
434 Stabilwax[®] column (2 m \times 0.250 mm inner diameter, 0.50 μ m film thickness; Restek Corporation) in
435 the second dimension (²D). A SilTite[™] μ -Union (SGE Analytical Science, Wetherill Park, NSW,
436 Australia) was used to connect the ¹D and ²D columns. The Rxi[®]-624Sil MS column is recognised as
437 being highly selective for VOCs. The suitability of the Rxi[®]-624Sil MS \times Stabilwax[®] column
438 combination has been assessed in a previous study for decomposition VOC profiling [32].
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445 Sample introduction was performed by desorbing the SPME fibre directly in the GC \times GC inlet at a
446 temperature of 250 $^{\circ}$ C for 5 min using splitless injection with a 30 s inlet purge time. High purity
447 helium (BOC, Sydney, NSW, Australia) was used as the carrier gas at a constant flow rate of
448 1.0 mL/min. The ¹D oven temperature program included a 5 min hold at 35 $^{\circ}$ C followed by a
449 temperature ramp to 240 $^{\circ}$ C at 5 $^{\circ}$ C/min before a final hold at 240 $^{\circ}$ C for 5 min (i.e. a total run time of
450 51 min). Relative to the ¹D oven, the ²D oven and modulator were programmed to have a constant
451 offset of +15 $^{\circ}$ C and +20 $^{\circ}$ C, respectively. A modulation period of 5 s was used with a 1.00 s hot pulse
452 time and a 1.50 s cool time between stages. The transfer line connecting the GC \times GC with the TOFMS
453 was maintained at 250 $^{\circ}$ C. The TOFMS detector was operated at a rate of 100 Hz between m/z 29 –
454 450. The ion source temperature was 200 $^{\circ}$ C and the electron ionisation energy was 70 eV. The
455 detector voltage was set at +200 V above the optimised detector voltage, which was determined daily
456 prior to sample analysis.
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463 2.4. Data Processing

464 Data processing was performed using ChromaTOF[®] (version 4.51.6.0; LECO). The baseline was
465 automatically smoothed with an 80% offset. The expected peak widths in the ¹D and ²D were set at
466 30 s and 0.15 s, respectively, based on the typical widths observed for the narrowest, non-saturated
467 peaks within the resultant chromatograms. A minimum signal-to-noise ratio (S/N) of 250 was used for
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base peak detection with a minimum of 2 apexing masses and sub-peak detection was cut-off at a minimum S/N of 20. A 65% mass spectral match was required to combine subpeaks with each other and their corresponding base peak. Peak identification was performed by a forward search to the 2011 National Institute of Standards and Technology (NIST) mass spectral library database with a minimum similarity match of >80%.

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Chromatographic alignment, normalisation and Fisher ratio computation were performed using the Statistical Compare software feature within ChromaTOF[®] (version 4.51.6.0; LECO). Samples were input into Statistical Compare and separated into two classes: control textiles ($n = 21$) and experimental textiles ($n = 18$). During chromatographic alignment, peak researching was performed with a minimum S/N cut-off of 20 in order to search for peaks not found during the initial peak finding step. A maximum retention time difference of 10 s (i.e. 2 modulation periods) in the ¹D and 0.6 s in the ²D was permitted during alignment to allow for retention time deviations between samples. In order for peaks to be identified as the same compound across chromatograms during alignment a mass spectral match >600 was required. Analytes that did not meet this mass spectral match threshold were removed from the final compound list. In addition, analytes were only retained in the final compound list if detected in at least two or more of the samples within a class. Following alignment, the analyte peak areas (calculated using unique mass) were normalised against the bromobenzene internal standard peak area. Fisher ratio filtering was performed in order to identify class-distinguishing compounds based on its success in previous studies [23,33–38]. Analytes with a Fisher ratio (i.e. the ratio of between-class variance to within-class variance) above the critical value ($F_{crit} = 4.11$) were exported as a *.csv file and imported into Microsoft Excel for further analysis. The critical value was computed in Microsoft Excel using the F -distribution based on the number of classes in the analysis, the degrees of freedom for each class and the significance level chosen ($\alpha = 0.05$). Chromatographic artefacts (e.g. column and fibre bleed) were manually removed in Microsoft Excel and the remaining compounds were sorted into one or more of the following chemical classes: alcohol, aldehyde, aromatic, carboxylic acid, ester, ether, halogenated, hydrocarbon, ketone, nitrogen-containing, sulfur-containing or “other” (i.e. compounds with functional groups that did not fit into any of the previously described chemical classes).

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Principal component analysis (PCA) was used to view and evaluate the multivariate structure of the data. Data pre-processing (i.e. mean centering, variance scaling and unit vector normalisation [39,40]) was carried out in The Unscrambler[®] X (version 10.3.31813.89; CAMO Software, Oslo, Norway) followed by PCA. The dataset was verified to contain no outliers by means of the Hotelling’s T2 95% confidence limit.

527 528 529 530 531 **3. Results and Discussion**

3.1. Visual Observations

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534 *3.1.1 Buried Remains*
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536 The pig carcasses buried clothed in 100% cotton t-shirts were exhumed after 1, 3, 6, 12, 18 and 24
537 months of burial for the observation and collection of textile samples. After one month, when the first
538 pig grave was exhumed, a large amount of soft tissue was present and the entire carcass could be
539 easily extracted as a single, intact specimen. The surrounding soil was very moist, even up to 40 cm
540 above the remains. Three months post-burial the remains of the second pig carcass were exhumed
541 intact with a large amount of soft tissue remaining. Again the surrounding soil was still wet. After
542 lifting the remains, the soil beneath was very dark in colour, appearing almost black. After six months
543 post-burial, the limbs of the pig carcass were fully skeletonized while the soft tissue remaining on the
544 torso had begun to break down and a large presence of white adipocere was observed. The grave
545 exhumed after 12 months contained significant amounts of adipocere, with large tissue sections that
546 were well preserved. The remains after 18 months post-burial were very dry and demonstrated a large
547 amount of tissue loss, with mostly skin and bone remaining. After 24 months, the remains were fully
548 skeletonized and the grave environment was very dry.
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555 *3.1.2 Textile Damage*
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557 The experimental textile sample exhumed with the first pig carcass after one month burial was very
558 discoloured with orange and brown staining. Large sections of tissue adhered to the surface of the
559 t-shirt and a strong odour was produced. The experimental textile from the grave after three months
560 burial attracted a large amount of flies after it was removed from the grave. After 6 (**Fig. 1a**) and 12
561 months of burial, the experimental textiles located underneath the pig carcasses were very well
562 preserved. The experimental textile associated with the pig carcass exhumed after 18 months burial
563 had only a small portion of the textile recovered (**Fig. 1b**). This remaining section was found to be
564 well preserved and covered in several layers of tissue, as was observed during the previous
565 exhumations after 6 and 12 months of burial. On the final sampling day, 24 months post-burial, the
566 experimental textile was almost completely disintegrated; however the seams and small sections of
567 textile attached to the seams still remained (**Fig. 1c**).
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582 **Fig. 1.** Visual damage to the experimental textiles samples exhumed from the pig graves after a) 6,
583 b) 18 and c) 24 months post-burial.
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593 The severe degradation of textile observed in the last two experimental graves (i.e. 18 and 24 months
594 post-burial) was most likely due to the distinct difference in moisture detected during these two post-
595 burial intervals when compared to the other graves. The increased moisture content preserved the
596 remains, especially in the 12 month graves; this was evident by the production of adipocere. It is
597 hypothesised that the presence of remains with soft tissue resulted in the preservation of the
598 experimental cotton samples in these graves.
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602
603 Control textile samples consisting of 100% cotton t-shirts were exhumed after 1, 3, 6, 12, 18 and 24
604 months post-burial. The control textile samples were found to degrade rapidly in the soil grave in the
605 absence of any remains and after 12 months there was virtually no part of the t-shirt left other than the
606 seams. The experimental textile samples on the other hand, showed a great deal of preservation
607 suggesting that the presence of decomposing remains prevents the natural bacterial consumption of
608 the cotton fabric. These very distinct visual differences demonstrate another benefit of the use of
609 textiles as training aids. The findings show that textile samples are still likely to be present (and
610 therefore recoverable by scene of crime officers) several years postmortem, even when the remains
611 have skeletonized.
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616 617 *3.2. VOC Profile*

618 The VOC profile of the textile samples was collected using HS-SPME and analysed using GC×GC-
619 TOFMS. **Fig. 2** displays GC×GC-TOFMS total ion current (TIC) contour plots obtained from the
620 control and experimental textiles exhumed and analysed 6 months post-burial. A scale of 0–20% of
621 the normalized signal intensity was required in order to assist with chromatographic visualisation of
622 trace components. These contour plots demonstrate the typical sample complexity and dynamic range
623 observed throughout this study. Overall, an average of 939 analytes were detected from the control
624 textiles and an average of 1116 analytes were detected from the experimental textiles. The overall
625 complexity exhibited in the samples analysed herein continues to support the use of GC×GC-TOFMS
626 for the forensic analysis of decomposition odour.
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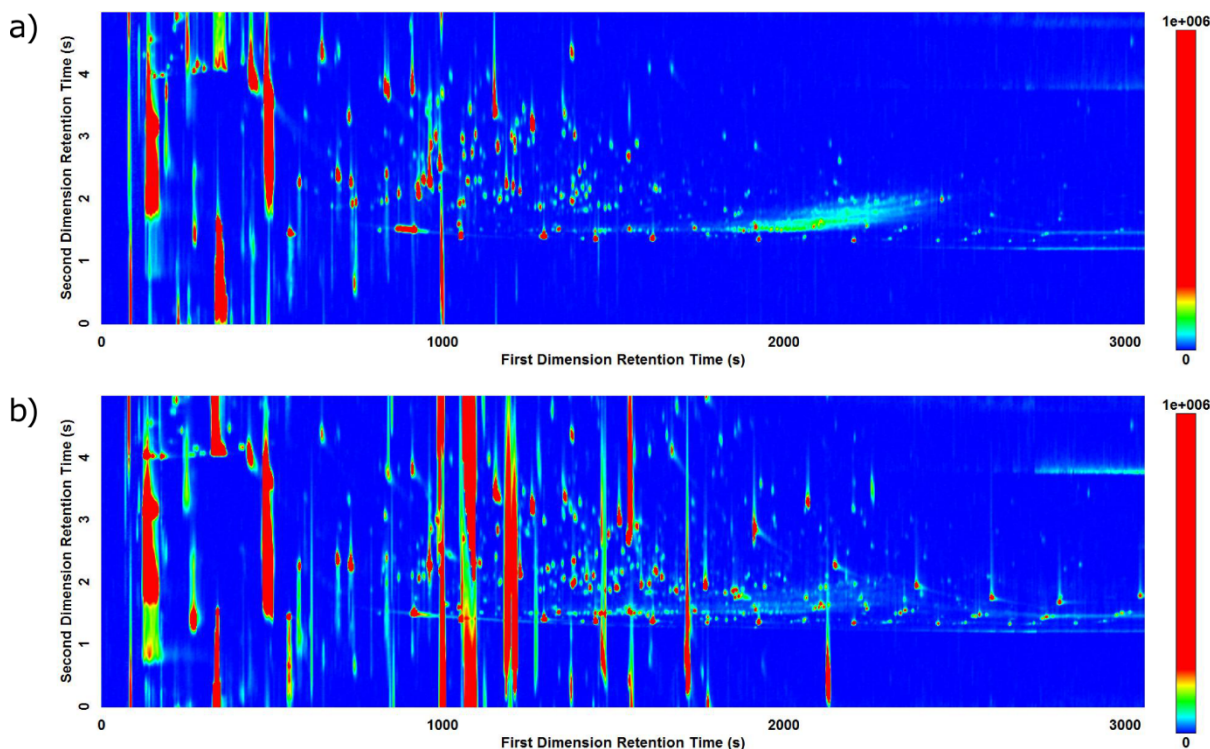


Fig. 2. GC×GC-TOFMS TIC contour plots of a) control and b) experimental textiles exhumed and analysed 6 months post-burial.

PCA was used to reduce data dimensionality and to provide a visual representation of the multivariate structure of the data using scores and loadings plots. A total of 297 analytes were chosen for submission to PCA. Analytes were chosen using the ChromaTOF® Statistical Compare software feature and Fisher ratio filtering (described in Section 2.4) in order to identify analytes present at significantly different concentrations/levels between the experimental and control textiles. In this case (**Fig. 3**), 43% of the variation within the dataset was captured within the first two principal components. Inspection of additional principal components (i.e. third and fourth principal components) revealed very little variation in the dataset (i.e. 7% and 6%, respectively), and were concluded to provide no further discriminatory information.

The scores plot (**Fig. 3a**) revealed discrimination between the experimental and control textiles horizontally along the first principal component (PC-1) for the majority of the study period, accounting for 31% of the variation within the dataset. Variation was also observed along PC-1 between the experimental textile samples collected at different postmortem intervals and was a result of numerous compounds detected in the profile rather than a few individual VOCs (**Fig. 3b**). While the experimental textile samples were separated horizontally along PC-1, the control textile samples were spread out displaying variation vertically along the second principal component (PC-2) axis, accounting for 12% of the variation within the dataset. This variability in the control textiles is a result of the interaction between the cotton textiles and microorganisms in the burial environment (e.g. fungi and bacteria in the soil) which produce enzymes that are capable of attacking the molecular structure

of the textile fibre [41,42]. This destruction of the textiles can lead to changes in colour or staining of the textile and unpleasant odours [41,43].

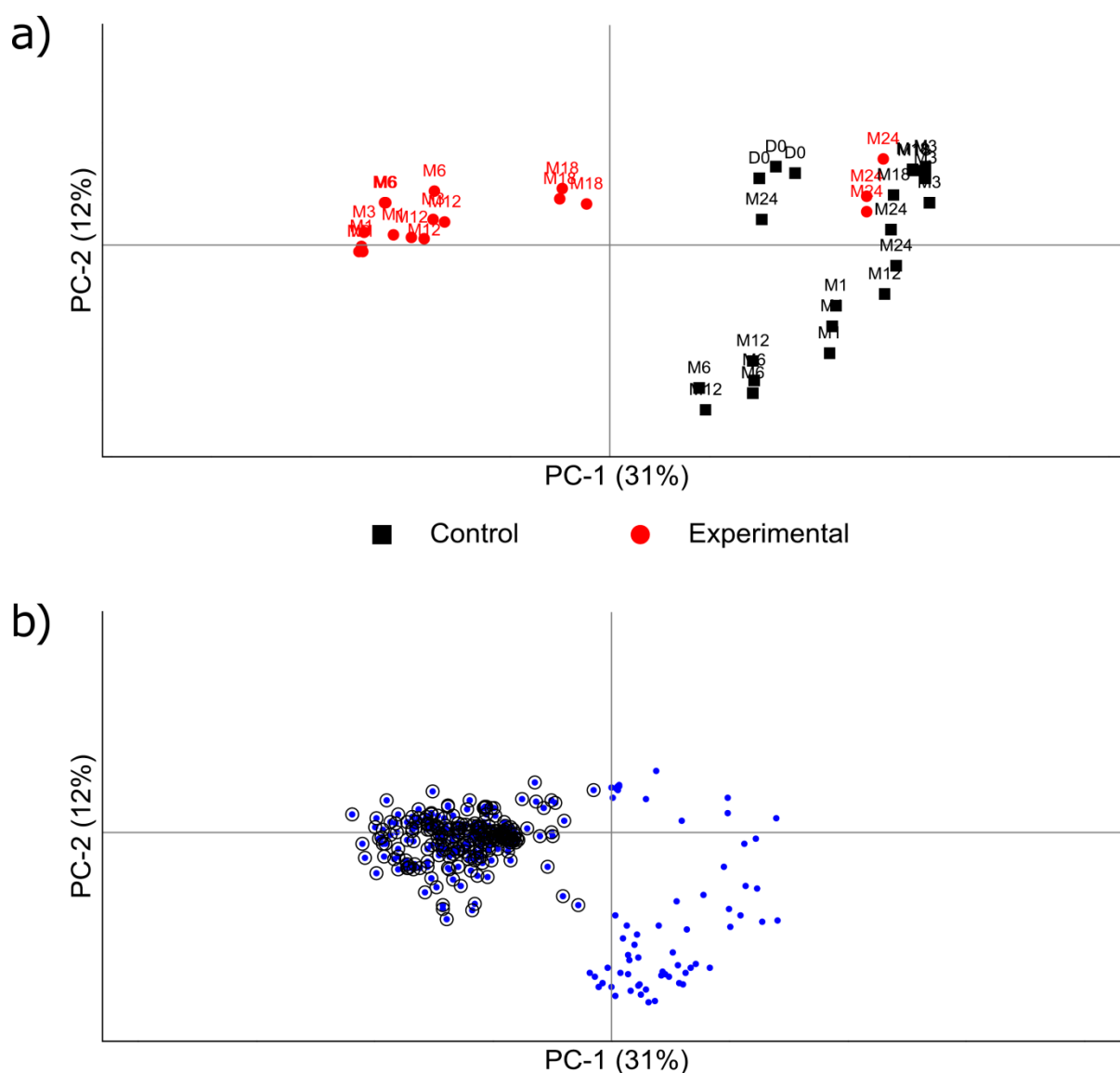
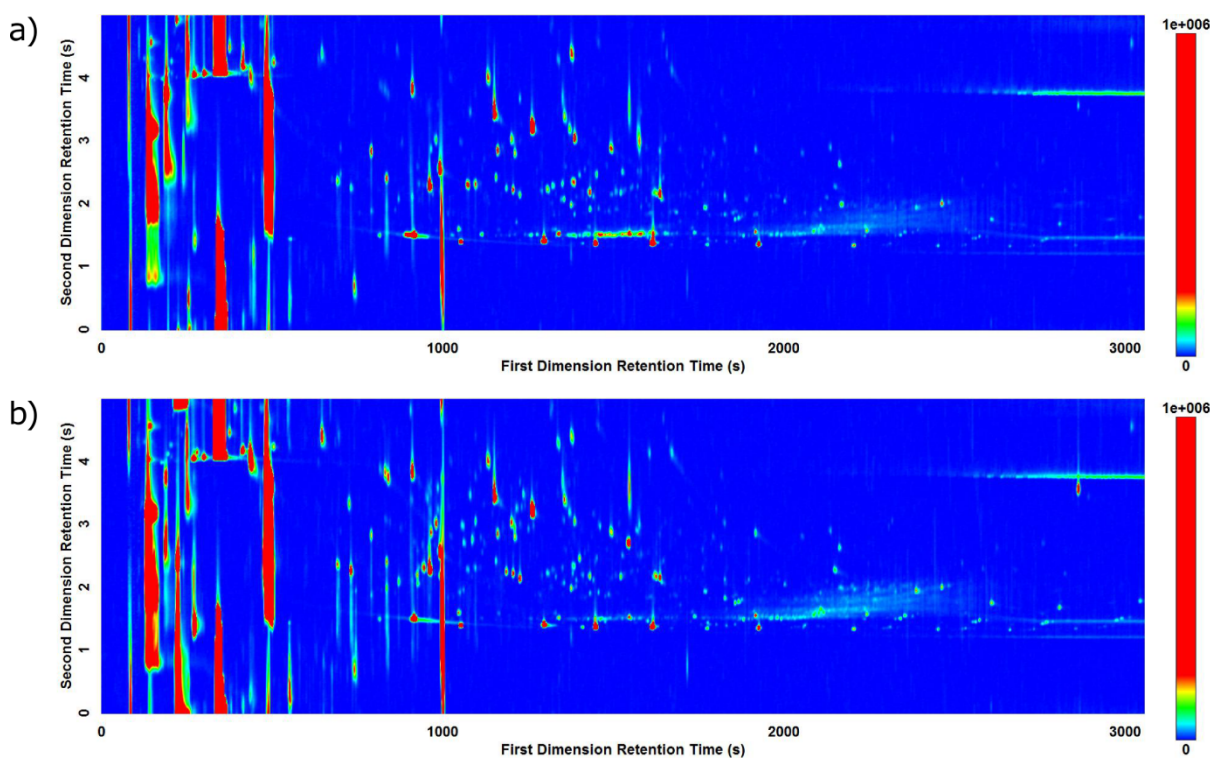


Fig. 3. Principal component analysis (PCA) a) scores and b) loadings plots calculated using pre-processed GC×GC-TOFMS peak area data for compounds detected with a Fisher ratio above F_{crit} in all experimental and control textiles investigated. Point labels in the a) scores plot denote the postmortem interval at the time of exhumation (D = day; M = month). Points circled in the b) loadings plot highlight the detected VOCs (listed in **Table A-1**) that were identified to discriminate the experimental textiles from the control textiles (i.e. *decomposition-related VOCs*).

The VOC profile produced from the experimental textiles collected 24 months postmortem did not appear to be differentiated statistically (**Fig. 3a**) or chromatographically (**Fig. 4**) from the control textiles. The authors recognize the low number of replicates in this study (i.e. $n = 1$ grave per postmortem interval) is a limitation and that the result obtained at 24 months could be an anomaly arising due to differences in decomposition/preservation of both the remains and the textile. As noted

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770 previously, the remains exhumed 24 months post-burial were fully skeletonized and the grave
771 environment was very dry with severe degradation to the textile observed. At this stage of
772 decomposition (i.e. skeletonization) the remains themselves would not be expected to produce an
773 odour as strong as remains that are still actively decomposing (i.e. bloat, active decay and advanced
774 decay stages). Although caution is taken when interpreting these results due to the lack of replicates, it
775 is possible that decomposition odour may not remain trapped within the 100% cotton textiles
776 indefinitely, and that as the postmortem interval increases (and decomposition progresses towards
777 skeletonization) the decomposition odour profile within the textile diminishes. Regardless, these
778 results demonstrate the importance of performing chemical analysis to verify that textiles recovered
779 from remains have retained an odour reflective of cadaveric decomposition before implementing the
780 textile as a training aid in cadaver-detection dog training.
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810 **Fig. 4.** GCxGC-TOFMS TIC contour plots of a) control and b) experimental textiles exhumed and
811 analysed 24 months post-burial.
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814 Insight into the chemical differences between the control and experimental textiles was gained by
815 examining the loadings plot displayed in **Fig. 3b**. Those analytes that appear grouped in the left-hand
816 quadrants of the loadings plot (**Fig. 3b**) were considered to strongly influence or contribute to the
817 placement of the experimental textile samples in the left-hand quadrants of the scores plot (**Fig. 3a**).
818 Together, these 231 analytes (circled in the loadings plot (**Fig. 3b**) and listed in **Table A-1**) were
819 identified as the VOCs contributing to the discrimination of the experimental textiles from the control
820 textiles and were therefore considered as *decomposition-related VOCs*. More than half (i.e. ~55%) of
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829 these VOCs have been previously identified in at least one other decomposition odour study published
830 in the literature for human remains or human analogues (see **Table A-1**). The remainder of the
831 discussion in this article will focus on these 231 analytes that make up the decomposition odour
832 profile detected in the experimental textiles investigated herein.
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836 **Fig. 5** displays the average VOC abundance ($n = 3$) for all 12 compound classes detected specific to
837 the experimental textiles at each postmortem interval. The overall class composition of the
838 decomposition odour profile was found to change over time and several interesting trends were
839 observed.
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842 The most prominent compound class in the first 12 months of sampling was carboxylic acids. These
843 compounds were detected in very high abundance, often streaking across the GC×GC TIC contour
844 plots (**Fig. 2b** – first dimension retention times of 1000 – 2000 s). The poor peak shapes observed are
845 a result of the high (and often overloaded) concentrations detected and the unfavourable interaction
846 between the GC column stationary phases and the –COOH functional group, which readily forms
847 intermolecular hydrogen bonds [25]. Notably, the carboxylic acid compound class was the only
848 compound class in which all compounds detected were reported in the literature in previous
849 decomposition odour studies (**Table A-1**). Carboxylic acids have previously been identified in fat
850 tissue and adipocere, as well as in muscle tissue [10]. The detection of these compounds may be
851 indicative of the presence of soft tissue on the remains. In the final two graves (18 and 24 months) the
852 abundance of carboxylic acids decreased dramatically, where both of these graves contained remains
853 that were almost void of soft tissue. For improved viewing of trends in other compound classes, **Fig. 5**
854 is also displayed with the carboxylic acid class removed (**Fig. 5b**).
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862 Sulfur-containing compounds (specifically polysulfides) are currently the most widely recognised
863 group of compounds within the decomposition odour profile, as they are the most consistently
864 reported across studies [18,19,44–49]. These sulfur-containing compounds are commonly reported in
865 the earlier stages, especially in the bloat stage [19,45,46]. However, research conducted in the same
866 location as the present study found that polysulfides were detected throughout all decomposition
867 stages in the air above pig carcasses that were allowed to decompose naturally on the soil surface
868 [25]. In the current study, sulfur-containing compounds were not detected as a major contributor to
869 the decomposition odour profile (**Fig. 5b**), rather the sulfur-containing compound class was the least
870 abundant compound class detected at all post-mortem interval investigated (with the exception of
871 ethers, a rarely reported class of decomposition VOCs). Sulfur-containing compounds were detected
872 mostly in the three month samples, at this stage there was a large amount of tissue present, however,
873 the skin had ruptured and the soil beneath the pig was stained black, thus the likelihood of seeing the
874 sulfur-containing compounds were higher than in the one month samples where the pig carcass was
875 fully intact with less purging of fluids into the soil. Beyond three months the remains were in a later
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stage of decomposition and the sulfur-containing compounds may have dissipated and diffused through the soil. Although this compound class may be present during all stages of decomposition, sulphur-containing compounds were not detected in great abundance in the textile samples. These compounds might therefore be less retained by the textiles during burial decomposition and might be preferentially retained in the soil instead. Forbes and Perrault [21] found that VOC samples taken from the soil beneath decomposing remains contained a larger number of sulfur-containing compounds than air VOC samples taken above the same remains, again demonstrating the soil's ability to capture and trap these compounds.

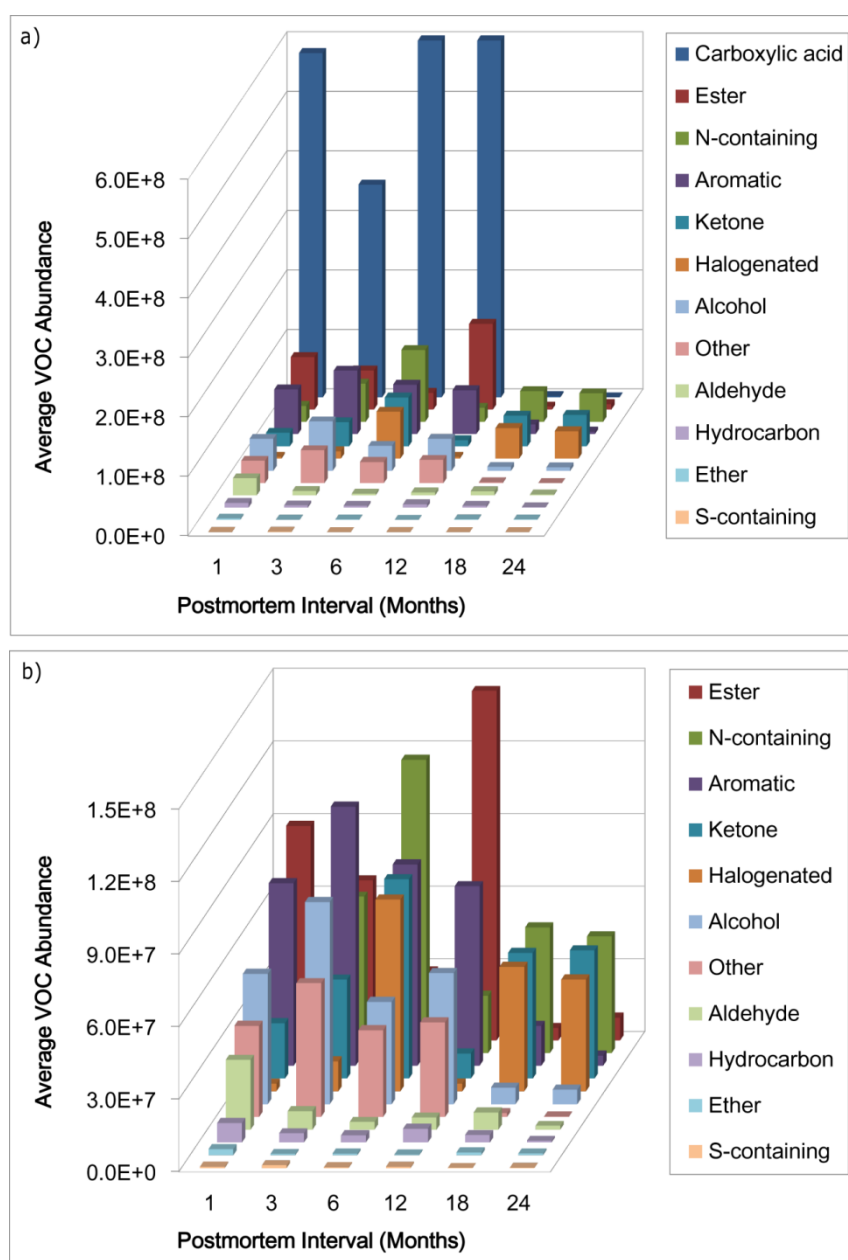


Fig. 5. Average VOC abundance ($n = 3$) of compound classes detected specific to the experimental textiles at each postmortem interval a) with and b) without carboxylic acids included for improved viewing of trends in other compound classes. Note VOCs were assigned to multiple classes when necessary (i.e. compounds with multiple functional groups).

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948 VOCs characterised as esters were initially high before decreasing after the first 6 months. Similar
949 results were reported by Forbes and Perrault [21], who found that when the VOC profile from soil
950 samples was used to distinguish the stages of decomposition, the PCA plot demonstrated that esters
951 were common in the earlier stages of decomposition, and most abundant during active decay. Esters
952 were found to be prevalent in the textile samples when the remains still had a presence of tissue. A
953 large increase in esters was identified in the 12 month samples, which could be due to the presence of
954 adipocere in the grave environment [10]. However, due to the lack of replicate pigs the spike in esters
955 could also be an anomaly and may simply be due to the initial fatty acid content in the pig from that
956 specific gravesite.
957

962 Nitrogen-containing compounds were found to increase steadily the first 6 months, before decreasing.
963 This corresponds to other studies where nitrogen-containing VOCs are known to appear in the early
964 stages of decomposition [47].
965

967 Aromatic VOCs (especially phenols) have commonly been reported in decomposition VOC studies
968 [18,19,29,46]. The abundance of aromatics was high for the samples collected within the first 12
969 months and low in the 18 and 24 month samples. These findings correspond to previous research as
970 aromatics are found in the active decay stage and early stages of advanced decay [45,46], the absence
971 of these compounds are then indicative of samples that are from a late stage of decomposition. When
972 looking solely at phenol, it was found in all samples except the 24 month samples (**Table A-1**), which
973 again corresponds to previous findings.
974

978 Aldehydes are found more commonly in the later stages of decomposition [18,19,46] and peak during
979 the transition from advanced decay to dry remains for surface studies. However, as the current study
980 involved buried specimens, which tends to promote the formation of adipocere [50], it is more likely
981 that the presence of aldehydes would be less significant as these are formed in aerobic conditions. The
982 one month post-burial grave was disturbed by scavengers on day 2 after burial, which resulted in the
983 remains being exposed. However, the remains were covered again and wire mesh was added to avoid
984 further burrowing. This soil disturbance likely introduced air in the grave. Maggots were also found
985 upon excavation and this might explain the presence of aldehydes in the one month textile samples as
986 some aerobic decomposition was likely to have occurred. The remaining sampling months had a
987 relatively consistent and low abundance of aldehydes.
988

994 Ethers are a rarely reported class of decomposition VOCs [51], which is consistent with the low
995 number and abundance of ethers detected at all postmortem intervals investigated throughout this
996 study. Hydrocarbons were most abundant in the samples collected 1 month post-burial, before
997 decreasing and remaining fairly stable. Ketones were found to increase for the first 6 months before a
998 decrease was seen in the 12 month samples; the abundance of ketones then increased and remained
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1006 consistent for the last two excavation times. There was a spike in halogenated compounds detected in
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1008 the 6 month post-burial samples, and a higher amount in the 18 and 24 month samples compared to
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1010 the other sampling days. Lastly, alcohols were determined to be higher in abundance in samples
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1012 where soft tissue was still present, similarly to aromatic compounds, carboxylic acids, esters and
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1014 aromatics.

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1016 Overall, there was a lack of sulfur-containing compounds detected in the textile samples; however,
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1018 this may be due to the fact that the remains were buried in a soil environment rather than being placed
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1020 on the soil surface during decomposition. Despite the reduced amount of sulphur-containing
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1022 compounds detected, the remaining compound classes commonly reported in decomposition odour
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1024 research (i.e. carboxylic acids, esters, nitrogen-containing compounds and aromatics) all produced
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1026 VOC profiles from the textile samples that were consistent with that of decomposing remains. This
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1028 demonstrates the ability of textiles to retain an odour comparable to that of decomposing remains and
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1030 suggests that although the profile may not be retained indefinitely, as determined by the PCA analysis
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1032 (**Fig. 3a**), textiles could be a viable option as a training aid for cadaver-detection dogs.

1028 **4. Conclusions**

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1030 This study provides evidence that 100% cotton textiles associated with decomposing remains may
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1032 represent a useful natural training aid for cadaver-detection dogs with a VOC profile reflective of a
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1034 large subset of cadaveric decomposition odour. Obtaining textiles associated with decomposing
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1036 remains from different postmortem intervals is useful for providing training aids that represent the
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1038 broad spectrum of decomposition odour cadaver-detection dogs are likely to encounter in the field
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1040 when searching for remains at varying stages of decomposition. Results suggest that decomposition
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1042 odour may not remain trapped in the 100% cotton textile indefinitely, and therefore chemical analysis
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1044 is valuable for the verification that textiles recovered from remains have retained an odour reflective
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1046 of cadaveric decomposition before the textiles are employed as a training aid. Chemical analysis could
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1048 likewise prove useful in confirming that the odour is retained overtime with prolonged use and
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1050 storage.

1048 **Acknowledgements**

1051
1052 The authors wish to thank all research group members and extended contacts, both past and present,
1053
1054 who have contributed to the planning and execution of field work including: Dr. Katelynn Perrault,
1055
1056 Dr. Rebecca Buis, Kate Trebilcock, LaTara Rust, Amanda Troobnikoff, Laura McGrath, Dr. Paul
1057
1058 Thomas, Chris Watson and Robert Chatterton. Laboratory technical staff, Dr. David Bishop, Dr.
1059
1060 Ronald Shimmon and Dr. R. Verena Taudte, are gratefully acknowledged for their ongoing technical
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support. The authors also wish to acknowledge the Australian Research Council and the University of Technology Sydney (UTS) for providing financial support for this work.

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1360 **Appendix A.**
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1362 **Table A-1**

1363 List of tentatively identified decomposition VOCs detected (×) in the experimental textiles grouped according to compound class. Literature references are
1364 provided for compounds that have been previously reported in decomposition odour research. Note: each VOC only appears once in the table (i.e. assigned to a
1365 single compound class); however, for the purposes of overall analysis, VOCs were assigned to multiple compound classes when necessary (i.e. compounds with
1366 multiple functional groups).
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Volatile Organic Compounds (VOCs)	Postmortem Interval (Months)						Literature References
	1	3	6	12	18	24	
<i>Alcohol</i>							
1,5-Hexadien-3-ol	×	×	×	×	×	×	[22,51]
1-Heptanol	×	×	×	×	×	×	[18,19,30,51–53]
1-Hexanol	×	×	×	×	×		[10,18,19,22,28,47,49,51,52,54–56]
1-Octanol	×	×	×	×	×	×	[10,18,19,21,29,30,51,52,56]
1-Octen-3-ol	×	×	×	×	×	×	[10,19,47,51–53,56]
1-Pentanol			×			×	[3,10,18,19,21,22,28,30,46,47,51,52,54,55]
2-Octanol, (S)-	×	×	×	×	×	×	
2-Pentanol				×			[21,22,30,32,47,51–53,55,57]
2-Propanol, 2-methyl-		×	×	×	×	×	
3-Octanol	×	×	×	×	×	×	
Isoborneol	×	×	×	×	×	×	[29]
<i>Aldehyde</i>							
(E)-4-Oxohept-2-enal	×	×	×		×		
2,4-Nonadienal, (E,E)-	×				×		[10,28]
2-Butenal, 2-ethenyl-	×		×				
2-Butenal, 3-methyl-	×	×	×	×	×	×	[18,51,52]
2-Heptenal, (Z)-	×	×	×	×	×	×	[10,19,22,28,51]
2-Nonenal, (E)-	×	×	×	×	×	×	[10,18,28,51]
2-Octenal, (E)-	×	×	×	×	×	×	[10,18,19,28,51]
2-Pentenal, (E)-	×	×	×	×	×	×	[47]
2-n-Butylacrolein	×	×	×	×	×	×	[22,38,51]
Decanal	×	×	×	×	×	×	[28,29,44,49,51]
Heptanal	×	×	×	×	×	×	[3,18,19,28,30,46,51–53,56,58]
Nonanal	×	×	×	×	×	×	[10,18,21,22,28–30,44,49,51,52,58]

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Volatile Organic Compounds (VOCs)	Postmortem Interval (Months)						Literature References
	1	3	6	12	18	24	
Octanal	×	×	×	×	×	×	[10,18,19,21,28,30,51,52,56,58]
Pentanal	×	×	×	×	×	×	[19,38,46,51,52,54,58]
<i>Aromatic</i>							
2-(5-Methyl-furan-2-yl)-propionaldehyde					×		
2-Ethylhexyl salicylate	×	×	×	×	×	×	
2-Heptylfuran	×	×	×	×	×	×	[30]
3-Phenylpropanol	×	×	×	×	×		
Acetic acid, 2-phenylethyl ester	×	×	×	×			
Acetophenone	×	×	×	×	×	×	[32,47,48,51]
Benzaldehyde	×	×	×	×	×	×	[3,10,18,19,28–30,32,44,46–48,51–53,55,56]
Benzene, (1,1-dimethylethoxy)-	×	×	×	×	×		
Benzene, (1-methylethyl)-	×	×	×	×	×	×	[18]
Benzene, 1,2,3,4-tetramethyl-	×	×	×	×	×	×	[29,57]
Benzene, 1,2,3-trimethyl-	×	×	×	×	×	×	[29,52,54,59]
Benzene, 1,2,4,5-tetramethyl-	×	×	×	×	×	×	
Benzene, 1,3,5-trimethoxy-	×	×	×	×	×	×	
Benzene, 1,3-dichloro-	×	×	×	×	×	×	
Benzene, 1,3-diethyl-	×	×	×	×	×	×	
Benzene, 1,3-dimethyl-	×	×	×	×	×	×	[52,55]
Benzene, 1-ethenyl-4-ethyl-	×	×	×	×	×	×	
Benzene, 1-ethyl-2-methyl-	×	×	×	×	×	×	[38,49,57,58]
Benzene, 1-ethyl-3-methyl-	×	×	×	×	×	×	[47,57]
Benzene, 1-methoxy-4-methyl-	×	×	×	×	×	×	[48]
Benzene, 1-methyl-3-(1-methylethyl)-	×	×	×	×	×	×	[32,51,52,55,57]
Benzene, 1-methyl-3-propyl-	×	×	×	×	×	×	
Benzene, 1-methyl-4-(1-methylpropyl)-	×	×	×	×	×	×	
Benzene, 1-methyl-4-propyl-	×	×	×	×	×	×	[57]
Benzene, 2-ethyl-1,3-dimethyl-	×	×	×	×	×	×	
Benzene, 4-ethyl-1,2-dimethyl-	×	×	×	×	×	×	
Benzene, hexyl-	×	×	×	×	×	×	
Benzene, pentyl-	×	×	×	×	×	×	[38]
Benzene, propyl-	×	×	×	×	×	×	[32,38,47,52,54,55]
Benzeneethanol, ð-methyl-	×	×	×	×	×		

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Volatile Organic Compounds (VOCs)	Postmortem Interval (Months)						Literature References
	1	3	6	12	18	24	
Benzenemethanol, à,à-dimethyl-							[23,44,49,58]
Benzenemethanol, à-methyl-, (R)-	×	×	×	×	×		[18]
Benzenepropanal, á-methyl-	×	×	×	×	×	×	
Benzenepropanoic acid, methyl ester	×	×	×	×	×	×	
Furan, 2-pentyl-	×	×	×	×	×	×	[3,10,18,21,28,30,32,51,52,55]
Furan, 2-propyl-	×			×	×		[38,55]
Furfural	×	×	×	×	×	×	[29]
Indan, 1-methyl-	×	×	×	×	×	×	
Indane	×	×	×	×	×	×	[54]
Naphthalene, 1,2,3,4-tetrahydro-	×	×	×	×	×	×	
Oxirane, 2-methyl-2-phenyl-	×	×	×	×	×	×	
Phenol	×	×	×	×	×		[19,21,29,30,46–48,51–53,55,56,59]
Phenol, 2,4-bis(1,1-dimethylethyl)-	×	×	×	×	×	×	
Phosphonic acid, (p-hydroxyphenyl)-	×	×	×	×	×	×	
Propanoic acid, 2-methyl-, 3-phenylpropyl ester	×	×	×	×			
Propanoic acid, 2-phenylethyl ester	×	×	×	×			
o-Cymene	×	×	×	×	×	×	[51,52]
o-Xylene	×	×	×	×	×	×	[18,29,38,47,51,55,59]
p-Cresol	×	×	×	×	×	×	[22,51]
p-Cymene	×	×	×	×	×	×	[18,46]
á-Phenylethyl butyrate	×	×	×	×			
Carboxylic acid							
Acetic acid	×	×	×	×			[19,21,29,30,47,51,53,56]
Butanoic acid	×	×	×	×	×		[3,10,18,19,21,22,30,46,51,53,56]
Butanoic acid, 2-methyl-	×	×	×	×	×		[18,19,21,22,28–30,46,51,53]
Butanoic acid, 3-methyl-	×	×	×	×	×		[18,19,21,22,28,30,46,51,53]
Hexanoic acid	×		×	×			[3,10,18,19,28–30,32,46,51,56]
Propanoic acid	×	×	×	×			[3,10,19,21,22,28,30,46,51,53,56]
Propanoic acid, 2-methyl-	×	×	×	×	×		[18,19,21,22,29,30,46,51,56]
Ester							
1,3-Propanediol, diacetate	×	×	×				
1-Butanol, 3-methyl-, propanoate	×	×	×	×	×		
2(3H)-Furanone, 5-ethylidihydro-	×	×	×	×	×	×	[38]

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Volatile Organic Compounds (VOCs)	Postmortem Interval (Months)						Literature References
	1	3	6	12	18	24	
2(3H)-Furanone, 5-hexyldihydro-	×	×	×	×			
2(3H)-Furanone, dihydro-	×	×	×	×	×	×	[56]
2(3H)-Furanone, dihydro-5-methyl-	×	×	×	×	×	×	[18]
2(3H)-Furanone, dihydro-5-pentyl-	×	×	×	×	×		
2(3H)-Furanone, dihydro-5-propyl-	×	×	×	×	×		[38]
Acetic acid, methyl ester	×	×	×	×	×	×	[22,30,32,51,52,55]
Acetic acid, pentyl ester	×	×	×	×			[38]
Butanoic acid, 1-methyl-, butyl ester	×	×	×	×	×	×	
Butanoic acid, 2-methyl-, hexyl ester	×	×	×	×	×	×	
Butanoic acid, 3-methyl-, butyl ester	×	×	×	×	×	×	[18,21,30]
Butanoic acid, 3-methyl-, hexyl ester	×	×	×	×	×	×	
Butanoic acid, 3-methyl-, pentyl ester	×	×	×	×	×	×	
Butanoic acid, hexyl ester	×	×	×	×	×	×	[30]
Butanoic acid, methyl ester	×	×	×	×	×	×	[21,28,30,32,51,52,55]
Butanoic acid, pentyl ester	×	×	×	×	×	×	[30]
Decanoic acid, ethyl ester	×	×		×			
Heptanoic acid, methyl ester	×	×	×	×	×	×	[30]
Hexanoic acid, 1-methyl-, ethyl ester	×		×	×			
Hexanoic acid, 2-methyl-, propyl ester	×	×	×	×			
Hexanoic acid, ethyl ester	×	×	×	×	×	×	[10,28,30]
Hexanoic acid, hexyl ester	×		×	×			[10,28]
Hexanoic acid, ethenyl ester	×						
Hexanoic acid, methyl ester	×	×	×	×	×	×	[28,30]
Hexanoic acid, pentyl ester	×	×	×	×			[10,28,30]
Octanoic acid, methyl ester	×	×	×	×	×	×	[28,30]
Pentanoic acid, ethyl ester	×	×	×	×	×	×	[21,30]
Pentanoic acid, methyl ester	×	×	×	×	×	×	[30]
Propanoic acid, 2,2-dimethyl-, 2-phenylethyl ester	×	×	×	×			
Propanoic acid, 2-methyl-, pentyl ester	×	×	×	×	×	×	
Propanoic acid, butyl ester	×	×	×	×	×		[18]
Propanoic acid, heptyl ester	×	×	×	×	×	×	
Propanoic acid, hexyl ester	×	×	×	×	×	×	
Propanoic acid, methyl ester	×	×	×	×	×	×	[32,51,52,55]

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Volatile Organic Compounds (VOCs)	Postmortem Interval (Months)						Literature References
	1	3	6	12	18	24	
<i>Ether</i>							
Furan, 2,3-dihydro-2,5-dimethyl-		×	×	×	×	×	[18]
Furan, 2-butyltetrahydro-	×	×	×				[18,21,30,52]
Oxirane, ethyl-	×		×		×	×	
Oxirane, hexyl-	×	×	×	×	×	×	
<i>Halogenated</i>							
Ethane, hexachloro-	×	×	×	×	×	×	
Pentane, 3-bromo-3-methyl-	×	×				×	
Tetrachloroethylene	×	×	×	×	×	×	[10,28,44,49,58]
<i>Hydrocarbon</i>							
1,3-Heptadiene, 5,5-dimethyl-	×	×	×	×	×	×	
5-Undecene, (E)-	×	×	×	×	×	×	
5-Undecene, (Z)-	×	×	×	×	×	×	
3-Dodecene, (Z)-	×	×	×	×	×	×	
1-Nonene	×	×	×	×	×	×	
8-Heptadecene	×	×	×	×	×	×	[21,30,51,52]
Aromandendrene	×	×	×	×	×	×	[22,52]
Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4Z,9S*)]-	×	×	×	×	×	×	
Cyclohexane, (2-ethyl-1-methylbutylidene)-	×	×	×	×	×	×	
Cyclohexane, butyl-	×	×	×	×	×	×	
Cyclohexane, hexyl-	×	×	×	×	×	×	
Cyclohexane, propyl-	×		×	×	×	×	[51,57]
Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	×	×	×	×	×	×	[51,53]
Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-					×	×	
Decane	×	×	×	×		×	[28,38,47,53,57-59]
Decane, 2,3,5,8-tetramethyl-	×	×	×	×	×	×	
Decane, 2,4,6-trimethyl-	×	×	×	×	×	×	[57]
Decane, 2,6-dimethyl	×	×	×	×	×	×	
Decane, 4-methyl-	×	×	×	×	×	×	
Decane, 5-methyl-	×	×	×	×	×	×	
Dodecane	×	×	×	×	×	×	[18,28,47,51]

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Volatile Organic Compounds (VOCs)	Postmortem Interval (Months)						Literature References
	1	3	6	12	18	24	
Dodecane, 2,7,10-trimethyl-	×	×	×	×	×	×	
Heptadecane	×	×	×	×	×	×	[29,30,51–53]
Nonane	×	×	×	×	×	×	[18,19,23,47,51–53,55,58]
Nonane, 2-methyl-	×	×	×	×	×	×	[57]
Nonane, 3-methyl-	×	×	×	×	×	×	[38,47]
Octane, 3-ethyl-	×	×	×	×	×	×	
Undecane	×	×	×	×	×	×	[18,28,29,44,49,51–53,55,57–59]
Undecane, 2,6-dimethyl-	×	×	×	×	×	×	[46,57]
Undecane, 2-methyl-	×	×	×	×	×	×	[47]
Undecane, 3-methyl-	×	×	×	×	×	×	[47]
Undecane, 4-methyl-	×	×	×	×	×	×	
á-Pinene	×	×	×	×	×	×	[18,21,22,30,32,47,51,52,54,55]
Ketone							
2,3-Pentanedione	×	×	×	×	×	×	[22,51,52]
2,5-Hexanedione		×	×	×	×	×	[18]
2-Decanone	×	×	×	×	×	×	[18,19,28,30,51]
2-Tridecanone	×	×	×	×	×	×	[48,53]
2-Dodecanone	×	×	×	×	×	×	
2-Nonadecanone	×	×	×	×	×		[21,30]
2-Nonanone	×	×	×	×	×	×	[18,19,21,28,30,46,48,51–53,59]
2-Pentanone, 4-hydroxy-4-methyl-	×	×	×	×	×	×	[51,57]
2-Tetradecanone	×	×	×	×	×		[51]
3,4-Hexanedione	×		×				
3,5-Octadien-2-one, (E,E)-	×	×	×	×	×	×	[28,30]
3-Hepten-2-one, 5-methyl-	×	×	×	×	×	×	
3-Nonen-2-one	×	×	×	×	×	×	
3-Octen-2-one	×	×	×	×	×	×	[19,30,51]
3-Penten-2-one, (E)-	×	×	×	×	×	×	[46]
4-Penten-2-one, 4-methyl-	×	×					[18]
Ethanone, 1-(3-butyloxiranyl)-	×	×	×	×	×	×	
N-containing							
1-Butanamine		×	×				
1-Butanamine, 3-methyl-N-(2-phenylethylidene)-	×	×	×				

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Volatile Organic Compounds (VOCs)	Postmortem Interval (Months)						Literature References
	1	3	6	12	18	24	
1-Butanamine, 3-methyl-N-(3-methylbutylidene)-		×	×				[51]
1-Butanol, 3-methyl-, nitrate	×	×	×	×	×		
1H-Indole, 3-methyl-		×	×	×	×	×	[18,19,53]
1H-Pyrrole, 1-methyl-	×	×	×				[51]
1H-Pyrrole, 2,4-dimethyl-		×	×		×	×	
1H-Pyrrole, 2,5-dimethyl-	×	×	×	×			[18,51]
1H-Pyrrole-2,5-dione, 1-methyl-	×						
1H-Pyrrole-2-carboxaldehyde, 1-methyl-	×	×	×				
2-Piperidinone	×	×	×	×			[18,21,30,46,51]
2-Pyrrolidineethanol, 1-methyl-	×						
5H-1-Pyridine	×	×	×	×	×	×	[22,52]
8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-	×	×	×				
Acetamide	×	×	×	×			[18,46]
Acetamide, 2,2,2-trifluoro-		×	×	×	×	×	
Acetamide, 2,2,2-trifluoro-N-(2-methylpropyl)-		×	×		×		
Acetamide, 2,2,2-trifluoro-N-(2-phenylethyl)-		×	×		×		
Acetamide, 2,2,2-trifluoro-N-propyl-			×				
Acetamide, N-(3-methylbutyl)-	×	×	×	×	×		
Acetamide, N-butyl-2,2,2-trifluoro-		×	×		×		
Amantadine	×	×	×				
Benzonitrile	×	×	×	×	×	×	[19,21–23,29,30,32,44,46,48,51–53,55]
Butanamide	×	×	×				[18,46,51]
Butanamide, 3-methyl-	×	×	×	×			[18,46,51]
Cyclohexanone, 3-methyl-	×	×	×		×	×	[48,51,52,55]
Hexanamide	×	×	×	×			[18]
Hexane, 1-nitro-	×	×	×	×	×	×	
Indole	×	×	×	×	×		[10,18,19,21,28,30,46,48,51–53,56,60]
Methanamine, N-heptylidene-	×	×	×				
Methylamine, N,N-dimethyl-	×	×	×	×	×	×	[22,51,52,57]
Pentanal, oxime	×	×	×	×			
Propanamide	×	×	×				[18,46]
Propanamide, 2-methyl-	×	×	×				[18]
Propanamide, N,2-dimethyl-	×	×	×		×		[18]

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Volatile Organic Compounds (VOCs)	Postmortem Interval (Months)						Literature References
	1	3	6	12	18	24	
Propanamide, N-hexyl-	×	×	×	×	×		
Propanamide, N-methyl-		×	×				[18,46]
Pyrazine, tetramethyl-	×	×	×	×	×	×	[18,53,56]
Pyrazine, trimethyl-	×	×	×	×	×	×	[18,28,46,51]
Pyridine, 2-methyl-	×	×	×	×	×	×	[18,51,52]
Pyridine, 2-pentyl-	×	×	×	×			
Pyridine, 2-methyl-6-propyl-	×	×	×	×			
Pyridine, 3-butyl-	×	×	×				
Pyridine, 3-ethyl-	×	×					
Pyridine, 3-propyl-	×	×					
Pyrrole, 1,2,5-trimethyl-		×	×				
<i>S-containing</i>							
2-Furanmethanethiol, 5-methyl-	×	×			×		
3-(Methylthio)propanoic acid methyl ester		×	×	×			
Dimethyl sulfone	×	×					[22,38,47,51,57]
Disulfide, methyl pentyl	×	×	×	×	×		[53]
Isothiocyanate, 2,5-dimethylphenyl	×	×	×		×		
Methanethiol	×	×	×	×	×	×	[22,46,47,51–53,57,60]
Pentane, 1-(methylthio)-	×	×		×			[51]
Sulfurous acid, isobutyl pentyl ester	×		×				
Thiophene, 2-pentyl-	×	×	×	×	×	×	[51,52]

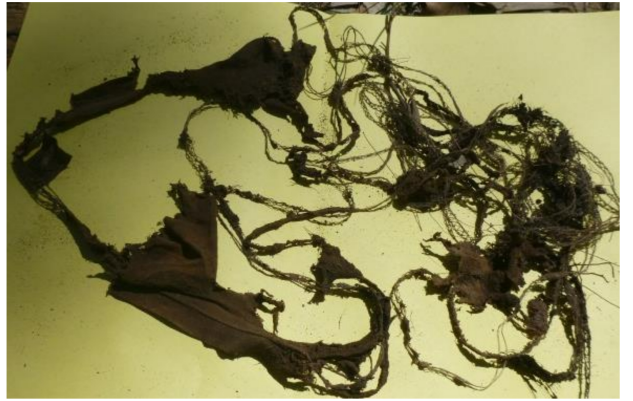
a)

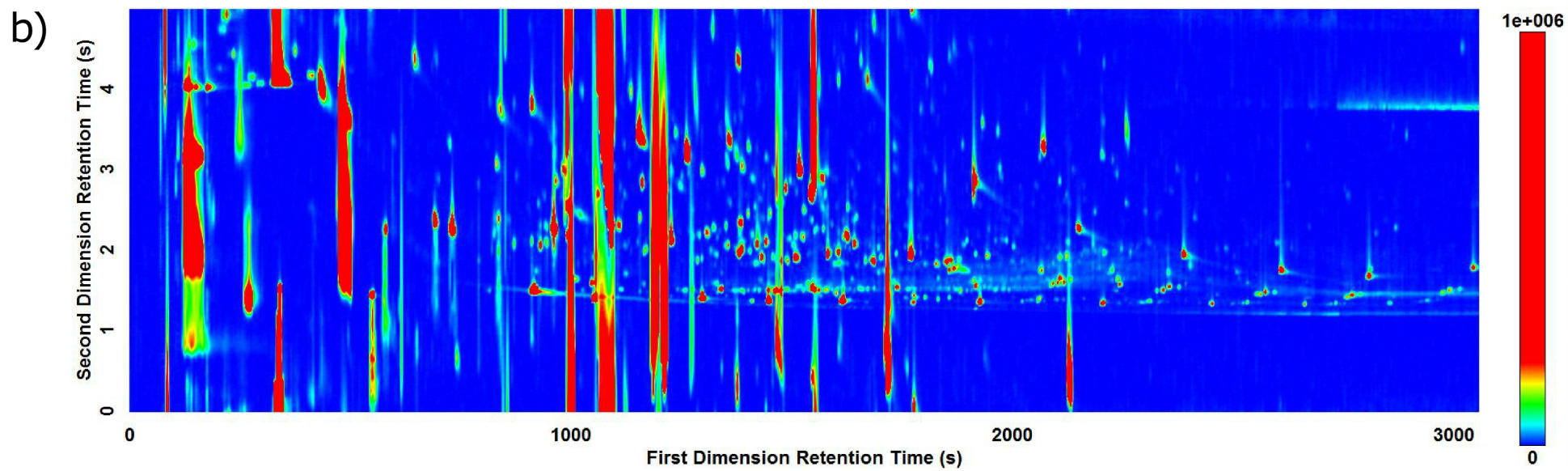
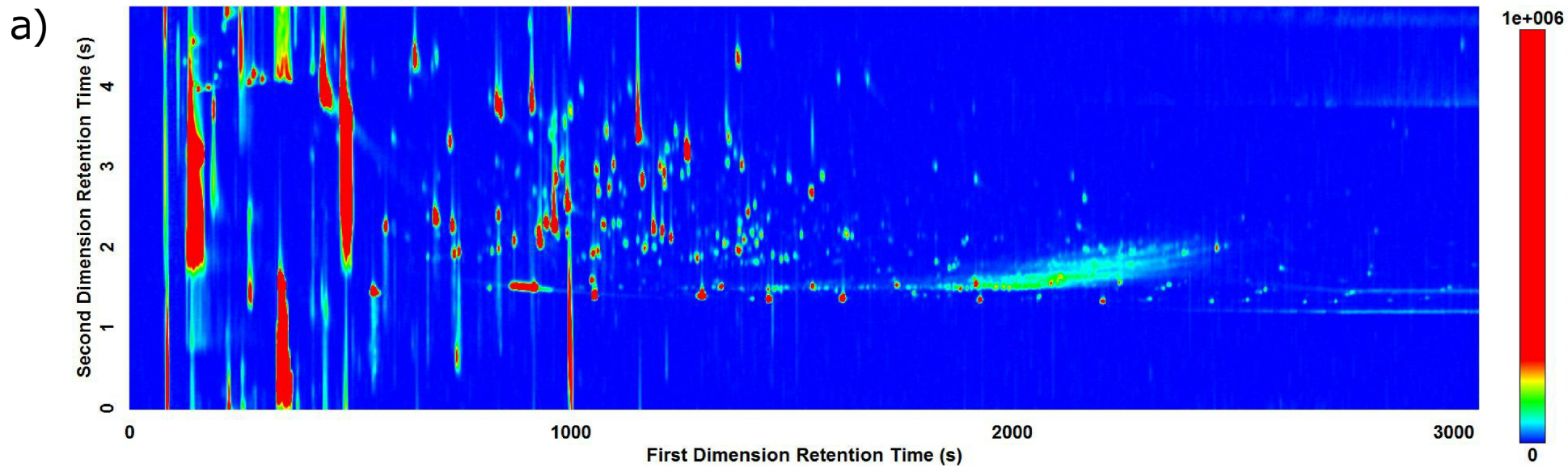


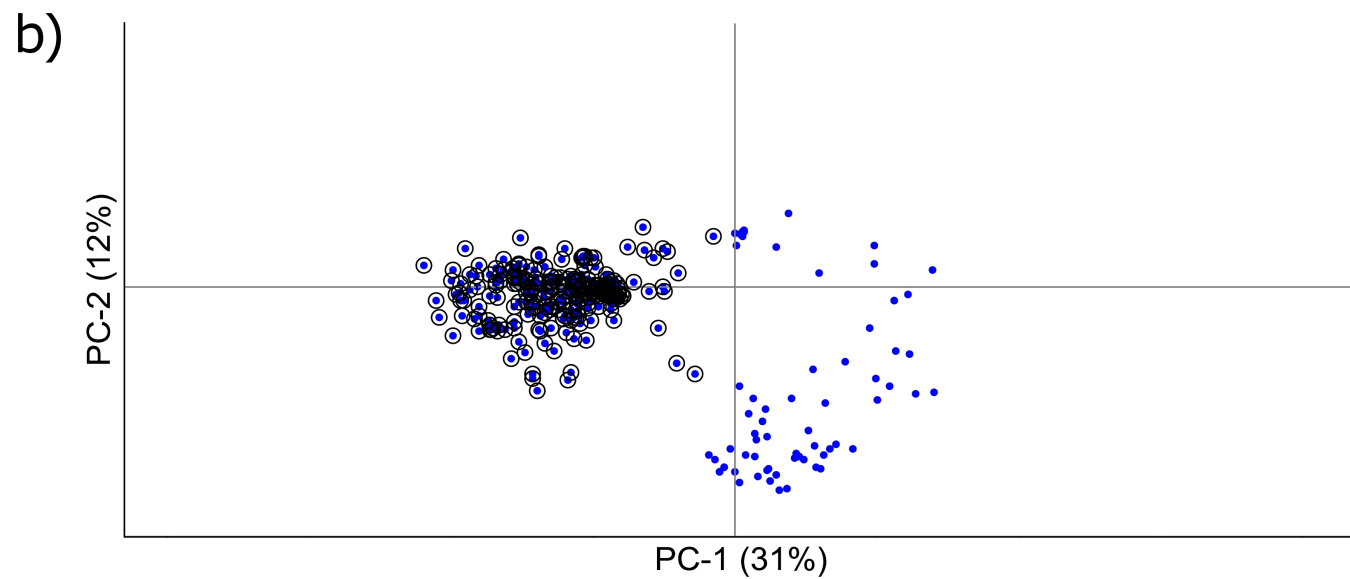
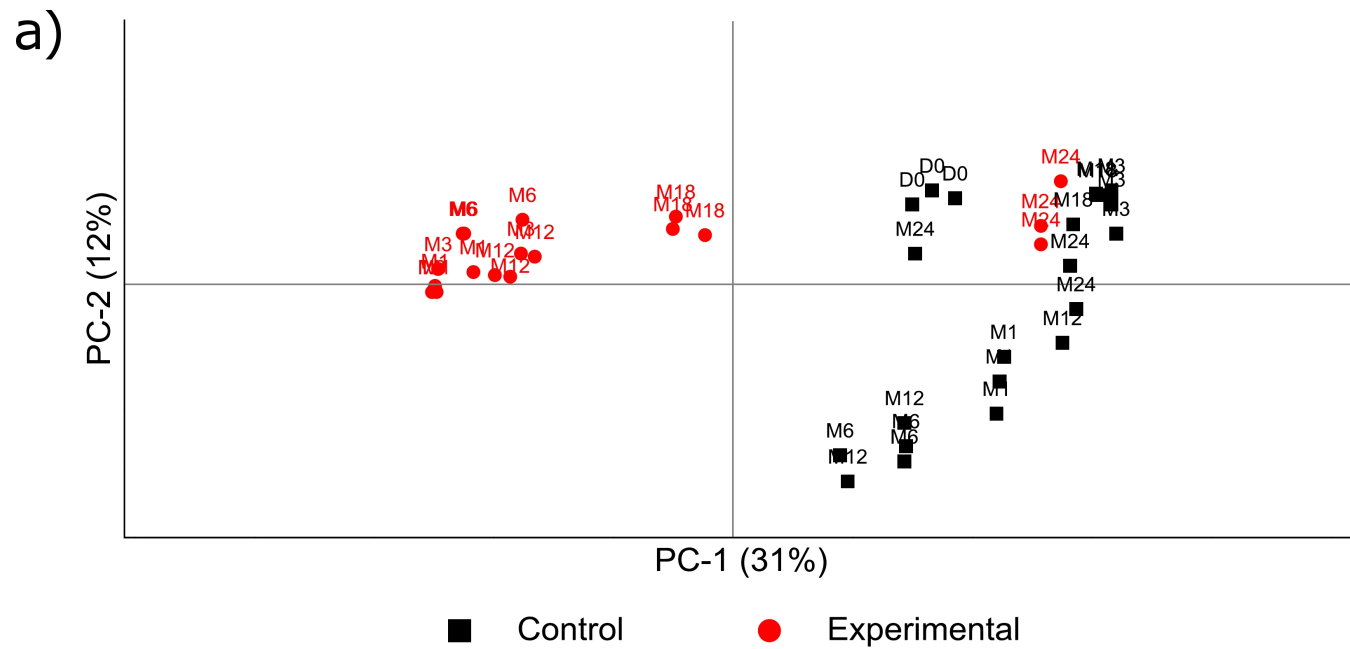
b)

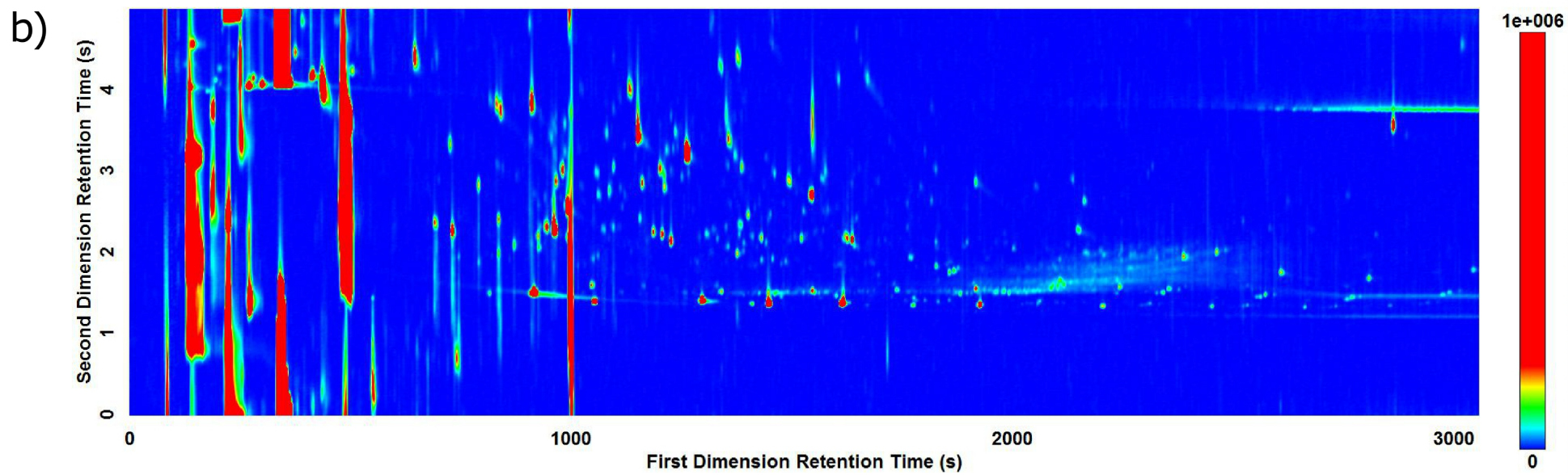
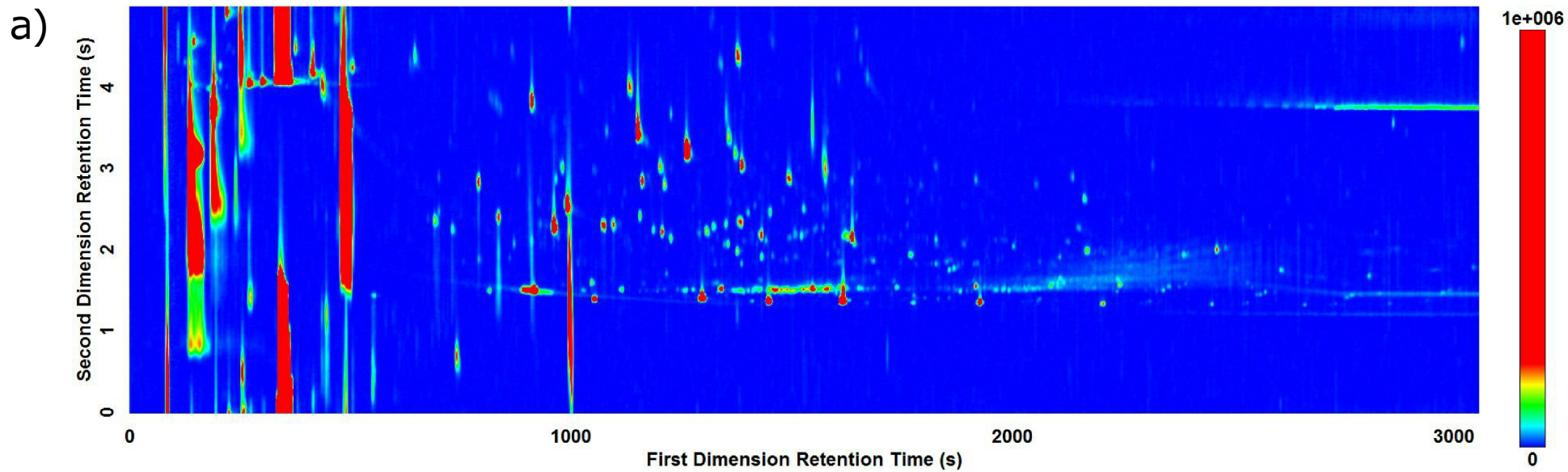


c)









Highlights:

- Odour profiles of textiles associated with decomposing remains were investigated
- Pig carcasses clothed in 100% cotton t-shirts were used as human odour analogues
- Textiles collected at different postmortem intervals exhibited variation in odour
- Overall odour profile reflected a large subset of cadaveric decomposition odour
- Results suggest decomposition odour may not remain trapped in textile indefinitely