A comparison of human and pig decomposition rates and odour profiles in an Australian environment

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Abstract

Cadaver-detection dogs are trained to locate victim remains; however, their training is challenging due to limited access to human remains. Animal analogues, such as pigs, are typically used as alternative training aids. This project aimed to compare the visual decomposition and volatile organic compound (VOC) profile of human and pig remains in an Australian environment, to determine the suitability of pig remains as human odour analogues for cadaver-detection dog training. Four human cadavers and four pig carcasses were placed in an outdoor environment at the Australian Facility for Taphonomic Experimental Research (AFTER) across two seasons. Decomposition was monitored progressively in summer and winter. VOCs were collected onto sorbent tubes and analysed using comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry. Visual observations highlighted the differences in decomposition rates, with pig remains progressing through all stages of decomposition, and human remains undergoing differential decomposition and mummification. Chemical and statistical analysis highlighted variations in the composition and abundance of VOCs over time between the odour profiles. This study concluded that the visual decomposition and VOC profile of pig and human remains was dissimilar. However, in cooler conditions the results from each species became more comparable, especially during the early stages of decomposition.

Keywords:

Forensic taphonomy; volatile organic compounds; decomposition; GC×GC-TOFMS; AFTER

*Corresponding Author: Dr Maiken Ueland. Email: maiken.ueland@uts.edu.au

1. Introduction

In cases where remains are concealed either naturally (i.e. disaster victims) or intentionally (i.e. homicides), investigators need a reliable search tool to assist in the search and recovery of victim remains. As a body decomposes, the organic components of the body are slowly broken down into smaller gas and liquid molecules[1]. Complex mixtures of volatile organic compounds (VOCs) represent many of the gaseous compounds released as by- and end-products of the decomposition process[1]. VOC mixtures form a dynamic odour profile that insects and canines can both utilise to track and locate remains[1,2]. Due to their superior olfactory systems (compared to humans), canines have an enhanced ability to detect specific odours. For example, cadaver-detection dogs are trained to detect the scent of decomposition in order to assist with victim recovery operations[1,2]. These dogs are trained using a range of natural and artificial training aids to continually imprint them with the variety of decomposition odour profiles that could be encountered in criminal and mass disaster investigations[1,2].

In many countries, ethical and legal restrictions prevent the use of human cadavers in the training of cadaver-detection dogs and in decomposition research studies generally[1]. This has led handlers and researchers alike to utilise either small samples of human remains (e.g. cadaveric blood, bone, soft tissue, or decomposition fluid) or human analogues[1]. Until 2016, decomposition studies in Sydney, Australia rarely used human remains, and particularly not outside of a mortuary setting. Studies[3,4,5,6,7,8,9,10] instead utilised pig carcasses as human analogues. Pigs were considered suitable human decomposition analogues due to the similarity of their internal anatomy and gut biota to humans. They are also more readily available and often do not require ethics approval (i.e. for adults not bred for research purposes)[11]. However, the recently established Australian Facility for Taphonomic Experimental Research (AFTER) is a licensed facility that meets the ethical and legal requirements necessary for research involving human cadavers, but such research can only be conducted in the local environment of Sydney. It is therefore important to compare the process of decomposition between the two species to determine the suitability of pig carcasses as human decomposition analogues. This is also important in understanding the applicability of using human analogues as training aids for cadaver-detection dogs.

While the decomposition process is a complex, variable and multi-faceted process, it is often classified into five stages: fresh, bloat, active decay, advanced decay, and dry remains or skeletonization[12,13,14]. However, the complex mechanisms involved in human decomposition can result in the occurrence of differential decomposition. This refers to the

presence of multiple stages of decomposition occurring concurrently on a body[15]. Factors that can influence differential decomposition include insect activity, differential temperatures, partial concealment of remains in soil or water, sun versus shade, etc.[15]. Due to the difficulty and subjectivity in assessing decomposition through conventional staging, several studies have attempted to provide a more objective method of determination[16,17]. Total Body Score (TBS) is a numeric system for quantifying the amount of decomposition at any given point in time[3,15,16,18]. The system works by classifying the remains into regions and then scoring each of these regions individually based on specific criteria[3,15,16,18]. The individual scores are combined to produce a final TBS. This system caters to the presence of differential decomposition across the body by evaluating each region of the body, rather than assigning an arbitrary classification that may not represent the overall state of the remains[3,15,16,18]. As each stage is characterized by its own set of distinct qualities, each stage is also known to have its own odour signature[23]. It is for this reason that dogs require a variety of training aids, to account for the dynamic nature of decomposition odour across all stages of the process.

This study involved the collection of a headspace sample from above human and pig remains onto sorbent tubes. The samples were analysed using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS). Sorbent tubes were used rather than other sample collection methods such as solid-phase microextraction (SMPE) fibres due to their suitability for use in field studies and based on a history of similar studies that have utilised this technique with success[8,9,10,19,20,21]. GC×GC provides a greater degree of separation which makes it more useful than one dimensional GC in comprehensive screening studies[7]. TOFMS is more suitable for non-target analyses and has a faster acquisition rate that can accommodate the narrower peaks produced by GC×GC[22]. GC×GC-TOFMS has been recognised as a successful method for decomposition odour analysis and has become common practice in a number of recent studies[7,8,9,21,23,24,25,26].

The aim of this study was to compare the decomposition process of human and pig remains in the Sydney environment, both visually and chemically through VOC analysis. A recent study[27] found that animal analogues were not accurate representations of human decomposition in a Tennessee environment. Specifically, the study reported a faster rate of decomposition for pig remains and much greater variability in the process of human decomposition. The current study intends to not only compare the rate of decomposition but to also compare the decomposition odour of pig and human remains in the natural Sydney environment and across the entire decomposition process.

2. Materials and method

2.1 Experimental Design

The study was conducted in a natural outdoor Australian environment in Western Sydney on land privately owned by the University of Technology Sydney (UTS). The human remains were located at AFTER while the pig carcasses were placed in the same location but outside the AFTER fence to comply with the licencing requirements of AFTER. The research area consisted of open eucalypt woodland, defined as Cumberland Dry Sclerophyll Forest. Soils at the site are broadly classified as sandy clay loam or gravelly sandy clay, with a pH range from 5.5—6.5. The site encompasses approximately 4.86 hectares of land surrounded by a high-security fence with closed-circuit television (CCTV) cameras operating continuously.

To compare the human and pig VOC profile, domestic pig carcasses (*Sus scrofa domesticus L.*) weighing 60 – 80 kg were compared to donated human cadavers weighing 60 – 90 kg. In order to account for potential seasonal differences, two experimental trials were conducted. The summer trial was conducted from February 2 – March 8, 2016 using two human cadavers (16-02 and 16-03) and two pig carcasses (SP1 and SP2), and the winter trial was conducted from July 27 – August 30, 2016, also using two human cadavers (16-17 and 16-18) and two pig carcasses (WP1 and WP2).

Conforming to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004), animal ethics approval was not required, as the pig carcasses used in this study did not include living or foetal subjects. The pig carcasses were purchased post-mortem from a licensed abattoir. All pigs were killed by captive-head bolt following the standard guidelines for Australian abattoir procedures. All carcasses were wrapped in large polyethylene tarpaulins for transportation to the site. The pig remains were placed directly onto the soil surface approximately 3 m apart within 1 h of death. No visible signs of decomposition were observed on the carcasses when they were placed at the site.

The four human cadavers used in this study were acquired through the UTS Body Donation Program overseen by the Surgical and Anatomical Science Facility (SASF) at UTS. Consent was provided by all donors to use their remains for the purposes of research at AFTER, in accordance with the NSW Anatomy Act (1977). The research project was approved under the UTS Human Research Ethics Committee Program Approval (UTS HREC REF NO. ETH15-0029). All donors were placed directly onto the soil surface at AFTER, in the centre of individual 5 m × 5 m plots. In the summer trial, the plots for donors 16-02 and

16-03 were approximately 10 m apart, while in the winter trial the plots for donors 16-17 and 16-18 were adjacent to one another, with the remains approximately 3 m apart. All four donors were male and arrived with no visible signs of decomposition, with the exception of donor 16-17 who demonstrated signs of early decomposition.

For all trials, a control site located a minimum of 3 m from the remains was established to collect control samples that would identify the background VOCs naturally produced in the surrounding environment. Anti-scavenging cages were placed over the remains when they were not being sampled. These were designed to discourage vertebrate animals from scavenging, while still allowing for invertebrate scavenging to occur and exposure to normal weather conditions.

Visual observations were recorded (i.e. written notes and photographs) once per sampling day. In addition to general observations, the remains were also assigned a TBS adapted from Megyesi et al.[16]. The summer trial was carried out for 34 experimental days and the winter trial for 79 experimental days to cover the range of decomposition processes typically experienced in this environment. Sample collection was performed at varying intervals depending on the expected decomposition rates with additional sampling days in the winter trial (n=15) compared to the summer trial (n=12) due to the slower decomposition rates observed during the early post-mortem period of the winter trial. The stage of decomposition was reported in experimental days (ED).

For each trial, a Hobo Weather Station equipped with a Hobo U30 No Remote Communication data logger (OneTemp, Marleston, NSW, Australia) was used to monitor temperature (°C) and rainfall (mm) at an hourly rate.

2.2 VOC sample collection

The method for sampling VOCs was adapted from headspace VOC collection used in previous research[7,8,9,21,23,24,25,26]. An aluminium hood was placed over the remains and left for 15 min to allow the VOCs to accumulate in the headspace. An ACTI-VOC low flow air sampling pump (Markes international Ltd., Llantrisant, UK) was connected to one end of a dual sorbent tube containing Tenax TA and Carbograph 5TD (Markes international Ltd.), with the other end of the tube attached to the sampling port on the aluminium hood. The pump was used to actively draw 1 L of headspace through the sorbent tube at a flow rate of 100 mL/min. All tubes were sealed with brass storage-caps after collection, wrapped in aluminium foil and placed in an airtight glass container for transportation and storage in the laboratory. The sorbent tubes were stored at 4 °C until the sample analysis was performed.

2.3 GC×GC-TOFMS analysis

To enable peak area normalisation, an internal standard consisting of 2 µl of 150 ppm bromobenzene (GC grade, Sigma-Aldrich, Castle Hill, NSW, Australia) in methanol (HPLC Grade, Sigma-Aldrich) was injected onto each sorbent tube prior to analysis.

A Markes Unity 2 Thermal Desorber and Series 2 ULTRA multi-tube autosampler (Markes International Ltd.) was used to perform thermal desorption of the sorbent tubes. Each sorbent tube was heated to 300 °C for 4 min to allow thermal desorption of the compounds before being collected onto a general-purpose cold trap (TenaxTA/Carbograph 1TD) at -10 °C. The trap was desorbed at 300 °C for 3 min with a split flow of 20 mL/min.

The thermal desorption unit was connected to a Pegasus® 4D GC×GC-TOFMS (LECO, Castle Hill, NSW, Australia) using a 1 m uncoated silica transfer line (Markes International Ltd.) held at 120 °C by way of an Ultimate Union Kit (Agilent Technologies, Mulgrave, NSW, Australia). A 30 m × 0.25 mm inner diameter (ID), 1.40 µm film thickness Rxi®-624Sil MS column (Restek Corporation, Bellefonte, PA, USA) was used as the first dimension column, and a 2 m × 0.25 mm ID, 0.50 µm film thickness Stabilwax® column (Restek Corporation) was used as the second dimension column. The columns were joined with a SilTite µ-Union (SGE Analytical Science). Helium (high purity, BOC, Sydney, NSW, Australia) was used as the carrier gas at a constant flow rate of 1.00 mL/min. The first dimension oven was set to 35 °C and held at this temperature for 5 min before increasing at a rate of 5 °C/min to 240 °C where it was held for a further 5 min. The offset for the modulator was +5 °C relative to the GC first dimension oven temperature and the offset for the second dimension column was +15 °C relative to the second dimension oven temperature. The modulation period was 5 s with a 1 s hot pulse. The transfer line between the second dimension column and the MS was held at 250 °C. An acquisition rate of 100 spectra/s was used to target a mass acquisition range of 29 – 450 amu. The ion source was held at 200 °C, the electron ionisation energy was 70 eV, and the detector voltage was programmed with a 200 V offset above the optimized detector voltage determined.

2.4 Data processing

ChromaTOF® (version 4.51.6.0; LECO) was used for data processing. A 150 signal-to-noise (*S/N*) ratio was used with a baseline offset of 0.8. The peak widths for the first and second dimensions were 30 s and 0.15 s, respectively. The National Institute of Standards and Technology (NIST) Mass Spectral Library was used to establish a list of compounds with a

mass spectral match threshold of 80%. Peak alignment was performed between samples using a mass spectral match threshold of 60% by utilising the Statistical Compare software feature within ChromaTOF®. Once aligned, analyte peak areas were normalised based on the peak area of the internal standard. The samples were organised into two classes per analysis: experimental (n=24 for the summer trial and n=30 for the winter trial), and control (n=12 for the summer trial and n=15 for the winter trial). This procedure was carried out separately for the human and pig samples; however, an additional analysis that combined the two datasets was also performed (i.e. pig vs. human). The additional analysis also consisted of two classes: experimental (n=48 for the summer trial and n=60 for the winter trial), and control (n=24 for the summer trial and n=30 for the winter trial). During alignment, analytes were only retained if found in at least 3 of the total samples for that trial or in 10% of the samples within a class.

The Statistical Compare software feature was used to calculate the Fisher ratio of each compound detected. A Fisher ratio threshold was established based on a critical F value (F_{crit}) that was calculated using the number of classes, the degrees of freedom per class, and a significance level of 0.05. Compounds that had a Fisher ratio lower than the F_{crit} value were excluded. Compounds that arose due to chromatographic artefacts or were a result of column/sorbent bleed were also removed. The final peak table was further processed using Microsoft Excel.

Unscrambler® X (version 10.5; CAMO Software, Oslo, Norway) was used to perform principal component analysis (PCA). Mean centering, variance scaling and unit vector normalisation were all applied to the datasets prior to PCA. The data was shown to contain no outliers by way of Hotelling's T2 95% confidence limit.

3. Results and discussion

3.1 Weather Conditions

During the summer trial, the average daily temperature was 23.5 °C with a total range of 14.7 °C – 40.6 °C. A total of 3 mm of rainfall was recorded during this period. In the winter trial, the average daily temperature was 11.1 °C with a total range of 1.1 °C – 27.3 °C. A total of 34.2 mm of rainfall was recorded during this period.

3.2 Visual Comparison

3.2.1 Summer Trial

On ED 0 of the summer trial, both human cadavers were defined as being in the fresh stage and the posterior of the torso for donor 16-02 continued to be scored as fresh until ED 16. Both donors developed mild bloat on ED 1, before entering full bloat on ED 6. Donor 16-03 developed mild bloat on ED 6 and entered full bloat on ED 10. Bloating began to subside on ED 14 for donor 16-02 and ED 16 for donor 16-03; with both sets of remains displaying post-bloat deflation by ED 21. Active decay was only observed internally and along the soil line/posterior aspect of the torso and upper limbs. In both donors, active decay started between ED 14 – 21. Both sets of remains began to transition into advanced decay in these regions as the trial ended from ED 28 – 35.

There was a clear trend towards differential decomposition and preservation exhibited by both sets of human remains. Desiccation occurred as early as ED 10 in donor 16-03. The head and neck region and the anterior aspect of both donors were desiccated by ED 14. The posterior aspect of the arms was the only area exhibiting skeletonization in donor 16-02, occurring around ED 21. Donor 16-03 showed the same skeletonization pattern, although the donor had additional skeletonization of the face due to heavy entomological scavenging around the nose. Donor 16-03 first showed signs of skeletonization of the face on ED 28, with skeletonization of the arms developing on ED 35.

The decomposition process was faster for the pig remains compared to the human remains, similar to the study reported in Tennessee[27]. On ED 0 of the summer trial, both carcasses were defined as being in the fresh stage. By ED 1, both carcasses had entered the bloat stage. Bloating had subsided by ED 6 and active decay was observed from ED 6 – 8. The progression of active decay was rapid causing skeletonization to occur in the head and limbs for both sets of remains during this time. The carcasses transitioned into advanced decay from ED 10 to ED 35, but also exhibited some desiccation during active decay (ED 6 – 8). This was likely due to the high temperatures exhibited during the summer trial. Desiccation persisted through advanced decay, however, the carcasses showed a more typical trend towards skeletonization, which began during active decay and increased over time until the end of the trial (ED 6 – 35).

Despite the use of anti-scavenging cages, some animal scavenging occurred as a result of burrowing underneath the cages. The presence of animal scavenging during this trial meant that some of the remaining desiccated tissue was removed manually, with both carcasses becoming fully skeletonised around ED 28 – 35. The warmer daily temperatures and minimal rainfall during the summer trial were likely responsible for the faster decomposition rates and desiccation observed compared to the winter trial. Differences

between the pig and human decomposition during this trial was likely due to biological dissimilarities between the two species such as distribution of body weight rather than environmental variables, since the remains were placed in the same location. Additionally, the regions accessed and degree of insect activity varied between species demonstrating greater soft tissue loss and skeletonization in pig remains.

3.2.2 Winter Trial

In the winter trial, human and pig remains were classified as being in the fresh stage of decomposition on ED 0. However, donor 16-17 had visibly undergone some decomposition and was defined as being in the late stages of fresh decomposition, with green discolouration and fluid blisters indicating that enzymatic decay processes had already commenced. Donor 16-18 maintained some areas with fresh tissue throughout the entire trial, with the feet and lower legs experiencing minimal decomposition. Bloat only occurred on one of the two human remains. Donor 16-17 displayed mild bloating on ED 27, though this had subsided by ED 34. Similar to the summer trial, active decay was confined internally and was only visually apparent in the head and neck regions. Active decay was observed during ED 13 – 34 for donor 16-17 and ED 16 – 34 for donor 16-18. The remains in the winter trial also demonstrated a trend towards differential decomposition and preservation. Desiccation was first observed on donor 16-18 on ED 16 and on donor 16-17 on ED 20. In both cases, the desiccation began in the head and neck region, followed by the arms, and progressed slowly across the anterior of the body until ED 79, marked by a slow continual darkening of the desiccated skin. Skeletonization was first observed on ED 16 of donor 16-18 around the lower jaw, due to entomological activity in this area. On ED 41, the upper torso of 16-17 began to show signs of skeletonization. By ED 50 this had extended to also include the posterior aspect of the arms and head on both 16-17 and 16-18, and the groin of 16-17 on ED 79.

The pig remains also progressed slower through the decomposition timeline in comparison to their summer trial counterparts. WP1 showed signs of bloating on ED 9, and full bloat from ED 13 – 27. WP2 showed signs of bloating on ED 6, and full bloat from ED 9 – 27. Both carcasses exhibited post-bloat deflation as they progressed into active decay on ED 34, fully deflating by ED 50. Unlike the human remains, the pig remains exhibited far less desiccation during the trial period. The skin of WP1 became darker and leathery on ED 64; however, it had not fully desiccated by ED 79. The pigs showed a greater precedence towards soft tissue loss and therefore skeletonization. Skeletonization of the head and neck

began on ED 41 in both carcasses, with the limbs also showing signs of skeletonization on ED 50. The areas of skeletonization increased and became more prominent until the end of the trial on ED 79. The cooler daily temperatures and increased prevalence of rainfall during the winter trial were likely responsible for the slower decomposition rates observed during the winter trial, and these findings were comparable to other studies in the same region using pig remains[9,10].

In both trials, the human remains demonstrated differential decomposition with the posterior progressing into active decay but the anterior becoming mummified. In contrast, the pig remains progressed through the more typical stages of decomposition resulting in skeletonization. These differences can be predominantly associated with the degree of entomological activity and regions accessed in the bodies. Insect activity was heavily localised to the head and groin for human remains during the earlier postmortem period allowing time for soft tissue to become mummified elsewhere on the remains. Ultimately, the anterior mummified tissue acted as a protective shell for the entomological activity which eventually led to active decay of the posterior in contact with the soil. In contrast, entomological activity although initiated in the head and groin of the pig remains, was observed across the entire remains leading to more rapid soft tissue loss and exposure of bone. This may be due to the different structure of the pig remains whereby the lack of a defined neck and shortened limbs provides a more uniform body mass for the insect larvae to move across and consume.

3.2.3 Total Body Scoring

Total body scoring was used to semi-quantify the degree of decomposition over time for all sets of remains. During the summer trial, TBS ranged from 3 – 32, while in the winter trial TBS ranged from 3 – 27 (Figure 1). Overall, the TBS of remains during winter were lower than those recorded in summer which correlates with visual observations. In summer, the pig remains scores were generally higher than those recorded for the human remains, with this difference increasing over time. In contrast, the TBS recorded in winter were comparable for human and pig remains throughout the trial. These trends indicate that decomposition rates vary greatly in warmer temperatures between human and pig remains; with the decomposition rates becoming more comparable for both species in cooler temperatures. This is likely because decomposition occurs at a slower rate in cooler temperatures and when rain is more prevalent[9,10]. These results also correlate with the study in Tennessee[27] reporting a faster rate of decomposition for pig remains. However, when insect activity was absent (as it

was for the first 100 days of the Tennessee study and for ED 0 - 34 of this study at AFTER during winter), the rate of pig and human decomposition was more comparable[27]. While providing some valuable insight, the original criteria and scoring system outlined by Megyesi et al.[16] was unsuitable for scoring decomposition of human remains in a temperate Sydney environment and a revised system is currently being developed.

3.3 Chemical VOC Comparison

The Statistical Compare software feature and Fisher ratio filtering was used to filter out compounds based on their individual F_{crit} values from the experimental classes (human and pig remains). The filtering process selects the compounds that are statistically relevant based on their Fisher ratio (i.e. the ratio of between-class variance to within-class variance) being higher than the F_{crit} (4.05 [Summer Trial] and 4.01 [Winter Trial]), meaning the compound is specific to a particular class of samples (i.e. experimental)[31-35]. Background VOCs from the environment were manually removed, as were VOCs known to originate from the experimental process, such as solvents and aerosols used at the field site. In the summer trial, a total of 77 VOCs were deemed statistically significant in differentiating the experimental from the control samples, with 74 of these believed to result from the decomposition process based on previous literature[1,4-10,20,21,23-26]. Seventy (70) of these VOCs were detected in the human decomposition odour profile, while all 74 were detected in the pig decomposition odour profile. Hence, there were no VOCs unique to human remains in the summer trial. In the winter trial, a total of 29 VOCs were deemed statistically significant in differentiating the experimental from the control samples, with 28 of these believed to result from the decomposition process based on previous literature[1,4-10,20,21,23-26]. Twenty-three (23) of these VOCs were detected in the human decomposition odour profile, while all 28 were detected in the pig decomposition odour profile. Hence, there were no VOCs unique to human remains in the winter trial.

An average abundance was calculated for each VOC detected from human remains and pig remains in summer and winter. These compounds were sorted into one or more of the following classes: alcohols, aldehydes, aromatics, carboxylic acids, esters, ethers, hydrocarbons, ketones, nitrogen-containing, sulfur-containing, and other (compounds not within these classes). The total class abundance (the sum of the abundances of all VOCs in a class) for each class was calculated and presented graphically in Figures 2 and 3.

In the summer trial (Figure 2), the most abundant compound class detected in human remains were hydrocarbons, followed by alcohols and aromatics. These classes were also

detected in similar odour studies using human remains conducted by Vass[25,36] and Cablk[26] who both reported hydrocarbons, alcohols and aromatics in their respective studies. For the pig remains in the summer trial, carboxylic acids were the most abundant class detected, followed by aromatics and hydrocarbons. Carboxylic acids, aromatics and hydrocarbons were also detected in several pig studies conducted by Perrault[4-7,10,20] and Forbes[8,9,28] in the same environment as the current study. In the winter trial (Figure 3), the most abundant class detected in human remains were esters, followed by hydrocarbons and aldehydes. Like those detected in the summer trial, the classes detected in the winter trial were also detected in similar odour studies using human remains conducted by Vass[25,36] and Cablk[26] who both reported esters and aldehydes in addition to hydrocarbons. Hydrocarbons were the most abundant class detected for pig remains in the winter trial, followed by sulfur-containing compounds and esters. These classes were also detected in several pig studies conducted by Perrault[4-7,10,20] and Forbes[8,9,28] in the same environment as the current study. Pig remains also demonstrated a higher abundance in the few halogenated compounds (in the 'other' class) that were detected during the winter trial. However this was rarely seen in other studies.

These results indicate that the two profiles share many of the same compounds; however, the composition and abundance of these compounds is rarely similar at any given point in time during the decomposition process. It was only during the early stages of the trials when human and pig decomposition rates were more comparable that the two odour profiles shared similarities in the composition and abundance of their odour profiles. While the specific compounds present play an important role in the overall odour profile, the ratio and abundance of the VOCs at any given point in time is also integral to this profile, particularly for training cadaver-detection dogs. This accounts for one of the major chemical differences between the decomposition odour profiles of pig and human remains.

PCA was performed to determine the statistical variation of human and pig decomposition odour profiles. The 74 compounds in the summer trial and 28 compounds in the winter trial that were determined to be of significance were used in the analyses. For the summer trial (Figure 4a), the first principal component (PC-1) accounted for the highest amount of variation (24%) in the dataset followed by the second principal component (PC-2), which explained 15% of the variance in the dataset. Samples showed intra-variability within an individual class which likely correlates with differences in the decomposition process between the two human donors as a result of factors such as their body mass, genetics, diet, and age. The samples also showed inter-variability due to differences in the rate of decomposition between human and pig remains. Samples collected on Day 16, 21, 28 and 35

when the human remains demonstrated an advanced decomposition stage formed a close cluster indicating minimal variation in the odour profile due to reduced amounts of soft tissue remaining. The Day 16, 21, 28 and 35 samples from both species are separated across both principal components showing a variation in the VOCs released from the individual samples of the pig and human remains.

For the winter trials (Figure 4b), PC-1 explains 29% of the variance while PC-2 explains 17% of the variance in the dataset. Despite the decomposition being more similar during the winter trial, there exists a notable spread of samples across both principal components. Intra-day variation in the VOC profiles of human and pig remains resulted in spread amongst the samples collected on the same day. This can be correlated with the difference in the visual comparison recorded during the field experimental trials.

The extent of statistical variation between the human and pig odour profiles suggests that, although the compounds detected using pig remains accounts for those detected using human remains, the ratio and abundance of these compounds over time demonstrates dissimilar odour signatures. This is likely due to the variation in decomposition rates observed whereby soft tissue loss was rapid in the pig remains resulting in skeletonization, but differential decomposition was observed in the human remains with mummified tissue forming on the anterior. Since there is little known about which aspects of the odour profile are utilized by the cadaver-detection dogs to recognise their target, the significance of this dissimilarity is not fully understood and needs to be further investigated involving cadaver-detection dog trials[2,29,30]. Based on the extent of analysis that was possible in this study, it is recommended that human remains be used as training aids where available. In regions where legal and ethical restrictions prevent the use of human cadaveric materials, the use of pig remains as training aids should be conducted with caution until an understanding of the significance of the difference in odour profiles between pig and human remains is achieved. Such information is important to further our understanding of the science of canine olfaction, and is particularly important for handlers who may be challenged in court on their testimony regarding training protocols of cadaver-detection dogs.

4. Conclusion

The visual findings of this study suggest that pig carcasses are not reliable analogues for describing human decomposition patterns after early decomposition in temperate Sydney environments. However, this cannot be confirmed based on the low number of replicates and

further replicate analyses are currently being performed to ascertain if these trends are repeatable. To date, the results of this study support recent findings from Tennessee[27] that reported a visual difference between the decomposition of human cadavers and animal analogues (namely pig and rabbit). If confirmed with future studies, these findings will have a significant impact on the use of pig remains to understand the process of human decomposition in the Sydney environment, and particularly their use in estimating time since death.

A comparison of the chemical odour profiles found that while the pig remains may account for the VOCs detected from human remains, the variation in ratio and abundance yield dissimilar odour profiles. This finding was supported by PCA analyses which demonstrated statistical variation between the human and pig decomposition odour profiles during both seasonal trials. The comparison of seasonal conditions for this study identified that during the cooler months, human and pig decomposition became more comparable than during the warmer months. This was also reflected in the VOC profiles, with the samples collected in winter being more comparable than those collected in summer. Until further replication is carried out to produce confirmatory findings, the results of this study suggest that cadaver-detection dogs in Sydney, Australia should continue to be trained on the odour of human remains, rather than pig remains to ensure enhanced capabilities when deployed in the field.

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Figure 1: Scatter plot of total body scores for all human and pig remains studied.

Figure 2: Total abundance of compound classes in the summer trial for average human (H) and pig (P) decomposition odour profiles.

Figure 3: Total abundance of compound classes in the winter trial for average human (H) and pig (P) decomposition odour profiles.

Figure 4: a) Principal component analysis for the summer trial, the circle shows clustering of the VOC profiles from the human donors on days 16, 21, 28 and 35. b) Principal component analysis for the winter trial. Note: a zoomed in region of the data points in the circle was added for easy viewing.

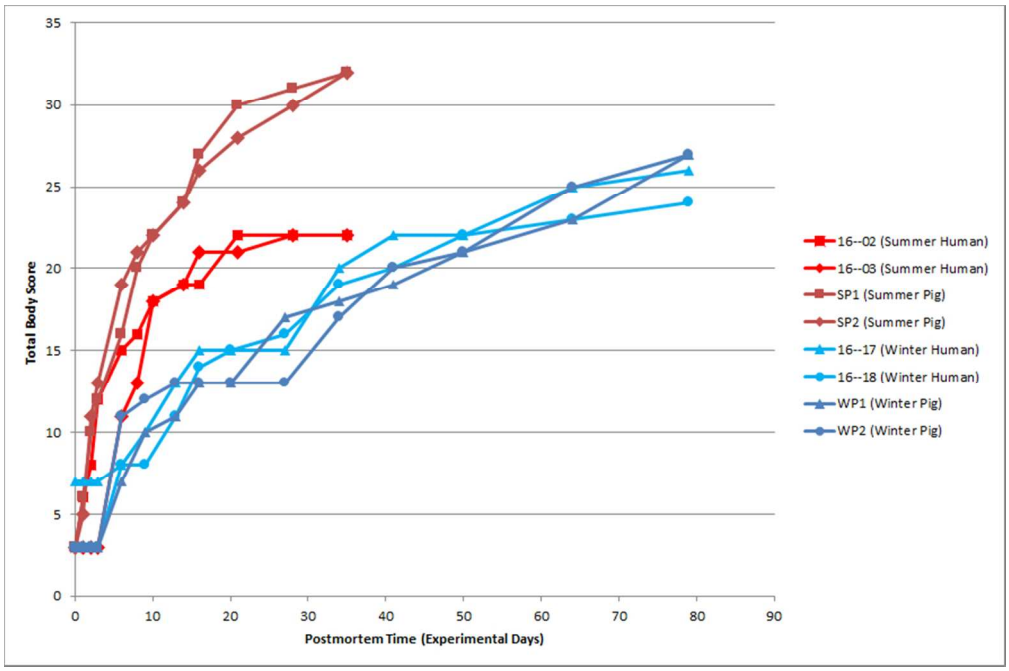

Figure 1: Scatter plot of total body scores for all human and pig remains studied.

234x155mm (96 x 96 DPI)

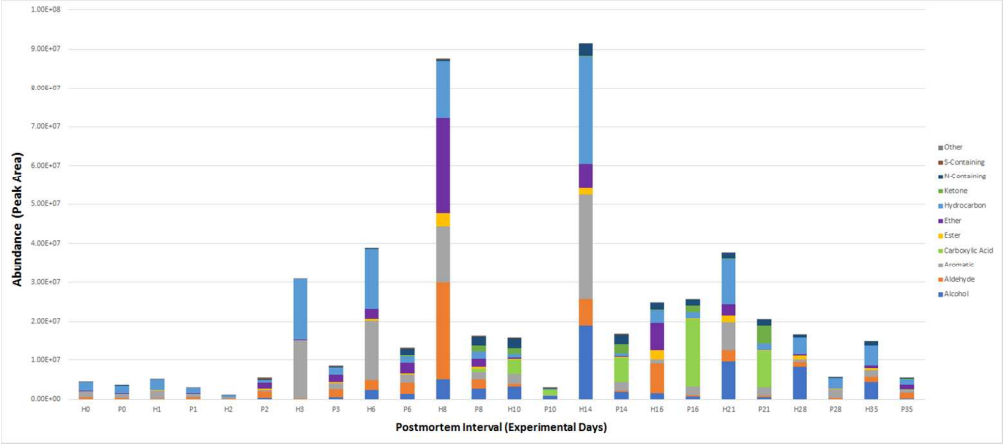

Figure 2: Total abundance of compound classes in the summer trial for average human (H) and pig (P) decomposition odour profiles.

404x179mm (96 x 96 DPI)

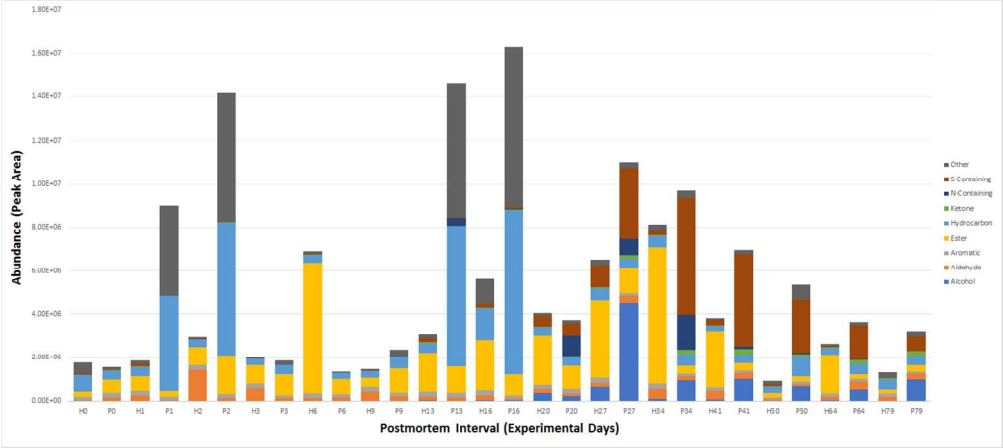

Figure 3: Total abundance of compound classes in the winter trial for average human (H) and pig (P) decomposition odour profiles.

404x180mm (96 x 96 DPI)

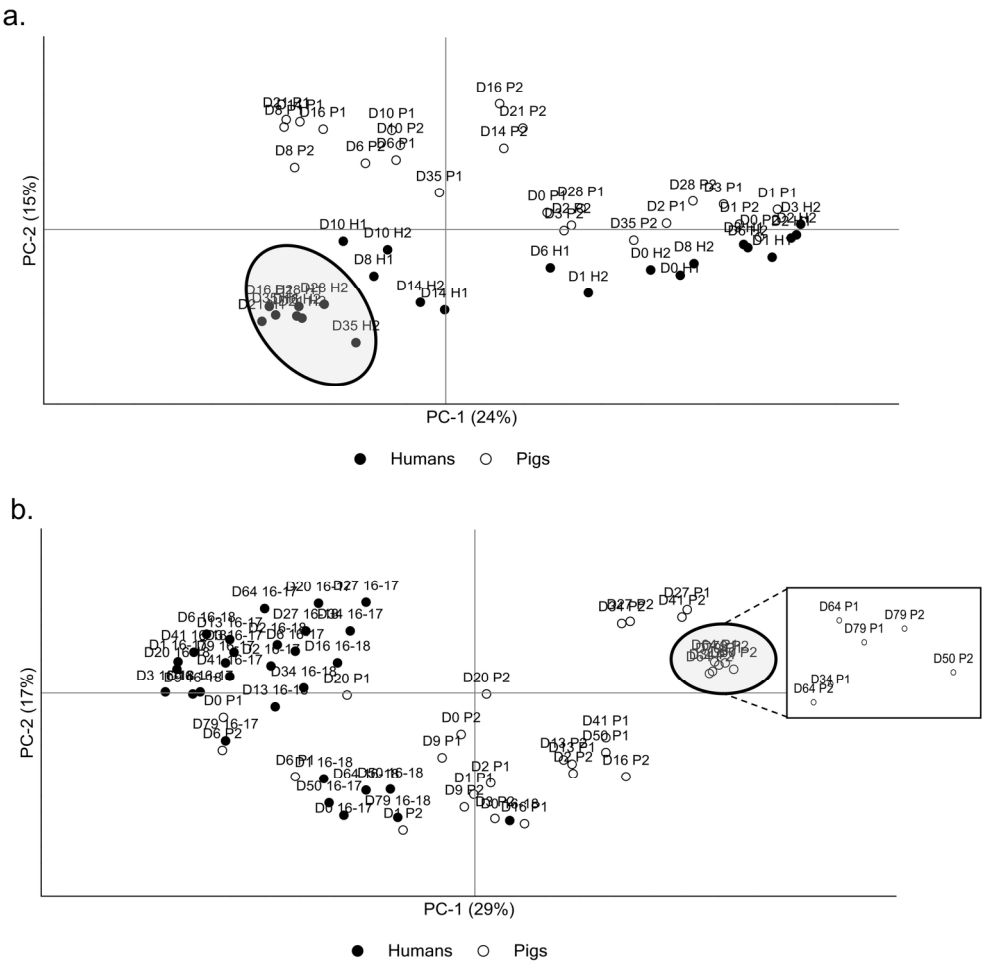

Figure 4: a) Principal component analysis for the summer trial, the circle shows clustering of the VOC profiles from the human donors on days 16, 21, 28 and 35. b) Principal component analysis for the winter trial. Note: a zoomed in region of the data points in the circle was added for easy viewing.

167x164mm (300 x 300 DPI)