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Profiling the scent of weathered training aids for blood-detection dogs

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ABSTRACT

At outdoor crime scenes, cadaver-detection and blood-detection dogs may be tasked with locating blood that is days, weeks or months old. Although it is known that the odour profile of blood will change during this time, it is currently unknown how the profile changes when exposed to the environment. Such variables must be studied in order to understand when the odour profile is no longer detectable by the scent-detection dogs and other crime scene tools should be implemented. In this study, blood was deposited onto concrete and varnished wood surfaces and weathered in an outdoor environment over a three-month period. Headspace samples were collected using solid phase microextraction (SPME) and analysed using comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC × GC–TOFMS). The chemical odour profiles were compared with the behavioural responses of cadaver-detection and blood-detection dogs during training. Data interpretation using principal component analysis (PCA) and hierarchical cluster analysis (HCA) established that the blood odour could no longer be detected using SPME–GC × GC–TOFMS after two months of weathering on both surfaces. Conversely, the blood-detection dogs had difficulty locating the blood samples after one month of weathering on concrete and after one week of weathering on varnished wood. The scent-detection dogs evaluated herein had not been previously exposed to environmentally weathered blood samples during training. Given that this study was conducted to test the dogs' baseline abilities, it is expected that with repeated exposure, the dogs' capabilities would likely improve. The knowledge gained from this study can assist in providing law enforcement with more accurate training aids for blood-detection dogs and can improve their efficiency when deployed to outdoor crime scenes.

1. Introduction

Blood has significant value when located at a crime scene. It can provide information to help identify: 1) the location of a primary or secondary crime scene; 2) persons of interest who were present at the crime scene; 3) potential murder weapons involved; or 4) other valuable evidence items. Blood evidence found at crime scenes is often present in small amounts and therefore may not be visible to the human eye; this is referred to as latent blood. As a result, appropriate detection techniques are required to locate latent blood present at a crime scene, often within a large search area. The technique used must also ensure the preservation of the evidence to allow for extraction and identification of DNA.

Luminol is a common chemical that has been used for years as a presumptive test in blood-detection; however, it requires direct contact with blood for chemiluminescence to occur, and a dark room to enhance this luminescence. This makes it inappropriate for outdoor settings where the search area is large or the location of the blood is unknown, or in cases where the luminol is likely to react with other interfering compounds present, such as bleach in a bathroom. It has also been treated with caution when used at crime scenes due to its health and safety risks such as being an irritant to eyes, respiratory systems and skin [1]. In recent years, Australia, Italy and the United Kingdom have substituted chemical blood-detection techniques with blood-detection canines, a specialised group of cadaver-detection canines trained to detect the scent of latent blood.

Scent-detection canines are employed by many law enforcement agencies to detect a specific class of contraband such as illicit drugs,

Abbreviations: BOM, Bureau of Meteorology; EDTA, ethylenediaminetetraacetic acid; F_{crit}, critical value; PC-1, first principal component; GC–MS, gas chromatography – mass spectrometry; GC × GC–TOFMS, comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry; HCA, hierarchical cluster analysis; K_3EDTA, tripotassium ethylenediaminetetraacetic acid; NIST, National Institute of Standards and Technology; PCA, principal component analysis; PDMS/DVB, polydimethylsiloxane/divinylbenzene; RSID™- Blood, Rapid Stain Identification of Human Blood; S/N, signal-to-noise ratio; (PC-2), second principal component; SPME, solid phase microextraction; TIC, total ion current; VOCs, volatile organic compounds

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firearms, explosives or accelerants, through tracking and identifying the substances' characteristic odour [2–6]. They are a desirable tool as they are minimally destructive and are highly sensitive, allowing them to easily reduce a search area even when only minute concentrations of the target odour are present [7,8]. As well as being highly sensitive, canines have high selectivity, being able to isolate and detect odours of trace amounts even in the presence of many other extraneous odours such as those naturally present in the environment [3,4,7–10]. This is particularly useful in outdoor scenes, such as bushland or other difficult terrain, where the canines can reduce the target area for further processing using more confirmatory (and costly) techniques such as Rapid Stain Identification of Human Blood (RSID™- Blood).

The canines detect their target odour through recognising its unique chemical makeup. All odours are comprised of different compounds known as volatile organic compounds (VOCs). As the canine sniffs, the air passes through the receptor cells in their nose where the VOCs bind, sending a chemical signal to the canine's brain to register the compounds present [8,11]. Canines have 20–60 times more receptor cells than humans, giving them the ability to detect a higher number of compounds at any one time, as well as those present in trace concentrations (e.g. in cases where a perpetrator may have washed away all visible blood) [4,5,12,13].

Cadaver-detection canines are primarily used to locate human remains, with some also trained to detect blood and other tissue samples. Blood-detection canines are a specialised group of cadaver-detection dogs, trained and employed to detect odours associated with human blood at both indoor and outdoor crime scenes [4,9]. At present, the main training aid used in the development of blood-detection dogs is fresh human blood deposited on bandages, soil or directly in tin cans [12]. Blood is advantageous as a training aid as it accurately represents the target odour and is able to be donated consensually, thus reducing ethical and legislative concerns [9,12].

Although they are primarily trained directly on their target odour, rather than synthetic training aids, blood-detection dogs are treated with caution when referred to in court, due to the insufficient research surrounding their abilities and limitations [14]. This is often the case when confirmatory tests are unable to validate the dog's findings. Anecdotal evidence suggests that the dogs are far superior to current technology but data to support this is currently not available in the literature. Therefore, in order for blood-detection canines to be valued in the forensic and law enforcement communities, further research is required to better understand both the VOCs liberated from blood under varying conditions and the corresponding canine response to the chemical composition of the sample, thus improving our understanding of the compounds and concentrations the canines are able to detect.

Previous research in the literature has demonstrated the consequences of utilising training aids that do not cover the full spectrum of blood odour a cadaver-detection or blood-detection dog is likely to encounter in the field [15]. By exposing cadaver-detection dogs to blood samples of varying ages, Degreeff et al. [15] demonstrated the difficulty of dogs trained exclusively on aged blood to locate fresh blood, with their accuracy increasing as the blood was aged. It has been theorised in other studies that the increase in complexity of the blood odour profile as it ages and degrades may influence the canine's ability to track and locate both fresh and degraded blood operationally [5]. The difficulty the canines experienced in locating fresh blood in the study by Degreeff et al. [15] is likely due to a difference in the odour profile of fresh and aged blood [5], and as a result, due to gaps in the canine's training.

Very little research has investigated the odour profile of blood in a forensic context, and even less research has focused on the effects of the outdoor environment. Previous studies have established that blood has a distinct odour compared to other human tissues [8], and that ageing and storage conditions also have a significant impact on the overall odour profile [5,8]. Although these studies are helpful in enhancing current training aids, and therefore increasing the dog's ability to recognise relevant VOCs, most research has been performed in a closed environment and the effects of the outdoor environment are still unknown [5,6,9]. It is important to study these environments as blood-detection canines can be employed in outdoor crime scenes and as a result, their training aids should be an accurate representation of potential casework environments.

Previous studies have collected the VOCs present in the blood odour profile from the headspace of samples using solid phase microextraction (SPME). SPME is a simple technique with minimal sample preparation and requires no contact with the sample making it ideal for extracting VOCs from biological materials. Once collected, the preferred analytical technique is gas chromatography – mass spectrometry (GC–MS), however, this technique often has insufficient resolution capabilities when analysing complex mixtures such as blood. In recent years, comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC × GC–TOFMS) has emerged as the preferred analytical technique as it provides improved separation due to its high peak capacity, allowing for a more comprehensive profile to be discovered in these complex matrices [5,16,17].

This study aimed to bridge the gap in research by identifying the effects of an outdoor environment on the odour profile of blood. The study identified VOCs present in the odour of blood deposited on concrete and varnished wood samples that were weathered in an outdoor environment over a three-month period using headspace SPME and GC × GC–TOFMS. The results from the chemical trials were compared with those from dog trials whereby cadaver- and blood-detection dogs were exposed to fresh and weathered blood on concrete and varnished wood surfaces for the first time in order to establish insight into their baseline abilities and limitations. The knowledge gained from this research will help to establish training aids that more closely replicate the odours the dogs are likely to encounter in the field, increasing their accuracy and reliability.

2. Materials and methods

2.1. Experimental design

Blood was collected following human ethics approval (HREC# 2013000132), and was collected from a single donor to eliminate the possibility of inter-subject variation across samples. The donor was a 22-year-old female who was not taking any medication and had no ongoing health issues at the time of collection. A regular routine of hygiene and diet was maintained by the donor prior to collection to ensure an accurate representation of natural biological conditions.

A qualified phlebotomist aseptically collected blood via venipuncture into BD Vacutainer® Tubes with Lavender BD Hemogard™ caps (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). BD Vacutainer® Tubes contain glass and spray-coated tripotassium ethylenediaminetetraacetic acid (K_3EDTA), which is used to prevent blood clots, allowing for an easier transfer of the blood onto the surfaces throughout the study [18,19]. The effect of K_3EDTA on the blood VOC profile has been previously tested and determined to have no significant effect above background VOC levels [5]. The vacutainers were inverted several times after blood collection to ensure adequate mixing of the EDTA and blood for effective anticoagulation [19]. Studies have shown that EDTA has no significant effect on blood cells if removed from the tubes 2–3 h after collection [19]. Therefore, the blood was transferred from the BD Vacutainer® Tubes within 1 h of collection.

Two weathering trials were performed during this study: one with blood deposited on concrete (Brighton Charcoal Concrete Pavers; Bunnings, Australia) and the other with blood deposited on varnished wood (Tarkett Bamboo; Bunnings, Australia). The local police dog unit recommended these surfaces, as they are both commonly encountered at outdoor crime scenes where blood is likely to be located. Tarkett bamboo wood was chosen as an alternative for hickory, the typical wood type used in hammer and shotgun handles, as it was accessible,

easy to cut, and provided the most reasonable replication. The concrete and wood surfaces were cut into 32 individual 4 × 4 × 4 cm cubes or 32 separate 4 × 4 cm tiles, respectively.

At the start of the first trial, 64 new 250 mL aluminium tins and lids (Morris McMahon & Co Pty Ltd., Sydney, NSW, Australia) were placed in an oven at 110 °C for 24 h to remove VOC contaminants. Directly after the tins were removed from the oven, the lids were hammered on to seal the tins and prevent headspace contamination from exposure to the laboratory air.

Due to time constraints, the start date of each surface trial was offset, as there was insufficient time to analyse both surfaces on the same day using manual SPME–GC × GC–TOFMS. The concrete trial started on 18 May 2016 and the wood trial commenced on 20 June 2016. At the start of each trial (i.e. day 0), 1 mL of blood was deposited in a drop wise fashion (to mimic a victim or perpetrator dripping blood at a crime scene) onto 24 of the 32 surfaces. 1 mL of blood was chosen based on a previously optimised method and to replicate the training procedures implemented by the local police dog unit [5]. The remaining eight surfaces were used as controls for the purpose of determining background VOCs naturally produced by the surface.

Three blood replicates and one control replicate were prepared for defined intervals over a three-month period to allow for the analysis of samples at different weathering stages (i.e. day 0, day 1, day 2, day 7, day 22, day 28, day 59 and day 84 for concrete, and day 0, day 1, day 2, day 7, day 14, day 28, day 59, and day 84 for wood). Day 0 samples (i.e. blood and control replicates) were placed directly into individual tins for sample analysis, and the remaining surfaces were placed outside on a wooden pallet on the roof. All surfaces were left to weather in the same environment and a layer of chicken wire was placed over the samples to prevent sample loss due to extreme conditions and scavengers. A Bureau of Meteorology (BOM) weather station, located ~4 km away from the sample location, was used for the collection of weather data daily, including rainfall (mm), temperature (°C) and humidity (%).

Samples prepared for headspace analysis were left to weather until the day of analysis and then discarded after VOC collection. An additional set of replicates of blood and controls were also prepared and left to weather on the roof in preparation for the canine trials. However, due to operational commitments, blood-detection and cadaver-detection dogs used in this study were not always available at the same weathering intervals as those samples analysed chemically. Thus, for some samples used in the canine trials that did not align with the VOC sample collection days, an additional set of replicates were prepared from subsequent blood collections from the donor to mimic the weathering ages analysed chemically. While potential differences in exposure to environmental factors limits the direct comparison of those samples with samples presented to the canines, these differences were noted by the researchers and taken into consideration for the interpretation of trends in this study. It is recommended that future research control variations in weathering of comparison samples where possible.

2.2. VOC sample collection

On the relevant VOC sample collection day, the surfaces were picked up directly from the outdoor wooden pallet and placed into individual 250 mL aluminium tins and sealed to allow for the accumulation of VOCs in the headspace of the tin for an average of 3 h at room temperature. Before VOC sample collection, an in-fibre internal standardisation was performed using 100 ppm bromobenzene (GC grade; Sigma-Aldrich, Castle Hill, NSW, Australia) in HPLC grade methanol (Sigma-Aldrich). The internal standard was heated to 40 °C and a polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65 μm SPME fibre was exposed to the headspace of the internal standard for 15 s as per a previously optimised method [5]. Prior to analysis, each sample tin was heated in a sand bath at 40 °C for 15 min to allow for additional accumulation of VOCs. Prior to extraction, the sealed lid of the aluminium tin was replaced with a punctured lid to allow for sampling. The same fibre was subsequently exposed to the headspace of the sample for 45 min [5]. Further details of the SPME parameters used for VOC sample collection can be found in [5].

2.3. VOC sample analysis

VOC analysis was conducted using a LECO Pegasus® 4D GC × GC–TOFMS (LECO, Castle Hill, NSW, Australia), following a method previously optimised by Forbes et al. [5]. Sample introduction was performed by desorbing the SPME fibre directly in the GC × GC inlet at a temperature of 250 °C for 5 min. A mid-polar Rxi®-624Sil MS column, 30 m in length with an internal diameter of 0.250 mm and a 1.40 μm film thickness (Restek Corporation, Bellefonte, PA, USA), was used in the first dimension. A polar Stabilwax column, 2 m in length with a 0.250 mm internal diameter, and a film thickness of 0.50 mm (Restek Corporation), was used in the second dimension.

High purity helium (BOC, Sydney, NSW, Australia) was used as the carrier gas and was operated at a constant flow rate of 1 mL/min with a split ratio of 2:1. The first dimension oven was held for 1 min at 35 °C, and then ramped to 240 °C at 5 °C/min and held for 10 min. The modulation period was 5 s with hot pulse durations of 1 s. The modulator temperature offset was + 5 °C relative to the second dimension oven, and the second dimension oven temperature offset was + 20 °C relative to the first dimension oven. The transfer line was held at 250 °C and a 70 eV electron ionisation energy was applied with the ion source maintained at a temperature of 200 °C. A + 200 V offset was applied above the optimised detector voltage. The TOFMS had an acquisition rate of 100 Hz across a mass range of 29–459 amu.

2.4. VOC data analysis

The data obtained from the SPME–GC × GC–TOFMS analysis were processed using ChromaTOF® (version 4.51, 6.0; LECO). An 80% offset was automatically applied to smooth the baseline and the expected peak widths were set to 15 s in the first dimension and 0.30 s in the second dimension. A signal-to-noise ratio (S/N) cut-off of 250 was set for the base peak, and a S/N cut-off of 20 was set for the sub-peaks. The peaks required a minimum match of greater than 800 with the 2011 National Institute of Standards and Technology (NIST) mass spectral library database for the corresponding compound to be tentatively identified.

The Statistical Compare software feature within ChromaTOF® was used to align and normalise peak areas (calculated using unique mass) in relation to the bromobenzene internal standard peak. Separate Statistical Compare files were created for both the concrete and wood chemical trials. In all statistical methods, the blood (n = 24) and control (n = 8) samples were grouped in separate classes. In order for a compound to be identified and added to the compound table it had to be detected in at least 3 out of the total 32 samples or found in 50% of the samples within a class (i.e. control or blood) to be reported in the final compound table. A maximum retention time difference of 5 s was applied to the first dimension and 2 s for the second dimension to allow for retention time deviation between samples during alignment. A larger retention time difference was necessary in the second dimension to allow for chromatographic alignment due to the installation of a new second dimension column mid-trial. The previous second dimension column had become shorter than that required for the method as a result of multiple trimmings during regular maintenance and column changes leading to a substantial retention time shift in the second dimension. A critical value (F_{crit} = 4.17) was calculated in Microsoft Excel® using the F-distribution, and Fisher ratios were calculated using the Statistical Compare software feature. The compound table was filtered by Fisher ratio using the F_{crit} value to determine compounds considered to be significant (i.e. those with a Fisher ratio higher than the F_{crit} value). The final peak tables were converted to .csv files and

exported into Microsoft Excel® to remove instrumental artefacts such as column and fibre bleed, and compounds associated to the breakdown of the bromobenzene internal standard used in this study.

Principal component analysis (PCA) was performed via The Unscrambler® (Version 10.3; CAMO Software, Oslo, Norway) and was used to group data using scores plots to show patterns/variation, and loadings plots to identify compounds responsible for the resulting patterns/variation. All data was confirmed to be free of outliers using the Hotelling's T^2 95% confidence limit. All compounds were grouped into their corresponding compound classes (i.e. alcohol, aldehyde, aromatic, carboxylic acid, ester, ether, hydrocarbon, ketone, nitrogen-containing, sulfur-containing, and other) for further data comparison and analysis.

R (The R Foundation for Statistical Computing, version 3.1.3, Vienna, Austria), the open source software for statistics, was used to perform hierarchical cluster analysis (HCA). HCA was used to produce a hierarchical tree whereby the samples started as a single cluster, and each split in the tree identified a variation between the samples. For all HCA analyses, Euclidean distances were calculated based on *z*-score normalised peak area values.

2.5. Canine trials

Cadaver-detection and blood-detection dogs are both regularly trained using blood samples. For the purposes of this study, both cadaver-detection and blood-detection dogs were evaluated to increase the number of dogs observed. Six canine and handler teams from a local police dog unit were evaluated including two blood-detection canines and four cadaver-detection canines (Table 1). Cadaver- and blood-detection dog evaluation was performed following animal ethics approval (ACEC #2014000213). The blood-detection canines had only been exposed to blood training aids during previous training, whereas the cadaver-detection canines were previously trained on a variety of training aids including blood, bone, grave soil and decomposition fluid.

The dog trials were conducted following the standard training procedures for odour accreditation of dogs and handlers within the local law enforcement agency. A total of 24 concrete cinder blocks were numbered and set up in a standard U-shaped scent line-up formation with each block holding two 1 L aluminium tins with perforated lids. Hence, each canine was exposed to a total of 48 samples during each run.

The study consisted of two separate trials: concrete and wood, both with three separate scent line-ups. Each of these line-ups involved six target tins: three unweathered positive controls containing fresh blood (< 24 h old) and three containing weathered blood surfaces (i.e. those weathered in an outdoor environment for 1 week, 1 month or 2 months). Eight tins containing unweathered and weathered surfaces without blood (i.e. control odours) were also placed in the line-up to establish the canines' ability in detecting blood VOCs, rather than the VOCs produced by the concrete or wood surfaces.

Each line in the U-shaped formation held one of each of the two target odours, four control odours, and two distractor odours (nitrile gloves or pipette tips), exposing each canine to a total of 6 blood odours, 12 control odours and 6 distractor odours per run. The remaining 24 tins in the scent line-up contained 1 mL of tap water following the typical training procedures conducted by the law enforcement agency. The position of every tin was determined through a random number generator, with the first blood odour being fresh blood in position 1–6 to reinforce the target odour.

Nitrile gloves and pipette tips were used as distractor odours to mimic materials used during sample preparation. The canines were exposed to 1 nitrile glove and 1 pipette tip per row in the line-up, therefore allowing exposure to three of each distractor odour throughout a single run. Exposing the canines to these odours provides insight into whether the canines were alerting to the odours present in the blood due to the use of these sample preparation materials, or due to the blood-related VOCs.

Each line-up within the two trials involved five runs. Five different dogs were requested for each line-up; however, due to operational availability of dogs and handlers, some line-ups involved fewer teams, in which case one or more dogs were run through twice. To reduce the complexity of the experimental design, and to maintain the normal training procedures used by the dog unit, the order of the samples exposed to the canines were not randomised which may have influenced the canines' ability to detect the more weathered samples due to their increased exposure to the surface types.

In the concrete trial, five different dogs were exposed to at least two differently weathered concrete samples (note: team F was absent at all concrete line-ups), and a minimum of three different dogs were exposed to every weathered concrete sample. In the wood trial, all six teams were exposed to at least two differently weathered wood samples with a minimum of three different dogs exposed to every weathered wood sample. Table 2 outlines the dates at which each weathered surface was presented to the canines and which canines were exposed to each sample.

Each canine training session was limited to a maximum of two line-ups to ensure the results accurately displayed the canines' natural or baseline capabilities. Numerous line-ups within one training session may result in the dogs being trained onto the odour, significantly improving their detection limits within a single training session. This may lead to inaccurate results as the canines are likely to have a higher detection rate in samples run at the end of the training session, due to their earlier pre-exposure.

The trials were performed as single-blind experiments, whereby the handler was unaware of the location of the target odours, but another trainer who held the reward was positioned at a distance from the handler and dog. The trainer knew the locations of the blood samples (i.e. target odours) for reward purposes but would only issue the reward when the handler called a positive alert by the dog. Single-blind experiments ensure the canine is not being influenced by changes in the handler's behaviour. Once the handler called a positive alert, the dog was required to hold the alert for a period of time, and then the trainer would approach to issue the play reward (e.g. ball or tug toy). This

Table 1
Summary of cadaver-detection and blood-detection dogs evaluated in this study.

	Team A	Team B	Team C	Team D	Team E	Team F
Detection category	Cadaver	Cadaver	Cadaver	Blood	Cadaver	Blood
Breed	English Springer Spaniel	English Springer Spaniel	German Shepherd	Labrador	English Springer Spaniel	Labrador
Age (years)	7	6	7	3	4	2
Sex	Female	Female	Male	Female	Male	Male
Experience (years)	5.5	4–4.5	5	1	2	1

Table 2
Surfaces presented and canine teams present at each training session.

Date	Surface	Weathered age	Canine team
15/07/16	Concrete	Fresh/unweathered	Team A
		2 months	Team B
	Wood	Fresh/unweathered	Team D
		1 month	Team E
12/08/16	Wood	Fresh/unweathered	Team A
		1 week	Team B
		2 months	Team C
			Team D
			Team F
13/09/16	Concrete	1 month	Team E

procedure followed the normal training practices of the collaborating dog unit which assisted the researchers in assessing baseline capabilities. Between runs, the target tins were rearranged to ensure the location of the tins remained unknown to the handler/canine teams, and all sample lids were removed, replaced, and wiped clean to remove contaminating odours that could result in the dogs cuing on the previous dog's alerts. All other tin lids were wiped with paper towels between runs to ensure a common odour across all tins as per standard training procedures.

The canines were trained to alert to target odours by demonstrating a passive 'freeze' alert, whereby the dog crouches in place, with its nose pointed at the location of the target odour. When the dog performed a freeze alert at a target tin, it was recorded as a positive alert. Other responses included false positive alerts (i.e. the dog alerted to a control or distractor odour), true negative alerts (i.e. the dog did not alert on a non-target odour) and a false negative alert (i.e. the dog did not alert to the target odour). All responses were recorded on odour accreditation sheets used by the trainers, and interpreted using Microsoft Excel®. It is important to note that the two surfaces experienced different weathering conditions, which may have influenced the behavioural responses of the canines, and therefore was considered during data interpretation.

3. Results and discussion

3.1. Concrete trial

The concrete trial was performed during the Australian winter from 18 May to 28 July 2016, and all weather data was recorded hourly. The weather conditions experienced throughout the concrete trial are listed in Table 3.

When applied to the concrete surfaces, the fresh blood immediately started soaking into the concrete due to its porous nature, and was fully absorbed within the first 24 h. After 2 weeks, the blood had significantly dried, and began to change colour from dark red to red/green. There was a distinct visual difference between the blood surfaces and the control surfaces throughout the entirety of the trial, with the blood remaining visible for the full period of weathering.

On day 0 (D0), a higher total number of compounds (150) were detected in the fresh blood on concrete sample in comparison to the corresponding concrete control sample (74); however, most of these blood-related compounds were lost within the first 24 h, leaving the fresh blood on concrete sample with a total of 89 compounds. On day 1 (D1), the total number of compounds detected in the blood odour profiles decreased, appearing similar to the control samples (89 and 81, respectively), and the number of compounds detected remained relatively consistent for the remainder of the trial.

The loss of VOCs after D0 is apparent in the GC × GC–TOFMS total ion current (TIC) contour plots in Fig. 1. The plots in Fig. 1 are zoomed in, focusing on the busiest region of the chromatogram, from 575 to 1915 s in the first dimension, to assist with chromatographic visualisation and comparison. The D0 concrete control sample (Fig. 1a) exhibited fewer compounds in comparison to the corresponding blood on concrete sample (Fig. 1b). On D1, following 24 h of weathering, most compounds in the blood odour profile were lost (Fig. 1d) and the D1 blood profile appeared similar to the control (Fig. 1c). By the end of the trial (i.e. 84 days of weathering), the GC × GC–TOFMS TIC contour plots for the blood and control samples were visually indistinguishable (Fig. 1e and f, respectively), and further statistical interpretation (i.e. PCA and HCA) of the data was required to determine if the samples could be differentiated statistically.

Table 3
Weather data recorded throughout the concrete and wood trials.

		Minimum	Maximum	Average	Total
Concrete trial (18 May–28 July 2016)	Temperature (°C)	5.4	26.8	14.8	–
	Humidity (%)	33	90.5	58.7	–
	Rainfall (mm)	–	–	–	505
Wood trial (20 June–12 September 2016)	Temperature (°C)	5.4	25.7	14.6	–
	Humidity (%)	33	90.5	57.4	–
	Rainfall (mm)	–	–	–	337

Statistical data processing resulted in the majority of compounds being excluded as their Fisher ratio was lower than the *F*-critical value (4.17), meaning the compound was not specific to a particular class of samples (i.e. they did not statistically differ in abundance between the blood on concrete and concrete controls). A total of 13 compounds were deemed statistically significant in differentiating the blood samples from the control surfaces, and all data interpretation herein is a representation of these compounds. The significant compounds were 2,4,4-trimethyl-1-pentene, 2,6-dimethyl-2,6-octadiene, 3-methyl-6-(1-methylethyl)-2-cyclohexen-1-one, 5-methyl-2-hexanone, 2-nonanone, 2,2,4-trimethyl-3-penten-1-ol, aniline, 1,3,3-trimethyl-bicyclo[2.2.1]heptan-2-one, methylcyclopentane, 2-pentylfuran, heptanal, nonanal, and octane. Unweathered blood on concrete (i.e. D0) had the highest number of blood-related compounds detected; however, these compounds reduced as the blood weathered over time.

PCA was performed to identify trends and distinguish between control and blood VOC profiles. The sample odours grouped together in the PCA plot are deemed similar in chemical composition, while the samples separated within or across quadrants demonstrate variation. The PCA plot for the concrete trial (Fig. 2a) shows grouping of most concrete control samples confirming there is reduced variation in their odour profiles, even after weathering in an outdoor environment. Two control samples did however separate from the group, which may be attributed to variation within the concrete, instrumental error or potential environmental contaminants.

The odour profile detected for fresh blood on concrete (B D0) was significantly different from the odour profile for the control concrete samples, accounting for 47% of the variability in the dataset as observed on the first principal component (PC-1) axis (Fig. 2a). As the blood weathered, the profile became less distinguishable from the concrete control profile, shown by the migration of the data points towards the control group. The odour profile of blood aged for 84 days on concrete was indistinguishable from the odour profile of the control concrete samples as demonstrated by their overlap on the PCA plot. This trend correlates with the GC × GC–TOFMS TIC contour plots, which were visually very similar for the blood weathered on concrete and the corresponding concrete control (Fig. 1e and f). Overall, PCA was able to identify variations between the VOC profiles of blood weathered on concrete and weathered concrete controls for up to 59 days of weathering. Therefore, under the conditions experienced in this study, 59 days of weathering was determined to be the limit of detection for blood weathered on concrete using SPME–GC × GC–-TOFMS.

There were seven compounds detected in fresh blood (B D0) which were shown to have high discriminatory power (Fig. 2b) namely 2,4,4-trimethyl-1-pentene, 3-methyl-6-(1-methylethyl)-2-cyclohexen-1-one, 5-methyl-2-hexanone, 2-nonanone, 2,2,4-trimethyl-3-penten-1-ol, aniline and methylcyclopentane. These compounds are responsible for the discrimination between the fresh blood on concrete odour profiles and control concrete odour profiles.

Methylcyclopentane has previously been cited as a compound present in the odour of blood, however, it is also used in the fuel industry [20]. It is possible that this compound was present in the fresh blood profile due to the donor's body filtering out the toxicant via the bloodstream. A similar assumption can be made about the detection of aniline in these samples, as it has previously been cited as a compound present in cigarette smoke [21].

The other five compounds have not previously been identified in blood odour, although cannot be excluded as blood-related compounds in this study. Their lack of detection in previous studies may be

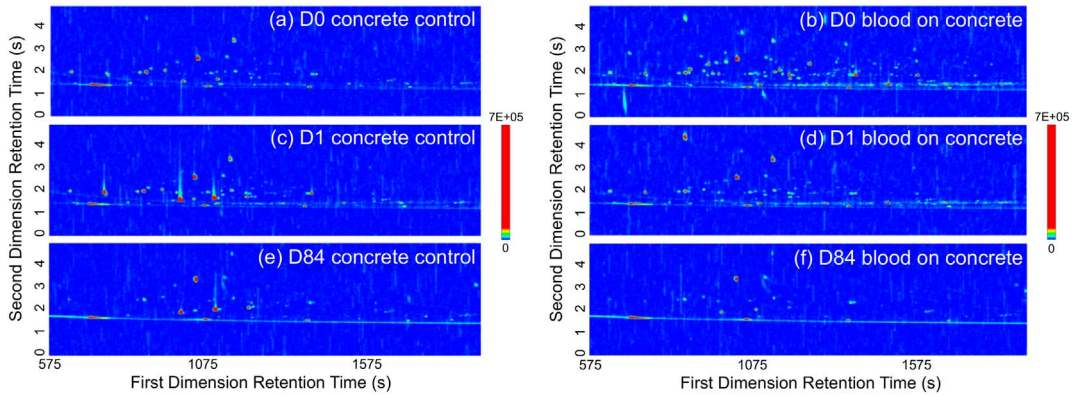

Fig. 1. GC × GC-TOFMS total ion current (TIC) contour plots (575–1915 s) of a) D0 concrete control, b) D0 blood on concrete, c) D1 concrete control, d) D1 blood on concrete, e) D84 concrete control, and f) D84 blood on concrete.

attributed to the use of instruments with lower sensitivity. For example, GC–MS is a commonly used technique throughout blood profiling research, however, GC–MS is considerably less sensitive than GC × GC–TOFMS, which was used in this study. GC × GC–TOFMS may therefore allow for a more comprehensive odour profile to be detected in these samples. The effect of EDTA on blood odour has not been investigated using two-dimensional gas chromatography, and therefore the possibility of these compounds being present due to the EDTA and blood interaction cannot be excluded.

Three compounds (i.e. 2-pentylfuran, heptanal and nonanal) had a high discriminatory value in the top right quadrant of Fig. 2b, corresponding to the blood samples weathered for 1, 2 and 22 days. These three compounds have been cited in previous studies as compounds present in the odour profile of blood [5,8,11,22–24]. These compounds were not found to be discriminatory in the unweathered blood samples; however, this may be because the compounds were only present at low

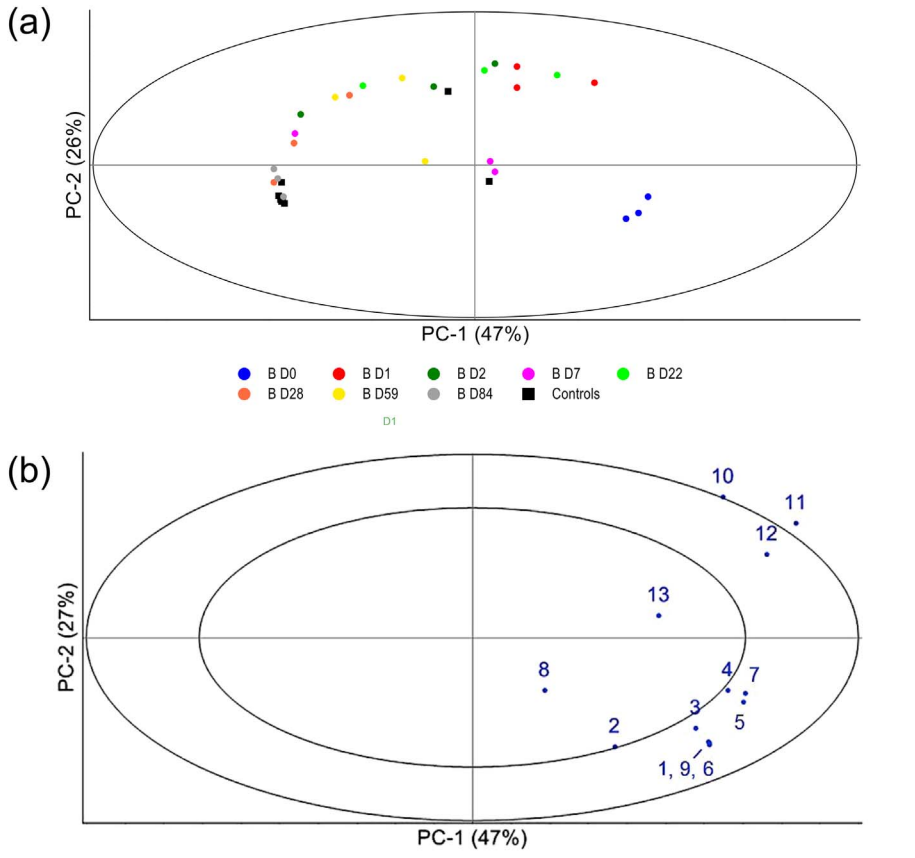

Fig. 2. a) PCA scores plot of blood (B) and control concrete samples weathered from day 0 (D0) to day 84 (D84) and b) PCA correlation loadings plot of compounds influencing the variability of the blood and control concrete samples

1: 2,4,4-trimethyl-1-pentene	8: 1,3,3-trimethyl-bicyclo[2.2.1]heptan-2-one
2: 2,6-dimethyl-2,6-octadiene	9: methylcyclopentane
3: 3-methyl-6-(1-methylethyl)-2-cyclohexen-1-one	10: 2-pentylfuran
4: 5-methyl-2-hexanone	11: heptanal
5: 2-nonanone	12: nonanal
6: 2,2,4-trimethyl-3-penten-1-ol	13: octane
7: aniline	

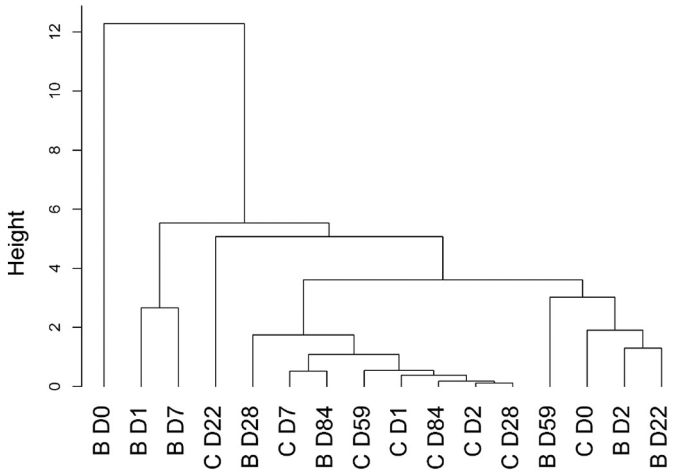

Fig. 3. HCA dendrogram showing segregation based on the VOC profiles of all blood (B) and control (C) concrete samples weathered from day 0 (D0) to day 84 (D84)

concentrations in the fresh blood samples or because they were produced as a product of weathering.

HCA was performed on the 13 compound dataset obtained from the concrete trial to improve visual interpretation and a HCA dendogram (cluster tree) was produced (Fig. 3). The dendogram is based on the Euclidian distances of *z*-score normalised peak areas for all significant compounds within the concrete samples. The cluster tree in Fig. 3 demonstrates that fresh blood on concrete (B D0) is the most distinct from all other samples, as it is separated from the remainder of the cluster. Blood weathered on concrete for 24 h and 7 days showed similarities in the odour profile, as well as blood weathered on concrete for 2 and 22 days. The cluster tree also confirms that blood weathered on concrete for 84 days (B D84) has a similar VOC profile to the control (C) concrete samples. This is demonstrated by the clustering of B D84 and C D7 samples, along with the C D59, C D84, C D2, and C D28 samples.

3.2. Wood trial

This study was performed during the Australian winter between 20 June and 12 September 2016 and all weather data was recorded hourly. The weather conditions experienced throughout the wood trial are listed in Table 3.

Being a non-porous surface, the varnished wood prevented the blood from being absorbed, instead creating a 'bead effect' where the blood remained in small droplets on the surface. Within the first 24 h of weathering, most of the blood had dried into flakes and is likely to have been lost due to wind or high levels of rainfall (38.8 mm). This considerably reduced the amount of blood on each surface leaving some surfaces with no blood visible, and other surfaces with only a few dried drops. After two weeks of weathering, there was no blood visible on the majority of the varnished wood surfaces, making them visually indistinguishable from the controls. These surfaces appeared unchanged for the remainder of the trial.

The total number of compounds detected in the odour profile of the fresh blood sample on wood (88) and control wood samples (107) increased after the first 24 h (170 and 166, respectively). Considering the 38.8 mm of rainfall experienced during this period, the increase was assumed to be due to the interaction of the blood with the varnish on the wood surfaces. The results show the interaction to have the most influential effect over the first 24 h, displaying a significantly higher number of VOCs produced.

After the first 24 h, the number of compounds detected in each blood sample on wood remained consistent. There was a slight decrease in compounds between 7 and 14 days of weathering, which may be attributed to higher temperatures during this period (maximum = 18.8 °C). The number of compounds then increased slightly between day 14 and 28, before decreasing again after day 59. The loss of compounds at this time of the trial can be assumed to be due to high levels of rain experienced between day 59 and 84 (97.8 mm), as well as higher temperatures (maximum = 24.3 °C). The total number of compounds detected in the blood on wood samples, however, was still higher on day 84 than in the D0 blood on wood and control wood surfaces.

The GC × GC–TOFMS TIC contour plots in Fig. 4 are zoomed in, focusing on the busiest region of the chromatogram, from 575 to 1915 s in the first dimension, to assist with chromatographic visualisation and comparison. Fig. 4a and b show the contour plots of a D0 wood control and a D0 blood on wood sample, respectively. The D0 chromatograms are visually similar, showing only a small difference in the number of compounds present. After 24 h of weathering, the total number of compounds increased for both the wood control and the blood on wood sample, and again the chromatograms appeared similar (Fig. 4c and d, respectively). Over the period of the trial, both the blood on wood samples (Fig. 4d and f), and the control wood samples (Fig. 4c and e), showed a decrease in the number of compounds detected.

Statistical data processing resulted in the majority of compounds being excluded as their Fisher ratio was below the F-critical value (4.17), meaning they were not specific to a particular class of samples. A total of 10 compounds were deemed to be statistically significant and subsequently used in all proceeding data interpretation methods. The significant compounds were 1-hepten-3-ol, 1-octen-3-one, (Z) 2-heptenal, 2-methylpropyl ester benzoic acid, cyclohexanone, *N*,*N*,-dibutylformamide, *N*-formyl-*N*-methylformamide, heptanal, methoxyphenyl-oxime, and phenol.

PCA was once again performed to gain a better understanding of the similarities and differences between the VOC profiles produced from the varnished wood controls and blood weathered on the varnished

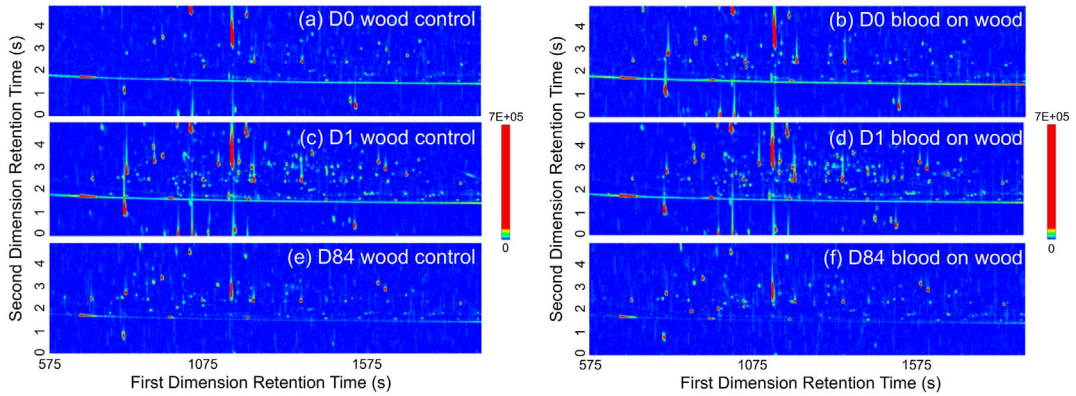

Fig. 4. GC × GC-TOFMS total ion current (TIC) contour plots (575–1915 s) of a) D0 wood control, b) D0 blood on wood, c) D1 wood control, d) D1 blood on wood, e) D84 wood control, and f) D84 blood on wood.

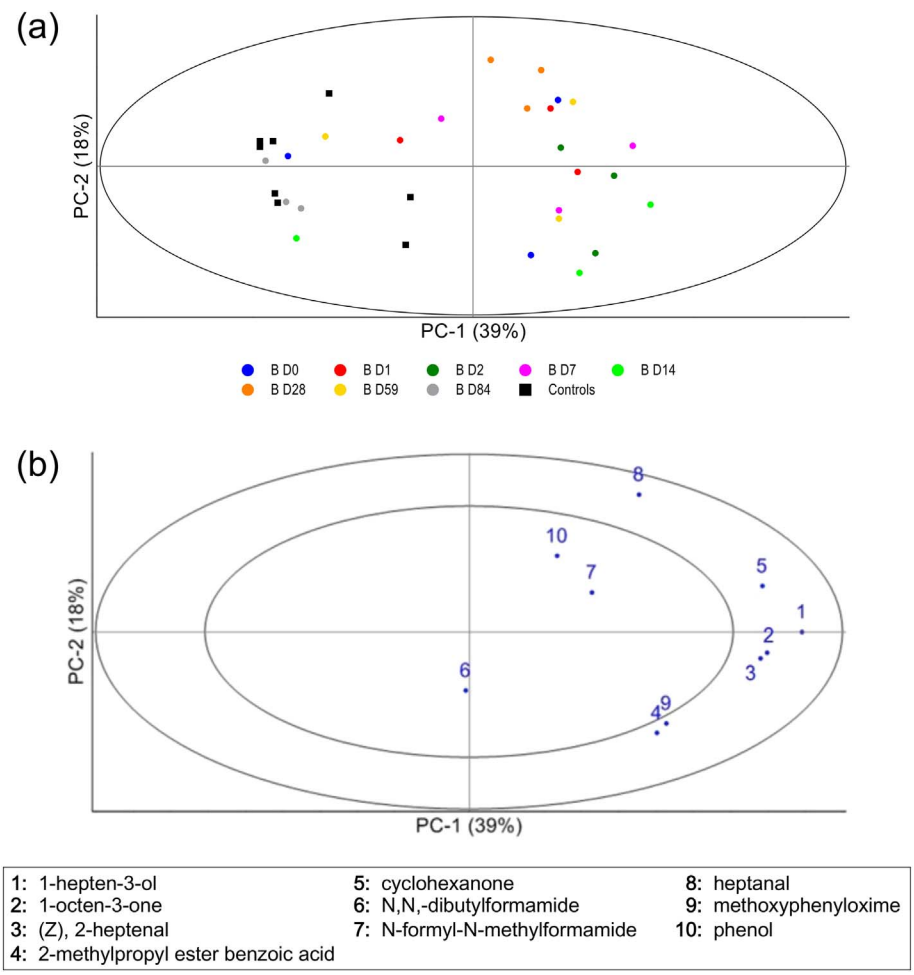

Fig. 5. a) PCA scores plot of blood (B) and control wood samples weathered from day 0 (D0) to day 84 (D84) and b) PCA correlation loadings plot of compounds influencing the variability of the blood and control wood samples.

wood surfaces. Whilst the data may not display a clear differentiation between each sampling day, PCA demonstrates a significant difference between the control surfaces, and the blood surfaces weathered up to 59 days (Fig. 5a). The wood control surfaces showed higher variability in comparison to the controls in the concrete trial, accounting for 18% of the variability across the second principal component (PC-2). This may be attributed to the type of wood, which is unable to absorb or trap the VOCs, and therefore the compounds detected are more easily influenced by variable weather conditions.

Within the blood surfaces there is a high degree of variability between the sampling days of the blood surfaces as well as the replicates of the same weathering period. The blood samples were separated based on the PC-2, accounting for 18% of the variability. The variability may be due to the inconsistencies of blood volumes on the surfaces after the flaking of the blood within the first 24 h.

The separation between the blood samples and the control samples, accounted for 39% of the variability within the dataset and is represented on PC-1. The differentiation of the blood samples from the control samples is due to the presence of 1-hepten-3-ol, (Z)-2-heptenal, 1-octen-3-one, benzoic acid, 1-methylpropyl ester, methoxy-phenyl-oxime, cyclohexanone and heptanal as demonstrated in the correlation loadings plot (Fig. 5b).

(Z)-2-heptanal, heptanal and cyclohexanone were detected in all wood controls and blood surfaces, and were the only significant compounds that were previously cited as being present in the odour profile of blood. (Z)-2 heptanal is commonly observed in the blood odour of healthy humans as a result of lipid peroxidation in the body [23,25]. Heptanal has previously been cited in degraded blood in a study by Forbes et al. [5]. It is also regarded as a lung cancer biomarker as has shown to be in higher concentrations in the blood odour of lung cancer patients than healthy human patients [23]. Cyclohexanone has also previously been observed in the odour of blood by Hoffman et al. and Mochalski et al. [8,26], as well as being a compound present in adhesives, solvents, and agricultural production paints and coatings [27].

1-hepten-3-ol, 1-octen-3-one, 2-methylpropyl ester benzoic acid, and methoxy-phenyl-oxime were all present in both the controls and the blood surfaces, however, have not been previously cited as being present in the odour profile of blood. 1-hepten-3-ol is common within the food industry and has previously been cited as a known food additive [28]. 1-octen-3-one is found in numerous foods including cranberry, melon, peas and breads [29]. It has also been suggested that 1-octen-3-one is responsible for the metallic smell when skin comes into contact with metals and blood, and is known to be a product of degradative reduction in the chemical reaction of skin lipid peroxides and Fe^{2+} [29]. Kusano et al. reported 1-octen-3-one as being a constituent of human breath and sweat, suggesting it to be a product of the oxidation of milk fat [11]. 2-methylpropyl ester, benzoic acid is present in alcoholic beverages including beer and cider, and is known within the food flavouring industry, in banana, sweet cherry, papaya and cacao [30].

The blood samples weathered for 84 days clustered with most of the controls indicating minimal differentiation between their odour profiles. Considering the location of the compounds in Fig. 5b, it is likely that the blood-related compounds (i.e. those shown in the correlation loadings plot) have been lost, and the resultant profile is predominantly the odour of varnished wood.

HCA assisted in identifying further variation and similarities between the blood and control surfaces and was the preferred statistical

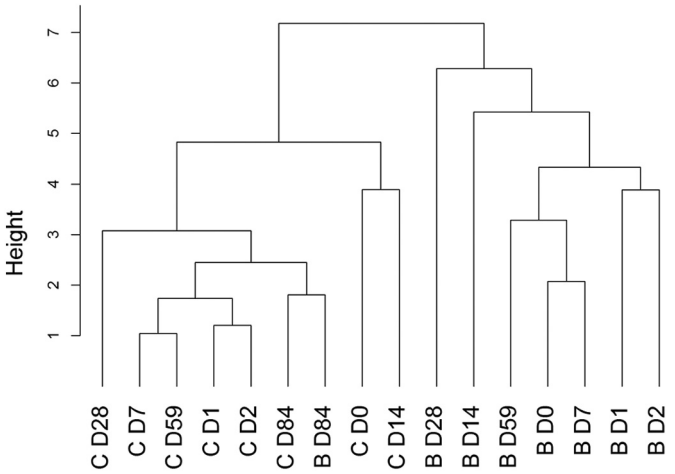

Fig. 6. HCA dendrogram showing segregation based on the VOC profiles of all blood (B) and control (C) wood samples weathered from day 0 (D0) to day 84 (D84).

tool for interpretation within the wood trial. The dendogram in Fig. 6 is based on the Euclidian distances of z-score normalised peak areas for all significant compounds detected within the wood trial samples. It displays two distinct clusters, one containing all control samples and the day 84 blood samples, and another containing all other blood samples, as shown in Fig. 6. Hence, the blood weathered on wood for up to 59 days demonstrates a different odour profile to the wood surface but by day 84 the blood odour profile is no longer detectable by SPME–GC × GC–TOFMS.

The HCA dendrogram exhibited similarities in the VOC profile of fresh blood and blood weathered for 7 days, as well as blood weathered for 1 and 2 days. The D0 and D7 blood cluster also demonstrated some similarity to the blood sample weathered for 59 days. Blood weathered for 14 and 28 days had a higher degree of variability, indicated by their separation from the other clusters. This reflects the high variation in the overall VOC abundances in their odour profile in comparison to all other samples as displayed in Fig. 6.

The findings observed for the wood trial were comparable to those of the concrete trial, demonstrating an inability to distinguish the odour profile of blood from the background surface once the samples were weathered for up to 84 days. There was higher variation between replicates in the wood trial compared to the concrete trial, which may be due to the inconsistency in the blood volume present on the wood surfaces after 24 h of weathering, or due to the different weather conditions experienced throughout each trial.

PCA was beneficial for determining variation and trends within the concrete trial (Fig. 2a and b), however was less discriminatory in the wood trial (Fig. 5a and b). The blood samples weathered up to 84 days appeared to be more random within the wood trial showing no consistent trends. In contrast, HCA was valuable in determining variation and trends within the wood trial (Fig. 6), but gave no additional information in the concrete trial (Fig. 3).

3.3. Dog trials

All results within this section are recorded as percentages to account for days in which teams were absent from training. The percentage of correct alerts and false positive alerts averaged for all dogs across the trial are summarised in Fig. 7a and b, respectively. In order to visualise trends associated with individual dogs, the percentage of correct alerts and false positive alerts have also been summarised by teams in Tables 4 and 5, respectively. Within the concrete trial, the canines alerted to 64% and 67% of the unweathered blood samples and 1 week weathered blood samples, respectively (Fig. 7a).

The influence of prior exposure is shown in the responses of the canines to the 1 month weathered blood on concrete (Fig. 7a) which showed the highest percentage of correct responses (87%). In contrast, the concrete blood samples with the lowest percentage of correct responses were those weathered for 2 months (33%) (Fig. 7a). The 2-month samples were the first set of samples that the canines were exposed to in the concrete trial (due to randomisation), and the VOC analysis of these samples detected few blood-related compounds, suggesting the odour profile was not distinguishable from the control surface.

The canines showed a higher percentage of detection for less weathered samples on wood, alerting to 78% of unweathered blood samples, and 87% of blood samples weathered for 1 week on wood (Fig. 7a). After one week of weathering, the canines only detected 33% of blood samples weathered for 1 month, and 40% of blood samples weathered for 2 months on wood (Fig. 7a). Overall, the canines demonstrated more correct alerts for blood on wood than blood on concrete, with the exception of those weathered for 1 month (Fig. 7a).

As the concrete controls were weathered, the canines gave fewer false positive alerts (Fig. 7b). Team E and F had the least exposure to blood odour prior to this study, and were not as successful at distinguishing the blood odour profiles from the control or distractor odour profiles. This caused the total false positive alert percentage to be heavily influenced by the team E and F results, as demonstrated in Table 5. For example, team E alerted to 83% of wood controls that had been weathered for 1 month and team F alerted to 67% of wood controls weathered for 1 week (Table 5). This resulted in the wood controls having the highest total false alert percentages displayed in Fig. 7b (27% and 47% for 1 week and 1 month weathered, respectively).

Across both trials, the canines distinguished the distractor odours from the blood odours for the majority of samples, shown in the low percentages of false positive alerts (Fig. 7b - most below 30%, with the exception of the 1 month weathered wood control). The small percentage of false positive alerts on these samples may have occurred due to these distractor odour tins being in close proximity to the target odour tins, or due to the canines alerting to any odour that appeared different to the odour of tap water. As the blood samples weathered, the VOC profiles of the blood on the surface became chemically indistinguishable from the VOC profiles of the control surface, which may explain the canines' responses. Additionally, there were numerous compounds detected in the VOC profiles of the control samples that have previously been identified in blood odour, for example 2-pentylfuran, heptanal and nonanal [5,8,11,22–24]. These compounds may have influenced the canines' ability to discriminate between the odours of the control and blood samples.

When interpreting the responses of the canines, it is important to consider the influence of a canine's personality and behaviour, as well as their past experience with similar target odours. For example, the behaviour of some canines is meticulous and slow during a scent line-up, whereas others are energetic and fast. Slow and meticulous canines (i.e. team A), are more likely to correctly alert to all target odours (see Table 4); however, their desire to double-check odours may cause them to false positive alert on more controls (see Table 5). In contrast, fast and less methodical canines such as those in most other teams involved in this study, are likely to miss target odours as well as non-target odours, reducing their percentage of both positive and false alerts.

The canines involved in this study all have varying levels of experience, which appeared to influence their accuracy and ability in recognising the odour of blood (refer to Table 1). Teams A, B and C had more than 4 years of experience detecting blood as a target odour, whereas teams D, E and F had less than 2 years. All teams were regularly trained on fresh blood samples deposited directly into the aluminium tins, and therefore had no prior exposure to blood on concrete or wood in a training environment before this study.

It is difficult to make a conclusion into the likely abilities of these canines at outdoor crime scenes as the only environment in which the canines were exposed to these samples was a controlled, indoor training

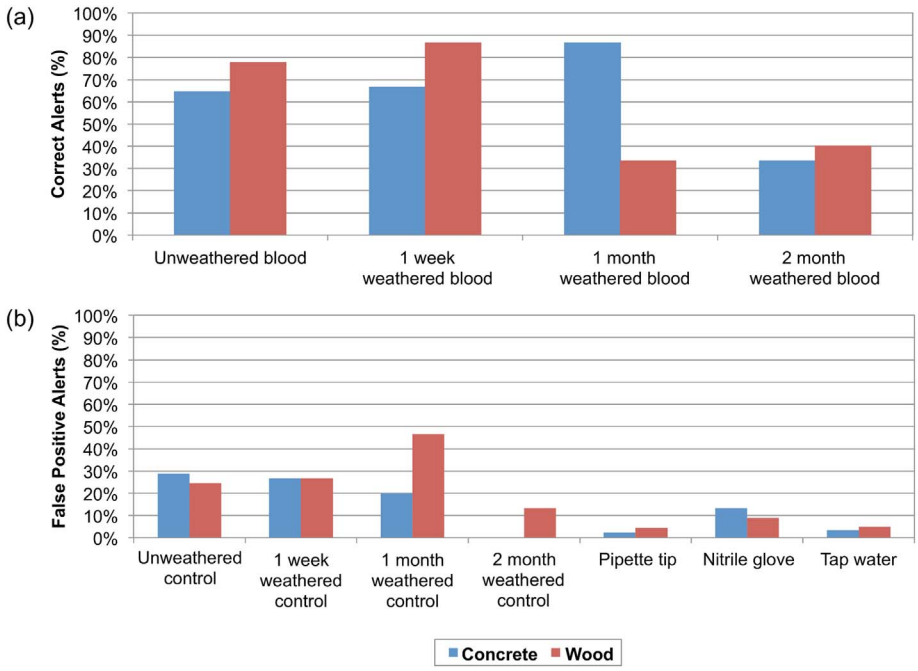

Fig. 7. a) Percentage of correct alerts averaged across all dogs for blood on concrete and wood at different stages of weathering and b) percentage of false positive alerts averaged across all dogs for concrete and wood controls distractor odours and tap water.

Table 4
Percentage of correct alerts shown by the canines within both the concrete and wood trials.

	Team	Unweathered blood	1 week weathered blood	1 month weathered blood	2 month weathered blood
Concrete	A	67%	[a]	[a]	33%
	B	56%	100%	100%	0%
	C	100%	50%	67%	[a]
	D	67%	[a]	[a]	17%
	E	40%	67%	100%	100%
	F	[a]	[a]	[a]	[a]
Wood	A	56%	100%	33%	33%
	B	89%	100%	0%	100%
	C	33%	33%	[a]	33%
	D	89%	100%	33%	0%
	E	100%	[a]	50%	[a]
	F	100%	100%	[a]	33%

[a] Canine was not exposed to this sample.

environment. This is not an accurate representation of the environments they will be deployed to and hence, it is recommended that the canines also undergo training in an outdoor environment using weathered samples. Prior to this study, the canines had no exposure to blood on concrete or wood during training, which was beneficial in that it gave an understanding into their natural capabilities, however, it also identified the need for more regular training on these surface types and others.

4. Conclusion

This study aimed to chemically analyse the VOC profile of blood on concrete and wood surfaces that had been weathered in an outdoor environment for different periods. The intent was to understand these VOC profiles, and to then expose the samples to blood- and cadaver-detection canines so as to gain an understanding of their abilities and limitations in blood detection.

The chemical aspect of the study displayed a significant decrease in the number of compounds present in the odour profile of blood on concreate, within the first 24 h. The VOC profiles for the blood on concrete surfaces and control concrete surfaces demonstrated similar chemical compositions after 24 h, with the majority of the odour profile consisting of nonanal, heptanal, and 2-pentyl-furan. Data interpretation by PCA and HCA allowed the VOC profiles of the blood surfaces to be distinguished from control surfaces for up to 59 days of weathering but were no longer distinguishable by 84 days of weathering.

Likewise, the VOC profile of blood on wood could be differentiated

Table 5
Percentage of false positives shown by the canines within both the concrete and wood trials.

	Team	Unweathered control	1 week weathered control	1 month weathered control	2 month weathered control	Pipette tips	Gloves	Tap water
Concrete	A	33%	[a]	[a]	0%	33%	0%	13%
	B	11%	0%	0%	0%	0%	0%	4%
	C	25%	0%	0%	[a]	0%	0%	0%
	D	0%	[a]	[a]	0%	0%	0%	3%
	E	53%	67%	50%	0%	0%	40%	3%
	F	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Wood	A	22%	33%	33%	33%	11%	11%	12%
	B	44%	0%	0%	33%	11%	22%	4%
	C	0%	0%	[a]	0%	0%	17%	2%
	D	11%	33%	33%	0%	0%	0%	2%
	E	50%	[a]	83%	[a]	0%	0%	3%
	F	17%	67%	[a]	0%	0%	0%	3%

[a] Canine was not exposed to this sample.

from the VOC profile of wood control samples for up to 59 days of weathering. However, the number of compounds and compound classes demonstrated different compared to the concrete trial. For example, within the first 24 h, the number of compounds present in both the blood and control surfaces increased.

The majority of compounds detected in the concrete and wood control surfaces, were also detected in the blood profiles, but in higher abundances. This was hypothesised to be due to: 1) the compounds being present in both the surface and the blood; or 2) the presence of blood influencing the surface VOCs causing them to be more readily released into the headspace. It is recommended that future studies investigate the influence of odourless liquids (i.e. water) on the surface odour to identify whether the presence of these liquids cause the surface odours to become more prevalent.

Both trials displayed variation between the blood replicate samples, likely due to the replicates consisting of separate surfaces that were influenced by the weather differently. For example, during the first 24 h of the wood trial, there were varying volumes of blood lost due to drying and flaking from the surfaces. The use of one control and three blood replicates per sampling day compounds the effect of this variation. As a result, additional replicates of both the control and blood surfaces are recommended for future studies. Increased replicates would better account for any variation in surfaces, and increase the accuracy and significance of the statistical analysis.

Scent-detection canines have been shown in previous studies to be more sensitive than analytical instrumentation. However, in this study the limit of detection for the dogs was blood weathered for 1 month on concrete and blood weathered for 1 week on wood. At a baseline level these results indicate that the canines are less sensitive than the GC × GC–TOFMS instrumentation used for chemical profiling of the blood samples. However, the canines had not previously been exposed to blood on these surfaces as part of their standard training procedure, nor had they been exposed to blood that had been weathered in an outdoor environment. There were also chemical similarities between the control and blood surfaces' VOC profiles, which may have influenced the canines' response. Regular training and further exposure to these samples may assist the canines in recognising these specific odours during training and improve their accuracy in detecting blood in outdoor operational scenarios.

This is the first time this study has been carried out and the first time the canines used in this study were exposed to weathered blood on concrete and wood during training. Unavoidable variables were experienced in this study due to limited supplies, time restraints and inconsistent training dates with the canines. It is recommended that future research attempts to control the variables experienced in this study, to enhance the validity of the results. However, the findings can assist in the training of cadaver- and blood-detection canines in order to increase their accuracy and reliability when deployed at outdoor crime scenes. The results provide baseline evidence of the canines' natural capabilities for blood-detection, which may help to assist courts and legal systems in understanding the validity of scent-detection canines as a preliminary search tool.

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