



Volabolomics' innovative evidence technique could have changed the outcome of the 'painter murder' sentence?

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ABSTRACT

In the context of forensic science, advances in trace identification are a crucial aspect. A recent advance in the investigation of Volabolomic science highlights the possibility of using Volatile Organic Compounds (VOCs) as evidence. In this paper, we applied experimental research on VOCs to forensic investigations, translating the method to a real murder case. Volabolomic data recordings were made using an electronic nose (e-nose) sensor. We tested, under conditions mimicking the true circumstances of a murder, the emission of VOCs from plastic bags and their permeability to the gases of cadaveric decay, *Volaboloma mortis*. All types of plastic bags used release VOCs, with slight differences depending on the material and processing method. In addition, all bags allow permeation to VOCs, in a consistent manner, enabling a higher permeation rate between 48 and 72 hours. These results highlight the importance of studying Volabolomic traces, which could enable a breakthrough in crime scene analysis by enriching it with a new set of measurable evidence.

1. Introduction

Death results in the shutdown of blood circulation and consequently the cessation of tissue oxygenation, progressively producing hypoxia and cellular anoxia. The physiology of this condition involves the swelling of cells with the disruption of cell membranes and the release of enzymes leading to autolytic soft tissue digestion [1]. Cell lysis promotes replication, transmigration, and activity of endogenous bacteria causing progressive gas bloating with soft tissue swelling [2]. Other intrinsic physiological factors contributing to gas production are the modality of death (e.g., trauma, pathology and type of pathology, etc.), body mass index, etc. [3–5]. In addition, extrinsic biotic factors, such as microorganisms outside the body (mainly aerobes), insects, micro- and macro-scavengers, together with extrinsic abiotic environmental parameters, such as humidity, temperature, sun exposure, ventilation, and

burial context, influence the rate of gas production [4–7].

Gases from a decomposing body are physiologically associated with the release of different types of volatile organic compounds (VOCs) [8, 9], which vary according to intrinsic and extrinsic factors, forming the *Volaboloma mortis* [10]. In line with the production of odorogenic molecules from the corpse is the creation of a decomposition odour database (DOA) to define the chemical fingerprints produced by VOCs during different decay process of human remains [11]. Consequently, VOCs are not only produced by the metabolism of the living and released through breath, sweat, urine and faeces, but also by the decay of the body [12]. VOCs are currently studied by a new specialty in biology, Volabolomics [12]; and used in human diagnostics [13–19] until 2024 when Mazzatenta et al. proved its application in forensic studies [12].

As a result, released VOCs can be considered traces and studied by forensic science, as in the case of DNA, for example. In this paper, we

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applied experimental research on *Volaboloma mortis* to forensic investigation mimic a real murder case.

2. A case history

The murder is known as the "painter" case referred to in sentence [20], which permanently charged her ex-husband, a 67-year-old pensioner who died by suicide in prison in 2024, and her unemployed son, 43 y.o., with more than 20 years for murder in conjunction. Son's motivation, according to the ruling, would have been contempt for the mother figure added to the economic motive because he did not want his father to pay alimony.

In summary, the painter, 64 y.o., a resident of Ancona (43°37'N 13°31'E), disappeared Oct. 9, 2017, after meeting her ex-husband and their son, at their home in Giulianova (42°45' 09.06"N 13°57'24.07"E), in the province of Teramo, which is about an hour's train ride south of its city also on the Adriatic coast. The body was found about a month later, on the banks of the Chienti river near the town of Tolentino (43°12'31"N 13°17'02.76"E), halfway between Ancona and Giulianova, in the countryside. In the sentence it was reconstructed that the cadaver was kept for about three days in the bathroom and then in the car, an old-fashioned Fiat 600 parked near the entrance of the apartment building at about 22 degrees average temperature. The 80 kg. dead body had been packed in a compostable garbage bag. Then the son and father would drive with the body in the passenger compartment, given the size of the car and the body, for more than 105 km and dump the body by removing the bags that wrapped it in a muddy area. One compatible car was reported from a single roadside image on the way to Tolentino, which supported the accusatory scaffolding. The next day would be taken for gearbox damage by the mechanic who detected no traces of mud or foul odour in the car. No "painter" genetic traces were found in either the apartment or the car, while others DNA were found [20].

3. Experimental forensic questions

The decaying body at 22 °C as average could emit VOCs and could VOCs permeate the compostable garbage bags used to pack the body? And finally, are such VOCs recordable?

4. Materials and method

The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee (COET No. 6065-04.03.2021). Family members of the deceased subjects signed an informed consent. Approximately one-gram samples of superficial and deep muscle tissue, thyrohyoid and ileopsoas, collected from cadavers of 40 (♂), 43 (♀), 45 (♂) and 71 (♀) years [for details regarding sampling, see Ref. 12,21]. In addition, the same amount of human tissue, one gram of animal muscle (cow, pig and chicken) under the same experimental conditions was used as an additional control.

Compostable low-density polyethylene (LDPE) garbage bags of three different brands Bizeta – maxy (50µm thickness), Spazzy - Maxi Strong (48µm thickness), Virosac - Sacco Forte (42µm thickness; this one was supposed to be used in the case [20]) was used. LDPE have densities ranging from 0.91 to 0.925 g/cm³, melting temperature T_m 110 °C and glass transition temperature T_g -130 °C. The FT-IR spectra of the bag samples were obtained using a Shimadzu IRAffinity-1S spectrophotometer (Shimadzu Italia S.r.l., Milan, Italy) equipped with a sealed and dried interferometer, a DLATGS (Deuterated Triglycine Sulphate Doped with L-Alanine) detector and an ATR diamond with single attenuated total reflection (QATR 10, Shimadzu Italia S.r.l., Milan, Italy). All spectra were recorded in the range of 4000–400 cm⁻¹ with the addition of 45 interferograms at a resolution of 4 cm⁻¹ with Happ-Genzel apodization. Before the analysis of each sample, a thorough crystal cleaning and background recording were performed. Spectra were made and processed with LabSolution IR software, version 2.27 (Shimadzu

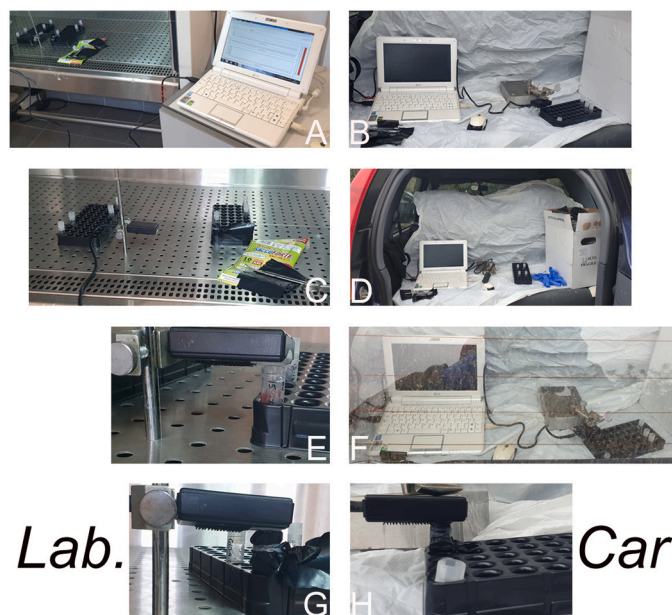


Fig. 1. Experimental settings in both laboratory (A) and car (B) conditions to mimic the real scenario of cadaver preservation (bathroom and Fiat 600 old fashion car); (C and D) background VOCs control recording; (E and F) human muscle VOCs emission control recording; (G and H) experimental recording of human muscle VOCs emission through permeation compostable garbage bag.

Italia, S.r.l., Milan, Italy).

Human tissues were also subjected to histopathological examination, as additional control, and showed no signs of tissue injury. Volabonomic data recordings were carried out using an electronic nose-type sensor (e-nose - iAQ-2000; Applied Sensor, CO2 Meter.com, Beach, FL, USA) based on a metal oxide semiconductor (MOS) sensor, according to the standard analytical method [13–19]. The e-nose is connected directly to the PC to acquire, at 1 Hz in real time, the signal generated by VOCs binding and stored for analysis. Volabonomic recordings were performed under controlled conditions at 22 °C in two environments compatible with homicidal reconstruction. The first is the laboratory, under biological fume hood, to mimic the first cadaver storage environment; the second in a car compatible with the size of the one under facts (Fig. 1). Continuously sampled VOCs were analysed at four points 0, 24, 48 and 72 hours. The choice of these time intervals is to compare the results with the case.

The raw data were transformed, normalized and analysed using MatLab v. R2024b, Jamovi 2.4.14 and Origin 2018b software (9.55). Statistical tests are MANOVA and one-way post-hoc ANOVA, with the alpha level set at 0.01. Finally, repeatability and reproducibility tests were conducted to assess the consistency and replicability of multiple measurements—three LDPE bags for three repeated measurements—for all tissues and conditions.

5. Results

The experiment involves real-time recording with e-nose during the decomposition of tissues, wrapped in plastic bags, from 0 to 72 hours. The objective is to sample VOCs, produced by the physiological behaviour of decomposition, that permeate the garbage plastic bags as hypothesized in the real case. This experiment will revolutionize crime scene investigations, introducing a new scientific element to be sought during criminalistic investigations by the forensic police: the Volabonomic trace.

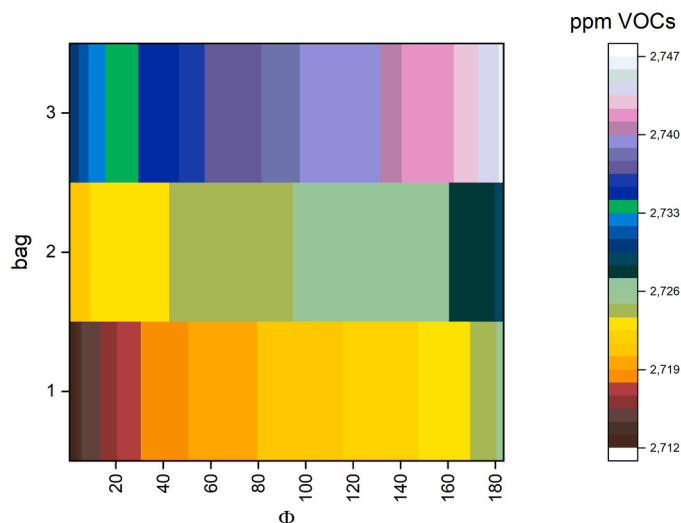


Fig. 2. Experimental results of ppm VOCs passive releases of the three compostable waste bags, Δ is 0.035, $T = 22^\circ\text{C}$. Frequencies are significantly different, MANOVA $p < .001$. False colours are used to magnify the differences.

Table 1
FT-IR peaks of low-density polyethylene (LDPE).

Band (cm^{-1})	Assignment
3200–3400	OH stretching
2914	CH ₂ asymmetric stretching
2846	CH ₂ symmetric stretching
1739	-
1472 and 1462	Bending deformation
1375	CH ₃ symmetric deformation
876	-
729 and 720	Split CH ₂ rocking deformation

5.1. Negative control: releases from compostable garbage bags

The preliminary negative control was to record the passive releases of the three compostable waste bags. All three bags release different frequencies of VOCs, limited to the range of 2.710–2.745 ppm, with a Δ of 0.035 (Fig. 2); the slight difference is probably due to the different materials and production procedures. Based on the FT-IR spectra, all three plastic bag samples are composed of non-pure LDPE (Table 1), confirming the specifications provided by the manufacturers. In fact,

LDPE has a single CH₂ oscillation peak at about 720 cm^{-1} and a CH₂ chain deformation peak at about 1470 cm^{-1} . In the three samples, both peaks appear doubled, characteristic of HDPE insertion; thus, the peak at 1375 cm^{-1} suggests the presence of linear low-density polyethylene (LLDPE) as well. The signals in 1739 and 876 cm^{-1} are due to dyes or additional chemical materials supporting LDPE, such as UV stabilizers. The peak between 3200 and 3400 cm^{-1} corresponds to the stretching of the OH group. The peaks at 2914 and 2846 cm^{-1} are attributed to the asymmetric and symmetric stretching of the intrachain CH₂ units, while the signals at 1472 and 1462 cm^{-1} are related to the asymmetric and symmetric deformations of the above units (Fig. 3).

The difference in VOCs among three bags is significant MANOVA $p < .001$ ($F_{(2546)} = 1982.88$) and all post-hoc one-way ANOVA return a $p < .001$ (bag 1 vs. bag 2 $F_{(1364)} = 415.62$; bag 2 vs. bag 3 $F_{(1364)} = 1869.34$; bag 1 vs. bag 3 $F_{(1364)} = 2864.87$), (Fig. 4).

5.1.1. Positive control: VOCs emission from animal remain

A preliminary control was performed on remaining animal tissues of chicken, pig and cow without and with LPDE barrier; the range of VOCs fluctuation in all conditions is small, $2.85 \pm .01$ ppm VOCs (Fig. 5).

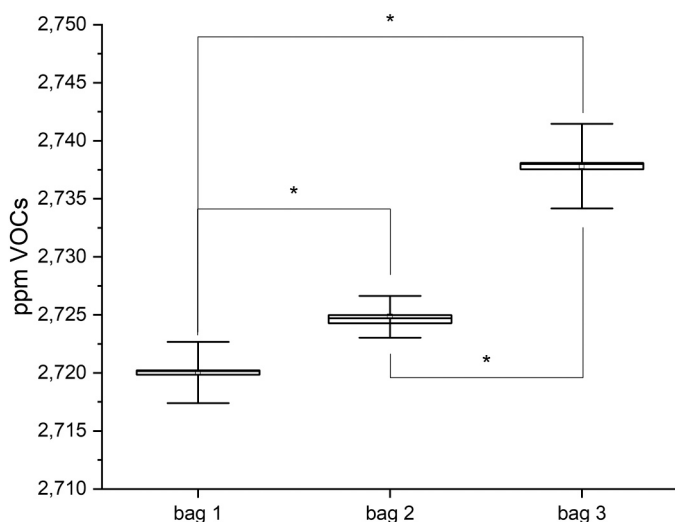


Fig. 4. VOCs releases by the three compostable waste is significant different, post-hoc one-way ANOVA $p < .001$, due to materials/manufacturing procedures.

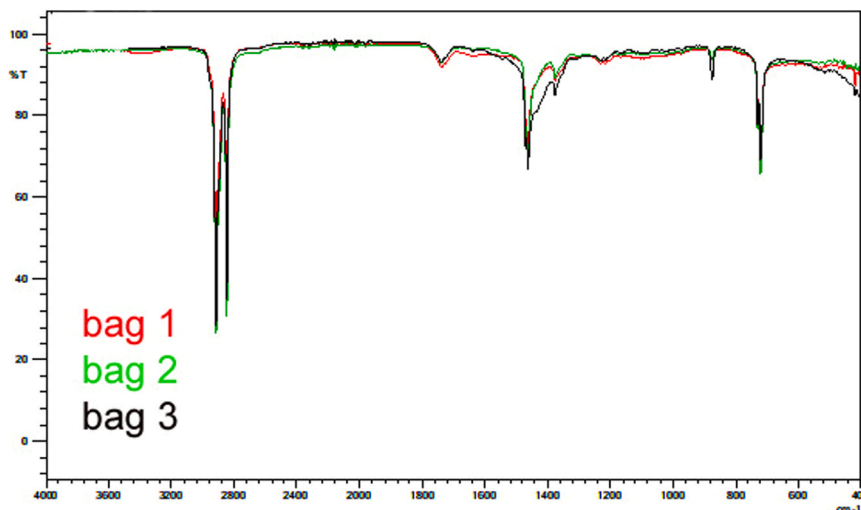


Fig. 3. Overlapping FT-IR spectra of three bags.

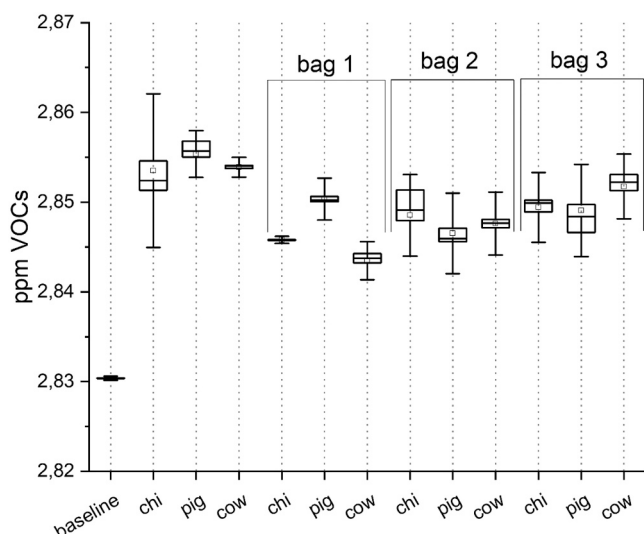


Fig. 5. A preliminary control was performed on remaining animal tissues of chicken, pig and cow without and with LPDE barrier.

Repeatability and reproducibility tests were conducted to assess the consistency and replicability of the measurements, $p < .001$ (Table 2).

5.1.2. Positive control: VOCs emission from human remain

The positive control was obtained by recording the emission of VOCs from human tissues. Human tissue releases different frequencies of VOCs during three days of decomposition process, ranging from 2.86 to 3.94 ppm Δ is 1.08 (Fig. 6). Repeatability and reproducibility tests were conducted on all trials, $p < .001$; and specifically, the repeatability test was $p = .000581$ and the reproducibility test $p = .001627$.

The difference in VOCs during three days of decomposition process is significant MANOVA $p < .001$ ($F_{(3796)} = 2124.70$) and all post-hoc one-way ANOVA return a $p < .001$ (0 h vs. 24 h $F_{(1398)} = 922.14$; 0 h vs. 48 h $F_{(1398)} = 1352.60$; 0 h vs. 72 h $F_{(1398)} = 3106.56$; 24 h vs. 48 h $F_{(1398)} = 77.90$; 24 h vs. 72 h $F_{(1398)} = 2034.36$; 48 h vs. 72 h $F_{(1398)} = 1748.52$), (Fig. 7).

5.2. Experiment: human VOCs permeate compostable garbage bags

Recording of VOCs emitted from human tissue during three days of decomposition process and permeating from compostable waste bags. Human VOCs of decomposition process permeate all three bags, ranging from 2.884 to 3.392 ppm Δ is 0.508, about 50 % of decrease imposed by physical barrier of bag (Fig. 8).

Repeatability and reproducibility tests return $p < .001$; specifically, the repeatability test was bag1 $p = .000879$, bag2 $p = .006601$, bag3 $p = .008995$; the reproducibility test bag1 $p = .002461$, bag2 $p = .018484$, bag3 $p = .025186$.

The difference in VOCs during three days of decomposition process and across bag 1 is significant MANOVA $p < .001$ ($F_{(3796)} = 2676.7$) and all post-hoc one-way ANOVA return a $p < .001$ (0 h vs. 24 h $F_{(1398)} = 146.92$; 0 h vs. 48 h $F_{(1398)} = 320.28$; 0 h vs. 72 h $F_{(1398)} = 4012.59$; 24 h vs. 48 h $F_{(1398)} = 306.87$; 24 h vs. 72 h $F_{(1398)} = 42786.18$; 48 h vs. 72 h $F_{(1398)} = 11861.89$), (Fig. 9A). The difference in VOCs during three

days of decomposition process and across bag 2 is significant MANOVA $p < .001$ ($F_{(3796)} = 10060.22$) and all post-hoc one-way ANOVA return a $p < .001$ (0 h vs. 24 h $F_{(1398)} = 48.35$; 0 h vs. 48 h $F_{(1398)} = 479.49$; 0 h vs. 72 h $F_{(1398)} = 21441.9$; 24 h vs. 48 h $F_{(1398)} = 501.9$; 24 h vs. 72 h $F_{(1398)} = 13714.58$; 48 h vs. 72 h $F_{(1398)} = 61252.66$), (Fig. 9B). The difference in VOCs during three days of decomposition process and across bag 3 is significant MANOVA $p < .001$ ($F_{(3796)} = 12443.08$) and

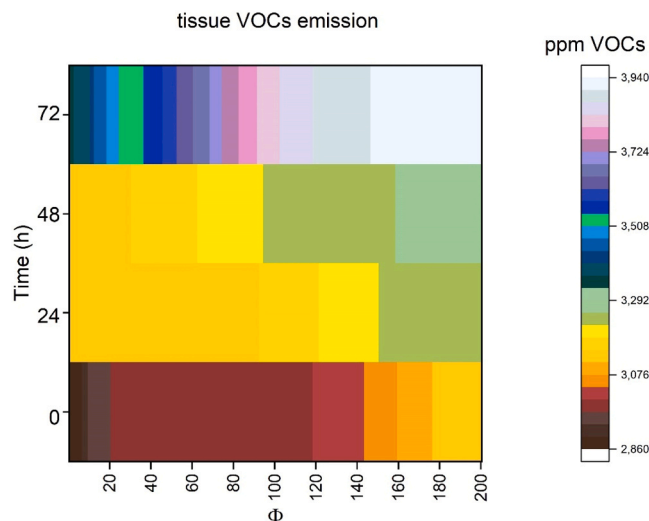


Fig. 6. Human tissue VOCs passive releases during three days of decomposition process at 22°C, Δ is 1.08. Frequencies are significantly different, MANOVA $p < .001$. False colours are used to magnify the differences.

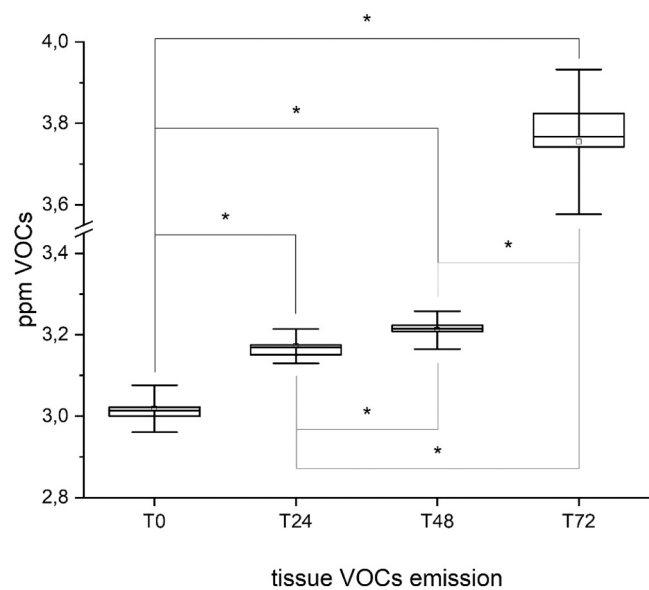


Fig. 7. Human tissue VOCs passive releases at 22°C is significant different, post-hoc one-way ANOVA return a $p < .001$, due to three days of decomposition process.

Table 2
Repeatability and reproducibility tests.

	Repeatability test results, $p =$				Reproducibility test result, $p =$			
	without LPDE	bag1	bag2	bag3	without LPDE	bag1	bag2	bag3
chicken	.000881	.000611	.000697	.000675	.002466	.001711	.001952	.001890
pig	.000640	.000634	.000695	.000720	.001792	.001776	.001946	.002015
cow	.000616	.000630	.000664	.000667	.001724	.001764	.001858	.001866

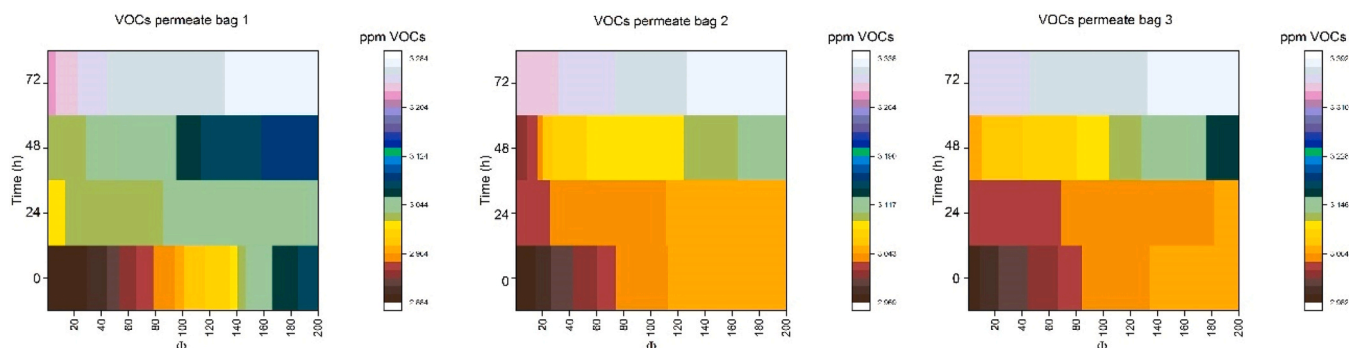


Fig. 8. Permeability of three different bags to human VOCs remains decomposition process at 22°C, Δ is 0.508. Frequencies are significantly different, MANOVA $p < .001$. False colours are used to magnify the differences.

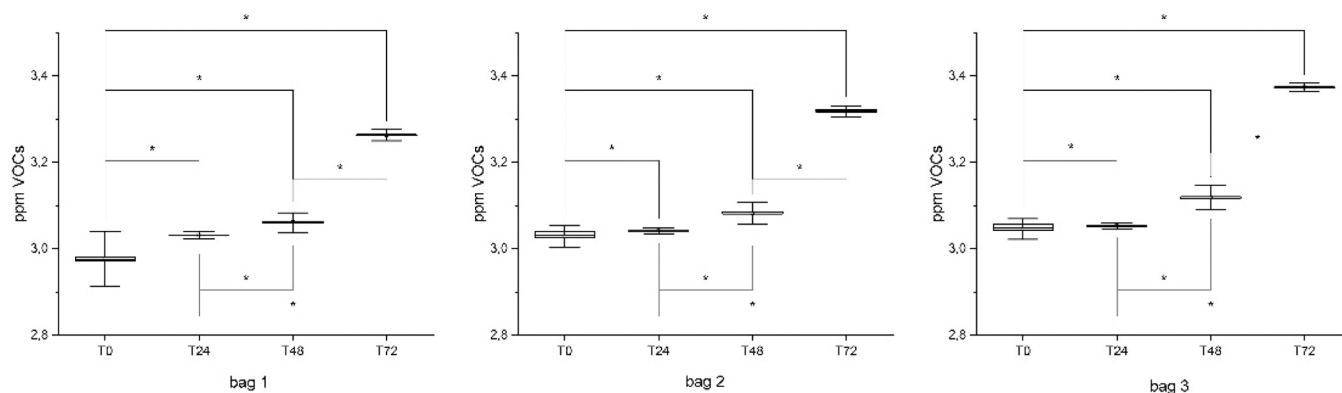


Fig. 9. Permeability of three different bags to human VOCs remains is significant different, post-hoc one-way ANOVA return a $p < .001$, at 22°C.

all post-hoc one-way ANOVA return a $p < .001$ (0 h vs. 24 h $F_{(1398)} = 17.53$; 0 h vs. 48 h $F_{(1398)} = 759.57$; 0 h vs. 72 h $F_{(1398)} = 31964.67$; 24 h vs. 48 h $F_{(1398)} = 995.78$; 24 h vs. 72 h $F_{(1398)} = 146804.60$; 48 h vs. 72 h $F_{(1398)} = 14341.16$), (Fig. 9C).

5.3. Fingerprint of human VOCs permeable in car experiment

The replicate experiment inside a car. The fingerprint of VOCs permeable in car experiment return the behaviour of molecules. Human remains show the natural fingerprint of VOCs emission (Fig. 10A). This fingerprint is modified by physical barrier imposed by bags as showed in laboratory controlled conditions (Fig. 10B,C,D).

6. Discussion

Physiologically, human remains release a complex bouquet of VOCs that fluctuate over time and depending on intrinsic and extrinsic factors [11, 22–26].

Volaboloma mortis is composed of VOCs, and like DNA they have trace value, so these markers must be considered and studied by forensic science. Here we explored the feasibility of using *Volaboloma mortis* in a real case, the murder of the “painter” [20]. The key to judgment is the storage of the body for three days in the house and car at an average of 22°C. [20] Following Locard’s “every contact leaves a trace” paradigm [27], was any volabolomic trace detectable in the house or vehicle?

To answer this question, we measured, using an e-nose as suggested by recent literature, the VOCs permeated through LDPE plastic bag human remains decay [12,28]. LDPE of bags is a semi-crystalline material with moderate elasticity, tends to permanently deform, is resistant to many solvents at room temperature, aromatic and chlorinated hydrocarbons cause it to swell, has excellent water impermeability. Nevertheless, as shown by our experimental demonstration and data in

the literature LDPE is permeable to gases, VOCs and vapours [29]. Portable technologies, such as electronic noses (e-noses), are an alternative to expensive benchtop equipment for VOCs detection. Further, e-noses are sensors that mimic the olfactory system in real time [28]. VOCs emitted from either three common plastic bags used for garbage, including the one allegedly used in the crime, and human remains were recorded for 72 hours. The analysis shows a slight significant difference between the VOCs emitted from the bags. Accordingly, plastic bags release VOCs related to the manufacture and materials used. However, these VOCs, on the one hand, are crime scene contaminants and, on the other hand, are useful in determining which brand was used in the crime. This result has two aspects of interest: one general, VOC analysis could support forensic chemistry and materials engineering in the study of functional properties of materials, such as LDPE plastic bags; and one for the case at hand, it could have helped investigators find traces of plastic bags and ascertain compatibility with a particular brand.

As in Mazzatenta et al. [12] decay of human tissues releases VOCs that are clearly recordable in relation to elapsed time and stages of decomposition, which is also important for determining the postmortem interval (PMI). Again, the result has two aspects of relevance: one general, VOC analysis could support legal medicine and forensic anthropology in studying the functional properties of human tissue decay; and one for the case at hand, it could have helped investigators find traces of the human cadaver inside the house and/or in the car.

Since polymer films used for garbage bags are permeable to VOCs [30,31]. We tested whether it was possible to record the human *Volaboloma mortis* permeated by these bags over time under two conditions that mimic the actual circumstances of murder. The experiment shows that all plastic bags allow permeation to VOCs; furthermore, all three bags behave compatibly allowing a higher permeation rate between 48 and 72 hours. However, bag 1 shows a slight difference in the VOCs permeation rate, probably because of its material

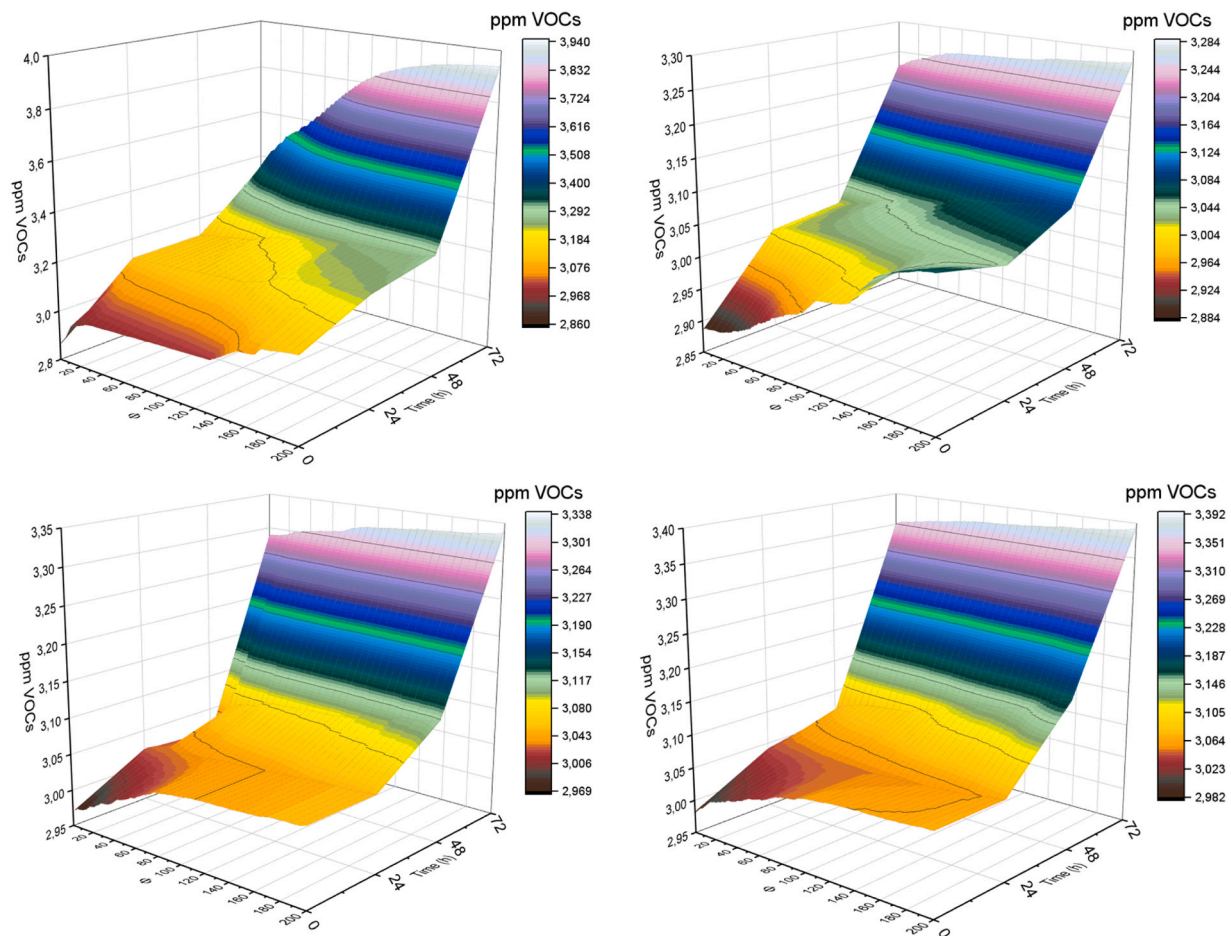


Fig. 10. Permeability of three different bags to human VOCs remains decomposition process at 22°C, Δ is 0.508. Frequencies are significantly different, MANOVA $p < .001$. False colours are used to magnify the differences.

composition/manufacturing. The results highlight the importance of this investigation, which could enable a breakthrough in crime scene analysis by enriching it with a new set of measurable traces.

Finally, we replicated the experiment in the complex environment of the automobile. The results suggest that the VOCs produced under these conditions vary with time. Observing the permeation, the bags clearly act as a barrier in the initial stage of permeation, while later increasing permeation; again, all bags behave similarly, with bag 1 showing a slight difference, similarly in the laboratory controlled conditions. The experiment demonstrates the feasibility of *Volaboloma mortis* as a trace in murderous crimes and related medico-legal and forensic applications.

7. Conclusion

In conclusion, the study of *Volaboloma mortis* is a new biomedical science applicable in criminal investigation. The power of VOC investigation is based on the ephemeral world of invisible volatile molecules that, however, pervade the entire environment and that all investigators remember when they first enter a crime scene, mainly a homicide ranging from decomposition gases of a corpse, body fluids, gunpowder, cleaning substances, etc. In the present experiment and our previous work, we studied the application of *Volaboloma mortis* in a real case with experimental evidence and for the study of PMI.

Today we are in an era like the time when DNA was hypothesized to be a forensic trace.

CRediT authorship contribution statement

Valentini Cristiana: Investigation, Conceptualization. **Demontis Roberto:** Writing – review & editing, Investigation. **Ciulla Michele:** Investigation, Formal analysis. **Berti Andrea:** Writing – review & editing, Methodology. **Locatelli Marcello:** Methodology, Formal analysis. **Di Profio Pietro:** Investigation, Formal analysis. **D'Ovidio Cristian:** Writing – review & editing, Validation, Supervision, Data curation. **Mazzatenta Andrea:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pietrangelo Tiziana:** Writing – review & editing, Conceptualization.

Declaration of Competing Interest

The authors declare that there are no financial or other relationships that could lead to a conflict of interest.

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