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Evaluating Attenuated Total Reflectance Fourier Transform Infrared to Identify and Delineate Gravesoil Post-Exhumation of a Murine Carrion

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1 INTRODUCTION

- **Mammalian carrion are significant nutrient-cycling resources. Decomposition and subsequent cadaveric seepage induce a succession of the soil's physical, microbial and biochemical properties known as the cadaver decomposition island (CDI).[1]**
- **Furthermore, the forensic applicability of biochemical and spectroscopic profiling of the surrounding cadaveric environment has been recognised for estimating time since death or post-mortem interval (PMI).[2,3]**
- **Despite demonstratable progress, there remains scope for development of models in terrestrial burial scenarios where remains may have been exhumed, relocated, or desiccated beyond taphonomic classification.[4,5]**
- **Moreover, the quantitative longevity of the chemical CDI landscape may enhance and complement the current body of literature and could aid in forming models at historical and cold-case crime scenes.**
- **Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy lends itself to forensic applications in that it requires only a small aliquot of sample material and little preparation while being non-destructive, rapid, cheap and reproducible.**

2 OBJECTIVE

The aim of this study was to utilise non-destructive attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy techniques to locate, identify and differentiate gravesoil post exhumation of *Mus Musculus* remains.

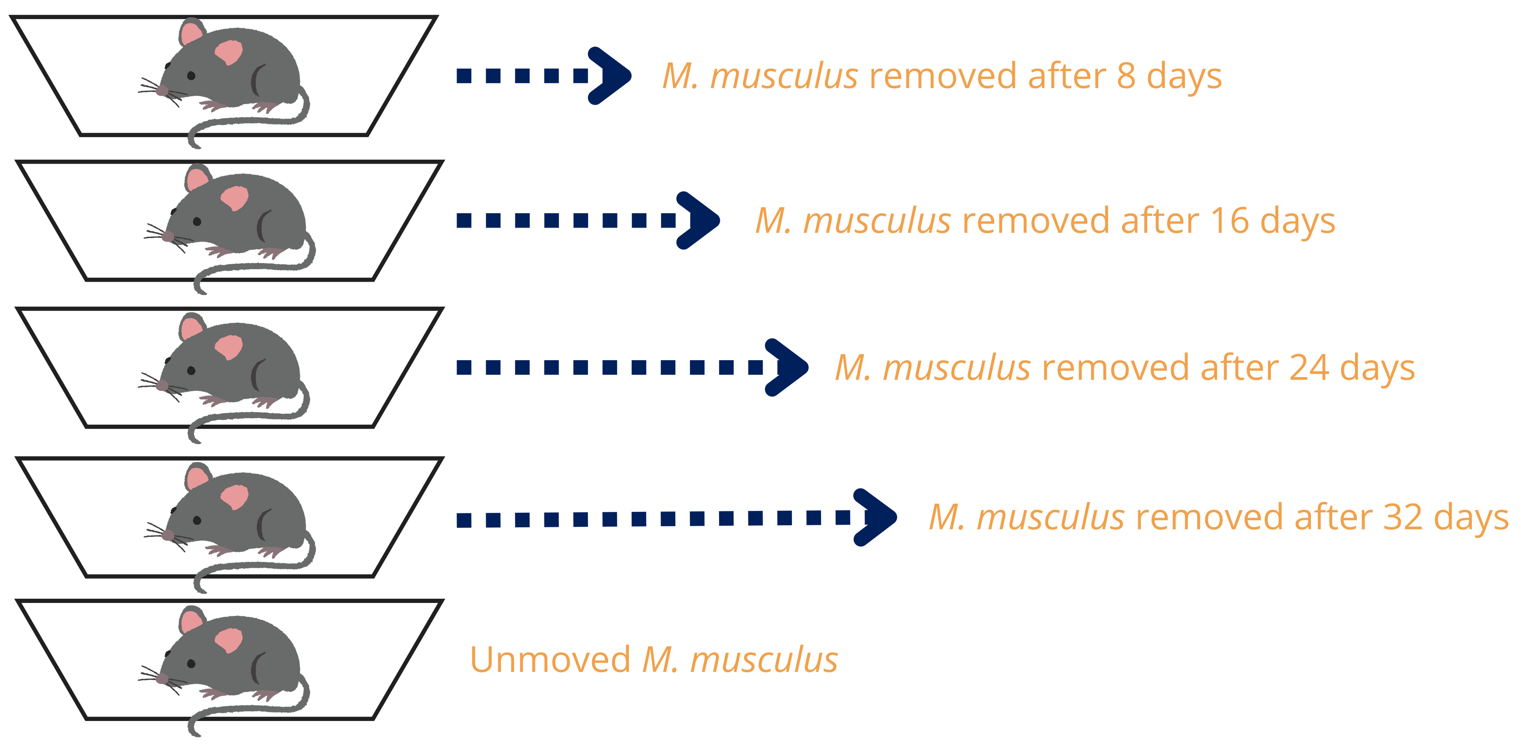
KEY

Day 0
Day 8
Day 16
Day 24
Day 32
Day 50
Day 80
Day 110

Control soil
Unmoved *M. musculus* soil
Experimental soil samples

Control
Unmoved *M. musculus*
Moved after 8 days
Moved after 16 days
Moved after 24 days
Moved after 32 days

3 METHODOLOGY



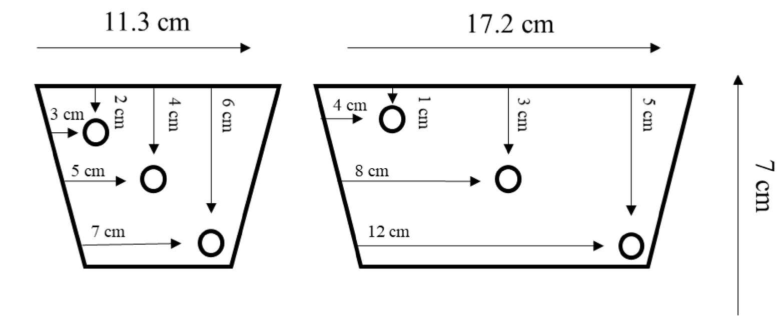


Figure 1. 1 cm perforations (n=12) created with a heated metal corning tool at: at 1 cm x 4 cm, 3 cm x 8 cm and 5 cm x 12 cm on two long sides and 2 cm x 3 cm, 4 cm x 5 cm and 6 cm x 7 cm on the alternate short sides.

M. musculus (~18.2 g) burial simulation microcosms, ran in triplicate maintained indoor at ambient temperature → ***M. musculus* remains removed after 8, 16, 24 or 32 days post burial** → **Soil samples were collected on days 0, 4, 8, 16, 32, 40, 50, 80, and 110 through perforations on the microcosm sides and stored at -20** → **Homogenised soil aliquots (1–2 mg) were measured directly on the Perkin Elmer BX FT-IR spectrometer within a range of 4000 – 400 cm^{-1} over 64 scans at a 4cm^{-1} resolution** → **Spectra were treated with Multiplicative and Extended Multiplicative Scatter Correction (MSC/EMSC) prior to applying the second derivative (D2) using the Savitzky-Golay algorithm with 10 smoothing points.**

4 SPECTRA

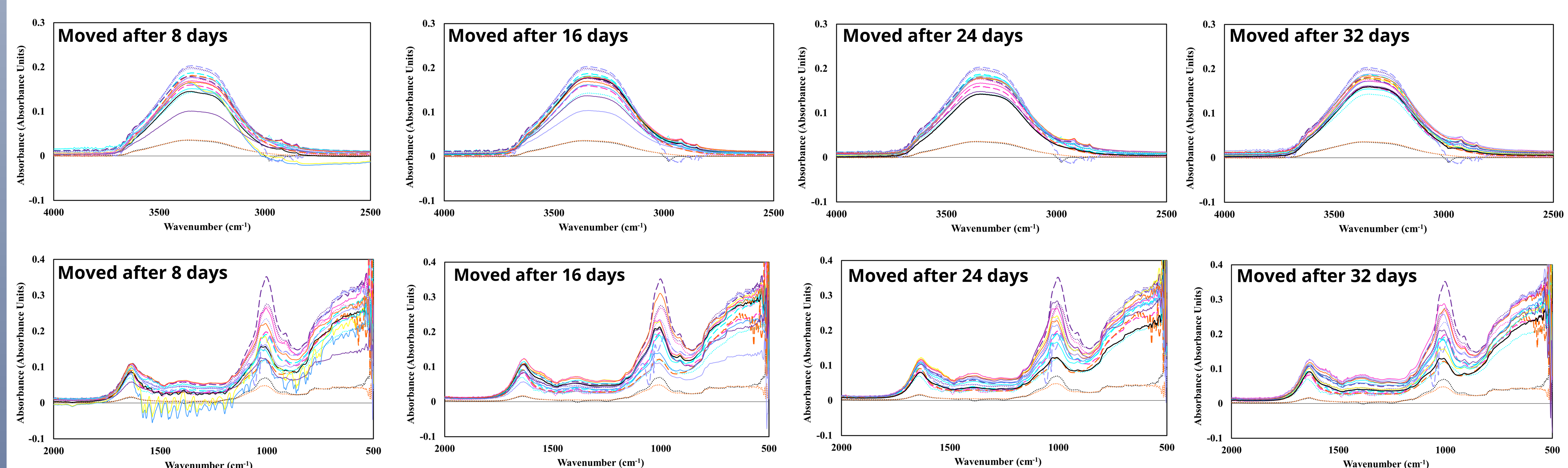


Figure 2. FTIR spectra of soil samples taken from microcosms after removal of *M. musculus* after 8, 16, 24 and 32 days of burial. Post-exhumation samples were taken over 170 days. **Top:** expanded 4000 cm^{-1} - 2500 cm^{-1} region **Bottom:** expanded 2000 cm^{-1} - 500 cm^{-1} region.

5 CHEMOMETRICS

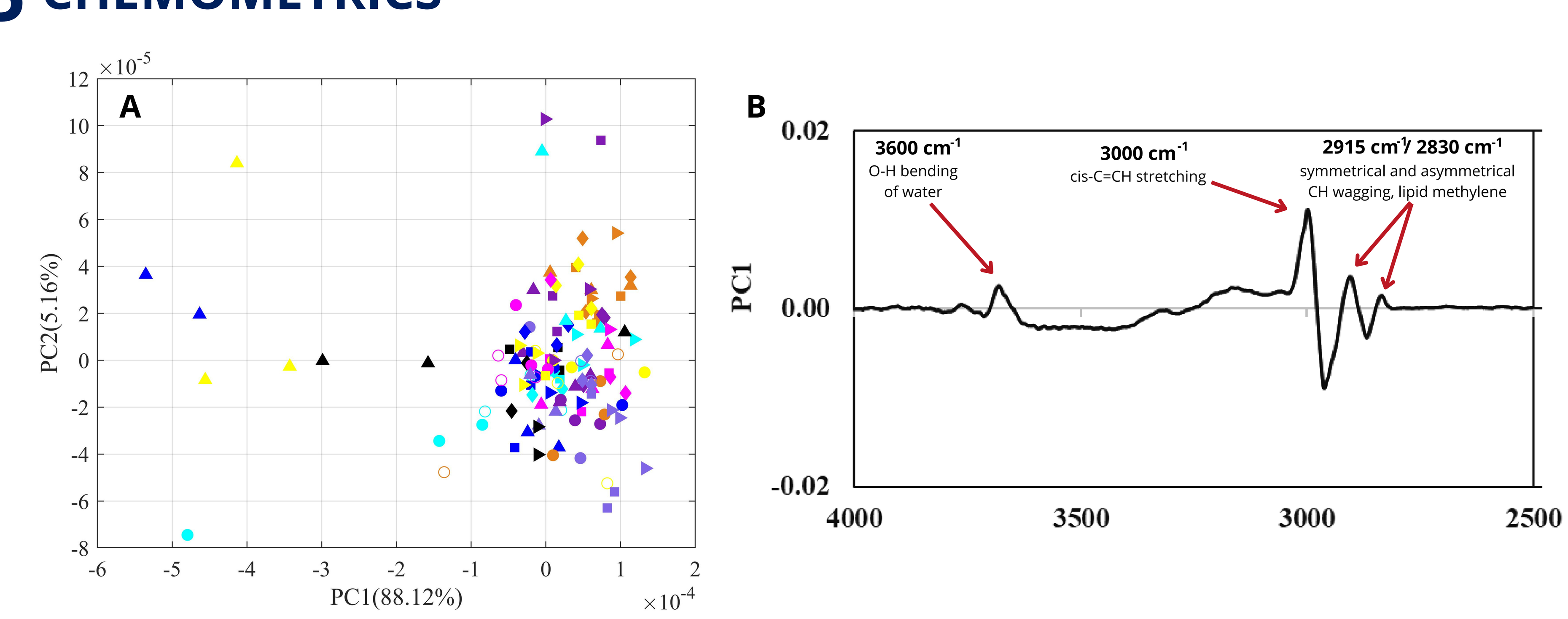


Figure 3. (A) PC scores plot of all soil MSC-D2 treated spectra within the selected region of 4000cm^{-1}- 2500 cm^{-1} sampled on days 0, 8, 16, 24, 32, 50, 80 and 110 **(B)** PC loadings plot of the expanded 4000cm^{-1} - 2500 cm^{-1} region.

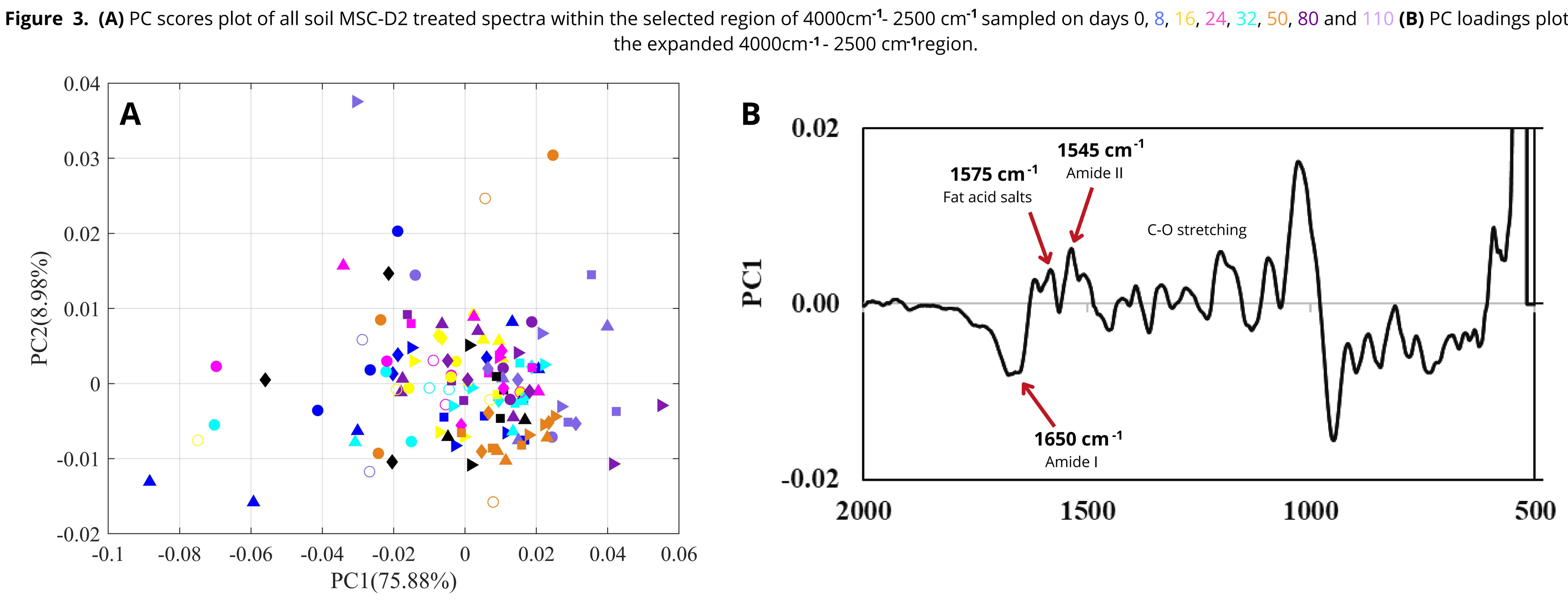


Figure 4. (A) PC scores plot of all soil MSC-D2 treated spectra within the selected region of 2000cm^{-1} - 500cm^{-1} sampled on days 0, 8, 16, 24, 32, 50, 80 and 110 **(B)** PC loadings plot of the expanded 2000cm^{-1} - 500 cm^{-1} region.

6 RESULTS

- **Chemometric analysis performed on MSC-D2 spectral data highlighted differentiation of grave soil for 16 days after exhumation of carrion left to decompose for 8 days.**
- **PC scores plot of both the expanded 4000 - 2500 cm^{-1} and 2000 - 500 cm^{-1} demonstrate little delineation between all of the exhumation parameters.**
- **Samples that contained murine remains, irrespective of exhumation or time of exhumation, began to cluster in the later sampling intervals (days 50, 80 and 110).**
- **The loadings plot of the 4000 - 2500 cm^{-1} region highlights that 88.12% of the variation between samples was due to changes in both symmetrical and asymmetrical CH_2 wagging present at 2915 cm^{-1} and 2830 cm^{-1} aliphatic chain lipids and O-H water bending at ~3600 cm^{-1}.[3]**
- **The loadings plot of the 2000 - 500 cm^{-1} region demonstrated that 75.88% of the observed variation between samples was a result of N-H stretching at 1650 cm^{-1} and 1545 cm^{-1}, amide I and II, respectively. Similarly, absorbance at 1575 cm^{-1} is indicative of salts of fatty acids when unsaturated[3].**

7 CONCLUSIONS

- **Observed variation between the time of exhumation soils was a result of the changes to the lipid methylene and amide profiles, concurrent with previous cadaveric fluid tracking literature.[3,6]**
- **Notable discrimination of 16-days burial gravesoil at 8 days after M. musculus exhumation.**
- **The inability to successfully delineate between exhumation parameters suggests that the time of exhumation has little impact on identifying the presence of a ~18.2 g murine carrion in a burial simulation scenario.**
- **Clustering of later sampling intervals (50, 80 and 110), particularly the 4000 - 2500 cm^{-1} region, irrespective of exhumation treatment suggest that CDI development began to homogenise.**
- **In contrast, the lack of temporal significance, as identified in our previous studies, highlights the impact of remains removal on dating the post-burial interval of *M. musculus.* Furthermore, this provides scope to investigate the impact of exhumation further with larger cadaver proxies and natural terrestrial environments, with insect and scavenger access. Adding to this model is pertinent for establishing a time elapsed at unattended death scene that may have been relocated.**

RELATED LITERATURE

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3. Collins S, Stuart B, Ueland M. Monitoring human decomposition products collected in clothing: an infrared spectroscopy study. Australian Journal of Forensic Sciences. 2020 Jul 3;52(4):428-38.
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