



UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI
SCIENZE DELLA TERRA "ARDITO DESIO"



The Biogeochemistry of Body Concealment: from Hypogeal CDI to Forensic Taphonomy

A dissertation submitted in partial fulfillment of the requirements for the degree of
Dottore di Ricerca in Scienze della Terra

Presented by

Giulia Tagliabue

R13855

ORCID: 0000-0002-8841-0957

Academic Year

2024-2025

PhD Cycle XXXVIII

Advisor *Prof. Luca Trombino*

Co-Advisor *Prof. Cristina Cattaneo*

Thesis Reviewers:

Prof. Vincent Varlet

Dr. Daniel Gaudio

Defense Committee:

Prof. Lucia Angiolini

Prof. Vincent Varlet

Dr. Daniel Gaudio

PhD Coordinator *Prof. Giovanni Muttoni*

*“I have observed that everything in Nature transforms,
and that from destruction new life arises.”*

— Maria Sibylla Merian (1647–1717)

*Preface to Der Raupen wunderbare Verwandlung und sonderbare Blumennahrung
(1679–1683)*

Table of Contents

ABSTRACT	1
CHAPTER 1: INTRODUCTION	7
1.1. THESIS STRUCTURE	7
1.2. OBJECTIVES	8
1.3. FORENSIC TAPHONOMY & THANATOLOGY	12
1.3.1. POSTMORTEM INTERVAL (PMI) & POST-BURIAL INTERVAL (PBI)	18
1.3.1.1. ADD, TBS and Other PMI Estimation Formulas	24
1.3.2. MICROTRACES DETECTION: INORGANIC GUNSHOT RESIDUES	30
1.4. FORENSIC BIOGEOCHEMISTRY & (HYPOGEAL) CADAVER DECOMPOSITION ISLAND	38
CHAPTER 2: THE EXPERIMENT – CONTEXT AND CONDITIONS OF THE STUDY	47
2.1. TICINO-LEAFs: THE FIRST (NON-HUMAN) FORENSIC TAPHONOMY FACILITY IN ITALY	47
2.1.1. OVERVIEW OF GEOSCIENCES APPLIED TO FORENSIC TAPHONOMY: THE INTERDISCIPLINARY APPROACH OF THE ITALIAN NON-HUMAN RESEARCH FACILITY (TICINO-LEAFs) PUBLISHED IN GEOSCIENCES	46
2.1.2. THE EXPERIMENT <i>TICINO 2023</i> – STUDY DESIGN	76
2.1.3. THE ETHICS BEHIND THE EXPERIMENT	81
2.2. ENVIRONMENTAL FRAMEWORK	83
2.2.1. SOILS OF THE <i>PARCO LOMBARDO DELLA VALLE DEL TICINO</i>	89
2.2.2. CLIMATIC AND MICROCLIMATIC FRAMEWORK	93

CHAPTER 3: THE EXPERIMENT – HYPOGEAL CADAVER DECOMPOSITION

ISLAND **100**

3.1. MATERIALS & METHODS **100**

3.1.1. MATERIALS 100

3.1.2. METHODS 102

3.1.2.1. Field Activity: Site and Soil Description 102

3.1.2.2. Laboratory Soil Characterization 103

3.1.2.3. Total Organic Carbon (Walkley-Black Method) 107

3.1.2.4. pH Analysis 109

3.1.2.5. Dissolved Organic Matter & Fourier-Transform Infrared Spectroscopy 110

3.1.2.6. Organic Acids (High-Performance Liquid Chromatography) 112

3.1.2.7. Magnetic Susceptibility & Hysteresis Analyses 115

3.2 RESULTS **119**

3.2.1. SITE & SOIL CHARACTERIZATION 119

3.2.2. TOTAL ORGANIC CARBON (TOC) 124

3.2.3. *DISSOLVED ORGANIC MATTER: UNRAVELING THE ROLE OF ORGANIC CARBON FROM THE HYPOGEAL CADAVER DECOMPOSITION ISLAND PUBLISHED IN FORENSIC SCIENCE*

INTERNATIONAL 128

3.2.4. MAJOR ORGANIC ACIDS: VFAs, LACTIC ACID, AND SUCCINIC ACID 142

3.2.5. SOIL MAGNETIC SUSCEPTIBILITY & HYSTERESIS 148

CHAPTER 4: THE EXPERIMENT – FORENSIC TAPHONOMY &

DECOMPOSITION **158**

4.1 MATERIALS AND METHODS **158**

4.1.1. MATERIALS	158
4.1.1.1. Piglets (<i>Sus scrofa domesticus</i>)	158
4.1.1.2. Associated Material & Treatments	164
4.1.2. METHODS	167
4.1.2.1. Recovery, Micro-excavation, and Post-burial External Examination of the Remains	167
4.1.2.2. Decomposition, Total Body Score (TBS) and PMI Estimation Formulas	170
4.1.2.3. Taphonomy of Gunshot Wounds: Macroscopic Analysis	172
4.1.2.4. SEM-EDX Analysis of Inorganic Gunshot Residue (IGSR)	175
4.2. RESULTS	177
4.2.1. ASSESSMENT OF DECOMPOSITION & TAPHONOMIC ALTERATIONS	177
4.2.2. POSTMORTEM & POST-BURIAL INTERVAL ESTIMATION	188
4.2.3. TAPHONOMIC AND CHEMICAL ANALYSES OF GUNSHOT-RELATED WOUNDS	211
<u>CHAPTER 5: DISCUSSION AND CONCLUSIONS</u>	<u>212</u>
5.1. THE HYPOGEAL CADAVER DECOMPOSITION ISLAND AS A TOOL FOR POST-BURIAL INTERVAL ESTIMATION	213
5.1.1. THE ROLE OF CARBON AS A MARKER FOR HYPOGEAL DECOMPOSITION: DISSOLVED ORGANIC MATTER CHARACTERIZATION FOR PMI ESTIMATION	214
5.1.2. HCDI BIOGEOCHEMICAL ANOMALIES AND THEIR RELATIONSHIP WITH PMI	224
5.1.3. MAGNETIC SUSCEPTIBILITY AND HYSTERESIS INVESTIGATION OF THE HCDI: A PROMISING LABORATORY TOOL FOR CLANDESTINE GRAVES DETECTION	232
5.2. CONCEALMENT METHODS AS TAPHONOMIC AGENTS	238
5.2.1. TBS OF BURIED BODIES: A COMPARISON OF THREE METHODS	242

5.2.2. CHALLENGES IN PMI ESTIMATION: INSIGHTS FROM ADD AND VASS' UNIVERSAL FORMULA	245
5.2.3. TAPHONOMIC AND CHEMICAL ANALYSES OF GUNSHOT-RELATED LESIONS ON BURIED PIGLET REMAINS: A COMPLEMENTARY APPROACH	250
5.3. CONCLUSIONS & FUTURE PERSPECTIVES	266
<u>CHAPTER 6: SIDE PROJECT - THE CRYPT OF THE CA' GRANDA HOSPITAL</u>	<u>273</u>
6.1. <i>THANATOGENIC ANTHROSOL: A GEOFORENSIC APPROACH TO THE EXPLORATION OF THE SEPOLCRETO OF THE CA' GRANDA (MILAN)</i> PUBLISHED IN <i>AAPP, CLASS OF PHYSICAL, MATHEMATICAL AND NATURAL SCIENCES</i>	276
<u>REFERENCES</u>	<u>298</u>
<u>ACKNOWLEDGEMENT</u>	<u>332</u>
<u>APPENDIX</u>	<u>334</u>
<u>PUBLICATIONS AND DISSEMINATION ACTIVITIES</u>	<u>509</u>

ABSTRACT

The present PhD project provides an integrated and methodologically exploratory investigation of the processes governing cadaver decomposition in clandestine burials, here conceptualized as the Hypogeal Cadaver Decomposition Island (HCDI). Conducted at Ticino-LEAFs (Ticino Park–LABANOF facility for Environmental Analysis in Forensic Sciences), the research brings together taphonomic observation, soil biogeochemistry, and macro- and microanalytical approaches, offering a broad perspective on how cadaver decomposition may interact with the surrounding environment. The study also considers the influence of concealment methods on these dynamics, as well as the potential for the hypogeal environment to preserve time-dependent information that may be relevant to forensic investigations.

Twenty-one piglets that had died of natural causes, completely independent of this research, were selected as human analogues and assigned to different burial treatments, including textile wrapping, quicklime application, and the simulation of gunshot traumas. They were subsequently exhumed at predetermined, progressively increasing PBIs. To support standardization and comparability between specimens, pre-burial radiographs were taken, weight matching within groups was carried out, and fixed burial positions were maintained. This controlled framework helped identify subtle shifts in both decomposition-derived anomalies and soil-mediated processes.

A multi-proxy analytical workflow was employed to characterize the HCDI. Soil texture, Total Organic Carbon (TOC), pH, Dissolved Organic Matter (DOM), Volatile Fatty Acids (VFA) and other organic acids, and soil magnetic properties were monitored throughout the experiment. DOM appeared to be one of the more sensitive markers of hypogeal decomposition: its concentrations increased during intermediate post-burial intervals, likely reflecting the release of soluble organic compounds during active decay, and decreased once decomposition processes slowed. FT-IR spectrometry contributed substantially to the interpretation of DOM composition, revealing functional groups associated with proteins (potentially useful for monitoring decomposition-related proteolysis), lipids (which may indicate the presence of adipocere), and other biomolecules. In trauma-bearing subjects, the soil surrounding gunshot wounds showed molecular signatures that may be linked to blood infiltration, suggesting that DOM can sometimes retain information not only on decomposition intensity but also on lesion-induced biochemical inputs.

Where multiple concealment methods were introduced, soil responses diverged considerably. Indeed, quicklime, rather than accelerating decay, preserved tissues and promoted adipocere formation. FT-IR spectrometry captured the sequential hydration and carbonation of lime, while DOM concentrations peaked abruptly before declining, suggesting that this analysis may help detect decomposition-linked anomalies in soil even when tissues remain preserved. Clothing also influenced the system by absorbing putrefactive fluids and altering soil-microbe interactions, at times affecting chemical anomalies that might otherwise help differentiate decomposition phases. Taken together, these observations indicate that concealment methods can function as active taphonomic agents and should therefore be considered when interpreting biogeochemical evidence from burial environments.

pH measurements complemented these observations and generally appeared to reflect increases associated with the release of alkaline decomposition products, namely NH_4^+ . However, the presence of lime or textile seemed to modify these trends, implying that pH may serve as a reliable first-line indicator only when soil-cadaver exchange is unimpeded. Similarly, VFA profiles obtained through HPLC were informative but inconsistent across groups, highlighting the value of multi-proxy interpretation rather than reliance on a single biomarker.

A novel aspect of the project was the exploratory application of magnetic susceptibility and hysteresis analyses to hypogeal decomposition. Although decomposition products apparently do not directly alter magnetic signatures, burial disturbance and the redox processes associated with organic-matter degradation appeared to produce measurable differences between control soils and HCDI samples. The latter tended to show more homogeneous magnetic behavior, with grain-size distributions that may indicate transformations of iron-bearing minerals under reducing conditions. These observations suggest that magnetic methods have potential as complementary laboratory tools for detecting or characterizing burial contexts, particularly in settings where traditional biochemical indicators may be altered, such as in the presence of clothing.

From a taphonomic perspective, decomposition progressed across post-burial intervals but showed substantial heterogeneity influenced by concealment methods. Indeed, quicklime appeared to cause extensive desiccation in early PBIs and later to induce mummification, whereas clothing absorbed decomposition fluids and reduced visible soft-tissue breakdown, ultimately contributing to humid mummification. These modifications resulted in inconsistent Total Body Score (TBS) values among subjects within the same interval and increasingly

widened inter-group variability as decomposition advanced. Consequently, the application of standard PMI-estimation methods, particularly the Accumulated Degree Days (ADD) method and Vass's universal formula for buried bodies, proved challenging. ADD tended to underestimate PMI for much of the experiment, while Vass's formula tended to overestimate it, suggesting that neither method is currently calibrated for buried remains exposed to heterogeneous taphonomic conditions.

In parallel, the study examined gunshot-related lesions on piglet remains, integrating taphonomic assessment with SEM–EDX microanalysis. While soft-tissue evidence degraded rapidly, bone appeared to retain key ballistic features even in advanced stages of decay, despite inherent limitations in their diagnosis. The analysis of Inorganic Gunshot Residue (IGSR) may help address some of these challenges. Firearm-related particles persisted, although in altered forms, reflecting environmental leaching and mineralogical interactions. Because particles considered *characteristic of* and *consistent with IGSR* tended to reduce with increasing burial time, the study suggests broadening diagnostic criteria when examining remains subjected to prolonged hypogeal conditions. This highlights the potential value of interdisciplinary approaches, indicating how anthropological and chemical analyses may be combined to support trauma reconstruction when soft tissues have been substantially altered by decomposition.

Overall, this research considers the burial environment as a responsive, multifactorial system shaped by cadaveric decay, soil chemistry, microbial activity, and anthropogenic disturbance. Even in coarse, rapidly draining soils such as those characterizing the experimental area, the HCDI appeared to preserve detectable anomalies in pH, DOM quantification and characterization, and magnetic signatures. At the same time, the experiment highlights the challenges involved in developing universal PMI or PBI estimation methods, given the strong influence of soil properties and concealment strategies on decomposition trajectories. By integrating taphonomic, biogeochemical, magnetic, and microanalytical approaches, this thesis proposes a holistic framework for interpreting clandestine burials. It suggests that multidisciplinary analysis may offer a more comprehensive understanding of the multifaceted interactions governing hypogeal decomposition, thereby potentially improving the accuracy, reliability, and contextual relevance of forensic interpretations.

– CHAPTER 1 –

INTRODUCTION**1.1. Thesis Structure**

The present PhD thesis is centered on the experiment entitled *Ticino 2023*, which constitutes the core of the doctoral project and whose design and principal outcomes are presented across five chapters. In addition, a sixth chapter is dedicated to a side project focused on the ancient crypt of the Ca' Granda Hospital (Milan, Italy).

Chapter 1 presents the introduction to the thesis and is organized into four main sections:

- **1.1** brief description of the thesis structure.
- **1.2** main objectives of this study and workflow of samples and main analyses.
- **1.3** state of the art of forensic taphonomy and thanatology, including their contribution to Postmortem Interval (PMI) and Post-Burial Interval (PBI) estimations. The section also introduces and defines Inorganic Gunshot Residues (IGSR), illustrating their potential for identifying firearm-related lesions in bodies at advanced stages of decomposition.
- **1.4** state of the art of forensic biogeochemistry and its application on the investigation of the Hypogeal Cadaver Decomposition Island (HCDI).

Chapter 2 describes the PhD project and is divided into two sections:

- **2.1** presentation of Ticino-LEAFs (Ticino Park-LABANOF facility for Environmental Analysis in Forensic sciences), the first non-human forensic taphonomy facility in Italy, and the latest taphonomy project carried out: the Experiment *Ticino 2023*. This section includes a manuscript published on *Geosciences* 2024, 14, 359 about the importance of an interdisciplinary approach in the analysis of forensic taphonomy. The paper also lists all the currently working Forensic Taphonomy Facilities (FTF) in the world, highlighting the need to conduct projects considering the cadaver and the surrounding (epigeal and/or hypogeal) environment as dynamic systems of mutual interaction, while also adhering to high ethical standards.
- **2.2** illustration of the study design, including environmental and climatic framework of the experimental area in the FTF, along with the description of the soil that characterizes it, and the field activities for the realization of the simulated clandestine graves.

Chapter 3 presents the PhD project *Ticino 2023* from the HCDI biogeochemical perspective. It is divided into two sections:

- **3.1** detailed description of materials and the methodologies applied in the study. In particular, the section shows field and laboratory methods used for the HCDI investigation: particle-size analysis for soil texture characterization; Walkley-Black method for Total Organic Carbon determination; pH analysis; Dissolved Organic Matter and Fourier-Transform Infrared Spectroscopy; High-Performance Liquid Chromatography for major decomposition-related organic acids (Volatile Fatty Acids, lactic acid, and succinic acid) detection; Magnetic Susceptibility and hysteresis for geomagnetic analysis.
- **3.2** presentation of the results of the analyses performed on the HCDI soil samples. The section includes a manuscript published in *Forensic Science International* regarding the results of DOM and FT-IR spectrometry analyses on HCDI and its relationship with soil pH and decomposition.

Chapter 4 focuses on the results of the analyses conducted on the buried remains. It includes two sections:

- **4.1** list of materials and methods included in this part of the project *Ticino 2023*. Specifically, the section describes the piglets and the associated material before their inhumations. Furthermore, the methodologies applied for recovery, micro-excavation, and post-burial forensic external examination are presented. Subsequently, the section illustrates the methods applied for the macroscopic description of the decomposition state of the piglets, the calculation of their Total Body Score (TBS) and the application of the main formulas for PMI estimation (namely through the calculation of Accumulated Degree Days (ADD) and the Vass “elusive universal formula” for buried bodies). Finally, the taphonomic, osteological and chemical analyses (SEM-EDX) conducted on gunshot wounds on the cadavers are as well described.
- **4.2** presentation of the results of the analyses conducted on the remains, especially focusing on PMI/PBI estimation. This section also includes the outcomes of taphonomic and IGSR analyses carried out on the gunshot lesions on piglets’ skulls.

Chapter 5 provides the main discussions and conclusions of the PhD experimental project *Ticino 2023*, including future perspectives. The chapter is divided into three sections:

- **5.1** discussion of the findings of the analyses conducted on the HCDI sample. The outputs allowed to observe how some biogeochemical evidence such as pH, DOM and organic compounds that characterized it could be considered potential tool for PMI and PBI estimation, while others like VFAs should be considered more informative about the characterization of a clandestine grave. Finally, the section presents the results of the application of Magnetic Susceptibility (MS) and Hysteresis on the HCDI, which was never conducted before.
- **5.2** influence of concealment methods and pre-deposition treatments on the chronology of decomposition phases. In particular, the section suggests that PMI estimation methods based on the macroscopic analysis of the cadaver decomposition state (whether validated on human or animal models) could be insufficient for a correct interpretation of the evidence, and that a specific environmental assessment should always be considered. Moreover, it discusses how decomposition in a hypogeal environment can render gunshot wounds unrecognizable and how a multidisciplinary approach based on osteological and chemical analyses may help address these challenges.
- **5.3** overview of the interaction between the two substrates, cadaver and hypogeal environment, outlining the possible future perspectives arising from the study.

Chapter 6 exhibits a side project conducted during the three PhD years: taphonomy and matrix analysis on samples from the ancient crypt of the Ca' Granda Hospital (XVII century – Milan, Italy). This chapter also includes a secondary section:

- **6.1** this section presents a manuscript published in *AAPP Journal 2023, 101 (S1 A8)* which introduces a new type of *Anthrosol*, mainly composed of bone microfragments and organic matter resulting from the decomposition of human remains, therefore named *Thanatogenic*.

Appendix includes raw data discussed in the thesis.

Publications and Dissemination Activities reports peer-reviewed manuscripts, the conference presentations and scientific dissemination produced during the three years of the PhD programme.

1.2. Objectives

Italian statistics indicate that, over the last ten years (2015–2024), an average of approximately 350 intentional homicides per year have been recorded (official statistics on causes of death reported by the Italian National Institute of Statistics [ISTAT], 2024; data from the Criminal Events Archive of the Italian Department of Criminal Police, Ministry of the Interior, 2025). A substantial proportion of these cases may remain unresolved or require extended investigative periods due to concealment of the victim (Cattaneo & Salsarola, 2009; Borrini, 2015; De Matteis et al., 2021; Delicato, 2025).

The term “concealment” describes the attempt of the murderers to destroy the valuable forensic evidence and/or prevent the identification of the offender or the victim itself (Rahim et al., 2018). The concealment, indeed, can hamper not only the analysis of trace evidence and the identification of the individuals involved, cause and manner of death, but also the determination of the postmortem interval (PMI), post-burial interval (PBI) and, overall, the dynamics of the crime act (De Matteis et al., 2021; Byard, 2024). As emerged in De Matteis et al. (2021), the majority of the currently published articles are focused on a single type of concealment, such as domestic interment, burning, dismembering (Toms et al., 2008; Dogan et al., 2010; Tümer et al., 2012), and therefore are limited to analyzing some peculiar aspect of it. Nevertheless, more recent studies (Schotsmans et al., 2014; Schotsmans & de Voorde, 2017; Rahim et al., 2018; Adams et al., 2019; Byard, 2024) show that the leading pattern of body disposal is represented by an overlapping combination of multiple methods of concealment with the most frequent being the disposal of the body in isolated outdoor areas (Caccianiga et al., 2021; Jones & Allsop 2023; Byard, 2024), often by burying it in soil (Pringle & Jervis, 2010; Larson et al., 2011; Mansegosa et al., 2021), and even with the use of lime and quicklime (Schotsmans et al., 2014; Schotsmans & de Voorde, 2017; Thurn et al., 2024). In this light, increasing attention is being directed toward examining how clothing or body-enveloping fabrics modulate decomposition rates and the associated release of cadaveric material into the surrounding environment (Kelly et al., 2009; Hill & Pokines, 2022). Similarly, recent studies have examined the fate of traces and microtraces, such as gunshot residue, deposited on a victim whose body has been clandestinely buried (Nguyen-Hong et al., 2024).

In this context, recent applications of forensic taphonomy have emphasized the importance of observing the interaction between the cadaver and the surrounding environment, along with decomposition processes and dynamics involving associated items. Nonetheless, most of the

field research is generally limited to forensic botany (Walsh-Haney et al., 2019; Jadhav et al., 2022), entomology (Kotzé et al., 2021; Bansode et al., 2025) and anthropology (Skinner et al., 2003; O'Reilly et al., 2023) neglecting the role of soil until very recent times.

According to Carter & Tibbett (2008) this is an aftermath of the traditional view of forensic science, which sees soil as a passive medium with its own independent properties (Fitzpatrick, 2008) and hence nothing more than associative evidence in a death investigation. Conversely, soil should in fact be regarded as a dynamic medium capable of responding rapidly to environmental changes, particularly when these involve a disturbance as profound and localized as the presence of a decomposing buried body (Carter & Tibbett, 2008). On the other hand, with the progression of decomposition processes, the cadaver and the surrounding environment merge, becoming a unique biogeochemical dynamic substrate characterized by a continuous, yet variable, exchange of organic material.

Therefore, correlating biogeochemical investigations with a comprehensive analysis of the taphonomic alterations affecting buried remains and any associated items constitutes a fundamental approach in forensic and medico-legal inquiries involving homicide and body concealment in outdoor hypogeal environments. Research on human decomposition and its interaction with the natural environment may be conducted either under uncontrolled conditions, primarily through case-based observations, or within controlled experimental settings, such as Forensic Taphonomy Facilities (FTFs, or Body Farms; Forbes, 2017), where human or non-human proxies are systematically studied.

The here-presented PhD project *Ticino 2023* focuses on the biogeochemical and taphonomic analyses of material exchange between buried bodies and the hypogeal environment, especially the Hypogeal Cadaver Decomposition Island (HCDI) in order to evaluate potential time-dependent markers helpful for the postmortem interval (PMI) or post-burial interval (PBI) estimation and the possibility to detect weapon-related microtraces on buried remains.

For this purpose, four groups of simulated clandestine graves containing piglet cadavers (subadult *Sus scrofa domesticus*) that had died from causes unrelated to the present study were established at the non-human Forensic Taphonomy Facility Ticino-LEAFs (Ticino Park–LABANOF Facility for Environmental Analysis in Forensic Sciences), University of Milan. The experimental design included both single-method concealment (simple soil burials) and multiple-method concealment (burials involving textiles and quicklime), as well as a set of

burials in which the piglets were subjected to different types of trauma, including gunshot wounds to the head.

Exhumations were performed at predetermined post-burial intervals, during which multiple soil samples were collected from the HCDIs. The preservation state of the bodies was assessed macroscopically and subsequently correlated with qualitative and quantitative variations in biomarker profiles. In addition, during the external examination, tape-lift sampling was performed on the firearm-inflicted wounds.

Overall, the doctoral research project *Ticino 2023* was conceived to address a series of questions arising from the observation of interaction dynamics between buried bodies and the surrounding hypogeal environment. A graphical overview of these research questions, together with the distribution of the experimental approaches designed to address them within this thesis, is provided in Figure 1.1.

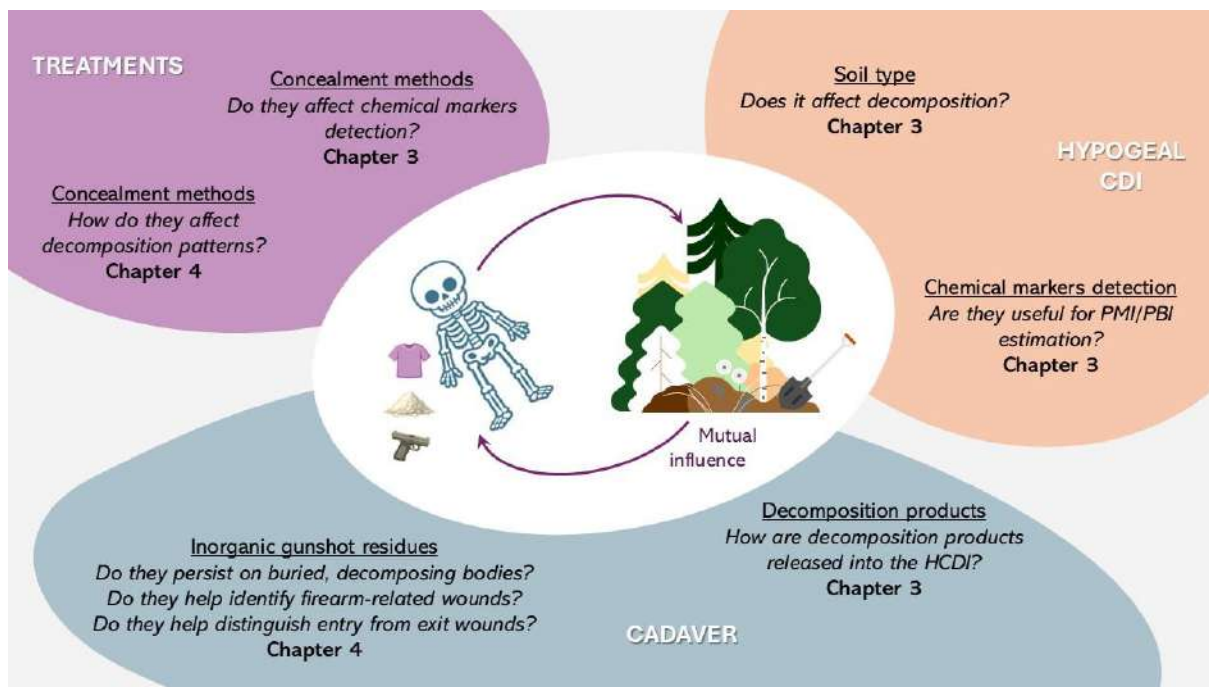


Figure 1.1: Graphical summary of the main research questions underlying the present PhD project and the chapters in which the corresponding experimental sections are presented. The image highlights the reciprocal interaction between a buried decomposing body and the surrounding hypogeal environment. The three colors represent the main elements on which the experiment focused: the cadaver, the treatments applied to it, and the cadaver decomposition island (CDI) formed as a result of decomposition.

In order to address these questions, a series of biogeochemical analyses and taphonomic and thanatological evaluations were carried out on samples of different nature (Figure 1.2).

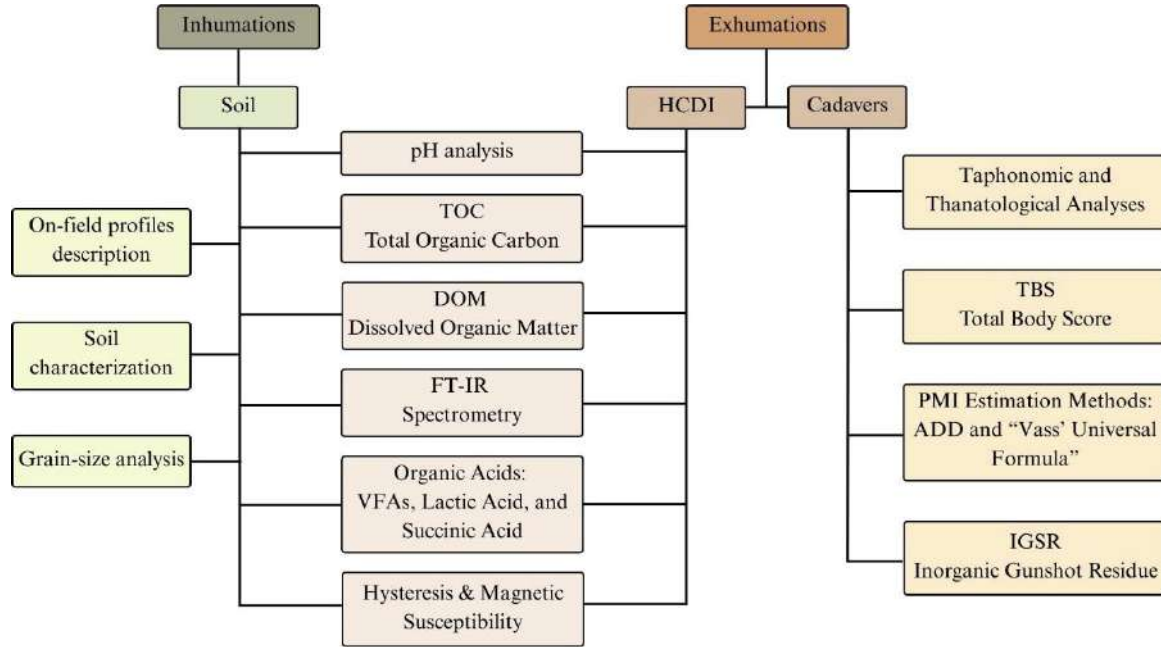


Figure 1.2: Workflow of the main field and laboratory analyses carried out in this PhD project.

Among the various analyses performed on HCDI soil samples, two represent an innovative approach to investigating decomposition-related environmental alterations. The first involves the application of Chemical Oxygen Demand (COD) testing to quantify Dissolved Organic Matter (DOM) concentrations. DOM constitutes the most mobile and soluble fraction of soil organic matter, properties that make it a promising proxy for detecting organic anomalies associated with cadaveric inputs. These analyses were conducted at DISAA (Department of Agricultural and Environmental Sciences – Production, Landscape, Agroenergy), University of Milan.

The second approach consists of assessing changes in the magnetic properties of soil ferromagnetic minerals following the substantial organic matter input associated with cadaver decomposition. These analyses were performed at LASA (Laboratory for Accelerators and Applied Superconductivity), INFN (National Institute for Nuclear Physics), University of Milan.

1.3. Forensic Taphonomy & Thanatology

The observation of postmortem processes affecting the cadaver represents a primary focus of several disciplines, foremost among them forensic taphonomy (Schotsmans et al., 2017). Like many others that bear the appellation “forensic,” taphonomy originates from a purely applicative intent of older and already consolidated disciplines within the investigative field. In this case, the term *taphonomy*, whose etymology derives from the Greek words *taphos* (burial) and *nomos* (laws) (Cadée, 1991; Lyman, 2010), first appeared in 1940 when the paleontologist Ivan Antonovič Efremov defined it as “*the study of the transition of animal remains from the biosphere into the lithosphere*” (Efremov, 1940). Subsequently, in the 1980s, paleobiologists Anna K. Behrensmeyer and Susan M. Kidwell (1985) expanded the concept of taphonomic research to include “*the study of processes of preservation and how they affect information in the fossil record.*” In other words, for the first time taphonomy was conceived not only as a tool for understanding what can be observed, but also what may be absent, due to environmental processes belonging to the past and interpretable by proxy. From this new, seminal intuition, the concept of taphonomy came to be valued in the archaeological domain as well, both conventional and funerary. An example is the definition provided by archaeologists Colin Renfrew and Paul G. Bahn (1991), who described taphonomy as the observation and evaluation of the factors that have affected an object or organism during the time elapsed between its deposition and recovery. It is precisely this definition, explicitly delineating the (relative) temporal window within which taphonomic investigation takes place, that made its translation into the forensic domain appear so natural. Among the various definitions in this latter context, the most comprehensive is arguably that provided by the two forensic taphonomy experts William D. Haglund and Marcella H. Sorg (1997): “*Forensic taphonomy [...] refers to the use of taphonomic models, approaches and analyses in forensic contexts to estimate the time since death, reconstruct the circumstances before and after decomposition, and discriminate the products of human behavior from those created by the earth’s biological, physical, chemical and geological subsystems.*”

This definition invites consideration of the cadaver from both taphonomic and ecological perspectives, highlighting the interactions among the multiple spheres involved in the development of a “*newly emerging microenvironment*” (Haglund & Sorg, 1997). Indeed, it is not difficult to envisage how the substantial nutrient input generated by a decomposing body exposed to a natural environment constitutes a valuable source of energy capable of triggering

a cascade of reactions within the trophic network (Haglund & Sorg, 1997; Tibbett & Carter, 2008), in addition to producing marked alterations in the biogeochemical balance of the surrounding environment (Aitkenhead-Peterson et al., 2012; Fancher et al., 2017; Mason et al., 2022). Moreover, as decomposition progresses, the body and the context tend to blend, as a consequence of the penetration and mobilization of putrefactive products (linked to the dynamics of the soil system, waters and groundwaters, the activity of macro- and microorganisms, etc.), and of the environment itself which, in turn, merges with the cadaver, such as minerals incorporated within tissues (Zangarini et al., 2016) and plant organisms (Haglund & Sorg, 1997; Caccianiga et al., 2021).

However, according to Haglund & Sorg (1997), the most critical phase of taphonomic investigation corresponds to the period during which soft tissues are still present, largely because of the rapid progression and consequences associated with early postmortem processes (Micozzi, 1991). For this reason, a detailed understanding of the changes affecting the body from the moment of death, along with their chronological sequence, is of fundamental importance for accurately reconstructing events, regardless of the environmental context in which the body is recovered. The discipline concerned with the study of these early processes is forensic thanatology, which can be defined as the investigation, through cadaveric examination, of all phenomena related to death (Franco et al., 2018). The ultimate objectives of the discipline include the reliable diagnosis of death (thanatodiagnosis), the determination of the time of death (thanatochronology), and the differentiation between postmortem and vital phenomena (Puccini, 2003).

The investigation begins with the external postmortem examination, whose primary aim is to ascertain death, although it may also constitute part of the autopsy when further investigation is required¹. Excluding immediate abiotic phenomena, which occur immediately after death and are scarcely appreciable to the naked eye, the first macroscopically observable postmortem changes are the so-called consecutive abiotic phenomena (or Early PMI; Puccini, 2003). These phenomena show patterns that can be correlated with the time since death (see subsection 1.3.1.) and may be briefly summarized as follows:

¹ Italian legislation, in its legal texts, distinguishes between autopsies performed on subjects whose death is suspected to be related to a criminal offence (*codice di procedura penale – Art. 116*) and postmortem examinations carried out for diagnostic purposes (*D.P.R. 10.9.1990 n. 285 – Art.37*).

- *Livor mortis* (cadaveric hypostasis): the development of lividity (ranging from pink to purple/blue discoloration) results from the gravitational pooling of blood and other body fluids following irreversible circulatory arrest (Madea & Kernbach-Wighton, 2013; Puccini, 2003). In the supine position, blood settles into the most dependent parts of the vascular system (towards the dorsal regions), with hypostatic staining becoming visible approximately 30 minutes postmortem (Knight, 2002). The process occurs in two sequential phases (Brooks & Sutton, 2018):
 1. Non-fixed livor mortis: vessels contain fluid blood. If the body is repositioned, blood may accumulate in newly dependent areas.
 2. Fixed livor mortis: blood fixated in tissues, and hypostatic patches become permanent and immobile.Consequently, not only the appearance of hypostatic staining, but also its color, mobility, and distribution are of diagnostic forensic relevance (Madea & Kernbach-Wighton, 2013).
- *Rigor mortis* (cadaveric rigidity): progressive muscular stiffening results from the rapid depletion of adenosine triphosphate (ATP). As a consequence, myosin heads bound to actin filaments cannot be detached to allow relaxation, leading to muscle rigidity (Puccini 2003; Madea, 2014). Rigor mortis first becomes evident in smaller muscles and then in larger groups, immobilizing joints associated with short muscles before those controlled by stronger ones, although it emerges simultaneously throughout the body (Puccini, 2003). Its resolution, caused by proteolysis and the consequent release of ammonia (NH₃), follows the same sequence as its onset (Knight, 2002).
- *Algor mortis* (cadaveric cooling): body cooling after death is due to the cessation of heat-generating homeostatic cellular activity (Brooks & Sutton, 2018), which normally maintains body temperature around 37°C (Knight, 2002). Following death, body temperature gradually equilibrates with the environment through heat dissipation. From a physical perspective, it may be intuitive to assume that body cooling follows a linear pattern, directly proportional to the temperature gradient between the body and the surrounding environment (assuming intrinsic and extrinsic variables are excluded). However, the cooling curve instead displays a characteristic delay, with an initial and terminal plateau, known as the temperature plateau effect (TPE), resulting in a sigmoidal decline (Henssge & Madea, 2004). Heat loss is slower during the first hours after death and in the final phase as equilibrium with the environment is approached, whereas it is more rapid during the intermediate stage (Puccini, 2003).

However, the rate of cooling is influenced by multiple variables, including environmental conditions, the subject's characteristics, posture, and clothing, among others (Madea, 2014). Core temperature measurement is commonly evaluated with rectal temperature (8-10 cm deep) serving as the international reference standard (Henssge, 1988; Henssge & Madea, 2004; Madea, 2023). However, hepatic temperature, measured via a small subcostal incision, has also been proposed as a stable index of deep core temperature, though it is less standardized and mainly adopted in research rather than routine casework (Madea, 2023; Kumar et al., 2013). French-speaking medico-legal centers have also validated external auditory canal temperature, with Baccino's formulae demonstrating accuracy comparable to rectal or brain-based models under controlled conditions (Baccino et al., 1996; Laplace et al., 2021, 2023). Ultimately, infrared tympanic thermometry has been explored as a rapid, non-invasive alternative at the scene, showing good agreement with probe-based measurements when appropriately calibrated (Cattaneo et al., 2009).

As soon as the first signs of decay appear, the subsequent phases (commonly referred to as *decomposition*) set in, though these cannot be separated strictly chronologically (Madea & Kernbach-Wighton, 2013). These processes aim to progressively break out the body tissues' biomolecules to their elemental atomic components. However, they may result not only in the decomposition of the tissues but, in certain circumstances, also in their preservation.

The process of decomposition begins on a molecular level with *autolysis* (or self-digestion) due to the failure to maintain the integrity of cellular membranes. The degradation and ultimate rupture of cells result in the liberation of proteolytic enzymes that further degrade exposed cell surfaces and connective tissue fibers, creating a degradative chain reaction (Brooks & Sutton, 2018). Autolysis creates ideal conditions for aerobic bacterial proliferation, which depletes the oxygen reserves in the tissues (Vass et al., 2002), thereby creating an anoxic environment optimal for the subsequent stages. Indeed, the anaerobic organisms normally present within the gastrointestinal tract are then able to decompose carbohydrates, fats, and proteins, and consequently produce organic acids (see Chapter 1.4; Vass, 2001; Dent et al., 2004). These transformations characterize the *putrefactive phase*, which ultimately leads to the partial reintroduction of oxygen molecules into the tissues (Janaway, 1996), restoring the environment to an aerobic state, through which skeletonization can begin (Mann et al., 1990).

From a macroscopically phenomenological point of view, the phases characterizing putrefaction have been extensively studied and categorized. Conventionally, the American

model classifies the phases of decomposition into fresh, bloat, decay, and dry (Galloway et al., 1989). However, the branch of Italian forensic medicine related to thanatology adopts a slightly different subdivision (Puccini, 2003), to be understood more as complementary to the American one rather than in contrast with it. These phases are briefly summarized below:

- *Discoloration phase*: the cadaver assumes a greenish coloration, due to the action of anaerobic bacteria, which create catabolism of the hemoglobin molecule, releasing the blood pigment and combining it with hydrogen sulfide (H_2S) resulting from proteolysis. This process causes changes in the sulfur bridges of the blood that alter its color, forming sulfmethemoglobin. Typically, this phenomenon begins with the putrefactive patch, which appears in the right iliac region and then extends to the whole body. In this phase, the phenomenon of marbling is also observable, in which the veins and arteries take on a red-to-bluish coloration.
- *Bloating phase*: bacterial degradative reactions (predominantly anaerobic) continue with development and accumulation of gases, which swell the tissues spreading from the intestine to the vascular network and then to the subcutaneous tissue and internal cavities. In this phase, phenomena of epidermolysis with formation of skin blisters can be observed: the epidermis of feet and hands detaches like a glove, and hair may loosen. From a chromatic point of view, a progressive darkening of the body occurs, taking on a brownish green tone. Furthermore, putrefactive fluids can be observed seeping upwards under gas pressure with possible outflow of foam.
- *Liquefaction phase*: with the progressive cessation of gas production, the cadaver drastically reduces its volume, and its coloration shifts from brownish green to brownish black. Organs and tissues disintegrate and undergo liquefaction, producing fluids that accumulate in anatomical cavities and then diffuse outward, releasing the characteristic odor of ammonia.
- *Skeletonization*: the preceding phases typically leave skeletal remains articulated only by joints and ligaments (Dent et al., 2004). From this point onward, skeletonization proceeds with the destruction of all soft parts, until only the harder resistant tissues of bones and teeth remain, which in turn undergo progressive dehydration and pulverization (inorganic chemical weathering; Behrensmeyer, 1978).

As mentioned before, decomposition processes could also be preservative, mainly due to peculiar environmental factors such as temperature, humidity, and oxygen availability. There are three main preservative decomposing processes:

- *Mummification*: it consists of the dehydration of tissues and a general loss of weight of the cadaver. This process allows the cellular structure to still be integrally preserved despite having lost its aqueous component. The skin in this case assumes a hard consistency and a brownish coloration. This process is favored by environments lacking humidity, ventilated and/or with high temperatures, such as highly draining soils in arid climates or dry and/or ventilated domestic environments with absorbent surfaces, like mattresses (Byard, 2025).
- *Humid mummification (corification)*: transformation of the tissues of the cadaver that becomes macroscopically similar to leather as a result of a modification of the connective tissue. This phenomenon is typically observed in cadavers decomposing in humid and confined environments, such as zinc coffins (Collini et al., 2014). The external appearance of the body may lead to confusion with mummification; however, in this condition tissues are not dehydrated through lyophilization-like processes and therefore do not preserve their cellular structures, but rather retain primarily the collagen framework. As a result, much of the biological information that could otherwise be recovered through rehydration techniques is lost. Furthermore, the high rate of humidity predisposes the surface to colonization by fungi and insects.
- *Adipocere formation*: it involves both the hydrolysis of adipose triglycerides into saturated and unsaturated fatty acids through the action of lipases and bacterial enzymes, and the subsequent hydrogenation of unsaturated fatty acids into saturated compounds (Magni et al., 2021). The material then becomes infiltrated by salts dissolved in the surrounding matrix, increasing its hardness and resulting in a whitish substance with a creamy to friable consistency capable of protecting underlying tissues. This phenomenon typically occurs in bodies that have remained for prolonged periods in aquatic or water-saturated, oxygen-poor environments (Forbes et al., 2021), although anatomical regions with adipocere may also be observed in cadavers buried in soils with significant water-retention capacity (Fiedler & Graw, 2003). The term “saponification” is sometimes used synonymously, but more precisely denotes the soap-forming reactions occurring within adipocere development (Ubelaker & Zarenko, 2011; Magni et al., 2021).

1.3.1. Postmortem Interval (PMI) & Post-Burial Interval (PBI)

The period that elapses between an individual's death and the discovery of their remains is defined as the postmortem interval (PMI) or time since death. Estimation of this interval represents a routine task in forensic casework, as it can support investigations by narrowing the temporal window of interest and assisting in the reconstruction of events surrounding a suspected criminal act (Pringle et al., 2010). However, although the value of PMI estimation is closely linked to its precision, its reliability ultimately constitutes an even more critical factor (Madea, 2013). Indeed, the fundamental principle in estimating the time since death is to derive a measurable parameter along a time-dependent curve and to trace it back to its origin. Since the features of these elements are modulated by both intrinsic and extrinsic factors, encompassing antemortem and postmortem influences, the estimation of the PMI can never provide an exact moment, but must necessarily be expressed as a temporal interval (Madea, 2013).

Regarding the estimation of the time since death in the early PMI and decomposition phases, pathologists and forensic practitioners rely on various methods, starting with the examination of postmortem changes in the body. Conversely, as decomposition progresses and the PMI increases, the indicators progressively lose their relevance to the body while gaining increasing significance in relation to the environment. Therefore, relying solely on the body as a witness of the time elapsed since death could be profoundly misleading. It is in this context that other disciplines, such as forensic anthropology, as well as environmentally oriented sciences like the natural sciences (including entomology, botany, soil science etc.), become the methods of choice, as the interaction ultimately becomes the only reliable measure of the time elapsed since death (Figure 1.3).

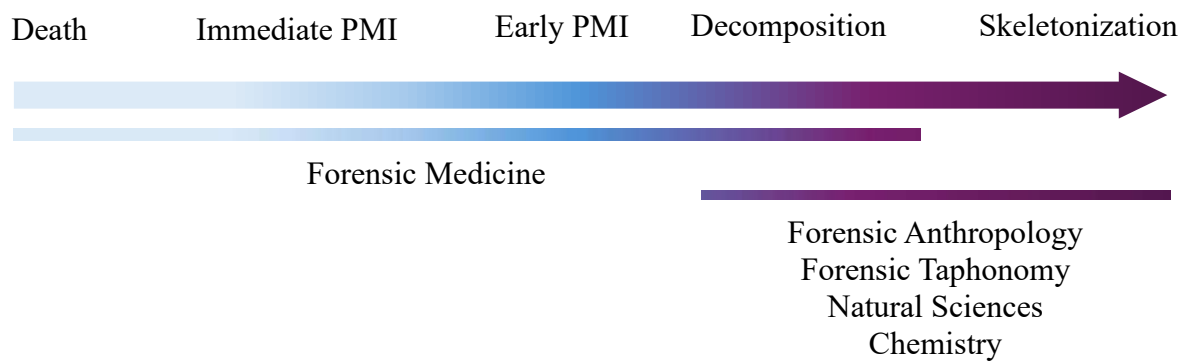


Figure 1.3: Main disciplines involved in the estimation of PMI based on the conservation state of the body.

Nevertheless, in order to be admissible as scientific evidence in court, PMI estimation, like any other form of expert testimony, must comply with the so-called *Daubert* criteria (Balouet et al., 2009). These criteria were established following the 1993 landmark decision of the United States Supreme Court in *Daubert v. Merrell Dow Pharmaceuticals* (Barry, 1997). The case brought significant changes in the standards for reliability and admissibility of expert opinions in federal courts, including some criteria that must be respected for evidence to be considered valid. The *Daubert* criteria can be described as follows:

- 1) The theory or method proposed must be testable and verifiable.
- 2) The method must be published to peer-reviewed journals or other forms of publications.
- 3) The method must have a known or potential rate of error.
- 4) Existence and maintenance of standards controlling its operation must be guaranteed.
- 5) The method must be widely accepted within a relevant scientific community.

Consequently, the main methods used for the estimation of the time since death in the early PMI and decomposition phases will be described below. These comply with almost all the *Daubert* criteria imposed, although the rate of error is not always quantified. This latter point will therefore be commented on for each of them.

Starting from the first appreciable postmortem findings on the body after death, as presented in section 1.3, the consecutive abiotic phenomena show patterns that can be correlated with the PMI (Puccini, 2003):

- *Livor mortis*: the time of onset of hypostasis is variable, although the first sign often appears around 30 minutes after death, becoming more evident 4-6 hpm (hours postmortem). They reach their maximum extension between 12 and 18 hpm. The fixation process is also gradual: hypostasis is completely mobile up to 6 hpm, partially migratable between 6 and 12 hpm, and absolutely fixed between 12 and 15 hpm (Madea, 2013). Several studies on livor mortis evaluation for PMI are published in peer-reviewed journals (Kaatsch et al., 1993, 1994; Vanezis & Trujillo, 1996; Usumoto et al., 2010; Madea et al., 2014; Romanelli et al., 2015), but none of them meet all the *Daubert* criteria. Indeed, methods are used to achieve blanching of livor mortis with the goal of objectifying the lividity color changes and ultimately estimating the PMI through it, but most of them are not validated and do not mention a standard error or 95% standard deviation concerning the PMI (Gelderman et al., 2021), with the only exception of Usumoto et al. (2010), who found a root mean square error of ± 4.76 h. This overall limitation is likely attributable to the fact that the development of livor mortis varies between individuals, depending on factors such as blood loss, cause and manner of death, and other physiological or environmental variables.
- *Rigor mortis*: generally, the phenomenon appears 2-3 hpm, extends to all muscles 12-24 hpm, and remains stationary for about 36-48 hpm. It usually resolves within 72-84 hpm. It should be emphasized, however, that the phenomenon is subject to multiple intrinsic factors (muscle development, age, dynamics of death) and extrinsic factors (mainly environmental). For example, low temperatures delay the onset of the phenomenon but prolong its duration (for two weeks or longer; Madea, 2013), while high temperatures anticipate their onset but accelerate their resolution (Puccini, 2003). However, the mean time in which complete rigidity or resolution occurs, including standard deviations and 95% confidence intervals, are derived from the manually assessed degree of muscular rigidity (Gelderman et al., 2021). The intrinsic subjective nature of the manual method makes it unreproducible and difficult to validate. However, methods based on measuring rigor mortis using a device (hence without breaking it) have been recently validated, as well as the re-establishment of rigor after breaking it (Krompecher et al., 2008; Anders et al., 2013; Crostack et al., 2017), which described the 95% confidence intervals for their results. The utilization of devices for measuring the force needed to break rigor mortis overcomes the subjectivity of manual

evaluation but appears to be unpracticable in forensic casework (Gelderman et al., 2021). Therefore, the data on which the timeline of rigor mortis is based would be evaluated as inadequate to be used in court.

- *Algor mortis*: as previously mentioned, the cooling curve of the cadaver follows a sigmoidal trend, with a progressive temperature decrease equivalent to 0.5°C/hour for the first 2-3 hpm, and 1°C/hour in the following 6-8 hours (10-12 hpm). After these two phases, heat loss becomes progressively less significant (from 0.75 to 0.5 to 0.25°C/hour and so on) until thermal equilibrium with the surrounding environment is reached (18-24 hpm). Clearly, this phenomenon is also closely related to intrinsic and extrinsic factors, which led to the development of PMI estimation methods based on algor mortis that also included some of these factors, such as Henssge's nomogram (Henssge, 1988). According to Madea (2013), in the best case a precision of ± 2.8 h around the mean value (95% confidence limits) can be achieved with this method. However, several studies (some of them conducted by Henssge himself) have focused on the limitation of the nomogram application, concerning the usage of correction factors in case of non-standard cooling conditions (Henssge, 1992; Henssge et al., 2000; Kaliszan et al., 2005; Kaliszan, 2011; Hubig et al., 2011). In particular, they questioned the reliability for application of the nomogram method, especially regarding its practical usefulness, subjective bias in choosing an appropriate correction factor and availability of limited data on choosing correction factors (Gelderman et al., 2021). Nonetheless, despite being criticized, Henssge's nomogram is considered the most reliable and accepted method for estimating the PMI in forensic casework.

As decomposition progresses, associating discrete putrefactive phases with a universally applicable post-mortem interval becomes increasingly problematic. During the *discoloration phase*, the greenish discoloration of the lower right abdomen could be commonly observed between 18 and 36 hpm in summer, whereas in winter it may not appear until approximately 3-4 days after death (Puccini, 2003; Madea, 2013). These intervals, however, should be regarded as indicative ranges rather than diagnostic thresholds, as their expression depends on environmental and individual variables. Similarly, the *bloating phase* generally develops 3-6 days after death in summer but may be delayed for several weeks in winter (Puccini, 2003), reflecting temperature-dependent microbial metabolism and gas accumulation rather than a strictly time-regulated transition. The *liquefaction phase*, although initiated by reactions that begin much earlier, typically becomes macroscopically appreciable after 2-3 weeks in summer and only after several months in winter (Puccini, 2003). *Skeletonization* further illustrates the

limits of chronological generalization: while it is usually completed within 3–5 years, documented cases indicate that this stage can be reached within a few weeks under particular taphonomic conditions (Mann et al., 1990). Overall, these observations suggest that decomposition stages represent overlapping biological processes modulated by context rather than sequential markers that can be reliably mapped onto fixed temporal intervals.

The situation becomes even more complex with the occurrence of preservative processes, under which establishing the time since death without considering environmental variables is virtually impossible. Moreover, it is unlikely that the entire body will be recovered in a uniform state of preservation or degradation; therefore, the investigation of environmental interactions, through entomological, botanical, and biogeochemical analyses, becomes essential. Even when attempting to define temporal ranges for the onset and duration of postmortem phases, substantial variability persists. This difficulty likely stems from the absence of clearly defined starting and ending points for early and late postmortem phenomena (Vass, 2011), which should not be considered strictly sequential but rather frequently overlapping, as well as from the significant influence of intrinsic and extrinsic factors on their rate of progression.

Among the environmental factors that play a decisive role in the rate of cadaveric decomposition, burial represents a key variable. To date, only a limited number of studies have quantitatively addressed the differences in the speed at which cadaver degradation progress in burials compared with those placed on the surface (the latter forming the basis of the most widely used methods for PMI estimation; Fiedler & Graw, 2003; Simmons et al., 2010; Marais-Werner et al., 2018). One of the earliest to examine putrefactive processes in bodies exposed to different environments was Professor Johann Ludwig Casper, who, drawing on his experience as a forensic physician and researcher, stated: *“At a tolerably similar average temperature, the degree of putrefaction present in a body after lying in the open air for one week (or month) corresponds to that found in a body [...] after lying in the earth in the usual manner for eight weeks (or months)”* (Casper, 1862).

This observation subsequently gave rise to the so-called “Casper’s rule” (Fiedler & Graw, 2003), according to which a buried body undergoes the same sequence of decomposition stages as an exposed body, but with a progression approximately eight times slower. Although the rigidity of this rule does not always correspond to empirical observations, it is now well established that decomposition in buried cadavers generally proceeds at a slower rate (Figure 1.4). These differences are attributable to the hypogeal microenvironment that develops around

the body, which is directly shaped by the soil type of the burial site. This environment not only modulates the abiotic parameters typically governing decomposition processes but also either restricts or facilitates the access of macro- and microorganisms to their nutrient source.

Indeed, according to Fiedler & Graw (2003) and Marais-Werner et al. (2018), the three primary factors contributing to the reduced rate of decomposition in buried remains, in the absence of significant environmental disturbances, are:

- The near absence of scavenger activity.
- Limited insect colonization.
- Reduced temperature fluctuations.

In addition, moisture and ability of the burial matrix to maintain stable humidity levels should be considered (Turner et al., 1999), as these conditions strongly influence the likelihood of preservative processes occurring, which may ultimately render the estimation of the post-burial interval in the hypogeal environment unreliable.

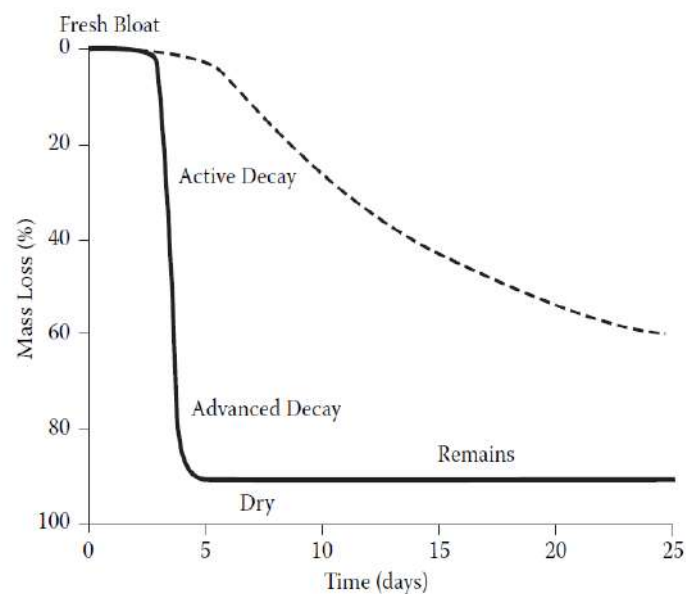


Figure 1.4: Representation of the decomposition curves typically associated with cadaver decomposition on the soil surface (—) and following burial in soil (- - -). From Carter & Tibbett, 2008.

The parameters described above can be correlated with the postmortem changes observed in a cadaver at the time of recovery or discovery, with the aim of developing models for estimating the postmortem interval (PMI). However, in cases involving bodies recovered from burial contexts (whether legitimate, clandestine, or experimental) the term post-burial interval (PBI) is often preferred. The PBI refers to the time elapsed between the deposition of the body and its recovery, and this terminology is widely adopted in the literature (Forbes, 2008; Pringle et al., 2010, 2015a; Singh et al., 2016; Dick and Pringle, 2018).

This distinction arises because the methods typically employed in burial contexts primarily assess the interaction between the cadaver and the surrounding burial environment, which begins at the time of deposition and does not necessarily coincide with the time of death. Consequently, conflating PMI and PBI may lead to misinterpretation of forensic evidence, including potential underestimation of the interval between death and discovery, thereby compromising the reconstruction of events. For this reason, in the presentation and discussion of the data collected within this PhD project, the term PBI will be used instead of PMI, although in the present experimental context the two intervals coincide.

1.3.1.1. ADD, TBS and other PMI Estimation Formulas

In 2005, Megyesi et al. (2005) proposed a method for estimating the PMI based on gross observation of the decay of soft tissue, as had traditionally been done by forensic anthropologists and pathologists, but with a pioneer quantitative approach. The ultimate aim of this method was to increase the statistical validity of the initial hypothesis (namely the relationship between decomposition and the time elapsed since death) and to present in court evaluations whose error could be known. To achieve this, the authors proposed to correlate the postmortem changes of a cadaver not only with the passage of time, but also with the variable that likely has the greatest influence on the rate of decomposition: temperature. This influence can be assessed through the calculation of Accumulated Degree Days (ADD). This is a formula widely used in fields such as agricultural sciences, botany, and entomology (Febler et al., 2018) to predict, at a known temperature, the amount of time required for a given phenological stage to occur, also taking into account the base temperature at which the process under examination ceases (Megyesi et al., 2005).

In the case of PMI estimation, the phenological stage under examination is the state of decomposition of the body at the time of its discovery. To express this observation in quantitative terms, Megyesi et al. introduced the concept of Total Body Score (TBS). This consists of assigning a score to the different body regions according to the degree of degradation they exhibit, distinguishing head and neck, trunk, and limbs, since not all decomposition stages apply universally to the entire body. The sum of these evaluations constitutes the TBS. Thus, since ADD is the variable dependent on the observation of TBS, which is therefore the independent variable, a regression curve is constructed whose simplified formula is:

$$ADD = 10^{(0.002 * TBS^2 + 1.81)} \pm 388.16$$

where 388.16 is the standard error, which can be doubled (776.32) to approximate the 95% prediction interval for a TBS.

At this point, it is necessary to trace back the daily mean atmospheric temperatures using the weather monitoring station closest to the recovery site and to sum them retroactively until values like those obtained with the formula are reached. It should be kept in mind that all negative daily mean atmospheric temperature values must be converted to 0 °C, since the freezing point is considered the base temperature at which the process under examination ceases (Vass et al., 1992; Megyesi et al., 2005).

As will be illustrated in subsection 1.5.1, many controlled forensic taphonomy studies aimed at estimating the PMI involve the use of animal models (primarily pigs, *Sus scrofa domesticus*) as human analogues. This could pose a problem in the application and evaluation of methods that are instead calibrated on the human species. The issue was addressed by Keough et al. (2017), who noted that the timing and manner in which the early stages of decomposition manifest in the two species when exposed on the surface are considerable, especially regarding the onset of bloating. Consequently, the authors proposed a modified version for scoring decay, based on results obtained from a series of pig decomposition studies conducted in South Africa (Myburgh et al., 2013; Sutherland et al., 2013; Keough et al., 2015). Given the geographical specificity of the experimental area, the method was also tested by Indra et al. (2024) at a different latitude, in a temperate Swiss forest. The results reported discrepancies with the model proposed by Keough et al. (2017), particularly with respect to the rate of insect colonization, suggesting the need for identification of regional decomposition patterns and drivers to ensure that such methods are applicable across different environments, both in forensic taphonomy research and

in forensic casework. For this reason, Indra et al. in turn suggested modifications to adapt the South African model to temperate forests. In order to summarize the differences characterizing the three studies (Megyesi et al., 2005; Keough et al., 2017; and Indra et al., 2024), these have been compared in the Appendix (Table A.1, p. 335).

Clearly, the usefulness of TBS lies in offering a unique perspective, where the four-stage model “fresh, bloat, decay, and dry” (Galloway et al., 1989) is further divided into more precise and descriptive measurement units, useful for identifying as precisely as possible the developmental stage of the process. Moreover, these studies have demonstrated that the postmortem interval is more accurately estimated when decomposition is associated not only with the passage of time but also with environmental and concealment conditions. However, the method is not yet refined enough for field application: although a TBS system has recently been developed by van Daalen et al. (2017) for the analysis of bodies recovered from aquatic environments, none has been specifically developed for use in buried remains (Brooks & Sutton, 2018).

Nevertheless, an effort in this direction was made by Vass (2011), who proposed an “elusive universal postmortem interval formula” which included environmental data such as temperature, moisture, and the partial pressure of oxygen, considered three of the four primary drivers of human decomposition (the fourth being pH). The study proposed two formulas for the direct calculation of the PMI (with results expressed in days): one for above-ground (aerobic) human decomposition and one for human burial decomposition (anaerobic/below ground). The latter will be illustrated below:

$$PMI_{\text{Anaerobic}} = \frac{1285 \times \left(\frac{\text{decomposition}}{100} \right) \times 4.6 \times \text{adipocere}}{0.0103 \times \text{temperature} \times \text{soil moisture}}$$

Where:

1285 is a constant representing the empirically determined ADD value at which volatile fatty acid (VFA) liberation from soft tissue ceases. According to Vass’ research (2017), when soft tissue decomposition ends, VFA production ends as well. This occurs at approximately 1285 ADDs, which indicates the onset of the post-skeletonization phase of decomposition, so the formula could be used only if soft, non-mummified tissues are still present.

Decomposition could be a value, or range, between 1 and 100 (%), representing the extent of total soft tissue decomposition. Since the use of the four-stage model is globally used in the field to describe decomposition, Vass proposed a table (Table 1.1) that correlates decomposition stages and degree of decomposition (expressed in %), under warm ($> 12^{\circ}\text{C}$) and cold conditions ($< 12^{\circ}\text{C}$).

<i>Stage</i>	<i>Decomposition range under warm conditions</i>	<i>Decomposition range under cold conditions</i>
<i>Fresh</i>	1-10 %	1-20 %
<i>Bloat</i>	11-35 %	21-45 %
<i>Decay</i>	36-85 %	46-85 %
<i>Dry</i>	86-100 %	86-100 %

Table 1.1: correlation between decomposition stages and extent of total body soft tissue decomposition (from Vass., 2011)

4.6 is a constant which represents a deceleration in the rate of decomposition as a consequence of a lack of oxygen. This value was determined by comparative experiments measuring the decomposition rate of surface and buried individuals taking into accounts temperature and moisture differences between the two categories of deposition.

Adipocere is a multiplicative value based on the estimation of the % of adipocere noticeable on the body. Its presence is widely present in buried remains, especially when soil has a noticeable water-retention capability. The percentage of adipocere estimated can then be converted to a corresponding multiplier (Table 1.2). This estimation is included in the formula since, according to Vass, the presence of adipocere can cause a substantial deceleration of the decomposition rate, reducing the availability of oxygen for aerobic microbial degradation of the remaining tissue.

<i>% Adipocere formation</i>	<i>Multiplier</i>
< 35	1
35	3
40 - 65	5
70	6
75	7
80	8
85	11
90	14

Table 1.2: Estimated multiplier values for the contribution of % adipocere formation during human burial decomposition (modified from Vass, 2011).

0.0103 is a constant, representing the effect of moisture on decomposition rate. It is due to the fact that, when comparing the % decomposition to the time averaged amount of moisture present, the effect of the latter on human decomposition can be represented by a line with a slope of 0.0103.

Temperature is the value in degrees Celsius (°C) of the soil temperature in the grave vault at the time of excavation and at the level of the corpse. Typically, a thermometer or thermocouple is placed in the grave vault wall if available. However, soil temperature can be estimated quite accurately if required from the atmospheric one, by subtracting 3.3 °C if the daily mean temperature is < 15 °C and adding 4.4 °C if the daily mean temperature is > 15 °C (Vass et al., 2004 and 2008).

Soil moisture is a value between 1 and 100, representing the % of soil moisture at the site on the day the body was discovered. It is normally determined using dry weight assays: 5-10 g of soil is collected and dried at 100°C to determine the pre vs. post drying weight of the soil. This reflects the amount of moisture present and can easily be converted into a percentage by weight.

Both ADD and Vass's formula for PMI estimation were published in peer-reviewed journals and validated. As previously mentioned, TBS has a substantial standard error of 388.16 ADD, which would make it hardly applicable in practice. Nonetheless, Moffatt et al. (2016) proposed a correction to the statistical approach of Megyesi et al. (2005), creating a model that was found to be more accurate in earlier decomposition stages while Megyesi's one resulted more accurate in later decomposition stages (Forbes et al., 2019) even on animal models (Keough et al., 2017; Indra et al., 2024). Moffatt's model offers 95% prediction intervals whilst using different total body scores. However, of all the methods, Megyesi's TBS/ADD method is still the most accepted internationally (Gelderman et al., 2021).

Regarding Vass' formula, it was validated in several experiments, resulting in underestimate (Marhoff-Beard et al., 2018), both over- and underestimate (Cockle & Bell, 2015), or partly accurately estimate (Maile et al., 2017) the PMI. However, a standard error is not mentioned in the original paper.

1.3.2. Microtraces Detection: Inorganic Gunshot Residues

The degree of tissue integrity in a decomposing body is of forensic relevance also with regard to the potential for detecting traces and microtraces that may be associated with the environment in which the criminal act (or part of it) took place, as well as with the weapons and tools employed. The potential of searching for these traces lies in Edmond Locard's (1930) exchange principle, according to which *"Every human action, and even more so a violent action such as a crime, cannot take place without leaving some trace."* This principle considers traces to be the inevitable result of interactions between two substrates, emphasizing that their presence is even more likely when generated by violent acts such as crimes.

An illustrative example is represented by gunshot residues (GSR). This term encompasses all materials generated and subsequently deposited during, or immediately after, the discharge of a firearm (Minzière et al., 2023a). To summarize the operating mechanism of a firearm: when the trigger is pulled, the firing pin strikes the primer, initiating a flash that ignites the gunpowder. The combustion process produces substantial volumes of gas, which in turn cause a rapid increase in both temperature and pressure inside the chamber, thereby enabling the bullet to be expelled from the muzzle (Minzière et al., 2023a). Ultimately, vapors (referred to as the plume) composed of gases as well as burnt and unburnt particles, are released through all available openings of the firearm (Feeney et al., 2021).

These gases and particles constitute GSR, which, originating from the plume, are subsequently deposited onto surrounding surfaces, such as the shooter's body (hair, face, nostrils, hands, forearms, clothing; Feeney et al., 2020), the surrounding environment (Black et al., 2021), and the target. The components of the firearm that give rise to these residues are the primer, the cartridge case, the projectile, the propellant (Figure 1.5), and the weapon itself.

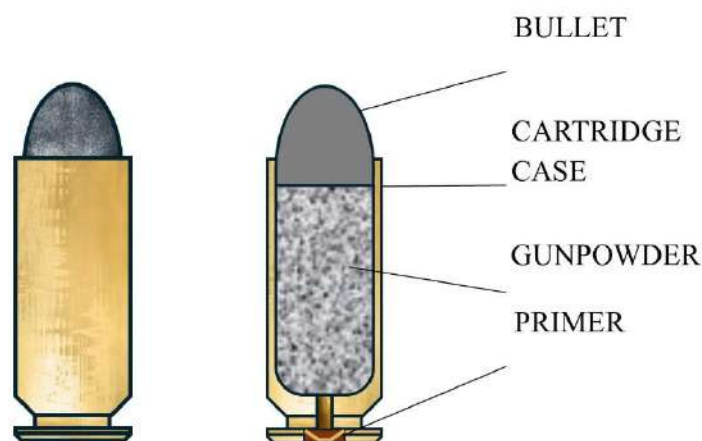


Figure 1.5: Representation of the main components of a firearm ammunition: the bullet is used as projectile; the gunpowder works as propellant; the cartridge case has the aim to hold the components together; the primer ignites the propellant.

Residues can be classified into inorganic (IGSR), namely burnt and unburnt particles originating from the primer (nitrates, nitrites, and metallic particles), and organic (OGSR), which derive from the incomplete combustion of the propellant. Additional residues may also be associated with other materials composing the cartridge case, the projectile, and, more broadly, other components of the firearm (Maitre et al., 2017). Tape lifting (particularly carbon-coated tapes for SEM-EDX analysis) represents the most common technique for sampling inorganic residues, whereas swabbing has primarily been employed for the collection of organic compounds. Nowadays, due to the growing concerns about health risks linked to human and environmental exposure to elevated concentrations of heavy metals, “lead-free” or “non-toxic” ammunition were introduced to the market (Benito et al., 2015; Minzière et al., 2023a). Nonetheless, this type of ammunition complicates the clear identification of GSR: in such situations detecting specific organic compounds derived from gunpowder combustion provides decisive evidence to distinguish GSR from environmental particles. Consequently, when “lead-free” ammunition is involved, it becomes crucial to examine both inorganic and organic residues within the same sample (Minzière et al., 2023b).

However, when the substrate under investigation is the victim’s body in an advanced state of decomposition, due to the fragility of the tissues and the high risk of external contamination, IGSR appear to represent the most reliable evidence for confirming a shooting event. As stated, the primary firearm component responsible for the formation of IGSR is the primer mixture, although such particles may also originate, to a lesser extent, from mechanical abrasion of the

firearm and the cartridge case during discharge (Gallidabino & Weyermann, 2020). The primer mixture comprises components with different functions, some of which are characterized by distinct compounds containing heavy metals (Cardinetti et al., 2004; Maitre et al., 2017; Feeney et al., 2020; Minzière et al., 2023a). These are summarized in Table 1.3.

Role	Function	Compounds	
Initiator	It initiates the primer ignition process when struck by the firing pin.	Lead styphnate	C₆H_N₃O₈Pb
		Lead azide	Pb(N ₃) ₂
		Copper thiocyanate	CuSCN
Oxidizer	It provides oxygen for the combustion of the primer mixture when the firing pin strikes the primer cap.	Barium nitrate	Ba(NO₃)₂
		Lead oxide and peroxide	PbO/PbO ₂
		Lead nitrate	Pb(NO ₃) ₂
		Aluminum oxide	Al ₂ O ₃
		Phosphoric acid	H ₃ PO ₄
		Potassium chlorate/nitrate	KClO ₄ /KNO ₃
		Sodium nitrate	NaNO ₃
		Silicon monoxide	SiO
		Stannic oxide	SnO ₂
		Strontium nitrate	Sr(NO ₃) ₂
		Tungsten trioxide	WO ₃
		Zinc peroxide	ZnO ₂

Reducing agent	It reacts with the oxidizer in the primer in a rapid redox reaction releasing the burst of heat, flame, and hot gases	Aluminum powder	Al
		Magnesium	Mg
		Titanium powder	Ti
		Zirconium powder	Zr
Fuel	It reacts with the oxidizer to produce the heat and gas that ignite the gunpowder inside the cartridge.	Antimony sulfide	Sb₂S₃
		Calcium silicate	CaSi ₂
		Bismuth sulfide	Bi ₂ S ₃
		Zinc sulfide	ZnS
		Metal powder	Mg, Ti, Zr, Al, Cr, Mn
Friction agent	It is added to the primer mix to make it more sensitive to the mechanical energy of the firing pin strike.	Glass powder	(SiO ₂) + (Na ₂ O) + (CaO) + others
Stabilizer	It reacts with the by-products of the mixture breakdown, preventing them from building up and making the mixture more stable.	Aluminum silicate	Al ₂ SiO ₅
		Sodium sulfate	Na ₂ SO ₄

Table 1.3: Examples of inorganic compounds from the primer and their role in the mixture. Components of the most common primers are in bold.

IGSR could also be generated from other components (Feeney et al., 2020) such as the projectile (Antimony (Sb), Tungsten (W), Iron (Fe), Chromium (Cr)), the cartridge case (brass (Cu, Zn and other elements), bronze (Cu, Sn and other elements), copper (Cu), nickel (Ni), tin (Sn), Zinc (Zn), and bullet casing (cupro-nickel - Cu, Ni).

According to the latest ASTM standard (2020), which provides a standardized protocol for GSR analysis through SEM-EDX (Scanning Electron Microscopy/Energy Dispersive X-ray spectrometry), the particles could be classified into three categories:

- *Characteristic of IGSR particles*: these are the most specific to IGSR as they are rarely encountered in the environment as contaminants and are thus strongly associated with the discharge of a firearm.

Composition: Pb-Ba-Sb condensed in a single particle.

- *Consistent with IGSR particles*: they could have some non-firearms sources

Composition: Pb-Ba, Pb-Sb, Sb-Ba, Pb-Ba-Ca-Si, Ba-Ca-Si-(S), Ba-Al-(S), Ba-Sb, Ti-Zn, or -Sr.

- *Commonly associated with IGSR particles*: they could have many alternative sources in the environment and are therefore less specific.

Composition: Pb, Ba(S), or Sb.

These particles could also contain silicon (Si), calcium (Ca), aluminum (Al), copper (Cu), iron (Fe), sulfur (S), phosphorus (P), zinc (Zn) (in combination with copper), and nickel (Ni) (rare and only in combination with copper and zinc), zirconium (Zr), tin (Sn), potassium (K) and chlorine (Cl; Maitre et al., 2017).

From a morphological point of view, a particle could be confirmed as being characteristic of IGSR if it has a spheroidal/non-crystalline morphology. However, it is noted that angular or crusty particles could also be encountered. Regarding their dimension, particles usually present a diameter between 0.5 and 5.0 μm (Maitre et al., 2017).

Given that the diagnostic criteria rely on both the chemical composition and the morphology and size of the particles, SEM–EDX represents the method of choice for detecting, chemically characterizing, and morphologically analyzing IGSR-related particles within the samples (Goldstein et al., 2017; ASTM International, 2020). Scanning Electron Microscopy is widely used across scientific disciplines to examine the surface morphology of material fragments (3D

compositional image), and when coupled with an Energy-Dispersive X-ray (EDX) system, it also provides information on the elemental composition of the sample, offering both qualitative and semi-quantitative data (Goldstein et al., 2017). Regarding the 3D compositional image, it appears as a high-magnification micrograph in which the grayscale intensity directly reflects the elemental composition of the object. Elements with a higher atomic weight (such as the metals typically found in IGSR particles) possess a greater number of positive charges within their nuclei and therefore emit a stronger signal, which is visually represented in the image as brighter (lighter) areas (Krinsley et al., 1998). Through qualitative and semi-quantitative chemical microanalyses provided by the EDX spectroscopy, it is possible to obtain the energy spectrum associated with the unknown sample and to identify the characteristic peaks corresponding to the emission lines of individual chemical elements. Observation of the spectrum also allows for semi-quantitative assessments, based on the correlation between the peak height and the percentage content of a given element, thereby indicating its relative abundance or mere trace presence (Krinsley et al., 1998).

The formalization of these diagnostic criteria is particularly useful for ruling out contamination from non-firearm sources of GSR-like aggregates. Among these are car-related activities, such as the abrasion of brake pads (Cardinetti et al., 2004), which exhibit a chemical composition analogous to that of GSR (Pb, Ba, Sb). Nevertheless, their morphology differs: particles from brake pads are typically angular, and brake dust is accompanied by a large number of particles not commonly encountered in gunshot residue (Maitre et al., 2017). Another example is represented by residues from the deployment of automotive airbags, since some manufacturers employ percussion primers containing Pb, Ba, and Sb as initiators of the explosion. However, even in this case, the resulting particle population includes many species not normally associated with firearm discharge (Berk, 2009). Finally, another potential source of GSR-like particles is represented by firework devices (Trimpe, 2003).

Although the risk of contamination from non-firearm sources is most commonly considered in relation to the suspected shooter (particularly through occupational exposure or frequent presence in contaminated environments) the search for IGSR on cadavers is also subject to similar limitations. Indeed, homicide and concealment victims are often discovered in abandoned or degraded environments containing waste, scrap metal, and debris, which may represent alternative sources of metallic particles that could be misidentified as IGSR. In cases of clandestine burial, particular attention must be paid to the surrounding soil, which may

already contain particles resembling IGSR but unrelated to the criminal event, as well as metallic particles of different origin that could potentially be misidentified as gunshot residues.

Furthermore, until the moment of recovery, buried cadavers are exposed to a range of taphonomic processes involving both physical factors (e.g., temperature, humidity, and precipitation) and biological agents (e.g., plant and animal activity). These processes may substantially alter soft tissues and skeletal appearance, potentially compromising the recognition and interpretation of traumatic lesions. In such contexts, forensic anthropological analysis of taphonomic alterations, particularly on bone tissue, can play a crucial role in assessing the etiology and timing (antemortem, perimortem, or postmortem) of skeletal trauma. This information may assist in the determination of the manner of death, especially when advanced decomposition has obscured or eliminated direct evidence of violent injury, such as gunshot wounds.

However, there are cases in which anthropological analysis alone is insufficient to confirm the presence of lesions attributable to firearm trauma. In such contexts, the detection and characterization of IGSR may represent a critical tool in forensic anthropology and pathology, as these residues may constitute the only remaining evidence of gunshot injury when soft tissues are destroyed or absent and skeletal lesions are no longer clearly recognizable. For instance, Gibelli et al. (2010) conducted experimental research involving pig heads shot with .38 caliber revolver and decomposed in open air or buried in soil for up to 16 weeks. The outcomes showed that even in advanced stages of decomposition, firearm-related elemental traces such as Sb can persist within putrefied tissue, including buried samples, even if Pb signal diminished in this latter case. These findings were further supported by the work of Taborelli et al. (2012), who performed SEM–EDX analyses on the same pig heads and compared the results with four human cranial samples from gunshot victims. Their study demonstrated the long-term persistence of Pb-, Sb-, and Ba-containing particles, confirming the diagnostic value of SEM–EDX in identifying firearm-related trauma even in the absence of soft-tissue evidence. The potential of SEM–EDX is also highlighted by investigations conducted by Nguyen-Hong et al. (2024), who analyzed cow feet shot with 7 mm semi-jacketed bullets and subsequently buried for up to 60 days. Their results showed that characteristic IGSR particles containing Pb, Ba, and Sb remained detectable despite prolonged burial and extensive soft-tissue decomposition, enabling reliable differentiation between entrance and exit wounds. Moreover, the persistence of IGSR has also been demonstrated in different conditions such as skeletonized, buried,

submerged, cremated, or otherwise degraded remains (Amadasi et al., 2012a, 2012b, 2015), and even after mechanical removal of soft tissue from bones (Berryman et al., 2010).

Moreover, in cases involving the investigation of lesions on bodies in an advanced state of decomposition, the detection of IGSR may not only serve as an indicator for lesion characterization, but also provide useful information for differentiating between bullet entry and exit wounds. This distinction has been proposed to rely on differences in the abundance and spatial distribution of characteristic IGSR particles, with higher particle deposition typically observed around or within entry wounds, whereas exit wounds tend to exhibit fewer particles or modified elemental signatures (Moussa et al., 2024; Nguyen-Hong et al., 2024). Moreover, quantitative assessment of total IGSR particle counts may reveal a differential pattern, generally showing greater concentrations at entry sites, thus supporting the use of distribution data as a discriminative parameter (Montoriol et al., 2021; Nguyen-Hong et al., 2024).

1.4. Forensic Biogeochemistry & (Hypogeal) Cadaver Decomposition Island

When operating within a forensic investigation context, forensic taphonomy assumes a specific role: applying knowledge of cadaveric decomposition dynamics to estimate the postmortem interval (PMI), to locate or identify concealment sites, and to contribute both to the determination of cause and manner of death and to the collection of evidence useful for victim identification (Haglund & Sorg, 1997; Carter & Tibbett, 2008).

However, most research in this field focuses on observations of decomposition when a body is deposited on the surface, whether in actual casework or in controlled experimental settings using human or non-human models. Such studies frequently emphasize the associated entomofauna (Campobasso et al., 2001; Kocárek, 2003; Dawson et al., 2020; Maisonhaute & Forbes, 2023), the activity of scavengers (Jeong et al., 2016; Steadman et al., 2018; Spies et al., 2020), and the biogeochemical alterations observable in the soil beneath and around the body (Aitkenhead-Peterson et al., 2012; Fancher et al., 2017; Barton et al., 2020; DeBruyn et al., 2021).

Although detailed investigations of biogeochemistry alterations are relatively recent, they have led to the formalization of the universally accepted concept of Cadaver Decomposition Island (CDI). A CDI can be defined as a localized zone beneath the body characterized by dark coloration and an accumulation of nutrients derived from decomposing tissues (Carter & Tibbett, 2008). This nutrient input profoundly disrupts the soil's natural biogeochemical equilibrium and influences the behavior of organisms interacting with the affected area, including plants (Caccianiga et al., 2012; Perrault & Forbes, 2016; Cholewa et al., 2022), insects (Barton et al., 2019; Voss et al., 2019), and microorganisms (DeBruyn et al., 2025). Within forensic taphonomy, CDI is highly informative precisely because of soil-focused biogeochemical investigations. In a forensic framework, biogeochemistry could be defined as the study of the chemical, geological, and biological processes governing natural environmental dynamics (Schlesinger & Bernhardt, 2020), given that cadaver decomposition is a critical driver of biogeochemical cycling in all ecosystems (DeBruyn et al., 2020). Particular attention is directed toward chemical elements such as carbon (C; Carter et al., 2007; Szelecz et al., 2018a), phosphorus (P; Benninger et al., 2008; Tagliabue et al., 2023), and various organic compounds (Perrault et al., 2015), which either regulate or respond to biological activity.

The application of biogeochemistry to forensic taphonomy has shown that nutrient concentrations within a CDI vary with the stage of cadaver decomposition at the time of discovery and, consequently, with the elapsed time since death. Despite its considerable potential, detailed biogeochemical study of the CDI remains relatively recent (Aitkenhead-Peterson et al., 2012; Silva-Bessa et al., 2025; Taylor et al., 2023), and investigations of CDI formation within burial contexts are virtually absent, apart from the work of Vass et al. (1992, 2004, 2008) and Vass (2012).

In this case, the CDI is hypogeal in nature. Accordingly, within the present project the term *Hypogeal Cadaver Decomposition Island* (HCDI) was introduced and will be used throughout this work to refer to the portion of soil underlying a buried body that is expected to become enriched in nutrients due to the leaching of decomposition products. The introduction of this term is intended to provide a more precise conceptual and operational framework for describing decomposition-related alterations occurring in subsurface burial environments, which differ substantially from those observed in surface CDIs in terms of taphonomic processes and soil-cadaver interactions.

To date, the soil beneath a decomposing buried body has not been formally defined as HCDI, although several studies have investigated decomposition dynamics and associated nutrient inputs in burial contexts. For example, Dent et al. (2004) reported that in-soil human decomposition is initially driven by aerobic conditions, followed by predominantly anaerobic conditions as decomposition progresses. These findings highlight the distinct biogeochemical processes occurring in burial environments and support the need for specific terminology to describe the localized zone of soil affected by subsurface decomposition.

Beginning in the early stage of decomposition (*autolysis*), the breakdown of carbohydrates, proteins, and lipids releases a variety of metabolic products through the action of aerobic bacteria and hydrolytic enzymes. These enzymes are primarily endogenous (already present within tissues such as the intestines and respiratory tract) but may also originate from aerobic microorganisms and fungi that colonize the soil (Polson et al., 1985; Dent et al., 2004).

The hydrolytic disruption of biological macromolecules proceeds via oxidative processes that require available oxygen, which is ultimately depleted. This depletion leads to a subsequent decomposition phase dominated by anaerobic microorganisms that, likewise, may derive from internal tissues (notably the intestinal tract) or migrate into the remains from the surrounding

soil (Dent et al., 2004). Oxygen availability in the burial environment is therefore a critical factor influencing the rate of body decomposition. In burial contexts, however, oxygen availability is limited, particularly compared with conditions experienced by bodies exposed to the atmosphere (Vass, 2011). Regardless of the grain size of the soil matrix, the act of creating a grave alters the normal infiltration and diffusion pathways of gases and water: excavation and interment initially allow oxygen to reach the bottom of the grave, while subsequent backfilling progressively displaces gases and water. The refilled soil remains looser than undisturbed soil, thereby exhibiting greater porosity and permeability (Dent et al., 2004).

Gases that are typically generated during decomposition (CO_2 , CH_4 , H_2S , NH_3 ; Ioan et al., 2017) then migrate upward through the pore spaces of the loosened soil, competing with oxygen for available voids. Over time, natural soil compaction occurs, aided by water infiltration and percolation (Dent et al., 2004). Consequently, decomposition within a burial environment becomes predominantly anaerobic after only a brief initial period.

Regarding the cadaveric macromolecules undergoing decomposition, proteins are the first to experience proteolysis. This process, however, is not uniform: proteins of neuronal and epithelial tissues (including the lining of the gastrointestinal tract and the pancreatic epithelium) are destroyed during the early stages, whereas more decomposition-resistant proteins such as those of the epidermis, reticulin, collagen, and muscle are decomposed during the *putrefactive phase* (Geissenberger et al., 2024). Although the rate at which putrefaction begins is highly dependent on the surrounding environment, Dent et al. (2004) identify it as the most probable in-grave decomposition process.

Among the various products released during protein degradation, and consequently detectable within the surrounding soil, are amino acids, together with the gases typically associated with putrefaction (Dent et al., 2004). Amino acids may undergo deamination, mediated by the enzyme deaminase, yielding organic acids such as valeric, succinic, and lactic acids (Swann et al., 2010). Alternatively, they can undergo decarboxylation, being reduced to compounds of carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) (Statheropoulos et al., 2005; Ioan et al., 2017). Some amino acids contain sulfur atoms, which during putrefaction are subject to bacterial desulfhydration, contributing to the production of several of the aforementioned gases as well as pyruvic acid ($\text{CH}_3\text{--CO--COOH}$). Sulfur is of particular importance in the study of the HC DI: soil is naturally rich in iron, and in its presence sulphaemoglobin reacts to form ferrous sulfide (FeS), the characteristic black stain beneath and around a cadaver that often aids in

identifying a CDI (Carter et al., 2007). In general, the limited oxygen within a burial promotes the accumulation of sulfide (the inorganic sulfur anion S^{2-}), and under anaerobic conditions these sulfide products are not further transformed (Dent et al., 2004). Nitrogen, being a constituent of amino acids, is readily utilized as an energy source by soil microorganisms (Rilling et al., 2007), releasing nitrogen primarily as ammonia (NH_3). The acidic conditions of grave soil convert ammonia to ammonium (NH_4^+), which serves as a nutrient for plants or microorganisms (Dent et al., 2004). The presence of NH_4^+ has been identified as a key factor responsible for pH increase within the CDI (Ioan et al., 2017; Vass, 2017), particularly during the bloating and active-decay stages of the body, after which soil pH decrease can occur (Rai et al., 2022; Tagliabue et al., 2026). According to Vass (2017), if the CDI pH is not alkaline, detection of decomposition-derived organic compounds is unlikely to be reliable. Alternatively, ammonia that is not converted to ammonium may undergo nitrification (oxidation of NH_3 to nitrite NO_2^- , and subsequently nitrate NO_3^-) under aerobic conditions, or denitrification (reduction of nitrate NO_3^- , to nitrite NO_2^- , gaseous nitrogen, or nitrous oxide N_2O) under anaerobic conditions (Dent et al., 2004; Hayatsu et al., 2008).

Adipose tissue (composed of approximately 60-85% lipids, of which 90-99% are triglycerides) also undergoes decomposition. Triglycerides consist of a single glycerol molecule bound to three fatty acids (Swann et al., 2010); among these, monounsaturated oleic acid is the most abundant, followed by linoleic and palmitoleic (unsaturated) and palmitic (saturated) acids (Dent et al., 2004). During decomposition, lipids are first hydrolyzed to triglycerides through the action of lipase enzymes and subsequently to saturated and unsaturated fatty acids (Ioan et al., 2017). Anaerobic conditions, such as those typical of a burial environment, favor hydrogenation of unsaturated fatty acids, producing saturated fatty acids (Dent et al., 2004). Some of these fatty acids are volatile (Volatile Fatty Acids, VFA), including acetic, propionic, butyric, and valeric acids. VFA are termed “volatile” because they form vapors under acidic conditions. However, in an alkaline environment (such as that arising during proteolysis within an HCDI) VFAs can react with sodium (Na^+) and potassium (K^+) ions to form their respective fatty acid soaps that migrate with putrefactive leachate, permeating the soil (Vass, 2017). Continued hydrolysis and hydrogenation of fatty acids increase the proportion of saturated fatty acids while reducing the relative abundance of unsaturated ones (Dent et al., 2004; Forbes et al., 2004). Following burial, however, sodium and potassium ions may be replaced by calcium (Ca^{2+}) and magnesium (Mg^{2+}) naturally present in the soil (Swann et al., 2010). This

transformation is especially common in buried remains due to low oxygen levels, high moisture, and the availability of free cations in soil (Vass, 2011).

Finally, during putrefaction the carbohydrates present within tissues also undergo breakdown. Glycogen, for example, is microbially degraded to glucose monomers, which under anaerobic conditions are incompletely oxidized by bacteria to produce organic acids such as lactic, butyric, and acetic acids. Additional gases generated through bacterial carbohydrate fermentation include methane, hydrogen, and hydrogen sulfide (Dent et al., 2004). It is important to note, however, the considerable difficulty in distinguishing carbohydrates in grave soil that originate from human decomposition from those already present in the matrix - for instance, polysaccharides produced by microbial decomposition of plant material (Soong et al., 2014).

As mentioned in section 1.3, at the conclusion of the putrefactive phase, the body softens and degenerates into a mass of unrecognizable tissue that, with continued decomposition, undergoes *liquefaction* (Dent et al., 2004). These transformations allow a partial reintroduction of oxygen molecules into the tissues (Janaway, 1996), restoring an aerobic environment that permits the onset of *skeletonization* (Pokines & Symes, 2013). Bone tissue itself can contribute nutrients to the CDI as it degrades. Bone is composed of a protein fraction (collagen), a mineral fraction (hydroxyapatite, $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$), and a ground substance of other organic compounds (Florencio-Silva et al., 2015). Collagen and hydroxyapatite are tightly bound through a protein-mineral matrix that imparts strength and elasticity while providing resistance to postmortem degradation. Initial degradation targets collagen, which is broken down by microbial activity, reducing proteins to peptides and then to amino acids that leach away (Dent et al., 2004). Subsequent deterioration involves the loss of hydroxyapatite through inorganic mineral weathering (Behrensmeyer, 1978). The dynamics of bone weathering are difficult to define, as they involve not only the leaching of ions such as calcium and phosphorus but also infiltration and adsorption of external elements that further compromise the structural integrity of the bone.

The extent and persistence of bone-degradation products within a HCDI remain poorly investigated (Fiedler et al., 2004; Tagliabue et al., 2023). Moreover, according to Vass et al. (1992), the decomposition of a human body releases such a substantial nutrient load that concentrations can remain elevated above background soil levels even after skeletonization is complete, potentially complicating their detection.

Given these nutrient-driven alterations to soil chemistry, biogeochemical analysis of the HCDI provides a valuable tool for detecting criminal activity (Carter & Tibbett, 2008). Indeed, an increasing number of studies have focused on the appearance and depletion trends of decomposition-related compounds in grave soil, with many supporting the utility of forensic biogeochemistry for estimating both postmortem interval and post-burial interval.

For example, Wilson et al. (2007) discussed how buried bodies themselves could sensitively modify their surroundings (raising soil pH, enriching microbes, and shifting moisture) creating measurable indicators of PBI. However, they also highlight the importance of interpreting the evidence in context of site-specific climate and soil conditions. Indeed, according to the authors, HCDI biogeochemistry and microbiology anomalies are indicators that a burial has undergone active decomposition, so they should be considered in order to reach a complete clandestine grave investigation.

Similarly, Fancher et al. (2017) highlighted how soil beneath cadavers retains a long biogeochemical fingerprint, useful for PMI estimation for nearly five years postmortem. Therefore, biogeochemistry can serve as a complementary tool to forensic entomology and taphonomy. This “long-term soil memory” of the cadaver was confirmed by von der Lühse et al., (2017). In their experiment, high levels of products of human fat tissue breakdown were detected in soil, even one year after the removal of the body from its original deposition site. These findings not only underline the importance of biogeochemistry applied to CDI, but they are also crucial for cases where a cadaver was relocated or removed before discovery.

Finally, Pringle et al. (2010) conducted an experiment to test whether measuring electrical conductivity of putrefactive leachate in soil water could provide a field-ready method to estimate PBI and, therefore, a minimum PMI for clandestine graves. They demonstrated how decomposition fluids leach salts and ions, driving soil water electrical conductivity far above background, with a pattern of increase that correlates with PBI: a rapid followed by a continued sharp rise in correspondence with the highest input of nutrients due to putrefaction, and a slower, less predictable rise after 300 days circa.

Nevertheless, all these processes are typically described in general terms, while it is essential to consider the factor that most influences decomposition in the case of burials: the burial environment itself. In particular, site conditions (such as soil properties, vegetation, geology, topography, wildlife presence, air circulation, humidity, and heat budget) exert a decisive

impact on the decomposition rate (Fiedler et al., 2003). Among these factors, soil plays a primary role since it completely surrounds the remains, creating a microenvironment to which they are continuously exposed and with which they interact. For example, soil grain size determines its capacity to retain water within pore spaces, thereby affecting both oxygen availability and the overall humidity of the hypogeal environment (Fitzpatrick & Donnelly, 2021). This principle was demonstrated by Tumer et al. (2013), who assessed that grave soil texture and moisture critically influence decomposition: loamy and organic soils promoted aerobic microbial activity, accelerating tissue breakdown, whereas clay-rich soils limited oxygen diffusion, favored anaerobic conditions, and slowed decay while promoting adipocere formation.

pH is also a critical factor, not only for tracking fluctuations associated with the stages of decomposition but also for accurately interpreting the taphonomic condition of tissues such as bone. In general, soils with a neutral to slightly alkaline pH promote better bone preservation, whereas acidic soil accelerates its degradation. Sandy, dry soils likewise favor bone preservation because bacterial activity is limited, while more permeable soils enhance decomposition through greater water and gas exchange (Dent et al., 2004).

In addition, according to Fiedler et al. (2013), when assessing the suitability of a site for interment in a cemetery context, the parent material is of primary importance. On loam-enriched moraine deposits, periglacial regoliths, poorly permeable substrates such as schist, and marl or clay stones, human remains are more likely to undergo preservative processes such as adipocere formation.

– CHAPTER 2 –

THE EXPERIMENT

STUDY CONTEXT AND CONDITIONS OF THE STUDY

This chapter aims to provide an overview of the experimental and naturalistic context in which the PhD research project *Ticino 2023* was conducted. Beginning with a description of the experimental area, the study was carried out at Ticino-LEAFs (Ticino Park–LABANOF Facility for Environmental Analysis in Forensic Sciences), the first non-human forensic taphonomy facility in Italy. Operational since 2009, the facility has hosted numerous experiments investigating body–environment interactions through the reconstruction of various scenarios involving the simulation of concealment of animal remains in natural settings.

2.1. Ticino-LEAFs: The First (Non-Human) Forensic Taphonomy Facility in Italy

In this section a published manuscript on the importance of an interdisciplinary approach in the analysis of forensic taphonomy is presented. Moreover, the paper introduces the birth and evolution of Ticino-LEAFs.

Subsequently, the latest forensic taphonomy project carried out at the facility, which began on March 3, 2023, and on which this thesis is based, will be presented.

2.1.1. *Overview of Geosciences Applied to Forensic Taphonomy: The Interdisciplinary Approach of the Italian Non-Human Research Facility (Ticino-LEAFs) published in Geosciences*

This manuscript, published in *Geosciences* 2024, 14, 359, is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Authors: Giulia Tagliabue (corresponding), Anna Masseroli, Giulia Caccia, Pasquale Poppa, Cristina Cattaneo and Luca Trombino.

DOI: <https://doi.org/10.3390/geosciences14120359>

Link: <https://www.mdpi.com/2076-3263/14/12/359>

The work reviews the integration of geosciences into forensic taphonomy, emphasizing their role in understanding decomposition and post-inhumation processes. It highlights how interdisciplinary approaches can enhance the accuracy of postmortem interval (PMI) estimations and improve contextual interpretation of body–environment interactions.

The paper also lists all the currently working Forensic Taphonomy Facilities (FTF) in the world and presents the first Italian non-human FTF Ticino-LEAFs, inaugurated in 2009 by the University of Milan. Research conducted there employs pigs and piglets as human analogs, underlining both anatomical similarities and inherent differences between the species. Projects at the facility adopt an interdisciplinary methodology, treating the cadaver and surrounding (epigeal and/or hypogeal) environment as dynamic systems of mutual interaction, while also adhering to ethical standards by using animals that died naturally or in clinical independent contexts.

Review

Overview of Geosciences Applied to Forensic Taphonomy: The Interdisciplinary Approach of the Italian Non-Human Research Facility (Ticino-LEAFs)

Giulia Tagliabue ^{1,2,*} , Anna Masseroli ³, Giulia Caccia ¹, Pasquale Poppa ¹, Cristina Cattaneo ¹ and Luca Trombino ² 

¹ LABANOF (Laboratorio di Antropologia e Odontologia Forense), Sezione di Medicina Legale, Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Via Mangiagalli, 37, 20133 Milano, Italy; giulia.caccia@unicampania.it (G.C.); pasquale.poppa@unimi.it (P.P.); cristina.cattaneo@unimi.it (C.C.)

² Dipartimento di Scienze della Terra “Ardito Desio”, Università degli Studi di Milano, Via Mangiagalli, 34, 20133 Milano, Italy; luca.trombino@unimi.it

³ Dipartimento di Scienze Agrarie e Ambientali-Produzione, Territorio, Agroenergia, Università degli Studi di Milano, Via Celoria, 2, 20133 Milano, Italy; anna.masseroli@unimi.it

* Correspondence: giulia.tagliabue@unimi.it

Abstract: The recent surge in the application of geosciences in forensics has yielded significant insights into taphonomy and decomposition processes, particularly with regard to the identification and interpretation of evidence pertaining to the interaction between the cadaver and the outdoor post-depositional environment. Given that a substantial proportion of current knowledge about decomposition dynamics can be attributed to forensic taphonomy experiments utilizing pigs as human models, a literature review was conducted, focusing on experiments incorporating geosciences into their investigative techniques. The results demonstrated the crucial role of this approach in ensuring accurate interpretation of data, both in the context of forensic cases and in experimental research. Furthermore, this manuscript presents the opening of the first Italian non-human forensic taphonomy facility, which was inaugurated by the LABANOF of the University of Milan in 2009 at the Lombardy Park of the Ticino (PV). The facility strives to integrate the use of non-human animals as human models while upholding a high ethical standard and minimizing the construction of artifacts, thereby striving for forensic realism. Furthermore, the most recent project conducted at the Italian FTF establishes a connection between the outcomes of preceding projects and underscores the significance of a pragmatic and intellectual disposition that embraces interdisciplinary collaboration.

Keywords: forensic taphonomy; geosciences; body concealment; clandestine graves; soil chemistry; forensic taphonomy facility; body farm; non-human models; decomposition; postmortem interval



Citation: Tagliabue, G.; Masseroli, A.; Caccia, G.; Poppa, P.; Cattaneo, C.; Trombino, L. Overview of Geosciences Applied to Forensic Taphonomy: The Interdisciplinary Approach of the Italian Non-Human Research Facility (Ticino-LEAFs). *Geosciences* **2024**, *14*, 359. <https://doi.org/10.3390/geosciences14120359>

Academic Editor: Fedor Lisetskii

Received: 16 October 2024

Revised: 28 November 2024

Accepted: 17 December 2024

Published: 23 December 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The application of geosciences in forensic investigations has increased markedly in recent times [1–4]. In such a scenario, soils, due to their inherent characteristics, appear to be suitable resources for the recovery of evidence and the detection of trace elements linked to a criminal act [5–13]. This is particularly evident when considering taphonomic analysis of remains discovered in natural environments [13–17], clandestine graves [18–21], or performing research activities in Forensic Taphonomy Facilities (FTF) [16,22–25] otherwise colloquially known as “body farms” (i.e., dedicated areas, often outdoor, belonging to institutes and universities, where typical forensic scenarios are recreated using experimental cadavers, whether human or animal models [26]). The principal objective of these facilities is to observe the taphonomic agents affecting the body in a controlled setting, monitoring the numerous variables that influence decomposition. An interdisciplinary approach to forensic taphonomy research could yield significant benefits for casework, including

the joint application of techniques from the medical and environmental sciences for the investigation of crime scenes. This would result in the identification of the reciprocal exchange of material between a decomposing body and its surrounding environment. Indeed, in contexts that are extremely complex, such as the natural environment, where numerous variables that are difficult to control and predict come into play, it is not possible to assess the state of preservation of the corpse without taking environmental parameters into account. Given that investigative work in body concealment is frequently oriented toward estimating the post-mortem interval (PMI), the recognition of time-dependent trends in the concentration of specific decomposition products within the associated matrix is of paramount importance in achieving this goal [15–17,21,25]. Moreover, the exposure of a decomposing body to a matrix that surrounds it, such as soil, results in the formation of organ–mineral compounds, which may be present in both substrates. These compounds may be indicative of a potential previous burial site [21]. It is, therefore, essential to consider the role of soil, given that its characteristics, on the one hand, are able to markedly affect the decomposition processes of an organism, and, on the other hand, they could undergo an alteration of which evidence remains. Disciplines closely related to natural and Earth sciences, such as ecology and geoecology, soil chemistry, geochemistry, geophysics, microbial activity, and geoactivity, are included among taphonomy studies [5,22,26,27]. Despite this, research conducted at FTFs still struggles to include soil analysis for the proper interpretation of decomposition processes and the resolutions of questions pertaining to post-mortem interval (PMI) and contextual taphonomy. Neglecting these characteristics and how they affect the rate of decomposition and taphonomic alterations may result in the loss of crucial elements for local investigations, also considering that the environment and the soil that defines and is defined by it have immense inherent variability. It is, therefore, evident that Forensic Taphonomy Facilities must be established in locations that can represent the full range of ecological realities characterizing our planet [28], with particular emphasis on locations that are typically affected by victim concealment activities. In addition to these extrinsic limiting factors, which result directly from the environment to which the body is exposed, intrinsic factors must also be considered. These can be identified as all those characteristics that define the body itself and consequently influence its own rate of decomposition, also taking into account that the decomposition process is an extremely variable phenomenon, even between human individuals with comparable characteristics, as every human being has a unique pattern of life [28]. This may result in a lack of repeatability, which, according to some studies, could be addressed by utilizing animal surrogates that mimic human remains. It is, therefore, crucial to ascertain the nature of the specimen in question, whether human remains or other animals (typically larger mammals, such as pigs). However, the debate surrounding this topic is still ongoing, especially concerning the use of pigs in experiments focused on forensic geosciences [29]. Indeed, although pigs are commonly used in forensic taphonomy research, some authors have proposed that they decompose at a different rate and pattern than humans, making them an inadequate substitute for decomposition studies [23,30,31]. Conversely, other papers emphasized the suitability of pigs as a model for forensic experimentation that includes the application of geosciences, citing the numerous anatomical and physiological similarities between the two species [32–34]. The aim of the present review is to provide a comprehensive, yet not exhaustive, overview of experiments in the literature that employ the use of pigs as human surrogates in the reconstruction of concealment scenarios, with a particular focus on geosciences, including geophysics, the analysis of soil biogeochemical characteristics, and their application as a tool to better interpret data from other forensic disciplines. Moreover, the present paper has the purpose of presenting the currently ongoing project at the LABANOF's Forensic Taphonomy Facility of the University of Milan, in Italy.

2. Methods

For the purpose of presenting a survey of the published works about experimental forensic scenarios involving pig cadavers and focused on the use of geosciences, a literature review was performed on ScienceDirect and GeoscienceWorld, using different keywords and combinations of them, such as forensic taphonomy pigs, forensic soil pigs, experiment body concealment, clandestine graves pigs, forensic geoscience experiment, soil decomposition chemistry, forensic simulation pigs, and soil forensic experiment. The selection criteria consisted primarily of the presence of simulations of the concealment of pig bodies by burial or by exposing them to the natural environment under controlled conditions (work that included the use of single processed parts of pig cadavers or the inclusion of other animal species within the experiment was excluded). In addition, studies were considered that included geological resources among the determinant analyses for the evaluation of the experiment, using techniques such as geophysics and soil chemistry, whether or not they were cases of simulated clandestine burials. The selected papers included reviews, technical notes, and research articles, while dissertations, books, and book chapters were excluded.

3. Literature Review

The criteria presented here led to a final selection of 39 papers. Even though most of them involved the use of techniques typical of geosciences, the papers were reviewed and categorized according to the scientific discipline (or combination of disciplines) on which each manuscript was based, namely geophysics, soil chemistry, forensic anthropology and taphonomy, forensic entomology, forensic botany, and microbiology (Table 1).

Table 1. List of the papers included in the review. Experimental design, duration, and applied geoscience techniques (if there are any) are specified.

First Author and Reference	Year	Field	Experimental Design	Included Geo-Environmental Analysis	Burial	Surface
Hopkins [9]	2000	Microbiology	3 piglets Buried (10 cm) 430 days	Soil pH Soil moisture Total C Total N NH_4^+ , NO_3^- Amino acids Soil microbial biomass	X	
Szelecz [15]	2018	Soil chemistry	5 hanged pigs 5 pigs Soil surface deposition 367 days	Soil pH Total C Total N NH_4^+ , NO_3^- Bioavailable P K^+		X
Dick [19]	2018	Soil chemistry	1 pig Buried (60 cm) 549 days	Soil pH Electrical conductivity Elemental analysis (leachate in soil)	X	
Benninger [27]	2008	Soil chemistry	5 pigs Soil surface deposition 100 days	Soil pH Soil moisture content Total C Total N Soil-extractable P Lipid-P		X
Sutherland [32]	2013	Forensic anthropology and taphonomy	15 pigs 15 piglets Surface deposition 90 days	/		X

Table 1. Cont.

First Author and Reference	Year	Field	Experimental Design	Included Geo-Environmental Analysis	Burial	Surface
Jervis [35]	2009	Geophysics	1 naked pig 1 pig wrapped in tarpaulin Buried (60 cm) 364 days	Electrical resistivity (ER) Soil porosity Soil moisture content	X	
Pringle [36]	2012	Geophysics	1 naked pig 1 pig wrapped in tarpaulin Buried (60 cm) 3 years	Electrical resistivity (ER) and Electrical Resistivity Imaging (ERI) Ground Penetrating Radar (GPR)	X	
Pringle [37]	2016	Geophysics	1 naked pig 1 pig wrapped in tarpaulin Buried (60 cm) 6 years	Electrical resistivity (ER) and Electrical Resistivity Imaging (ERI) Ground Penetrating Radar (GPR)	X	
Booth [38]	2016	Geophysics	1 naked pig 1 pig wrapped in tarpaulin Buried (60 cm) 5 years	Ground Penetrating Radar (GPR)	X	
Pringle [39]	2020	Geophysics	1 naked pig 1 pig wrapped in tarpaulin Buried (60 cm) 10 years	Electrical resistivity (ER) and Electrical Resistivity Imaging (ERI) Ground Penetrating Radar (GPR)	X	
Pringle [40]	2021	Geophysics	1 naked pig 1 pig wrapped in tarpaulin Buried (60 cm) 10 years	Electrical resistivity (ER) and Electrical Resistivity Imaging (ERI) Ground Penetrating Radar (GPR)	X	
Jervis [41]	2014	Geophysics	1 naked pig 1 pig wrapped in tarpaulin Buried (60 cm) 3 years	Electrical Resistivity (ER) Soil moisture content	X	
Schultz [42]	2006	Geophysics	6 buried pigs (50–60 cm) 6 buried pigs (100–110 cm) 21 months	Ground Penetrating Radar (GPR)	X	
Schultz [43]	2008	Geophysics	6 buried piglets (50–60 cm) 6 buried piglets (100–110 cm) 21 months	Ground Penetrating Radar (GPR)	X	
Schultz [44]	2012	Geophysics	6 pig burials: 1 naked pig (50 cm) 1 naked pig (100 cm) 1 pig covered in small rocks (100 cm) 1 pig wrapped in tarpaulin (100 cm) 1 pig with lime (100 cm) 1 pig wrapped in a cotton blanket (100 cm) 1 year	Ground Penetrating Radar (GPR)	X	
Van Belle [45]	2009	Soil chemistry	6 pigs Buried (40 cm) 1 year	Ninhydrin reactive nitrogen (NRN)	X	
Meyer [46]	2013	Soil chemistry	6 pigs Soil surface deposition 60 days	Soil pH Total carbon C Total NNinhydrin-reactive nitrogen (NRN) NH_4^+ , NO_3^-		X

Table 1. Cont.

First Author and Reference	Year	Field	Experimental Design	Included Geo-Environmental Analysis	Burial	Surface
Anderson [47]	2013	Soil chemistry	6 pigs Soil surface deposition 3 years	Soil pH Total C Total N Ninhydrin-reactive nitrogen (NRN) NH_4^+ , NO_3^-		X
Brasseur [48]	2012	Soil chemistry	2 pigs Buried (40 cm) 6 months	Soil Volatile organic compounds (VOCs)	X	
Schotsmans [49]	2012	Forensic anthropology and taphonomy	6 pairs of pigs (covered in hydrated lime, quicklime, and naked) Buried (34–38 cm) 6 months	Soil pH	X	
Schotsmans [50]	2014	Forensic anthropology and taphonomy	1 pig Stainless steel chamber deposition	/		X
Schotsmans [51]	2014	Forensic anthropology and taphonomy	6 pairs of pigs (hydrated lime, quicklime, naked) Buried (34–38 cm) 17 and 42 months	Soil temperature Soil pH Soil microbiology	X	
Kontopoulos [52]	2016	Forensic anthropology and taphonomy	4 pigs mass buried (60 cm) 3 piglets clothed and wrapped Soil surface deposition 7 years	/	X	X
Marais-Werner [53]	2018	Forensic anthropology and taphonomy	25 buried pigs (75 cm) 10 months	/	X	
Buekenhout [54]	2018	Forensic anthropology and taphonomy	2 piglets Buried (120 and 106 cm) 9 months	Soil pH Soil texture	X	
Cogswell [55]	2021	Forensic anthropology and taphonomy	3 pigs deposition on concrete 3 pigs deposition on gravel 5 pigs deposition on the grass 50 days	/		X
Ururahy-Rodrigues [56]	2008	Forensic entomology	6 pigs Soil surface deposition 1 month	/		X
Valdes-Perezgasga [57]	2010	Forensic entomology	7 pigs Soil surface deposition 70 days	/		X
Dekeirsschieter [58]	2009	Forensic entomology	3 pairs of pigs Soil surface deposition (forest, agricultural site, urban site) 6 months	/		X

Table 1. Cont.

First Author and Reference	Year	Field	Experimental Design	Included Geo-Environmental Analysis	Burial	Surface
Horensteinn [59]	2010	Forensic entomology	8 pigs Soil surface deposition 7 weeks	/		X
Anton [60]	2011	Forensic entomology	8 pigs Concrete ad grass surface deposition From 20 to 133 days	/		X
Bonacci [61]	2011	Forensic entomology	1 pig Soil surface deposition 25 days	/		X
Payne [62]	1965	Forensic entomology	10 piglets Soil surface deposition 100 days	Soil moisture Soil temperature		X
Payne [63]	1972	Forensic entomology	Piglets Water	/		
Payne [64]	1968	Forensic entomology	5 piglets Buried in coffin or wooden boxes (50–100 cm) 6 months	/	X	
Turner [65]	1999	Forensic entomology	3 piglets Buried (10 cm) 17 weeks	Soil pH Soil temperature Soil texture	X	
Rai [66]	2022	Forensic entomology	3 pigs Buried (10–22 cm) 3 years	Soil pH Soil temperature Soil texture	X	
Perrault [16]	2016	Forensic botany	7 pigs Soil surface deposition 96 days	Soil pH Soil Water holding capacity (% WHC) Soil-available P Ca, Mg, K, Na (soil and vegetation)		X
Silván-Cárdenas [67]	2021	Forensic botany	16 pigs Buried (109–150 cm) 174 days	Hyperspectral/multispectral imaging UAV-based photogrammetry Thermal imaging	X	

3.1. Geophysics

As expected, most of the literature considered for this paper is based on experiments conducted in the field of geoscientific research applied to forensics, primarily geophysics. A great example is the consistent experimentation performed at Keele University [35–40]. The study started in 2007 [35] to investigate how electrical resistivity (ER) surveys could be a tool to detect homicide burials; the researchers created an experimental setting based on the realization of clandestine graves. Specifically, they simulated five burials, three of which were used for resistivity surveys, while two other graves were created to sample soil and groundwater, with the goal of determining whether a burial could affect the porosity and moisture content of the soil, along with groundwater conductivity. The results of the study indicated that ER effectively revealed the presence of the pig cadavers, but it was inefficient in the detection of the control grave, leading to the hypothesis that putrefaction leachate could be responsible for a localized increase in conductivity (and a subsequent decrease in resistivity). Indeed, as expected, the wrapped pig grave was more challenging to discern, as the wrapping could function as a barrier to the decomposition

fluids. The data collection continued for another three years [36], including multifrequency ground-penetrating radar (GPR) among the search techniques. This study found interesting links between the depth of the clandestine burials and the setting of the ER electrodes, suggesting wider separations in addition to the standards when the burial depth is not known. Additionally, they recommended ER over GPR surveys in clay-rich soils, firstly because of the higher probability of finding conductive putrefaction leachate and secondly for the known poor penetration depths of GPR in such soils. Furthermore, the authors assessed that GPR surveys were also found to be suboptimal for detecting targets in cases of advanced decomposition, even if skeletal material would still be imaged. To determine the period following the interment during which geophysical surveys would be unable to detect a clandestine burial and to determine the timeframe within which grave soil conductivity would return to background levels, the study site was monitored on an ongoing basis. Indeed, the ER and GPR survey results were also presented in 2016, when Booth et al. [38] performed semblance analysis (a quantitative method) to assess the data collected during the first five years of experimentation and reflected on long time period data [37]. Findings from these studies revealed no evident long-term temporal trend observed with the GPR data over the survey period, although Pringle et al. [36] previously noted a reduction in the hyperbolae amplitude as burial time increased, giving confidence that GPR surveys could detect targets well up to five years after burial at specific site conditions. Regarding ER, according to this study, buried naked victims should be able to be located within the first four years of burial, after which the body would be more difficult to detect due to the migration of the leachate. Finally, between 2020 and 2021, the final results of the 10-year-long experiment were published by Pringle et al. [39,40]. The key insights from this study included GPR as a very effective method for the detection of wrapped buried victims at both low, medium, and high frequencies, even ten years after body concealment, whereas ER seemed to be successful only after a certain amount of time (one to two years). Conversely, the naked pig was detectable by GPR surveys within the first year of burial, after which the signal would decrease, while ER results were satisfactory up to four years after burial. While this well-performed study provided insight into the simulation of clandestine graves using pigs as analogs and the application of geosciences to forensic scenarios, the interpretation of the results could be questioned. Indeed, the study did not consider several variables that could affect the data, such as the depositional environment (even though they considered the seasonal effects on ER [41]), the size of the victim, the burial method, and the depth or the presence of wounds on the body. Moreover, by not including more variables in the experimental setting (namely the presence of associated material), the results are difficult to incorporate into real forensic investigations, which are often more complicated.

A further study, based on the utilization of GPR, was conducted by Schultz et al. [42] over a 21-month period with the aim of monitoring 12 large pig burials, realized at different depths and in different soil horizons. The results were found to be consistent with those reported in previous studies [36–40], whereby the presence of cadavers in sandy horizons was readily discernible throughout the duration of the study. In contrast, burial in clay resulted in progressively reduced detectability of the pig cadavers over the first year. Nevertheless, the analysis demonstrated that it was still possible to discern the soil disturbance resulting from the grave construction in the clay horizon, even when the cadaver response was minimal. Conversely, the investigation of the control graves in sandy soil revealed that the hyperbolic anomaly was predominantly attributable to the decomposing body or skeleton rather than the disturbed soil.

Schultz et al. performed similar research, burying 12 piglets in the same site [43]. The findings of this study indicate that it may prove challenging to detect small cadavers interred in sand following skeletonization. This is due to the fact that the surrounding area of the body may not have the necessary contrast to be detected by the GPR. Additionally, the burial depth proved to be a significant factor in the detection of graves. Bodies buried at greater depths may be detected for a longer period due to reduced decomposition rates.

Finally, the same site hosted research by Schultz and Martin focused on monitoring six burials containing a small pig carcass in different burial conditions [44]. GPR data, obtained with both 250 and 500 MHz dominant frequency antennae, were collected monthly over the course of a 12-month monitoring period. The findings indicated that the acquisition of superior images was more successful for deep graves in comparison to shallow graves. The 250 MHz antenna yielded superior images for the detection of deep graves, as the production of non-target anomalies was low. It also demonstrated consistent detection of the disturbed soil in the deep control grave. Conversely, the 500 MHz antenna presented superior performance in the detection of shallow pig graves but a limited detection of disturbed soil. Overall, the studies Schultz et al. conducted [42–44] yielded promising results, as several burial variables that could affect the surveys were considered, including the size of the targets, the soil type, the depth of the grave, the body wrapping, items added to the grave, and the conservation state of the cadaver, as well as subsurface soil moisture.

3.2. Soil Chemistry

In the field of geosciences, soil chemistry is another fairly often utilized discipline applied in forensic research. This is due to the fact that it is well-established that the biogeochemical condition of the soil matrix can influence the rate of decomposition of a cadaver that has been concealed through clandestine burial; additionally, soil chemistry can serve to record alterations that are attributable to the presence of a body. A comprehensive understanding of the decomposition processes and their impact on soil conditions can assist forensic investigators in estimating the post-mortem interval (PMI), which represents the minimum and maximum time frame for which an individual may have been deceased [68], identifying potential concealment locations, and generally reconstructing the sequence of events that transpired during the commission of the crime. In this particular area, much research is focused on the analysis of the biogeochemical characteristics of the soil following the deposition of pig carcasses on its surface at different PMIs, rarely shorter than 60 days [15,27,45–47].

For instance, Benninger et al. [27] examined the dynamics of various chemical elements and compounds in soil beneath five pig cadavers placed on the soil surface over a 100-day period. The findings of the study revealed a notable increase in the concentration of grave soil nutrients, with a maximum PMI of 43 days (lipid-P), 72 days (total nitrogen), and 100 days (soil-extractable phosphorus). While this study provides insights into the potential of soil-based methods to act as a tool for the estimation of extended post-mortem interval (PMI), it fails to address the importance of shorter PMIs. Indeed, a carcass can release a significant pulse of nutrients into the soil, some of which (total nitrogen, soil extractable phosphorus, and lipid phosphorus) are significantly elevated above background levels up to 100 days post-mortem. However, the study demonstrated that the most significant increase in these nutrients was observed early in the decomposition trial (first three weeks).

A considerably shorter PMI (60 days) was employed in the study conducted by Meyer et al. [46], which concentrated on the impact that seasonal fluctuations can exert on decomposition rates and soil biogeochemistry. In this case, the experiment was performed by placing a pig carcass in a plot in winter and one in summer and repeating the operations three times, resulting in a total of six carcasses analyzed in three years. As was to be expected, in summer, pigs decomposed three to seven times faster than in winter, and, in accordance with the findings of other seasonal studies [27], some nutrients showed the most significant increase in the shorter PMI (total nitrogen, ninhydrin reactive nitrogen (NRN), and ammonium), regardless of season. Finally, the study found interesting correlations between ammonium and pH (positive correlation) and between nitrate and pH (negative correlation).

A comparable experiment was conducted on the same site by Anderson et al. [47], in which soil samples were collected and subjected to chemical analysis at intervals of one and three years following the deposition of pig cadavers on the soil surface. The analysis showed noticeable increases in NRN, pH, total nitrogen, and nitrate in soil one-year post-

mortem, whereas no significant differences were detected after three years, contributing to the existing knowledge in this field. However, the study yielded new insights into the vegetation associated with the soil beneath a decomposing body. In fact, unlike previous research [69,70], which found that the input of nutrients and moisture, coupled with enhanced aeration and redistribution of resources (resulting from the realization of the grave), could facilitate enhanced plant growth, the current study demonstrated that surface decomposition could hinder plant growth for a minimum of one year. This could be due to chemical toxicity and reduced gas diffusion through the soil matrix. However, by the three-year sampling, the area occupied by the now skeletonized remains had become overgrown with new vegetation, likely because of the soil chemistry returning to its original baseline levels.

Moreover, studies have examined soil chemical composition in relation to different simulated concealment scenarios employing pigs as a human model. In this regard, the objective of Szelec et al. [15] was to investigate the effects of decomposing pigs on soil chemistry over a one-year period, whether placed on the ground or hung one meter above. During the first two months, the presence of the cadavers resulted in notable elevations in ammonium, nitrogen, phosphorus, and potassium levels. Conversely, nitrate concentrations exhibited a marked increase around the conclusion of the study. Soil pH showed an increase at first but decreased significantly at the end of the experiment, even if with a predictable weaker concentration in the soil under the hanging pig. Similar to the work of Anderson et al. [47], after one year, some markers returned to basal levels, but conversely, others were still significantly different, namely nitrate, total nitrogen, and phosphorous. Considering these findings, the authors outlined three categories of chemical markers based on their response pattern to decomposition products over time: early peak markers (EPM), late peak markers (LPM), and late decrease markers (LDM). The marker categories may prove useful in enhancing our understanding of soil processes, particularly when changes in soil chemistry are associated with alterations in the composition of soil organism communities.

Another example of scientific experimentation in soil chemistry that relates different conditions of simulated concealment is that of Van Belle et al. [45], who investigated the release and lateral extent of NRN following both burial and soil surface decomposition of pig cadavers. In accordance with the cited literature [15,27,46,47], the results of the study demonstrated a significant increase in NRN concentration in grave soil collected from the center of graves during the initial two to six months of burial, with the rate of increase depending on the sampling depth. In contrast, carcasses undergoing decomposition on the soil surface were found to be associated with higher NRN values up to 97 days of decomposition. Thus, the authors assessed that the analysis of soil NRN can serve as a presumptive test for grave soil, provided that the burial occurs within two months and the surface deposition is limited to 97 days. This last finding is not consistent with the results of previous research work [15,27,46,47]. Regarding the lateral extent of the NRN, the study indicated it as relatively narrow. The grave wall samples collected at carcass level demonstrated an increase at three months, whereas the edge samples (60 cm from the center of the grave) taken from the surface to a depth of 20 cm showed no significant increase. In any case, the authors emphasized the necessity of considering the influence of cadaver size and soil texture on the lateral and vertical extent of decomposition fluids.

Research focused exclusively on the detection of clandestine graves through soil chemistry analysis was also carried out. For instance, Dick and Pringle [19] presented the results of an inorganic elemental analysis conducted on grave soil from a simulated homicide burial of a pig cadaver over an 18-month period. The results demonstrated that potassium, sulfate, and sodium are key detectable elements and that inorganic elements could provide both detection and potential dating of clandestine burials, even when physical remains have decayed. Such a research approach to clandestine burials could be criticized for the choice of chemical elements it aims to detect. In fact, there is a gap regarding the fate of the nutrients released in the grave soil during the decomposition

processes, which, on the contrary, are widely considered in surface studies [15,27,45–47], which are total carbon, total nitrogen, soil extractable phosphorus, lipid phosphorus, NRN, ammonium, nitrate, and pH. This aspect should be deepened since the cadaveric decomposition island (CDI) [25,71] manifests itself similarly in a burial since the post-mortem processes are generally the same as those of an exposed cadaver, even if typically slower [72]. In fact, the limited environment of a grave could contribute to a more durable preservation of the evidence, depending on the biogeochemical characteristics of the soil grave. Therefore, the monitoring of these elements and compounds must be considered in order to carry out a complete investigation of a clandestine burial and to provide a method that could be proposed as an investigative tool in real scenarios.

Differently, Brasseur et al. [48] focused on the study of volatile organic compounds (VOCs) in the grave soil of two experimental burials involving pig cadavers over a period of six months. Indeed, during decomposition, a buried cadaver releases several VOCs in the soil that could be analyzed through gas chromatography coupled to mass spectrometry (GC-MS) for the purpose of collecting information about the PMI. The work led to the identification of two main groups of analytes: a first group of 20 compounds specifically located below the carcass (ketones, nitriles, sulfurs, heterocyclic compounds, and benzene derivatives), and a second group of compounds present in all grave soil samples (methyl-branched alkane isomers). The study permitted the observation of a trend in the relative presence of these alkanes across the various soil horizons, emphasizing an increase in the specific alkanes as they transitioned from the carcass to the surface. Such soil sample screening for cadaveric decomposition could differentiate soils in proximity to buried decaying cadavers from undisturbed soils.

3.3. Forensic Anthropology and Taphonomy

Several experiments simulating body concealment in natural environments have been conducted with a particular focus on the dynamics of decomposition and decay of the cadavers. This type of research could potentially be complementary to that focused on geotaphonomy [48] and soil chemistry for forensic investigations. Nevertheless, very few studies have addressed the question of the effect of the specific characteristics of the environment on a concealed cadaver, including its decomposition rate and, therefore, the right interpretation of taphonomy. An illustrative example of a study that places a significant emphasis on the influence of the depositional environment on taphonomic alterations is that conducted by Schotsmans et al. [49–51], who buried pig cadavers as human analogs with and without lime (whether hydrated or quicklime) and recovered them after six months [49], 78 days [50], and up to 42 months [51]. The use of lime is due to the fact that the concealment of a body is often associated with the assistance of caustic substances, although the evidence on the effects of hydrated and quicklime on the decomposition rate is conflicting. Indeed, a common fallacy is the assumption that lime is able to accelerate the rate of decay, reduce the probability of identifying a victim, destroy evidence, and ultimately result in the rapid and total destruction of human remains. In fact, the substance in question has, rather, been observed to transform biological tissues and influence the rate of decomposition, even preserving some key evidence during investigation. According to the research of Schotsmans et al. [49], which also took into account environmental and microenvironmental conditions in addition to the pH of each grave, both hydrated lime and quicklime are able to retard the rate of decomposition of buried remains, at least after six months of burial. The findings indicated that the pigs treated with lime exhibited superior preservation compared to those not subjected to the same treatment, probably because lime served as a barrier. Thus, the effects of the soil biogeochemical characteristics and the taphonomic agents could be partially obliterated or reduced. The histological analysis also demonstrated that the tissue on the limed surfaces of the pigs was more well preserved than that on the undersurfaces, showing that quicklime appeared to have desiccating effects. A second experiment was conducted by Schotsman et al. [50] in a laboratory-simulated environment. In this case, the pigs were placed in

a small stainless steel chamber with limited access by insects, covered in hydrated lime or quicklime, and monitored for 78 days. This “laboratory microcosm experiment” [50] allowed observing the effects of the substance in the early stages of decomposition, showing that even though the pig covered in hydrated lime had well-preserved skin, it followed a similar pattern of changes to the uncovered pig, with the internal organs slightly better preserved. Conversely, the application of quicklime resulted in an initial acceleration of the decay process, causing a rapid rupture and poor internal preservation, likely due to the exothermic hydration effect of quicklime, which creates an environment conducive to bacterial growth that accelerates putrefactive change. In light of these findings, it can be argued that the exothermic reaction has implications for the estimation of the PMI when a cadaver is concealed by means of quicklime. Nevertheless, it must be taken into account that these results are from a laboratory simulation. As mentioned by the authors themselves [49], it is important to exercise caution when translating data obtained from this kind of study to real (or experimental) forensic scenarios in a field environment. Finally, Schotsmans et al. [51] repeated the previously performed experiment [49] and buried three pairs of pigs, respectively, without lime, with hydrated lime, and quicklime in shallow graves and recovered them after 17 and 42 months of burial. In this case, the analyses of the soil, lime, and carcasses were joined by entomological, pH, moisture content, microbial activity, histological, and lime carbonation investigations. The findings of this study indicated that the process of decomposition is indeed slowed down by burial with both hydrated lime and quicklime. However, as the decay process advances, the degree of liquefaction between the lime-covered and uncovered remains becomes increasingly similar, both ultimately resulting in skeletonization.

Otherwise, much of the literature on forensic taphonomy experiments using pigs as human analogs in concealment scenarios only considers the state of preservation of the remains [32,52,53]. This is a major limitation in the interpretation of the results, especially since these experiments often compare biological samples from different environments without considering the determinant factors of decomposition, such as soil characteristics, microbial community, temperature, precipitation, presence of scavengers, etc. On the other hand, other studies [54,55] based on the macroscopic observation of the state of decomposition of cadavers exposed to different conditions provide a general framework for the environment but lack the consideration of specific parameters that are fundamental for the correct interpretation of their results. As complex as it may be, the best strategy would be to take into account the environmental data (both those characterizing an area as well as the microenvironment of the concealment site) and the possible presence of scavengers and relate them to the state of preservation of the body, especially in the late PMI when thanatological parameters are more difficult to collect. Such an approach implies interdisciplinarity and an overview that should be the basis of the design of an experiment as well as of a real forensic investigation.

3.4. Forensic Entomology

Experimentation with pigs in forensics is also particularly active in life sciences studies, such as entomology, botany, and microbiology. In the field of forensic entomology, there are studies that neglect the geoenvironmental and microenvironmental data to focus on the relationship between necrophagous insects and decomposing bodies exposed in natural environments, such as how they interact [56,57] or how insects are attracted by the cadaver [58]. Conversely, there are others that relate the ecosystem that characterizes the experimental site and the presence of certain insect species useful for PMI estimation or for contextual taphonomy. Some of them, such as Horenstein et al. [59], Anton et al. [60], and Bonacci et al. [61], tried to determine the ease of access and the consequent presence of specific families of arthropods in relation to the exposure environment of the body, its state of preservation, and the season. However, they tended to neglect the characteristics of the soil, which inevitably influences the ecosystem, starting with the vegetation, with consequences on the entomofauna.

Differently, there are several forensic entomological studies that consider the soil characteristics of the experimental site in order to interpret their results correctly and as fully as possible. In fact, the pioneer of carrion ecology and forensic entomology, Professor Jerry A. Payne, was the first to use pig carcasses in his innovative experiments on decomposition [34,62,63], including burial scenarios [64]. These experiments were followed by other forensic entomological studies that increasingly included soil characteristics among the observed data, emphasizing their importance and influence on the ecology of the environment and the body. Turner et al. [65] presented an experiment focused on solving an inconsistency that resulted from a real case. In a murder investigation with a buried victim partially exposed by scavengers, the PMI estimated from entomological analysis did not correspond to the one determined with other techniques. The experiment, aimed to reproduce the conditions of the recovery of the victim, consisted of the realization of three shallow graves, each containing a pig cadaver, in the same site characterized by heavy clay soils and in the same period of the year. The analysis showed that the cadavers remained in a relatively fresh state for three whole months before beginning to show signs of decay. This should be due to the particular type of soil, being able to maintain a low temperature inside the grave for a certain amount of time and creating an anoxic environment. These conditions strongly influenced the decomposition rate of the cadaver, attracting scavengers only after several months and, thus, delaying the exposure of the pigs to Diptera and other insects. As a result, the species of flies that are typically recognized as some of the earliest to find freshly dead individuals and, therefore, used to determine the PMI were detected after a much longer period of time. Clearly, the combination of cold weather and specific soil conditions effectively inhibited the decomposition process and the associated odor production so that the carcasses remained undetected by scavengers, and consequently by arthropods, for a very long time, despite the shallowness of the burial. Regardless of the excellent insight provided by the study, it fails in some of the methods chosen to monitor the decomposition state of the carcasses. During the time of the experimentation, the authors performed partial exhumations of the cadavers by digging some holes down to the cadavers and exposing them for up to 30 min, then replacing and treading down the grave soil. This operation, however brief and necessary for the experiment, could alter the burial environment, causing some disturbance in the data. A solution could be including more burials in the experimental setting and monitoring and abandoning every grave at every sampling.

More recently, another paper has been published by Rai et al. [66] looking at the impact a buried decomposing carcass can have on the soil mesofauna and how this can be used to estimate PMI when access to the carcass is difficult for insects. The study showed how the decomposition of three pig carcasses buried in shallow graves caused a significant increase in the initially neutral soil pH during decomposition. This condition attracted an abundance of mites (Acari), which rapidly became more abundant in grave soils than in control soils after the fresh stage of decomposition. Therefore, increasing soil pH had a positive effect on mite abundance in graves, whereas aboveground variations in temperature, relative humidity, and precipitation had no significant direct effect on mite abundance despite the graves being only 10–20 cm deep. The results allowed them to consider soil pH as the most important environmental factor affecting mite abundance in shallow graves.

3.5. Forensic Botany

The practice of including geosciences in research areas like the life sciences is also evident in work on forensic botany. One example is the study of Perrault and Forbes [16], who focused on elemental analysis of the soil and vegetation surrounding several pig carcasses placed on the ground surface for 96 days. The results were then confronted with the state of decomposition of the carcasses, allowing them to provide a general guideline for a complete estimation of the PMI. The study reported a significant increase in soil percent water holding capacity (%WCH), pH, soil-available phosphorus, and sodium over three months within a 20-cm radius and occasionally at 50 cm. Conversely, potassium, calcium,

and magnesium concentrations remained unaltered throughout the study. The findings showed how anomalies related to the abundance of nutrients are exclusively located inside the CDI perimeter. However, such an input into the environment results in a surge of nutrients into the surrounding soil, affecting local plant dynamics. Indeed, the study highlighted that phosphorus was detected concurrently between two trials conducted in different seasons, and sodium constituted an important source of nourishment, determining plant dynamics around carcasses and potentially contributing to the regrowth of *Agrostis gigantea*. In addition to showing that nutrient cycling is a large-scale process involving inputs from many reservoirs, the study demonstrated that small perturbations in the cycles can be introduced in the form of cadaveric leachate and that this feature could be used by forensic experts to detect, for instance, the original deposition site of a victim.

Similarly, Silván-Cárdenas et al. [67] proposed the combination of geosciences (especially geophysics) and botany as an effective and rapid tool for the detection of clandestine graves. Specifically, the study consisted of testing three optical remote sensing techniques: hyperspectral/multispectral imaging, UAV-based photogrammetry, and thermal imaging. The overall objective was to evaluate their effectiveness in detecting anomalies associated with burials and to understand if spectral indices could be used to detect vegetation anomalies related to cadaveric nitrogen enrichment. Indeed, nitrogen content in plants is one of the most studied botanical chemical characteristics because it is closely related to leaf chlorophyll content, which in turn has well-defined spectral characteristics. According to the authors, the relationship between nitrogen and chlorophyll concentrations can be observed as a positive correlation with leaf reflectance values between 430 and 960 nm, with variability depending on the plant species. Overall, the results showed that both hyperspectral and multispectral sensors have the potential to detect a burial site using some spectral indices that are sensitive to the nitrogen content of vegetation, but enough time must be allowed for the nutrients to be absorbed by plants. Therefore, the duration of the effects of graves on vegetation would require further investigation. Moreover, the authors found that thermal imagery was also a good method for detecting thermal anomalies caused by burials that, after decomposition of the body, may create the voids that can be detected by differential thermography, although it seemed to be effective only after vegetation had been mechanically removed. Finally, the use of the UAV photogrammetry technique efficiently allowed the reconstruction of the vegetation regrowth over the graves from oblique aerial photographs, but the subsidence of the terrain was not successfully detected since aerial photography is a passive sensor able to detect only the upper vegetation canopy and cannot penetrate the upper layer of the trees.

3.6. Microbiology

Concerning microbiology, Hopkins et al. [9] presented a paper in which the grave soil is considered an interface between soil microbiology and forensic science and, therefore, an essential tool for the investigation of clandestine burials. For the experiment, the authors observed some selected chemical and microbial characteristics of soils from the graves of three pigs 430 days after burial. The results showed that grave soils contained more total carbon, microbial biomass carbon, and total nitrogen than control soils and showed increased rates of respiration and nitrogen mineralization. In addition, soil pH increased significantly as a result of the abundant supply of ammonium (NH_4^+) from the decomposing pigs. Nevertheless, the naturally poorly draining, acidic soil created an environment unfavorable for nitrification, which was not detected in any of the soils, and a consequent restriction in the supply of nitrates (NO_3^-) from nitrification. As a result, these conditions led to a general restriction in soil microbial activity despite the fact that the addition of a large amount of nutrients to the grave soil due to cadaveric decomposition led to an increase in soil biomass, as expected. Nevertheless, the low bacterial activity at least partially explained the good preservation of the body at the time of the exhumation. The study, conducted in 2000, provided a snapshot of the dynamics of the interaction between the soil microbial community and a decomposing body in a clandestine grave scenario at a

time when very little was known about the microbiology of graves. Moreover, because the analyses were performed at the end of another experiment, there are no data on the evidence of decomposition processes in the grave soil during the 430 days prior to sample collection, even though the chemical soil analysis indicated that decomposition was still occurring. However, in the absence of other soil microbiological studies of graves, the authors have produced potentially useful preliminary information of interest to archaeologists, forensic scientists, and those involved in clandestine grave investigations.

3.7. Proposing Applications to Forensic Casework

Overall, all these studies belonging to different disciplines inherent in forensic science have emphasized how hazardous it could be to exclude geoenvironmental data among the evidence needed for a correct reconstruction of the events characterizing a criminal act, such as a victim concealment in an outdoor area (Figure 1). In particular, with respect to the study of clandestine burials, it was pointed out that there is a strong need to recreate simulations capable of providing biogeochemical data at different time intervals so that evidence related to the state of decomposition of cadavers, the presence of insects at certain stages of their growth, and vegetation anomalies can be properly interpreted. This section of the present review focuses on soil chemistry, showing that there is little research in this area at a short PMI of less than 60 days. This can be a major limitation since, at a stage when the cadaver is generally well preserved, investigators rely mainly on thanatological parameters for estimating the time of death. The condition of the burial can greatly delay the rate of decomposition, leading to misinterpretation of the data and serious errors later in the consultation. Consequently, a thorough understanding of the biogeochemical dynamics affecting the hypogeal environment is indispensable in the study of clandestine burials, and the only way to achieve this is to conduct a long-term experimental study based on the combination of geosciences, forensic anthropology, forensic medicine, and the life sciences in general, focusing also on the early post-mortem period and including as many variables as possible in its setting.

On the other hand, it would be possible to make a point of reflecting on the application of the aforementioned geoscientific disciplines to forensic casework. Indeed, the use of specific techniques by the works considered in this review is emblematic of how some analyses are chosen more frequently than others (Figure 1). Referring, for example, to the use of geophysical survey techniques for the detection of clandestine burials, it became evident that ER is much more efficient in detecting burials containing a corpse in an advanced state of decomposition than GPR. The latter, on the other hand, is capable of detecting burial-related anomalies in their early stages or when the material associated with the victim does not allow for the dispersal of putrefaction products into the soil. Both, however, perform better in the search for deep burials rather than shallow ones. All of these limitations and potentials of geophysical techniques should be kept in mind by investigators and police officers from the earliest stages of surveying or searching a potential crime scene. Another example, also highlighted in Figure 1, concerns the approach to soil science in forensic contexts. Firstly, the investigation of pH in environments concerned with or suggestive of corpse concealment could be a potential screening tool for confirming or not confirming such suspicion. Indeed, as evidenced by the reviewed publications here, it is a method that is widely used (likely because it is relatively inexpensive and quick to perform) and successfully reflects any anomalies in the chemical balance of the matrix, always having the background soil as a reference. Therefore, pH can be considered a reliable proxy for identifying specific areas where subsequent and more targeted analyses can be applied with satisfactory results and minimizing the expenditure of time and resources. In this way, localized analyses of soil biogeochemistry, such as the search for characteristic organic compounds or specific ions traceable to decomposition dynamics, can be applied. Such a protocol, although generic, would contribute to a comprehensive answer to questions specific to the actual casework, such as the estimation of post-burial interval or the localization of previous abandoned clandestine burials. In contrast, the present

review has shown that analyses of total carbon in grave soils are, in fact, inconclusive. Indeed, although one would intuitively expect an anomaly in the carbon concentration due to the input from the decomposing body, the highlights of the studies considered do not show significant post-burial fluctuations. Consequently, the application of this method of biogeochemical analysis to forensic cases may not be particularly indicated, except in special cases where this analysis is explicitly requested (i.e., crimes against the environment). Finally, the review showed that an understanding of the intrinsic dynamics of the soil at the site of investigation is key to interpreting evidence related to decomposition processes in the individual case. It follows that the chemical and physical characteristics of the soil should be included in a hypothetical protocol for the death investigation of victims concealed by burial or exposed to the natural environment. However, collaborators of the judiciary and prosecutors are not always fully aware of these dynamics and would, therefore, not consider involving soil specialists at the scene. In fact, the specialist is often involved in the later stages of the investigation, perhaps when the samples have already been taken by third parties, and thus without direct access to the scene as it was presented during the inspection. As a result, the interpretation of the results may be distorted or difficult to interpret, thus compromising the efficiency of the inquiry. Undoubtedly, such a scenario represents one of the main obstacles to an agile transition of forensic taphonomic knowledge acquired in the experimental setting to actual investigations.

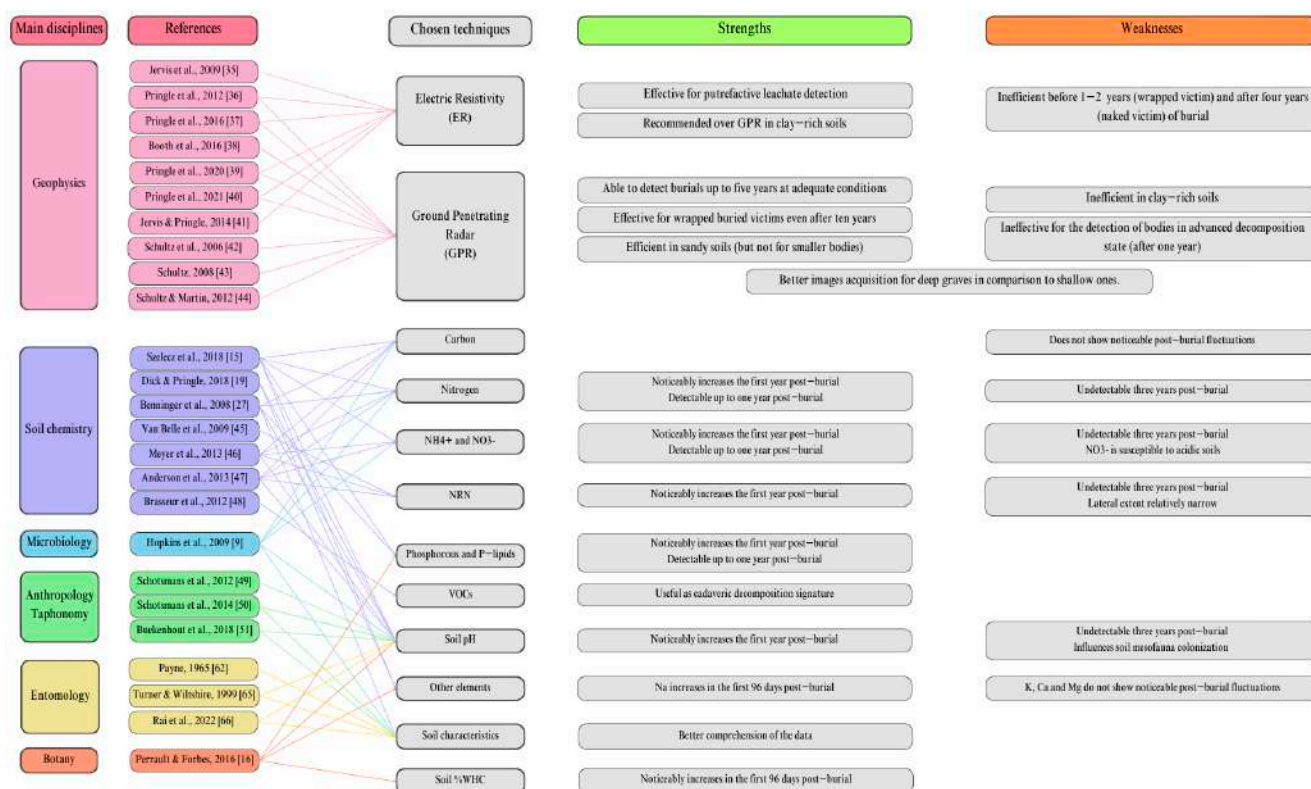


Figure 1. Resuming image of the geoscience techniques applied in the papers presented. The figure also shows their main strengths and weaknesses [5,9,15,16,19,27,35–51,62,65,66].

4. The Italian Non-Human Forensic Taphonomy Facility: Ticino-LEAFs

Since 1981, when Professor William Bass established the first Human Forensic Taphonomy Facility at the University of Tennessee in Knoxville [73], the study of forensic taphonomy and human decay in outdoor controlled environments started to spread in the specialized scientific community. However, before that time and thus far, such studies were conducted with the employment of non-human animals as models for decomposition processes [28], especially pigs. Moreover, some of the currently operating facilities (also colloquially named “body farms”) are carrying out projects focused on the monitoring of

decomposition processes in both species, as shown in Table 2 and Figure 2. The human and non-human FTFs worldwide provide an invaluable opportunity to apply interdisciplinary methods in the context of forensic experimentation to identify, in a controlled environment, mechanisms capable of influencing decomposition processes. The results derived from these frameworks allow the formulation of interpretation techniques that can be applied to real forensic cases to obtain information such as PMI estimation, victim or perpetrator identification, reconstruction of events as they occurred, and many others.

Table 2. List of Forensic Taphonomy Facilities in the world, with a focus on the species of the specimens.

Name of the Facility	Institution	Country	Year of Foundation	Human	Non-Human	Reference
FAC (Forensic Anthropology Center)/ARF (Anthropology Research Facility)	University of Tennessee	Tennessee (USA)	1981	X		[74]
FORest (Forensic Osteology Research Station)	Western Carolina University	North Carolina (USA)	2006	X	X	[75]
FARF (Forensic Anthropology Research Facility)	Forensic Anthropology Center (Texas State University)	Texas (USA)	2008	X		[76]
STAFS (Southeast Texas Applied Forensic Science facility)	Sam Houston State University	Texas (USA)	2009	X		[77]
TRACES (Taphonomic Research in Anthropology Centre of Experimental Studies)	University of Central Lancashire	UK	2009		X	[78]
Taphos-m Facility	Universitat Autònoma de Barcelona	Spain	2011		X	[30]
CFAR (Complex for Forensic Anthropology Research)	Southern Illinois University	Illinois (USA)	2012	X	X	[26,77]
FIRS (Forensic Investigative Research Station)	Colorado Mesa University	Colorado (USA)	2013	X	X	[30]
AFTER (Australian Facility for Taphonomic Experimental Research)	University of Technology, Sydney (UTS)	Australia	2016	X	X	[28]
ARISTA (Amsterdam Research Initiative for Sub-surface Taphonomy and Anthropology)	Amsterdam's Academic Medical Center	The Netherlands	2017	X		[79]
FIRST (Florida Forensic Institute for Research, Security, and Tactical Training)	University of South Florida and Pasco Sheriff's Office	Florida (USA)	2017	X		[26]
Buckingham Environmental Forensics Facility	Florida Gulf Coast University	Florida (USA)	2018	X		[80]
FROST (Forensic Research Outdoor Station)	Northern Michigan University	Michigan (USA)	2018	X		[26]
REST[ES] (Recherche en Sciences Thanatologiques [Expérimentales et Sociales])	University of Québec Trois Rivières	Canada	2020	X		[80]
Murdoch University Body Farm	Murdoch University	Australia	2023		X	[81]

With this intention, in 2009, thanks to a collaboration between the Ticino Park Authority and the Institutes of Forensic Medicine and Veterinary Medicine of the University of Milan, the first phase of Ticino-LEAFs (Ticino Park-LABANOF Facility for Environmental Analysis in Forensic Sciences) was inaugurated. This non-human forensic taphonomy facility, located in the Lombardy Park of the Ticino, is made up of different outdoor areas where simulations of body concealment are organized using specimens of domestic pigs (*Sus scrofa domesticus* L.).

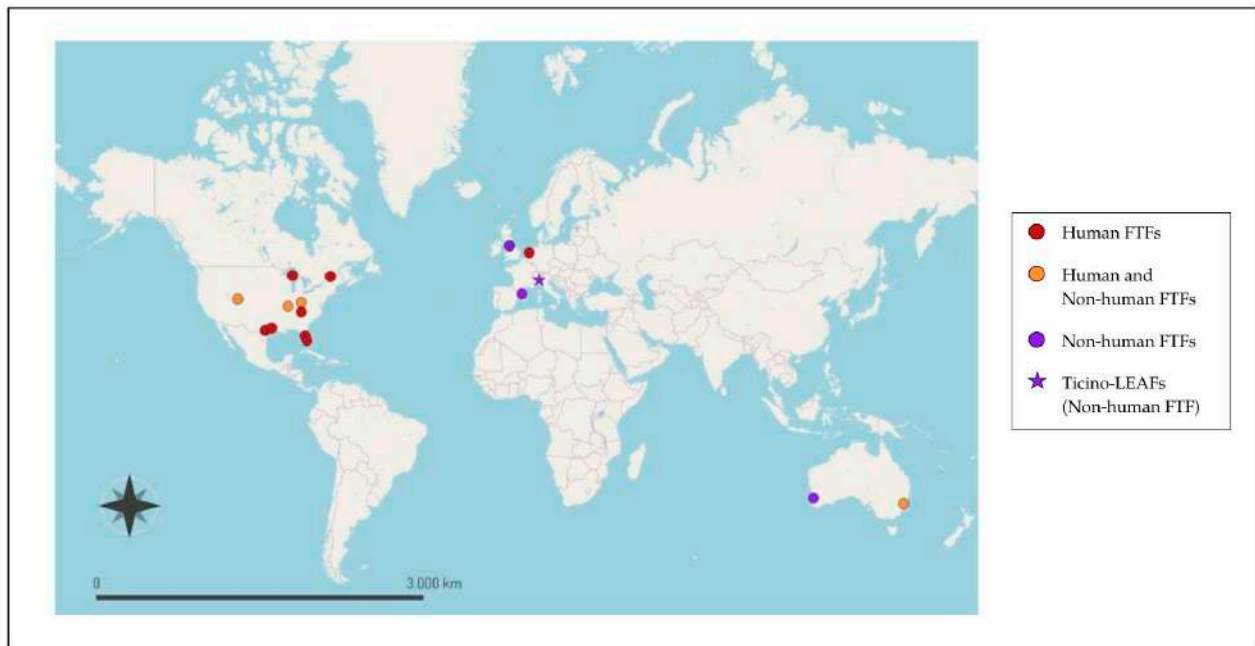


Figure 2. Map showing the distribution of the Forensic Taphonomy Facilities in the world, including the Italian Facility Ticino-LEAFs (OpenStreetMap scale 1:175,000,000 modified with QGIS version 3.34.12).

4.1. The Projects at Ticino-LEAFs

The first research project conducted at Ticino-LEAFs in 2009 was focused on the investigations of clandestine graves in natural areas [82–84]. Especially, the ten pigs used in this experimental plan were provided by the Veterinary Medicine Institute of the University, as they had previously been part of a study project on artificial heart valves [83]. Therefore, the pigs died from causes independent of the work. The specimens were buried in two different environments and exhumed at predetermined PMI, namely after 7 weeks, 29 weeks, 56 weeks, 102 weeks, and 130 weeks. The research activities first included a framework of the experimental area from a geographical, geological, geopedological, botanical, zoological, and climatic point of view. An exhaustive understanding of the characteristics of the field was, in fact, of fundamental importance for the correct interpretation of the data collected during the two-and-a-half years of the project. The investigative analyses of the burials included geophysical techniques (in particular GPR), forensic archaeological excavation activities, geopedological analysis, soil chemistry and forensic botany studies, and medico-legal, taphonomic, and microtaphonomic observations.

The remarkable innovation of this structure lies in its interdisciplinarity [21,69,82,85–88]. The choice to involve experts from many different disciplines, first of all, natural scientists, who are able not only to interpret the environment but also to quickly and efficiently recover the documentation to complete the classification of the areas, could make it easier for anthropologists and pathologists to interpret the taphonomic signs on the cadavers. Furthermore, the familiarity with the sources, which is a canonical part of a natural scientist's training, allows one to easily determine the temperatures and climatic conditions of the period of interest, the dominant plant species, the wildlife that is a potential scavenger, the type of soil, and the biogeochemical characteristics that can influence the rate of decomposition. In short, the natural scientist is accustomed to thinking of the post-depositional environment not as a passive receptacle but as an agent that actively interacts and captures or transforms the evidence and provides proxies. This fact is often overlooked both in the design of a field experiment and in the choice of experts to be involved in the investigation of real cases, where the observation of the evidence is often exclusively left to the pathologist. Therefore, not only has the project underlined how the collaboration between specialists of different areas (first that between natural scientists, archaeologists, and pathologists/anthropologists)

is an essential prerogative for the investigation of clandestine burials, but it has also allowed us to explore different areas of research using a limited number of animal samples, limiting their sacrifice.

Later, from 2019 to 2021 (organized in two series due to the COVID-19 pandemic), another project started at Ticino-LEAFs with simulations of cadaver surface deposition in two different natural areas [89]. In this case, the samples consisted of 16 piglets not older than four months, dead for natural causes, therefore not sacrificed nor previously selected for the study. The analysis was extended to biothanatological observations, including the interaction between the environment and the body, along with its substrates (such as skin and hair) and the biological traces that may be present on them [90]. Throughout the experiment, the cadavers were observed, and specific samples were taken from each, with greater frequency in the first few days after placement, when entomofaunal intervention and changes at the body level were most evident, and progressively less frequency as the time interval progressed, until monthly. Sampling covered an interval of eight to nine months for each of the two series and was carried out in all four seasons. For the first time in the facility, scientists started to consider the body as a microenvironment itself, able to interact with the surrounding environment, intended, in the strictest sense of the word, as an evidence provider and alteration agent [89].

Finally, in 2023, another project started, which included four different groups of six simulated clandestine graves each. Here, too, samples were taken from piglets not older than four months, not yet weaned, and weighing between 3 and 5 kg. The distribution of the samples into the four groups was based on the homogeneity of the sample in terms of body mass. Specifically, the four groups consisted of one set of burials with naked piglets, one with piglets dressed in cotton shirts, one with piglets covered with quicklime, and one with naked piglets that had suffered post-mortem injuries. The latter consisted of two gunshot wounds to the head, two stab wounds to the thoracic-abdominal area, and two blunt force wounds to the lower limbs for each piglet of the group. The exhumations of one piglet per group were performed circa 20, 50, 100, 200, 400, and 800 days after burial. During the exhumations, soil samples were taken for soil chemical analysis, and the recovered bodies were subjected to autopsy, osteological, and microtaphonomic analysis, following in the footsteps of previous projects at Ticino-LEAFs. Indeed, the main goal of the project is to study the interaction between the remains and the post-depositional environment, as it may alter or obscure features necessary for effective analysis of post-mortem interval (PMI), trauma, pathology, and biological profile, thus affecting analytical conclusions and the quality of information obtained. Specifically, the design of the experiment, which is still in progress, considers as the main substrate the hypogeal cadaveric decomposition island (CDI), linking the groundbreaking results of the two previous projects, which focused mainly on the hypogeal environment, the former, and the cadaver as environment, the latter. It should be noted, however, that neither of the two previous works excluded their counterparts and once again succeeded in emphasizing the importance of a practical and intellectual attitude that welcomes interdisciplinarity.

4.2. The Use of Pigs (And Piglets) as Human Analogs

The Ticino-LEAFs opted for the use of non-human animals, namely pigs and piglets, as human proxies. As reported by Matuszewski et al. [34], in the late 1800s, most studies of vertebrate decomposition used non-human cadavers, later focusing on pigs [62–64]. The choice of swine specimens as surrogates for humans in forensic experimentation is primarily justified by the biological and anatomical similarities between the two species, especially those endogenous features that directly influence the decomposition pattern [33]. For example, the early stages of decomposition are characterized by tissue autolysis, for which the bacteria that make up the intestinal flora are partly responsible. In this respect, pigs and humans share the status of monogastric omnivores, with well-defined small and large intestines, in addition to being omnivorous [33]. However, it should be noted that there is a difference in intestinal length between the two species, with that of pigs being

on average more than three times longer than that of humans, and a different microbial intestinal community [30], despite the greenish discoloration of the skin of the anterior abdominal wall being the first sign of putrefaction in both. Another characteristic that has strong consequences for the rate of decomposition is the presence of adipose tissue. Again, the two species are similar, especially regarding the percentage of body fat (for both, on average, around 20%) and the distribution of subcutaneous and visceral fat [91,92]. Of course, even in this case, there are differences; humans and pigs share the same groups of fats introduced through the diet but with different proportions [33]. Finally, since outdoor Forensic Taphonomy Facilities base part of their observations on the interaction between the decomposing body and the surrounding environment, the external characteristics of the sample must also be evaluated. Pigs, in terms of quantity and distribution of skin appendages, are undoubtedly the mammal species that most closely resemble humans, so much so that pig skin is normally used for transplants [93,94].

On this basis, Beary and Lyman [95] and Matuszewski et al. [34] highlighted how the inclusion of non-human animals in experimental taphonomy research provided a more experimental attitude for forensic taphonomy and laid the foundation for the understanding of some major decomposition processes. Especially with the beginning of the outdoor human FTFs, a comparison of the trends of decomposition in human and non-human experiments was finally possible, and since then, a debate has arisen both about the reliability of the use of animals for taphonomy studies and the need for a universal experimental and ethical protocol [34]. About the first point, some studies criticize the use of pigs (or other non-human species) in forensic experimentation. For instance, works such as those by Connor et al. [30] and Dautartas et al. [23] consider animal models incapable of reflecting human decomposition patterns based on the results of their field research. Nevertheless, the authors' interpretation of the data can be questioned since the two groups of specimens not only differed in the species but also in the sex, the body mass, and the surface-to-volume ratio, providing some confounded variables [34], as defined by Quinn and Keough [96]. Moreover, according to Matuszewski et al. [34], the difficulties associated with experimenting with human cadavers can lead to results that are far more misleading and difficult to apply in real-field scenarios. Indeed, the authors highlight how the source of the population contributing to HFTFs worldwide is often complex and difficult to predict, being that human donations usually vary and often consist of cadavers that deeply differ from the individuals generally involved in forensic scenarios for age and cause of death. This bias has consequences for the lack of control over the experimental setting and repeatability, making the findings obtained from human-based experiments difficult to extrapolate to forensic cases. In fact, the use of an inappropriate population makes it impossible to compare the results with a control sample and even to structure a defensible experiment because of too many variables. On the contrary, experiments with *Sus scrofa domesticus* L. cadavers can be replicated repeatedly, even in large numbers, with relatively few resources. Indeed, according to Matuszewski et al. [34], an extended common choice inherent in the use of pigs in experiments could also be advisable because there is a huge variation between different species of animals, which can lead to misinterpretation of results, especially if the setting of an experiment is not sufficiently robust. On the other hand, intraspecific variability, for example, related to the mass of samples of different ages, can be an advantage, allowing the sample to be modulated according to the specific question. In fact, the only way to meet the requirements of the current project at Ticino-LEAFs, which is to replicate a large amount of data over an extended period of time, is to use piglets, which are easier to handle and available in large quantities. Paradoxically, current knowledge of human decomposition is derived from experiments conducted on non-human animals, primarily pigs and piglets. The widespread use of this species is also due to the fact that many of the variables that constrain an eventual forensic scenario are instead known, such as the characteristics of the specimen when it was alive, the time of death, the possible presence of disease or use of medical therapies, post-mortem treatments, and many others. In addition, it is possible to have access to a large number

of subjects who are likely to be homogeneous in body mass and age [33]. Moreover, there are also some studies that specifically validated the choice of pigs as human analogs for taphonomy studies, focusing on the interaction between remains and the post-depositional environment [30]. For instance, if we focus on forensic entomology, Rodriguez and Bass [97] compared insect succession data collected by Reed [98] from non-human animal cadavers to that observed in humans, finding significant overlap. In addition, some carrion ecology experimental works (namely the one of Schoenly et al. [99]) showed that the majority of forensically important species of insects colonize human and pig remains similarly, with no significant differences found until after skeletonization occurred, validating the choice of pigs as human analogs.

Thus, it is clear that pigs are not a perfect proxy for understanding the taphonomic dynamics of a decomposing human cadaver in the open environment, but they do have a fair amount of availability and intraspecific variability on their side, which is less limiting for experimentation than donated human cadavers. The latter clearly have specific characteristics similar to those of real victims in investigative cases, but the data collected are then difficult to apply because of all the difficulties inherent in the donation system. Moreover, it must be considered that in several countries, like Italy, the current legislation does not allow involving human cadavers in experimental taphonomy research, making the use of animals as proxies the only way to conduct these kinds of projects. Indeed, according to Forbes [28], although the use of human subjects in HTFTs is still preferred in order to reproduce as reliably as possible the scenarios that may be investigated, human variability actually plays a lesser role in this type of experiment.

However, it must be remembered that there is a universal limitation that accumulates in all experiments conducted in the field, whether they involve human or non-human cadavers: there will always be a certain margin of error that must necessarily be quantified so that it can be efficiently presented at trial and meet the requirements imposed by the Daubert standards [100]. However, according to Matuszewski et al. [34], *“the possibility that a model animal and the humans that it models decompose differently does not make that model useless; it depends on the specific question being addressed”*. The authors also stated how recent work, on the one hand, highlighted the possibility of consolidating the insights from animal models in taphonomy studies and, on the other hand, suggested deepening the implications of what has been learned from that to design progressively better and more accurate field experiments.

4.3. Forensic Taphonomy Experiments: A Realistic and Interdisciplinary Approach

A robust and accurate field project should aim to keep as many variables under control as possible but, at the same time, to fully reflect the forensic case history so that they can provide guidelines that are useful in investigative practice. For this reason, it is necessary for forensic research to respect what Miles et al. [33] call “forensic realism”. In particular, the authors focus on certain aspects underlying the design of a forensic taphonomy experiment: the voluntary exclusion of scavengers, the use of clothing, and the number of specimens to be included. With regard to restricting access to exposed bodies, this is generally accomplished by using protective cages with wide enough mesh to allow weather and insects to interact with them instead. According to Miles et al. [33], although the exclusion of scavenging is the simplest way to control for this variable, the results of studies using this strategy should be taken with caution, especially with regard to the applicability of any guidelines to real cases, which can only be considered in cases where there is no doubt that the victim of the case under study was not scavenged. Such an assessment is often difficult to make since traces left by small animals on bones are often difficult to detect, if not impossible, as in the case of other taphonomic factors that may have left traces that may obscure them. In addition, it is possible to find skeletal remains that show no signs of scavenging, but it cannot be ruled out that there was activity in the now decomposed or poorly preserved soft tissues at the time of recovery. In all likelihood,

it is advisable to allow the scavengers access to the corpses, as this is the safest way to get relevant data [101,102] that is applicable to real cases.

This is what was done at Ticino-LEAFs in the latest experimental project. In this case, the pigs were buried, which in itself constitutes a natural obstacle to the intervention of surface wildlife. However, during the first months of the experiment, the particular characteristics of the soil (already considered in the design phase) and the intense rainfall caused the filling of one of the burials to collapse, allowing volatile organic compounds to emerge and attracting scavengers. As a result, the grave was dug by the animal, and the body was removed in the period between the first and the subsequent exhumation. This could be interpreted as a path error in the context of the research, and in a certain sense, it is, but on the other hand, it also represented a great opportunity for further investigation, allowing the research group to monitor the concentration of certain nutrients at the bottom of the burial (which was left open following the discovery) as the PMI advanced. The fact that the removal of the body was done through scavenging made the results of the analysis even more plausible and, consequently, applicable in a dynamically similar real case of death and concealment investigation.

The second question reported by Miles et al. [33] concerns the use of clothing, which is known to be able to influence the rate of decomposition of the corpse differently depending on the material and the environment [103]. Despite this, there remains a lack of understanding about how, on the contrary, the products of decomposition alter the clothing and other textile materials associated with the corpse, even if the interest in this topic is rapidly spreading [104–107]. This is a remarkably interesting perspective aimed at considering the opportunity to use this type of evidence as a tool for the estimation of the PMI or in the evaluation of contextual taphonomy. In fact, this intuition was also applied in the case of Ticino-LEAFs, in which the simulation and the analyses were set up in such a way as to consider the clothes worn by the buried pigs not as a barrier for the exchange of material between the body and the hypogeal environment but as a further substrate to be considered in the evaluation of the evidence of concealment.

Finally, Miles et al. [33] addressed the issue of the number of carcasses to include in a taphonomy experiment. In this case, the authors focused on simulations of bodies exposed on the surface at the mercy of scavengers and insects, emphasizing that it is advisable to consider a single corpse to obtain results applicable in real contexts. However, this can constitute a huge limitation in the design of an experiment, especially if one wishes to evaluate the evolution of the evidence manifestation over time, as in the last Ticino-LEAFs project case. Renouncing the involvement of multiple specimens would imply repeated manipulation of the same specimen, disturbing the ecology of the environment and the carrion, thus creating a bias that would make the observations inapplicable.

In general, the reduction of artifacts to a minimum is one of the cornerstones of the experimentation in progress at Ticino-LEAFs, and it is considered, in this context, a possible further piece of forensic realism to be added to the ones presented by Miles et al. [33]. For example, the graves were dug in an inhospitable and irregular undergrowth, which was deemed suitable for imitating the environments typically considered for the construction of one or more clandestine burials [108]. The perimeter of the graves was not outlined or marked, and consequently, after only a few months, they were difficult to locate without applying forensic archaeology research techniques. Since the areas used for the experimentation are known, geophysical investigation methods were not used below the surface, while the operations typical of forensic archaeology were carried out aimed at identifying the perimeter of the burials under the layer deposited after their realization [109]. Furthermore, the limitation of the artifacts also allowed the environment surrounding the study area to interact with the burials, even regarding extraordinary atmospheric events. In fact, during the experiment, there was an intense rainstorm with extraordinarily strong winds that caused the outrooting of some trees (especially *Quercus robur* and *Robinia pseudoacacia*), including one with a root system that had damaged one of the walls of a burial. The result was a dragging of the wall with a dislocation of a few

centimeters of the body, which, however, was not exposed. Once again, the environmental dynamics made it possible to observe the effect of phenomena capable of altering and upsetting an experimental setting that can also be reflected in real cases. Firstly, carrying out the excavation and recovery of the remains in such a particular situation allowed us to observe the anomalous behavior of the stratigraphy, providing tools to be able to interpret similar evidence in investigations of death and concealment. Secondly, by tracing the meteorological data, it was also possible to evaluate the evolution of the underground environment after the strong disturbance and, therefore, formulate observations regarding the state of conservation of the cadaver.

However, although forensic realism could be considered one of the strengths of the projects at Ticino-LEAFs, it shares some limitations with other projects presented in this review. One above all is the type of soil that characterizes the study area. The soil, due to its intrinsic variability, is extremely peculiar to a specific area, and consequently, the results obtained can find applicability exclusively in areas with similar geoenvironmental characteristics [21]. However, such specificity can be considered an advantage. Indeed, the aim of these facilities is not to provide universal tools that adapt to numerous situations but to propose increasingly efficient guidelines to be applied in the investigative practice in the territories of competence. Furthermore, a future perspective that we hope for is to create similar projects with a standardized investigation protocol in different biogeographical contexts to facilitate the production of globally applicable data. Moreover, a further limitation may be that of time, inevitably constrained by the resources provided by the institutions and by bureaucratic implications. However, it is important to underline how building a project with solid foundations is essential to renew and perpetuate the frequentation of an experimental area, as in the case of the research conducted at Keele University [35–40], and thus evaluating the long-term taphonomic alterations, always considering the risk of contamination due to previous experiments [22]. As regards Ticino-LEAFs, it was possible to observe how the in-depth knowledge of the area, its ecological characteristics, and its limiting factors made it possible to carry out continuous activities for 15 years, allowing the errors and obstacles encountered in the previous phases to contribute to building the foundations of the subsequent ones.

4.4. The Ethics Behind the Ticino-LEAFs Projects

The decision to use non-human animals as human models does not exempt those involved in research from the need for ethical evaluation. Indeed, the use of animals as surrogates for humans in forensic research is a legacy of clinical and biomedical studies. In fact, it is common practice in this context to rely on animal models (mainly mammals) and later conduct experiments on humans, adhering to the safety and ethical dogmas imposed by the scientific community. Of course, animal experimentation, although fundamental in the clinical setting, is also strictly regulated and cannot be performed in the presence of other methods. In 1992, Professor Knight [110] affirmed that in the context of clinical medicine, animal experimentation should be conducted in accordance with a cost (to be intended as “harm”)–benefit evaluation carried out by a highly rigorous ethics committee, which is conducted prior to the allocation of any funding. Naturally, the objective of such research is to obtain direct benefits, whether in the diagnostic or therapeutic field. Professor Knight then posed the question of how forensic research should be conceptualized and why animal experimentation in this field should be subject to a different regulatory framework. Indeed, since the discipline was created to support operations for the purpose of justice and not for questions of therapeutic benefit, the sacrifice of non-human animals for these applications should follow an even more rigorous procedure, especially when the potential benefits in the medico-legal sphere are only speculative.

Moreover, as Cattaneo et al. [111] stressed, even including practices that anesthetize the animal before killing, their sacrifice for forensic research is consciously difficult to justify. In fact, the authors point out that the protection of animals is one of the moral achievements of the civilized world and that the forensic scientist, who is likely to have been trained in

the natural, life, and biomedical sciences, should have among his or her automations that of protecting other species, criticizing their use as proxies, especially when the research does not have saving lives, human or non-human, as one of its objectives.

Currently, the fact that animal tissue is often easier to obtain and that there are perceived fewer ethical issues associated with its use may increase the number of animal studies performed. However, another issue that must be considered is that animals, who should not be treated as mere resources but as beings with moral status, cannot give their consent, so it is up to animal care and ethics committees to decide whether or not a particular animal study is justified [112]. The respect and care given to animals used in research should be comparable, if not equal, to that given to human tissue donors since the value derived from animal experimentation can affect the lives of both humans and animals, even in forensics. Moreover, as stressed by Mole and Heyns [112], tissue, regardless of its origin, is a scarce resource and should, therefore, be used with appreciation and understanding of the sacrifice and suffering involved in obtaining it.

Therefore, one solution that allows the use of animals as models in forensic scientific experiments while maintaining a high ethical standard is to use specimens that have already died of natural or unrelated causes. Mole and Heyns themselves affirm that the reuse of animals can save costs (intended as animal sacrifice) and limit the number of animals used in research, provided that the previous research and method of euthanasia will not cause unknown interactions in the newly proposed study [112]. This is particularly true in the study areas of decomposition, taphonomy, post-mortem interval, and body detection, which typically use animals that have already died or part of animals already used for other forensic studies [113–115].

All the projects conducted at Ticino-LEAFs used samples from adult pigs that had already died in clinical trials (the first) or from piglets that had died of natural causes (the others). One criticism that could be made, especially in the case of piglets, is that the sample could be inhomogeneous because the specimens could have died at random sizes. This is not really the case since these are animals that died under the same conditions (during lactation), and their masses are, therefore, within a range that still makes them sufficiently representative, while the extreme values have been excluded from the experiment and involved in other projects. However, it is always assumed that this is a different species from humans, and therefore, any findings from the study must be contextualized and understood within a trend and not as an absolute value. What we are focusing on are the biogeochemical anomalies related to the extraordinary input of nutrients that the underground environment receives. This should be interpreted as part of an evolution pattern that reflects the succession of decomposition states that any mammal exposed to the same environment passes through. The slight difference in mass between the piglets is, therefore, negligible in the context of such a research project. Another criticism that could be leveled at the choice of using already deceased specimens is the infliction of post-mortem lesions. In fact, these would have radically different histopathologic characteristics than those inflicted peri-mortem, making their experimental observation completely useless for research purposes.

The project currently ongoing at Ticino-LEAFs does not provide for any type of histological or histochemical examination of the lesions, except for the search for residues of the weapon used on the ground adhering to the soft tissues. Consequently, the fact that the tissues were no longer vital at the time of the infliction of the lesion does not create any type of bias. Furthermore, it is necessary to remember, as Professor Knight [110] himself states, that violent and painful practices on conscious animals, often in an attempt to date the lesions, can have only a tenuous potential benefit to any medico-legal problem. It would, therefore, be gratuitous cruelty that brings no benefit to forensic practice.

5. Conclusions

The incorporation of geosciences into forensic taphonomy has allowed a radically deeper understanding of many of the dynamics that influence the decomposition processes

of a body. In particular, it made it possible to identify and interpret evidence of interaction between the cadaver and the post-depositional environment when the former is concealed or found in a natural outdoor area. Much of the knowledge we have today about the dynamics of decomposition in open environments is due to forensic taphonomy experiments conducted using pigs as human models within simulations of body concealment. Surprisingly, not only the disciplines belonging to the earth and soil sciences have based their studies on these typical techniques, but also work from the life sciences applied to forensics, such as entomology and botany, has included the study of soil characteristics in their research. In fact, it has been shown that this approach is fundamental to the correct interpretation of the data. However, many of the experimental designs presented in this review have shortcomings, as they often neglect to consider the environment as a conditioning agent of decomposition, thereby increasing the risk of bias in the interpretation of the findings. These considerations are particularly apparent when one considers the ultimate objective of forensic taphonomy, which is to comprehend the mechanisms that regulate the decomposition of the human body and their interaction with the environment, with a view to developing an overarching approach for forensic casework. Indeed, in complex scenarios of cadaver analysis, such as in the case of a body found in an advanced state of decomposition in an open environment, a range of disciplines parallel to forensic medicine become unavoidable (these include anthropology, archaeology, entomology, botany, soil chemistry, microtrace analysis, and others). This is particularly the case when the number of variables to be considered is substantial, and it is, therefore, essential to have experts from different backgrounds who are able to recognize and interpret them.

However, the present review brought to light how these investigative strategies are applied in the context of experiments with buried or exposed pigs, which replicate simulations of body concealment in a natural environment, with their strengths and limitations. As previously noted, the research activities themselves face the challenge of coordinating the various disciplines necessary for the proper interpretation of the data. It is, therefore, understandable that translating this approach into practice may be difficult.

Nevertheless, the Italian non-human forensic taphonomy facility Ticino-LEAFs (Ticino Park-LABANOF facility for Environmental Analysis in Forensic Sciences) inaugurated by the University of Milan in 2009, emphasizes the active interaction between the remains and the post-depositional environment, which can alter the evidence necessary for effective forensic analysis. In addition, the facility seeks to incorporate the use of non-human animals as human models while maintaining a high ethical standard and minimizing the construction of artifacts, thereby striving for forensic realism. Moreover, the latest project carried out at Ticino-LEAFs links the results of the previous projects and succeeds in emphasizing the importance of a practical and intellectual attitude that welcomes interdisciplinarity.

Author Contributions: Conceptualization, G.T., A.M. and L.T.; formal analysis, G.T., G.C. and P.P.; investigation, G.T., A.M., G.C., P.P., C.C. and L.T.; resources, C.C. and L.T.; writing—original draft preparation, G.T.; writing—review and editing, G.T., A.M., G.C., C.C. and L.T.; visualization, G.T., A.M. and L.T.; supervision, C.C. and L.T.; project administration, C.C. and L.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical approval received from the University of Milan Ethics Committee.

Data Availability Statement: The authors declare that all data supporting the findings of this study are available within the paper.

Acknowledgments: The authors would like to thank the Lombardy Ticino Park, Cassolnovo (PV), Italy.

Conflicts of Interest: We all declare that there are no conflicts of interest, and this article has neither been sent to nor published in any other journal.

References

1. Pringle, J.K.; Jeffery, A.J.; Ruffel, A.; Stimpson, I.G.; Pirrie, D.; Bergslien, E.; Madden, C.; Oliver, I.; Wisniewski, K.D.; Cassella, J.P.; et al. The use of portable XRF as a forensic geoscience non-destructive trace evidence tool for environmental and criminal investigations. *Forensic Sci. Int.* **2022**, *332*, 111175. [[CrossRef](#)] [[PubMed](#)]
2. Liu, Y.; Wei, W. Forensic geophysics: A new trend of research on applied geophysics. *Prog. Geophys.* **2023**, *38*, 1824–1841. [[CrossRef](#)]
3. Berezowski, V.; Mallett, X.; Simyrdanis, K.; Kowlessar, J.; Bailey, M.; Moffat, I. Ground Penetrating Radar and Electrical Resistivity Tomography Surveys with a Subsequent Intrusive Investigation in Search for the Missing Beaumont Children in Adelaide, South Australia. *Forensic Sci. Int.* **2024**, *357*, 111996. [[CrossRef](#)]
4. Ruffell, A.; Rocke, B.; Powell, N. Geoforensic Search to Crime Scene: Remote Sensing, Geophysics, and Dogs. *J. Forensic Sci.* **2023**, *68*, 1379–1385. [[CrossRef](#)] [[PubMed](#)]
5. Tibbett, M.; Carter, D.O. Research in Forensic Taphonomy: A Soil-Based Perspective. In *Criminal and Environmental Soil Forensics*; Springer Netherlands: Dordrecht, The Netherlands, 2009; pp. 317–331. [[CrossRef](#)]
6. Fitzpatrick, R.W. Soil: Forensic Analysis. In *Wiley Encyclopedia of Forensic Science*; John Wiley & Sons, Ltd.: West Sussex, UK, 2009. [[CrossRef](#)]
7. Fitzpatrick, R.W. Nature, Distribution, and Origin of Soil Materials. In *The Forensic Comparison of Soils in Soil Analysis in Forensic Taphonomy: Chemical and Biological Effects of Buried Human Remains*; Tibbett, M., Carter, D., Eds.; CRC Press: Boca Raton, FL, USA, 2008; Chapter 1.
8. Murray, R.C. Forensic Examination of Soils. In *Forensic Chemistry Handbook*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2012; Volume 1, pp. 109–130.
9. Hopkins, D.W.; Wiltshire, P.E.J.; Turner, B.D. Microbial characteristics of soils from graves: An investigation at the interface of soil microbiology and forensic science. *Appl. Soil Ecol.* **2000**, *14*, 283–288. [[CrossRef](#)]
10. Fiedler, S.; Schneckenberger, K.; Graw, M. Characterization of soils containing adipocere. *Arch. Env. Contam. Toxicol.* **2004**, *47*, 561–568. [[CrossRef](#)] [[PubMed](#)]
11. Procter, F.A.; Swindles, G.T.; Barlow, N.L.M. Examining the transfer of soils to clothing materials: Implications for forensic investigations. *Forensic Sci. Int.* **2019**, *305*, 110030. [[CrossRef](#)]
12. Chauhan, R.; Kumar, R.; Sharma, V. Soil forensics: A spectroscopic examination of trace evidence. *Microchem. J.* **2018**, *139*, 74–84. [[CrossRef](#)]
13. Dent, B.B.; Forbes, S.L.; Stuart, B.H. Review of human decomposition processes in soil. *Environ. Geol.* **2004**, *45*, 576–585. [[CrossRef](#)]
14. Ruffell, A.; Pringle, J.; Cassella, J.; Morgan, R.; Ferguson, M.; Heaton, V.; Hope, C.; McKinley, J. The use of geoscience methods for aquatic forensic searches. *Earth Sci. Rev.* **2017**, *171*, 323–337. [[CrossRef](#)]
15. Szelec, I.; Koenig, I.; Seppey, C.V.W.; Le Bayon, R.C.; Mitchell, E.A.D. Soil chemistry changes beneath decomposing cadavers over a one-year period. *Forensic Sci. Int.* **2018**, *286*, 155–165. [[CrossRef](#)] [[PubMed](#)]
16. Perrault, K.A.; Forbes, S.L. Elemental analysis of soil and vegetation surrounding decomposing human analogues. *J. Can. Soc. Forensic Sci.* **2016**, *49*, 138–151. [[CrossRef](#)]
17. Quaggiotto, M.-M.; Evans, M.J.; Higgins, A.; Strong, C.; Barton, P.S. Dynamic soil nutrient and moisture changes under decomposing vertebrate carcasses. *Biogeochemistry* **2019**, *146*, 71–82. [[CrossRef](#)]
18. Berezowski, V.; Moffat, I.; Shendryk, Y.; MacGregor, D.; Ellis, J.; Mallett, X. A multidisciplinary approach to locating clandestine gravesites in cold cases: Combining geographic profiling, LiDAR, and near surface geophysics. *Forensic Sci. Int.* **2022**, *5*, 100281. [[CrossRef](#)] [[PubMed](#)]
19. Dick, H.C.; Pringle, J.K. Inorganic elemental analysis of decomposition fluids of an in situ animal burial. *Forensic Sci. Int.* **2018**, *289*, 130–139. [[CrossRef](#)] [[PubMed](#)]
20. Pringle, J.; Ruffell, A.; Jarvis, J.; Donnelly, L.; McKinley, J.; Hansen, J.; Morgan, R.; Pirrie, D.; Harrison, M. The use of geoscience methods for terrestrial forensic searches. *Earth Sci. Rev.* **2012**, *114*, 108–123. [[CrossRef](#)]
21. Tagliabue, G.; Masseroli, A.; Ern, S.I.E.; Comolli, R.; Tambone, F.; Cattaneo, C.; Trombino, L. The Fate of Phosphorus in Experimental Burials: Chemical and Ultramicroscopic Characterization and Environmental Control of Its Persistency. *Geosciences* **2023**, *13*, 24. [[CrossRef](#)]
22. Damann, F.E.; Tanittaisong, A.; Carter, D.O. Potential carcass enrichment of the University of Tennessee Anthropology Research Facility: A baseline survey of edaphic features. *Forensic Sci. Int.* **2012**, *222*, 4–10. [[CrossRef](#)] [[PubMed](#)]
23. Dautartas, A.; Kenyhercz, M.W.; Vidoli, G.M.; Jantz, L.M.; Mundorff, A.; Steadman, D.W. Differential Decomposition Among Pig, Rabbit, and Human Remains. *J. Forensic Sci.* **2018**, *63*, 1673–1683. [[CrossRef](#)]
24. Barton, P.S.; Reboldi, A.; Dawson, B.M.; Ueland, M.; Strong, C.; Wallman, J.F. Soil chemical markers distinguishing human and pig decomposition islands: A preliminary study. *Forensic Sci. Med. Pathol.* **2020**, *16*, 605–612. [[CrossRef](#)]
25. Fancher, J.; Aitkenhead-Peterson, J.; Farris, T.; Mix, K.; Schwab, A.; Wescott, D.; Hamilton, M.D. An evaluation of soil chemistry in human cadaver decomposition islands: Potential for estimating postmortem interval (PMI). *Forensic Sci. Int.* **2017**, *279*, 130–139. [[CrossRef](#)] [[PubMed](#)]
26. Varlet, V.; Joye, C.; Forbes, S.L.; Grabherr, S. Revolution in death sciences: Body farms and taphonomics blooming. A review investigating the advantages, ethical and legal aspects in a Swiss context. *Int. J. Leg. Med.* **2020**, *134*, 1875–1895. [[CrossRef](#)] [[PubMed](#)]

27. Benninger, A.L.; Carter, D.O.; Forbes, S.L. The biochemical alteration of soil beneath a decomposing carcass. *Forensic Sci. Int.* **2008**, *180*, 70–75. [\[CrossRef\]](#)
28. Forbes, S. Body farms. *Forensic Sci. Med. Pathol.* **2017**, *13*, 477–479. [\[CrossRef\]](#)
29. Berezowski, V.; Moffat, I.; Seckiner, D.; Crebert, I.; Ellis, J.; Mallett, X. The suitability of using domestic pigs (*Sus* spp.) as human proxies in the geophysical detection of clandestine graves. *J. Forensic Sci.* **2024**, *69*, 316–328. [\[CrossRef\]](#)
30. Connor, M.; Baigent, C.; Hansen, E.S. Testing the Use of Pigs as Human Proxies in Decomposition Studies. *J. Forensic Sci.* **2018**, *63*, 1350–1355. [\[CrossRef\]](#)
31. Dawson, B.M.; Wallman, J.F.; Barton, P.S. How does mass loss compare with total body score when assessing decomposition of human and pig cadavers? *Forensic Sci. Med. Pathol.* **2022**, *18*, 343–351. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Sutherland, A.; Myburgh, J.; Steyn, M.; Becker, P.J. The effect of body size on the rate of decomposition in a temperate region of South Africa. *Forensic Sci. Int.* **2013**, *231*, 257–262. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Miles, K.L.; Finaughty, D.A.; Gibbon, V.E. A review of experimental design in forensic taphonomy: Moving towards forensic realism. *Forensic Sci. Res.* **2020**, *5*, 249–259. [\[CrossRef\]](#)
34. Matuszewski, S.; Hall, M.J.R.; Moreau, G.; Schoenly, K.G.; Tarone, A.M.; Villet, M.H. Pigs vs people: The use of pigs as analogues for humans in forensic entomology and taphonomy research. *Int. J. Leg. Med.* **2020**, *134*, 793–810. [\[CrossRef\]](#)
35. Jervis, J.R.; Pringle, J.K.; Tuckwell, G.W. Time-lapse resistivity surveys over simulated clandestine graves. *Forensic Sci. Int.* **2009**, *192*, 7–13. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Pringle, J.K.; Jervis, J.R.; Hansen, J.D.; Jones, G.M.; Cassidy, N.J.; Cassella, J.P. Geophysical monitoring of simulated clandestine graves using electrical and ground-penetrating radar methods: 0–3 Years after burial. *J. Forensic Sci.* **2012**, *57*, 1467–1486. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Pringle, J.K.; Jervis, J.R.; Roberts, D.; Dick, H.C.; Wisniewski, K.D.; Cassidy, N.J.; Cassella, J.P. Long-term Geophysical Monitoring of Simulated Clandestine Graves using Electrical and Ground Penetrating Radar Methods: 4–6 Years After Burial. *J. Forensic Sci.* **2016**, *61*, 309–321. [\[CrossRef\]](#)
38. Booth, A.D.; Pringle, J.K. Semblance analysis to assess GPR data from a five-year forensic study of simulated clandestine graves. *J. Appl. Geophys.* **2016**, *125*, 37–44. [\[CrossRef\]](#)
39. Pringle, J.K.; Stimpson, I.G.; Wisniewski, K.D.; Heaton, V.; Davenward, B.; Mirosch, N.; Spencer, F.; Jervis, J.R. Geophysical monitoring of simulated homicide burials for forensic investigations. *Sci. Rep.* **2020**, *10*, 7544. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Pringle, J.K.; Stimpson, I.G.; Wisniewski, K.D.; Heaton, V.; Davenward, B.; Mirosch, N.; Spencer, F.; Jervis, J.R. Geophysical monitoring of simulated clandestine burials of murder victims to aid forensic investigators. *Geol. Today* **2021**, *37*, 63–65. [\[CrossRef\]](#)
41. Jervis, J.R.; Pringle, J.K. A study of the effect of seasonal climatic factors on the electrical resistivity response of three experimental graves. *Appl. Geophys.* **2014**, *108*, 53–60. [\[CrossRef\]](#)
42. Schultz, J.J.; Collins, M.E.; Falsetti, A.B. Sequential monitoring of burials containing large pig cadavers using ground-penetrating radar. *J. Forensic Sci.* **2006**, *51*, 607–616. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Schultz, J.J. Sequential Monitoring of Burials Containing Small Pig Cadavers Using Ground Penetrating Radar. *J. Forensic Sci.* **2008**, *53*, 279–287. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Schultz, J.J.; Martin, M.M. Monitoring controlled graves representing common burial scenarios with ground penetrating radar. *J. Appl. Geophys.* **2012**, *83*, 74–89. [\[CrossRef\]](#)
45. Van Belle, L.E.; Carter, D.O.; Forbes, S.L. Measurement of ninhydrin reactive nitrogen influx into gravesoil during aboveground and belowground carcass (*Sus domesticus*) decomposition. *Forensic Sci. Int.* **2009**, *193*, 37–41. [\[CrossRef\]](#)
46. Meyer, J.; Anderson, B.; Carter, D.O. Seasonal variation of carcass decomposition and gravesoil chemistry in a cold (Dfa) climate. *J. Forensic Sci.* **2013**, *58*, 1175–1182. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Anderson, B.; Meyer, J.; Carter, D.O. Dynamics of ninhydrin-reactive nitrogen and pH in gravesoil during the extended postmortem interval. *J. Forensic Sci.* **2013**, *58*, 1348–1352. [\[CrossRef\]](#)
48. Brasseur, C.; Dekeirsschieter, J.; Schotsmans, E.M.; de Koning, S.; Wilson, A.S.; Haubruge, E.; Focant, J.-F. Comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry for the forensic study of cadaveric volatile organic compounds released in soil by buried decaying pig carcasses. *J. Chromatogr. A* **2012**, *1255*, 163–170. [\[CrossRef\]](#)
49. Schotsmans, E.M.; Denton, J.; Dekeirsschieter, J.; Ivaneanu, T.; Leentjes, S.; Janaway, R.C.; Wilson, A.S. Effects of hydrated lime and quicklime on the decay of buried human remains using pig cadavers as human body analogues. *Forensic Sci. Int.* **2012**, *217*, 50–59. [\[CrossRef\]](#)
50. Schotsmans, E.M.; Fletcher, J.N.; Denton, J.; Janaway, R.C.; Wilson, A.S. Short-term effects of hydrated lime and quicklime on the decay of human remains using pig cadavers as human body analogues: Laboratory experiments. *Forensic Sci. Int.* **2014**, *238*, 142.e1–142.e10. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Schotsmans, E.M.; Fletcher, J.N.; Denton, J.; Janaway, R.C.; Wilson, A.S. Long-term effects of hydrated lime and quicklime on the decay of human remains using pig cadavers as human body analogues: Field experiments. *Forensic Sci. Int.* **2014**, *238*, 141.e1. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Kontopoulos, I.; Nystrom, P.; White, L. Experimental taphonomy: Post-mortem microstructural modifications in *Sus scrofa domesticus* bone. *Forensic Sci. Int.* **2016**, *266*, 320–328. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Marais-Werner, A.; Myburgh, J.; Becker, P.J.; Steyn, M. A comparison between decomposition rates of buried and surface remains in a temperate region of South Africa. *Int. J. Leg. Med.* **2017**, *132*, 301–309. [\[CrossRef\]](#)

54. Buekenhout, I.; Cravo, L.; Vieira, D.N.; Cunha, E.; Ferreira, M.T. Applying standardized decomposition stages when estimating the PMI of buried remains: Reality or fiction? *Aust. J. Forensic Sci.* **2018**, *50*, 68–81. [\[CrossRef\]](#)
55. Cogswell, G.C.; Cross, P.A. The effects of surface variation on the decomposition of pig carcasses. *J. Forensic Leg. Med.* **2021**, *79*, 102108. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Ururahy-Rodrigues, A.; Rafael, J.A.; Wanderley, R.F.; Marques, H.; Pujol-Luz, J.R. *Coprophanaeus lancifer* (Linnaeus, 1767) (Coleoptera, Scarabaeidae) activity moves a man-size pig carcass: Relevant data for forensic taphonomy. *Forensic Sci. Int.* **2008**, *182*, e19–e22. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Valdes-Perezgasga, M.T.; Sanchez-Ramos, F.J.; Garcia-Martinez, O.; Anderson, G.S. Arthropods of forensic importance on pig carrion in the coahuilan semidesert, Mexico. *J. Forensic Sci.* **2010**, *55*, 1098–1101. [\[CrossRef\]](#)
58. Dekeirsschieter, J.; Verheggen, F.; Gohy, M.; Hubrecht, F.; Bourguignon, L.; Lognay, G.; Haubruge, E. Cadaveric volatile organic compounds released by decaying pig carcasses (*Sus domesticus* L.) in different biotopes. *Forensic Sci. Int.* **2009**, *189*, 46–53. [\[CrossRef\]](#)
59. Horenstein, M.B.; Linhares, A.X.; De Ferradas, B.R.; García, D. Decomposition and dipteran succession in pig carrion in central Argentina: Ecological aspects and their importance in forensic science. *Med. Vet. Entomol.* **2010**, *24*, 16–25. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Anton, E.; Niederegger, S.; Beutel, R.G. Beetles and flies collected on pig carrion in an experimental setting in Thuringia and their forensic implications. *Med. Vet. Entomol.* **2011**, *25*, 353–364. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Bonacci, T.; Brandmayr, T.Z.; Brandmayr, P.; Vercillo, V.; Porcelli, F. Successional patterns of the insect fauna on a pig carcass in southern Italy and the role of *Crematogaster scutellaris* (Hymenoptera, Formicidae) as a carrion invader. *Entomol. Sci.* **2011**, *14*, 125–132. [\[CrossRef\]](#)
62. Payne, J.A. A Summer Carrion Study of the Baby Pig *Sus Scrofa* Linnaeus. *Ecology* **1965**, *46*, 592–602. [\[CrossRef\]](#)
63. Payne, J.A. Insect succession and decomposition of pig carcasses in water. *J. Ga. Entomol. Soc.* **1972**, *7*, 153–162.
64. Payne, J.A.; King, E.W.; Beinhart, G. Arthropod Succession and Decomposition of Buried Pigs. *Nature* **1968**, *219*, 1180–1181. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Turner, B.; Wiltshire, P. Experimental validation of forensic evidence: A study of the decomposition of buried pigs in a heavy clay soil. *Forensic Sci. Int.* **1999**, *101*, 113–122. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Rai, J.K.; Pickles, B.J.; Perotti, M.A. The impact of the decomposition process of shallow graves on soil mite abundance. *J. Forensic Sci.* **2022**, *67*, 605–618. [\[CrossRef\]](#) [\[PubMed\]](#)
67. Silván-Cárdenas, J.; Caccavari-Garza, A.; Quinto-Sánchez, M.; Madrigal-Gómez, J.; Coronado-Juárez, E.; Quiroz-Suarez, D. Assessing optical remote sensing for grave detection. *Forensic Sci. Int.* **2021**, *329*, 111064. [\[CrossRef\]](#) [\[PubMed\]](#)
68. Megyesi, M.S.; Nawrocki, S.; Haskell, N. Using Accumulated Degree-Days to Estimate the Postmortem Interval from Decomposed Human Remains. *J. Forensic Sci.* **2005**, *50*, 1–9. [\[CrossRef\]](#)
69. Rodriguez, W.; Bass, W. Decomposition of Buried Bodies and Methods That May Aid in Their Location. *J. Forensic Sci.* **1985**, *30*, 11017. [\[CrossRef\]](#)
70. Caccianiga, M.; Bottacin, S.; Cattaneo, C. Vegetation Dynamics as a Tool for Detecting Clandestine Graves. *J. Forensic Sci.* **2012**, *57*, 983–988. [\[CrossRef\]](#) [\[PubMed\]](#)
71. Ioan, B.G.; Manea, C.; Hanganu, B.; Statescu, L.; Solovastru, L.G.; Manoilescu, I. The chemistry decomposition in human corpses. *Rev. Chim.* **2017**, *68*, 1450–1454. [\[CrossRef\]](#)
72. Carter, D.; Tibbett, M. Cadaver Decomposition and Soil. In *Soil Analysis in Forensic Taphonomy*; CRC Press: Boca Raton, FL, USA, 2008; pp. 29–51. [\[CrossRef\]](#)
73. Byard, R.W. Body farms—characteristics and contributions. *Forensic Sci. Med. Pathol.* **2017**, *13*, 473–474. [\[CrossRef\]](#)
74. Vidoli, G.M.; Steadman, D.W.; Devlin, J.B.; Jantz, L.M. History and Development of the First Anthropology Research Facility, Knoxville, Tennessee. In *Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment*; Wiley: Hoboken, NJ, USA, 2017; pp. 461–475. [\[CrossRef\]](#)
75. George, R.L.; Zejdlik, K.; Messer, D.L.; Passalacqua, N.V. The John A. Williams Human Skeletal Collection at Western Carolina University. *Forensic Sci.* **2022**, *2*, 362–370. [\[CrossRef\]](#)
76. Gocha, T.P.; Mavroudas, S.R.; Wescott, D.J. The Texas State Donated Skeletal Collection at the Forensic Anthropology Center at Texas State. *Forensic Sci.* **2021**, *2*, 7–19. [\[CrossRef\]](#)
77. Hart, K.; Ainsworth, D.; Williams, A. Human Taphomy Facility Aka ‘The Body Farm’. *J. Forensic Sci. Crim. Investig.* **2017**, *5*, 555662.
78. Cross, P.; Simmons, T.; Cunliffe, R.; Chatfield, L. Establishing a Taphonomic Research Facility in the United Kingdom. *Forensic Sci. Policy Manag. Int. J.* **2010**, *1*, 187–191. [\[CrossRef\]](#)
79. Oostra, R.-J.; Gelderman, T.; Groen, W.M.; Uiterdijk, H.G.; Cammeraat, E.L.; Krap, T.; Wilk, L.S.; Lüschen, M.; Morriën, W.E.; Wobben, F.; et al. Amsterdam Research Initiative for Sub-surface Taphonomy and Anthropology (ARISTA)—A taphonomic research facility in the Netherlands for the study of human remains. *Forensic Sci. Int.* **2020**, *317*, 110483. [\[CrossRef\]](#)
80. Pecsí, E.L.; Bronchti, G.; Crispino, F.; Forbes, S.L. Perspectives on the establishment of a canadian human taphonomic facility: The experience of REST[ES]. *Forensic Sci. Int. Synerg.* **2020**, *2*, 287–292. [\[CrossRef\]](#) [\[PubMed\]](#)
81. Bridges, A. *Perth Body Farm Study on Piglets’ Decomposition to Help Forensic Scientists Analyse Crime Scenes*; ABC News: Perth, Australia, 2023.

82. Poppa, P.; Gibelli, D.M.; Mazzarelli, D.; Cappella, A.; Magli, F.; Cattaneo, C. From the search to the autopsy: The multidisciplinary approach to the assessment of bodies buried in soil: The Italian bodyfarm. In Proceedings of the IAFS WORLD Meeting and WPMO TRIENNIAL Meeting and MAFS Meeting, Funchal, Portugal, 12–17 September 2011.
83. Poppa, P. *L'individuazione e la Caratterizzazione dei Siti di Occultamento di Resti Umani in Ambito Forense: Applicazioni Geopedologiche e Geoarcheologiche*; Università degli Studi di Milano: Milano, Italy, 2010.
84. Ern, S.I.E. *Scienze Naturali in Ambito Forense: L'apporto di Studi Geopedologici e Botanici*; Milan University: Milan, Italy, 2012.
85. Ern, S.I.E.; Trombino, L.; Cattaneo, C. Micromorphological Aspects of Forensic Geopedology: Can vivianite be a marker of human remains permanence in soil? In Proceedings of the EGU General Assembly, Vienna, Austria, 2–7 May 2010.
86. Ern, S.I.E.; Trombino, L.; Gibelli, D.; Cattaneo, C. Application of geopedology to forensic anthropology: Can vivianite be a marker of burial in soil? Three case reports. In Proceedings of the Annual Scientific Meeting AAFS, Seattle, WA, USA, 22–27 February 2010.
87. Salsarola, D.; Poppa, P.; Amadasi, A.; Mazzarelli, D.; Gibelli, D.; Zanolli, E.; Porta, D.; Cattaneo, C. The utility of ground-penetrating radar and its time-dependence in the discovery of clandestine burials. *Forensic Sci. Int.* **2015**, *253*, 119–124. [[CrossRef](#)]
88. Zangarini, S.; Trombino, L.; Cattaneo, C. Micromorphological and ultramicroscopic aspects of buried remains: Time-dependent markers of decomposition and permanence in soil in experimental burial. *Forensic Sci. Int.* **2016**, *263*, 74–82. [[CrossRef](#)] [[PubMed](#)]
89. Caccia, G. *Interazione Corpo Ambiente: Il Fil Rouge Tra Analisi Tafonomiche e Tracce in Contesto Forense*; University of Milan: Milan, Italy, 2022.
90. Caccia, G.; Maregrande, L.; Canesi, M.; Caccianiga, M.S.; Cattaneo, C. The 'body farm' in the Ticino Park—Botanical Evidence on Skin and Hair. In *International Students Forensic Conference: 50 Sides of Crime*; AIR Unimi: Poznan, Poland, 2020.
91. Poklucar, K.; Čandek-Potokar, M.; Lukač, N.B.; Tomažin, U.; Škrlep, M. Lipid Deposition and Metabolism in Local and Modern Pig Breeds: A Review. *Animals* **2020**, *10*, 424. [[CrossRef](#)]
92. Meeuwssen, S.; Horgan, G.; Elia, M. The relationship between BMI and percent body fat, measured by bioelectrical impedance, in a large adult sample is curvilinear and influenced by age and sex. *Clin. Nutr.* **2010**, *29*, 560–566. [[CrossRef](#)] [[PubMed](#)]
93. Yamamoto, T.; Iwase, H.; King, T.W.; Hara, H.; Cooper, D.K. Skin xenotransplantation: Historical review and clinical potential. *Burns* **2018**, *44*, 1738–1749. [[CrossRef](#)] [[PubMed](#)]
94. Sykes, M.; Sachs, D.H. Transplanting organs from pigs to humans. *Sci. Immunol.* **2019**, *4*, eaau6298. [[CrossRef](#)]
95. Beary, M.O.; Lyman, R.L. The Use of Taphonomy in Forensic Anthropology: Past Trends and Future Prospects. In *A Companion to Forensic Anthropology*; Wiley: Hoboken, NJ, USA, 2012; pp. 499–527. [[CrossRef](#)]
96. Quinn, G.P.; Keough, M.J. *Experimental Design and Data Analysis for Biologists*; Cambridge University Press: Cambridge, UK, 2002.
97. Rodriguez, W.; Bass, W. Insect Activity and its Relationship to Decay Rates of Human Cadavers in East Tennessee. *J. Forensic Sci.* **1983**, *28*, 423–432. [[CrossRef](#)]
98. Reed, H.B. A Study of Dog Carcass Communities in Tennessee, with Special Reference to the Insects. *Am. Midl. Nat.* **1958**, *59*, 213. [[CrossRef](#)]
99. Schoenly, K.G.; Haskell, N.H.; Hall, R.D.; Gbur, J.R. Comparative Performance and Complementarity of Four Sampling Methods and Arthropod Preference Tests from Human and Porcine Remains at the Forensic Anthropology Center in Knoxville, Tennessee. *J. Med. Entomol.* **2007**, *44*, 881–894. [[CrossRef](#)] [[PubMed](#)]
100. Grivas, C.R.; Komar, D.A. *Kumho, Daubert*, and the nature of scientific inquiry: Implications for forensic anthropology. *J. Forensic Sci.* **2008**, *53*, 771–776. [[CrossRef](#)]
101. Olson, Z.H.; Beasley, J.C.; Rhodes, O.E. Carcass Type Affects Local Scavenger Guilds More than Habitat Connectivity. *PLoS ONE* **2016**, *11*, e0147798. [[CrossRef](#)] [[PubMed](#)]
102. Indra, L.; Errickson, D.; Young, A.; Lösch, S. Uncovering Forensic Taphonomic Agents: Animal Scavenging in the European Context. *Biology* **2022**, *11*, 601. [[CrossRef](#)]
103. Ueland, M.; Nizio, K.D.; Forbes, S.L.; Stuart, B.H. The interactive effect of the degradation of cotton clothing and decomposition fluid production associated with decaying remains. *Forensic Sci. Int.* **2015**, *255*, 56–63. [[CrossRef](#)]
104. EBostock, E.; Parkes, G.; Williams, G. Effect of decomposition on clothing damage evidence: A preliminary study. *Crime Secur. Soc.* **2018**, *1*, 1–4.
105. Ueland, M.; Forbes, S.L.; Stuart, B.H. Understanding clothed buried remains: The analysis of decomposition fluids and their influence on clothing in model burial environments. *Forensic Sci. Med. Pathol.* **2019**, *15*, 3–12. [[CrossRef](#)]
106. Collins, S.; Stuart, B.; Ueland, M. Monitoring human decomposition products collected in clothing: An infrared spectroscopy study. *Aust. J. Forensic Sci.* **2020**, *52*, 428–438. [[CrossRef](#)]
107. Collins, S.; Stuart, B.; Ueland, M. Anatomical location dependence of human decomposition products in clothing. *Aust. J. Forensic Sci.* **2023**, *55*, 363–375. [[CrossRef](#)]
108. Wilson, A.S.; Janaway, R.C.; Holland, A.D.; Dodson, H.I.; Baran, E.; Pollard, A.M.; Tobin, D.J. Modelling the buried human body environment in upland climes using three contrasting field sites. *Forensic Sci. Int.* **2007**, *169*, 6–18. [[CrossRef](#)]
109. Haglund, W.D. Archaeology and forensic death investigations. *Hist. Archaeol.* **2001**, *35*, 26–34. [[CrossRef](#)]
110. Knight, B. Forensic science and animal rights. *Forensic Sci. Int.* **1992**, *57*, 1–3. [[CrossRef](#)]
111. Cattaneo, C.; Maderna, E.; Rendinelli, A.; Gibelli, D. Animal experimentation in forensic sciences: How far have we come? *Forensic Sci. Int.* **2015**, *254*, e29–e35. [[CrossRef](#)] [[PubMed](#)]

112. Mole, C.G.; Heyns, M. Animal Models in Forensic Science Research: Justified Use or Ethical Exploitation? *Sci. Eng. Ethic* **2019**, *25*, 1095–1110. [[CrossRef](#)] [[PubMed](#)]
113. Kasper, J.; Mumm, R.; Ruther, J. The composition of carcass volatile profiles in relation to storage time and climate conditions. *Forensic Sci. Int.* **2012**, *223*, 64–71. [[CrossRef](#)] [[PubMed](#)]
114. Tumer, A.R.; Karacaoglu, E.; Namli, A.; Ketten, A.; Farasat, S.; Akcan, R.; Sert, O.; Odabaşı, A.B. Effects of different types of soil on decomposition: An experimental study. *Leg. Med.* **2013**, *15*, 149–156. [[CrossRef](#)] [[PubMed](#)]
115. Waterhouse, K. The effect of victim age on burnt bone fragmentation: Implications for remains recovery. *Forensic Sci. Int.* **2013**, *231*, 409.e1–409.e7. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

2.1.2. The Experiment *Ticino 2023* – Study Design

The project conducted at Ticino-LEAFs was based on the experimental re-enactment of real cases of body disposal, consisting of a combination of multiple methods of concealment, all of them including the inhumation of piglet cadavers (*Sus scrofa domesticus*) in a woodland setting at the Ticino Regional Park, in northern Italy (45°23'N 8°50'E).

The experimental area was divided into four sections, each hosting a separate group of burials. Each group comprised six inhumations, for a total of 24 actual burials plus one empty burial for soil characterization (Figure 2.1). The graves were dug by means of a mini excavator, at a depth between 40 and 50 cm, representing the range values encountered in most forensic scenarios (Manheim, 1997; Schultz et al., 2008 – Figure 2.2 a,b).

The piglets involved in the experiment, whose deaths were due to natural or accidental causes entirely independent of this project, weighed between 2.5 and 6.9 kg (see Chapter 4). Their selection as human analogues, to reproduce the decomposition pattern of a buried human body, was based on their similarity to certain human characteristics (Dekeirsschieter et al., 2009; Van Belle et al., 2009; White and Booth, 2014; Matuszewski et al., 2020; Miles et al., 2020). All the specimens involved were buried on the same day (3 March 2023). In particular, six piglets were inhumated unclothed (group “GN”); six piglets were subjected postmortem to three types of injuries: two gunshot wounds to the head, two knife wounds to the abdominal and thoracic regions, and two blunt force traumas inflicted with a hammer on the posterior legs (group “GW”); six piglets were buried in quicklime (CaO–group “GQ”); six piglets were buried clothed in cotton garments (group “GC”). At the same time as the excavation of the graves, a field description of the site location was carried out, along with soil characterization. Finally, soil profiles were described, and soil samples were collected. Regarding the exhumations, these were performed at predetermined PBIs, as summarized in Table 2.1.

2. The Experiment – Study Context and Conditions of the Study

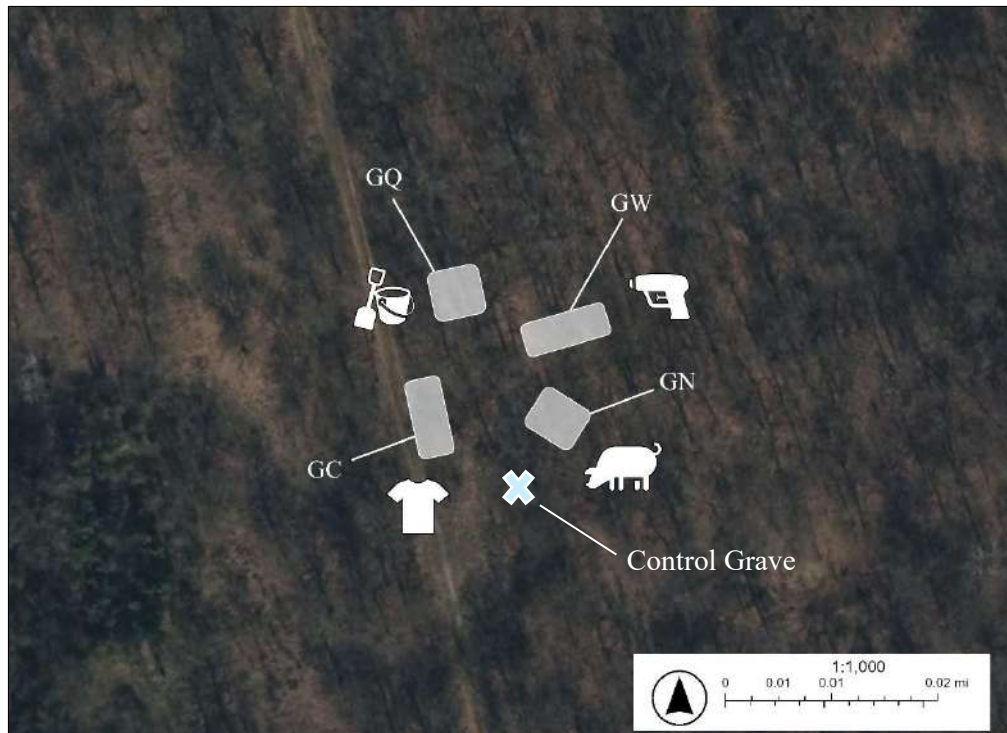


Figure 2.1: Schematic representation of the experimental area with the four groups of inhumations and their characteristics (satellite picture from Geoportale della Lombardia - 1:1000 scale).

2. The Experiment – Study Context and Conditions of the Study



Figure 2.2: Pictures of the realization of the experimental burials at Ticino-LEAFs. **a-** on the left the mini excavator is digging the graves of group “GC”; **b-** on the right the group “GW”.

2. The Experiment – Study Context and Conditions of the Study

<i>Grave ID</i>	<i>PBI (#)</i>	<i>PBI (Days)</i>	<i>Date</i>	<i>Activity</i>
All of them	0	0	March 3, 2023	Inhumations
GN1	1	18	March 21, 2023	Exhumation
GW1	1	18	March 21, 2023	Exhumation
GQ1	1	18	March 21, 2023	Exhumation
GC1	1	18	March 21, 2023	Exhumation
GN2*	2	/	/	/
GW2	2	49	April 21, 2023	Exhumation
GQ2	2	49	April 21, 2023	Exhumation
GC2	2	49	April 21, 2023	Exhumation
GN3	3	101	June 12, 2023	Exhumation
GW3	3	101	June 12, 2023	Exhumation
GQ3	3	101	June 12, 2023	Exhumation
GC3	3	101	June 12, 2023	Exhumation
GN4	4	206	September 25, 2023	Exhumation
GW4	4	220	October 9, 2023	Exhumation
GQ4	4	220	October 9, 2023	Exhumation
GC4	4	206	September 25, 2023	Exhumation
GN5	5	399	April 5, 2024	Exhumation
GW5	5	406	April 12, 2024	Exhumation

2. The Experiment – Study Context and Conditions of the Study

GQ5	5	467	June 12, 2024	Exhumation
GC5	5	397	April 3, 2024	Exhumation

Table 2.1: Chronological resume of the inhumations/exhumations field activities of the experiment. The subject from GN2 (*) was lost due to scavenging activity by local wildlife, therefore data are missing (/).

Although, as mentioned, each group consisted of six burials, in this thesis only the results of the first five exhumation rounds will be presented and discussed (20 buried piglets). At each exhumation, the grave fill was removed following the stratigraphic method (Harris, 2014). Once the HC DI had been identified, the soil beneath the cadaver was observed, documented, and sampled as duplicate bulk specimens: one subsample was air-dried, while the other was stored frozen at -18°C . The cadavers were then removed from the grave according to standard forensic archaeology procedures (Miller et al., 2013; Blau, 2016). In order to represent the group characteristics and the PBI at which the exhumations were going to be performed, the grave ID was projected as follows: *Group acronym + PBI#*.

Example: *GN1*

That identifies the grave for the group GN (inhumated naked) interested in the exhumation at PBI1 (18 Days).

Once recovered and documented, both cadaver and soil specimens were transferred to the respective laboratories at the University of Milan for the analyses. As for soil samples (Chapter 3), these were analyzed at the Earth Sciences Laboratory of the University of Milan to characterize them and to determine their grain size, pH, and Total Organic Carbon (TOC) content (for selected PBIs), both before and after the exhumations. The same samples were subsequently analyzed at the DISAA Laboratories (Department of Agricultural and Environmental Sciences–Production, Landscape, Agroenergy) of the University of Milan, in order to identify organic compounds (dissolved organic matter) and biomarkers (VFA and other organic acids), whose presence could be linked to the decomposition of a cadaver within the HC DI. Furthermore, only the soil samples from group GC were analyzed at the LASA (Applied Superconductivity and Accelerators Laboratory) of INFN (Italian National Institute for Nuclear Physics) in Milan. These analyses aimed to investigate the possible alteration of the magnetic

field of ferromagnetic minerals present in the soil matrix, caused by the strong and localized input of organic matter resulting from the decomposing bodies. Regarding the piglet remains (Chapter 4), once exhumed, they underwent external postmortem examination to assess their state of decomposition. The GW group was also subjected to post-burial radiographic examination (X-rays) and assessment of lesions on the skeletal remains. During the examination, swabs were taken from the gunshot wounds using carbon-coated tape for SEM-EDX analysis, to detect the possible presence of IGSR on the cadavers. At the current stage of the experiment, this represents the only weapon-related trace analyzed on the piglets from the GW group.

2.1.3. The Ethics behind the Experiment

As stated in Section 2.1.1 (Tagliabue et al., 2024), the use of non-human animals as human analogues requires rigorous ethical evaluation. Although derived from clinical and biomedical research traditions, where mammalian models precede human studies under strict ethical constraints, animal experimentation is permitted only when no alternatives exist. Professor Knight (1992) argued that clinical animal research must undergo a stringent harm-benefit assessment by an independent ethics committee, aimed at securing clear diagnostic or therapeutic benefits. He further questioned why forensic research, which serves judicial rather than therapeutic purposes, should justify animal sacrifice under a less stringent framework, particularly when its potential medico-legal benefits remain speculative. Cattaneo et al. (2015) likewise emphasized that even with anesthesia, killing animals for forensic research is ethically difficult to justify. Moreover, they argue that protecting animals is a key moral achievement and that forensic scientists, typically trained in life sciences, should critically assess the use of animals as proxies when research does not aim to save both human and non-human lives. Although animal tissues are often more accessible and perceived as less ethically problematic, animals possess moral status and cannot consent; thus, ethics committees must determine the legitimacy of each study. Respect for animals should be parallel to that afforded to human tissue donors, and all tissues (being scarce resources) must be used with full appreciation of the sacrifice involved. A more ethically defensible approach is the use of animals that have died of natural or unrelated causes, which would reduce the number of animals sacrificed (Mole & Heyns, 2019).

2. *The Experiment – Study Context and Conditions of the Study*

In the present case, current Italian legislation does not allow the establishment of human body donation programs for experimental research in the field of taphonomy studies. Consequently, the animal model is the only one currently applicable. In the case of Ticino-LEAFs, all projects conducted at the facility since its inauguration have used specimens that had died either during clinical trials or naturally and independently of the experiments, as in the experiment presented here, *Ticino 2023*. This was made possible through partnerships established with a local breeder, who preserves the piglets in a freezer (-24°C), rather than discarding them, in the unfortunate event that they die from natural causes (comparable to sudden infant death) or are accidentally crushed when the sow lies on them during nursing. The consequent absence of a systematic selection of the specimens involved in the experiment resulted in some variability in body size. Although this could be considered a limitation, all subjects died under comparable conditions closely related to their age, and therefore size, resulting in a sufficiently homogeneous sample. Moreover, it should be emphasized that the present research focuses on biogeochemical anomalies arising from the substantial nutrient input to the subsurface environment, reflecting decomposition processes common to mammals. Minor differences in body mass among the piglets are therefore unlikely to have significantly influenced the results.

Another potential limitation associated with the use of animals that had already died concerns the infliction of postmortem injuries, like in the case of group GW, which would differ histopathologically from perimortem trauma. However, the present project did not require histological or histochemical analyses; therefore, the non-vital state of the tissues did not introduce any methodological bias. Moreover, as emphasized by Knight (1992), the deliberate infliction of painful injuries on conscious animals for medico-legal purposes provides limited scientific benefit and constitutes unjustifiable cruelty. Accordingly, the projects conducted at Ticino-LEAFs show adherence to regulatory standards, including the Animals (Scientific Procedures) Act 1986 (UK Animal Acts, 1986), EU Directive 2010/63 (European Union, 2010), the NIH Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1986), and the more recent ARRIVE 2.0 guidelines for transparent reporting of animal studies (Percie du Sert et al., 2020). Locally, the experiment was conducted in respect of the Animal Welfare Organization instituted by the University of Milan (Legislative Decree no. 26/2014), whose tasks include applying the “Principle of the 3Rs”: Replacement (replace a model which uses an animal with an alternative model, when possible); Reduction (reduce the number of animals used in a given experiment); Refinement (take action to improve the techniques used to raise, house, care for, and utilize animals).

2.2. Environmental Framework

The experimental area where the project was conducted at Ticino-LEAF(s) is located within the Lombardy section of the Ticino Park (Province of Pavia), at 45.38971° N, 8.83536° E (WGS84), with an elevation of approximately 93 m a.s.l. (Figure 2.3).

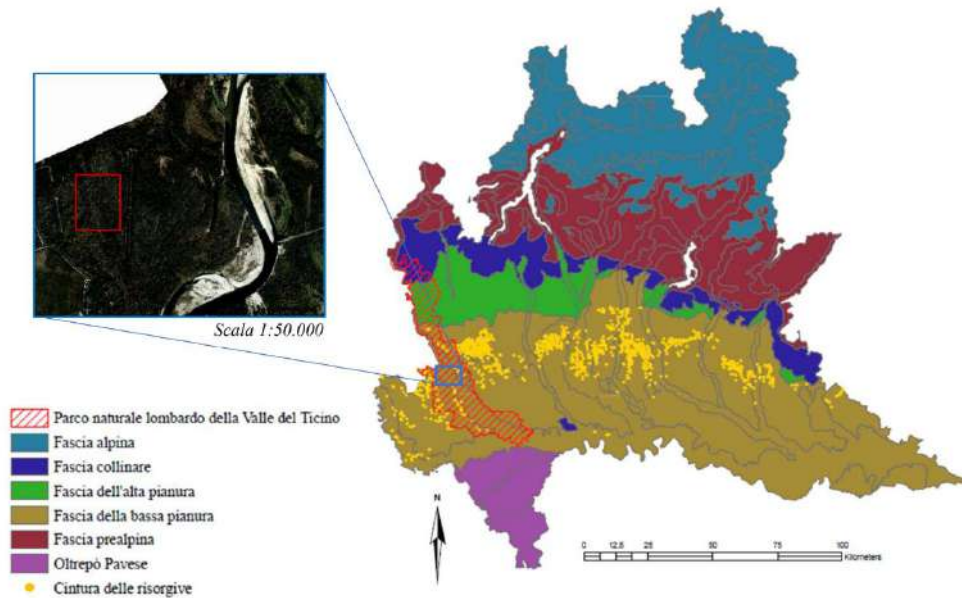


Figure 2.3: Geographic framework of the experimental area at Ticino-LEAF(s). Right: representation of Lombardy region 1:1,250,000 scale divided into landscape zones; Left: an excerpt from the 2018 orthophoto, at 1:50,000 scale, of Ticino-LEAF(s). The experimental area is located in the red square. From Geoportale della Lombardia – Modified with ArcMap.

The Ticino Valley lies partly in Switzerland and partly in Italy, spanning the Lombardy and Piedmont regions. Its Lombard portion is protected by the Lombard Ticino Valley Park, while the Piedmontese section is managed by the Ticino Natural Park authority. Lombardy Park covers about 91,800 ha, encompassing all 47 municipalities situated along the Ticino River between Lake Maggiore and the Po River in the provinces of Varese, Milan, and Pavia. Among these, the municipality of Cassolnovo (PV) has hosted several experimental projects of LABANOF (Laboratory of Forensic Anthropology and Odontology) and the University of Milan, including the research discussed in this PhD thesis. The study area lies close to the Ticino River and includes high-value natural sites that largely coincide with the riverbed and valley, often up to the edge of the main terrace. These areas preserve the last remnants of lowland forest, which form the zoning of the Ticino Natural Park. Cassolnovo belongs to the Lomellina

district, specifically to the lower plain or rice-growing landscape unit. This sector is typified by extensive rice cultivation and abundant surface water, particularly evident in the spring floods essential to the local environment. The lower Po plain (*bassa pianura lombarda*) is located south of the spring-line, characterized by finer silty-clay deposits (Pellegrini & Castaldi, 2008).

Regarding the geology of the area, according to the Italian Geological Map, Sheet 44–Novara (1:100,000), the site belongs to the domain of recent and active gravelly alluvial formations of abandoned and active channels (Holocene; Figure 2.4). Overlying surfaces above the Fundamental Level of the Po Plain (LfP) consist of late Pleistocene fluvioglacial and fluvial Würmian deposits later reworked by incision and deposition, forming terrace *alluvium*. The principal stratigraphic units, from oldest to youngest, include:

- fg^M** Fluvioglacial cobbly, coarse alluvium, weathered into typical *ferretto* up to a thickness of 3 meters, with deeply weathered pebbles of reddish-brown color (*Mindel*).
- fg^R** Fluvioglacial gravelly alluvium, weathered into yellow-ochre clayey soils, with a maximum thickness of 3 meters (*Riss*).
- fg^{WR}** Fluvioglacial gravelly alluvium, locally very coarse, with a thin yellowish-reddish clayey paleosol, sometimes covered by more recent silts (*Würm and Riss*).
- fg^W** Fluvioglacial gravelly-cobbly alluvium (upper terraces of the Ticino) and predominantly sandy-silty fluvioglacial deposits (downstream of the northern limit of the spring line, with a weak weathered layer) (*Würm*);
Gravelly, sandy, and silty alluvium confined to valley-bottom channels and not connected to morainic systems (Pluvial–Würm).
- a¹** Terraced cobbly and gravelly-sandy alluvium (early Holocene). The Holocene alluvial deposits are found along the main watercourses of the region, marking their ancient, abandoned channels.
The boundary with the fluvial-Würm deposits is usually marked by a terrace, particularly evident in the Ticino area.

- a² Terraced gravelly-sandy or silty alluvium, either stabilized or not, formed by the major watercourses (recent/current Holocene). These deposits occur along the main rivers, where they constitute the present riverbeds.

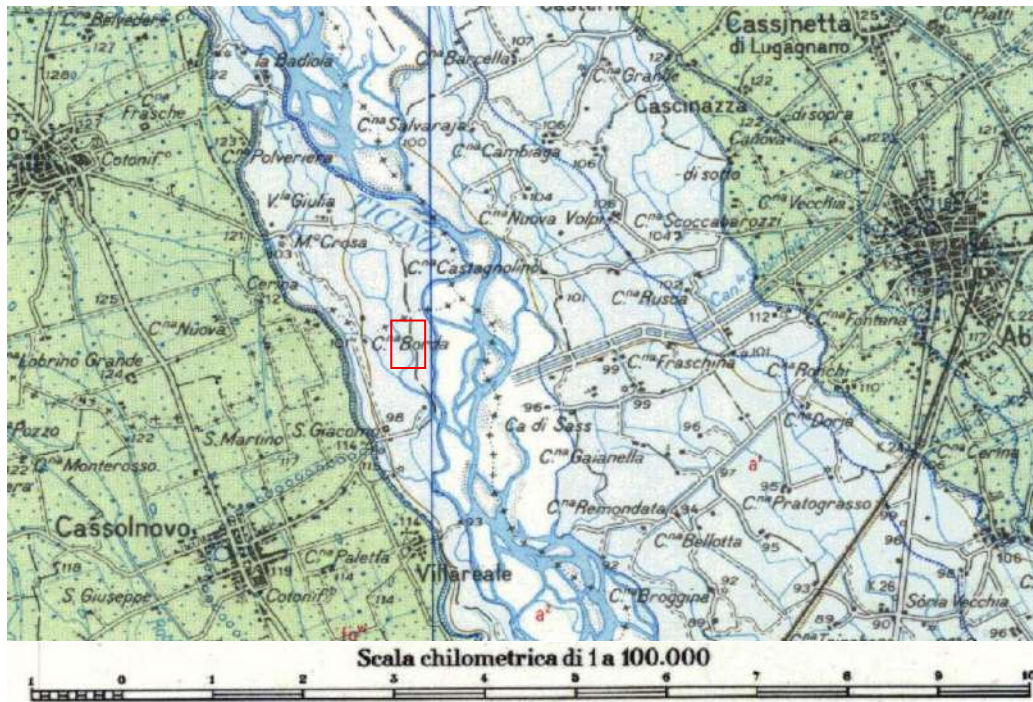


Figure 2.4: Excerpt from the Geological Map of Italy at a 1:100,000 scale. The red box indicates the study area (not to scale).

Regarding the geomorphological setting, the Geomorphological Map of the Po Plain at a 1:250,000 scale (Castiglioni et al., 1997) identifies the experimental area as being located to the west of the Ticino River floodplain (Figure 2.5). The territory, mostly flat, lies within the portion of the alluvial plain bounded by the paleo-channel of the river, whose ancient trace is well preserved and easily recognizable on maps. The surface sediments are predominantly gravelly in nature, defining an area naturally lacking the superficial weathering layer that characterizes the surrounding plain (Borsani, 2016). The wide Ticino Valley causes the landscape to be divided into three distinct geomorphological sectors:

- To the west of the river, where the experimental sector of the Park is located, the area consists of a broad, well-defined valley with elevations ranging between 70 m and 125 m a.s.l.

2. The Experiment – Study Context and Conditions of the Study

The valley floor is bordered by steep scarps, with height differences ranging from 20 m to 15 m on the western side (along the north–south axis) and from 25 m to 15 m on the eastern side, downstream of the city of Pavia (Borsani, 2016).

- On the eastern slope of the valley, a fluvial ridge with a steep longitudinal gradient can be observed, resulting in a general increase in slope (Castiglioni et al., 1997). Indeed, topographic maps of the area show a change in the orientation of contour lines, reflecting the shift toward the north–south direction of the Po River’s perpendicular drainage system.
- Finally, the third geomorphological sector is represented by the Ticino valley floor, which widens on the western bank up to the height of Vigevano, then narrows due to active erosional processes that continue to reduce the extent of its intermediate terraces. To the east, the valley broadens again toward the first spring belt (*fascia delle risorgive*), where wetland and peaty areas are found along the traces of the river’s abandoned meanders. These meanders exhibit morphometric features similar to those of the present channel, attesting to the ancient southwestward migration phase of the river (Castiglioni et al., 1997).

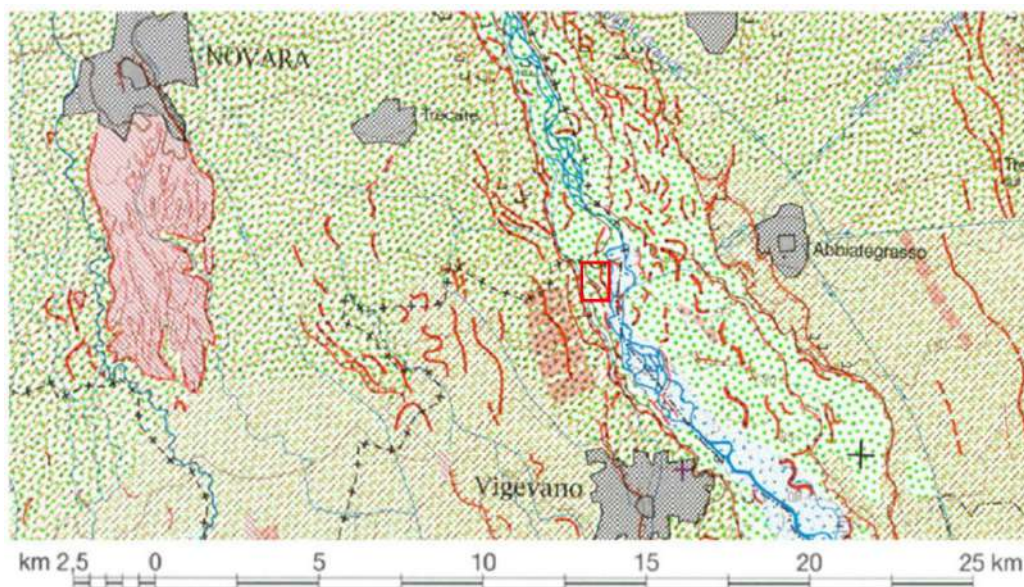


Figure 2.5: Excerpt from the Geomorphological Map of the Po Plain at a 1:250,000 scale. The red box indicates the study area (not to scale).

2. The Experiment – Study Context and Conditions of the Study

From a vegetational point of view, the Ticino Park area features vegetation types of considerable interest: it is occupied by almost 55 % agricultural areas, 22 % forests, 20 % urbanized areas, and 3 % hydrographic network. Specifically, the forested area (which also includes the experimental site) covers about 11,000 hectares. The main forest type of the park is the valley-bottom oak woodland, characterized by the presence of *Quercus robur* (pedunculate oak), *Galeopsis pubescens* (downy hemp-nettle), and *Aristolochia clematitis* (birthwort). These are young woods, and only certain areas show good structural development due to the intense exploitation of the land (ERSAL, 1996).

To describe the vegetation of the study area more specifically, the *Destinazione d'Uso dei Suoli Agricoli e Forestali* map (DUSAF 7.0-2021) at a scale of 1:20,000 was consulted (Figure 2.6). This allowed the vegetation of the area (green colored on the map) to be identified as “broadleaf forests of medium to high density, managed as coppice.”

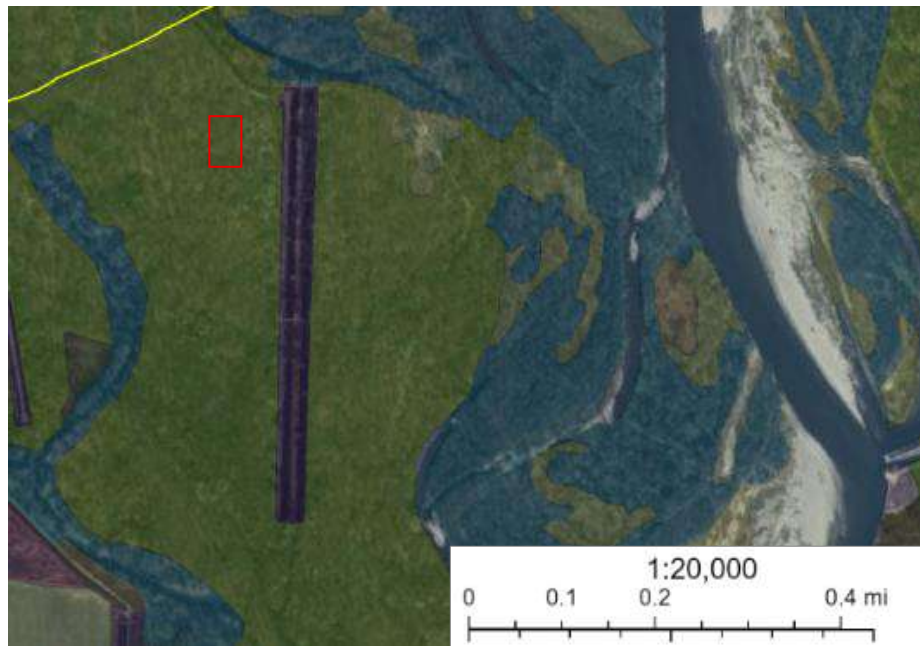


Figure 2.6: Excerpt from the DUSAF 7.0 (2021) map at 1:20,000 scale, with related legend. The red rectangle identifies the study area, not to scale.

2. The Experiment – Study Context and Conditions of the Study

Based on previous naturalistic characterization studies carried out at the experimental site (Caccia, 2022), the plant species were identified more precisely:

- Trees: *Quercus robur*, *Robinia pseudoacacia*, *Crataegus monogyna*.
- Shrubs: *Corylus avellana*, *Ailanthus altissima*, *Frangula alnus*.
- Various bramble species: *Rubus* spp., and herbaceous plants such as *Oxalis* spp. and *Brachypodium rupestre*.
- Climbing species: *Hedera helix*, climbing on the aforementioned tree and shrub species.

Finally, the presence of a rich and diverse array of ecosystems ensures that the Park contains a heritage of biodiversity unmatched in Po Valley (VV.AA, 2002). Concerning the animal kingdom, 4,037 species have been recorded to date:

- Fishes (72 species): 38 native (including *Cyprinus carpio*, considered para-native) and 17 non-natives. The most representative species include trout (including the marble trout *Salmo marmoratus*), pike (*Esox lucius*), and chub (*Squalius* spp.).
- Birds (327 species): the most abundant are the crane (*Grus grus*), the nightjar (*Caprimulgus europaeus*), the cormorant (*Phalacrocorax carbo*), and various pelecaniforms.
- Reptiles and amphibians (25 species): including the green whip snake (*Hierophis viridiflavus*), the European pond turtle (*Emys orbicularis*), the common toad (*Bufo bufo*), and the fire salamander (*Salamandra salamandra*).
- Invertebrates: the most represented clade, with a total of 3,551 recorded species.
- Mammals (62 species): including the gray squirrel (*Sciurus carolinensis*), the coypu (*Myocastor coypus*), the red fox (*Vulpes vulpes*), the roe deer (*Capreolus capreolus*), the brown hare (*Lepus europaeus*), and the rabbit (*Oryctolagus cuniculus*). The stone marten (*Martes foina*), the pine marten (*Martes martes*), and the badger (*Meles meles*) are also present, while the wild boar (*Sus scrofa*) is reported as a significant problem for the park ecosystem.

2.2.1. Soils of the *Parco Lombardo della Valle del Ticino*

To provide a general soil characterization of the area two small-scale maps were used: the Soil Map of Italy (1:1,000,000) which allows to identify the soil province, and the Soil Database reconnaissance map (1:250,000) for WRB classification (European Soil Bureau Network, 2005). The national map places the site in *Region D* (Po Plain and associated hills; Figure 2.7), characterized by *Cambisols*, *Calcisols*, *Luvisols*, *Vertisols* and *Fluvisols*, more specifically in the province defined by *Dystric Cambisol*; *Chromic Luvisol*; *Haplic Alisol* (*Cutanic*, *Fragic*) and (*Cutanic*, *Fragic*). Given the recent alluvial setting, *Cambisols* (young, weakly developed soils) are most probable.

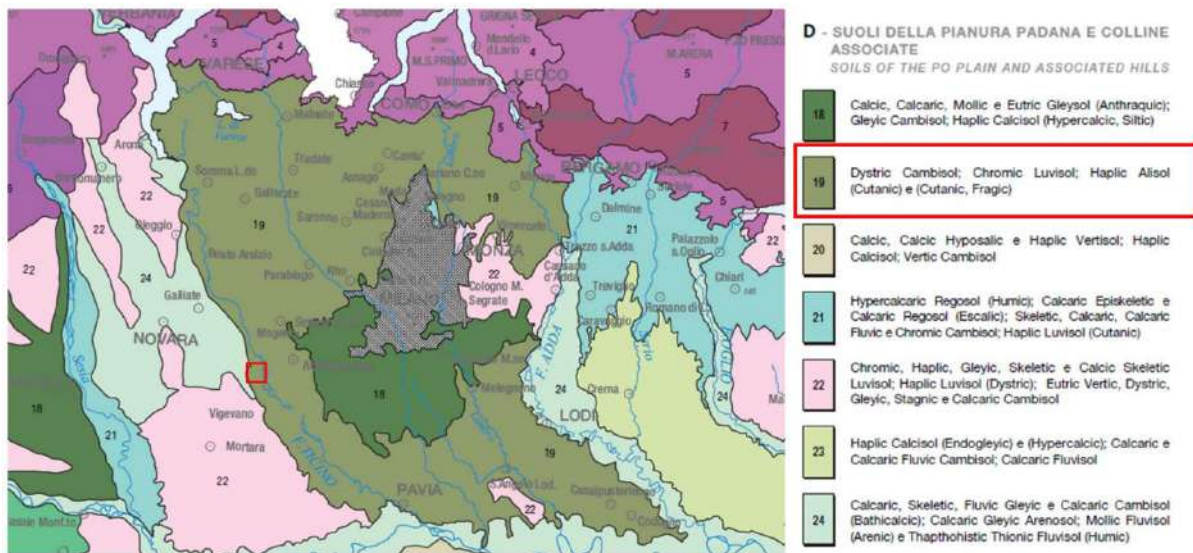


Figure 2.7: excerpt from the Soil Map of Italy (scale 1:1,000,000). The red box indicates the study area (not to scale). On the right, the corresponding legend.

The WRB classification based on the Soil Database reconnaissance map (1:250,000) assigns the area to the Lombard Plain (Padano-Veneta plain) - Ticino Valley district, province of the left-Po valleys and low terraces. The area is dominated by *Regosols* (poorly developed soils on loose material). Moreover, the functional properties of this specific typological unit (UTS) are very shallow profiles, with organic carbon ~0.275 % in the topsoil. The texture is sandy-loam, while particle class is skeletal-loam. Finally, the pH in the first meter is sub-acid (pH ≈ 5.6).

Greater detail is provided by the 1:50,000 semi-detailed Soil Map, which is provided by LOSAN Soil Database (*Sistema Informativo dei Suoli di Regione Lombardia*) and ERSAL/ERSAF (*Ente Regionale di Sviluppo Agricolo della Lombardia*) survey documentation

(ERSAL, 1996). The documentation focuses on land-use capability, water-protection value and environmental value (Figure 2.8).

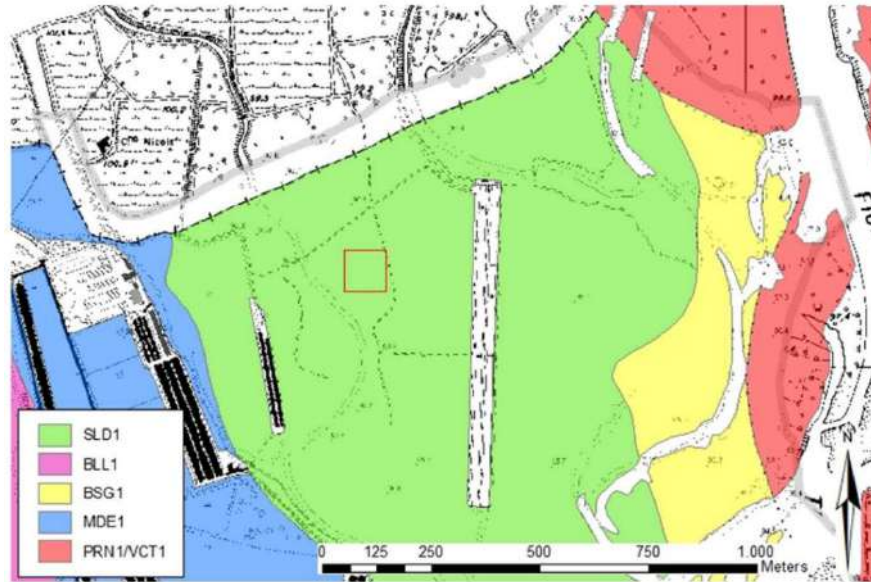


Figure 2.8: Excerpt from the semi-detailed Soil Map of the Lombard plain and foothill area at a scale of 1:50,000. The red box indicates the experimental area. Source: LOSAN Soil Database – Modified with ArcMap.

According to the survey, the experimental site belongs to cartographic unit VI6, a consociation consisting of flat-lying surfaces located at the same elevation as the watercourse, or only slightly raised, which can be flooded during major high-water events (with absent or minimal water retention). They occur along the middle to upper reaches of rivers and in mountain plains, in an intermediate position between the terraced floodplain and the more frequently inundated areas adjacent to the watercourses. The code indicates that the unit belongs to System V (fluvial valleys corresponding to the active or fossil meander belts of the present hydrographic network-Holocene) and more specifically to Subsystem VI, which identifies fluvial plains with predominantly depositional dynamics, partly flood-prone and composed of recent or current sediments. Furthermore, according to LOSAN Database, cartographic unit VI6 consists of 11 delineations distributed throughout the Ticino Valley, covering an area of approximately 2,988 hectares. It is characterized by nearly level surfaces corresponding to alluvial plains, partially floodable, with an average elevation of about 96 m a.s.l. and virtually zero average slope. This unit shows moderate surface stoniness and a moderate flood risk. The parent material is

composed of coarse alluvial deposits, while the substrate consists mainly of poorly sorted gravel with coarse sand. The principal land uses are coppice woodlands and rotational arable crops.

The soil classes that characterize these areas belong to the SLD (*Salvadorino*) series, which presents an A horizon about 25 cm deep, dark grayish brown in color, with a loam or sandy-loam texture and a skeleton from scarce to frequent, non-calcareous and acidic. Conversely, substrate is observable starting from 20–30 cm depth, and appears olive-gray in color, sandy, non-calcareous and subacid (neutral at 50 cm), with abundant coarse fragments. These soils have a base saturation rate lower than 35 % (very low).

More specifically, the soil typological unit identified for the area in question is SLD1 (*Salvadorino franca-loam*). These are very shallow soils, limited by layers with strongly contrasting texture and coarse horizons, which have high permeability and rapid drainage, sandy texture in the first meter and abundant coarse fragments. These are non-calcareous soils, with an acidic reaction at the surface and subacid–neutral at depth, with a very low base saturation rate and a very low available water capacity (AWC).

As for the typical profile, the A horizons generally have a thickness between 10 and 40 cm, and a moist matrix color of 10YR 3/3 and 2.5Y, with Value from 3 to 4 and Chroma 2. The structure is granular and subangular blocky, medium to large, weakly developed. The textural classes can be defined as sandy loam (FS), loamy sand (SF) and loam (F) and the particle-size classes as skeletal sandy (SKS) and coarse loam (FGR). The skeleton is common to abundant, with a sand content from 50 to 70 %. The C horizons, on the other hand, have the upper limit from 10 to 40 cm and a thickness between 30 and 95 cm. The moist matrix color is 2.5Y and 10YR, with Value from 4 to 6 and Chroma from 2 to 3, mottles 10YR 6/6. The textural classes identified are sand (S) and sandy loam (SF), while they also fall into the skeletal sandy (SKS) particle-size class. The skeleton is from scarce to very abundant, with a concentration of sand-sized grains from 85 to 99 %.

Ultimately, as regards the practical properties of these soils, those belonging to the SLD1 unit are unsuitable for agriculture and, although they do not show signs of erosion, they present limitations that are difficult to eliminate, linked to negative soil characteristics, restricting their use to pasture, forestry, or natural habitat. They are suitable for the spreading of livestock effluents, with moderate limitations due to permeability and particle size, while they are not suitable for the spreading of sewage sludge because of limitations related to drainage, particle

2. *The Experiment – Study Context and Conditions of the Study*

size, and cation exchange capacity (*Capacità di Scambio Cationico* - CSC). SLD1 soils have moderate protective capacity with respect to surface waters due to limitations linked to floodability, and low protective capacity with respect to groundwater due to limitations related to permeability and particle size. Finally, their environmental value is low.

In conclusion, it can be stated that the strong fluvial dynamics and the young age of the sediments characterizing this area are the main factors responsible for the limited development of the soils within the experimental area. In this sector of the Ticino Park, AC-profile soils (*Regosols*) prevail, that are thin and rich in coarse fragments, highly draining or with low water retention, and still reflecting the physical-chemical characteristics of the parent material.

2.2.2. Climatic and Microclimatic Framework

The climatic conditions of the study area were defined, in this case as well, using documentation produced by ERSAL (1996) for the project “The Soils of the Ticino Park – Southern Sector.” The publication reports thermometric and pluviometric data from two meteorological stations located at the southern extremities of the Park: Abbiategrasso and Pavia (Figure 2.9), both situated at elevations between 70 and 90 m a.s.l. According to ERSAL data, the mean annual air temperature ranges from 12.5 °C to 12.9 °C, with a mean annual thermal range, defined as the difference between the mean temperature of the warmest month (July) and that of the coldest month (January), of 22.1 °C to 23.8 °C. These values classify the climate as continental, with Abbiategrasso exhibiting a more pronounced degree of continentality.

Precipitation is not abundant: the mean annual total at Pavia is 912 mm, with a standard deviation of 207 mm, while at Abbiategrasso rainfall is higher (1007 mm yr⁻¹), confirming an increase in daily cumulative precipitation toward the north. Regarding rainfall distribution, Pavia shows a well-marked maximum in autumn (October-November), a secondary peak in August, and minimum in January, July, and September. This pattern classifies the pluviometric regime as *sublittoral*, intermediate between the Po Valley type and the Apennine type (Ottone & Rossetti, 1980). At Abbiategrasso, by contrast, there is a maximum in November and a winter minimum between January and February equal to roughly half of the autumn maximum. Consequently, the regime is classified as *padano sublittoral*.

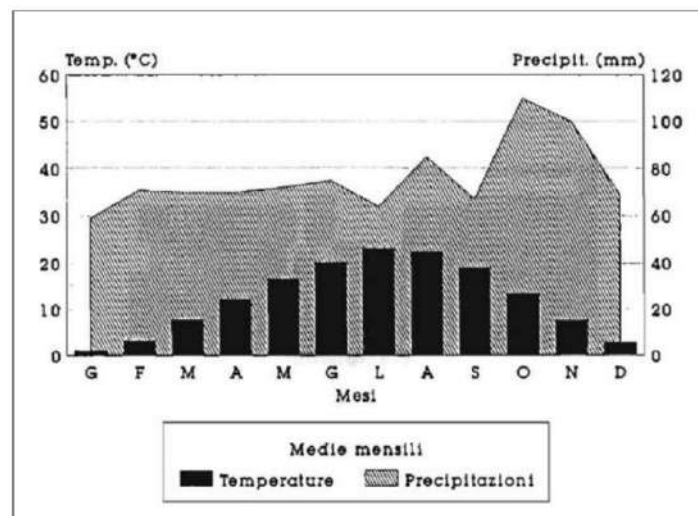


Figure 2.9: Monthly trend of temperature and precipitation for the Pavia station. Data source: ERSAL, 1996

According to the most recent update of the global Köppen-Geiger climate classification map (Kottek et al., 2006), the experimental area falls within the zone of humid temperate climates with hot summers: *Cfa*. The letter “C” identifies the class comprising mild, humid (mesothermal) climates in which the mean temperature of the coldest month ranges between -3°C and 18°C and at least one month has a mean temperature above 10°C . The second letter “f” denotes the subgroup of temperate climates without a dry season, where precipitation is evenly distributed throughout the year, while the final letter “a” indicates climatic regimes in which the mean temperature of the warmest month exceeds 22°C .

An additional climate classification method is that of Thornthwaite (Feddema, 2005), which requires calculation of the aridity index (Ia), humidity index (Ih), and overall humidity index (Im). For the Pavia station, these indices were calculated as 17.18, 40.36, and 23.19, respectively. Based on these values, the climate can be defined as humid, mesothermal, with moderate summer water deficiency. As for Abbiategrasso, the climatic pattern is similar, except that the water deficiency is very limited in this case.

For what concerns specifically the period in which the experiment was conducted (March 2023–June 2024), the temperature trend (whose data were collected from the Cerano (NO) weather station located 3 km north of Ticino-LEAF(s) 125 m a.s.l.) is shown in Figure 2.10.

From the reported data, it is possible to observe an irregular trend in temperature variations, with a strong increase occurring between May and September 2023, reaching the maximum temperature for the period of 35.8°C (28 August 2023). This increase characterized the period between PBI3 and PBI4. Subsequently, the temperature decreases rapidly, though with an irregular pattern, until recording the lowest temperature on 22 January 2024 (-5.1°C), between PBI4 and PBI5. Finally, a second increasing trend is observable starting from February 2024 and involves the entire sampling period of PBI5. The mean daily temperature for the entire sampling period was 14.8°C .

Regarding daily precipitation trends during the experiment period, Figure 2.11 shows the highest amount was recorded on May 15, 2024 (62.3 mm), while several dry periods (0 mm) occurred, notably in March, April and September 2023. Especially, intense rainfall events were observed between PBI3 and PBI4 and again between PBI4 and PBI5.

2. The Experiment – Study Context and Conditions of the Study

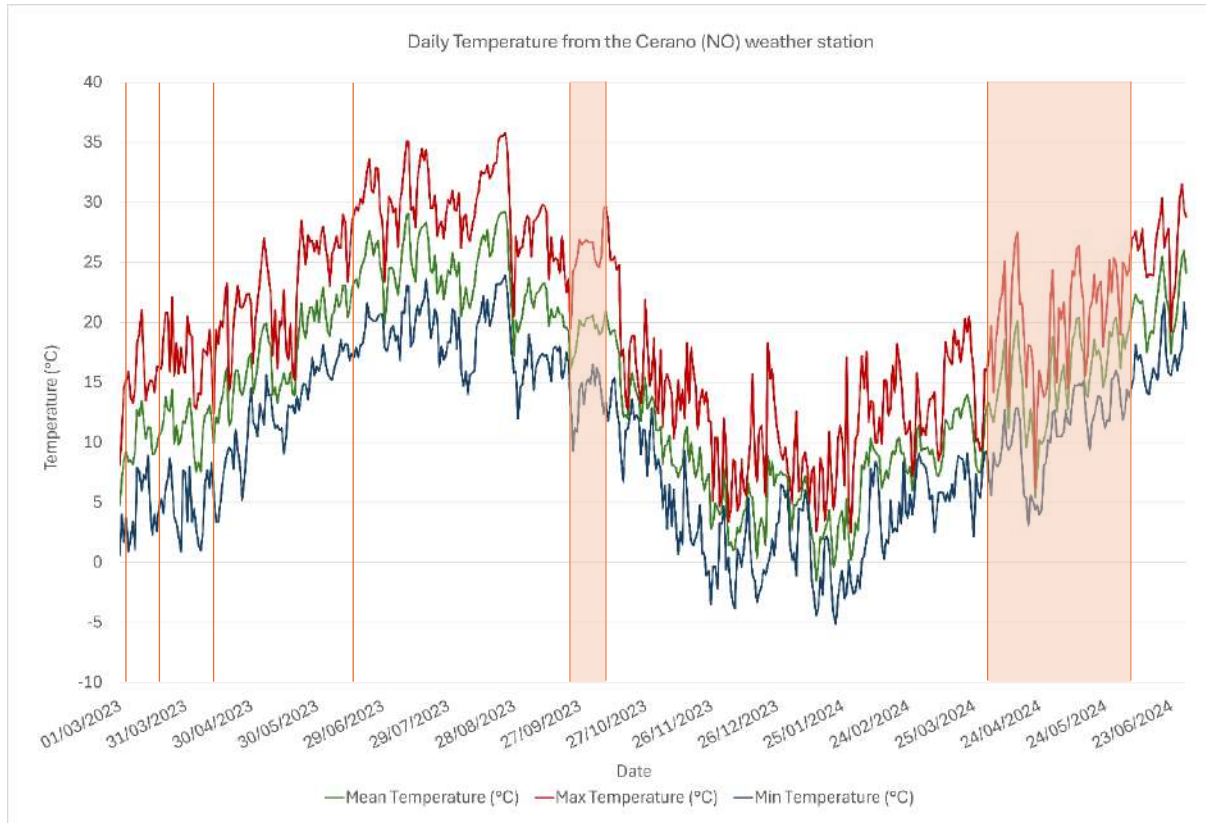


Figure 2.10: Daily atmospheric temperature in the experiment period. The red line represents the trend of the mean maximum temperatures; the green line represents the trend of the mean temperatures, and the blue line represents the trend of the mean minimum temperatures. The orange lines/rectangles indicate the specific exhumation and sampling days for the respective PBIs. The last field operations needed more time due to complications related to the FTF practicability issues.

2. The Experiment – Study Context and Conditions of the Study

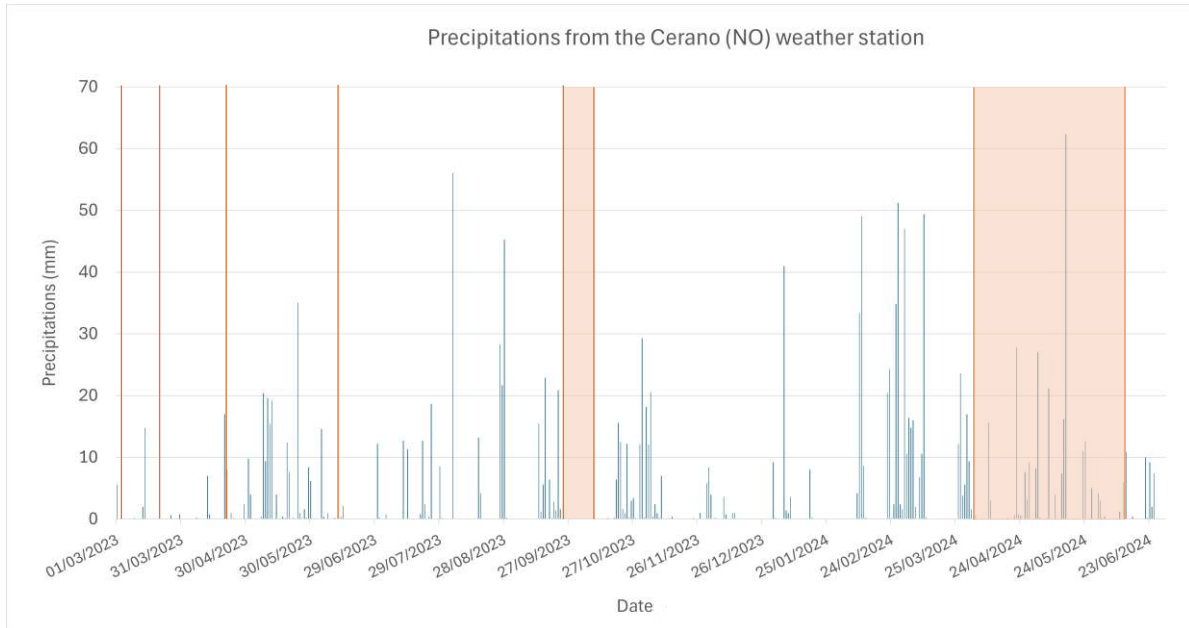


Figure 2.11: Daily precipitations (mm) for the experiment period. The columns represent the cumulative rainfall for each day. The orange lines/rectangles indicate the specific exhumation and sampling days for the respective PBLs. The last field operations needed more time due to complications related to the FTF practicability issues.

2. The Experiment – Study Context and Conditions of the Study

Starting from June 2023, three Data Loggers (Lascar EL-GFX-2) were installed at ground level, capable of recording soil temperature ($^{\circ}\text{C}$) and relative humidity (%) at strategic points of the experimental area (Figure 2.12):

- Data Logger “GN-GC”: 45.38969 N, 8.83599 E (WGS84) – 100 m a.s.l.
- Data Logger “GW-GN”: 45.38975 N, 8.83590 E (WGS84) – 99 m a.s.l.
- Data Logger “GQ-GW”: 45.38975 N, 8.83588 E (WGS84) – 109 m a.s.l.

The devices recorded the parameters every 20 minutes and data were elaborated through EasyLog USB software version 7.7. Unfortunately, due to several malfunctions of the instruments, only a fraction of the operational period was monitored (Figures 2.13 and 2.14 - The data has been processed as daily average temperatures). Nevertheless, the collected data, especially those related to soil temperature, were used for the application of formulas for the calculation of the PMI (Chapter 4).

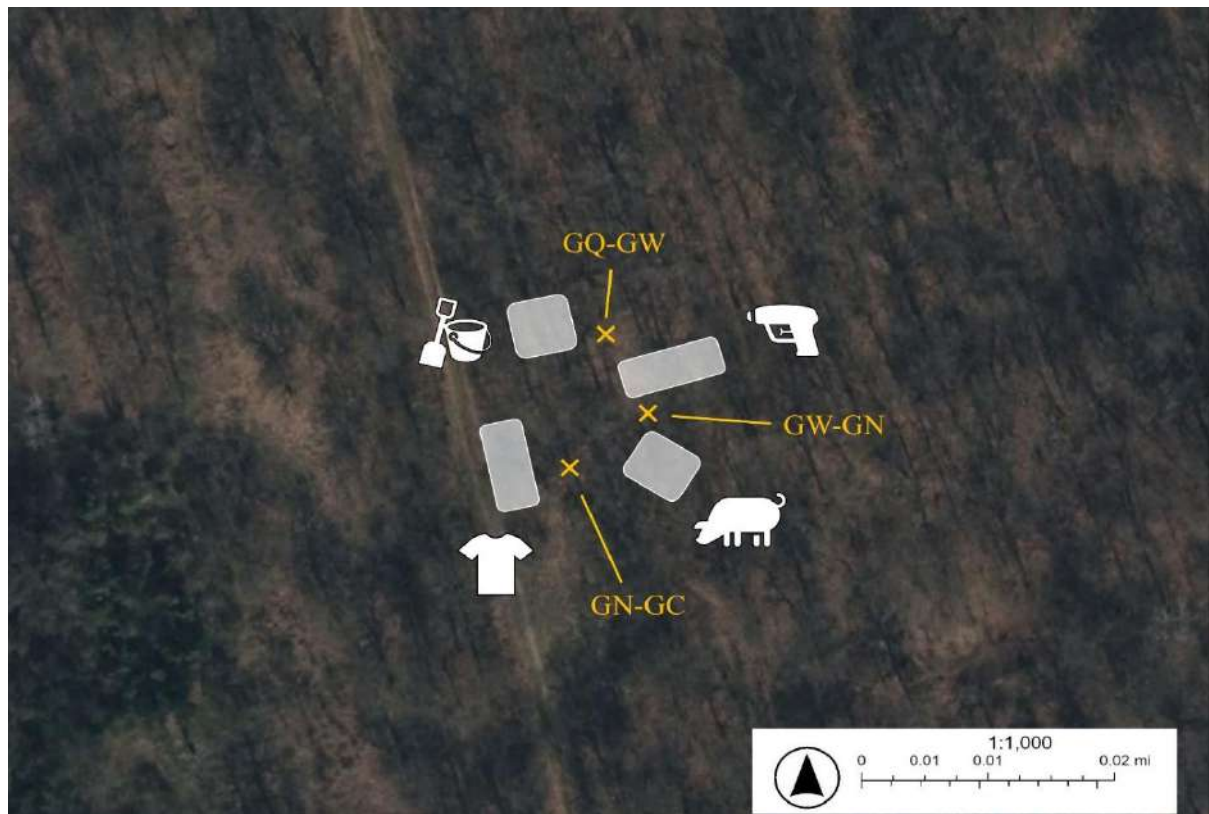


Figure 2.12: Position of the three Data Loggers located in the experimental area. Satellite picture from Geoportale della Lombardia - 1:1000 scale).

2. The Experiment – Study Context and Conditions of the Study

By comparing the daily mean atmospheric temperature patterns recorded at the Cerano (NO) weather station with the ground temperatures measured by the data loggers (Figure 2.13), largely overlapping trends are observed, except for the period between November and January 2023, during which the ground temperatures exhibited a smaller thermal range (10.3°C in the atmosphere vs. 6.1°C at the surface).

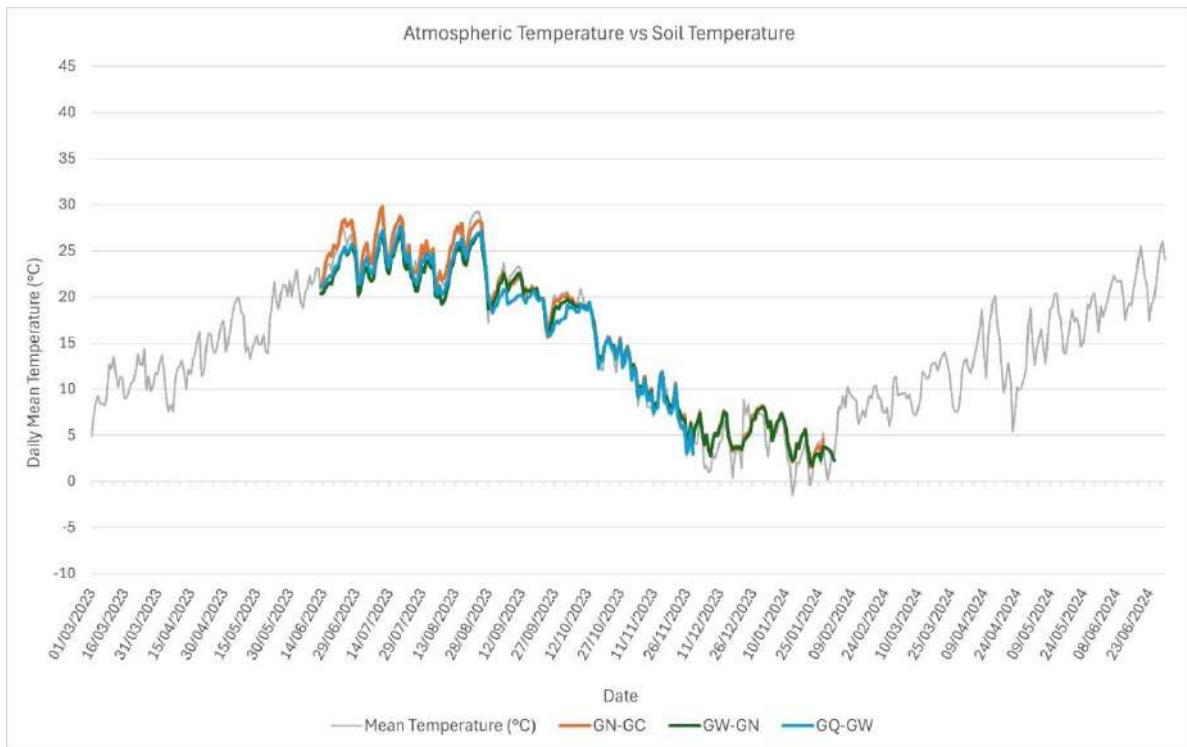


Figure 2.13: Comparison of the daily mean atmospheric temperatures recorded by the weather station and the daily mean soil temperature recorded by the three Data Logger.

2. The Experiment – Study Context and Conditions of the Study

Regarding the comparison between daily total precipitation recorded by the weather station and near-surface relative humidity (Figure 2.14), the data show an almost parallel pattern between the two parameters. In June 2023, when rainfall events markedly decreased, the lowest relative humidity values were recorded by data loggers GN-GC and GW-GN. Conversely, progressive increases in relative humidity were observed during intense summer precipitation events. Nevertheless, the data reveal considerable variability in the values reported by the different instruments in response to the same rainfall events. Such heterogeneity may be attributable to the varying degree of tree canopy cover, which provides different levels of protection to the ground surface.

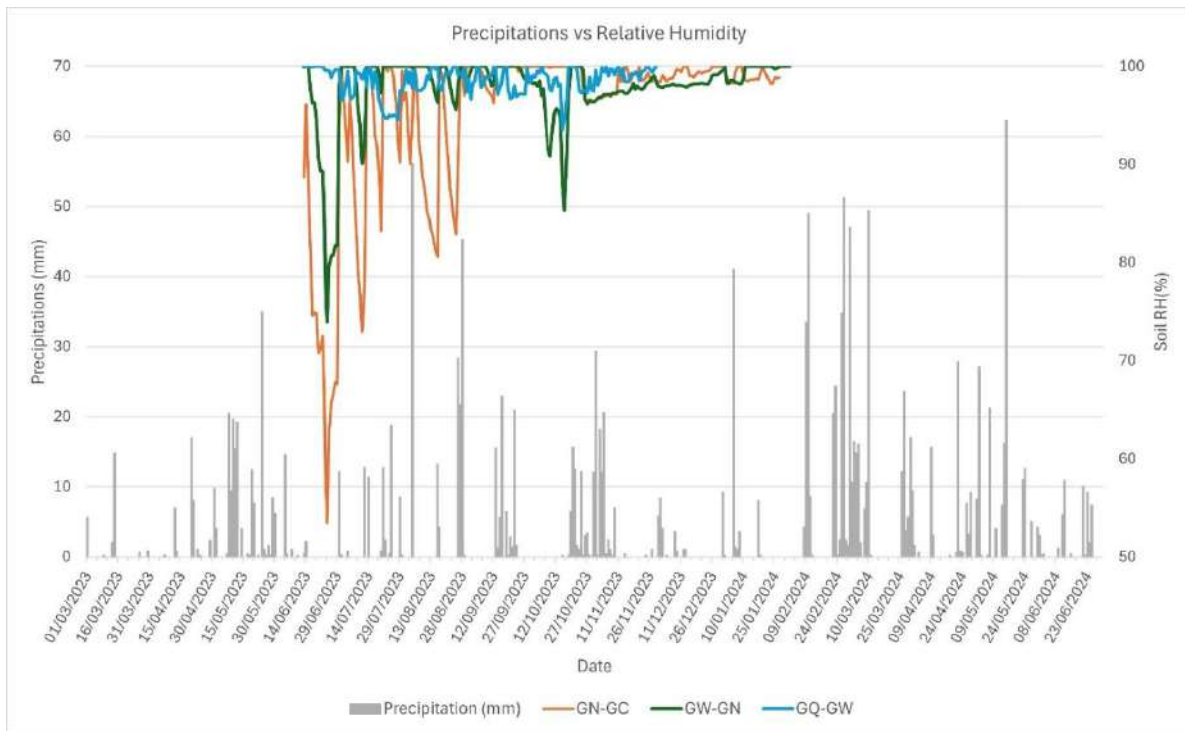


Figure 2.14: Comparison of the daily precipitations recorded by the weather station and the daily mean soil relative humidity recorded by the three Data Logger.

THE EXPERIMENT

HYPOGEAL CADAVER DECOMPOSITION ISLAND

This chapter presents the materials and methods adopted for sampling and for the analyses conducted on the HCDIs associated with the burials, together with the results obtained. Particular attention was devoted to the characterization of the hypogeal matrix, namely the soil, in order to better understand how the soil type defining the experimental area influences the decomposition processes of buried bodies and its capacity to retain associated biomarkers. The HCDI was investigated to assess the effective presence of such markers and to evaluate their potential role in supporting PMI/PBI estimation.

3.1. Materials & Methods

3.1.1. Materials

The investigation of the HCDI was conducted through analyses of soil samples collected beneath the cadavers during each exhumation. The latter were carried out with pairs of operators assigned to each grave, each using their own trowels, which were employed exclusively for that single grave in order to avoid cross-contamination. After each recovery, soil samples were collected from the HCDI, below the cadavers. Owing to the small size of the bodies (Chapter 4) and their “crouched” position, it was not possible to subdivide the HCDI by anatomical districts. Consequently, soil samples were taken from the center of the chromatically altered area at the bottom of the graves (Figure 3.1a), identified as potentially rich in biomarkers and measurable biogeochemical alterations (Vass, 2017).

For each HCDI, two soil samples of approximately 900 g each (including gravel and pebbles) were collected and stored in clean plastic bags sealed hermetically: one was air-dried at room temperature for pH, DOM, FT-IR, and electromagnetic analyses while the other was frozen at -18°C for organic acids HPLC analysis. Electromagnetic analyses were performed exclusively on samples from the GC group. This choice reflects the exploratory nature of the experiment, for which an initially limited sample set was preferred. Samples from the GW and GQ groups

3. The Experiment - Hypogeal Cadaver Decomposition Island

were excluded due to the potential influence of the applied treatments on the measurements, whereas the GN group was excluded because specimen GN2 was not recovered.

To assess the anomalies induced by the presence of the decomposing body, control soil samples were collected from the bottom of the graves prior to deposition using the same procedures. Conversely, for TOC analysis, soil samples were collected every 10 cm (0-50 cm depth) from the burial walls before inhumation and from the grave fill during recovery of the remains, in order to detect TOC concentrations throughout the entire profile (Figure 3.1b). Ultimately, control grave was dug to assess the natural soil profile of the area.

Therefore, the analyses were performed on:

- 40 air-dried soil samples: 20 from the bottom of the graves prior to burial + 20 from the HCDIs for the decomposition-related analyses.
- 40 frozen soil samples: 20 from the bottom of the graves prior to burial + 20 from the HCDIs for HPLC analyses.
- 99 air-dried soil samples: 46 from the burials wall (inhumations) + 53 from the fill (exhumations) for TOC analysis.
- 2 soil samples from the control grave for site characterization.

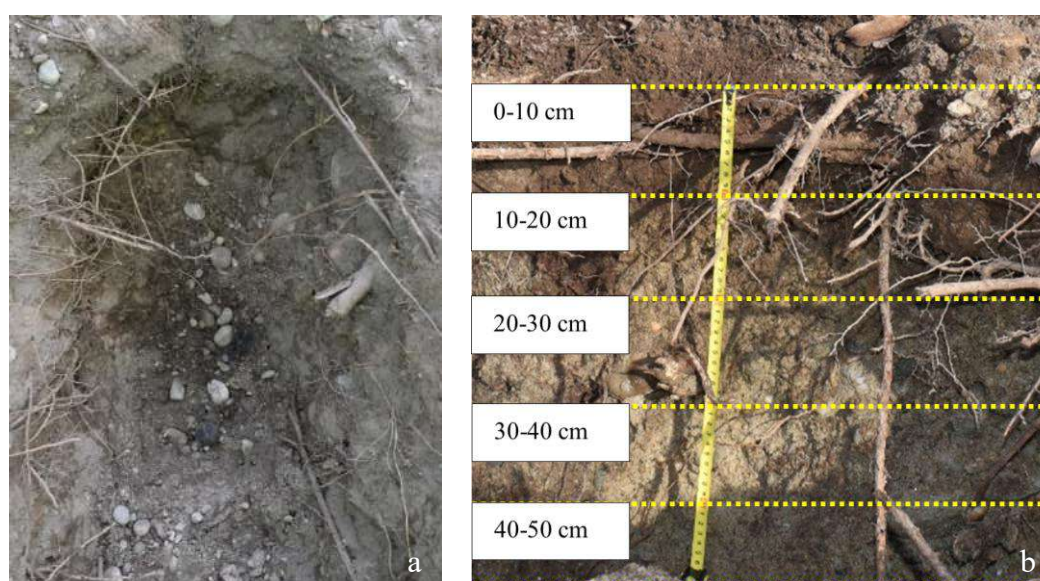


Figure 3.1: *a-* on the left, black-stained area detected as HCDI from the bottom of one of the graves (GW4); *b-* on the right, sampling scheme for TOC analysis.

3.1.2. Methods

3.1.2.1. Field Activity: Site and Soil Description

For the characterization of the site and soils in the experimental area, the profile of the control grave was observed and described in the field following the FAO Guidelines for Soil Description (FAO, 2006). The characteristics of the site and its surface features were considered common to all four burial groups. Their description involved the preliminary collection of data on the geographical location of the study area, integrated with the coordinates of the sampling site and its main geomorphological features. Subsequently, surface data were gathered either through direct field observations or derived from published sources. These data included rockiness, stoniness, soil erosion, land use, vegetation, parent material, geological formation, geomorphology, and pedology.

The next step involves the description of the soil profile. For this purpose, it is first necessary to identify any variations that allow the distinction of macro-units, such as the presence of an undecomposed litter layer in the uppermost portion of the profile, as well as differences in color, texture, and structure. These macro-units are then subdivided into horizons. Finally, each horizon is documented and described in detail.

In particular, the following parameters are considered: thickness, shape of the lower boundary, degree of moisture (assessed by finger manipulation and observation of porosity), color and mottling, fine earth texture, presence of coarse fragments (> 2 mm) in terms of frequency, size, lithology, degree of weathering, and shape, degree of particle aggregation, porosity, and presence of roots. The horizons thus defined are designated by single capital letters; however, when two different types of horizon properties are simultaneously present, two capital letters may be used together, with the first indicating the dominant characteristic.

3.1.2.2. Laboratory Soil Characterization

Laboratory analyses were carried out in the Sediments and Soils Laboratory of the *Ardito Desio* Department of Earth Sciences at the University of Milan. Specifically, particle-size analysis and color determination were performed on the soil samples collected from the control grave (Gale & Hoare, 1991; Ministero per le Politiche Agricole, 1999).

For the particle-size analysis, it was necessary to separate the mineral fraction into conventional grain-size classes, namely:

- *Gravel*: particles with a diameter greater than 2000 μm
- *Sand*: particles with a diameter between 2000 μm and 63 μm
- *Silt*: particles with a diameter between 63 μm and 2 μm
- *Clay*: particles with a diameter smaller than 2 μm

Before this procedure, the samples were air-dried, after which their weight was determined using a precision balance. The coarse fraction ($> 2000 \mu\text{m}$) was then separated from the fine earth ($< 2000 \mu\text{m}$) using a 2 mm stainless-steel sieve. Because aggregates were present, they were manually broken to facilitate sieving, while the coarse fraction was washed under running water to remove fine particles and subsequently weighed after drying.

The resulting fine earth was subjected to quartering, a technique used to divide the material into homogeneous sub-samples weighing between 90 g and 150 g, representative of the original sample. The quartered samples were then transferred to a beaker and treated with hydrogen peroxide (130-volume concentration). This treatment was necessary because the presence of organic matter would promote particle aggregation, thereby interfering with the particle-size analyses.

Following this treatment, wet sieving was performed to separate the sand fraction (2000 μm -63 μm). This technique consists of passing the sample through a stack of ten stainless-steel sieves with progressively smaller mesh openings: 1400 μm , 1000 μm , 710 μm , 500 μm , 355 μm , 250 μm , 180 μm , 125 μm , 90 μm , and 63 μm .

A stream of water and gentle pressure applied with the fingertips facilitated the passage of material from one sieve to the next. Once all material was sieved, each fraction was oven-dried

3. The Experiment - Hypogeal Cadaver Decomposition Island

at approximately 40°C. The finer material passing through the 63 µm sieve was then collected, allowed to settle in a basin placed at the base of the column, and then air-dried.

Subsequently, the samples underwent a second sieving, this time dry, by reassembling the sieve stack. The dry sieving was carried out using a mechanical shaker for 20 minutes (Figure 3.2).

At the end of the procedure, the material retained on each sieve was weighed individually, while the fraction passing through ($< 63\text{ }\mu\text{m}$), consisting of silt and clay, was subjected to particle-size analysis by sedimentation (aerometry). Initially, 3 g of sodium hexametaphosphate, a deflocculating agent, were added to each sample to prevent the formation of flocs that develop among clay particles when placed in suspension. Each sample was then agitated for 20 minutes. The aerometric procedure is based on Stokes' law, which relates the settling velocity of suspended particles to their size: larger particles settle more rapidly, whereas smaller ones settle more slowly (Gale & Hoare, 1991; Mazzoldi et al., 2010). As time progresses, the density of the suspension decreases as an increasing proportion of particles settle. Consequently, the settling velocity can be inferred indirectly by measuring the density of the suspension.



Figure 3.2: Sieve stack placed on the mechanical shaker.

3. The Experiment - Hypogeal Cadaver Decomposition Island

After approximately 24 hours, and prior to analysis, the samples were agitated for an additional 5 minutes and then transferred into 2 L graduated cylinders, which were brought to volume with demineralized water. The samples were then manually mixed with a plunger (to resuspend any particles that had settled), and density measurements were initiated.

The measurements were accompanied by temperature readings taken with a thermometer, in order to correct for any density measurement errors caused by temperature variations in the suspension. Both parameters were recorded at predetermined time intervals starting from the end of agitation: 1 min, 2 min, 5 min, 10 min, 20 min, 40 min, 1 h 20 min, 4 h, and 24 h.

The values collected were then entered into conversion tables capable of providing the percentage of each grain-size class for the sample.

Conversely, color analysis was performed only on the fine fraction. Color is a soil characteristic used as a diagnostic criterion because it reflects various soil properties and conditions. To determine soil color, the *Munsell Soil Color Charts* were employed (Figure 3.3 - Munsell Color, 1994).



Figure 3.3: *Munsell Soil Color Charts used for the color determination.*

3. *The Experiment - Hypogeal Cadaver Decomposition Island*

This classification system is based on three elements:

- *Hue*: the primary color, indicated by a capital letter (R for red, Y for yellow, G for green, B for blue, YR for intermediate yellow-red hues, etc.) preceded by numbers from 2.5 to 10.
- *Value*: the lightness, ranging from 0 to 10. Lighter colors are indicated by numbers from 10 to 5, and darker colors from 5 to 0. The number is placed after the hue designation.
- *Chroma*: the index of color purity, expressed by numbers from 0 to 8. The higher the number, the more vivid the color. The chroma value follows the value number and is preceded by a “/”.

The use of this system allows soil colors to be identified objectively. However, as color varies with moisture content, it was determined for each sample under both dry and moist conditions, the latter achieved by lightly moistening the sample with water.

3.1.2.3. Total Organic Carbon (Walkley-Black Method)

Among the decomposition-related analyses performed on soil samples collected from the bottom of the graves prior to burial and from the HCDIs after exhumation was the measurement of Total Organic Carbon (TOC). These analyses were conducted at the Sediments and Soils Laboratory of the *Ardito Desio* Department of Earth Sciences and were limited to samples collected at PBIs 1 and 2. This choice reflected an attempt to employ TOC as a preliminary proxy to assess the vertical distribution of organic matter within the soil profile before burial and to identify potential anomalies associated with the presence of a decomposing body following exhumation. However, as illustrated in Section 3.3 and discussed in Chapter 5, this approach proved ineffective from the outset; consequently, the analyses were discontinued.

Various methods are available for measuring carbon, including the so-called indirect methods, which determine the reducing power of the soil using an oxidizing agent. The Walkley & Black method (1933) is one such procedure and is based on the assumption that organic carbon is the principal reducing agent in soil, with other reducing substances present in smaller and relatively constant amounts. Because indirect methods do not achieve complete oxidation, the values obtained typically represent only about 70-80% of the TOC. The Walkley & Black method is a wet-chemistry procedure in which the reagents are in aqueous solution and the organic matter (particularly the organic carbon) is removed by chemical oxidation. The oxidizing reagents are added in fixed, excess amounts relative to those required for complete oxidation of the sample, so that the residual oxidant, equal to the difference between the initial amount added and the amount that reacted with the organic matter, can subsequently be quantified by titration. Since the amount of oxidant added is constant, the variable that must be adjusted according to the expected organic matter content is the weight of the sample to be analyzed. The method therefore requires an approximate estimation of the organic matter content based on the recovery context, weighing a smaller amount of sample when a high organic content is expected and, conversely, a larger amount when the material is more mineral-rich. Sample weights typically range from about 0.2 g for highly organic soils to up to 1.5 g for mineral-rich ones (Figure 3.4). Blank tests (performed without soil samples) were carried out beforehand to calibrate the system. The soil samples to be analyzed were weighed into flasks, after which the reaction was initiated by adding the oxidizing agent to each sample.

3. The Experiment - Hypogeal Cadaver Decomposition Island



Figure 3.4: Flasks containing soil samples from the HCDIs used for TOC determination.

After 30 minutes, the oxidation process was stopped by lowering the temperature through the addition of water. The residual oxidant not consumed in the reaction was then titrated using a reducing agent and a redox indicator; the titration was considered complete when the solution turned bright green.

The reagents used for these analyses are as follows (Figure 3.5):

For the oxidation:

- 10 mL of aqueous potassium dichromate ($K_2Cr_2O_7$) solution
- 20 mL of 96% sulfuric acid (H_2SO_4)
- Water to stop the reaction, for a total volume of 200 mL

For the titration:

- Aqueous ammonium iron(II) sulfate solution $(NH_4)_2Fe(SO_4)_2 \cdot 6 H_2O$
- 5 mL of 85% phosphoric acid (H_3PO_4)
- 0.5 mL of diphenylamine solution in sulfuric acid $[(C_6H_5)_2NH]$

3. The Experiment - Hypogeal Cadaver Decomposition Island



Figure 3.5: Reagents used for the analyses. On the left potassium dichromate (orange); on the right ammonium iron(II) sulfate solution (green).

3.1.2.4. pH Analysis

Soil reaction (pH) is another parameter that was considered in this study to evaluate decomposition-related processes in HCDI.

Before the actual analysis, performed as well at the Sediments and Soils Laboratory of the *Ardito Desio* Department of Earth Sciences, a suspension was prepared consisting of 10 g of soil sample sieved to 2 mm and 25 mL of double-distilled water (1:2.5 soil-to-water ratio). The samples were then shaken for 20 minutes using a mechanical shaker (Figure 3.6a) and, after resting for approximately 24 hours, shaken again for 5 minutes before analysis.

The pH determination was carried out with a benchtop multiparameter instrument, model PC8 + DHS Stirrer (Zetalab; Figure 3.6b), previously calibrated at pH 4 and 7, by immersing two probes in the suspension: one for pH measurement and one for temperature. Every measurement was repeated three times per sample.

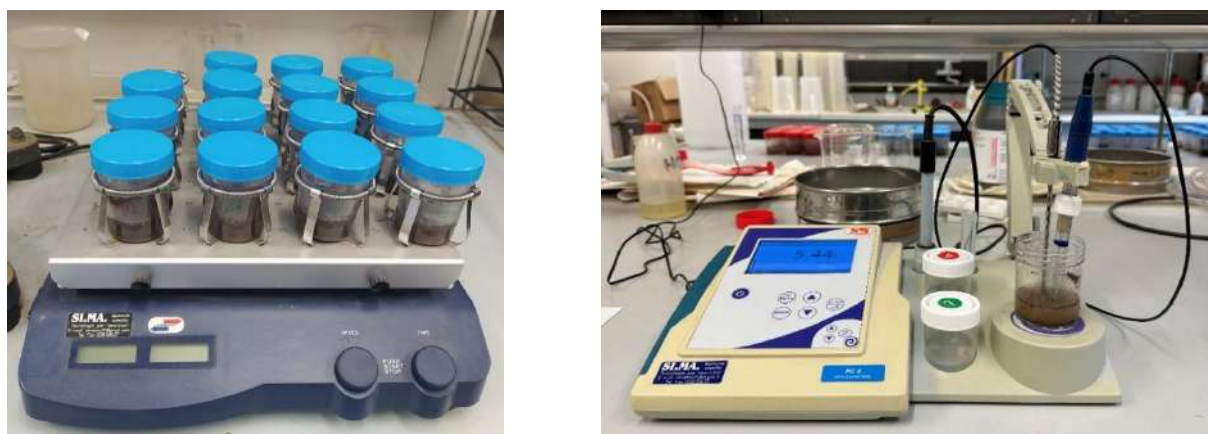


Figure 3.6: Laboratory equipment used for pH analysis. **a-** on the left, samples placed in the mechanical shaker; **b-** on the right, benchtop multiparameter model PC8+DHS Stirrer (Zetalab).

3.1.2.5. Dissolved Organic Matter & Fourier-Transform Infrared Spectroscopy

Dissolved organic matter (DOM) was also considered as one of the proxies potentially related to the presence of a decomposing body in a clandestine grave. The analysis was carried out at the DISAA laboratories (Department of Agricultural and Environmental Sciences – Production, Landscape, Agroenergy) of the University of Milan, as described in Bona et al. (2023).

For this procedure, 20 g of soil sieved to 2 mm were placed in a Falcon tube, to which 30 mL of deionized water were added. The mixtures were then agitated on a rotary shaker for 30 minutes, after which the tubes were centrifuged for 15 minutes at 6000 rpm. The resulting supernatant was filtered through a 0.45 μm vacuum filter (Büchner) to isolate only the dissolved organic matter (DOM) fraction present in the sample.

To analyze its concentration, 2 ml of solution were transferred in the Tube tests NANOCOLOR COD (Macherey-Nagel). The system implies the use of a kit of potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) and sulfuric acid (H_2SO_4) which reacts with the sample solution, leading to decomposition of the organic matter at 160°C for 30 (by means of a heating block). The final solution was ultimately analyzed with photometer EST-903 PF-12Plus for calculating the concentration of organic carbon (mg L^{-1}), which was finally converted to DOM concentration.

3. The Experiment - Hypogeal Cadaver Decomposition Island

Moreover, the DOM samples were freeze-dried and underwent Fourier Transform Infrared (FT-IR) Spectroscopy by means of IRAffinity-1S (Shimadzu; Figure 3.7 a,b) in order to detect the organic compounds that composed the organic matter in the sample (Gigliotti et al., 1997). Finally, the spectra were manipulated with LabSolutions IR Advanced Software.

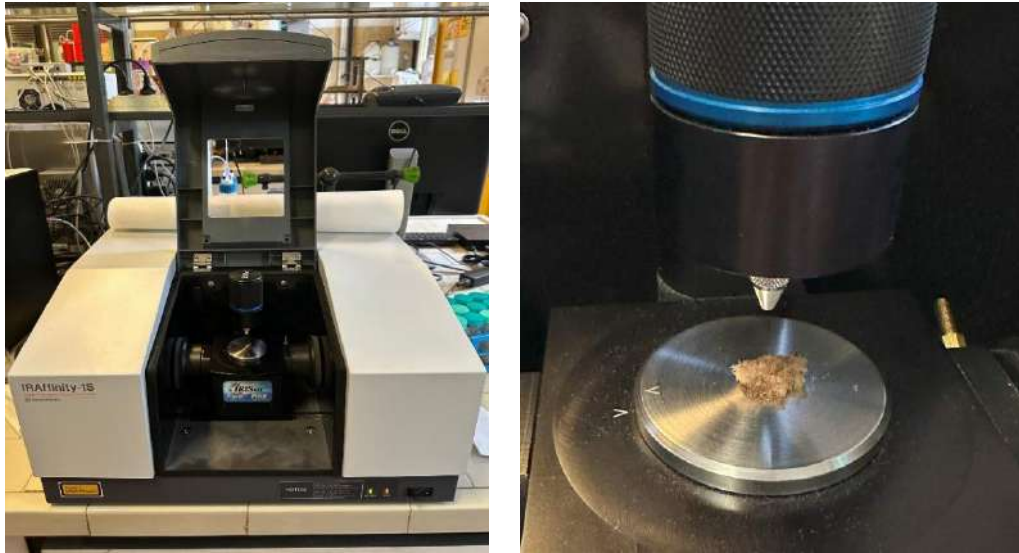


Figure 3.7: *a- on the left the FT-IR Spectrophotometer IRAffinity-1S (Shimadzu); b- on the right a freeze-dried DOM sample in the spectrophotometer.*

3.1.2.6. Organic Acids (High-Performance Liquid Chromatography)

Other analyses aimed at detecting decomposition-related organic acids, such as volatile fatty acids (VFA), lactic and succinic acids, were carried out at the DISAA laboratories on HCDI soil samples preserved by freezing, following the method proposed by Canale et al. (1984).

For sample preparation, 5 g of fresh (non-dried) soil were placed in Falcon tubes, to which 10 mL of 0.05 M sulfuric acid (H_2SO_4) were added, maintaining a silage/ H_2SO_4 extraction ratio of 1:2 (w/w).

The samples were then shaken for 10 minutes and centrifuged for 10 minutes at 7000 rpm. Subsequently, the solution was filtered through a 0.22 μm filter, and 2 mL of the filtrate were transferred into vials for analysis by High-Performance Liquid Chromatography (HPLC) coupled with UV detection at 210 nm using a CTO-20A column oven (Shimadzu; Figure 3.8).

The method was based on external calibration using pure analytical standards. An internal standard was not employed, as it is generally not required when working with HPLC-UV systems and uniformly treated samples; moreover, instrumental performance remained stable throughout all analytical sessions.



Figure 3.8: The HPL Chromatograph used for the analysis. On the right, CTO-20A column oven.

3. The Experiment - Hypogeal Cadaver Decomposition Island

The analytical standards for the selected organic acids were prepared, injected into vials, and analyzed with the chromatograph. The retention time (RT) for each acid was determined as follows:

Volatile Fatty Acids:

- Acetic acid [$\text{CH}_3\text{--COOH}$]: 18 min
- Propionic acid [$\text{CH}_3\text{--CH}_2\text{--COOH}$]: 20–21 min
- Butyric acid [$\text{CH}_3\text{--CH}_2\text{--CH}_2\text{--COOH}$]: 26–27 min
- Isovaleric acid [$(\text{CH}_3)_2\text{CH--CH}_2\text{--COOH}$]: 28–31 min

Other organic acids:

- Succinic acid [$\text{HOOC--CH}_2\text{--CH}_2\text{--COOH}$]: 11 min
- Lactic acid [$\text{CH}_3\text{--CH(OH)--COOH}$]: 12–13 min

Reagent:

- Sulfuric acid (H_2SO_4): 8 min

Calibration curves for organic acids were also established accordingly. The best statistical fit for all analytes was obtained using a linear regression with intercept, with coefficients of determination $R^2 > 0.98$.

For each sample, the instrument produces a spectrum showing peaks at specific retention times (RT) characteristic of the organic compounds, with each peak area representing their relative abundance (Figure 3.9).

3. The Experiment - Hypogeal Cadaver Decomposition Island

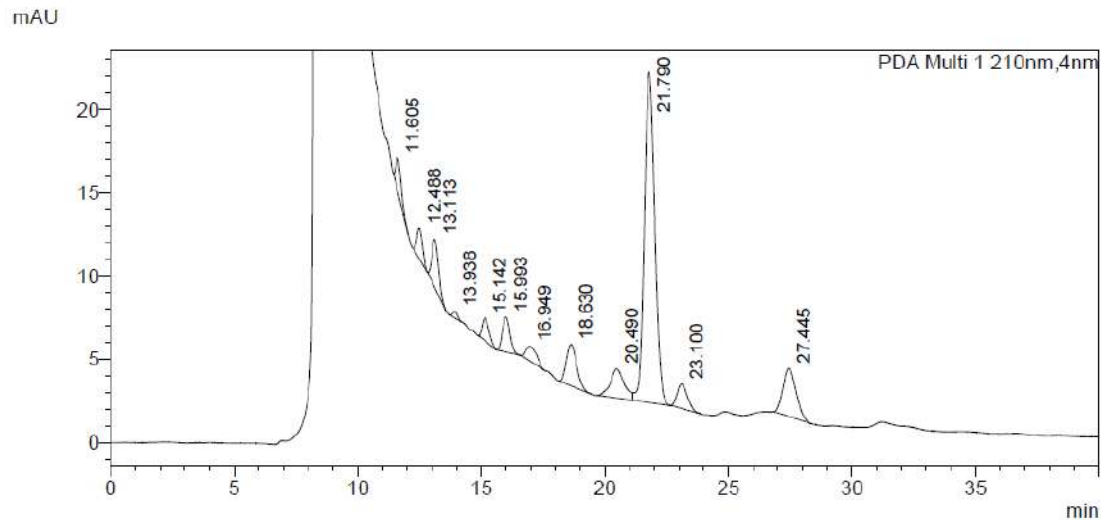


Figure 3.9: Example of organic acids spectrum.

To convert these data into absolute concentrations relative to the sample, it was first necessary to determine the dry matter content by oven-drying them. 2 g of fresh soil samples were placed in an oven at 100°C for 24 hours, then reweighed, and the percentage of dry matter was calculated.

The dry soil weight was then obtained using the following formula:

$$\text{dry soil (g)} = \text{sample weigh (\sim 5g)} \times \frac{\% \text{ dry matter}}{100}$$

In parallel, the gross concentrations (mg soil kg⁻¹ or ppm) of the organic acids were calculated from the peak areas and normalized using the calibration curve for each acid:

Succinic acid

$$\text{concentration} = \frac{(\text{peak area})+318.75}{1013.4}$$

Lactic acid

$$\text{concentration} = \frac{(\text{peak area})+1078.8}{1252.9}$$

Acetic acid

$$\text{concentration} = \frac{(\text{peak area})+12869}{1245.7}$$

Propionic acid

$$\text{concentration} = \frac{(\text{peak area})+10450}{866.28}$$

3. The Experiment - Hypogeal Cadaver Decomposition Island

<i>Butyric acid</i> concentration = $\frac{(\text{peak area})-9898.9}{440.27}$	<i>Isovaleric acid</i> concentration = $\frac{(\text{peak area})-14446}{321.16}$

Finally, the correct concentration (mg soil kg⁻¹ or ppm) of each analyte, corrected for dry matter, was calculated as follows:

$$\text{Corrected concentration (mg soil kg}^{-1} \text{ or ppm)} = \text{gross concentration} \times \frac{10}{\text{dry soil (g)}}$$

3.1.2.7. Magnetic Susceptibility & Hysteresis Analyses

Magnetic analyses were carried out at LASA (Applied Superconductivity and Accelerators Laboratory) of INFN (Italian National Institute for Nuclear Physics), University of Milan.

For these analyses, 40 g of soil were collected from the HCDIs of the GC group only. This choice was made because of the pioneering and highly experimental nature of applying these techniques to HCDI. The GQ and GW groups were excluded on the assumption that the treatments applied to the piglets could alter the results, while the GN group was excluded due to the loss of one specimen, an event that would have compromised a complete assessment of the evolution of these parameters.

In particular, magnetic susceptibility (χ) measures the ease with which a material becomes magnetized when subjected to an external magnetic field (Day et al., 1977). In this study, frequency-dependent magnetic susceptibility was determined using the Bartington MS2-B sensor, which allows precise and rapid measurements at both low and high frequencies.

For sample preparation, a coarse manual homogenization was first performed to reduce intrinsic variability. Then, 10 g of each sample were collected and pressed into sample holders with a capacity of approximately 20 cc (Figure 3.10).

3. The Experiment - Hypogeal Cadaver Decomposition Island



Figure 3.10: two samples of soil from the HCDIs prepared for the susceptibility analysis.

For the magnetic susceptibility analysis, three measurements were performed for each sample, for a total of 30 measurements (3×5 control soil + 3×5 HCDI).

By contrast, the magnetic hysteresis curve describes the relationship between the magnetization (M) of a material and the applied magnetic field (H), showing the sample's response to increasing and decreasing field strength. When analyzing a magnetic hysteresis curve, it is essential to distinguish between the uncorrected (raw) curve and the corrected (manipulated) curve. The raw curve includes paramagnetic contributions (e.g., pyroxenes, amphiboles, micas) and diamagnetic contributions (e.g., calcite and quartz), which affect the slope of the curve. These effects are superimposed on the ferromagnetic response that constitutes hysteresis. Conversely, the corrected (manipulated) hysteresis curve is free of para- and diamagnetic contributions.

The corrected hysteresis curve provides four key parameters (Basavaiah & Khadkikar, 2004; Figures 3.11a-d):

- Saturation magnetization (Ms)
- Saturation remanence (Mr)
- Coercivity (Hc)
- Remanence coercivity (Hcr)

The magnitude of these parameters indicates the nature of the particles present in the sample (Maher, 1988). Moreover, the coercivity of remanence (Hcr) is derived from the Direct Current

3. The Experiment - Hypogeal Cadaver Decomposition Island

Demagnetization (DCD) curve, which allows a clearer distinction of the domain state of the grains.

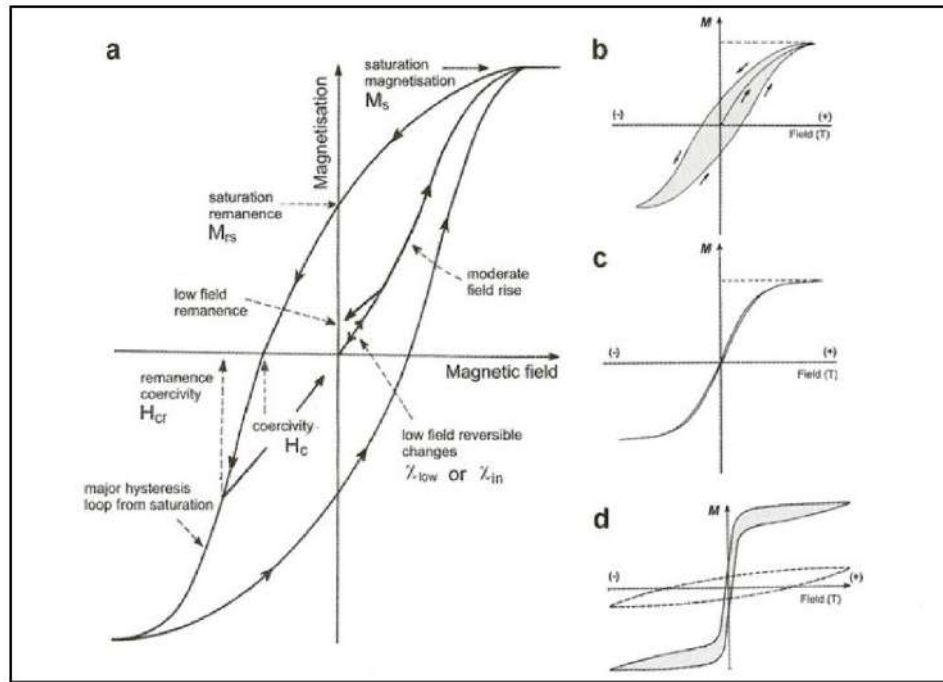


Figure 3.11: Examples of hysteresis curves from Basavaiah & Khadkikar, 2004: **a-** general hysteresis curve showing the parameters measured during mineral magnetic experiments; **b-** hysteresis curves for SSD magnetite; **c-** MD; **d-** hematite (dotted line) and two components (hematite and magnetite) hysteresis curves.

For the acquisition of hysteresis and DCD curves, a vibrating sample magnetometer (VSM) MicroSense EZ7 (Figure 3.12) was used. This instrument measures magnetization as a function of the applied magnetic field up to saturation and provides accurate calculations of the associated parameters (M_s , M_r , H_c , H_{cr}). For sample preparation, approximately 1 cc of soil was collected and pressed into dedicated sample holders, carefully eliminating any voids between particles to prevent vibration during analysis.

The sample holder was then secured to a quartz rod and positioned between the instrument's two dipoles. Ultimately, the software was configured to a saturation range (in this case, from -1.5 to 1.5 T). This procedure generated two hysteresis curves for each sample: an uncorrected (raw) curve and a corrected (manipulated) one.

3. The Experiment - Hypogeal Cadaver Decomposition Island



Figure 3.12: Vibrating sample magnetometer (VSM) MicroSense EZ7. Top left: sample mounted on the quartz rod and positioned between the instrument dipoles (image by Ottavia Gigliotti).

The data obtained from the hysteresis curves can be used to classify the magnetic domain state of the samples using the Day diagram (Day et al., 1977), which is based on two ratios derived from the magnetic hysteresis curve:

- The x-axis shows the ratio of coercivity of remanence (H_{cr}) to coercivity (H_c): H_{cr}/H_c
- The y-axis shows the ratio of saturation remanence (M_r) to saturation magnetization (M_s): M_r/M_s

For constructing the diagram, the magnetic parameters should be taken from the corrected (manipulated) hysteresis curve, which is free of para- and diamagnetic contributions, so as to represent only the behavior of the ferromagnetic minerals. The position on the diagram provides information about the magnetic domain state of the grains present in the sample.

3.2. Results

Chapter 3 focuses on the biogeochemical response of HCDI to the presence of a decomposing cadaver buried in a clandestine grave. Therefore, in the following sections, the collected data are presented starting with the description and characterization of the site and soil of the experimental area, followed by the results of chemical and magnetic analyses performed on soil samples.

3.2.1. Site and Soil Characterization

This section presents the field description of the experimental area in the FTF followed by the results of grain-size, color and pH analyses on soil samples from the control grave.

Site and surface data are common to all four burial groups and are presented as follows:

Site data

Location: Ticino-LEAFs (Parco Lombardo della Valle del Ticino)

Survey area: Cassolnovo (PV)

Date: 03/03/2023

Slope: flat

Coordinates (WGS84): Lat. 45°23'22.6"N, Long. 8°50'09.2"E

Elevation: 100 m a.s.l.

Type of excavation: backhoe excavation

Validity range of observations: tens of meters

3. The Experiment - Hypogeal Cadaver Decomposition Island

Surface data

Rockiness: absent

Stoniness: absent

Soil erosion: absent

Land use: deciduous forest of medium to high density managed as coppice

Vegetation: Pedunculate oak woodland (*Quercus robur*), often replaced by communities dominated by *Robinia pseudoacacia* (black locust) and *Prunus serotina* (black cherry). Canopy cover: 50–75%

Parent material: coarse alluvial deposits

Geological formation: Terraced gravelly-sandy or silty alluvium (recent/modern Holocene)

Geomorphology: Alluvial plain; gravelly fluvioglacial deposits; well-preserved traces of an extinct river channel and slightly raised fluvial ridges

Geopedology: Soil Taxonomy (1992): *Typic Udorthens sandy-skeletal mixed, mesic*; WRB (2014): *Dystric Leptosols* (from Carta Pedologica scale 1: 37.500)

Profile description

Substrate: easily accessible

Litter: abundant, composed of pedunculate oak and black locust leaves

Macrounit distinction: single unit

3. The Experiment - Hypogeal Cadaver Decomposition Island

With regard to the description of the control grave profile documented in the field, it was recorded as follows:

OA 0-20 cm	Clear, linear lower boundary; moist; matrix color: dry 10YR3/3, moist 10YR2/2; no mottling; texture: sandy silt; skeleton: absent; fine granular structure; porosity: macro- and micropores; roots from very fine (common) to coarse (frequent).	
C 20-50+ cm	Moist; matrix color: dry 2.5Y4/2, moist 2.5Y3/2; no mottling; texture: sandy; skeleton: dominant pebbles and cobbles; structure: loose; porosity: macropores; medium and coarse roots frequent.	

Within the profile, two horizons were identified: a surface horizon extending from 0 to 20 cm depth and a second horizon from 20 cm to the bottom of the pit at approximately 55 cm.

The sample from the 0-20 cm horizon had a total mass of 670.53 g, of which 5.37 g consisted of gravel (0.8%), while the remaining 665.16 g (99.2%) was fine earth. The fine fraction comprised 37.35% sand, 57.63% silt, and 4.22% clay, giving a sandy-silt texture.

In contrast, the sample from the 20–55 cm horizon had a total mass of 3643.31 g, with 2481.08 g of gravel (68.10%) and 1162.23 g of fine earth (31.90%). The fine earth consisted of 31.26% sand and 0.64% silt, with no clay detected, resulting in a sandy texture (Figure 3.13).

3. The Experiment - Hypogeal Cadaver Decomposition Island

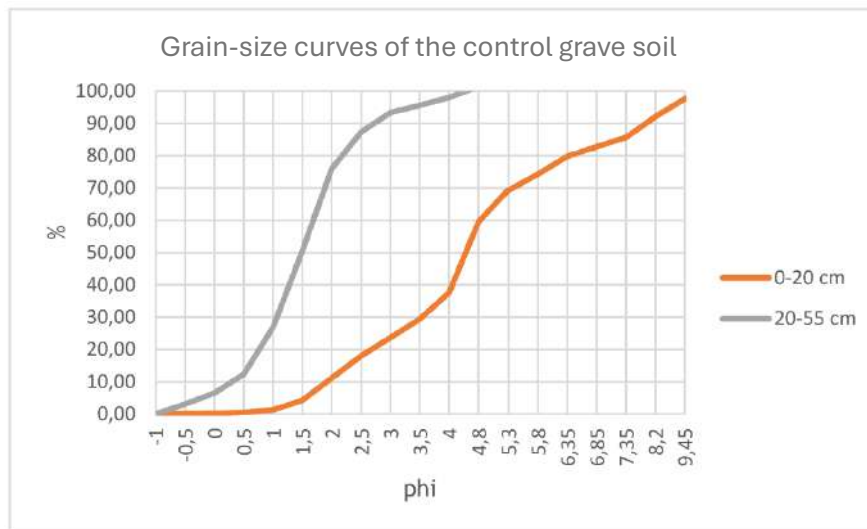


Figure 3.13: Graph showing the cumulative particle-size distribution curves of the fine earth from both horizons.

Moreover, the 20–55 cm horizon consisted predominantly of gravel, with the remaining portion composed mainly of sand and an extremely small silt fraction (less than 1%).

In contrast, the surface horizon (0–20 cm) contained a higher proportion of fine material, composed primarily of sand and silt with a smaller percentage of clay. In this upper horizon, gravel accounted for less than 1%.

Overall, these data indicated that gravel content increased with depth, while sand and silt decreased accordingly (Figure 3.14).

3. The Experiment - Hypogeal Cadaver Decomposition Island

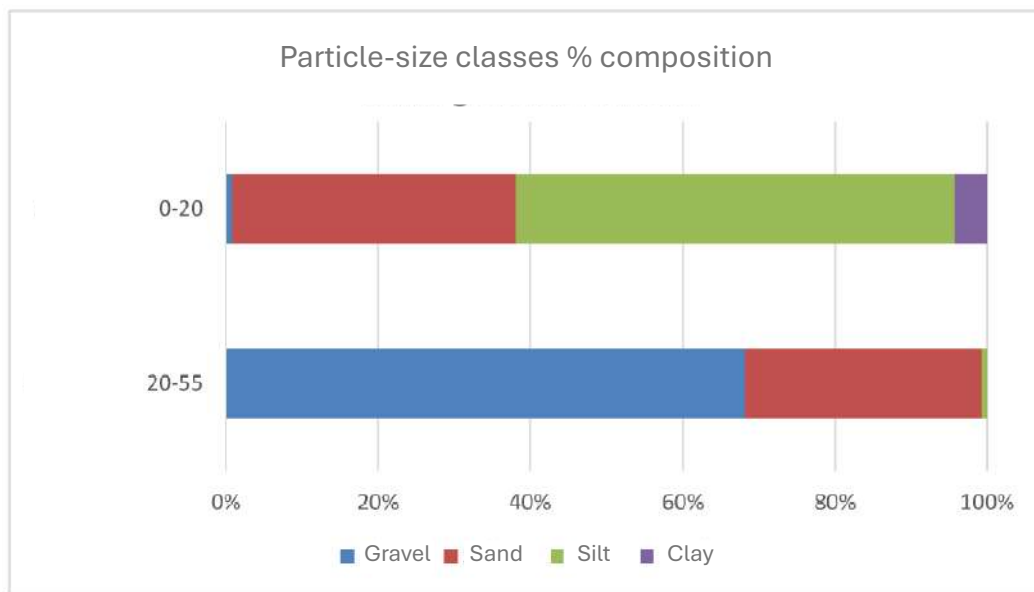


Figure 3.14: Graph showing the relative abundances of the particle-size classes for both horizons.

Ultimately, the Munsell Soil Color Charts allowed us to correct the color analysis on the samples from the two horizons: 0-20 cm horizon was described as 10YR 3/2 (dry) and 10YR 2/1 (moist), while 20-55 cm horizon was described as 2.5Y 7/2 (dry) and 2.5Y 5/3 (moist).

Regarding pH analysis, the surface horizon had $\text{pH } 3.9 \pm 0.4$ while the 20–55 cm horizon had $\text{pH } 5.5 \pm 0.6$. The soil could therefore be described as acid/subacid.

3.2.2. Total Organic Carbon (TOC)

This section presents the results of the TOC analysis performed on the samples from PBIs 1 and 2 only. It provides a general description of TOC trends across the two PBIs, supported by graphical representations, while the raw data are reported in the Appendix (Tables A.2–A.5, pp. 342–345).

Below are the results of the analyses comparing PBI0 (before inhumations - *IN*) and PBI1 (first set of exhumations – 18 days; *EX*) from the same grave, presented by burial type.

In IN GN1, the soil profile showed a sharp decrease in carbon within the first 20 cm of depth, from 91.54 g/kg to 13.56 g/kg, followed by a further decline to just above 4 g/kg in the last 30 cm. Conversely, EX GN1 displayed a more gradual decrease, from 48.50 g/kg to 26.48 g/kg in the first 40 cm, and a slight increase in the final three samples, including the Pig and HCDI samples, with values rising from 10.13 g/kg to 16.26 g/kg (Figure 3.15a).

In IN GW1, carbon content decreased sharply from 70.04 g/kg to 0.92 g/kg between 0 and 40 cm, followed by a slight increase in the last 20 cm, from 1.40 g/kg to 2.42 g/kg. In contrast, in EX GW1, values declined from 15.92 g/kg to 4.22 g/kg between 0 and 50 cm, while in the final 20 cm—corresponding to the Pig and HCDI samples—carbon increased again to 7.64 g/kg and 12.98 g/kg, respectively (Figure 3.15b).

In IN GQ1, carbon content decreased from 74.09 g/kg to 1.32 g/kg between 0 and 30 cm, then showed a slight increase between 30 and 50 cm (1.32 g/kg to 5.79 g/kg), before decreasing again in the last 10 cm to 1.96 g/kg. Conversely, EX GQ1 exhibited a gradual increase from 13.57 g/kg to 46.42 g/kg between 0 and 50 cm, followed by a decrease in the Pig and HCDI samples to 26.03 g/kg and 17.90 g/kg, respectively (Figure 3.15c).

Finally, IN GC1 showed a decrease in carbon from 91.68 g/kg to 5.73 g/kg in the first 50 cm, with a slight increase to 9.03 g/kg in the final 10 cm. In EX GC1, values varied little, ranging from a maximum of 28.45 g/kg in the sample taken between 20 and 30 cm to a minimum of 17.45 g/kg in the HCDI sample (Figure 3.15d).

3. The Experiment - Hypogeal Cadaver Decomposition Island

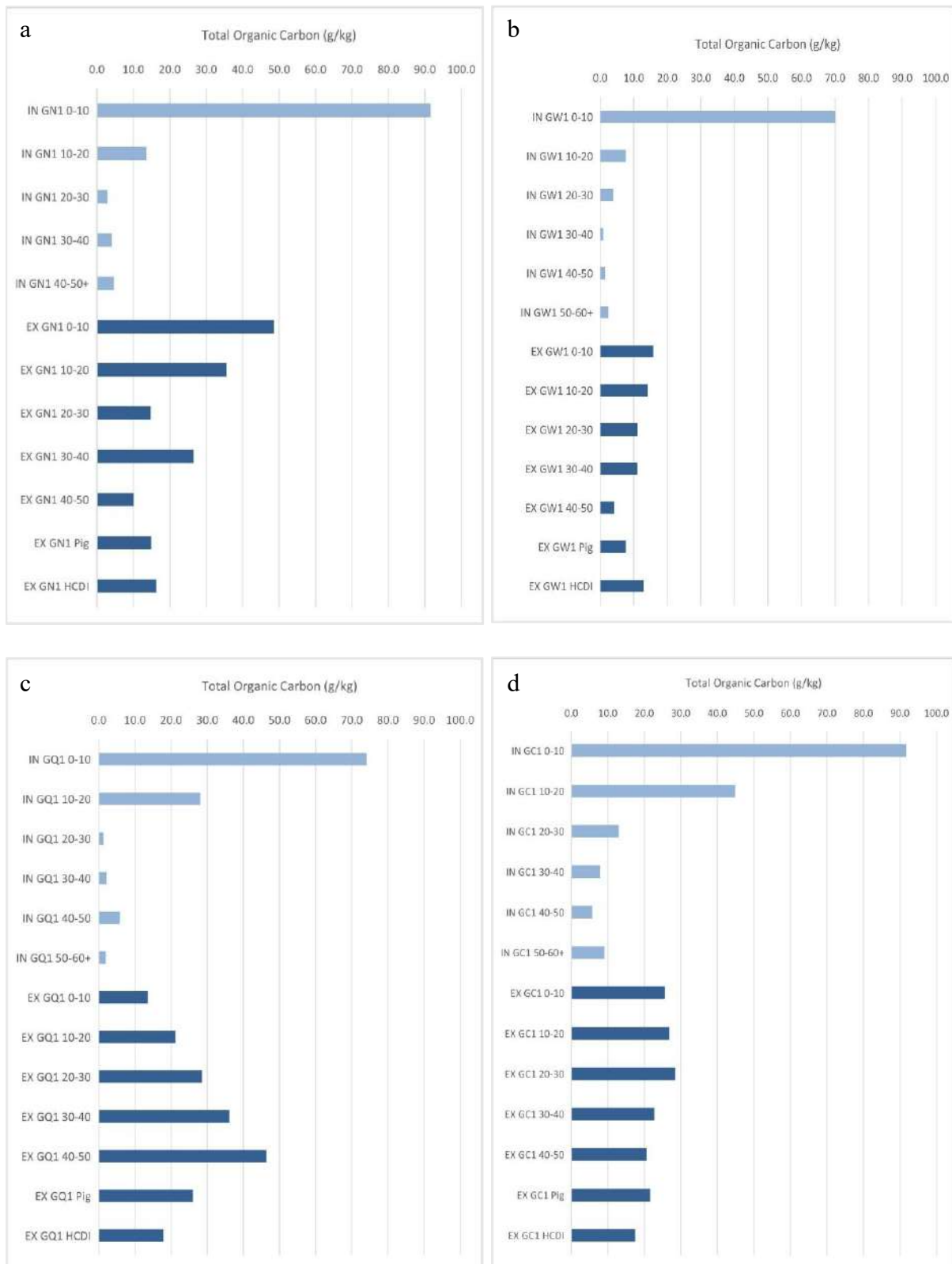


Figure 3.15: Soil TOC concentration in the same grave before the inhumations (IN) and during the first set of exhumations (EX) for each group: **a-** GN; **b-** GW; **c-** GQ; **d-** GC.

3. The Experiment - Hypogeal Cadaver Decomposition Island

Below are the results of the analyses comparing PBI0 (before inhumations - *IN*) and PBI2 (second set of exhumations – 49 days; *EX*) from the same grave, presented by burial type.

In IN GN2, TOC analysis showed a decrease in carbon content from 0 to 50 cm depth, with values dropping from 44.69 g/kg to 2.88 g/kg, and a slight increase in the sample taken between 30 and 40 cm. In the exhumation samples (EX GN2), carbon also decreased down to 50 cm depth, from 19.91 g/kg to 11.58 g/kg, with an increase to 30.85 g/kg in the sample collected between 10 and 20 cm. In the final 10 cm, carbon content rose again to 16.80 g/kg (Figure 3.16a).

In IN GW2, a decrease in carbon content was observed from 0 to 40 cm depth, from 61.37 g/kg to 0.78 g/kg, followed by a slight increase in the final 20 cm, from 1.77 g/kg to 4.08 g/kg. In contrast, EX GW2 showed decreasing values from 41.94 g/kg to 13.12 g/kg between 0 and 40 cm, followed by an increase in the Pig sample to 26.52 g/kg and a subsequent decrease in the HCDI sample to 12.96 g/kg (Figure 3.16b).

In IN GQ2, carbon content decreased from 42.88 g/kg to 3.07 g/kg within the first 50 cm, followed by a slight increase in the last 10 cm to 5.90 g/kg. Conversely, in EX GQ2, carbon increased in the first 20 cm, from 20.49 g/kg to 25.68 g/kg, then decreased down to the Pig sample (reaching 9.80 g/kg), and increased again in the HCDI sample to 13.78 g/kg (Figure 3.16c).

Finally, IN GC2 showed a decrease in carbon content from 60.58 g/kg to 2.50 g/kg in the first 40 cm, followed by a slight increase in the last 20 cm to 3.40 g/kg and 3.43 g/kg. In EX GC2, carbon increased from 8.22 g/kg to 17.25 g/kg within the first 20 cm, then gradually decreased through the subsequent layers down to the Pig sample (17.25 g/kg to 9.20 g/kg), before rising again in the HCDI sample to 15.03 g/kg (Figure 3.16d).

3. The Experiment - Hypogeal Cadaver Decomposition Island

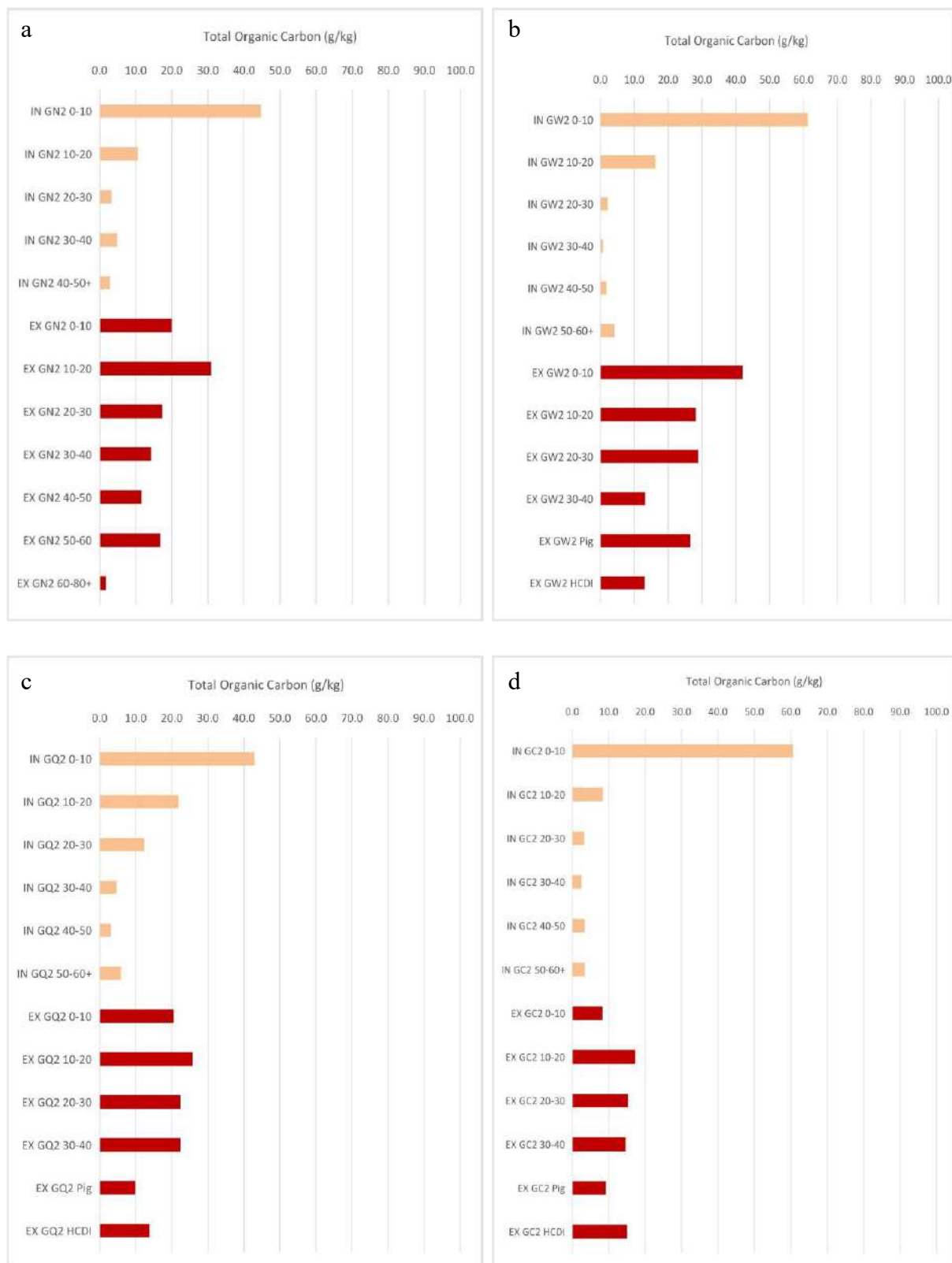


Figure 3.16: Soil TOC concentration in the same grave before the inhumations (IN) and during the second set of exhumations (EX) for each group: **a-** GN; **b-** GW; **c-** GQ; **d-** GC.

3.2.3. Dissolved Organic Matter: Unraveling the Role of Organic Carbon from the Hypogeal Cadaver Decomposition Island published in *Forensic Science International*

This section consists of a manuscript published in *Forensic Science International*, 2026, 112887. It is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Authors: Giulia Tagliabue (corresponding), Anna Masseroli, Giulia Caccia, Pasquale Poppa, Cristina Cattaneo and Luca Trombino.

DOI: <https://doi.org/10.1016/j.forsciint.2026.112887>

Link: <https://www.sciencedirect.com/science/article/pii/S0379073826000745>

The article addresses the challenges of using carbon as a proxy for identifying a decomposing body within a burial. The paper then proposes monitoring changes in pH and dissolved organic matter (DOM) concentrations in the HCDI as potential time-dependent markers for estimating the postmortem interval (PMI) and post-burial interval (PBI). It also presents the use of FT-IR spectrometry to identify the main organic compounds characterizing the organic matter within the HCDI and to explore their relationship to the state of body decomposition. The individual FT-IR spectra are provided in the Appendix (pp. 346–384).



Dissolved organic matter: Unraveling the role of organic carbon from the hypogeal cadaver decomposition island

Giulia Tagliabue^{a,b,*}, Anna Masseroli^c, Alex Golinelli^c, Fulvia Tambone^c, Cristina Cattaneo^a, Luca Trombino^b

^a LABANOF (Laboratorio di Antropologia e Odontologia Forense), Legal Medicine Division, Department of Biomedical Sciences for Health, University of Milan, Milan, Italy

^b Department of Earth Sciences "Ardito Desio", University of Milan, Milan, Italy

^c Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, University of Milan, Milan, Italy

ARTICLE INFO

Keywords:

Forensic taphonomy
Clandestine graves
Dissolved organic matter
HCDI

ABSTRACT

The biogeochemical processes occurring in soils beneath buried cadavers, here defined as hypogeal cadaver decomposition islands (HCDIs), remain poorly understood, particularly with respect to the role of organic carbon. This study investigated temporal variations in dissolved organic matter (DOM) within HCDI to evaluate its potential as a time-dependent decomposition marker. Twenty piglets were buried under controlled conditions at the non-human Forensic Taphonomy Facility Ticino-LEAF(s) (University of Milan, Italy), using four different concealment scenarios: unclothed, wounded, clothed in cotton, and covered with quicklime prior burial. Soil samples collected at successive post-burial intervals (PBIs) up to 467 days were analyzed for pH, DOM concentration, and molecular composition through Fourier Transform Infrared (FT-IR) spectroscopy. Results revealed consistent correlations between decomposition stage, soil pH, and DOM dynamics. In most groups, pH increased during early decay and declined in advanced stages, while DOM concentrations peaked at intermediate intervals before returning to baseline levels. FT-IR spectra highlighted molecular markers of lipids, fatty acids, and protein degradation, with adipocere-associated signals detectable at later stages. Quicklime-treated burials exhibited atypical chemical patterns, though DOM analyses remained informative. Other burial conditions, such as the presence of lesions or clothing, significantly influenced both decomposition processes and the expression of biochemical markers. This pilot study suggests that combined monitoring of DOM concentration and molecular characterization, supported by pH measurements, may contribute to a better understanding of decomposition processes. The approach shows potential for estimating the post-burial interval, suggesting a potentially rapid, cost-effective, and context-sensitive support tool for forensic investigations.

1. Introduction

The study of biogeochemistry related to the decomposition of a cadaver exposed to a natural environment and in contact with soil typically focuses on what is called cadaver decomposition island (CDI). It is defined as a dark-colored area immediately below the body characterized by a significant concentration of nutrients derived from putrefactive leachate and capable of affecting the normal physiology of the environmental matrix in a pointwise manner [1]. Despite the extensive chemical observation of these processes in both experimental [2,3] and uncontrolled studies [4,5] on surface depositions, numerous aspects

remain ambiguous, particularly concerning the dynamics of CDI in cadavers buried in clandestine graves (henceforth Hypogeal Cadaver Decomposition Island, or HCDI), which seem to be still poorly explored.

Among the various chemical markers considered diagnostic for understanding the dynamics affecting the matrix beneath a body are pH [6, 7], nitrogen [8,9] and two of its specific chemical species, nitrites and nitrates [8,10], phosphorus and phospholipids [9,11], and total organic carbon (TOC – [7,10]). However, the interaction between these compounds and both the mineral and organic components of the soil must also be considered, as it may lead to ambiguous results that are difficult to interpret, thereby limiting their utility for estimating the postmortem

* Corresponding author at: LABANOF (Laboratorio di Antropologia e Odontologia Forense), Legal Medicine Division, Department of Biomedical Sciences for Health, University of Milan, Milan, Italy.

E-mail address: giulia.tagliabue@unimi.it (G. Tagliabue).

<https://doi.org/10.1016/j.forensiint.2026.112887>

Received 4 October 2025; Received in revised form 10 February 2026; Accepted 11 February 2026

Available online 14 February 2026

0379-0738/© 2026 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

interval (PMI) or the post-burial interval (PBI). Among the parameters mentioned, variations in soil organic carbon content would logically be expected to provide a more accurate representation of the changes occurring within the context of a CDI. Nevertheless, most studies that have focused on monitoring organic carbon in association with a CDI have either failed to detect significant variations in the concentration of this element [7–10] or, when quantitative variations have been observed, have been unable to determine the origin of such changes [12].

For instance, Benninger et al. [9] investigated the impact of pig cadaver deposition on soil biogeochemistry over a 100-day period. Their results showed no significant differences in total carbon content between CDI and control soil samples. Similarly, Meyer et al. [10] examined the decomposition of swine carcasses during summer and winter, hypothesizing that more rapid and pronounced changes in grave-soil chemistry would occur during the summer. However, decomposition did not significantly affect total carbon concentrations, which were even higher during the winter period.

Another example is provided by Anderson et al. [7], who focused on chemical evidence of pig cadaver decomposition in CDI soils one and three years postmortem. Their study showed no significant differences in total carbon concentrations at either time point. Furthermore, Szelec et al. [8] sought to characterize the potential impact of pig cadavers (placed directly on the ground or suspended one meter above ground) on selected soil chemical markers over a one-year period. In this case as well, carbon content remained stable across all treatments, with no significant differences observed.

Finally, Martin et al. [12] analyzed chemical changes in loam and sandy loam soils surrounding buried animals after two months and found that organic carbon content was more strongly dependent on soil type than on decomposition products. Consequently, this marker was considered unreliable.

This issue may be attributed to the naturally high background levels of organic carbon in soils, largely resulting from inputs of plant-derived biomass, as well as to the limited ability of this parameter to reflect alterations associated with the presence of a decomposing body over the short to medium term. Furthermore, the low relevance of organic carbon as a marker in CDI studies may be hypothetically explained by the fact that carbon introduced into the soil undergoes multiple transformation processes, both biotic (e.g., reduction and oxidation) and abiotic (e.g., volatilization, leaching, and adsorption onto soil colloids).

Since the release of organic matter from a decomposing body is well documented by several chemical proxies [2,9], most notably through alterations in the natural soil pH [6,8], excluding the study of organic carbon may result in the omission of a key component in reconstructing decomposition dynamics. Consequently, it may be preferable to focus on a specific fraction of soil organic matter, namely dissolved organic matter (DOM), which has been proposed as an effective indicator of trends associated with the time-dependent input of decomposition products into the soil.

Soil DOM is operationally defined as the fraction of soil organic matter that can be extracted with water or other extracting solutions and passes through a 0.45 µm filter [13,14]. It consists of a complex mixture of organic compounds, including humic- and fulvic-like substances, phenolic compounds, carbohydrates, and nitrogen-containing molecules [13]. Despite its relatively small proportion in total soil organic matter, DOM is the most mobile and reactive fraction, playing a crucial role in nutrient cycling, microbial activity, and the transport of pollutants in soils [15]. From a dynamic perspective, putrefactive leachate originating from a buried decomposing body partially follows the same migration pathways as soil fluids. This observation supports the consideration of dissolved organic matter (DOM) as potential evidence of body concealment in clandestine graves.

Indeed, several previous studies have proposed focusing on dissolved organic matter (DOM) and dissolved organic carbon (DOC) as markers for the temporal evolution of a CDI beneath a decomposing cadaver. For

example, Fancher et al. [2] investigated the effects of surface human decomposition on soil characteristics over an approximately five-year period, suggesting that such changes may assist in estimating the post-mortem interval (PMI). In their study, DOC concentrations were higher in CDI soils than in control soils, although the observed abundance trends did not appear to be clearly time dependent.

Another application of this approach was reported by Aitkenhead-Peterson et al. [6], who evaluated the lateral spatial extent of decomposition products from the CDI of two human cadavers, depending on whether scavengers had access to the remains. Their results showed that mean DOC concentrations after approximately one year and eight months were significantly higher than those in control soils, differing by approximately one order of magnitude.

DOM was more recently investigated by Pecs et al. [16], who examined its relationship with soil microbial activity in CDI contexts. In their experiment, three adult domestic pigs were placed on the soil surface, and soil samples were collected at varying distances from the CDI up to 324 days post-deposition. Although the chemical analyses primarily focused on DOC concentrations, the results highlighted a localized increase in carbon associated with the decomposition of surface-deposited pig cadavers, extending to depths of up to 14 cm.

However, as also noted by the authors, the mere quantification of DOM within a soil sample is insufficient to distinguish cadaver-derived molecules from those naturally present in the soil system. For this reason, it may be more appropriate to perform a qualitative characterization of DOM, for example through Fourier transform infrared spectroscopy (FT-IR). This technique can facilitate a more detailed understanding of decomposition-related changes, as specific absorption bands can be associated with molecular subunits characteristic of individual biopolymers, such as lipids and proteins [17]. Moreover, variations in the intensity of these bands, driven by different stages of cadaver decomposition, may enable a semiquantitative assessment of the temporal enrichment or depletion of the corresponding biopolymers [16].

The treatment that the body may have undergone during concealment should also be taken into consideration: the presence or absence of clothing, the number and extent of injuries, or the use of caustic-acting substances may be able to alter normal decomposition processes [18–22] and, consequently, the influence that DOM may have on soil bacterial community [16].

Therefore, the present study aimed to detect the variation in DOM quantity and quality from the HCDI through time in four groups of simulated clandestine graves containing one piglet each at the non-human Forensic Taphonomy Facility (or FTF) Ticino-LEAF(s) (University of Milan, Italy). In particular, the goal of the experiment is to observe the relationship between the decomposition state of the cadavers and the corresponding pH anomalies, DOM concentrations, and organic compounds presence over time. The experiment underlined the effectiveness of the combined use of pH, DOM quantification, and spectroscopic characteristics (FT-IR spectroscopy) in monitoring decomposition processes, showing its potential forensic application in PMI estimation. A highly precise analytical approach capable of monitoring even minimal fluctuations in the concentration of dissolved organic matter (DOM) within the cadaveric decomposition island (CDI) over time shows promising potential in various forensic contexts.

In particular, in cases of clandestine burials, an increase in DOM concentration relative to the baseline biogeochemical conditions of the surrounding environment may support the estimation of the post-mortem interval (PMI). Broader application of this method alongside experimental research could also allow for the identification of consistent relationships between the physiological DOM levels of an environment and those observed within the CDI, serving as reliable indicators of time since death. Given its rapid execution and relatively low cost, this method could be effectively applied in real forensic investigations, providing timely information on the period a body has remained in a burial site or natural setting; an interval that can be cross-referenced with reports of missing persons or last known sightings.

2. Material and methods

2.1. Ticino-LEAF(s) and experimental setting

The experiment was conducted at the non-human Forensic Taphonomy Facility Ticino-LEAF(s) (Ticino Park-LABANOF facility for Environmental Analysis in Forensic Sciences) founded by LABANOF (Laboratory of Forensic Anthropology and Odontology) from the University of Milan, in collaboration with the Lombardy Park of the Ticino (PV – [23]).

The area is located in northern Italy (45°23' N 8°50' E) at 95 m a.s.l. The climate is sub-oceanic with the highest rainfall in spring and autumn and with an average rainfall of 1050 mm/year [11]. According to the Köppen & Geiger system [24], the experimental area could be defined as *Cfa*, which indicates humid temperate climates, characterized by long and hot summers, and cool to mild winters.

The vegetation within the study area is predominantly composed of lowland oak forests (*Quercus robur*), accompanied by a well-defined herbaceous layer including *Galeopsis pubescens* and *Aristolochia clematitis*. These woodlands are relatively young and situated in a landscape subject to significant anthropogenic use, which limits the structural development of the vegetation to specific zones. According to the "Land Use Map for Agricultural and Forest Soils," (DUSAF 7.0–2021) the area is classified as *broadleaved forests with medium to high density, managed as coppices*. Further floristic characterization was carried out based on previous ecological surveys related to the burial site, allowing for a more detailed identification of the local plant community [25]. Tree and shrub species identified include *Robinia pseudoacacia*, *Crataegus monogyna*, *Corylus avellana*, *Ailanthus altissima*, *Frangula alnus*. Additional plant species observed include *Rubus*, herbaceous taxa such as *Oxalis* spp. and *Brachypodium rupestre*, and climbing species like *Hedera helix*. The soils of the area are poorly developed on coarse fluvial deposits composed of crystalline rocks [11]. These soils are currently classified as Typic Udorthents sandy—skeletal, mixed, and mesic [26,27], characterized by highly permeable horizons with rapid drainage and sandy texture in the first meter.

Four sets of five simulated clandestine burials were established in a wooded area, each containing a piglet. Graves measuring 1 × 1.5 m were excavated to a depth of 50–60 cm and spaced 1.5 m apart. The entire experimental plot covered a compact area of approximately 950 m². All the specimens involved in this experiment, and in general all those used at Ticino-LEAF(s), had already died from natural causes or causes completely unrelated to the project [23]. This was made possible through partnerships established by the laboratory with a local breeder, who preserves the piglets in a freezer (–24 °C), rather than discarding them, in the unfortunate event that they die from natural causes (comparable to sudden infant death) or are accidentally crushed when the sow lies on them during nursing. The experiment was conducted in respect of the Animal Welfare Organization instituted by the University of Milan (Legislative Decree no. 26/2014), whose tasks include applying the "Principle of the 3Rs": Replacement (replace a model which uses an animal with an alternative model, when possible); Reduction (reduce the number of animals used in a given experiment); Refinement (take action to improve the techniques used to raise, house, care for, and utilize animals).

In the present case, current Italian legislation does not allow the establishment of human body donation programs for experimental research in the field of taphonomic studies; consequently, the animal model is the only one currently applicable. However, there is no scientific evidence supporting the necessity of causing the death of the animal in order for the simulation to be reliable; on the contrary, such a practice is widely considered unnecessarily violent and cruel [23,28,29]. Therefore, one approach that enables the use of animal models in forensic scientific research while upholding high ethical standards is the use of specimens that have already died from natural or otherwise unrelated causes. As stated by Mole and Heyns [30], the reuse of animals

can reduce costs (understood as the need for animal sacrifice) and help limit the overall number of animals used in research.

Prior to the burial, each of the 20 piglets was weighed (3–6 kg) and subjected to external and radiological examination to attest their integrity and suitability for the experiment. Each of the four groups of burials represented a method of body concealment: one group included five piglets buried naked (group GN); one group with five naked piglets wounded after death by gunshots, blunt-force wounds and stabbing; all three types of lesions were inflicted on the same subjects, in the ongoing effort to use the smallest possible number of animal specimens (group GW); one group including five piglets wrapped in 100 % cotton clothes (group GC); one group of five piglets completely covered with quicklime after deposition (CaO – group GQ). All piglets were inhumated on the same day in March 2023, while one piglet for group was exhumated at predetermined PBIs of 18, 49, 101, 206–220 and 397–467 days. Unfortunately, one of the piglets from the group GN was taken by the local wildlife, therefore data from the HCDI for PBI 2 (49 days) are missing.

2.2. Weather conditions during the experiment

To assess the potential influence of weather conditions on DOM concentrations in the HCDI, meteorological variables were monitored using data from the ARPA Piemonte weather station in Cerano (NO), located approximately 3 km north of the Ticino-LEAF(s) site at an elevation of 125 m a.s.l. Although temperature and humidity at a depth of 50–60 cm may differ from surface conditions, these parameters are generally less variable and buffered against short-term rainfall events and heat waves. Atmospheric parameters were therefore monitored using meteorological station data in order to identify any particularly intense events that may have altered the hypogeal microclimate during the experimental period.

During the experimental period (March 2023–June 2024), the mean daily air temperature (Fig. 1) was 14.8 °C, with a maximum of 35.8 °C recorded on August 28, 2023, and a minimum of –5.1 °C recorded on January 22, 2024. As shown in Fig. 1, a pronounced increase in air temperature was observed between PBI 3 and PBI 4, whereas the lowest temperatures occurred between PBI 4 and PBI 5.

Regarding precipitations (Fig. 2), the highest amount was recorded on May 15, 2024 (62.3 mm), while several dry periods (0 mm) occurred, notably in March, April and September 2023. As shown in Fig. 2, intense rainfall events were observed between PBI 3 and PBI 4 and again between PBI 4 and PBI 5.

2.3. Soil-HCDI sampling and chemical analyses

During the fieldwork phase preceding burial, approximately 1000 g of soil was collected from the bottom of each grave, taking care not to exceed a sampling depth of 1–2 cm. Control soil samples were collected from the bottom of each individual grave rather than relying on a single generic control sample. This approach was adopted to account for the intrinsic spatial variability of soils, particularly in a woodland environment such as the experimental area considered here, which could otherwise reduce the precision of result interpretation. These samples were used as references for baseline pH values and DOM concentrations of the experimental area, unaffected by disturbances associated with grave excavation or cadaver decomposition.

Excavation for the exhumations was carried out following a stratigraphic method, with pairs of specialists assigned to each grave, each using their own trowels, which were employed exclusively for that single grave in order to avoid cross-contamination. After each recovery, soil samples were collected from the HCDI, below the cadavers. Due to the small dimensions of the piglets, it was not possible to divide the HCDI into sampling areas corresponding to the anatomical portions of the bodies, therefore only the center of it was considered, collecting circa 1000 g of soil. The samples collected at each phase were stored in clean plastic bags sealed hermetically.

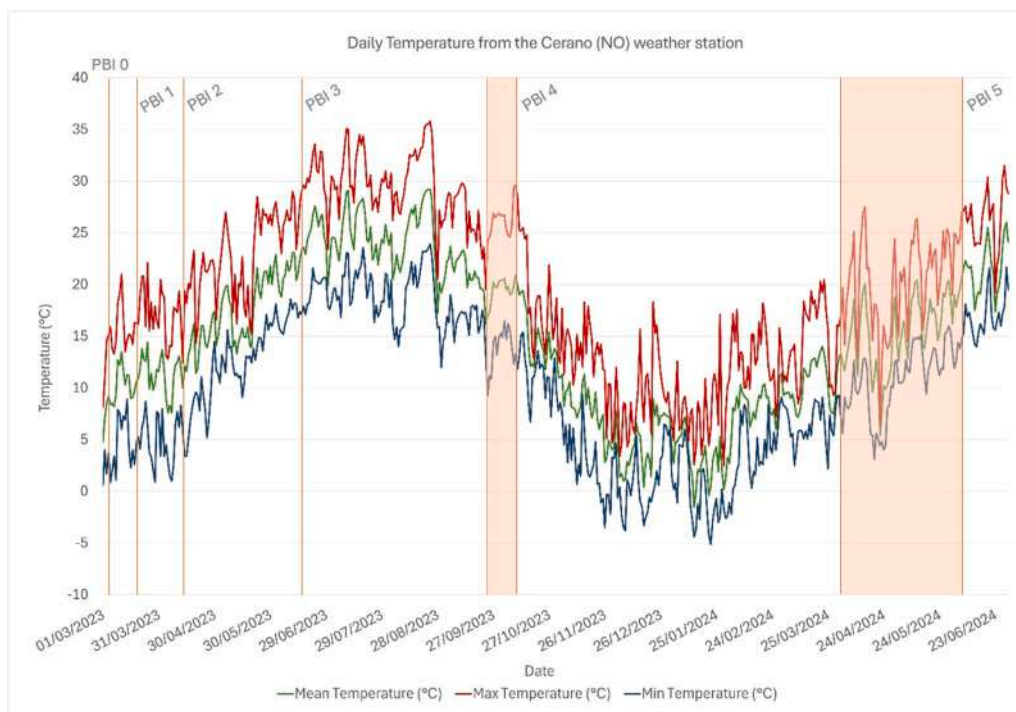


Fig. 1. Daily air temperature (°C) during the experimental period. The red, green, and blue lines indicate the trends of the mean maximum, mean, and mean minimum temperatures, respectively. Orange lines/rectangles mark the exhumation and sampling days for the corresponding PBIs (post-burial intervals). The final field operations required additional time due to accessibility issues at the FTF (Forensic Taphonomy Facility).

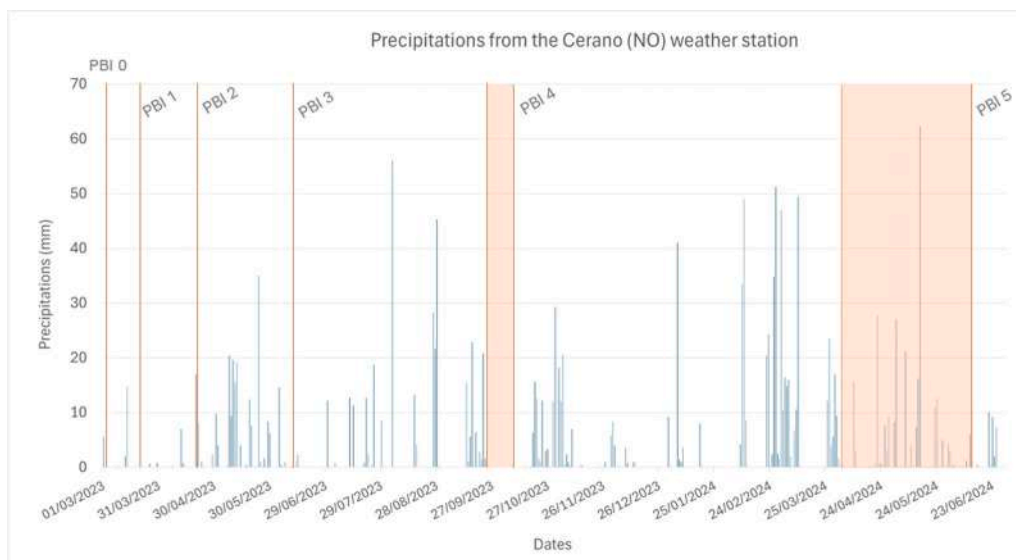


Fig. 2. Daily precipitation (mm) during the experimental period. Bars show cumulative rainfall for each day. Orange lines/rectangles mark the exhumation and sampling days for the corresponding PBIs (post-burial intervals). The final field operations required additional time due to accessibility issues at the FTF (Forensic Taphonomy Facility).

Once collected, the soil samples were air-dried in a protected environment in the laboratory within individual bags and kept separate from one another in order to avoid contamination. Once completely dried, the samples were prepared for chemical analyses. The samples' pH was determined using an aqueous solution prepared using a 1:2.5 soil-to-water ratio and measured with a benchtop multiparameter model PC8 +DHS Stirrer (Zetalab). Every measurement was repeated three times per sample.

DOM was extracted from all samples with distilled water using a solid-to-water ratio of 1:1.5 (w/v), on a fresh matter basis, shaking for

2 h at 25°C. The suspension was then centrifuged at 7000 rpm for 15 min, and the supernatant was filtered under vacuum through a 0.45 µm membrane. The organic carbon content in DOM extracts was determined using the dichromate method on an aliquot of the extracted volume and the final concentration was detected by a photometric detector, at around 620 nm, with PF-12Plus (Macherey-Nagel, Düren, DE; [31]). The remaining DOM extracts were frozen at −80 °C and subsequently lyophilized. Spectroscopic characterization was performed on the samples in powder form using Fourier Transform Infrared (FT-IR) spectroscopy. Spectra were acquired in Attenuated Total Reflectance

(ATR) mode with a Shimadzu IRAffinity-1S spectrometer equipped with a Miracle Pike ATR device (Shimadzu Italia srl, Milan, Italy). The wavenumber range investigated was 4000–400 cm^{-1} , with a resolution of 4 cm^{-1} and 32 scans per sample. The FTIR spectra were processed using LabSolutions IR Advanced Software (Shimadzu) for standard data treatment, including baseline correction, normalization, and smoothing, to improve spectral quality and facilitate interpretation without altering the original data.

3. Results

3.1. Decomposition state of the piglets

Following the various exhumation sessions of the piglet carcasses, an observation of their state of preservation was carried out. The results are summarized in Table 1.

For the GN group, after 18 days (PBI 1) the body appeared bloated, with slight green discoloration. By 101 days (PBI 3), active decay was evident, characterized by an abundance of putrefactive fluids beneath the carcass and the presence of adipocere. An advanced stage of decomposition was observed after 206 days (PBI 4), marked by the presence of skeletonized elements, such as the skull and limbs, and a substantial loss of mass in the remaining soft tissues. Adipocere was also present at this stage, and plant roots were more abundant and more extensively integrated with the body. Finally, after 399 days (PBI 5), the dry remains stage was observed, with only limited residues of adipocere still present, and the cadaver was completely enveloped by roots.

The decomposition pattern of the piglets in the GW group was similar to that observed in the previous group at PBI 1 (18 days) and PBI 5 (406 days). However, after 49 days (PBI 2), the carcasses exhibited clear signs of active decay, and by 101 days (PBI 3) the remains had already reached an advanced stage of decomposition. At this stage, anatomical structures were no longer recognizable during field recovery, and extensive liquefaction was observed, with some roots in direct contact with the torso. After 220 days (PBI 4), the soft tissues were extensively liquefied, with the presence of adipocere and roots enveloping the remains.

The GQ group, characterized by the presence of quicklime, showed a decomposition pattern that differed from the other groups. The formation of a lime cast was observed, which appeared to promote dehydration of the remains and may have contributed to partial physical protection. Progressive mass loss was recorded up to PBI 5 (467 days), at which point exposure of bone and cartilage was noted. At PBI 1 (18 days), bloating and epidermolysis were observed, with the skin presenting a desiccated appearance, increased rigidity, and detachment from the underlying epidermal layer. This layer exhibited a pronounced

reduction in thickness, becoming nearly transparent. From PBI 2 (49 days) onward, the external appearance of the skin remained broadly similar, although a gradual thickening was noted. Mass loss continued steadily over time, reaching its maximum at PBI 5, when the remains were dry, while cartilage and desiccated skin were still present.

Ultimately, the group GC presented a peculiar progression in the conservation state through time: at PBI 1 (18 days), the remains were in the full bloated stage, with slight discoloration in the abdominal area. At PBI 2 (49 days), on the other hand, the piglet's cadaver was in the active decay stage, with the skull and limbs already partially skeletonized and a large putrefactive stain in the abdominal region. In contrast, at PBI 3 (101 days), the cadaver was well preserved, with widespread discoloration but intact skin, although leathery. These characteristics are associated with humid mummification or corification (i.e., defined as a preservative decomposition process occurring in environments with high moisture and limited air exchange, resulting in arrested putrefaction and brownish, leathery skin with retained moisture; [32–34]).

At PBI 4 (206 days), the cadaver was already in an advanced stage of decay, with most body regions skeletonized and widespread presence of adipocere, while at PBI 5 (397 days), the remains were completely skeletonized. In both of these last two cases, the cotton t-shirts were mostly destroyed.

3.2. pH analysis

pH analysis of soil samples collected from the bottom of each grave prior to burial allowed the soils of the experimental area to be classified as acidic to subacidic, with values ranging from 4.2 to 6.1. Analysis of the HCDC soils (shown in Fig. 3 and summarized in Supplementary Material, Table S1) generally indicated an increase in pH starting at PBI 2, with a possible further rise at PBI 3, as observed for groups GN (Fig. 3a) and GW (Fig. 3b). Subsequently, pH values decreased, in some cases falling below those of undisturbed control soils. In contrast, group GQ (Fig. 3c) exhibited a marked increase in pH, exceeding twice the value of the control soil, although a decrease was observed at the final PBI. Finally, pH values in the HCDC soils of group GC (Fig. 3d) showed a peak at PBI 2, followed by a gradual decrease and stabilization over time at levels comparable to those of the control soils, until PBI 5, when pH declined to values lower than those measured in the control soils.

3.3. Dissolved organic matter (DOM) analysis

The mean DOM concentrations (shown in Fig. 4 and summarized in Supplementary Material, Table S2) generally increased relative to control soils starting at PBI 3, followed by a progressive decrease, eventually returning to values comparable to those considered physiological for the experimental area (37.39–74.85 mg/kg). This trend was observed in groups GN (Fig. 4a), GW (Fig. 4b), and GQ (Fig. 4c), in which DOM concentrations increased between PBI 3 and PBI 4, reaching values up to nearly eight times higher than those of the control soil (GW, PBI 4).

The only exception was group GC (Fig. 4d), for which DOM concentrations were generally comparable to those of the control soil, except at PBI 3. A pronounced peak was also observed at PBI 2, reaching a value nearly one hundred times higher than baseline levels (4647.95 vs 42.58 mg/kg).

3.4. Fourier transform infrared (FT-IR) spectroscopy

FT-IR analysis of the control soil DOM from the area revealed 20 superimposable spectra, represented by the example shown in Fig. 5, corresponding to the control sample from grave GQ1. These spectra are characterized by a broad absorption band in the single-bond region (4000–2500 cm^{-1}), with peaks around 3100 and 3030 cm^{-1} , attributable to C–H stretching vibrations in aromatic compounds. Additionally, two small sets of peaks were detected in the double-bond region (2000–1500 cm^{-1}), corresponding to aromatic ring vibrations, followed

Table 1

Decomposition state of the piglets as observed after the exhumations. GN=naked piglets; GW=wounded naked piglets; GC=clothed piglets; GQ=piglets covered with quicklime. PBI 1 = 18 days; PBI 2 = 49 days; PBI 3 = 101 days; PBI 4 = 206–220 days; PBI 5 = 397–467 days. “/” indicates that data of PBI 2 for the group GN is missing due to wildlife disturbance.

Group	Decomposition state				
	PBI 1	PBI 2	PBI 3	PBI 4	PBI 5
GN	Bloated	/	Active decay	Active decay Adipocere	Dry remains Adipocere
GW	Bloated	Active decay	Advanced decay	Advanced decay Adipocere	Dry remains Adipocere
GQ	Bloated	Desiccated	Desiccated Mass loss	Desiccated Mass loss	Dry remains Cartilage
GC	Advanced Bloating	Active decay	Humid mummification	Advanced decay Adipocere	Dry remains

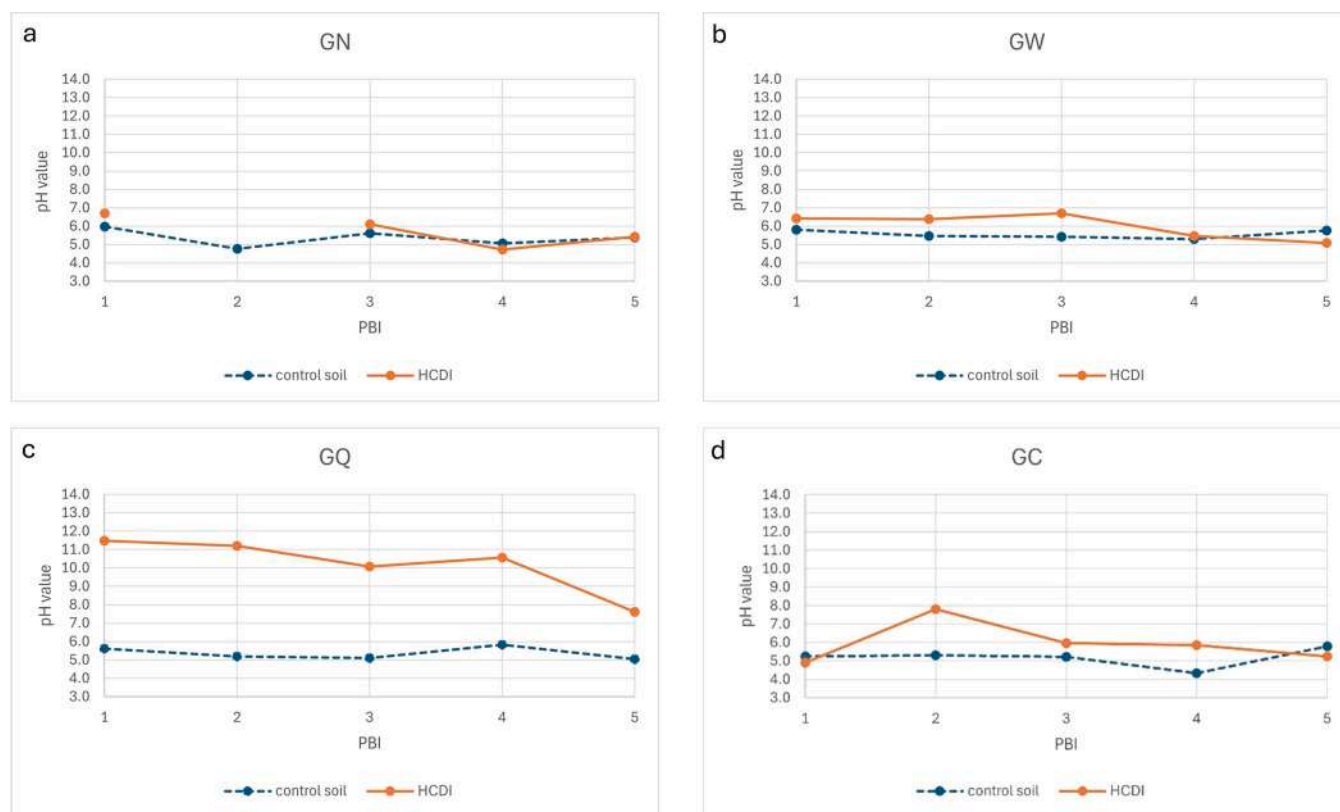


Fig. 3. Mean soil pH measured at the bottom of each grave for each group. The blue dotted line indicates the control soil pH, collected from each grave prior to inhumation, whereas the red line indicates the soil pH from each HCDI at different PBIs (post-burial intervals). GN = naked piglets; GW = wounded naked piglets; GC = clothed piglets; GQ = piglets covered with quicklime. PBI 1 = 18 days; PBI 2 = 49 days; PBI 3 = 101 days; PBI 4 = 206–220 days; PBI 5 = 397–467 days. Data for PBI 2 in group GN are missing due to wildlife disturbance.

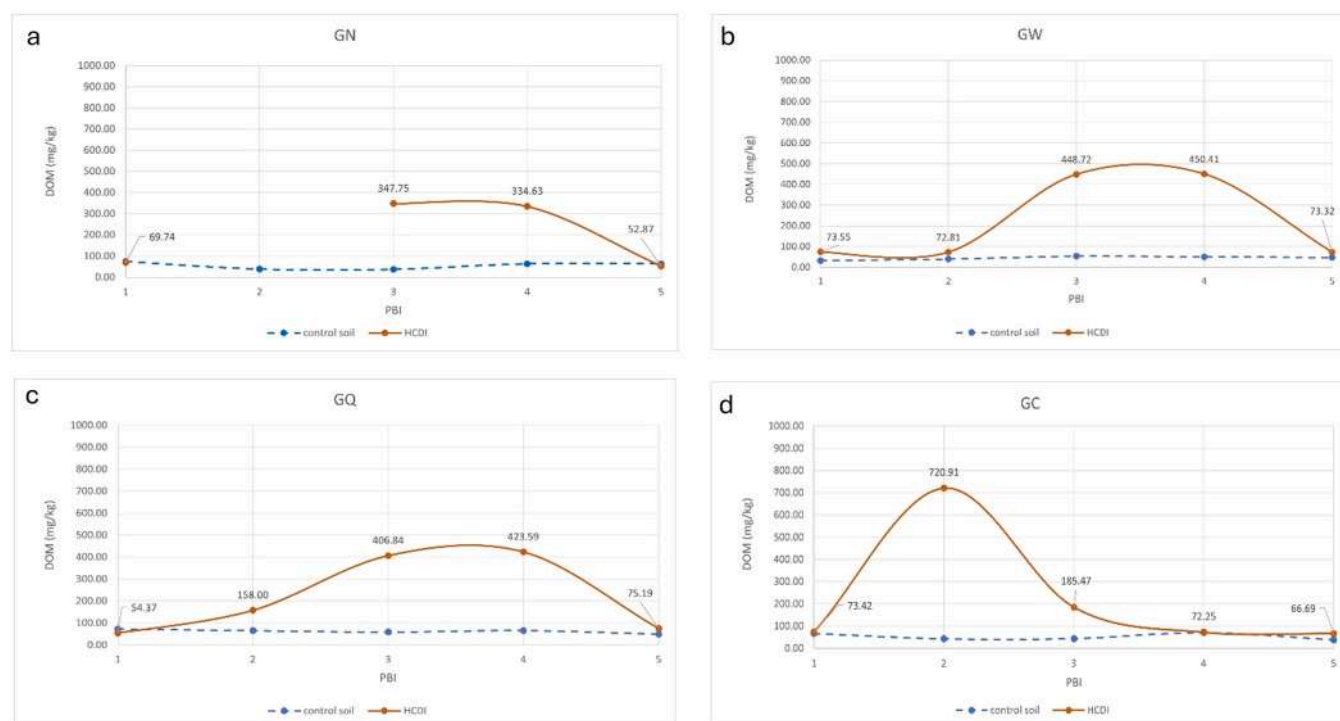


Fig. 4. Soil DOM concentrations measured at the bottom of each grave for each group. The blue dotted line indicates the DOM concentration in the control soil, collected from each grave prior to inhumation, whereas the red line indicates the DOM concentration in HCDI soil at different PBIs (post-burial intervals). GN = naked piglets; GW = wounded naked piglets; GC = clothed piglets; GQ = piglets covered with quicklime. PBI 1 = 18 days; PBI 2 = 49 days; PBI 3 = 101 days; PBI 4 = 206–220 days; PBI 5 = 397–467 days. Data for PBI 2 in group GN are missing due to wildlife disturbance.

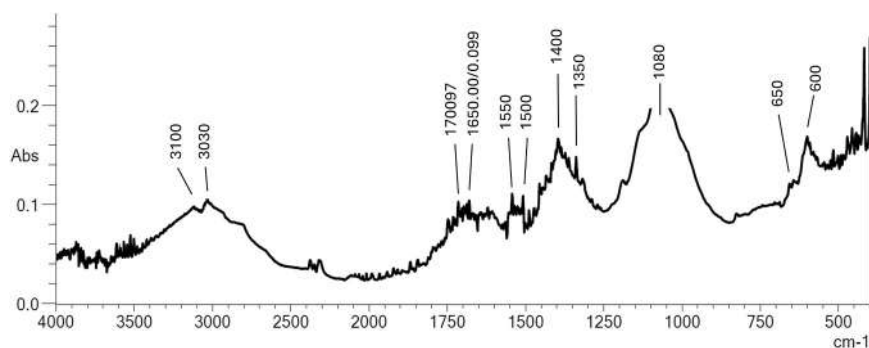


Fig. 5. Representative FT-IR spectrum of control soil DOM from the study area (GQ1), collected before inhumation.

by several very weak bands between 2000 and 1750 cm^{-1} . In the fingerprint region (1500–600 cm^{-1}), a prominent peak at approximately 1400 cm^{-1} , followed by another at around 1350 cm^{-1} , indicates the presence of three methyl groups ($-\text{CH}_3$), suggesting saturated aliphatic compounds such as trimethylamine ($\text{N}(\text{CH}_3)_3$). Finally, a strong peak near 1080 cm^{-1} , attributable to C–O stretching vibrations, suggests a substantial presence of polysaccharides or carbohydrates, while a

medium-intensity band between 650 and 600 cm^{-1} may be ascribed to C–H out-of-plane bending vibrations, further indicating the presence of organic material.

The FT-IR spectra of the group GN (Fig. 6a) showed no relevant alterations compared to the control soil, besides a progressive shifting of the absorption band in the single bond region towards higher wave-numbers, until reaching a splitting of the band in two separated peaks

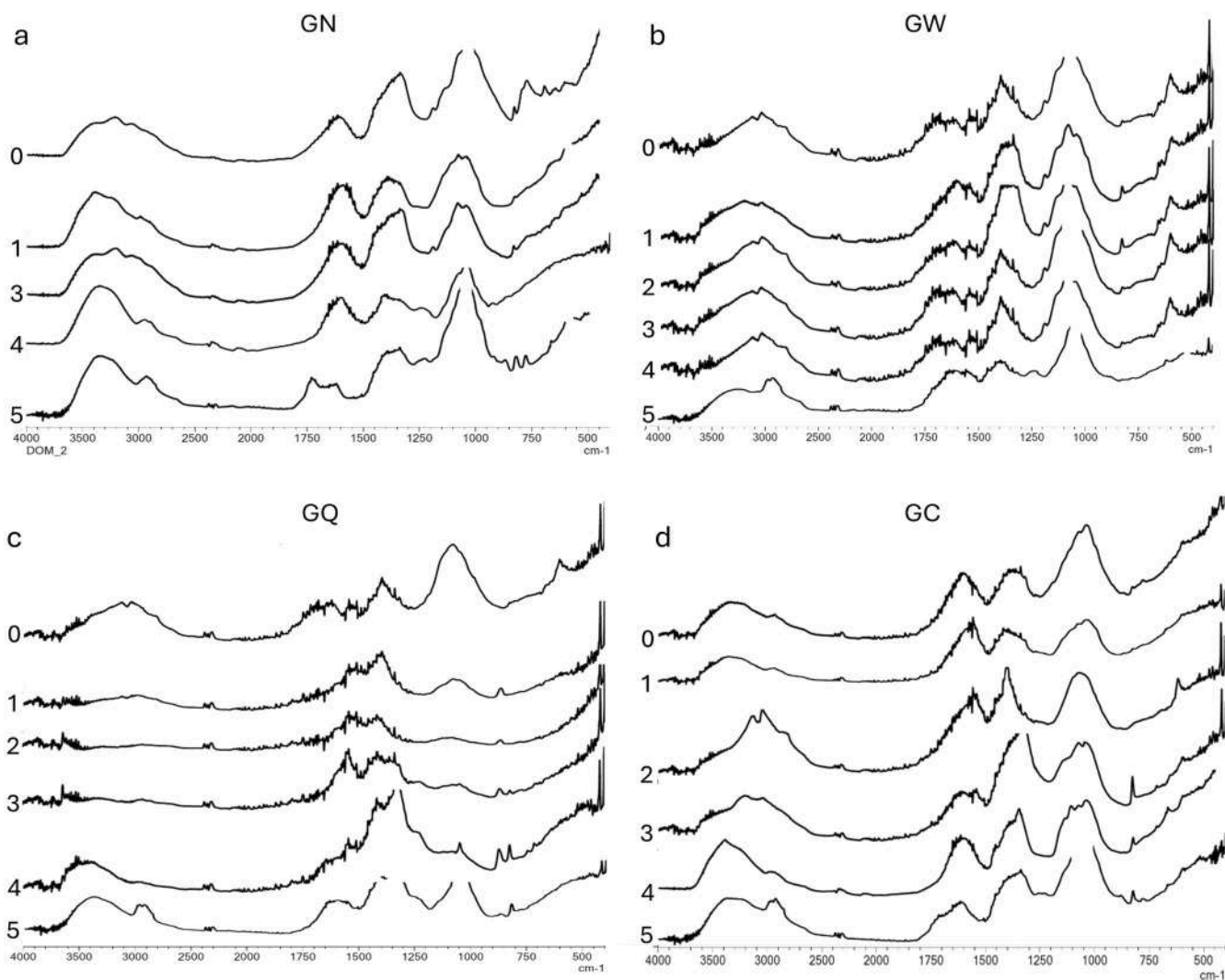


Fig. 6. FT-IR spectra of DOM samples collected from HCIDI soil for the four burial groups. Numbers to the left of each spectrum indicate the PBI (post-burial interval). PBI 0 = control soil spectrum from one grave prior to inhumation. PBI 1 = 18 days; PBI 2 = 49 days; PBI 3 = 101 days; PBI 4 = 206–220 days; PBI 5 = 397–467 days. GN = naked piglets; GW = wounded naked piglets; GC = clothed piglets; GQ = piglets covered with quicklime. Data for PBI 2 in group GN are missing due to wildlife disturbance.

(PBIs 4–5) attributable to unsaturated fatty acids ($3500\text{--}3000\text{ cm}^{-1}$) and hydroxy-fatty acids (2800 cm^{-1}). Moreover, from PBI 3 to PBI 5, two bands linked to amide I and II of proteins (around 1700 cm^{-1} and 1550 cm^{-1} respectively), and the emergence of a band near 1600 cm^{-1} , between the amide bands, and ascribable to free carboxylate groups are detectable. For group GW (Fig. 6b), a variation could be noticed in the intensity of the band around $1400\text{--}1250\text{ cm}^{-1}$ which increased from PBI 2 to later decrease and that could be linked to the C–N stretching of amide III. Moreover, from PBI 5 the spectra showed a different pattern of shifting to the right of the band in the single bond region, leading to the formation of a variation in the expression of lipids signals ($3500\text{--}2800\text{ cm}^{-1}$): O–H stretching bands (around $3400\text{--}3200\text{ cm}^{-1}$) attributed to fatty acids, and the well-defined absorbance bands around $3000\text{--}2800\text{ cm}^{-1}$ linkable to C–H stretching bands. On the other hand, group GQ (Fig. 6c) showed none to poor bands in the single bond region, with the only exception of PBIs 4 and 5, with a wide band ($3500\text{--}3100\text{ cm}^{-1}$) due to O–H stretching band of fatty acids and a well-defined absorbance band ($3000\text{--}2800\text{ cm}^{-1}$) attributed to C–H stretching bands of fats. Moreover, a small peak around 3630 cm^{-1} is observable from PBI 2 to PBI 4, detecting the O–H bond from water molecules. Conversely, triglycerides (1750 cm^{-1}) and unsaturated fatty acids (1670 cm^{-1}) absorbance bands were detected in samples from all the PBIs, until PBI 5 when they decrease. Furthermore, absorption bands around 1450 cm^{-1} , 1080 cm^{-1} and 870 cm^{-1} could be assigned to different vibration modes C–O between the oxygen atom of carbonate. However, the band in the area around 1450 cm^{-1} is characterized by a dense overlapping of absorbance bands, which could also be attributed to proteins or phospholipids. Finally, the FT-IR spectra of the group GC (Fig. 6d) presented a pattern similar to the ones of the groups GN and GW for what concerns the shifts of the band in the single bond region, attributed to =C–H stretching of lipids (3130 , 3050 , and 2800 cm^{-1}). Furthermore, bands related to the O–H stretching bands of fatty acids (around $3400\text{--}3200\text{ cm}^{-1}$) and the absorbance bands around $3000\text{--}2800\text{ cm}^{-1}$ linkable to C–H stretching bands, well detectable at PBI 5, are showing. In the double bonds region, two peaks around 1580 and 1550 cm^{-1} followed by modes between 1500 and 1400 cm^{-1} appeared at PBI 5, respectively ascribed to carboxylate bands of fatty acid calcium salts and lipid CH_2 scissoring. About the fingerprint region, the spectrum is characterized by the presence of a wide band between 1400 and 1250 cm^{-1} attributed to C–N stretching of amide III and, at PBI 2, another wide and strong absorbance band between 1080 and 1040 cm^{-1} , related to phospholipids. Ultimately, from PBI 3 it is possible to detect a fragmentation of the band between 1080 and 1020 cm^{-1} in three small peaks of C–O stretching of cotton cellulose, and a sharp peak at 850 cm^{-1} , attributable to the CH_2 rocking of lipids.

4. Discussion

This study aimed to investigate the concentration and temporal variation of DOM quantity and quality within the HCIDI across four groups of simulated clandestine graves employing different concealment methods. The results indicate possible associations between the decomposition state of the remains, soil pH measured within the HCIDI, and variations in both the concentration and molecular characteristics of DOM beneath the buried cadavers.

Considering the pH values observed in the GW group (Fig. 3b), a mean increase relative to control soils was recorded in samples collected from the HCIDI of each grave during PBI 1, PBI 2, and PBI 3, followed by a decrease to average values comparable to, or even lower than, those of the control soils at PBI 4 and PBI 5. With the exception of PBI 2, for which data were unavailable, the GN group appeared to exhibit a comparable pH trend (Fig. 3a). The initial alkaline phase in both groups coincided with the exhumation periods corresponding to the bloating and active decay stages of decomposition. This pattern is consistent with findings from previous studies [8,9,35], which reported increases in soil pH during the early to intermediate stages of cadaver decomposition,

followed by a decline during the later stages of tissue breakdown. This trend was attributed to the release of ammonium ions (NH_4^+) resulting from protein ammonification processes occurring during autolysis and putrefaction phase, which includes bloating and active decay [1,9]. Similarly, in the more advanced decomposition stage (dry remains), a decrease in soil pH within the HCIDI has been documented, as reported in other studies [8,35–37]. The secondary acidification may be attributed to the depletion of ammonium ions (NH_4^+) and other organic compounds [35], as a result of nitrification processes and the consequent production of H^+ [6,8,9], potentially indicating a shift in the microbial community inhabiting the CDI [38].

Accordingly, the DOM graphics of the samples from the two groups (Figs. 4a and 4b) showed a comparable trend with an increase of its concentration at PBIs 3 and 4, with a subsequent regression to values similar to the ones from the control soil (PBI 5). On the contrary, the FT-IR spectra revealed the presence of different compound signatures. In particular, the absorbance spectrum of group GW at PBI 2 (Fig. 6b) showed an increase in the intensity of the band in the $1400\text{--}1250\text{ cm}^{-1}$ region, which has been attributed to C–N stretching vibrations of the amide III band of blood in studies focusing on FT-IR characterization of human body fluids [39] and tissues [40]. Although these studies are not specifically related to forensic taphonomy or putrefactive leachates in soil, the presence of such molecular components in the present study cannot be excluded, particularly given that the GW group comprised individuals with multiple lesions that may have led to blood release into the surrounding environment. The absence of comparable spectral features at PBI 1 may be attributed to an insufficient accumulation of body fluids to produce signals distinguishable from background organic compounds, which may also exhibit band overlaps in the amide III region. It is therefore plausible that sufficient accumulation occurred only at PBI 2, after which these signals were progressively attenuated or lost as decomposition advanced or as compounds were leached by precipitation during subsequent PBIs.

In addition, it is possible to detect, at PBI 5, a variation in the expression of lipids signals ($3500\text{--}2800\text{ cm}^{-1}$): O–H stretching bands (around $3400\text{--}3200\text{ cm}^{-1}$) could be attributed to fatty acids [41,42] and the well-defined absorbance bands around $3000\text{--}2800\text{ cm}^{-1}$ could be linkable to C–H stretching bands, which were used as an indicator of the increase in the amount of lipid as the decomposition process proceeded, and of the presence of adipocere in other studies focused on the same topic [41,43,44].

In contrast, the spectra for group GN (Fig. 6a) showed no significant alterations until PBI 4, when a noticeable splitting of the absorption band between 3500 and 2800 cm^{-1} was observed. This could suggest the presence of hydroxy-fatty acids (2800 cm^{-1}) and the progressive formation of unsaturated fatty acids ($3500\text{--}3000\text{ cm}^{-1}$), better distinguishable at PBI 5, as evidence of the conversion to adipocere [41,44]. Furthermore, the evolution of the bands in the area $1700\text{--}1300\text{ cm}^{-1}$, attributable to proteins, was detectable from PBI 3 to PBI 5. According to Ueland et al. [22] two bands detected in the area could correspond to amide II (around 1550 cm^{-1}) and amide I (around 1700 cm^{-1}) and the respective evolution of the two through time or treatments could be useful for monitoring any change in protein of the decomposition fluid. Indeed, while the amide I peak is supposed to be sensitive to the protein conformation, which influences its location and intensity, amide II is normally more stable [22]. This relationship allows their ratio to be considered a marker of protein transformation [45]. The variation in the relative intensities and the broadening of the amide bands observed across the PBIs suggest that protein degradation and proteolysis occur throughout the decomposition process [43]. A reduction in the intensity of the amide I peak, accompanied by an increase in the intensity of the amide II, could serve as an indicator of protein breakdown, that could be employed to track the progress of proteolysis [46]. Additionally, as found in Collins et al. [43], the emergence of a band near 1600 cm^{-1} (situated between the amide bands) attributable to the formation of free carboxylate groups in the shorter peptide fragments

generated by proteolysis, could further contribute to the broadening of the amide bands as decomposition advances. In principle, the presence of analogous bands in the FT-IR spectra of the GW group was anticipated, given its comparable burial characteristics to those of the GN group. However, this was not observed. This discrepancy may be explained by the presence of a broad absorption band in the 1370–1200 cm^{-1} region in the GW spectra, attributed to C–N stretching vibrations of the amide III band of blood. Overlap of this band with the region of interest may have masked the corresponding signals, thereby hindering their detection over time.

About the group with quicklime (GQ), the pH graph (Fig. 3c) showed an atypical trend, reporting very high, despite decreasing values. This could be interpreted as the expression of the peculiar alkalinity of CaO, that being a basic oxide could have obliterated the pH signals attributable to NH_4^+ fluctuations due to putrefaction. The progressive acidification could suggest a corresponding hydration of the quicklime, obtaining lime ($\text{Ca}(\text{OH})_2$), which is also typically alkaline. From this state on, once the hydration process is concluded, the carbonation due to the contact with CO_2 (being environmental or decomposition-related) leads to the formation of calcium carbonate (CaCO_3), whose pH is lower [47,48].

The cadavers of this group were recovered in a dehydrated state, potentially attributable to the hygroscopic capacity of quicklime, which may have created a microenvironment with reduced humidity, at least until complete hydration occurred. Nevertheless, the DOM analysis (Fig. 4c) appeared to be as effective as in the GN and GW groups, exhibiting a comparable temporal trend. These outcomes could be interpreted as suggestive of the analytical technique's potential to detect and monitor organic matter concentration, even in scenarios in which the amount of putrefactive leachate reaching the soil is minimal.

Accordingly, the FT-IR spectra (Fig. 6c) showed absorbance bands in the lipid-related region (3500–2800 cm^{-1}) at PBIs 4 and 5, similarly to those observed in groups GW and GN. In contrast, absorbance bands associated with triglycerides (1750 cm^{-1}) and unsaturated fatty acids (1670 cm^{-1}) were detected in GQ samples at all PBIs, with relatively constant intensity until PBI 5, when a decrease was observed. Forbes et al. [44] reported that the persistence of these compounds is indicative of minimal decomposition, which is consistent with the altered decay processes induced by lime. This interpretation is in agreement with the main findings of the present study.

Moreover, the same experiment reported by Forbes et al. [44] indicated that an absorbance band around 1450 cm^{-1} can be attributed to C–O stretching vibrations associated with lime. Although a signal in this region was observed in the FT-IR spectra of the GQ group, this spectral area was characterized by dense band overlaps, which may also reflect contributions from protein or phospholipid degradation products [39]. Nevertheless, it was possible to hypothesize trends related to the hydration and carbonation phases of quicklime. In particular, a small peak around 3630 cm^{-1} was observed from PBI 2 to PBI 4, corresponding to O–H stretching vibrations of water molecules [49], which may indicate progressive hydration of CaO, with a decrease in intensity over time. Furthermore, absorption bands around 1450, 1080, and 870 cm^{-1} can be assigned to different vibrational modes of carbonate (CO_3^{2-}) groups [50, 51]. The presence of these bands at later PBIs (4 and 5) may indicate a relative increase in CaCO_3 compared to $\text{Ca}(\text{OH})_2$.

Finally, pH analysis of soils from group GC (Fig. 3d) revealed a markedly different trend, characterized by a pronounced peak at PBI 2 followed by very low pH values. The latter were hypothetically attributed to the absorptive capacity of the cotton T-shirts in which the bodies were wrapped. The garments may have absorbed putrefactive leachate or acted as a physical barrier between the soil bacterial community and the soft tissues; however, the role of textiles as an obstacle to the diffusion of decomposition products remains unclear. According to Ueland et al. [22], whose study focused on the effects of decomposition fluids on textile degradation, cotton fabrics are capable of absorbing putrefactive leachate, which may also inhibit or delay textile

degradation. Similarly, Collins et al. [43] identified lipids, proteins, and other decomposition products within fluids retained in cotton clothes, demonstrating that textiles can absorb these substances throughout different stages of decomposition. In the present experiment, variations in decomposition rate were also observed for this group compared with unclothed individuals (Table 1), suggesting a potential influence of cotton clothing on decomposition dynamics. However, findings in the literature remain mixed. For example, Card et al. [52] reported that unclothed piglet cadavers decomposed significantly faster than those clothed in cotton, although clothing provided additional sites for Diptera colonization. Conversely, Matuszewski et al. [19] suggested that cadaver mass is a more influential factor in decomposition than clothing, with the latter exerting only a minor effect. Nevertheless, the authors emphasized that, even if textiles have a limited impact on decomposition rates, they should still be regarded as a key evidentiary element in forensic investigations, particularly due to their capacity to retain information on decomposition by-products that may be useful for PMI estimation.

The only exception to this assessment is the cadaver from the group GC exhumated at PBI 2: at that point, the decomposition state of the piglet was evaluated as active decay phase, being the cadaver worst preserved than the one analyzed at PBI 3, which underwent a conservative decomposition process, accentuating the steepness of the decline in pH values and DOM concentration between the two exhumations.

Accordingly, FT-IR analysis at PBI 2 (Fig. 6d) revealed strong C–H stretching bands, represented by a prominent double peak at 3130 and 3050 cm^{-1} , with a shoulder around 2800 cm^{-1} attributable to =C–H stretching vibrations. These features suggest the presence of unsaturated fatty acids [41,44]. Moreover, a wide and strong absorbance band between 1080 and 1040 cm^{-1} , utilized as a reference band for degradation products from phospholipid decomposition [53], was also present. Conversely, FT-IR spectrum of the sample from PBI 3 did not show an intensification of the signal in the single bond region, highlighting a weaker input of fatty acids in the grave soil, until PBI 5 when two sharp bands at 1580 and 1550 cm^{-1} appeared. They could be considered as characteristic of the carboxylate bands of fatty acid calcium salts [41, 43], while the modes expressing between 1500 and 1400 cm^{-1} could be attributed to lipid CH_2 scissoring [41,54].

The decomposition trends observed in the GC group suggest variable interactions between buried bodies wrapped in cotton clothing and the surrounding hypogeal environment. GC2 showed an advanced decomposition state, accompanied by corresponding chemical signals, which may reflect localized alterations in grave conditions, potentially driven by atmospheric events that increased air and water permeability. In contrast, GC3 was recovered in a preservative state consistent with wet mummification, indicative of sustained moisture and restricted air exchange, possibly facilitated by the close adherence of clothing and the maintenance of an anoxic microenvironment. GC4 exhibited a decomposition state comparable to GW4 but with substantially lower DOM concentrations, likely related to the absorbent properties of the cotton garment. Collectively, these observations emphasize the inherent variability of burial environments and the importance of a comprehensive, multidisciplinary assessment of factors influencing decomposition.

Moreover, from PBI 3 to PBI 5, a progressive fragmentation of the absorption band between 1080 and 1020 cm^{-1} was observed, resulting in three smaller peaks that shifted toward higher wavenumbers and became more clearly defined as the PBI increased. This trend may be tentatively attributed to the progressive degradation of the 100 % cotton T-shirts wrapping the piglets in this group, which may have resulted in the release of cellulose degradation products into the HCIDI soil, as suggested by Collins et al. [43]. As the spectral signal associated with fabric degradation became more pronounced, other bands related to decomposition products also became more detectable, including those associated with O–H stretching vibrations of fatty acids (approximately 3400–3200 cm^{-1} ; [41,42]) and C–H stretching vibrations of lipids (approximately 3000–2800 cm^{-1} ; [43,44]).

Considering the overall results of the experiment (summarized in Table 2), a correlation appeared to exist across all groups between trends in soil pH within the HCDI and changes in DOM concentration. However, deviations from this pattern were observed in groups GQ and GC, where the method of concealment appeared to significantly influence the chemical state of the soil matrix, particularly pH. Previous studies investigating biomarkers within CDIs as tools for PMI estimation [55] have proposed assessing soil alkalinity as an indicator of successful sampling, based on the alkaline effect exerted by ammonium (NH_4^+) ions on soil pH, as also discussed in the present study. Therefore, it is crucial to consider both cadaver treatment and concealment methods, as these factors may substantially alter soil chemical signals and potentially lead to erroneous interpretations.

Another example highlighting the extent to which body treatment and concealment methods can influence taphonomic analyses is provided by the FT-IR spectroscopy results presented here. Despite the

expectation of detecting comparable decomposition-derived organic compounds across the groups, with differences hypothesized primarily in terms of timing of appearance or absorbance band intensity, the findings instead revealed distinct molecular profiles. These differences appear to be driven more by the specific treatments applied than by time-dependent decomposition processes.

Nevertheless, it must be acknowledged that this is a pilot study conducted on a relatively small sample size, and further investigation is necessary. Conversely, what is consistent across all the experimental groups is the detection of spectral bands attributable to lipids and fatty acid salts, compounds typically found in adipocere [56], which was also macroscopically observed during examination of the remains in PBI 4 and 5. This finding could be significant, not only because it shows how these molecules are capable of passing through the DOM filter threshold ($0.45 \mu\text{m}$) and thus remain detectable even in analyses conducted for different purposes, but also because they may serve as useful indicators

Table 2

Resume of the highlights of the study. DOM and pH values are to be intended as compared to the control soil values. GN=naked piglets; GW=wounded naked piglets; GC=clothed piglets; GQ=piglets covered with quicklime. PBI 1 = 18 days; PBI 2 = 49 days; PBI 3 = 101 days; PBI 4 = 206–220 days; PBI 5 = 397–467 days. “/” indicates that data of PBI 2 for the group GN is missing due to wildlife disturbance.

		PBI 1	PBI 2	PBI 3	PBI 4	PBI 5
GN	Decomp. state	Bloated	/	Active decay	Active decay	Dry remains
	pH	+ 0.7	/	+ 0.5	-0.4 ± 0.0	Adipocere
	DOM (mg/kg)	-5.11	/	+ 310.36	+ 271.08	+ 0
	FT-IR	/	/	1700 cm^{-1} : amide I 1550 cm^{-1} : amide II (start of proteolysis)	Splitting of: 3500–3000 cm^{-1} : unsaturated fatty acids AND 2800 cm^{-1} : hydroxy-fatty acids.	3500–3000 cm^{-1} : unsaturated fatty acids (adipocere).
					Advanced decay	
GW	Decomp. state	Bloated	Active decay	Advanced decay	Adipocere	Dry remains
	pH	+ 0.6	+ 0.9	+ 1.3	+ 0.2	Adipocere
	DOM (mg/kg)	+ 40.9	+ 33.09	+ 395.16	+ 399.93	-0.8
	FT-IR		1400–1250 cm^{-1} : C–N stretching of amide III (blood)			+ 27.89
						3400–3200 cm^{-1} : O–H stretching bands of fatty acids. 3000–2800 cm^{-1} : C–H stretching bands of lipids (adipocere).
GQ	Decomp. state	Bloated	Desiccated	Desiccated	Desiccated	Dry remains
	pH	+ 5.9	+ 6.0	+ 5.0	+ 4.8	Cartilage
	DOM (mg/kg)	+ 18.96	+ 92.72	+ 348.08	+ 357.88	+ 2.6
	FT-IR	1750 cm^{-1} : triglycerides 1670 cm^{-1} : unsaturated fatty acids	3630 cm^{-1} : O–H bond of water molecules 1750 cm^{-1} : triglycerides 1670 cm^{-1} : unsaturated fatty acids	3630 cm^{-1} : O–H bond of water molecules 1750 cm^{-1} : triglycerides 1670 cm^{-1} : unsaturated fatty acids	3630 cm^{-1} : O–H bond of water molecules. 3500–3100 cm^{-1} : O–H stretching of fatty acids 1750 cm^{-1} : triglycerides. 1670 cm^{-1} : unsaturated fatty acids. 1450 cm^{-1} , 1080 cm^{-1} and 870 cm^{-1} : vibration modes C–O of carbonate groups CO_3^{2-} .	3000–2800 cm^{-1} : C–H stretching bands of lipids (adipocere). 1450 cm^{-1} , 1080 cm^{-1} and 870 cm^{-1} : vibration modes C–O of carbonate groups CO_3^{2-} .
					Advanced decay	
GC	Decomp. state	Advanced Bloating	Active decay	Humid mummification	Adipocere	Dry remains
	pH	-0.3	+ 2.5	+ 0.8	+ 1.6	-0.6
	DOM (mg/kg)	+ 7.21	+ 678.33	+ 141.93	+ 2.25	+ 28.41
	FT-IR		3130 and 3050 cm^{-1} : C–H stretching bands. Shoulder at 2800 cm^{-1} : =C–H stretching of unsaturated fatty acids. 1080 and 1040 cm^{-1} : Phospholipid degradation products.	1400–1250 cm^{-1} : C–N stretching of amide III (proteins/phospholipid degradation). 3 small peaks between 1080 and 1020 cm^{-1} : cotton cellulose. 850 cm^{-1} : CH_2 rocking of lipids.	3400–3200 cm^{-1} : O–H stretching bands of fatty acids. 3 small peaks between 1080 and 1020 cm^{-1} : cotton cellulose. 850 cm^{-1} : CH_2 rocking of lipids.	3400–3200 cm^{-1} : O–H stretching bands of fatty acids. 3000–2800 cm^{-1} : C–H stretching bands of lipids (adipocere). 1580 and 1550 cm^{-1} : carboxylate bands of fatty acid calcium salts. Modes 1500 and 1400 cm^{-1} : lipid CH_2 scissoring. 3 small peaks between 1080 and 1020 cm^{-1} : cotton cellulose. 850 cm^{-1} : CH_2 rocking of lipids.

for identifying a CDI that is no longer macroscopically visible. For example, in cases where a body is recovered in a skeletonized state, the chromatic alterations typically associated with the CDI may no longer be discernible. However, the application of chemical analyses may still yield signals attributable to cadaveric material, as observed in the case of FT-IR analyses. Furthermore, in scenarios involving the transfer of a body to a secondary deposition site, the original burial location may nonetheless remain informative. In such contexts, an interdisciplinary approach is essential, involving archaeologists capable of identifying the original grave bottom through stratigraphic interpretation and pedology experts able to recognize the natural soil profile of the area.

Finally, the potential influence of atmospheric conditions on the results must be considered. As shown in Figs. 1 and 2, marked temperature fluctuations and several episodes of heavy precipitation occurred during different sampling phases. These factors may have influenced the results, with precipitation in particular potentially affecting soil chemical dynamics, especially given the characteristics of the soils in the Ticino-LEAF(s) experimental area. These soils are shallow and derived from unconsolidated parent material and are therefore characterized by high porosity, which may predispose them to fluid leaching.

However, based on the observed trends in pH and DOM concentrations within the HCDCI, the overall pattern appears to reflect an initial peak in organic matter input followed by a gradual depletion, suggesting a limited influence of precipitation events on the measured parameters. A possible explanation may be related to burial depth (50–60 cm) and to soil disturbance associated with grave excavation and backfilling, which likely displaced the organic soil horizon, typically characterized by lower porosity, into deeper layers. Nevertheless, these interpretations remain speculative and warrant further investigation.

5. Conclusions

The study of biogeochemical processes associated with cadaver decomposition in natural environments and in contact with soil has traditionally focused on the cadaver decomposition island (CDI); however, several underlying mechanisms remain insufficiently explored. Among these, the behavior of organic carbon has yielded ambiguous and, at times, difficult-to-interpret results, leading previous studies to suggest limited utility of bulk organic carbon measurements for estimating the post-burial interval (PBI) of cadavers concealed in clandestine graves, largely due to the naturally high background levels of organic carbon in soils. In this context, the present study suggests that analyzing both quantitative and qualitative variations in dissolved organic matter (DOM), assessed through FT-IR spectroscopy within the HCDCI over time, may provide a potentially informative, time-dependent, and decomposition stage-dependent indicator for PBI estimation.

Across most experimental groups, an initial alkaline phase was observed, which appeared to correspond to active decay and the release of ammonium ions, followed by a gradual acidification during the later stages of decomposition. Trends in DOM concentration generally mirrored this progression, with increases during intermediate PBIs (3–4) followed by subsequent decreases, likely reflecting both the input of decomposition-derived organic matter and its progressive depletion or transformation. The group treated with quicklime exhibited atypical chemical behavior, likely attributable to the strong alkalinity of CaO and its reaction products, which may have masked typical pH signals and altered decomposition processes. Nevertheless, DOM remained detectable in this group, suggesting that the analytical approach may retain sensitivity even under chemically altered conditions. In contrast, the group of piglets wrapped in cotton T-shirts yielded results that diverged from those observed in the other groups, raising questions regarding the role of clothing as a potential barrier to the diffusion of putrefactive fluids into the surrounding environment.

FT-IR spectroscopy further revealed group-specific molecular features. Lipid- and fatty acid-related signals, often associated with adipocere formation, were consistently detected during later stages of

decomposition across all groups, alongside spectral indicators of protein degradation and blood-derived compounds. Variations in body treatment and concealment conditions appeared to influence decomposition dynamics and the expression of biochemical markers, underscoring the importance of considering burial context when interpreting forensic soil data.

Overall, the combined application of pH measurements, DOM quantification, and FT-IR spectroscopy appears to represent a promising approach for monitoring decomposition processes and may hold forensic relevance for PMI and PBI estimation. The findings highlight the potential value of high-resolution analytical techniques for tracking temporal changes in DOM within CDIs, thereby providing additional insight into decomposition-related soil alterations.

Further experimental investigation is needed to determine whether reproducible DOM concentration patterns can be reliably associated with specific postmortem intervals, thereby improving the robustness and forensic applicability of this approach. Given its relative speed, low cost, and operational simplicity, the proposed method may be suitable for real-case forensic contexts, where it could complement existing investigative tools by providing additional temporal information. Nevertheless, considering the pilot nature of this study and the limited sample size, the results should be interpreted with caution, and further research is required to validate and refine the proposed methodology.

CRedit authorship contribution statement

Fulvia Tambone: Writing – review & editing, Resources, Methodology, Investigation, Formal analysis. **Alex Golinelli:** Methodology. **Anna Masseroli:** Writing – review & editing, Investigation. **Giulia Tagliabue:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cristina Cattaneo:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Luca Trombino:** Writing – review & editing, Supervision, Resources, Funding acquisition.

Institutional review board statement

Ethical approval received from the University of Milan Ethics Committee.

Funding

This research received no external funding.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for this journal and was not involved in the editorial review or the decision to publish this article.

Acknowledgments

The authors would like to thank the Lombardy Ticino Park, Cassolnovo (PV), Italy.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.forsciint.2026.112887](https://doi.org/10.1016/j.forsciint.2026.112887).

Data availability

The authors declare that all data supporting the findings of this study are available within the paper.

References

- [1] D.O. Carter, D. Yellowlees, M. Tibbett, Cadaver decomposition in terrestrial ecosystems, *Naturwissenschaften* 94 (2007) 12–24, <https://doi.org/10.1007/s00114-006-0159-1>.
- [2] J.P. Fancher, J.A. Aitkenhead-Peterson, T. Farris, K. Mix, A.P. Schwab, D. J. Wescott, M.D. Hamilton, An evaluation of soil chemistry in human cadaver decomposition islands: Potential for estimating postmortem interval (PMI), *Forensic Sci. Int.* 279 (2017) 130–139, <https://doi.org/10.1016/j.forsciint.2017.08.002>.
- [3] P.S. Barton, A. Reboldi, B.M. Dawson, M. Ueland, C. Strong, J.F. Wallman, Soil chemical markers distinguishing human and pig decomposition islands: a preliminary study, *Forensic Sci. Med. Pathol.* 16 (4) (2020) 605–612, <https://doi.org/10.1007/s12024-020-00297-2>.
- [4] L.M. Dubois, P.H. Stefanuto, L. Heudt, J.F. Focant, K.A. Perrault, Characterizing decomposition odor from soil and adipocere samples at a death scene using HS-SPME-GC× GC-HRTOFMS, *Forensic Chem.* 8 (2018) 11–20, <https://doi.org/10.1016/j.forc.2018.01.001>.
- [5] Larizza, M. and Forbes, S.L. "Detection of fatty acids in the lateral extent of the cadaver decomposition island" in *Environmental and Criminal Geoforescences*, Editor (s) Pirrie, D., Ruffell, A., Dawson, L.A., Geological Society of London (2013). <https://doi.org/10.1144/sp384.11>.
- [6] J.A. Aitkenhead-Peterson, C.G. Owings, M.B. Alexander, N. Larison, J.A. Bytheway, Mapping the lateral extent of human cadaver decomposition with soil chemistry, *Forensic Sci. Int.* 216 (1–3) (2012) 127–134, <https://doi.org/10.1016/j.forsciint.2011.09.007>.
- [7] B. Anderson, J. Meyer, D.O. Carter, Dynamics of ninhydrin-reactive nitrogen and pH in gravesoil during the extended postmortem interval, *J. Forensic Sci.* 58 (2013) 1348–1352, <https://doi.org/10.1111/1556-4029.12230>.
- [8] I. Szelec, I. Koenig, C.V.W. Seppey, R.C. Le Bayon, E.A.D. Mitchell, Soil chemistry changes beneath decomposing cadavers over a one-year period, *Forensic Sci. Int.* 286 (2018) 155–165, <https://doi.org/10.1016/j.forsciint.2018.02.031>.
- [9] A.L. Benninger, D.O. Carter, S.L. Forbes, The biochemical alteration of soil beneath a decomposing carcass, *Forensic Sci. Int.* 180 (2008) 70–75, <https://doi.org/10.1016/j.forsciint.2008.07.001>.
- [10] J. Meyer, B. Anderson, D.O. Carter, Seasonal variation of carcass decomposition and gravesoil chemistry in a cold (Dfa) climate, *J. Forensic Sci.* 58 (2013) 1175–1182, <https://doi.org/10.1111/1556-4029.12169>.
- [11] G. Tagliabue, A. Masseroli, S.I.E. Ern, R. Comolli, F. Tambone, C. Cattaneo, L. Trombino, The fate of phosphorus in experimental burials: chemical and ultramicroscopic characterization and environmental control of its persistency, *Geosciences* 13 (2) (2023) 24, <https://doi.org/10.3390/geosciences13020024>.
- [12] C. Martin, P. Maesen, D. Minchilli, F. Francis, F. Verheggen, Forensic taphonomy: characterization of the gravesoil chemistry using a multivariate approach combining chemical and volatile analyses, *Forensic Sci. Int.* 318 (2021) 110569, <https://doi.org/10.1016/j.forsciint.2020.110569>.
- [13] M.H. Chantigny, D.A. Angers, K. Kaiser, K. Kalbitz, Extraction and characterization of dissolved organic matter, *Soil Sampl. Methods Anal.* 2 (2008) 617–635.
- [14] A. Nebbioso, A. Piccolo, Molecular characterization of dissolved organic matter (DOM): a critical review, *Anal. Bioanal. Chem.* 405 (1) (2013) 109–124, <https://doi.org/10.1007/s00216-012-6363-2>.
- [15] B. Smreczak, A. Ukalska-Jaruga, Dissolved organic matter in agricultural soils, *SOIL Sci. Annu.* 2021 72 (1) (2021) 132234, <https://doi.org/10.37501/soilsa/132234>.
- [16] E.L. Peci, S. Forbes, F. Guillemette, Organic matter composition as a driver of soil bacterial responses to pig carcass decomposition in a Canadian continental climate, *J. Geophys. Res. Biogeosci.* 129 (2024) e2024JG008355, <https://doi.org/10.1029/2024JG008355>.
- [17] H. Reuter, J. Gensel, M. Elvert, D. Zak, Evidence for preferential protein depolymerization in wetland soils in response to external nitrogen availability provided by a novel FTIR routine, *Biogeosciences* 17 (2) (2020) 499–514, <https://doi.org/10.5194/bg-17-499-2020>.
- [18] P. Cross, T. Simmons, The influence of penetrative trauma on the rate of decomposition, *J. Forensic Sci.* 55 (2) (2010) 295–301, <https://doi.org/10.1111/j.1556-4029.2009.01277.x>.
- [19] S. Matuszewski, S. Konwerski, K. Frątczak, et al., Effect of body mass and clothing on decomposition of pig carcasses, *Int. J. Leg. Med.* 128 (2014) 1039–1048, <https://doi.org/10.1007/s00414-014-0965-5>.
- [20] E.M. Schotsmans, J.N. Fletcher, J. Denton, R.C. Janaway, A.S. Wilson, Long-term effects of hydrated lime and quicklime on the decay of human remains using pig cadavers as human body analogues: field experiments, *Forensic Sci. Int.* 238 (2014) 141–e1, <https://doi.org/10.1016/j.forsciint.2013.12.046>.
- [21] A.C. Smith, The effects of sharp-force thoracic trauma on the rate and pattern of decomposition, *J. Forensic Sci.* 59 (2014) 319–326, <https://doi.org/10.1111/1556-4029.12338>.
- [22] M. Ueland, K.D. Nizio, S.L. Forbes, B.H. Stuart, The interactive effect of the degradation of cotton clothing and decomposition fluid production associated with decaying remains, *Forensic Sci. Int.* 255 (2015) 56–63, <https://doi.org/10.1016/j.forsciint.2015.05.029>.
- [23] G. Tagliabue, A. Masseroli, G. Caccia, P. Poppa, C. Cattaneo, L. Trombino, Overview of geosciences applied to forensic taphonomy: the interdisciplinary approach of the Italian non-human research facility (Ticino-LEAFs), *Geosciences* 14 (12) (2024) 359, <https://doi.org/10.3390/geosciences14120359>.
- [24] Kottek, M., Grieser, J., Beck, C., Rudolf, B., & Rubel, F. World map of the Köppen-Geiger climate classification updated (2006). <https://doi.org/10.1127/0941-2948/2006/0130>.
- [25] G. Caccia, *Interazione Corpo Ambiente: Il Fil Rouge Tra Analisi Tafonomiche e Tracce in Contesto Forense*, Ph.D. Thesis. University of Milan, Italy, 2022.
- [26] ERSAL, I suoli del Parco del Ticino settore meridionale, Progett. "Carta Pedol. (1996).
- [27] Soil Survey Staff. *Keys to Soil Taxonomy*, 13th edition., USDA Natural Resources Conservation Service, 2022.
- [28] C. Cattaneo, E. Maderna, A. Rendinelli, D. Gibelli, Animal experimentation in forensic sciences: how far have we come? *Forensic Sci. Int.* 254 (2015) e29–e35.
- [29] B. Knight, Forensic science and animal rights, *Forensic Sci. Int.* 57 (1) (1992) 1–3.
- [30] C.G. Mole, M. Heyns, Animal models in forensic science research: justified use or ethical exploitation? *Sci. Eng. Ethics* 25 (4) (2019) 1095–1110.
- [31] N. Chavhan, G. Gawande, Emerging chemical oxygen demand detection techniques for wastewater: advances in sustainable analytical methods, *Water Air Soil Pollut.* 236 (13) (2025) 1–40.
- [32] F. Collini, S.A. Andreola, G. Gentile, M. Marchesi, E. Muccino, R. Zoja, Preservation of histological structure of cells in human skin presenting mummification and corification processes by Sandison's rehydrating solution, *Forensic Sci. Int.* 244 (2014) 207–212.
- [33] B. Madea, *Handbook of forensic medicine* (Ed.), John Wiley & Sons, 2014.
- [34] P. Saukko, B. Knight, *Knight's forensic pathology*, CRC press, 2015.
- [35] J.K. Rai, B.J. Pickles, M.A. Perotti, The impact of the decomposition process of shallow graves on soil mite abundance, *J. Forensic Sci.* 67 (2) (2022) 605–618, <https://doi.org/10.1111/2F1556-4029.14906>.
- [36] E.M. Schotsmans, J. Denton, J. Dekeirschlieter, T. Ivaneanu, S. Leentjes, R. C. Janaway, et al., Effects of hydrated lime and quicklime on the decay of buried human remains using pig cadavers as human body analogues, *Forensic Sci. Int.* 217 (1–3) (2012) 50–59, <https://doi.org/10.1016/j.forsciint.2011.09.025>.
- [37] A.A. Vass, W.M. Bass, J.D. Wolt, J.E. Foss, J.T. Ammons, Time since death determinations of human cadavers using soil solution, *J. Forensic Sci.* 37 (5) (1992) 1236–1253, <https://doi.org/10.1520/JFS13311J>.
- [38] L.S. Taylor, A.R. Mason, H.L. Noel, M.E. Essington, M.C. Davis, V.A. Brown, J. M. DeBruyn, Transient hypoxia drives soil microbial community dynamics and biogeochemistry during human decomposition, *FEMS Microbiol. Ecol.* 100 (10) (2024) fae119.
- [39] C.M. Orphanou, The detection and discrimination of human body fluids using ATR FT-IR spectroscopy, *Forensic Sci. Int.* 252 (2015) e10–e16, <https://doi.org/10.1016/j.forsciint.2015.04.020>.
- [40] Z. Movasaghi, S. Rehman, I. ur Rehman, Dr. Fourier transform infrared (FTIR) spectroscopy of biological tissues, *Appl. Spectrosc. Rev.* 43 (2) (2008) 134–179, <https://doi.org/10.1080/05704920701829043>.
- [41] A. Lerchi, T. Krap, P. Eppenberger, A. Pedergrana, Implementation of adipocere fingerprinting in archaeology by applying a forensic approach, *TrAC Trends Anal. Chem.* 157 (2022) 116801, <https://doi.org/10.1016/j.trac.2022.116801>.
- [42] S.J. Notter, B.H. Stuart, R. Rowe, N. Langlois, The initial changes of fat deposits during the decomposition of human and pig remains, *J. Forensic Sci.* 54 (1) (2009) 195–201, <https://doi.org/10.1111/j.1556-4029.2008.00911.x>.
- [43] S. Collins, B. Stuart, M. Ueland, Monitoring human decomposition products collected in clothing: an infrared spectroscopy study, *Aust. J. Forensic Sci.* 52 (4) (2019) 428–438, <https://doi.org/10.1080/00450618.2019.1593504>.
- [44] S.L. Forbes, B.H. Stuart, B.B. Dent, The effect of the burial environment on adipocere formation, *Forensic Sci. Int.* 154 (1) (2005) 24–34, <https://doi.org/10.1016/j.forsciint.2004.09.107>.
- [45] J. Hopkins, L. Brenner, C.S. Tumosa, Variation of the amide I and amide II peak absorbance ratio in human hair as measured by Fourier transform infrared spectroscopy, *Forensic Sci. Int.* 50 (1) (1991) 61–65, [https://doi.org/10.1016/0379-0738\(91\)90134-5](https://doi.org/10.1016/0379-0738(91)90134-5).
- [46] S.G. Wubshet, I. Måge, U. Böcker, D. Lindberg, S.H. Knutsen, A. Rieder, N. K. Afseth, FTIR as a rapid tool for monitoring molecular weight distribution during enzymatic protein hydrolysis of food processing by-products, *Anal. Methods* 9 (29) (2017) 4247–4254, <https://doi.org/10.1039/C7AY00865A>.
- [47] C.B. Andersen, Understanding carbonate equilibria by measuring alkalinity in experimental and natural systems, *J. Geosci. Educ.* 50 (4) (2002) 389–403, <https://doi.org/10.5408/1089-9995-50.4.389>.
- [48] Y. Song, H. Li, X. Gan, M. Zhang, M. Xue, J. Li, Comparative experimental study on calcium oxide, calcium hydroxide, and calcium carbonate solidified zinc-contaminated red clay, *Geofluids* 2022 (1) (2022) 8428982, <https://doi.org/10.1155/2022/8428982>.
- [49] T. Ilani, E. Schulz, B. Chefetz, Interactions of organic compounds with wastewater dissolved organic matter: role of hydrophobic fractions, *J. Environ. Qual.* 34 (2) (2005) 552–562, <https://doi.org/10.2134/jeq2005.0552>.
- [50] S.I. Kuriyavar, R. Vetrivel, S.G. Hegde, A.V. Ramaswamy, D. Chakrabarty, S. Mahapatra, Insights into the formation of hydroxyl ions in calcium carbonate: temperature dependent FTIR and molecular modelling studies, *J. Mater. Chem.* 10 (8) (2000) 1835–1840, <https://doi.org/10.1039/B001837F>.
- [51] M.J. Blesa, J.L. Miranda, R. Moliner, Micro-FTIR study of the blend of humates with calcium hydroxide used to prepare smokeless fuel briquettes, *Vib. Spectrosc.* 33 (1–2) (2003) 31–35, [https://doi.org/10.1016/S0924-2031\(03\)00089-4](https://doi.org/10.1016/S0924-2031(03)00089-4).

- [52] A. Card, P. Cross, C. Moffatt, T. Simmons, The effect of clothing on the rate of decomposition and Diptera colonization on *Sus scrofa* carcasses, *J. Forensic Sci.* 60 (4) (2015) 979–982, <https://doi.org/10.1111/1556-4029.12750>.
- [53] M. Deng, H. Wu, Z. Zhu, S. Xie, G. Wang, X. Liang, Z. Wang, New perspective: an in vitro study on inferring the post-mortem interval (PMI) of human skeletal muscle based on ATR-FTIR spectroscopy combined with machine learning, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* (2025) 126284, <https://doi.org/10.1016/j.saa.2025.126284>.
- [54] K. Yu, H. Zhang, Y. Liu, H. Wu, W. Cai, X. Wei, Z. Wang, Adipose tissue estimates the postmortem interval based on ATR-FTIR spectroscopy, *Microchem. J.* 164 (2021) 105977, <https://doi.org/10.1016/j.microc.2021.105977>.
- [55] A.A. Vass, The Use of Volatile Fatty Acid Biomarkers to Estimate the Post-Mortem Interval (Editor(s)), in: E.M.J. Schotsmans, S.L. Márquez-Grant, Forbes (Eds.), *Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment: Forensic Analysis of the Dead and the Depositional Environment*, 2017, pp. 385–393, <https://doi.org/10.1002/9781118953358.ch25> (Editor(s)).
- [56] B.H. Stuart, S. Forbes, B.B. Dent, G. Hodgson, Studies of adipocere using diffuse reflectance infrared spectroscopy, *Vib. Spectrosc.* 24 (2) (2000) 233–242, [https://doi.org/10.1016/S0924-2031\(00\)00097-7](https://doi.org/10.1016/S0924-2031(00)00097-7).

3.2.4. Major Organic Acids: VFAs, Lactic Acid, and Succinic Acid

HPLC analysis conducted on soil collected from the control grave before inhumation (control soil) and from the HCDIs after each exhumation allowed us to detect the VFAs (acetic, propionic, butyric, and isovaleric) and other carboxylic-related acids (namely lactic and succinic) concentration through time. Below are the acids concentrations illustrated, followed by the corresponding graphical representations. The raw data are provided in the Appendix (Table A.6, p. 385), followed by the spectra produced by the instrument, also in the Appendix (pp. 393-446). Control samples were found to be naturally devoid of the analyzed organic acids at every depth, with the only exception of 0-10 depth sample, which recorded 66.15 ppm of acetic acid and 71.80 ppm of propionic acid. Conversely, soil samples from all the HCDIs showed a common increase in organic acids quality and quantity around PBIs 2-3 (Table 3.1).

<i>Group ID</i>	<i>Fatty Acid</i>	<i>PBI 1</i>	<i>PBI 2</i>	<i>PBI 3</i>	<i>PBI 4</i>	<i>PBI 5</i>
GN	succinic	0.00	\	0.00	15.59	16.60
	lactic	0.00	\	176.35	0.00	20.06
	acetic	0.00	\	0.00	0.00	0.00
	propionic	200.55	\	552.60	0.00	73.44
	butyric	137.18	\	234.16	0.00	0.00
	isovaleric	0.00	\	808.75	0.00	30.62
GW	succinic	0.00	68.76	0.00	0.00	22.82
	lactic	0.00	98.50	54.83	53.24	0.00
	acetic	0.00	155.38	83.95	212.95	0.00
	propionic	0.00	1562.06	269.29	669.6	89.42
	butyric	0.00	501.72	0.00	0.00	0.00
	isovaleric	0.00	0.00	0.00	0.00	0.00
GQ	succinic	0.00	0.00	0.00	0.00	0.00
	lactic	63.95	29.02	0.00	9.69	0.00
	acetic	204.74	111.98	140.48	92.52	46.98
	propionic	526.37	90.54	277.90	0.00	52.30

3. The Experiment - Hypogeal Cadaver Decomposition Island

	butyric	107.12	0.00	149.46	0.00	0.00
	isovaleric	1007.56	130.14	1300.58	170.25	989.20
GC	succinic	0.00	1393.32	23.42	0.00	0.00
	lactic	0.00	0.00	66.30	0.00	0.00
	acetic	0.00	284.36	0.00	0.00	0.00
	propionic	126.01	3678.59	79.38	0.00	115.70
	butyric	44.04	2343.80	0.00	0.00	0.00
	isovaleric	0.00	655.74	0.00	0.00	44.68

Table 3.1: Resume of the concentration (mg soil kg⁻¹ or ppm) of VFAs, succinic, and lactic acids in the HCDIs through PBIs 1-5.

Unfortunately, the absence of the GN2 sample made it difficult to reconstruct the temporal trend for this group (Figure 3.17a). Nonetheless, an increase in propionic and butyric acid values can already be observed at PBI1 (200.55 and 137.18 ppm, respectively), followed by a general quantitative and qualitative rise across the classes of organic acids. With the exception of acetic acid (which was never detected in this group) all analyzed fatty acids were identified at PBI3, where they also reached their highest concentrations relative to the subsequent PBIs. High concentrations of isovaleric acid (808.75 ppm) and propionic acid (552.60 ppm) were detected, together with medium concentrations of butyric (234.16 ppm) and lactic (176.35 ppm) acids. The HCDI sample collected at PBI4 showed only low concentrations of succinic acid (15.59 ppm), whereas at PBI5, lactic, propionic, and isovaleric acids were also detected, albeit at relatively low concentrations (< 75 ppm).

For the GW group (Figure 3.17b), no organic acids were detected until PBI2, when the sample showed the presence of all analytes except isovaleric acid. At this interval, propionic acid reached a very high concentration (1562.06 ppm), butyric acid was present at high concentration (501.72 ppm), and acetic (155.38 ppm) and lactic (98.50 ppm) acids were detected at lower levels. PBI3 and PBI4 exhibited similar concentrations of the same acids, with even an increase in acetic acid (from 83.95 to 212.95 ppm) and propionic acid (from 269 to 669 ppm) at the latter interval. Finally, only succinic and propionic acids were detected at PBI 5, at low concentrations (22.82 and 89.42 ppm, respectively). Isovaleric acid was never detected in this group.

3. The Experiment - Hypogeal Cadaver Decomposition Island

The GQ group showed the most anomalous trend compared with the rest of the sample set (Figure 3.17c), with very high concentrations of some acids (1007.56 ppm of isovaleric acid and 526.37 ppm of propionic acid) and medium concentrations of acetic and butyric acids (204.74 and 107.12 ppm, respectively), together with lactic acid at 63.95 ppm. At PBI2, a general decrease in acid concentrations was observed, with all values falling below 150 ppm. This was followed by an increase at PBI3, where the concentrations of all analytes rose again, except for lactic acid, which disappeared. Notably, isovaleric acid showed a marked increase, reaching 1300.58 ppm, while the other analytes, although more abundant than in the previous interval, remained at levels similar to those recorded at PBI1. A second decrease occurred at PBI4, accompanied by the disappearance of propionic and butyric acids. Finally, the sample collected at PBI5 displayed a third peak in isovaleric acid concentration (989 ppm) and low concentrations of acetic and propionic acids (46.98 and 52.30 ppm, respectively). Succinic acid was never detected in this group.

Finally, the GC group (Figure 3.17d) showed the presence of only propionic and butyric acids at PB1, both with concentrations below 150 ppm. In contrast, the sample collected at PBI2 exhibited very high concentrations of propionic acid (3678.59 ppm), butyric acid (2343.80 ppm), and succinic acid (1393.32 ppm), as well as high concentrations of isovaleric acid (665.74 ppm) and acetic acid (284.36 ppm). At PBI3, a sharp decrease in the concentrations of all analytes was recorded, with values falling below 80 ppm, followed by a complete absence of organic acids at PB4 and a slight increase at PBI5, where propionic acid reached 115.70 ppm and isovaleric acid 44.68 ppm.

3. The Experiment - Hypogeal Cadaver Decomposition Island

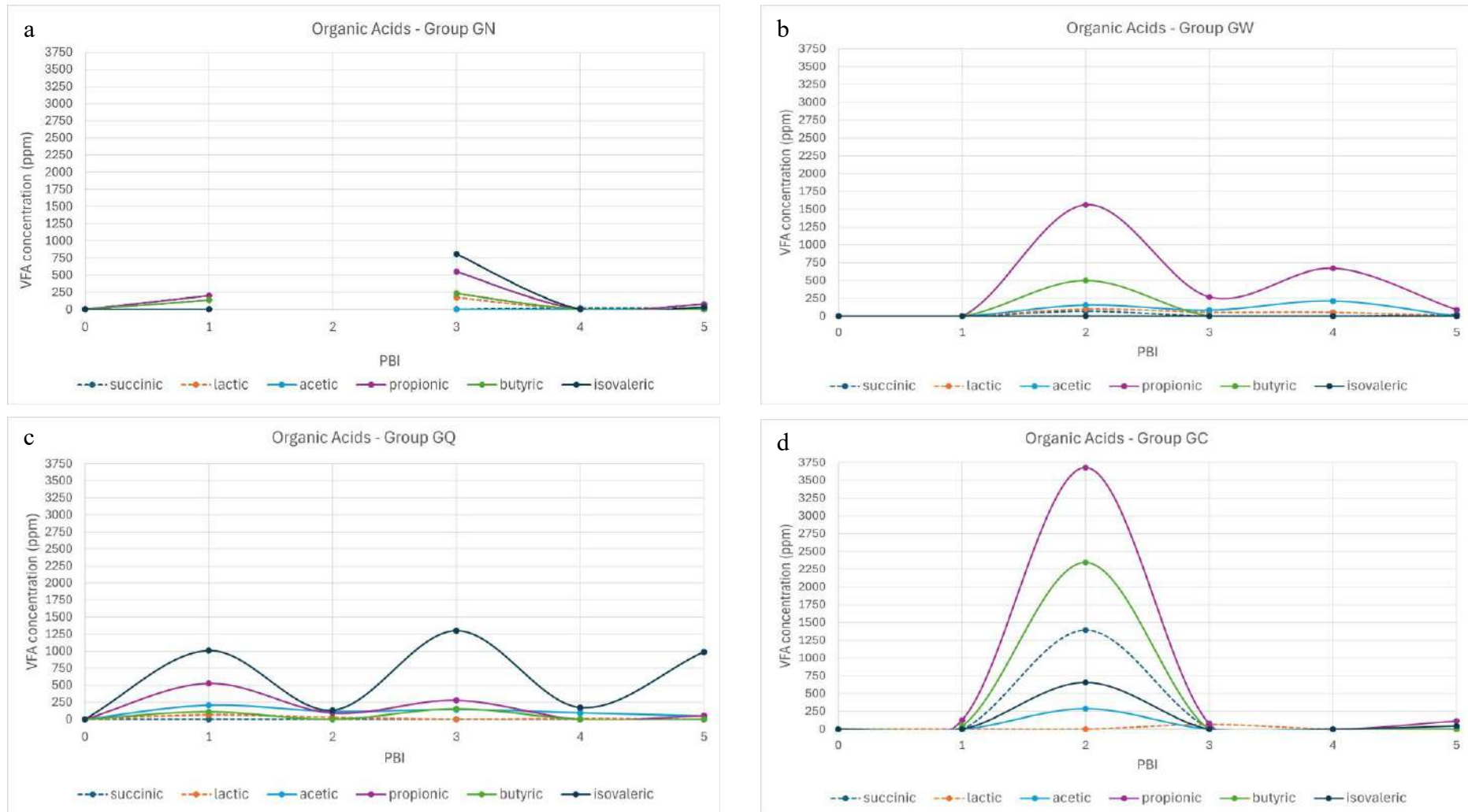


Figure 3.17: VFAs and succinic and lactic acids concentrations in the HCDI of the four groups of burials at different PBIs. The lines indicating the trends of lactic and succinic acid are dashed, in order to distinguish them from the VFA trend. **a-** GN; **b-** GW; **c-** GQ; **d-** GC.

3.2.5. Soil Magnetic Susceptibility and Hysteresis

This section presents the results of the magnetic susceptibility and hysteresis analysis performed on soil samples collected before inhumation from the bottom of the graves (control soil) and after the exhumations from the HCDI, for the GC group only, in which the piglets were buried wrapped in cotton T-shirts.

Regarding the first analysis, the results of frequency-dependent susceptibility at low frequency (Lf), high frequency (Hf), and χ_{fd} (%) are reported below. Three measurements were taken for each sample, labeled “A,” “B,” and “C.” The results are summarized in Table 3.2.

<i>Sample ID</i>	<i>Lf ($\times 10^{-4}$)</i>	<i>Hf ($\times 10^{-4}$)</i>	<i>$\chi_{fd}\%$</i>
GC1 Control soil - A	5.58	5.57	2.85×10^{-1}
GC1 Control soil - B	6.03	5.97	1.06
GC1 Control soil - C	4.71	4.67	9.57×10^{-1}
GC1 HCDI - A	5.02	4.94	1.51
GC1 HCDI - B	5.31	5.25	1.12
GC1 HCDI - C	6.04	6.00	6.63×10^{-1}
GC2 Control soil - A	5.32	5.25	1.30
GC2 Control soil - B	6.89	6.86	3.32×10^{-1}
GC2 Control soil - C	5.98	5.88	1.67
GC2 HCDI - A	3.82	3.78	9.87×10^{-1}
GC2 HCDI - B	3.58	3.55	9.43×10^{-1}
GC2 HCDI - C	4.70	4.66	9.52×10^{-1}
GC3 Control soil - A	5.01	4.99	3.07×10^{-1}
GC3 Control soil - B	4.02	4.00	4.49×10^{-1}
GC3 Control soil - C	5.64	5.63	3.38×10^{-1}
GC3 HCDI - A	3.38	3.34	1.13
GC3 HCDI - B	4.73	4.71	3.64×10^{-1}
GC3 HCDI - C	4.85	4.81	9.93×10^{-1}
GC4 Control soil - A	4.99	4.98	3.13×10^{-1}
GC4 Control soil - B	5.74	5.71	5.36×10^{-1}
GC4 Control soil - C	6.83	6.81	3.60×10^{-1}

3. The Experiment - Hypogeal Cadaver Decomposition Island

GC4 HCDI - A	4.22	4.20	$6.19 \cdot 10^{-1}$
GC4 HCDI - B	4.22	4.20	$3.53 \cdot 10^{-1}$
GC4 HCDI - C	4.58	4.55	$7.86 \cdot 10^{-1}$
GC5 Control soil - A	4.56	4.49	1.48
GC5 Control soil - B	5.53	5.43	1.89
GC5 Control soil - C	6.34	6.33	$1.70 \cdot 10^{-1}$
GC5 HCDI - A	4.37	4.36	$3.02 \cdot 10^{-1}$
GC5 HCDI - B	4.77	4.75	$5.00 \cdot 10^{-1}$
GC5 HCDI - C	4.48	4.42	1.35

Table 3.2: Low Frequency (L_f) and High Frequency (H_f) values expressed in SI (International System of Units) and Percentage of Frequency-Dependent Magnetic Susceptibility ($\chi_{fd}\%$).

The data were plotted in a diagram representing the L_f/H_f ratios of all samples (Figure 3.18). The x-axis shows the L_f values, while the y-axis displays the H_f values, both expressed in SI (International System of Units).

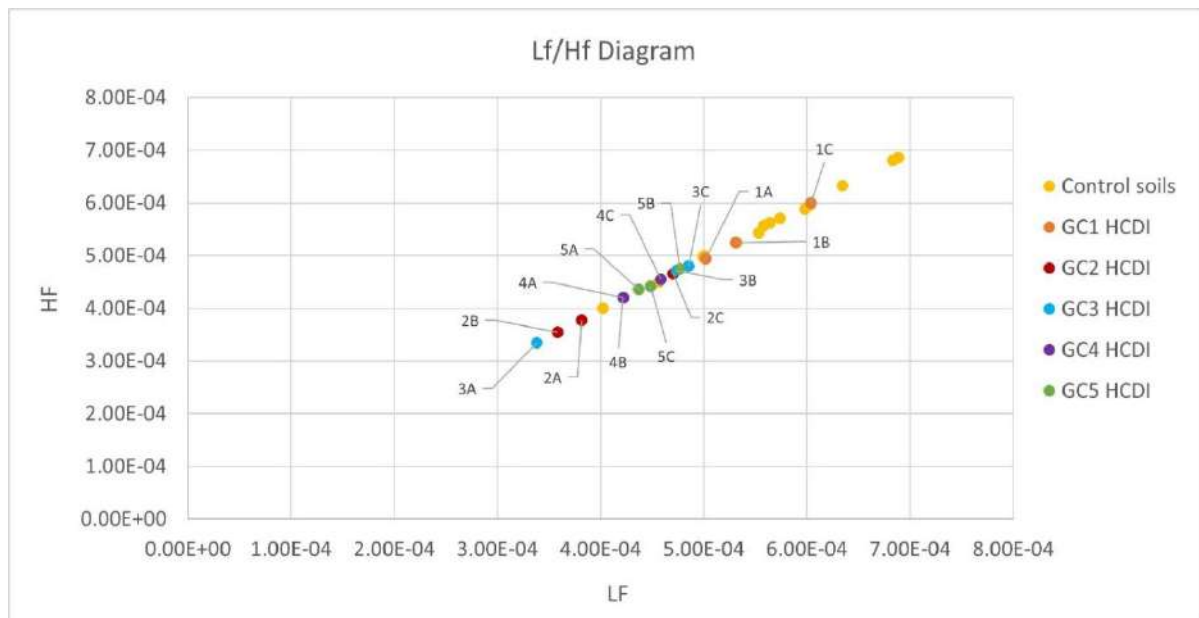


Figure 3.18: Low Frequency (L_f)/High Frequency (H_f) Diagram. Control soil samples are in yellow; HCDI samples follow a color scale and are indicated with a number detecting the PBI followed by a letter A, B, C that detects the sample.

3. The Experiment - Hypogeal Cadaver Decomposition Island

Data analysis showed that the control soil samples exhibited slightly higher frequency-dependent susceptibility values for both L_f and H_f compared with the samples collected from the HCDI. The control samples were also more evenly distributed across the Cartesian space, particularly at higher L_f and H_f values, ranging between 4.02×10^{-4} and 6.89×10^{-4} . Conversely, the HCDI samples appeared more clustered at lower intensities, showing reduced L_f/H_f ratios. Their values fell within a narrower and lower range, between 3.34×10^{-4} and 5.25×10^{-4} , with a single outlier corresponding to sample GC1 HCDI-C, which recorded an L_f value of 6.00×10^{-4} . In both cases, however, the data displayed a spatial distribution along an oblique trend, indicating a relationship between L_f and H_f close to 1.

Hysteresis analysis allowed the identification of a wide range of magnetic parameters that characterized each sample, including saturation magnetization (M_s), saturation remanence (M_r), coercivity (H_c), and remanence coercivity (H_{cr}). The results are first presented graphically and then discussed in detail, with consideration given only to the corrected (manipulated) data. Below are the hysteresis curves, beginning with the uncorrected curves (Figures 3.19a-c), which included paramagnetic and diamagnetic contributions, followed by the corrected curves (Figures 3.20a-c). In these graphs, the x-axis represents the applied magnetic field (H), ranging from -1.5 T to 1.5 T, while the y-axis represents magnetization, expressed in $\text{Am}^2 \text{kg}^{-1}$.

Overall observation of the graphs showed that the specific saturation magnetization (M_s) values were comparable among the samples, although the control soil samples displayed a wider range (up to approximately $0.17 \text{ Am}^2/\text{kg}$), while the HCDI samples exhibited a narrower range (up to about $0.13 \text{ Am}^2/\text{kg}$). Figure 3.19 shows how control soil samples are more dispersed within the plot compared to the HCDI samples, which appear more homogeneous, with narrower M_s ranges. Finally, the hysteresis loops are visibly thinner in the HCDI samples than in the control soil samples.

3. The Experiment - Hypogeal Cadaver Decomposition Island

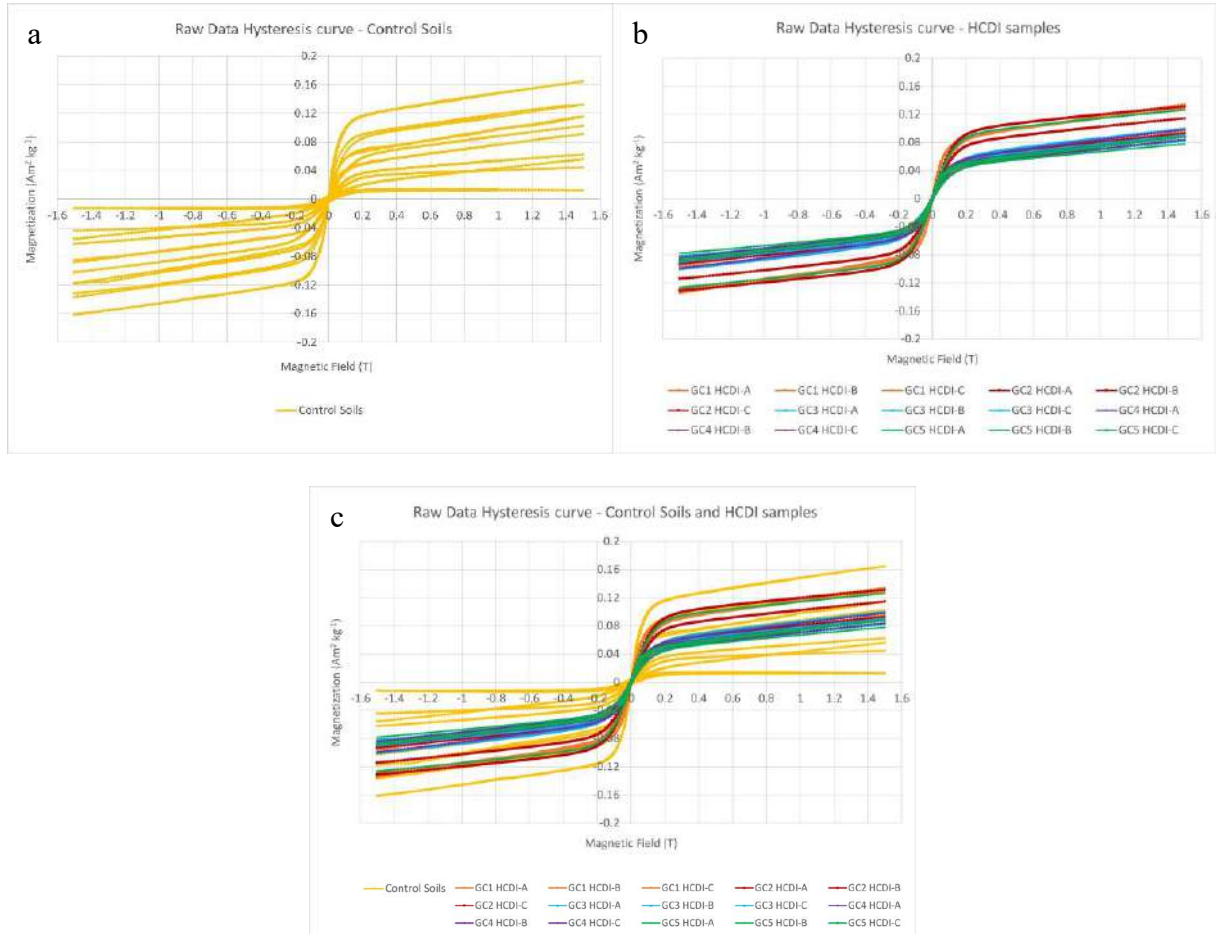


Figure 3.19: Raw data hysteresis curves. **a-** control soils; **b-** HCDI samples; **c-** superimposition of the two curves.

3. The Experiment - Hypogeal Cadaver Decomposition Island

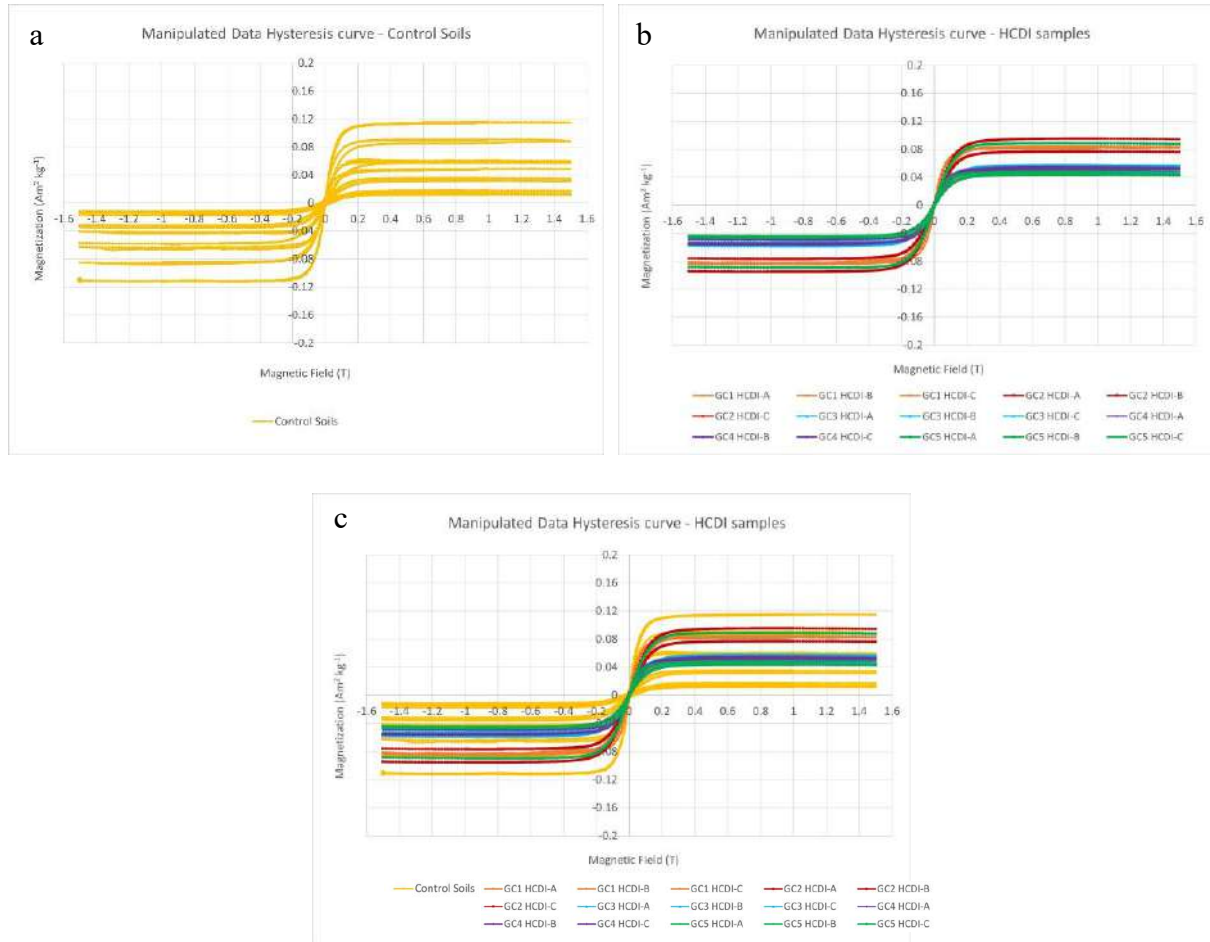


Figure 3.20: Manipulated data hysteresis curves. **a-** control soils; **b-** HCDI samples; **c-** superimposition of the two curves.

3. The Experiment - Hypogeal Cadaver Decomposition Island

In the case of the manipulated data curves, the hysteresis cycles obtained for the control soil samples displayed a generally linear trend, with higher magnetization values (except in a few cases) when compared to those obtained from the HCDI samples. The latter, in fact, showed a closed loop with lower saturation magnetization values.

In the corrected curves as well, this pattern was evident: the HCDI samples exhibited a more clustered distribution of magnetization values, whereas the control soil samples were more widely dispersed.

To quantify the differences between the control soil and HCDI samples, the results for saturation magnetization (M_s , $\text{Am}^2 \text{kg}^{-1}$) were extracted, and both the mean and standard deviation were calculated for raw (Table 3.3) and manipulated (Table 3.4) data.

	<i>Control soil</i>	<i>GC1 HCDI</i>	<i>GC2 HCDI</i>	<i>GC3 HCDI</i>	<i>GC4 HCDI</i>	<i>GC5 HCDI</i>
<i>Saturation magnetization ($\text{Am}^2 \text{kg}^{-1}$)</i>	$1.23 \cdot 10^{-2}$	$1.29 \cdot 10^{-1}$	$1.14 \cdot 10^{-1}$	$8.89 \cdot 10^{-2}$	$8.39 \cdot 10^{-2}$	$7.79 \cdot 10^{-2}$
	$4.44 \cdot 10^{-2}$	$1.35 \cdot 10^{-1}$	$9.24 \cdot 10^{-2}$	$8.15 \cdot 10^{-2}$	$9.88 \cdot 10^{-2}$	$1.26 \cdot 10^{-1}$
	$5.55 \cdot 10^{-2}$	$9.38 \cdot 10^{-2}$	$1.31 \cdot 10^{-1}$	$1.01 \cdot 10^{-1}$	$8.73 \cdot 10^{-2}$	$8.69 \cdot 10^{-2}$
	$8.49 \cdot 10^{-2}$					
	$1.60 \cdot 10^{-1}$					
	$1.02 \cdot 10^{-1}$					
	$1.18 \cdot 10^{-1}$					
	$1.32 \cdot 10^{-1}$					
	$6.23 \cdot 10^{-2}$					
	$1.24 \cdot 10^{-2}$					
<i>Mean values</i>	$7.84 \cdot 10^{-2}$	$1.19 \cdot 10^{-1}$	$1.12 \cdot 10^{-1}$	$9.03 \cdot 10^{-2}$	$9.00 \cdot 10^{-2}$	$9.70 \cdot 10^{-2}$
<i>S.D. (s)</i>	$4.98 \cdot 10^{-2}$	$2.21 \cdot 10^{-2}$	$1.94 \cdot 10^{-2}$	$9.57 \cdot 10^{-3}$	$7.79 \cdot 10^{-3}$	$2.56 \cdot 10^{-2}$

Table 3.3: Raw saturation magnetizations values of the samples. Below are mean and S.D. values.

3. The Experiment - Hypogeal Cadaver Decomposition Island

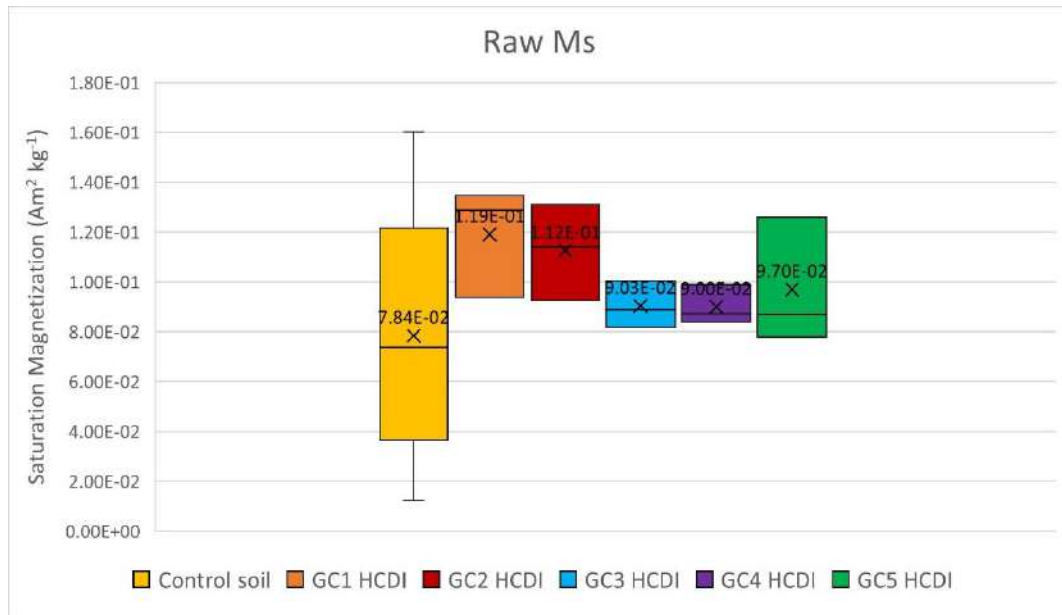


Figure 3.21: Boxplots representing the raw Ms values of control soil and HC DI samples.

For raw hysteresis curves, the values were more or less similar, with a comparable order of magnitude. However, the calculation of the standard deviation showed a larger value for the control soil samples compared to those from the HC DI at the various PBIs. These observations can also be appreciated through the boxplots (Figure 3.21).

3. The Experiment - Hypogeal Cadaver Decomposition Island

	<i>Control soil</i>	<i>GC1 HCDI</i>	<i>GC2 HCDI</i>	<i>GC3 HCDI</i>	<i>GC4 HCDI</i>	<i>GC5 HCDI</i>
<i>Saturation magnetization ($\text{Am}^2 \text{kg}^{-1}$)</i>	$1.46 \cdot 10^{-2}$	$8.43 \cdot 10^{-2}$	$7.69 \cdot 10^{-2}$	$5.37 \cdot 10^{-2}$	$4.43 \cdot 10^{-2}$	$4.41 \cdot 10^{-2}$
	$3.19 \cdot 10^{-2}$	$8.36 \cdot 10^{-2}$	$5.60 \cdot 10^{-2}$	$4.75 \cdot 10^{-2}$	$5.44 \cdot 10^{-2}$	$8.91 \cdot 10^{-2}$
	$1.77 \cdot 10^{-2}$	$4.65 \cdot 10^{-2}$	$9.53 \cdot 10^{-2}$	$5.83 \cdot 10^{-2}$	$5.04 \cdot 10^{-2}$	$4.81 \cdot 10^{-2}$
	$4.64 \cdot 10^{-2}$					
	$1.14 \cdot 10^{-1}$					
	$5.95 \cdot 10^{-2}$					
	$6.47 \cdot 10^{-2}$					
	$8.92 \cdot 10^{-2}$					
	$3.52 \cdot 10^{-2}$					
	$1.19 \cdot 10^{-2}$					
<i>Mean values</i>	$4.85 \cdot 10^{-2}$	$7.15 \cdot 10^{-2}$	$7.61 \cdot 10^{-2}$	$5.31 \cdot 10^{-2}$	$4.97 \cdot 10^{-2}$	$6.05 \cdot 10^{-2}$
<i>S.D. (s)</i>	$3.37 \cdot 10^{-2}$	$2.16 \cdot 10^{-2}$	$1.97 \cdot 10^{-2}$	$5.44 \cdot 10^{-3}$	$5.09 \cdot 10^{-3}$	$2.49 \cdot 10^{-2}$

Table 3.4: Manipulated saturation magnetizations values of the samples. Below are mean and S.D. values.

Similarly to the previous case, the corrected hysteresis curves showed values of comparable order of magnitude for all samples. In this case as well, although less pronounced, the standard deviation and the boxplots (Figure 3.22) indicated greater variability in the saturation magnetization values of the control soil samples.

3. The Experiment - Hypogeal Cadaver Decomposition Island

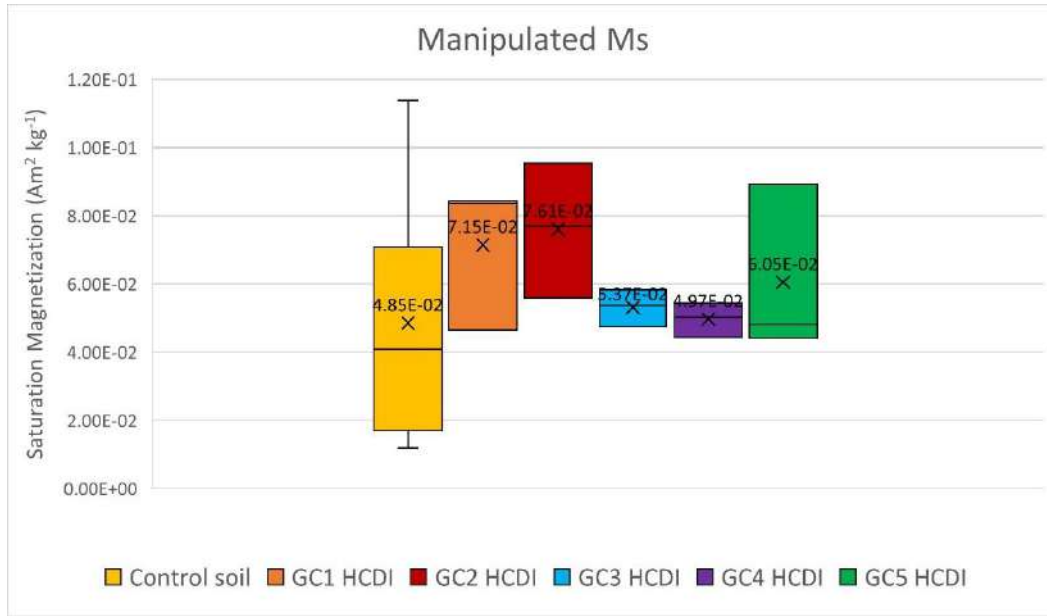


Figure 3.22: Boxplots representing the manipulated M_s values of control soil and HCDI samples.

The hysteresis analysis also allowed observation of the coercivity of remanence. The data are represented in the Direct Current Demagnetization (DCD) graph (Figure 3.23), where the x-axis shows the increasing demagnetization field, $H(T)$, and the y-axis represents the normalized residual magnetization, $M_r (A \cdot m^2/kg)$.

As in the previous cases, a more dispersed distribution was observed for the control soil samples, while the HCDI samples displayed a more regular and compact distribution.

Some traces showed greater signal fluctuation, which may be attributed to instrumental noise.

3. The Experiment - Hypogeal Cadaver Decomposition Island

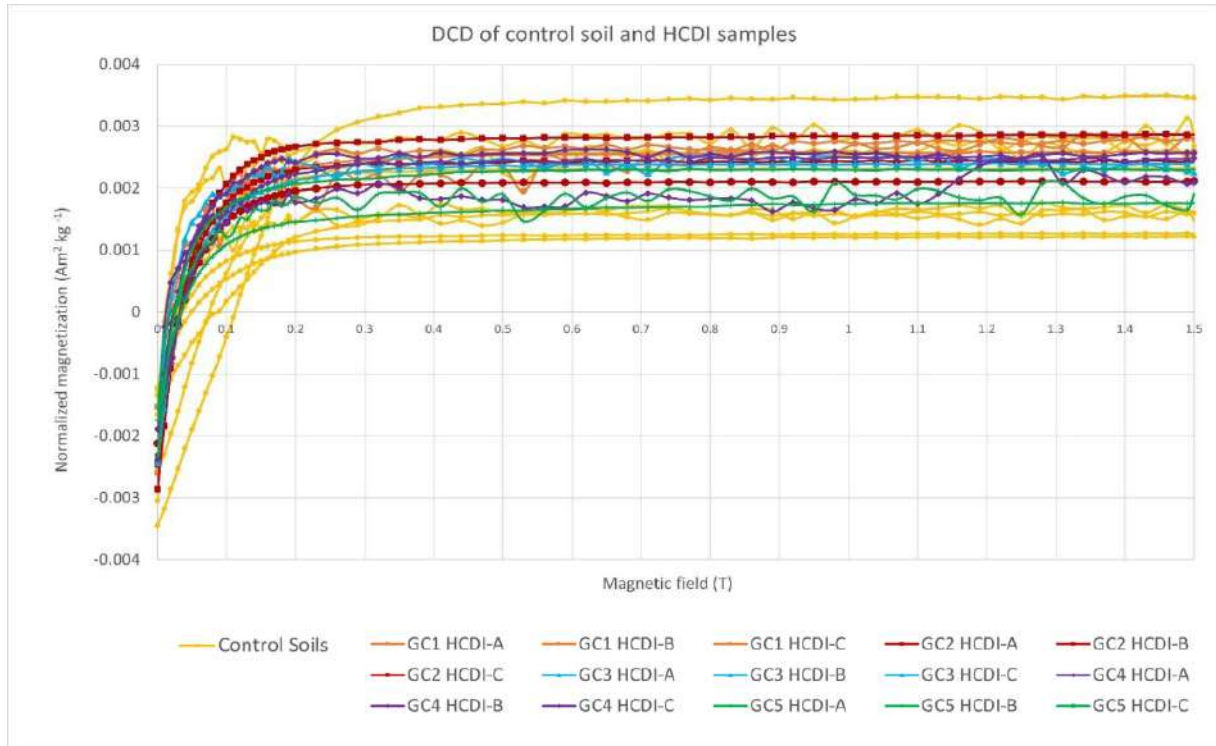


Figure 3.23: DCD curves of control soil and HCDI samples.

Finally, the parameters obtained from the hysteresis analyses on the manipulated curves only (Table 3.5) were used to construct the Day diagram (Figure 3.24).

Specifically, the ratios M_r/M_s and H_{cr}/H_c were used, plotted on the x-axis and y-axis, respectively.

3. The Experiment - Hypogeal Cadaver Decomposition Island

<i>Sample ID</i>	<i>Ms</i> (Am^2kg^{-1})	<i>Mr</i> (Am^2kg^{-1})	<i>Mr/Ms</i>	<i>Hc</i> (T)	<i>Hcr</i> (T)	<i>Hcr/Hc</i>
GC1 CS - A	$1.46 \cdot 10^{-2}$	$3.48 \cdot 10^{-3}$	$2.38 \cdot 10^{-1}$	$3.30 \cdot 10^{-2}$	$1.12 \cdot 10^{-1}$	3.41
GC1 CS - B	$3.19 \cdot 10^{-2}$	$1.32 \cdot 10^{-3}$	$4.13 \cdot 10^{-2}$	$5.80 \cdot 10^{-3}$	$3.66 \cdot 10^{-2}$	6.32
GC2 CS - A	$1.77 \cdot 10^{-2}$	$1.71 \cdot 10^{-3}$	$9.65 \cdot 10^{-2}$	$1.33 \cdot 10^{-2}$	$8.68 \cdot 10^{-2}$	6.50
GC3 CS - A	$4.64 \cdot 10^{-2}$	$2.21 \cdot 10^{-3}$	$4.76 \cdot 10^{-2}$	$3.22 \cdot 10^{-3}$	$1.93 \cdot 10^{-2}$	5.98
GC3 CS - B	$1.14 \cdot 10^{-1}$	$3.14 \cdot 10^{-3}$	$2.75 \cdot 10^{-2}$	$1.68 \cdot 10^{-3}$	$1.20 \cdot 10^{-2}$	7.16
GC3 CS - C	$5.95 \cdot 10^{-2}$	$2.49 \cdot 10^{-3}$	$4.18 \cdot 10^{-2}$	$5.05 \cdot 10^{-3}$	$3.16 \cdot 10^{-2}$	6.25
GC4 CS - A	$6.47 \cdot 10^{-2}$	$1.12 \cdot 10^{-3}$	$1.73 \cdot 10^{-2}$	$1.37 \cdot 10^{-3}$	$2.79 \cdot 10^{-2}$	$2.05 \cdot 10^{-1}$
GC4 CS - B	$8.92 \cdot 10^{-2}$	$4.64 \cdot 10^{-3}$	$5.20 \cdot 10^{-2}$	$3.79 \cdot 10^{-3}$	$1.58 \cdot 10^{-2}$	4.18
GC5 CS - A	$3.52 \cdot 10^{-2}$	$1.20 \cdot 10^{-3}$	$3.42 \cdot 10^{-2}$	$4.10 \cdot 10^{-3}$	$4.95 \cdot 10^{-2}$	$1.21 \cdot 10^{-1}$
GC5 CS - B	$1.19 \cdot 10^{-2}$	$2.60 \cdot 10^{-3}$	$2.19 \cdot 10^{-1}$	$2.60 \cdot 10^{-2}$	$3.42 \cdot 10^{-2}$	1.32
GC1 HCDI - A	$8.43 \cdot 10^{-2}$	$2.70 \cdot 10^{-3}$	$3.20 \cdot 10^{-2}$	$3.98 \cdot 10^{-3}$	$3.19 \cdot 10^{-2}$	8.00
GC1 HCDI - B	$8.36 \cdot 10^{-2}$	$2.03 \cdot 10^{-3}$	$2.43 \cdot 10^{-2}$	$1.85 \cdot 10^{-3}$	$1.89 \cdot 10^{-2}$	$1.02 \cdot 10^{-1}$
GC1 HDCI - C	$4.65 \cdot 10^{-2}$	$3.28 \cdot 10^{-3}$	$7.06 \cdot 10^{-2}$	$6.33 \cdot 10^{-3}$	$3.26 \cdot 10^{-2}$	5.15
GC2 HCDI - A	$7.69 \cdot 10^{-2}$	$2.71 \cdot 10^{-3}$	$3.52 \cdot 10^{-2}$	$4.40 \cdot 10^{-3}$	$3.25 \cdot 10^{-2}$	7.38
GC2 HCDI - B	$5.60 \cdot 10^{-2}$	$2.19 \cdot 10^{-3}$	$3.91 \cdot 10^{-2}$	$4.83 \cdot 10^{-3}$	$3.52 \cdot 10^{-2}$	7.28
GC2 HDCI - C	$9.53 \cdot 10^{-2}$	$2.99 \cdot 10^{-3}$	$3.14 \cdot 10^{-2}$	$4.00 \cdot 10^{-3}$	$3.32 \cdot 10^{-2}$	8.29
GC3 HCDI - A	$5.37 \cdot 10^{-2}$	$2.99 \cdot 10^{-3}$	$5.57 \cdot 10^{-2}$	$4.10 \cdot 10^{-3}$	$1.72 \cdot 10^{-2}$	4.19
GC3 HCDI - B	$4.75 \cdot 10^{-2}$	$1.90 \cdot 10^{-3}$	$4.01 \cdot 10^{-2}$	$2.82 \cdot 10^{-3}$	$1.43 \cdot 10^{-2}$	5.07
GC3 HDCI - C	$5.83 \cdot 10^{-2}$	$2.46 \cdot 10^{-3}$	$4.21 \cdot 10^{-2}$	$5.04 \cdot 10^{-3}$	$3.44 \cdot 10^{-2}$	6.83
GC4 HCDI - A	$4.43 \cdot 10^{-2}$	$2.57 \cdot 10^{-3}$	$5.80 \cdot 10^{-2}$	$6.11 \cdot 10^{-3}$	$3.39 \cdot 10^{-2}$	5.55
GC4 HCDI - B	$5.44 \cdot 10^{-2}$	$2.57 \cdot 10^{-3}$	$4.73 \cdot 10^{-2}$	$3.93 \cdot 10^{-3}$	$2.37 \cdot 10^{-2}$	6.02
GC4 HDCI - C	$5.04 \cdot 10^{-2}$	$2.60 \cdot 10^{-3}$	$5.15 \cdot 10^{-2}$	$3.68 \cdot 10^{-3}$	$1.33 \cdot 10^{-2}$	3.62
GC5 HCDI - A	$6.74 \cdot 10^{-2}$	$2.80 \cdot 10^{-3}$	$4.15 \cdot 10^{-2}$	$2.63 \cdot 10^{-3}$	$1.47 \cdot 10^{-2}$	5.60
GC5 HCDI - B	$4.41 \cdot 10^{-2}$	$1.84 \cdot 10^{-3}$	$4.17 \cdot 10^{-2}$	$4.39 \cdot 10^{-3}$	$3.42 \cdot 10^{-2}$	7.78
GC5 HDCI - C	$4.81 \cdot 10^{-2}$	$2.02 \cdot 10^{-3}$	$4.21 \cdot 10^{-2}$	$2.95 \cdot 10^{-3}$	$1.97 \cdot 10^{-2}$	6.70

Table 3.5: Main parameters obtained from the hysteresis cycles. CS = Control Soil.

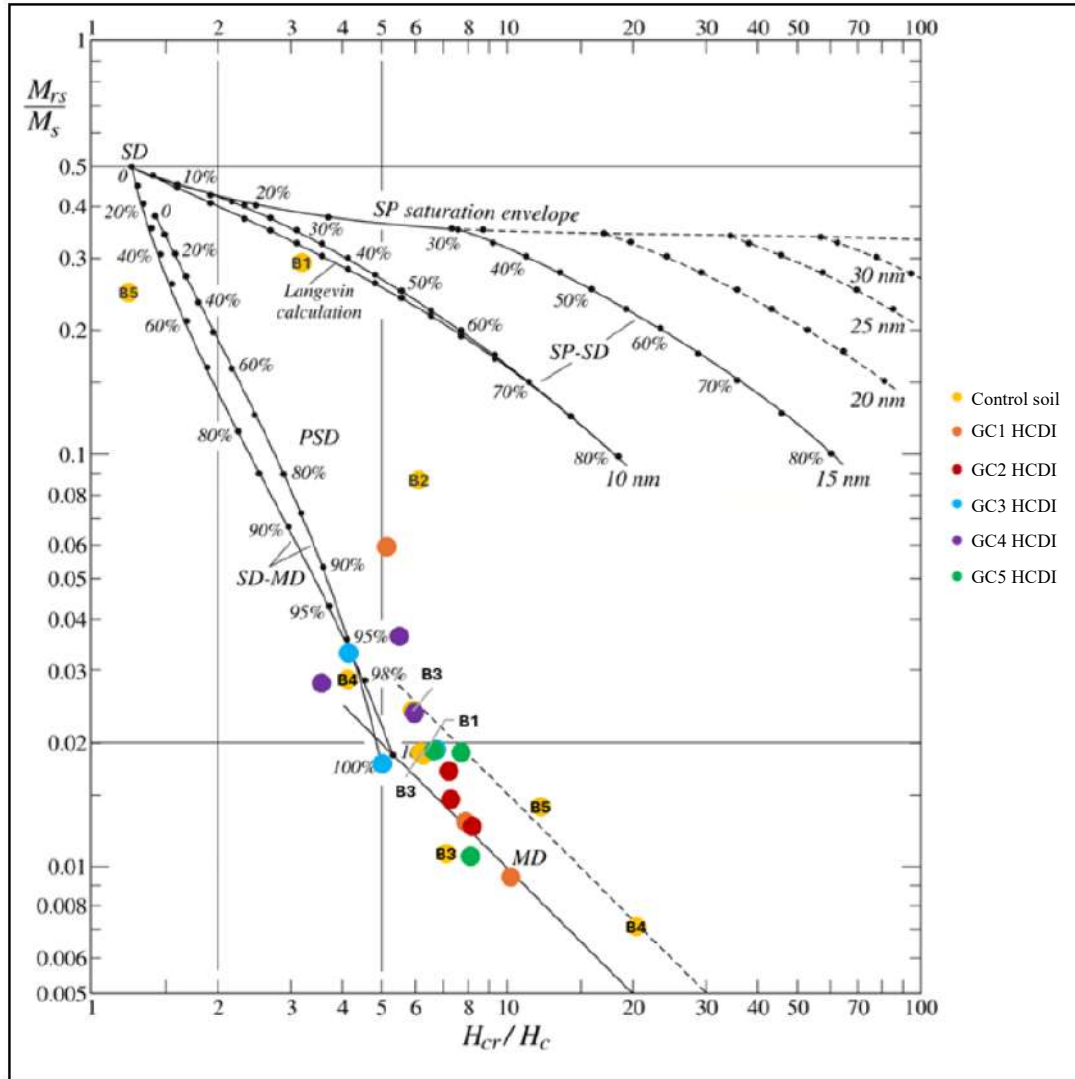


Figure 3.24: Day diagram. The samples marked in yellow represent the control soil, while those marked in different colors correspond to the HC DI samples. For graphical clarity, the HC DI samples were not visually categorized but are differentiated only by color, as shown in the legend on the right.

From the Day diagram, it could be observed that most of the samples were distributed within the multi-domain (MD) sector or the SD–MD transition region. No samples, however, fell within the single-domain (SD) or superparamagnetic (SP) fields.

Once again, the control soil samples were more widely distributed, occupying multiple regions of the Day diagram, whereas the HC DI samples appeared more homogeneous and concentrated within the MD and SD-MD fields.

– CHAPTER 4 –

THE EXPERIMENT

FORENSIC TAPHONOMY & DECOMPOSITION

This chapter presents the materials and methods, as well as the results collected during the experiment to investigate the interaction between the cadavers and the surrounding environment from the former point of view. The following sections describe the piglets employed as human analogues for decomposition observations, together with the associated materials, treatments, and concealment methods. Subsequently, the results concerning PMI estimations, wound taphonomy, and IGSR detection are presented.

4.1. Materials and Methods

4.1.1. Materials

4.1.1.1. Piglets (*Sus scrofa domesticus*)

The experiment presented in this PhD thesis involved 20 subadult domestic piglets (*Sus scrofa domesticus*) that died of natural causes or for reasons entirely unrelated to this research. Immediately after death was confirmed at the farm, the subjects were frozen and subsequently transferred to the morgue facilities of the Milan City Mortuary, where an external examination was conducted to assess their actual state of preservation. They were then subjected to lateral–lateral radiographic examination using a Poscom portable X-ray unit (model PXM-40BT) to exclude the presence of any wounds not macroscopically detectable, after which they were weighed.

The 20 piglets were divided into four burial groups (five piglets per group) to ensure the greatest possible homogeneity in body mass within each group (Table 4.1). Additionally, a twenty-first piglet was selected as a control for the GW group (GW0) to observe lesion patterns and recover weapon residues from a never-buried body. Finally, all piglets were placed at the center of the graves, lying on their right side (Figures 4.1-4), on the same day.

4. The Experiment – Forensic Taphonomy & Decomposition

Table 4.1: List of the piglets involved in the experiment and the corresponding weight. The ID “GW0” indicates the control pig that was added as a control (unburied) for the gunshot wounds.

<i>Burial group</i>	<i>Piglet ID</i>	<i>Weight (kg)</i>
GN	GN1	3.8
	GN2	3.6
	GN3	3.7
	GN4	3.5
	GN5	3.7
GW	GW0	4.8
	GW1	6.9
	GW2	4.2
	GW3	4.1
	GW4	6.9
	GW5	4.9
GQ	GQ1	3.1
	GQ2	3.0
	GQ3	2.9
	GQ4	3.1
	GQ5	2.7
GC	GC1	3.3
	GC2	3.4
	GC3	3.4
	GC4	3.5
	GC5	3.4

4. The Experiment – Forensic Taphonomy & Decomposition



Figure 4.1: Deposition of the piglets, group GN. **a-** GN1; **b-** GN2; **c-** GN3; **d-** GN4; **e-** GN5.

4. The Experiment – Forensic Taphonomy & Decomposition



Figure 4.2: Deposition of the piglets, group GW. **a-** GW1; **b-** GW2; **c-** GW3; **d-** GW4; **e-** GW5.

4. The Experiment – Forensic Taphonomy & Decomposition

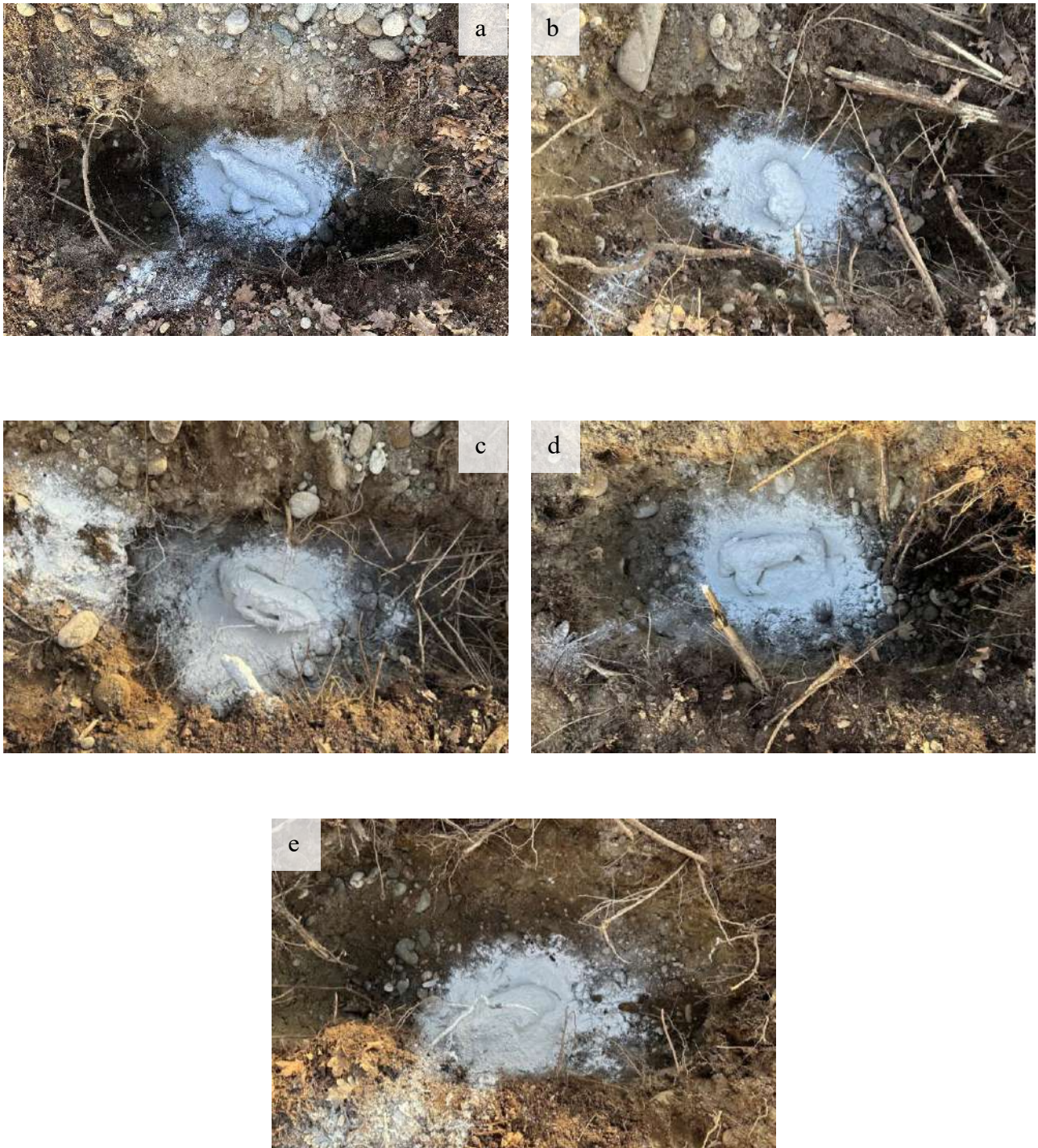


Figure 4.3: Deposition of the piglets, group GQ. **a-** GQ1; **b-** GQ2; **c-** GQ3; **d-** GQ4; **e-** GQ5.

4. The Experiment – Forensic Taphonomy & Decomposition

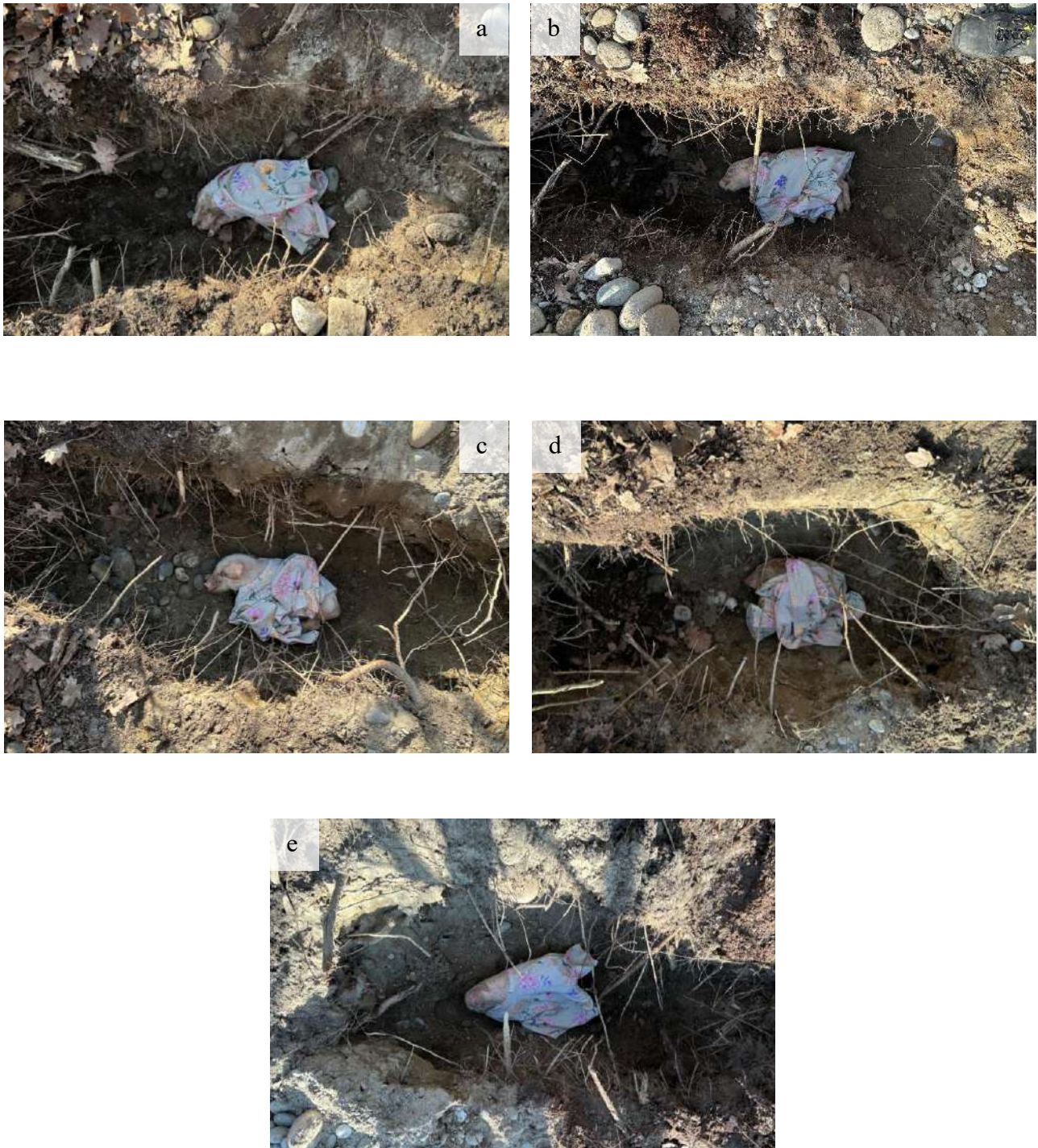


Figure 4.4: Deposition of the piglets, group GC. **a-** GC1; **b-** GC2; **c-** GC3; **d-** GC4; **e-** GC5.

4.1.1.2. Associated Material & Treatments

The four burial groups were characterized by different treatments and materials associated with the bodies. The GN group served as the control, with piglets buried naked and without any additional treatment.

Conversely, the piglets in the GW group were harmed post-mortem using different types of weapons, all hypothetically capable of leaving inorganic traces on the body. In particular, the subjects underwent:

- Two sharp-force wounds, both inflicted with the cutting edge of a steel, single-edged kitchen knife, oriented toward the operator and directed parallel to the main axis of the ribs. The piglets were lying on their right side during the procedure. The two lesions (one ventral and one dorsal) were approximately 4 cm apart (Figures 4.5a,b).

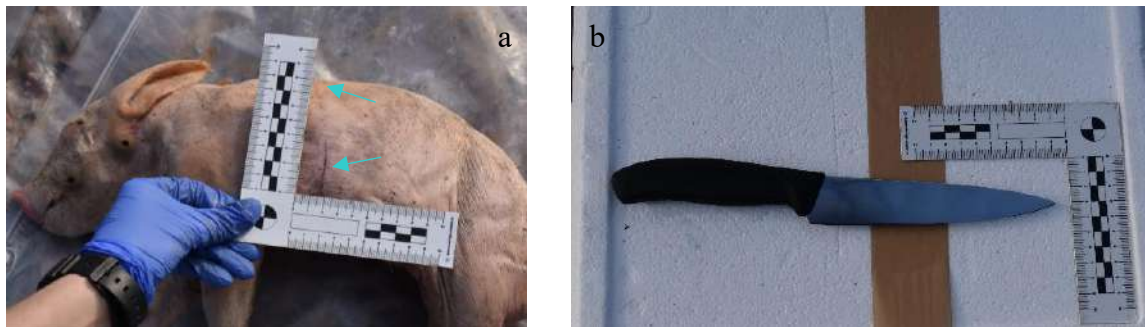


Figure 4.5: *a-* detail of the two lesions on piglet GW2 (arrows); *b-* the knife.

- Two blunt-force wounds affecting the hind limbs at the tarsus and metatarsus of the foot; one inflicted in a medio-lateral direction on the right limb and one in a latero-medial direction on the left limb. A ball-peen blacksmith's hammer with a carbon-steel head and wooden handle was used (Figures 4.6a,b).

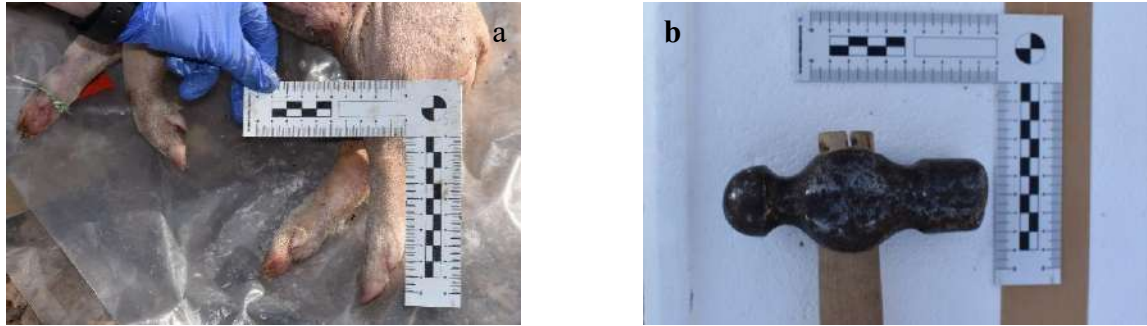


Figure 4.6: *a- the two lesions on piglet GW2; b- the hammer.*

- Two perforating gunshot wounds to the head, fired from left to right, from a distance of approximately 5 cm. One shot was located anteriorly in the maxillary region of the skull, while the other one was shot posteriorly in the cranial region, about 5 cm apart. During the firing procedure, the subjects were wrapped in several layers of plastic film, leaving only the head exposed, to prevent the spread of gunshot residues over the rest of the body. The firings were conducted at the USD Uboldo Shooting range with a Walther PP semi-automatic pistol, caliber 7.65 mm Browning loaded with Fiocchi brand full metal jackets bullet (Figures 4.7a,b). All wounds were documented with photographs including an ABFO metric reference scale.

As previously mentioned, only the lesions and residues related to gunshots are considered in this PhD thesis. Furthermore, due to extensive fragmentation, it was not possible to recover the skull of piglet GW3; consequently, this sample was excluded from the study.



Figure 4.7: *a- detail of the two entry wounds on piglet GW2 (arrows); b- the shooting.*

4. The Experiment – Forensic Taphonomy & Decomposition

Conversely, the subjects belonging to the GQ group were completely covered with a layer of quicklime (CaO) before burial, whereas those in the GC group were dressed in beige 100% cotton T-shirts with a printed floral pattern and the inscription “ALWAYS CREATIVE,” brand H&M (Figure 4.8).



Figure 4.8: *one of the T-shirts used for group GC.*

4.1.2. Methods

4.1.2.1. Recovery, Micro-excavation, and Post-burial External Examination of the Remains

The exhumations consisted of recovering one piglet from each burial group, all exhumed on the same day or, when this was not possible, under similar climatic conditions, at predetermined PBIs. To achieve this, a topsoil removal operation was first carried out to eliminate the superficial deposits that had accumulated on the ground surface after burial (such as litter and sediments; Figure 4.9a). This procedure allowed the burial-cut perimeter to be identified, as it became recognizable through chromatic and grain-size anomalies compared with those typically expected in a surface horizon (Figure 4.9b).

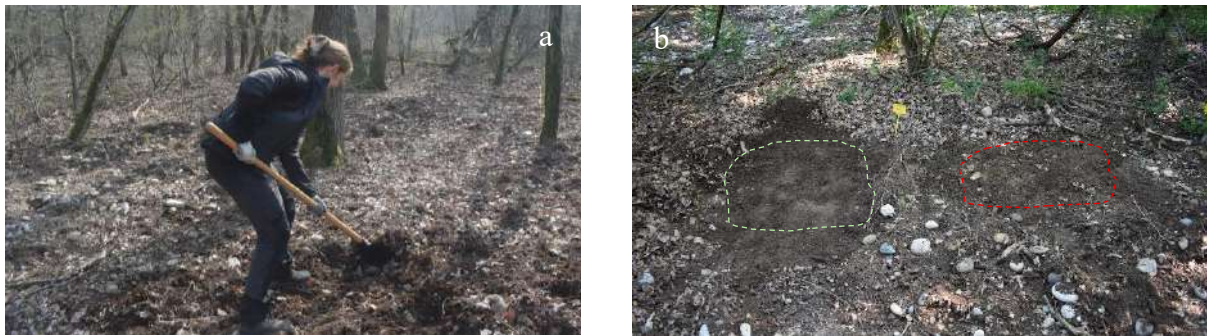


Figure 4.9: *a-* litter and topsoil removal operations; *b-* the result of topsoil removal in group GN. The picture presents an example of natural subsoil (green dotted line) and the perimeter of the grave GN3 showing anomalies in color and texture compared to the surrounding soil (red dotted line).

Once the burial was identified, an archaeological excavation was carried out using a stratigraphic method (Evis et al., 2016). After the outline of the body was recognized, it was isolated from the surrounding matrix through *pedestalling* (Leighton, 2015; Cheetham & Hanson, 2016 – Figure 4.10a). This technique consists of excavating the grave by removing the matrix around the body or artefact, leaving it resting on an artificially formed platform (Cheetham & Hanson, 2016). In this case, due to the difficulty of maintaining the integrity of the bodies, especially during the colliquative phase (PBIs 3-4), a tray covered with transparent plastic film was used for recovery. The tray was gently slid under the bodies and then wrapped around them (Figures 4.10b,c). The bodies were subsequently transported to the Milan City Mortuary for external examination and, for the GW group only, post-burial radiographic analysis. Every operation, from exposure to removal of the remains, was thoroughly documented photographically.



Figure 4.10: *a- example of pedestalling technique applied for the excavation of GQ4; b- Use of the tray for recovery of the remains of GN3; c- Remains of GW2 wrapped in plastic film for transport to the Milan City Mortuary.*

Once at the mortuary, the bodies were stored in refrigerated cells (-20°C).

The exhumations carried out during PBI5, however, followed a different procedure: since the buried bodies were mostly skeletonized, it was not possible to conduct a full recovery of the remains. Consequently, the *pedestalling* technique was applied around the block of soil containing them, which was then wrapped in transparent plastic film and transferred to LABANOF for micro-excavation. Since no standardized methodology for this operation is provided, the procedure followed the approach described by Higgins et al. (2020): the micro-excavation was performed in horizontal layers from top to bottom, following artificial stratigraphy, removing approximately 2 cm of soil at a time until reaching the skeletonized remains.

Once recovered, either through conventional archaeological excavation or micro-excavation, the bodies underwent external examination. In this case, the examination consisted of assessing

4. The Experiment – Forensic Taphonomy & Decomposition

the state of decomposition of the bodies, following an anatomical district-based approach (Section 4.1.2.2).

At the same time, for the GW group only, lateral-later radiographic analyses were performed using a Poscom portable X-ray equipment model PXM-40BT (Section 4.1.2.4), along with the collection of samples for SEM-EDX analysis of gunshot residues (Section 4.1.2.5).

4.1.2.2. Decomposition, Total Body Score (TBS) and PMI Estimation Formulas

Following each exhumation, the external examination was conducted in collaboration with a team of forensic pathologists and trainees in legal medicine to document the decomposition state of the bodies. The widely used four-stage model proposed by Galloway et al. (1989; Fresh, Bloat, Decay, and Dry) served as the initial framework, which was then adapted according to the classification proposed by Megyesi et al. (2005) in order to identify the developmental stage of the process as precisely as possible. Based on these descriptions, a specific evaluation form was created (Figure A.1, available in the Appendix, page 447) to divide the piglet bodies into anatomical regions and assign a score corresponding to their state of decomposition (Total Body Score, TBS). For this operation, the *categories* and *stages of decomposition* from three TBS methods were considered: Megyesi et al. (2005), Keough et al. (2017), and Indra et al. (2024), which have already been presented in Section 1.3.1.1 and summarized in the Appendix (Table A.1, page 335).

Once the TBS data were collected using different methods, they were entered into Excel spreadsheets for the application of the formula for calculating Accumulated Degree Days (ADD) proposed by Megyesi et al. (2005):

$$ADD = 10^{(0.002 * TBS^2 + 1.81)} \pm 388.16$$

For this purpose, the following function was entered into the spreadsheet:

$$= 10^{(0.002 * F2^2 + 1.81)}$$

In this syntax, cell F2 contains the TBS value assigned to the analyzed body. To this value, the standard deviation (S.D.) 388.16 was added and subtracted to define the ADD range within which the value most likely corresponding to the subject's decomposition stage is located.

Subsequently, the same spreadsheet was updated with the daily mean temperature values (in °C) provided by the Cerano (NO) weather station, entered in reverse order starting from the exhumation date and extending backward until reaching the ADD values included in the calculated range. These values were obtained through the cumulative sum of daily temperatures.

Finally, the PMI estimated using this method was compared with the actual PMI. An example of an Excel spreadsheet used for ADD calculation and PMI estimation is provided in the Appendix (Figure A.2, page 448). In addition, the formula proposed by Vass (2011) for the calculation of PMI in buried individuals was applied, as previously presented in Section 1.3.1.1:

$$PMI_{\text{Anaerobic}} = \frac{1285 \times \left(\frac{\text{decomposition}}{100} \right) \times 4.6 \times \text{adipocere}}{0.0103 \times \text{temperature} \times \text{soil moisture}}$$

In this case as well, an Excel spreadsheet was created, in which the following function was entered:

$$= (1285 * (C2 / 100) * 4.6 * D2) / (0.0103 * E2 * F2)$$

In this syntax, cells C2, D2, E2, and F2 contain, respectively, the data related to the decomposition score assigned to the subject, the multiplier associated with the presence of adipocere, the temperature, and the soil moisture percentage. Regarding temperature values, it was not possible to directly measure the temperature at the vault of the grave at the time of exhumation, as suggested in the original article by Vass (2011). According to Vass (2004), shallow burial temperatures tend to mimic surface temperatures, but lag behind them by about 12h. The suggested cutoff is approximately 15°C: burial temperatures would tend to be 3.3°C cooler than atmospheric temperatures above the cutoff and 4.4°C warmer below the cutoff. Vass therefore proposes deriving the burial temperature by subtracting or adding one of the two indicated values to the mean daily temperature on the day the body is discovered. However, these values were calculated on the basis of phenomena observed at the University of Tennessee's Anthropology Research Facility in Knoxville (Tennessee, USA) and may therefore hold validity only in specific environments that share characteristics similar to those of the facility.

Consequently, in the case of Ticino-LEAFs, a comparison was made between the daily mean atmospheric temperatures recorded by the Cerano (NO) weather station and those from the data loggers placed at ground level (Section 4.1.2). Given the near-perfect correspondence between the two datasets, the temperatures provided by the Cerano station were used directly for PMI calculation.

Finally, soil moisture (%) was determined using dry weight assays: 2 g of each soil sample was dried at 100 °C for 24 hours to determine its pre- and post-drying weight. The difference was then converted into a percentage by weight (as described in Section 4.2.2.5).

An example of an Excel spreadsheet used for applying Vass's formula for PMI estimation is provided in the Appendix (Figure A.3, page 449).

4.1.2.3. Taphonomy of Gunshot Wounds: Macroscopic Analysis

The analysis of the taphonomic alterations affecting the gunshot wounds inflicted postmortem on the subjects of the GW group was carried out in the dissection room of the Institute of Legal Medicine of Milan, in collaboration with a team of forensic pathologists and trainees in legal medicine. Contextually, lateral–lateral radiographs were taken using a Poscom portable X-ray equipment model PXM-40BT. An external examination was then performed, including metric–referenced photography of all wounds and swabbing (Section 4.1.2.4).

Subsequently, to observe the lesions affecting the bone tissue, careful defleshing of the muscles and major soft tissues was carried out as a preliminary step prior to maceration. The maceration phase consisted of immersing the bodies in plastic containers filled with cold water, which was partially renewed periodically to maintain the bacterial pool responsible for soft-tissue degradation. This procedure was applied only to the subjects whose soft tissues were still present at the time of the external examination (namely GW0, GW1, and GW2) which required 15, 10, and 5 days of maceration, respectively. At the end of this phase, the remaining soft tissues were carefully removed from each sample using a brush, metal tweezers, plastic tweezers, and a scalpel. The bone elements were then placed on a plastic tray to dry completely (Figure 4.11).

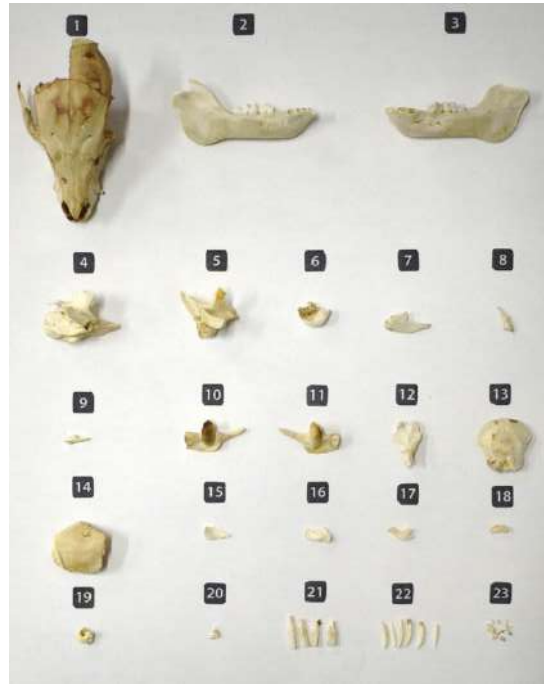


Figure 4.11: Cranial bone elements of GW1 drying after the water maceration phase. Photo by Dr Ludovico Re.

Regarding subject GW4, although the skull was largely skeletonized at the time of examination, some residual soft tissues were still present. These tissues were dried in an oven at 40 °C for 48 hours and subsequently removed using plastic tweezers. In contrast, the skull of subject GW5 was already fully skeletonized at the time of recovery, and therefore no further intervention was required.

The restoration phase was then carried out, consisting of the reversible reassembly of bone elements that could be positively associated, using a hot glue gun, preferably applied to the endocranial surface whenever possible to minimize alteration of external morphological features.

Once restoration was completed, a macroscopic analysis of the lesions was conducted. Each lesion was described and photographed individually with an ABFO metric reference. Specifically, the macroscopic analysis on bone examined peculiar characteristics of wounds' margins (such as morphology and internal or external beveling), and the presence or absence of gunshot-associated cranial bone lesions, such as radiating fractures, concentric fractures,

4. The Experiment – Forensic Taphonomy & Decomposition

deformed bone fragments and bone plugs (Ortner, 1981; Smith et al., 1987; Berryman & Symes, 1998; Quatrehomme & Işcan, 1998; Symes et al., 2012; Berryman, 2019; Stefanopoulos et al., 2025). Finally, the lesions were re-evaluated to identify the injury mechanism responsible for their production, both in absolute terms and in terms of compatibility with experimentally known wounding mechanisms, such as distinguishing entry from exit wounds.

This latter part of the study was conducted concurrently with the preparation of Dr. Ludovico Re's specialization thesis in Legal Medicine. He was responsible for the reconstruction of the skulls and the description of firearm-related lesions.

4.1.2.4. SEM-EDX Analysis of Inorganic Gunshot Residue (IGSR)

The analysis of IGSR was carried out using a *Jeol JSM-IT510* Scanning Electron Microscope equipped with an Energy-Dispersive X-ray system (SEM-EDX). For this purpose, double-sided carbon conductive tape was used to collect swabs from the lesions.

Specifically, before inhumation, the tape was applied by completely covering the posterior entrance and exit wounds (Figure 4.12a) on each cadaver, while during the post-burial external examinations, swabs were taken from the anterior entrance and exit wounds (Figure 4.12b). In both cases, the tape was mounted on a glass microscope slide and stored at -20°C .

In addition, soil samples were collected from both horizons of the control grave (see Chapter 3) to assess any potential risk of environmental or excavator-related contamination, particularly from the metallic components of the excavator bucket. Dedicated specimen carbon-coated stubs were used for sample mounting.

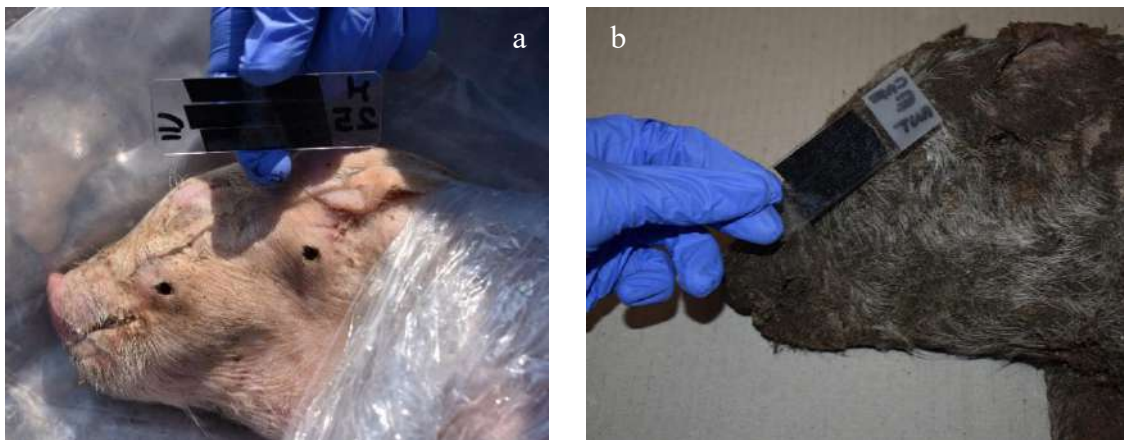


Figure 4.12: IGSR sampling with double-sided carbon conductive tape on piglet GW2. **a-** swabbing operations on the posterior entrance wound of GW2 before the inhumation; **b-** swabbing operations on the anterior entrance wound during the post-burial external examination.

4. The Experiment – Forensic Taphonomy & Decomposition

Once IGSR particles were detected on the samples collected from the wounds, they were classified according to the categories suggested by the latest ASTM standard (2020): “*characteristic of IGSR*” if composed of Pb–Ba–Sb condensed in a single particle; “*consistent with IGSR*” if composed of combinations such as Pb–Ba, Pb–Sb, Sb–Ba, Ba–Ca–Si(S), Ba–Al(S), Ti–Zn, or –Sr; and “*commonly associated with IGSR*” if only Pb, Ba, or Sb were present. For the purposes of this study, an additional category, “*other IGSR*”, was created to include particles potentially associated with firearm discharge that did not fit into the ASTM-defined groups.

Regarding the morphological criteria, particles were confirmed as characteristic of IGSR when they exhibited spheroidal or non-crystalline morphology. However, angular or crusty GSR particles may also be encountered (Brožek-Mucha, 2014; Maitre et al., 2017).

4.2. Results

Chapter 4 aims to present the macroscopically observable effects on body decomposition and taphonomy associated with concealment through clandestine burials. In the following sections, the results of these observations are presented, beginning with the description of the decomposition state of the bodies at exhumation, followed by the attribution of the TBS and the application of Megyesi's ADD and Vass's PMI estimation formulas. Finally, a focus on the taphonomy of gunshot wounds and on IGSR detection in the piglets from the GW group is presented.

4.2.1. Assessment of Decomposition & Taphonomic Alterations

This section reports the descriptions of the decomposition stages observed during the post-burial external examination. The results are presented for each experimental group, from PBI1 to PBI5, and are summarized in Table 4.2.

<i>Piglet ID</i>	<i>PBI (days)</i>	<i>Head & Neck</i>	<i>Trunk</i>	<i>Limbs</i>
PBI 1				
GN1	18	The head is presenting brownish shades on face, ears, and neck. Ears are still fresh. Prominent bloating of neck is observable (<i>Bloated</i>). Slight purging of fluids from the mouth.	The abdomen presents gray-purple discoloration with marbling. General bloating (<i>Bloated</i>).	The limbs show brown-green discoloration with bloating (entire limb- <i>Bloated</i>). No skin slippage.
GW1	18	Face, ears and neck show dark red/brownish discoloration. The neck is bloated	The abdominal area presents purple discoloration with marbling. General	Limbs' skin has glossy appearance and brown-purple discoloration.

4. The Experiment – Forensic Taphonomy & Decomposition

		(<i>Bloated</i>). Purging fluids from the mouth. Initial dry of ears.	bloating (<i>Bloated</i>).	Limbs are bloated, especially in the proximal region (<i>Bloated</i>).
GQ1	18	Face presents purple discoloration, with skin drying and slippage. Drying of lips, nose, and ears. Purging fluids from the mouth. Bloating of neck (<i>Bloated/Decay</i>).	Bloating with purple-black discoloration (<i>Bloated/Decay</i>). Skin drying and slippage.	Limbs present general brown-purple discoloration, skin drying and slippage. Anterior limbs are slightly bloated, while posterior limbs are drying (<i>Bloated/Decay</i>).
GC1	18	Slight dark red discoloration on the head, with prominent bloating of face and neck (<i>Bloated</i>).	Gray-green discoloration and marbling of abdomen with prominent bloating (<i>Bloated</i>).	Purple discoloration on limbs. Bloating of proximal region and skin slippage in distal region (<i>Bloated</i>). Initial loss of posterior hooves.
PBI 2				
GW2	49	Head shows green-gray discoloration, with purging fluids and caving in the flesh of the eyes. Bone exposure in the parietal/occipital region of the skull is observable (<i>Decay</i>).	Trunk appears to be in post-bloating phase, with grey discoloration. Production of sagging of flesh and caving in the abdomen in noticeable (<i>Decay</i>), with slight bone	Limbs show brown to black discoloration with signs related to moist decomposition (<i>Decay</i>). Exposure of feet and legs bones is observable (conversely, soft tissues of tights are still present).

4. The Experiment – Forensic Taphonomy & Decomposition

			exposure in the ventral region.	
GQ2	49	Brown-black discoloration of the head, with caving in the flesh of the eyes. Desiccation of ears and nose, with dry skin slippage is also noticeable. Rostrum bones are exposed (<i>Decay</i>).	Purple-black discoloration of the abdomen with severe dry skin slippage is observable (<i>Decay</i>).	Posterior legs present brown-black discoloration, with dry skin slippage and phalanges exposure. Anterior legs show grey-black discoloration with bone exposure, from forearm on (<i>Decay/Dry</i>).
GC2	49	Head presents brown-black discoloration with bone exposure for more than a half of the skull. Some moist flesh is still present (<i>Decay/Dry</i>).	Purple-black discoloration and purging of decomposition fluids. Skin slippage and probable fungi colonization under the T-shirt (<i>Decay</i>).	Proximal region of the limbs presents grey-green discoloration. Conversely, complete bone exposure from forearm on is noticeable (<i>Decay/Dry</i>).
PBI 3				
GN3	101	On skull, signs related to moist decomposition are observable, with presence of purging fluids. Less than a half of the skull shows bone exposure (<i>Decay</i>).	The trunk presents black discoloration, with decomposition of tissue producing sagging of flesh (<i>Decay</i>).	Limbs bone exposure is almost complete. However, some decomposed greasy tissue and fluids are still present (<i>Decay/Dry</i>).
GW3	101	Only some bones from the skull were	Shreds of moist soft tissues in the	Almost complete bone exposure.

4. The Experiment – Forensic Taphonomy & Decomposition

		recovered. They showed presence of moist decomposed tissue with purging fluids (<i>Decay/Dry</i>).	dorsal region, with large bone exposure (<i>Decay/Dry</i>).	Scarce decomposed moist tissue and fluids are present (<i>Decay/Dry</i>).
GQ3	101	Head shows brown-black discoloration of skin, with no bone exposure. Drying of nose and ears, and dry skin slippage are noticeable. Caving in the tissues of eye and throat (<i>Decay</i>).	Purple-black discoloration under the epidermis, with dry skin slippage. Caving in the abdominal cavity (<i>Decay</i>).	Limbs show purple-black discoloration and dry skin slippage with partial loss of hooves (<i>Decay</i>). No bone exposure.
GC3	101	Purple discoloration of the head. Skin has leathery appearance (<i>wet mummification</i>).	Purple-black discoloration, especially in the abdominal region. Skin has leathery appearance (<i>wet mummification</i>).	Purple-black discoloration of the limbs. Skin has leathery appearance (<i>wet mummification</i>).

PBI 4

GN4	206	Skull is fully skeletonized with abundant presence of roots and some greasy substance (<i>Dry</i>).	High amount of preserved fat tissue (adipocere) is noticeable. Body fluids are present. Diffused bone exposure (<i>Decay/Dry</i>).	Limbs show bone exposure, with some greasy decomposed tissue and body fluids (<i>Dry</i>).
GW4	220	Bone exposure of more than half of the skull, with greasy substances (adipocere) and	Trunk presents high amount of preserved fat tissue (adipocere). Body	Bone exposure is observable, with some greasy decomposed tissue

4. The Experiment – Forensic Taphonomy & Decomposition

		decomposition tissue fluids are also and body fluids (<i>Dry</i>). present, with (<i>Dry</i>). partial bone exposure (<i>Decay/Dry</i>).	
GQ4	220	Bone exposure of more than half of the skull with desiccated or mummified tissue. Bones are retaining some grease (<i>Dry</i>).	Trunk presents skin desiccation, with caving in the abdominal cavity. No bone exposure (<i>Decay</i>). Apparent mummification. No bone exposure and hooves are still present (<i>Decay/Dry</i>).
GC4	206	Skull exposure with some mummified and grease tissue (<i>Dry</i>).	Trunk shows mummified tissue and high amount of preserved fat tissue (adipocere). Bone exposure (<i>Decay/Dry</i>). Limbs show bone exposure, with some greasy decomposed tissue and body fluids (<i>Dry</i>).

PBI 5

GN5	399	Skull is fully skeletonized. Some roots are present. Very scarce mummified tissue and hair preserved (<i>Dry</i>). Small amount of adipocere.	Dry bones (<i>Dry</i>) wrapped in roots and dirt. Very scarce mummified tissue and hair are preserved. Dry bones (<i>Dry</i>) wrapped in roots and dirt. Very scarce mummified tissue and hair preserved. Scarce presence of adipocere.
GW5	406	Skull is largely dry but retaining some grease (<i>Dry</i>). Abundance of adipocere.	Bones are largely dry, but some decomposed tissue is still present (abundant adipocere; <i>Dry</i>). Presence of small Limbs bones are largely dry but retaining abundant adipocere (<i>Dry</i>). Presence of fallen hair.

4. The Experiment – Forensic Taphonomy & Decomposition

				roots and fallen hair.	
GQ5	467	Skull fully skeletonized. Some roots are present. Very scarce mummified tissue and hair preserved (<i>Dry</i>).		Dry bones (<i>Dry</i>), covered by mummified tissue and hair preserved.	Dry bones covered in mummified tissue for more than half of the limbs. Hooves still present (<i>Dry</i>).
GC5	396	Skull fully skeletonized. Some roots are present. Very scarce mummified tissue and hair preserved (<i>Dry</i>).		Dry bones (<i>Dry</i>). Very scarce mummified tissue and hair preserved	Dry bones (<i>Dry</i>). Very scarce mummified tissue and hair preserved (<i>Dry</i>).

Table 4.2: Summary table of the decomposition and taphonomic observations recorded during the post-burial external examination of the piglets from the different groups. In italics and in parentheses, the decomposition stage assigned to each subject is reported according to the four-stage model proposed by Galloway et al. (1989): *Fresh, Bloat, Decay, and Dry*.

Observation of the decomposition stage and taphonomic alterations in the subjects exhumed at the first PBI (18 days) revealed a generally homogeneous condition across all groups. All piglets were in the bloating phase, showing generalized swelling of the neck, abdomen, and limbs, as well as discoloration ranging from brown to purple.

The only exception was subject GQ1 which, although exhibiting postmortem changes similar to those observed in the other groups, also showed skin drying and slippage across the entire body. All subjects were assigned to the Bloat stage, although GQ1 was considered borderline between the *Bloat* and *Decay* stages.

At PBI2 (49 days), all examined subjects showed partial exposure of the skull and limb bones, along with a general purple-black discoloration. Once again, a variation from the rest of the sample was observed in the lime-treated group: subject GQ2 was the only one to display dry skin slippage and completely desiccated skin, appearing in a more advanced decomposition stage (Figure 4.13a). However, although it does not fall within the standard procedures of an external examination, the preservation state of the internal tissues was also assessed during the

4. The Experiment – Forensic Taphonomy & Decomposition

dismemberment of the individuals for maceration. In the case of subject GQ2, the muscles of the right hind limb were found to be remarkably well preserved, despite the time elapsed since death (Figure 4.13b). Nevertheless, the overall chosen decomposition stages were *Decay* or *Decay/Dry*.



Figure 4.13: *a-* piglet GQ2: *b-* detail of the right hind limb of GQ2.

In contrast, the piglets exhumed at PBI3 (101 days) showed marked heterogeneity in their state of preservation. GN3 exhibited partial exposure of the skull and limb bones, with abundant moist decomposed tissue and purging fluids, whereas GW3 showed extensive bone exposure with only small remnants of moist decomposed tissue. Conversely, GQ3 displayed no signs of skeletonization, retaining dried tissues and showing skin slippage, while GC3 underwent conservative decomposition processes that resulted in the transformation of the skin into leather-like tissue (wet mummification or corification).

At PBI4 (206–220 days), observation of the decomposition state once again revealed a generally homogeneous condition among the groups. Specifically, GN4, GW4, and GC4 all showed almost complete skeletonization of the skull and limbs, with residues of greasy material and large amounts of adipocere in the trunk region. Subject GQ4, however, differed from the rest of the sample, displaying only partial skeletonization of the skull, while the postcranial region appeared desiccated but well preserved, with the hooves still intact and articulated to the limbs.

Finally, at PBI 5 (396–467 days), a generally homogeneous state of decomposition was again observed among the subjects. GN5 was found to be almost completely skeletonized, with remnants of mummified skin, hair, and adipocere around the joints. In contrast, GW5 was only partially skeletonized, still showing decomposed tissue and abundant adipocere across all body regions. GQ5, on the other hand, exhibited preserved skin on the trunk and limbs due to mummification processes, with the hooves still intact and anatomically articulated to the limbs. GC5 was entirely skeletonized, with only small fragments of mummified skin and hair strands entangled in the T-shirt.

In conjunction with the exhumations and post-burial external examinations, observations were also collected regarding the associated material and its interaction with the bodies. For the GQ group, it was possible to document the progressive changes in the appearance and consistency of the quicklime covering the piglets. During the exhumation and external examination of GQ1, the substance still exhibited a powdery consistency, although centimeter-sized aggregates were already present (Figure 4.14a). In contrast, in GQ2 (Figure 4.13a), the lime showed clear signs of compaction, forming hydrated blocks adhering to the cadaver (particularly in the abdominal region) while retaining a thin encrusted layer (plaster-like in appearance) that easily crumbled upon contact (Figure 4.14b). GQ3 displayed a further transformation: the lime had completely lost its powdery nature and had formed a rigid shell around the cadaver, causing the dried epidermis to detach entirely when handled (Figure 4.14c). In GQ4, the progressive loss of body mass allowed the lime to occupy some of the internal spaces in the abdominal and limb regions, positioning itself beneath the skin (Figure 4.14d). This process was even more pronounced in GQ5, which still exhibited preserved skin on the trunk and limbs due to mummification processes, with parts of the skeleton partially encased in lime (Figures 4.14e,f).

For the GC group, the T-shirt in which all subjects were wrapped (Figure 4.15a) exhibited progressively more advanced degradation with increasing PBI. The T-shirt recovered from GC1 after exhumation showed no notable alterations apart from soil staining attributable to burial and a strong odor of decomposition (Figure 4.15b). In contrast, the T-shirt from GC2 displayed a brownish stain on the right side of the anterior surface (Figure 4.15c), while the collar contained tufts of hair mixed with fragments of decomposed skin. The T-shirt from GC3 exhibited black staining on both the anterior and posterior surfaces, together with numerous taphonomic tears, particularly in correspondence with the stained areas (Figure 4.15d). In GC4,

4. The Experiment – Forensic Taphonomy & Decomposition

only limited remnants of textile fibers were recovered, held together by intact stitching and the garment label (Figure 4.15e). A substantial number of roots were also found entwined around the body and the remaining fabric of this partially skeletonized subject. Finally, during the micro-excavation of GC5, only the T-shirt stitching, label, and small fragments of printed lettering were retrieved. Entangled within these were remnants of mummified skin and hair (Figure 4.15f).

4. The Experiment – Forensic Taphonomy & Decomposition



Figure 4.14: Observations on the interaction between quicklime and decomposing bodies. *a-* GQ1; *b-* GQ2. The green arrow indicates the thin encrusted layer of lime; *c-* GQ3; *d-* GQ4; *e-* trunk and posterior limbs of GQ5; *f-* detail of the left posterior limb of GQ5.



Figure 4.15: State of preservation of the T-shirts from the GC group subjects. **a-** appearance of the shirts before being worn by the subjects; **b-** T-shirt of GC1; **c-** T-shirt of GC2; **d-** T-shirt of GC3. White arrows indicate some of the numerous tears; **e-** textile fragments mixed with roots; **f-** stitching from the GC5 T-shirt wrapped around remnants of mummified skin and hair.

4.2.2. Postmortem & Post-burial Interval Estimation

For the application of the Accumulated Degree Days (ADD) formula for PMI estimation, the Total Body Score (TBS) was first calculated for each subject. To this end, the methods proposed by Megyesi et al. (2005), Keough et al. (2017), and Indra et al. (2024) were applied, as summarized in the Appendix (Table A.1, p. 335). The results, reported in Table 4.3, showed a progressive increase in TBS with increasing PBI.

Specifically, the piglets exhumed at PBI1, when evaluated using the method of Megyesi et al. (2005), all yielded a TBS of 13 points, despite the slight differences described in the previous paragraph. These differences were, however, captured by the other two methods (Keough et al. and Indra et al.), which returned the same scores for GN1 (10 points) and GC1 (11 points), but slightly different values for GW1 (11 and 10 points, respectively) and GQ1 (13 and 12 points, respectively).

For PBI2, the calculation of the TBS using the method of Megyesi et al. again yielded fairly homogeneous results for all exhumed cadavers, with a TBS of 20 points for GW2 and GC2, and 21 points for GQ2. In this case, the other two methods (which propose modifications to the original only up to 7 points) also assigned a score of 21 points to subject GQ2.

From PBI3 onward, a certain heterogeneity in the results began to emerge. The highest TBS calculated using Megyesi et al. was recorded for GW3 (25 points), followed by GN3 (22 points), while GQ3 was consistently assigned 18 points across all methods. The TBS of subject GC3 could not be evaluated due to the conservative decomposition processes affecting the body (*humid mummification* or *corification*).

The heterogeneity of the results persisted at PBI4: subjects GN4 and GW4 both achieved a score of 27, which was still lower than that of GC4 (28 points). Conversely, GQ4 was assigned 22 points using the method of Megyesi et al. and 21 points using the methods of Keough et al. and Indra et al.

Finally, the subjects exhumed at PBI5 were also assigned heterogeneous scores. In this case, the highest score was attributed to GC5, which totaled 35 points with the method of Megyesi et al. and 36 points with that of Indra et al. Subject GN5 also received high scores with both methods, with a TBS of 32 and 33 points, respectively. Conversely, GW5 achieved 29 points

4. The Experiment – Forensic Taphonomy & Decomposition

using Megyesi et al. and 30 points using Indra et al. Subject GQ5, on the other hand, was assigned a TBS of only 27 points (Megyesi et al. method).

4. The Experiment – Forensic Taphonomy & Decomposition

	Days	Piglet ID	Megyesi et al., 2005				Keough et al., 2017				Indra et al., 2024			
			Head & neck	Trunk	Limbs	TBS	Head & neck	Trunk	Limbs	TBS	Head & neck	Trunk	Limbs	TBS
PBI 1	18	GN1	5	4	4	13	4	3	3	10	4	3	3	10
		GW1	5	4	4	13	5	3	3	11	4	3	3	10
		GQ1	5	4	4	13	5	4	4	13	4	4	4	12
		GC1	5	4	4	13	4	3	4	11	4	3	4	11
PBI 2	49	GW2	8	6	6	20	-	-	-	-	-	-	-	-
		GQ2	9	5	7	21	9	5	7	21	9	5	7	21
		GC2	8	6	6	20	-	-	-	-	-	-	-	-
PBI 3	101	GN3	8	6	8	22	-	-	-	-	-	-	-	-
		GW3	8	9	8	25	-	-	-	-	-	-	-	-
		GQ3	7	6	5	18	7	6	5	18	7	6	5	18
		GC3*	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d

4. The Experiment – Forensic Taphonomy & Decomposition

PBI 4	206	GN4	10	9	8	27	-	-	-	-	-	-	-	-
	220	GW4	10	9	8	27	-	-	-	-	-	-	-	-
	220	GQ4	11	6	5	22	11	6	4	21	11	6	4	21
	206	GC4	11	9	8	28	-	-	-	-	-	-	-	-
PBI 5	399	GN5	12	11	10	33	-	-	-	-	12	11	11	34
	406	GW5	12	9	10	31	-	-	-	-	12	8	11	32
	467	GQ5	12	8	7	27	-	-	-	-	-	-	-	-
	397	GC5	13	12	10	35	-	-	-	-	13	12	11	36

Table 4.3: Results of the TBS calculation according to the three selected methods. TBS for piglet GC3 (*) was unevaluable (n/d).

4. The Experiment – Forensic Taphonomy & Decomposition

The ADD formula for PMI estimation was applied using all calculated TBS values, including, where applicable, those derived from each of the methods considered. The results comprise both the estimated absolute PMI and the corresponding PMI range, calculated by adding and subtracting ± 388 accumulated degree days (ADD) from the absolute ADD value. The resulting intervals were subsequently compared with the actual PMI (Table 4.4). The outcomes are presented below.

For PBI1 (18 days), the ADD calculation yielded an estimated PMI of one day for subjects GN1 and GW1 (TBS calculated using Keough et al. and Indra et al.), while in all other cases, a PMI of two days was estimated. The calculated range was between 0 and 9 days for GN1, GW1, and GC1 (TBS calculated using Keough et al. and Indra et al.) and between 0 and 10 days for all subjects whose TBS was assigned using the method of Megyesi et al. In general, therefore, the PMI for the first set of exhumations was underestimated by 8–9 days.

In the case of PBI2 (49 days), a PMI of 7 days was estimated for subjects GW2 and GC2, and 8 days for GQ2. The PMI range was found to be between 0 and 14 days for GW2 and GC2, and between 1 and 16 days for GQ2. Also in this case, the PMI was significantly underestimated (by approximately 40 days).

The ADD-based calculation for PMI estimation at PBI3 (101 days) yielded heterogeneous results. Specifically, a PMI of 8 days was estimated for GN3, 16 days for GW3, and only 3 days for GQ3. Regarding the PMI range, it was between 2 and 14 days for GN3, between 10 and 22 days for GW3, and between 0 and 9 days for GQ3, underestimating the real PMI by up to tenfold.

Regarding the PMI estimation at PBI4 (206 and 220 days), a PMI of 26 and 27 days was estimated for subjects GN4 and GW4, respectively (with a range of 21–32 days for both). Conversely, GC4 yielded an estimated PMI of 33 days, with a range of 28–38 days. Finally, subject GQ4 resulted in an estimated PMI of 8 days (TBS calculated using the method of Megyesi et al.) and 7 days (TBS calculated using the methods of Keough et al. and Indra et al.), with corresponding PMI ranges of 2–14 days and 0–13 days, respectively. Moreover, for PBI4, a strong underestimation of PMI was observed.

4. The Experiment – Forensic Taphonomy & Decomposition

Finally, PMI values were estimated for the subjects exhumed at PBI5. Subject GN5 (PBI of 399 days) yielded an estimated PMI of 160 days (TBS calculated using the method of Megyesi et al.) and 199 days (TBS calculated using the method of Indra et al.), with corresponding ranges of 152–167 and 193–205 days, respectively. The analysis of GW5 (PBI of 406 days) resulted in estimated PMIs of 60 and 84 days (for the two methods, respectively), with ranges of 48–68 and 73–94 days. An even greater underestimation was recorded for piglet GQ5 (PBI 567 days), with an estimated PMI of 28 days and a range between 22 and 34 days. Conversely, subject GC5 (PBI of 397 days) produced an estimated PMI of 312 days, with a range of 307–318 days when the TBS was calculated using Megyesi et al., and a PMI of 453 days (range 442–461) when the TBS was calculated using Indra et al. In this case, the estimate exceeded the real PMI by at least 45 days.

4. The Experiment – Forensic Taphonomy & Decomposition

<i>Piglet ID</i>	<i>TBS (Megyesi)</i>	<i>TBS (Keough)</i>	<i>TBS (Indra)</i>	<i>Estimated ADD</i>	<i>ADD range</i>	<i>Estimated PMI (Days)</i>	<i>PMI range (Days)</i>	<i>Actual PMI (Days)</i>
GN1	13	-	-	140.60	0 – 528.76	2	0 – 10	18
	-	10	10	102.33	0 – 490.49	1	0 – 9	
GW1	13	-	-	140.60	0 – 528.76	2	0 – 10	18
	-	11	-	112.72	0 – 500.88	2	0 – 9	
	-	-	10	102.33	0 – 490.49	1	0 – 9	
GQ1	13	13	-	140.60	0 – 528.76	2	0 – 10	18
	-	-	12	125.31	0 – 513.47	2	0 – 10	
GC1	13	-	-	140.60	0 – 528.76	2	0 – 10	18
	-	11	11	112.72	0 – 500.88	2	0 – 9	

4. The Experiment – Forensic Taphonomy & Decomposition

GW2	20	-	-	407.38	19.22 – 795.54	7	0 – 14	49
GQ2	21	-	-	492.04	103.88 – 880.20	8	1 – 16	49
GC2	20	-	-	407.38	19.22 – 795.54	7	0 – 14	49
GN3	22	-	-	599.79	211.63 – 987.95	8	2 – 14	101
GW3	25	-	-	1148.15	759.99 – 1536.31	16	10 – 22	101
GQ3	18	-	-	287.08	0 – 675.24	3	0 – 9	101
GC3	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
GN4	27	-	-	1853.53	1465.47 – 2241.69	26	21 – 32	206
GW4	27	-	-	1853.53	1465.47 – 2241.69	27	21 – 32	220
GQ4	22	-	-	599.79	211.63 – 987.95	8	2 – 14	220
	-	21	21	492.04	103.81 – 880.20	7	0 – 13	
GC4	-	-	-	2387.81	1999.65 – 2775.97	33	28 – 38	206

GN5	33	-	-	9727.47	9339.31 – 10115.63	199	193 – 205	399
	-	-	34	13243.42	12855.26 – 13631.58	247	240 – 252	
GW5	31	-	-	5395.11	5006.95 – 5783.27	116	109 – 130	406
	-	-	32	7211.08	6822.92 – 7599.24	160	151 – 167	
GQ5	27	-	-	1853.53	1465.37 – 2241.69	28	22 – 34	467
GC5	35	-	-	18197.01	17808.84 – 18585.17	312	307 – 318	397
	-	-	36	25234.81	24846.65 – 25622.97	453	442 – 461	

Table 4.4: ADD calculation based on the TBS calculated through the three methods considered.

4. The Experiment – Forensic Taphonomy & Decomposition

Finally, the Vass PMI estimation formula was applied, which required assessing the decomposition stage and the amount of adipocere for each individual, as well as the temperature and soil moisture conditions measured from the HCDI samples. Unlike the previously illustrated method (ADD calculation), the results (summarized in Table 4.5) revealed a substantial overestimation of the PMI.

Specifically, evaluations conducted on the subjects exhumed at PBI1 (18 days) yielded PMI estimates of 265 days for GN1, 293 days for GW1, 404 days for GQ1, and 631 days for GC1.

For PBI2 (49 days), the estimates increased even further, with calculated PMIs of 669 days for GW2, 492 days for GQ2, and 687 days for subject GC2.

As for the subjects exhumed at PBI 3 (101 days), individuals GQ3 and GC3 were deemed not assessable due to the particular preservation state in which they were found. Conversely, the analysis of subject GN3 yielded an estimated PMI of 1,131 days, while piglet GW3 produced an estimate of 1,093 days.

The bodies exhumed at PBI 4 (206-220 days) showed the presence of adipocere, which led to a further increase in the overestimation of PMI compared to the real values. In fact, subject GN4 resulted in an estimated PMI of 16,769 days, surpassed only by GC4, with a PMI of 17,829 days. Both individuals shared the same PBI (206 days) and exhibited a higher abundance of adipocere compared to the other two groups (with a PBI of 220 days). The latter yielded estimated PMIs of 1,103 days for GW4 and 687 days for GQ4.

Finally, PMIs were estimated for the subjects exhumed at PBI 5. In this case, despite the more advanced stage of decomposition, piglets GN5 and GW5 showed lower estimated PMIs compared to those calculated for the previous PBI, 1,886 days (actual PMI: 399 days) and 5,409 days (actual PMI: 406 days), respectively. Conversely, the estimated PMIs for the other two groups were higher than those obtained at PBI4, with values of 1,751 days for GQ5 (actual PMI: 467 days) and 2,304 days for GC5 (actual PMI: 397 days).

4. The Experiment – Forensic Taphonomy & Decomposition

<i>Piglet ID</i>	<i>Decomposition (%)</i>	<i>Adipocere (Multiplier)</i>	<i>Soil Temperature (°C)</i>	<i>Soil Moisture (%)</i>	<i>Estimated PMI (Days)</i>	<i>Actual PMI (Days)</i>	<i>Error (Days)</i>
GN1	10	1	16.2	14.68	241	18	+223
GW1	10	1	16.2	13.32	266	18	+248
GQ1	10	1	16.2	9.64	367	18	+349
GC1	10	1	16.2	11.22	316	18	+298
GW2	35	1	16.3	18.95	650	49	+601
GQ2	40	1	16.3	26.48	532	49	+483
GC2	35	1	16.3	18.46	668	49	+619
GN3	55	1	27.5	11.07	1037	101	+936
GW3	60	1	27.5	13.37	937	101	+836
GQ3*	n/d	n/d	27.5	21.08	n/d	101	n/d
GC3*	n/d	n/d	27.5	12.44	n/d	101	n/d
GN4	80	3	21.7	7.57	8385	206	+8179
GW4	80	1	25.3	15.43	1176	220	+956
GQ4	45	1	25.3	11.55	884	220	+664
GC4	80	3	21.7	7.12	8914	206	+8708

4. The Experiment – Forensic Taphonomy & Decomposition

GN5	90	1	18.4	14.88	1886	399	+1487
GW5	90	5	22.3	21.41	5409	406	+5003
GQ5	85	1	21.9	12.72	1751	467	+1284
GC5	95	1	16.1	14.7	2304	397	+1907

Table 4.5: Results of the application of Vass' Elusive PMI formula (2011). In this case, both GQ3 and GC3 (*) decomposition state were not valuable.

4.2.3. **Taphonomic and Chemical Analyses of Gunshot-Related Wounds**

This section reports the results of the taphonomic analysis carried out on gunshot-related lesions found on the skulls of the piglet from the GW group after their restoration. The lesions were described with particular attention to the features typically associated with firearm-related traumas to the skull and to the characteristics considered capable of suggesting a distinction between entrance and exit wounds.

Subsequently, outcomes related to IGSR detection via SEM-EDX analysis will be presented.

Wounds Taphonomy

During the external examination performed after freezing for subject GW0 and after exhumation for all the other subjects, lateral–lateral radiographs and the assessment of cranial wounds were carried out. Pre- and post-burial radiographies of the piglets are presented in the Appendix (Figures A.4a-j, pp. 450-451).

The analysis of gunshot-related lesions became increasingly challenging with increasing PBIs. Except for subject GW0, which was never buried, defects involving the skin and soft tissues of the buried subjects were already difficult to identify at early PBIs, with progressive obliteration of evidence due to putrefactive processes that led to the disintegration of anatomical structures and severe fragmentation of the skull. However, following maceration and restoration procedures, it was possible to reconstruct lesions and fractures affecting the bone tissue. Entry and exit gunshot-related skeletal lesions are summarized in Table 4.6, primarily to illustrate the difficulty of lesion characterization and to allow comparison with the results obtained from SEM–EDX analysis of GSR.

All photographs and analyses related to the morphological characteristics of the lesions were produced by Dr. Ludovico Re, forensic pathologist.

4. The Experiment – Forensic Taphonomy & Decomposition

	Lesion	Photography	Morphology	Internal Beveling	External Beveling	Radiating fractures	Concentric fractures	Deformed bone fragment	Bone plug
GW0	EnWa		Irregular	✓	X	✓	X	X	X
	ExWa		Quadrangular	X	✓	✓	✓	X	X
	EnWp	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	ExWp	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
GW1	EnWa		Irregular	X	✓	✓	X	X	X
	ExWa		Irregular	X	✓	✓	X	X	X

4. The Experiment – Forensic Taphonomy & Decomposition

	EnWp	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	ExWp	N.D.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
GW2	EnWa		Irregular	✓	X	✓	X	X	X
	ExWa		Stellate	X	✓	✓	X	X	X
	EnWp		Oval	✓	✓	✓	X	X	X
	ExWp		Irregular	X	✓	✓	X	X	X

4. The Experiment – Forensic Taphonomy & Decomposition

GW4	EnWa		Oval	✓	X	✓	X	X	X
	ExWa		Irregular	X	✓	✓	X	X	X
	EnWp		Oval	✓	X	✓	X	X	X
	ExWp		Irregular	X	✓	✓	X	X	X
GW5	EnWa		Irregular	X	X	✓	X	X	X

ExWa		Irregular	X	✓	✓	X	X	X
EnWp		Keyhole	✓	X	✓	X	✓	X
ExWp		Irregular	X	X	✓	X	X	X

Table 4.6: Gunshot-related bone entry and exit wounds detected for each sample. The entry anterior wounds are indicated with “EnWa”, while the exit anterior wounds are indicated with “ExWa”. The entry posterior wounds are indicated with “EnWp”, while the exit posterior wounds are indicated with “ExWp”. “✓” stands for “present”, “X” stands for “absent”. “N.D” stands for “not detectable”, “N.A” stands for “not assessable”.

IGSR Analysis

The SEM-EDX analyses for IGSR detection on the lesions on piglets' heads were conducted before (posterior wounds) and after their inhumations (anterior wounds). Compositional 3D images and semi-quantitative analyses results are presented in the Appendix (pp. 452-506).

The IGSR analyses revealed changes in both the quantity and composition of the detected particles when comparing pre- and post-burial samples. The results of the chemical analyses are summarized in Table 4.7 and illustrated in Figures 4.16 and 4.17. Control soil samples did not show the presence of particles composed of metals attributable to firearm discharge, either individually or in combination, nor evidence of contamination from other potential sources, including the metal bucket of the mechanical excavator used for grave excavation.

As the IGSR analyses performed on the lesions were conducted not only to assess particle alteration and persistence over the burial interval, but also to evaluate their potential as a complementary tool in forensic pathology and anthropology for the identification of gunshot wounds, the results are presented below in separate subsections. The findings related to particle morphology are presented first, followed by those concerning the classification and abundance of particles detected on the lesions before and after burial.

Before burial/examination (Posterior wounds)	GW0				GW1				GW2				GW4				GW5			
	EN		EX		EN		EX		EN		EX		EN		EX		EN		EX	
	Char.	16	1		3		/		5		1		8		/		5		/	
	Cons.	PbBa	5	PbBaIr	1	PbBa	1		PbBa	7	PbBa	1	PbBa	1			PbBa	4		
		PbSb	1								PbSb	2	PbSb	2						
						BaSb	1						BaSb	1	/		BaSb	1		TiZn
Comm. Ass.	Pb	8			Pb	1	Pb	1			Pb	5	Pb	1	Pb	1			Pb	2
									Sb	1	PbIr	2					Sb	1	PbIr	7
			Ba	2							Ba	1	Ba	1	Ba	1				
Other			Fe	5			FeTi	1			Fe	2			Fe	2			Fe	2
			Zn	1			Zn	1			Zn	2							Zn	1
	/		CuZn	2	/		CuZn	3	/		CuZn	1	/		CuZn	2	/		CuZn	1
			Ti	1							FeCrNi	1			FeCrNi	1			FeCrNi	1
			Cu	1															Cu	1
Total	30	14		6		7		13		18		14		7		11		16		

4. The Experiment – Forensic Taphonomy & Decomposition

After burial/examination (Anterior wounds)	Char.	3	/	/	/	/	/	/	/	/	/
	Cons.	PbBa 9		PbBa 11							
		PbBaIr 1			/	/	/	/	/	/	/
		PbSb 1	PbSb 1								
		BaSb 1									
	Comm. Ass.	Pb Ir 3 Ba 1	/	Pb Ir 4 Ba 2	/		Pb 1 Ba 1 Sb 1	Pb 1 Ba 7 Sb 1	Pb 5 Pb Ir 4	/	Pb 2 Pb Ir 1 Ba 5
Other		CuZn 3	CuZn 4	CuZn 1	CuZn 4			CuZn 4	CuZn 1		
		FeCrNi 1	FeCrNi 6	FeCrNi 1	FeCrNi 1	FeCrNi 1	/	FeCrNi 6	FeCrNi 1		FeCrNi 1
		Fe 1	Fe 10	Fe 3	Fe 6	Fe 1		Fe 9	Fe 7	Fe 7	Fe 6
								Zn 1	Zn 2	Sn 1	Zn 2
Total		24	21	22	11	3	2	29	20	8	17

Table 4.7: IGSR-related particles detected for each sample. The entry wounds are indicated with “EN”, while the exit wounds are indicated with “EX”. In the second column “Char.” stands for “characteristic of IGSR”, “Cons.” stands for “consistent with IGSR”, “Comm. Ass.” stands for “commonly associated with IGSR”.

4. The Experiment – Forensic Taphonomy & Decomposition

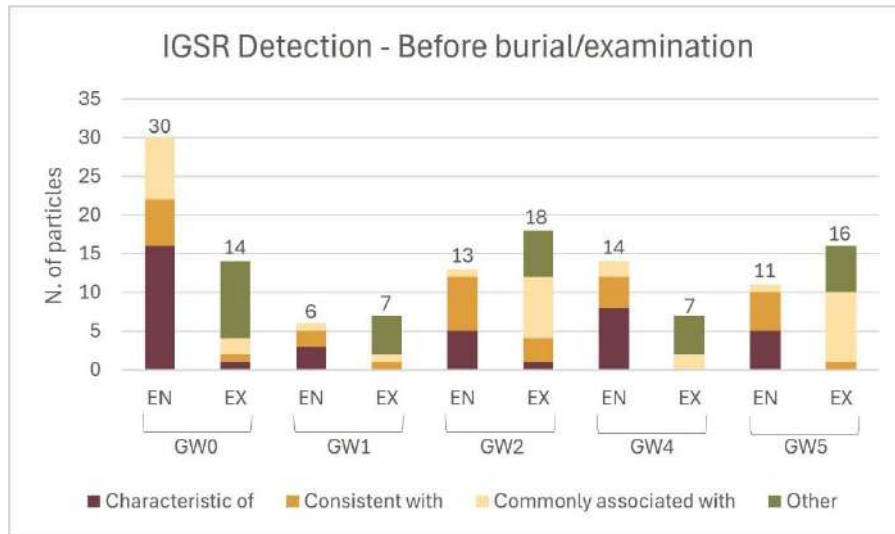


Figure 4.16: Stacked column chart showing the abundance (in particle counts) of IGSR-related particles for the entrance (“EN”) and exit (“EX”) bullet holes of each subject, prior to burial or, in the case of GW0, before the external examination. The numbers at the top of each column indicate the total number of particles detected in the corresponding sample.

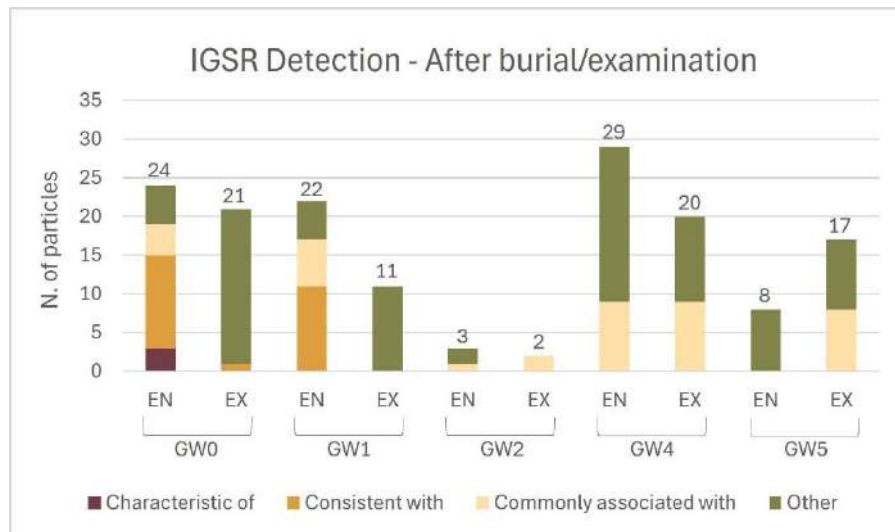


Figure 4.17: Stacked column chart showing the abundance (in particle counts) of IGSR-related particles for the entrance (“EN”) and exit (“EX”) bullet holes of each subject, after exhumations or, in the case of GW0, during the external examination. The numbers at the top of each column indicate the total number of particles detected in the corresponding sample.

Particles morphology

From a morphological point of view, the entrance holes of the posterior gunshot wounds, examined before burial, yielded the highest number of spherical particles (15 in total), mainly composed of Pb-Ba-Sb, Ba-Sb, or Pb associated with other metals, whereas the exit holes yielded a drastically smaller number (5), but when present, they were mainly composed of Ba or Pb-Ir. Conversely, the analyses carried out on the anterior lesions allowed the identification, for GW0, of only six spherical particles (composed of Pb-Ir, Pb-Ba, or Fe-Cr-Ni) on the entrance hole, and four on the exit hole (consistent in Pb-Sb, Fe-Cr-Ni, or Fe). Finally, regarding the lesions observed post-burial, spherical particles were found on all subjects. In particular, GW1 yielded three particles presenting this morphology on the entrance hole, composed of Pb-Ba, Ba, and Fe-Zn, and three on the exit hole, all composed of Fe, in one case associated with Zn. GW2 yielded only one spherical particle, located on the entrance hole and composed of Fe, while in GW4 four spherical particles were observed on the entrance hole (Ba and Fe-Cr-Ni) and three on the exit hole, composed of Fe-Cr-Ni or Fe-Cu. Subject GW5, on the other hand, showed the presence of two particles of this morphology only on the exit hole, composed of Fe-Cr and Fe-Zn.

In all other cases, the particles identified as IGSR constituents exhibited irregular shapes, resembling aggregates, fragments, or crusts. These were taken into consideration, as also illustrated in Brozek-Mucha (2014). However, the particles never showed crystalline morphologies, unlike those observed in the control soil.

Before burial – Posterior wounds analysis

The particles considered *characteristic of IGSR* were found in correspondence with all the entrance holes of the posterior gunshot wounds, sampled shortly after the firing tests. They were found to be more abundant at the entrance holes than at the exit holes and, for the latter, only subjects GW0 and GW2 showed the presence of one particle each, composed of Pb-Ba-Sb. Regarding the particles *consistent with IGSR*, they were identified in correspondence with all the posterior entrance holes with a relatively homogeneous distribution (between two and seven particles per stub). Among these, those composed of Pb and Ba were found in all entrance holes,

4. The Experiment – Forensic Taphonomy & Decomposition

whereas Pb and Sb, and Ba-Sb appeared randomly among the samples. Differently, particles *consistent with IGSR* on the exit holes were scarcer (from one to four) and showed different compositions, with the presence of Ir in subject GW0 and of Ti and Zn in subjects GW1 and GW5. Particles *commonly associated with IGSR* were found on the entrance holes and they were mostly composed of Pb, except for subjects GW2 and GW5, in which the only particle of this category was composed of Sb. On the contrary, the exit holes showed a heterogeneous distribution of these particles, composed of Pb and Ba. It is also noteworthy to the presence of particles composed of Pb associated with Ir in subjects GW2 and GW5. Finally, all the particles potentially related to the explosive event but originating from components of the firearm other than the primer were included in the *other IGSR* class. They were found to be present in none of the entrance holes, while they were frequent in the samples taken from the exit holes. In all these samples the presence of particles composed of Cu-Zn, Fe (which, in the case of subject GW1 only was associated with Ti) and Zn alone was observed, with the exception of GW4. Moreover, particles composed of Fe-Cr-Ni were found in subjects GW2, GW4, and GW5.

As shown in Figure 3.34, the highest number of particles were found on the entry bullet hole on subject GW0 (30), followed by the exit bullet holes of GW2 (18), GW5 (16) and GW0 itself (14), as well as on the entry wounds of GW4 (14), GW2 (13), and GW5 (11). In all other cases, the number of particles was found to be less than 10. Regarding their relative abundance, the particles identified as *characteristic of IGSR* were generally more numerous on the entrance gunshot wounds than on the exit ones, as were those classified as *consistent with IGSR*. In contrast, the particles *commonly associated with IGSR* showed a more random distribution between entrance and exit bullet holes, whereas those classified as *other IGSR* were found exclusively on the exit wounds in all subjects.

After burial – Anterior wounds analysis

As for the particles considered *characteristic of IGSR*, only three were found in correspondence with the anterior entrance hole of the GW0 lesion, which had never been buried. About the *particles consistent with IGSR*, these were abundantly found on the entrance hole of GW0, with the presence of Pb-Sb, Ba-Sb, and one particle of Pb-Ba associated with Ir, while one particle composed of Pb-Sb was found on the exit hole. Particles from this class were also detected on

4. The Experiment – Forensic Taphonomy & Decomposition

the entrance hole of GW1, all composed of Pb-Ba. Conversely, the *particles commonly associated with IGSR* were more abundant within the samples: these were found on the entrance holes of all subjects except GW5, often composed of Pb, but also of Ba or Sb. The presence of Pb associated with Ir in the entrance bullet holes of subjects GW0 and GW1 were also noted. As for the exit holes, this class of particles were present only in subjects GW2, GW4, and GW5. The latter two showed the presence of particles composed of Pb associated with Ir. Finally, the particles classified as *other IGSR* were generally more abundant, except for the exit hole of GW2, in which none were found. In general, particles composed of Cu-Zn, and of Fe-Cr-Ni were frequently detected in all subjects, as well as particles composed solely of Fe or of Fe associated with other trace metals (such as Cu or Zn).

Generally, the presence of particles attributable to IGSR was relatively homogeneous across samples collected after exhumation (Figure 4.35), with total counts ranging between 20 and 29 particles. A few exceptions were recorded: the exit hole of GW1 exhibited 11 particles, and the entrance hole of GW5 contained only eight. Subject GW2 showed notably fewer residues, with only three particles detected on the entrance hole and two on the exit hole.

In terms of the relative abundance of the different particle classes, particles considered *characteristic of IGSR* were detected exclusively on the entrance hole of GW0 (three particles), while particles *consistent with IGSR* were identified on both the entrance and exit holes of GW0 (12 and one particles, respectively) and on the entrance hole of GW1 (11 particles). Conversely, *commonly associated with IGSR* particles were observed on the entrance holes of GW0 (four) and GW1 (six), as well as on both the entrance and exit holes of GW2, albeit in very low numbers (one and two, respectively). In contrast, this same class was well represented on both holes of GW4 (nine particles in each case) and on the exit hole of GW5 (eight particles). *Other IGSR* appeared more frequently than in the samples collected prior to burial, suggesting possible post-depositional processes or environmental contamination. These particles were found on all entrance holes, showing an increase in their number with higher PBI values: five on the entrance holes of GW0 and GW1, two on that of GW2, and as many as 20 and eight on the entrance holes of GW4 and GW5, respectively. Regarding the exit holes, where present, *other IGSR* particles were identified in considerable amounts: 20 on GW0, 11 on both GW1 and GW4, and nine on GW2. No such particles were found on the exit hole of GW3.

– CHAPTER 5 –

DISCUSSION & CONCLUSIONS

The PhD project *Ticino 2023* presented in this thesis sought to investigate the exchange of material between a decomposing body and its surrounding environment. In the case of buried remains, this environment corresponds to the subsurface context, defined here as the Hypogeal Cadaver Decomposition Island (HCDI). Evidence concerning the interaction between these two substrates may contribute to addressing questions relevant to forensic investigations, particularly in cases involving homicide victims whose bodies have been concealed through clandestine burials. For example, exploring time-dependent chemical and biochemical markers in soil, together with observing how their concentrations may vary with increasing postmortem interval (PMI), could assist in time of death estimation, especially in situations where concealment strategies (such as lime or textile wrappings) complicate the interpretation of decomposition. Likewise, a careful understanding of the surrounding environment, both hypogeal and epigeal, may support a more informed assessment of the taphonomic alterations affecting the buried body. Such transformations can, in some cases, obscure or even eliminate key forensic indicators, including lesions associated with violent acts.

To discuss the findings from the perspective of both contributing substrates, the HCDI and the cadaver, this chapter is organized into three sections:

- **Section 5.1** focuses on the importance of the characteristics of the burial environment for a correct interpretation of the biogeochemical dynamics resulting from decomposition. Furthermore, the section introduces HCDI as a tool for PMI and PBI estimation.
- **Section 5.2** tests the application of the most common methods for PMI estimation based on the macroscopic observation of the cadaveric decomposition state, which have been validated on both human and animal models. This section also considers the various concealment treatments simulated in the experiment as complex taphonomic agents, alongside an evaluation of their potential influence on the interpretation of thanatological data.
- **Section 5.3** provides an overview of the interaction between the two substrates, outlining the possible future perspectives arising from the study.

5.1. The Hypogeal Cadaver Decomposition Island as a Tool for Post-Burial Interval Estimation

The HCDI, understood as the portion of soil beneath the buried body in which nutrients derived from decomposing tissues accumulate, is by definition the most informative substrate regarding specific postmortem dynamics. Indeed, the input of nutrients can disrupt the normal biogeochemical balance of the matrix (Carter & Tibbett, 2008; Aitkenhead-Peterson et al., 2012; DeBruyn et al., 2020; Silva-Bessa et al., 2025; Taylor et al., 2023), and consequently, the observation of related anomalies may help address some of the fundamental questions in forensic investigations, including the estimation of the time of death or burial. However, the success of this approach depends on the ability of the hypogeal substrate to capture and retain the anomalies associated with the introduction of cadaveric material into the system. Contributing to this ability, from a physical standpoint, is the soil grain size, which determines the capacity to retain water within pore spaces, thereby affecting both oxygen availability and the overall humidity of the hypogeal environment (Fitzpatrick & Donnelly, 2021).

The soils of the Ticino-LEAFs experimental area were identified as young, weakly altered soils, whose typical profile is characterized by two horizons: a OA horizon, the uppermost layer, approximately 20 cm deep and consisting of 0.8% gravel and 99.2% fine earth (itself composed of 37.35% sand, 57.63% silt, and 4.22% clay, giving a sandy-silt texture); and a C horizon, located deeper (20–55 cm), composed of 68.10% gravel and 31.90% fine earth, the latter consisting of 31.26% sand and 0.64% silt, with no detectable clay, resulting in a sandy texture. Given the burial depth simulated in the facility for this PhD project (50 cm circa), the HCDI was expected to lie at the same depth as the C horizon, or at its lower boundary. This would imply a limited capacity to retain putrefactive fluids released by the cadaver during decomposition, as water drains more rapidly through the large pores of sandy soils, which therefore exhibit low water storage capacity and a high infiltration rate (Huntley, 2023). However, the results of the biogeochemical investigations conducted appeared to suggest a different scenario.

5.1.1. The role of Carbon as a marker for Hypogeal Decomposition: Dissolved Organic Matter Characterization for PMI Estimation

The results collected in this project suggested the presence of noticeable anomalies associated with the cadaver within the HC DI, despite the occurrence of intense meteorological events between PBIs that may have contributed to the removal of relevant organic elements and compounds. This pattern may be partly explained by the mixing of the soil removed during grave excavation and later used as backfill, as well as by the downward movement of upper layers during the excavation process itself. On the one hand, such mixing could imply that part of the surface horizon material (typically richer in litter, silt, and clay, and therefore possessing greater retention capacity) was transferred to the area beneath the cadavers after deposition. On the other hand, this backfill may have created an alternation of structures more or less resistant to meteoric water infiltration, potentially preserving deeper layers that directly interacted with the body. This interpretation appears to be supported by the results of the Total Organic Carbon (TOC) analyses, performed using the Walkley-Black method on the samples collected during the burials and at PBIs 1 and 2 (Figures 3.27 and 3.28).

In general, all the soil samples collected from the trench profiles prior to burial showed an organic carbon distribution fully consistent with expectations: higher TOC concentrations in the surface horizons and progressively lower amounts in the deeper, more mineral horizons, clearly illustrating how decomposition and mineralization increase with depth (Jobbágy & Jackson, 2000). In contrast, the analysis conducted on the samples collected during the exhumations showed an organic carbon distribution within the backfill that was heterogeneous and not easily predictable. Based on the observed pattern, it may be hypothesized that the organic carbon content in the backfill above the cadavers reflected the mixing of the original horizons, namely, the natural organic matter from the topsoil combined with the deeper horizons, which are comparatively poor in organic material.

The TOC values recorded in the layers affected by the presence of the piglet (samples *Pig* and *HC DI*), which did not differ substantially from those of the immediately overlying horizons, made it difficult to determine whether the decompositional fluids released by the cadaver had actually altered these values or whether they simply reflected the soil mixing previously described. This uncertainty becomes even more evident when considering the analyses of the samples from burial GN2, from which the body had been removed between PBI1 and PBI2: in this case, the distribution of organic carbon does not show concentrations distinct from those

observed in the other burials investigated at the same PBI, where the cadavers were still present at the time of exhumation.

The analyses performed on the soil samples from the GW group (that is, the piglets to which postmortem lesions had been inflicted) yielded similarly ambiguous results. In the literature, several studies address the possible influence that the presence of lesions may have on the timing of the decomposition process and on the consequent release of substances into the surrounding environment. Indeed, the possibility that lesions may serve as attractive sites for insects has led some researchers, for example, Vass et al. (1992) and Campobasso et al. (2001), to suggest that the presence of open wounds could accelerate the decomposition process. These claims contrast with the conclusions of Cross & Simmons (2010), who found no direct evidence that the decomposition of bodies bearing lesions was in fact accelerated. However, these studies concern the decomposition dynamics of bodies deposited on the surface of the soil, with particular attention to insect access. This factor cannot be considered in the case of burials, since the soil itself acts as a physical barrier to entomofauna (Tibbett & Carter, 2008).

Regarding the trend observed in the layers above the level of cadaver deposition, higher organic carbon values were recorded at PBI2 compared with PBI1 at the same depth. For the soil in direct contact with the cadaver, the organic carbon content at PBI2 was generally higher than that measured at PBI1, although the two HCDIs exhibited similar concentrations, providing no clear evidence that can be directly linked to the more advanced stage of decomposition. In contrast, the organic carbon concentration detected in the soil sample in direct contact with subject GW2 was markedly higher than both the immediately overlying layer and the corresponding HCDI sample. In this case, one may hypothesize a greater lateral diffusion of decomposition fluids (compared with that occurring beneath the cadaver) possibly related to the presence of the lesions. These may have increased fluid release and facilitated microbial access. However, it cannot be excluded that this pattern may instead reflect, even in this group, the random distribution of organic carbon resulting from soil mixing.

In the case of the GQ group burials, in which the piglets were interred beneath a layer of quicklime (CaO), the organic carbon concentration in the horizons located above the cadaver (up to 40–50 cm) increased with sampling depth and then showed a decrease at the level of the cadaver at PBI1. At PBI2, however, the distribution of organic carbon appeared substantially uniform, comparable to that observed in the other groups. The use of quicklime derives from the observation that body concealment is often associated with the application of caustic

substances, although the evidence regarding the effects of hydrated lime and quicklime on the rate of decomposition remains somewhat contradictory. A common misconception is that quicklime accelerates decay, hinders victim identification, destroys evidence, and ultimately leads to the rapid destruction of human remains (D'Errico et al., 2011). In practice, however, quicklime has also been reported to transform biological tissues and influence decomposition rates, in some cases even contributing to the preservation of key forensic evidence. An illustrative example is the work by Schotsmans et al. (2012, 2014a, 2014b), who buried pig cadavers with and without lime (hydrated or quicklime) and recovered them after predetermined PBIs. According to their results, both hydrated lime and quicklime may slow the decomposition of buried remains, and pig cadavers treated with lime showed better preservation than untreated ones, possibly because lime acts as a physical barrier. Calcium hydroxide ($\text{Ca}(\text{OH})_2$), produced through quicklime hydration, reacts with atmospheric and cadaver-derived carbon dioxide (CO_2) to form a protective layer of calcium carbonate (CaCO_3) on the body surface (Schotsmans et al., 2012). In addition, the hygroscopic properties of quicklime can contribute to absorbing decomposition fluids, preventing their immediate dispersion into the surrounding soil. However, none of these expected effects were evident in the organic carbon analyses conducted on the GQ samples, as the TOC concentrations detected in the HCDI were comparable to those of the other groups. With regard to the distribution of organic carbon in the layers above cadaver GQ1, the observed accumulation may tentatively be attributed to the barrier effect created by the lime, although this effect appears to diminish by PBI2.

A further suggestion of the limitations of TOC as an indicator of the presence of decomposing bodies is provided by the analyses of the samples from the GC group, in which the piglets were buried wrapped in cotton T-shirts. Studies such as those by Hauteo et al. (2013) and Collins et al. (2020) suggest that clothing may absorb decomposition fluids throughout various stages of decay, forming a barrier around the cadaver that could reduce access by soil decomposer microorganisms and bacteria. However, in the present study, the organic carbon analyses did not show lower concentrations of this element in the HCDI compared with the other groups at either PBI. It should nevertheless be noted that, although textiles can act as substantial reservoirs for decomposition products, absorption by clothing may be not able to completely retain them; fluids may still escape and interact with the surrounding environment (Ueland et al., 2015), particularly given the highly degradable nature of the garments used in this project. Moreover, in this experiment, anomalies in the decomposition rate were observed in this group compared with the unclothed piglets, suggesting that cotton clothing may have influenced these

dynamics to some degree. The literature on this topic remains mixed. Card et al. (2015), for instance, reported that in their experiment unclothed piglet cadavers decomposed significantly faster than those dressed in cotton garments, although clothing provided additional sites for *Diptera* colonization. In contrast, Matuszewski et al. (2014) argued that cadaver mass exerts a stronger influence on decomposition than clothing, with the latter having only a minor effect. Nonetheless, these authors emphasized that even when textiles exert limited influence on decomposition, they still constitute important evidence in forensic investigations, for example, through their capacity to retain decomposition by-products that may aid PMI estimation. Therefore, although it remains uncertain whether clothing functions as an effective barrier to the diffusion of putrefactive leachates into the surrounding soil, textiles do appear to act as important reservoirs for these products (Ueland et al., 2015; Collins et al., 2019), at least as long as they are preserved. The only exception to this overall pattern was the cadaver from the GC group exhumed at PBI 2. At that time, the decomposition state of the piglet was assessed as active decay, and the body was more degraded than the one examined at PBI 3, which underwent corification. This difference likely accentuated the steep decline in values observed between the two exhumations.

Overall, the TOC analyses conducted in this experiment suggest that the variability in organic carbon concentrations across the different burial layers is largely influenced by the soil mixing that occurred during the backfilling of the graves, resulting in a random redistribution of the material originally present in the natural soil horizons. Under these conditions, it is difficult to draw firm conclusions about whether the amounts of organic carbon detected at the horizons where the bodies were deposited can be directly attributed, through a clear cause-and-effect relationship, to the release of cadaveric material into the soil.

This may appear counterintuitive, as variations in soil organic carbon content would, logically, seem to offer a more accurate representation of the changes occurring within the context of an HCDI. However, most studies focusing on the monitoring of organic carbon in CDI have either failed to detect any variation in the concentration of this chemical element (Benninger et al., 2008; Anderson et al., 2013; Meyer et al., 2013; Szelecz et al., 2018a) or, when quantitative variation has been observed, researchers have been unable to determine the origin of such change (Martin et al., 2021), in agreement with the present experiment. For instance, Benninger et al. (2008) observed the impact of pig cadavers' deposition on soil biogeochemistry for 100 days, showing that no significant difference was detected between the TOC content of the CDI and control soil samples. Similarly, Meyer et al. (2013) described the decomposition of swine

carcasses during summer and winter, hypothesizing faster noticeable changes in gravesoil chemistry in the former. However, body decomposition did not have a significant effect on the concentration of total carbon, which was even higher during the winter. Another example comes from Anderson et al. (2013) who focused on chemical evidence of decomposition of pig cadavers in CDI soil one- and three-years postmortem. In their study, the authors highlighted no significant difference in TOC concentration after either one or three years of deposition. Furthermore, Szelecz et al. (2018a) tried to characterize the possible impact of pig cadavers on selected soil chemical markers over a one-year period, demonstrating that the range of carbon content was stable during the entire experiment. Ultimately, in the work of Martin et al. (2021), who analyzed chemical changes in loam and sandy loam soils surrounding buried animals after two months, organic carbon content seemed to be more soil-type dependent than related to the decomposition products. Therefore, the marker was considered unreliable.

This limited significance is likely related to the fact that organic carbon introduced into the soil undergoes numerous transformation processes, both biotic (e.g., reduction, oxidation) and abiotic (e.g., volatilization, leaching, adsorption by soil colloids). Nevertheless, since the release of organic matter by a decomposing body is well established through a range of chemical proxies, excluding organic carbon from consideration might risk overlooking a potentially relevant aspect in reconstructing decomposition dynamics. For this reason, the present PhD project strategy focused specifically on a fraction of soil organic matter, namely Dissolved Organic Matter (DOM), which was hypothesized to more effectively reflect trends associated with the time-dependent input of decomposition products into the environment. Despite constituting only a small proportion of total soil organic matter, DOM represents the most mobile and reactive fraction, playing a key role in nutrient cycling, microbial activity, and pollutant transport in soils (Smreczak and Ukalska-Jaruga, 2021). From a dynamic perspective, putrefactive leachate originating from a buried decomposing body partially follows the same migration pathways as soil fluids. This observation supports the hypothesis of DOM being a potential indicator of body concealment in clandestine graves.

Previous studies proposed to focus on DOC (Dissolved Organic Carbon) as a marker for the evolution of the CDI under a deposited cadaver through time. For instance, in the study of Fancher et al. (2017) DOC concentrations appear to be higher in the soil from the CDI compared to the control soil, even if the trend of abundance seemed not to be time related. Another example of the application of this method comes from Aitkenhead-Peterson et al. (2012) who focused on evaluating the lateral spatial extent of decomposition products from the CDI of two

human cadavers. The highlights showed that mean DOC concentration in the CDI was significantly higher than that of the control soil, with a ratio of one order of magnitude. Regarding DOM specifically, it was only recently investigated in one study of Pecsí et al. (2024) which aimed to evaluate the relationship of the marker with soil microbial activity in CDI. The chemical analysis underlined a localized increase of organic carbon, following the decomposition of surface deposited pig cadavers up to depths of 14 cm. Therefore, while pioneering in nature, the decision to focus the investigation of soil anomalies associated with cadaveric material on DOM concentration proves to be a promising and potentially effective approach. However, the mere quantification of DOM within a soil sample is inadequate for distinguishing cadaver-derived molecules from those naturally present in the soil system. For this purpose, it may instead be worthwhile to perform a qualitative characterization analysis of DOM, such as FT-IR Spectrometry, which may assist in achieving a more detailed understanding of decomposition-related changes (Reuter et al., 2020; Pecsí et al., 2024).

The potential of DOM monitoring in elucidating the role of organic carbon within the HCDI has likewise been substantiated by the findings of this PhD research. Indeed, HCDI samples from the two groups GN and GW showed an increase of DOM concentration at PBIs3 and 4, with a subsequent regression to values comparable with the ones from the control soil (PBI5). Conversely, the FT-IR spectra of the DOM samples of the two groups reported the presence of different compounds. Indeed, the absorbance spectrum for the group GW showed, already at PBI2, a progression in the intensity of the band at $1400\text{--}1250\text{ cm}^{-1}$, which was attributed to the C–N stretching of amide III of blood in studies focused on the FT-IR characterization of human body fluids (Orphanou, 2015) and tissues (Movasaghi et al., 2008). Despite the non-specific nature of these studies on forensic taphonomy and putrefactive leachate in soil, the presence of such molecules in the present work cannot be ruled out, considering that the sample GW is composed of subjects characterized by the presence of lesions that could have caused blood to leach into the surrounding environment. Furthermore, the absence of such evidence at PBI1 could be attributed to an insufficient accumulation of body fluid to become more prominent than the existing organic compounds, which could exhibit band overlapping with amide III. Instead, it is possible that such an accumulation occurred exclusively at PBI2, only to be obliterated by decomposition or leached by precipitation as PBIs increased. In addition, at PBI5 a variation in the expression of lipids signals ($3500\text{--}2800\text{ cm}^{-1}$) was observable: O–H stretching bands (around $3400\text{--}3200\text{ cm}^{-1}$) could be attributed to fatty acids (Notter et al., 2009; Lerchi et al., 2022) and the well-defined absorbance bands around $3000\text{--}2800\text{ cm}^{-1}$ could be linkable to

C–H stretching bands, which were used as an indicator of the increase in the amount of lipid as the decomposition process proceeded, and of the presence of adipocere in other studies focused on the same topic (Forbes et al., 2005; Collins et al., 2019; Lerchi et al., 2022).

In contrast, the spectra for group GN showed no significant alterations until PBI4, when a noticeable splitting of the absorption band between 3500 and 2800 cm^{-1} was observed. This could suggest the presence of hydroxy-fatty acids (2800 cm^{-1}) and the progressive formation of unsaturated fatty acids (3500-3000 cm^{-1}), better distinguishable at PBI5, as evidence of the conversion to adipocere (Forbes et al., 2005; Lerchi et al., 2022). Furthermore, the evolution of the bands in the area 1700-1300 cm^{-1} , attributable to proteins, was detectable from PBI3 to PBI5. According to Ueland et al. (2015) two bands detected in the area could correspond to amide II (around 1550 cm^{-1}) and amide I (around 1700 cm^{-1}) and the respective evolution of the two through time or treatments could be useful for monitoring any change in protein of the decomposition fluid. Indeed, while the amide I peak is supposed to be sensitive to the protein conformation, which influences its location and intensity, amide II is normally more stable (Ueland et al., 2019). This relationship allows their ratio to be considered a marker of protein transformation (Hopkins et al., 1991): a reduction in the intensity of the amide I peak, accompanied by an increase in the intensity of the amide II, could serve as an indicator of protein breakdown, that could be employed to track the progress of proteolysis (Wubshet et al., 2017). Hypothetically, with further investigation, monitoring these two peaks could be considered a potential tool for PMI estimation, since the variation in their relative intensities and the broadening of the amide bands observed across the PBIs in this experiment suggested that protein degradation and proteolysis occurred throughout the decomposition process (Collins et al., 2019). Finally, the emergence of a band near 1600 cm^{-1} , situated between the amide bands and attributable to the formation of free carboxylate groups in the shorter peptide fragments generated by proteolysis, further contributed to the observed broadening of the amide bands as decomposition advanced. In principle, the presence of analogous bands in the FT-IR spectra of the GW group was expected, given their comparable burial characteristics to those of the GN group, with no discernible influence from associated materials on decomposition processes. However, this is not, in fact, the case. This condition could be explained by the broad absorption band (1370-1200 cm^{-1}), which was previously attributed to the C–N stretching of amide III of blood, originating from the wounds. This band may have overlapped with the region of interest, thereby preventing its detection over the long term.

Regarding group GQ, despite the peculiar state in which the cadavers of this group were found, the DOM analysis surprisingly resulted to be as effective as in the groups GN and GW, showing a progressive increase, with maximum concentrations at PBI3 (406.86 mg/kg) and PBI4 (423.59 mg/kg) and a following decrease. These outcomes could be interpreted as indicative of the analytical technique's capacity to detect and monitor the concentration of DOM, even in scenarios where the quantity of putrefactive leachate reaching the soil is minimal. This is not to be overlooked, given that DOM constitutes the most mobile and labile fraction of organic matter, being capable of presenting variations in its concentration even at an extremely small scale. However, the FT-IR spectra showed some variations compared to the other groups. Firstly, the region $3500\text{--}2500\text{ cm}^{-1}$ was characterized by no signals until PBI4, when a wide band ($3500\text{--}3100\text{ cm}^{-1}$) associable to O–H stretching band of fatty acids appeared (Notter et al., 2009; Lerchi et al., 2022), and PBI5, with a well-defined absorbance band ($3000\text{--}2800\text{ cm}^{-1}$) attributed to C–H stretching bands, identifying the presence of adipocere (Forbes et al., 2005; Collins et al., 2019; Lerchi et al., 2022), when the lime crust on the cadaver was only limited in confined area of the body. Conversely, absorbance bands relating to triglycerides (1750 cm^{-1}) and unsaturated fatty acids (1670 cm^{-1}) were detected in samples from all the PBIs, with a constant intensity until PBI5, when they decreased. Forbes et al. (2005) stated that the presence of these compounds suggests minimal decomposition, which is consistent with the alteration of decay caused by lime. This finding aligns with the highlights of the present study. Forbes et al. also signaled that an absorbance band around 1450 cm^{-1} could be attributed to the C–O stretching of lime. Although a signal in this region is present within the group's FT-IR spectra, the band in the area is characterized by a dense overlapping of absorbance bands, which could also be ascribed to the degradation of proteins, or phospholipids (Orphanou, 2015). Nonetheless, it is also possible to hypothesize some FT-IR trends attributable to the phases of hydration and carbonation of quicklime. For instance, a small peak around 3630 cm^{-1} was present from PBI2 to PBI4, which could indicate the O–H bond from water molecules (Ilani et al., 2005), testifying the hydration process of CaO, which decreased through time. Furthermore, the absorption bands around 1450 cm^{-1} , 1080 cm^{-1} and 870 cm^{-1} could be assigned to different vibration modes C–O of carbonate groups CO_3^{2-} (Kuriyavar et al., 2000; Blesa et al., 2003) between the oxygen atom of carbonate. Their presence at later PBIs (4 and 5) suggested an abundance of CaCO_3 compared to Ca(OH)_2 . Hence, while the presence of bands related to lime absorption can overlap with signals attributable to organic compounds produced by decomposition, FT-IR spectrometry remains a valuable tool for the examination of HCDI. In fact, this technique has proven capable of detecting features that support the atypical

preservation state of the remains under quicklime, thereby providing evidence of preservative decomposition process, culminating in the presence of adipocere in the final stage.

Ultimately, the results for the GC group showed a markedly different pattern of DOM concentration over time: a pronounced peak (720.91 mg/kg, more than 16 times higher than the DOM concentration found in the control soil from that grave) was detected at PBI2, followed by a sharp decrease. This was the only evidence of punctual input of organic matter in soil for this group. Moreover, by focusing on the FT-IR analysis performed on this HCDI sample for GC2 some interesting results were highlighted: the presence of strong C–H stretching bands (represented by the double high peak at 3130 and 3050 cm^{-1}) with a shoulder at 2800 cm^{-1} linkable to =C–H stretching, suggested the presence of unsaturated fatty acids (Forbes et al., 2005; Lerchi et al., 2022), as for groups GN and GW. Moreover, the spectrum of PBI2 presented a wide and strong absorbance band between 1080 and 1040 cm^{-1} , which was utilized as a reference band for degradation products from phospholipid decomposition (Deng et al., 2025). Accordingly with the decomposition state observed on the cadavers (Chapter 4), the FT-IR spectrum of the sample GC3 did not show an intensification of the signal in the single bond region, highlighting a weaker input of fatty acids in the grave soil, until PBI5 when two sharp bands at 1580 and 1550 cm^{-1} appeared. They could indicate the carboxylate bands of fatty acid calcium salts (Collins et al., 2019; Lerchi et al., 2022), while the modes between 1500 and 1400 cm^{-1} could be attributed to lipid CH_2 scissoring (Yu et al. 2021; Lerchi et al., 2022). On the contrary, the spectrum from PBI3 was characterized by the presence of a wide band between 1400 and 1250 cm^{-1} , which was not previously detected, attributed by Orphanou (2015) to C–N stretching of amide III, as evidence of the degradation of proteins, or phospholipids. This discrepancy is most likely attributable to the preservation state in which subject GC3 was found, namely, humid mummification (or corification), which will be discussed in detail in Section 5.2. Moreover, from PBI3 to PBI5 it was possible to notice a fragmentation of the band between 1080 and 1020 cm^{-1} in three small peaks, shifting towards right, which also became more defined as the PBI increases. Hypothetically, this trend could be attributed to the progressive fragmentation of the 100% cotton T-shirts wrapping the piglets of the group, that consequently released cellulose decomposition products in the HCDI soil, as suggested by Collins et al. (2019). Indeed, as the signals linked to the degradation of the fabric becomes more defined, other bands related to the products of decomposition become more detectable, such as the ones related to the O–H stretching bands of fatty acids (around 3400–3200 cm^{-1} ; Notter et al., 2009; Lerchi et al., 2022) and the absorbance bands around 3000–2800 cm^{-1} linkable to C–H stretching

bands of lipids, well detectable at PBI5, as in group GW (Forbes et al., 2005; Collins et al., 2019). Ultimately, from PBI3, a sharp peak at 850 cm^{-1} appeared, attributable to the CH_2 rocking of lipids forming adipocere (Stuart et al., 2000), which was indeed present in the latter PBIs. However, with the exception of the results obtained from sample GC2, the DOM concentration graphs for this group did not adequately represent the release of putrefactive products into the soil from HCDI. Instead, the presence and definition of these products could be obtained by observing the FT-IR spectrum.

Overall, the search for anomalies related to organic matter within CDIs and HCDIs cannot be considered inherently unreliable, provided that the fraction of organic matter investigated is capable of responding even to minor variations in nutrient input. According to the results of the present PhD project and recent scientific literature on the topic, monitoring TOC in soil does not appear to be the most suitable approach for this purpose, as its concentrations include contributions from all organic sources, including plant material and microorganisms, whose abundance may obscure anomalies associated with decomposition processes. By contrast, the results of the present experiment suggest that DOM is sufficiently sensitive to reflect variations associated with the nutrient input resulting from the percolation of putrefactive fluids. The sensitivity of this approach is particularly supported by the results obtained for the GQ group, in which, despite the dehydrating effect exerted by quicklime, anomalies in the concentration of this fraction of organic matter were nevertheless detected.

However, it must be acknowledged that this is a pilot study conducted on a relatively small sample size, and further investigation is therefore required. What appears consistent across all experimental groups, however, is the detection of spectral bands attributable to lipids and fatty acid salts, compounds typically associated with adipocere formation, which was also macroscopically observed during the examination of the remains at PBIs 4 and 5. This finding may be significant, not only because it demonstrates that these molecules are capable of passing through the DOM filtration threshold ($0.45\text{ }\mu\text{m}$), but also because they may represent useful indicators for identifying a CDI that is no longer macroscopically visible, for example in cases where a body is recovered in a skeletonized state. Furthermore, in scenarios involving the transfer of a body to a secondary deposition site, the original burial location may nevertheless retain valuable information. In such contexts, an interdisciplinary approach becomes essential, involving archaeologists capable of identifying the original grave bottom through stratigraphic interpretation and pedology experts able to recognize the natural soil profile of the area.

5.1.2. HCDI Biogeochemical Anomalies and Their Relationship with PMI

In parallel with the analysis of the HCDI, pH represented another important factor, not only for interpreting the taphonomic condition of soft and hard tissues (Dent et al., 2004), but also for tracking fluctuations associated with different stages of cadaver decomposition.

Starting with the pH values observed in the GW group, a mean increase was recorded in comparison to the control soils in samples collected from the HCDI during PBI1, PBI2, and PBI3. Following this phase, a decrease in pH values was observed, reaching average values comparable to those of the control soil sample at PBI4, or even lower, as in the case of PBI5.

About GN group, except for the data corresponding to PBI2, which are missing, the trend in pH values appeared similar to that observed for the previous group: an increase in pH was recorded in the HCDI soil samples collected at PBI1 and PBI 3, followed by a subsequent decrease at PBI4 and PBI5. This initial alkaline phase in both groups coincides with the exhumation periods during which bloating and active decay phases of decomposition were observed. This observation aligns with findings from previous studies (Benninger et al., 2008; Szelecz et al., 2018; Rai et al., 2022), which reported similar increases in soil pH during the early to intermediate stages of cadaver decomposition, followed by a decrease during the final stages of tissue breakdown. This trend has been attributed to the release of ammonium ions (NH_4^+) resulting from protein ammonification processes, during the and putrefaction, characteristic of the bloating and active decay phases (Carter et al., 2007; Benninger et al., 2008). Similarly, in the more advanced decomposition stage (dry remains), a decrease in soil pH within the HCDI has been documented, as reported in other studies (Vass et al., 1992; Schotsmans et al., 2012; Szelecz et al., 2018a; Rai et al., 2022). This secondary acidification may be attributed to the depletion of ammonium ions and other organic compounds (Rai et al., 2022), potentially indicating a shift in the microbial community inhabiting the CDI.

About GQ group, the pH analysis showed an atypical trend, reporting elevated, despite decreasing, values from 11.5 ± 0.2 (PBI 1) to 7.6 ± 0.3 (PBI 5). This could be interpreted as the expression of the peculiar alkalinity of CaO, that being a basic oxide completely obliterated the possible pH signals attributable to NH_4^+ fluctuations due to putrefaction. However, the decrease in the pH mean value of the HCDI through time is worth being commented on. Indeed, it could suggest a corresponding hydration of the quicklime (due to the putrefactive fluids or the precipitations), obtaining lime ($\text{Ca}(\text{OH})_2$), which is also typically alkaline. From this state on, once the hydration process is concluded, the carbonation due to the contact with CO_2 (being

environmental or decomposition-related) leads to the formation of calcium carbonate (CaCO_3), whose pH is lower. Additionally, the formation of CO_2 , which reacts with water to produce carbonic acid (H_2CO_3), combined with concurrent leaching processes that remove OH^- and Ca^{2+} , could contribute to a general decrease in soil pH. This hypothesis would explain the preservation status of the remains, which were evaluated as desiccated and characterized by mass loss from PBI3. Moreover, although pH may not be the most reliable tool for assisting PMI estimation in this context, its measurement in the presence of quicklime/lime could still be useful for interpreting the decomposition state of a cadaver and its relationship to the hydration state of the substance.

Finally, the pH analysis on the HCDI of the group GC highlighted a completely different trend which reflected the outcomes of the DOM analysis. The pH analysis results reported a noticeable peak at PBI2. Conversely, pH values from PBI3 and PBI4 were similar, being 6.0 ± 0.2 and 5.9 ± 0.2 respectively and followed by a slight decrease at PBI5. This trend, which differed from the previous groups, could be attributed to the absorptive capacity of the cotton T-shirts in which the bodies were wrapped. The garments may indeed have absorbed putrefactive sewage or acted as a barrier between the soil bacterial community and the soft tissues, although, the role of textiles as an obstacle to diffusion of decomposition products is not clear (Hauteo et al., 2013; Ueland et al., 2015; Collins et al., 2020).

Nevertheless, the sensitivity of pH to nutrient inputs within the HCDI makes it a useful parameter, including when searching for additional biomarkers. Indeed, according to Vass (2017), even in situations where a previously concealed cadaver is no longer present, measuring the pH of the soil solution should be the first analytical step. If the results do not show a higher pH than that of the control soil, it is unlikely that decomposition-related organic compounds can be detected with any degree of reliability.

Vass et al. (1992) also proposed a method based on the analysis of decomposition fluids released into the soil, suggesting that certain short-chain volatile fatty acids (VFAs) could serve as potential time-dependent biomarkers, contributing to PMI estimation in a complementary manner to the macroscopic taphonomic methods typically applied in forensic investigations. The potential of these acids derives from the fact that, after death, they are released from triglycerides by tissue lipases via enzymatic hydrolysis, and during the later stages of decomposition they undergo hydrolysis and hydrogenation (Queirós et al., 2023). This latter transformation results in the saturation of fatty acids, meaning that most forensic-relevant

biomarkers belong to the group of saturated VFAs, such as acetic, propionic, butyric, and isovaleric acids (Swann et al., 2010; Vass, 2017). Despite their volatile nature, in the alkaline environment created within the HCDI these acids may form salts that permeate into the soil, thus becoming detectable (Vass, 2017). In addition, other non-volatile organic compounds have been identified as biomarkers of the early stages of decomposition, such as lactic and succinic acids (Dent et al., 2004), produced through postmortem glycolysis and anaerobic fermentation (Paczkowski & Schütz, 2011), although their relationship with PMI is still not fully understood.

Regarding the HPLC analyses carried out within this PhD project (Table 3.1; Figure 3.29), the results were contradictory. For the GN group, in this case too it was not possible to evaluate the temporal trend in organic acid concentrations because subject GN2 had been removed by wildlife. Nevertheless, at PBI1 the presence of only butyric and propionic acids were detected, with a slight predominance of the latter. This result is partially consistent with the literature: propionic acid is generally attributed to the fermentation of carbohydrates and amino acids by anaerobic bacteria during the putrefactive phase, which includes the bloating stage (Dent et al., 2004; Ioan et al., 2017) observed for the piglet. In contrast, butyric acid typically indicates the butyric fermentation stage carried out by anaerobic bacteria acting on lactate, glucose, and other substrates (Paczkowski & Schütz, 2011). This stage characterizes more advanced phases of putrefaction, when the body begins to dry but still contains substantial soft tissue (Swann et al., 2010). Thus, the presence of propionic acid aligns with the decomposition state in which the remains were found, as does its higher concentration relative to butyric acid, although the detection of the latter remains difficult to fully explain. At PBI3, by contrast, notable concentrations of isovaleric and propionic acid were detected, whereas butyric acid showed only a slight increase. Isovaleric acid is typically produced by the fermentation of branched-chain amino acids (Thierry et al., 2002) by proteolytic bacteria, which are particularly present during the active and advanced stages of decomposition, producing VFAs alongside other protein putrefaction by-products such as biogenic amines, indoles, and phenols (Ioan et al., 2017). These conditions correspond well to the preservation state of GN3 and could also explain the increase in propionic acid. Conversely, the concentration of butyric acid was again unexpectedly low relative to expectations. Regarding the remaining organic acids, lactic acid was present (although in low amounts) while succinic acid was not detected. Both acids typically appear immediately after death, when cessation of cardiac activity and oxygen depletion trigger physiological changes such as glycogen degradation into lactic acid within skeletal muscle. They may also be produced by bacteria colonizing the body internally and

externally (Paczkowski & Schütz, 2011), and lactic acid can continue to be generated by intestinal microflora during early putrefaction. Reconciling these mechanisms with the low lactic acid concentrations detected in GN3 is, however, challenging given the advanced state of preservation observed. From PBI4 onward, a marked decline in the concentrations of all analyzed organic acids was observed, followed by a slight overall increase at PBI5. This pattern may indicate that some short-chain VFAs, particularly propionic and isovaleric acid, can persist in the soil for relatively long periods.

A markedly different trend was observed for the organic acids analyzed in the samples taken from the HCDI of the GW group. None of the analytes were detected at PBI1, whereas a pronounced peak of propionic acid was recorded at PBI2, followed by butyric and acetic acid, and lower concentrations of lactic and succinic acids. As previously discussed, propionic acid typically originates from the bacterial fermentation of carbohydrates, a process that also leads to the release of other VFAs such as acetic and butyric acid (Ioan et al., 2017). These compounds appear primarily during the bloating and active decay stages of the cadaver and are subsequently detected in the soil during advanced decomposition (Ioan et al., 2017; Vass, 2017). The results are therefore once again consistent with the decomposition state of GW2, which was in active decay. Lactic and succinic acids were also present, although at relatively low concentrations. This may be due to the fact that during active decay, the intestinal and tissue bacterial flora also produce these two organic acids as part of the mixed fermentation of pyruvate (Paczkowski & Schütz, 2011; Özcelik et al., 2016). Surprisingly, at PBI3, a drastic general decline in all VFAs was observed, while lactic acid showed only a slight decrease. This was difficult to interpret, as GW3 was in an advanced state of decomposition and produced a strong odor, conditions that would normally be associated with high concentrations of VFAs in the HCDI (Vass, 2017). One possible explanation is that the biomarkers were removed by meteoric water percolation and leaching, following the intense rainfall episodes that occurred between PBIs 2 and 3.

However, this hypothesis is not fully consistent with the observations from the GN group, where analyte concentrations at PBI3 were higher. This discrepancy may be related to the greater canopy cover above the GN burials, which could have influenced local environmental conditions. At PBI 4, a second peak in propionic acid was observed, together with a concomitant increase in acetic acid. The latter is produced through the bacterial fermentation of carbohydrates and through the degradation of lipids and proteins during active decay. However, even in the more advanced stages of decomposition, residual bacterial flora and soil microorganisms may continue to produce acetic acid as a fermentation by-product (Caballero-

Moreno et al., 2024; Ismail et al., 2025). Finally, at PBI 5, concentrations of all organic acids showed a general decline, although succinic and propionic acids remained detectable despite the remains being fully skeletonized.

The GQ group displayed yet another distinct trend, with generally lower concentrations than the other groups and three peaks of isovaleric acid at PBIs 1, 3, and 5, as well as two smaller peaks of propionic and butyric acid at PBIs 1 and 3. The analytes showed consistently low concentrations throughout the experiment, with a decreasing trend over time. This group was characterized by the presence of quicklime (CaO), which was also historically used to mask the odour of decomposing bodies (Schotsmans et al., 2014b; Thurn et al., 2024). Indeed, as stated, the hydration of quicklime can result in an increase in soil pH, which in turn slows down normal bacterial activity, altering the metabolism of anaerobic microorganisms (Chen et al., 2024) and consequently leading to a reduced production of organic compounds, including the volatile ones, which are responsible of part of the *odor mortis* (Vass, 2012 and 2019). Moreover, as already stated, in such an alkaline environment VFAs form their respective calcium salts, which are less volatile and therefore less odorous. This may explain the lower concentrations of acids relative to the other groups. Regarding the distribution of isovaleric acid peaks, the results are once again only partially aligned with the literature, as its presence is generally associated with the active and advanced stages of decomposition, being a product of proteolysis (Ioan et al., 2017). Similarly, propionic acid is typically released starting from the bloating stage (Dent et al., 2004; Ioan et al., 2017), whereas butyric acid release characterizes the more advanced stages of putrefaction (Swann et al., 2010; Paczkowski & Schütz, 2011). It is therefore possible that the lime, despite its hygroscopic properties, altered but did not prevent the release of VFAs and their precipitation and persistence as salts within the HCDI. This hypothesis is further supported by the burial conditions: the bodies were covered with quicklime on their left side, but their right side was in direct contact with the soil. Such a configuration may have allowed for the deposition of biomarkers (as also suggested by the DOM results), although their quality and quantity were likely altered by the caustic action of the substance. The increase in isovaleric acid concentration at PBI5, however, remains difficult to explain. It is worth noting that during the summer between PBIs 4 and 5 a series of severe storms occurred, strong enough to uproot several trees within the facility. One of these had developed roots around the body of subject GQ5; when the tree fell, it displaced the remains, which were subsequently recovered through careful archaeological excavation using a stratigraphic approach (Figure 5.1). Such disturbance

of the burial context may have introduced external contamination, possibly associated with decomposing plant organic matter.

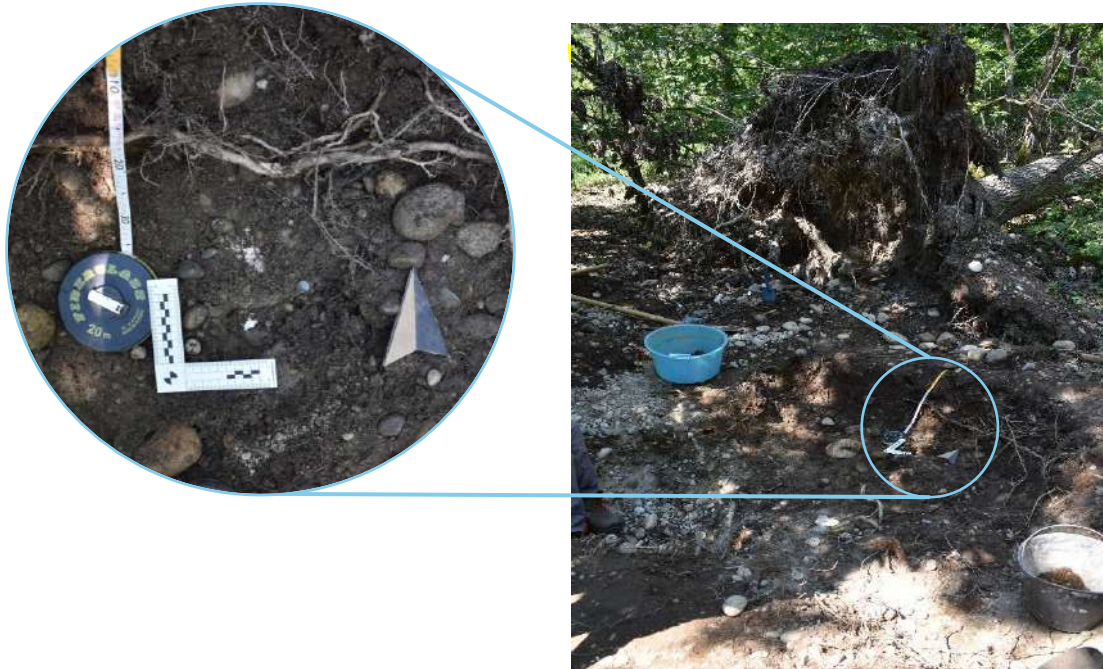


Figure 5.1: One of the recovery stages of subject GQ5. On the right, the fallen tree and the section of the new stratigraphic excavation. On the left, a detail of the first traces of quicklime that led to the remains, found precisely along the section. The burial had therefore been displaced toward the base of the tree.

Regarding the analyses conducted on the GC group, once again they revealed an atypical trend, although one that perfectly reflects the patterns observed in the analyses of DOM concentration and pH values. A clear peak in all analytes (except for lactic acid) was observed at PBI 2. Indeed, GC2 was in the active decay stage, with the skull and limbs already partially skeletonized and a large putrefactive stain in the abdominal area, while the cotton T-shirt was heavily soaked with decomposition fluids. In contrast, at PBI3 the cadaver was corificated, a condition that would explain the sharp decline in analyte concentrations between the two PBIs, given the conservative decomposition process affecting the remains. However, the exceptionally high concentration of organic acids at PBI2 still requires explanation, especially when compared with the other groups. In this case, contamination was ruled out, as consistent results were obtained across multiple analyses performed on different samples from the same HCDI. Among

the organic acids detected, propionic acid was the most abundant. Conventionally, this compound marks the phase of endogenous and exogenous bacterial colonization of the cadaver, which includes, among other processes, the degradation of amino acids (Paczkowski & Schütz, 2011; Ioan et al., 2017; Vass, 2017). Butyric acid, on the other hand, is more typically associated with the fermentation of fats and proteins during active decay (Paczkowski & Schütz, 2011; Vass, 2017). Consequently, the substantial input of VFAs into the soil in this case appears to reflect the decomposition state of GC2, although their concentration is exceptionally high, far exceeding expectations. Of particular interest is the detection of succinic acid, which, in this case, exhibited the highest concentration recorded in the entire experiment. Its presence further supports the hypothesis of an unusually abundant production of cadaveric fluids during this stage, the underlying cause of which remains to be clarified. The near absence of analytes at the other PBIs may be explained by the presence of cotton clothing, which, as previously discussed, could potentially absorb putrefactive fluids (Ueland et al., 2015; Collins et al., 2019). These conditions may not have occurred in the case of GC2, possibly due to a detachment of the garment from the cadaver's skin during or after burial, preventing the clothing from absorbing the decomposition fluids.

Ultimately, it was observed that in all groups (except for GW) a slight increase in analytes concentrations occurred at PBI5, following the marked decline recorded at PBI4, particularly for propionic and isovaleric acid. This pattern may be explained by the fact that during the PBI5 exhumations, numerous colonies of *Collembola*, with individuals at various developmental stages, were found on the nearly fully skeletonized bodies. This would indicate the presence of a self-sustaining population within the cadaveric microenvironment (Pohjoismäki et al., 2010). Similar findings have been reported in literature, documenting the presence of *Collembola* colonies on remains approaching skeletonization (Merritt et al., 2007; Vanin & Huchet, 2017; Szelecz et al., 2018b; Taleb, 2018). All studies agree that *Collembola* are most abundant in the soil beneath and around remains during the advanced stages of decomposition, as they are attracted both by the high moisture content accumulated in the HCDI and by the frequent fungal growth and microbial biofilms present on bones, residual tissues, and clothing. Therefore, the massive presence of these hexapods on bodies exhumed at PBI5 raised the possibility that organic compounds resulting from their ecological activities were being introduced into the hypogeal environment. Unfortunately, while the organic compounds produced by necrophagous and cadaver-colonizing insects have been well characterized (Frederickx et al., 2012; Stavert et al., 2014; Martin et al., 2019), the same cannot be said for *Collembola*. The literature documents

only a limited number of compounds produced directly by this clade, including aromatic and defensive compounds, cuticular hydrocarbons, and volatile carboxylic acids, the latter only in small quantities (Dekeirsschieter et al., 2009; Frederickx et al., 2012; Martin et al., 2019). Consequently, the presence of large populations during the later decomposition stages is likely to have only a minor impact on the VOC profile typically associated with a decomposing cadaver, especially when compared with the overwhelming influence of necrophagous and cadaver-colonizing insects during active decay. However, this does not exclude the possibility that compounds associated with *Collembola* might still be detected in the soil through HPLC analysis.

Overall, unlike what was observed for the DOM analyses, the concentration trends of the VFAs and the other organic acids examined here do not appear to show a consistent relationship with the conventionally defined stages of decomposition, nor a direct proportionality with increasing PMI. On the contrary, they seem to represent a highly effective tool for confirming the presence of a decomposing body in the hypogeal environment, even when the cadaver is not in the phase of maximal release of decomposition products. Moreover, the results suggest that additional concealment methods applied in conjunction with burial may exert a greater influence on tissue decomposition patterns than the PBI alone, potentially disrupting the expected trends.

5.1.3. Magnetic Susceptibility and Hysteresis Investigation of the HCDI: a Promising Laboratory Tool for Clandestine Graves Detection

The final technique employed to investigate the dynamics affecting the HCDI was the analysis of magnetic susceptibility (MS) and hysteresis on samples from the GC group only. Magnetic susceptibility analyses are not new within forensic research focused on the detection of clandestine burials, although this remains a relatively underexplored field. Pringle et al. (2015) described magnetic susceptibility as a cost-effective, rapid, and reliable surface method capable of detecting ground disturbance in archaeological and forensic research. In their experiments, the authors observed MS consistently increasing above disturbed soils, reflecting enhanced near-surface magnetic mineral concentrations produced by soil overturning and aeration. According to the study, simulated graves, burnt clothing, and buried weapons all produced positive MS anomalies, typically several times greater than background values and persist over time (up to one year). However, in these cases MS detected the disturbed soil matrix rather than the cadaver itself. This confirmed that MS is sensitive to modifications in the magnetic mineral fraction induced mainly by digging, which settles its potential as an early-stage screening tool to identify areas requiring more detailed geophysical or intrusive investigation. Another experiment was conducted by Molina et al. (2016). In this case the authors used MS to monitor simulated graves containing pig cadavers, skeletonized human remains, burnt remains, and empty control graves for up to 22 months post-burial. MS proved successful in identifying the graves, showing higher susceptibility values over disturbed soil compared to undisturbed background, even long after burial. The increase in MS was attributed to both the physical disruption of the soil (exposing magnetic minerals to oxidation) and the introduction of anthropogenic materials in some burials. In this case too, the study showed how MS anomalies reflected soil disturbance rather than decomposition products, meaning that the presence of a cadaver did not directly increase susceptibility. The results obtained by Pringle et al. (2015) and Molina et al. (2016) are consistent with the findings previously reported by Fenning & Donnelly (2004) who, in a broader geophysical review, emphasized both the potential and the limitations of magnetic methods in forensic applications: MS contributes by identifying contrasts in magnetic mineral content, which can help detect disturbed ground, metallic clothing accessories, or anthropogenic magnetic enhancement in soils around burial sites; on the other hand buried bodies themselves possess negligible magnetic signatures. Therefore, MS can be effective when soil contains ferromagnetic minerals or when burial processes introduce magnetic artifacts, being typically higher where soils have been reworked.

Thus, although MS does not identify cadavers directly, it could assist in delimiting likely grave locations, especially when used in combination with magnetometry and other geophysical techniques (Fenning & Donnelly, 2004). Conversely, Guedes et al. (2013) investigated MS primarily as a laboratory tool for forensic soil comparison rather than as a field search technique. Their outcomes showed that magnetic susceptibility is highly reproducible, sensitive to small sample sizes, and capable of distinguishing soils from different geological settings based on their magnetic mineral content. Although the study does not involve graves or cadavers research, its findings are relevant to forensic investigation since they confirm that MS can respond strongly to variations in mineralogy linked to natural, anthropogenic, or disturbance processes. According to the authors, disturbed or mixed soils from burial sites would likely exhibit elevated or modified MS values due to changes in magnetic mineral concentration caused by excavation, burning, or the introduction of foreign materials. Thus, MS can support both field detection of burial-related disturbance and laboratory comparison of soil trace evidence.

About its utilization as laboratory tool and its relationship with decomposition, according to Roberts (2015) magnetic susceptibility in sediments is strongly controlled by the microbial degradation of organic matter, which drives a cascade of redox reactions that progressively alter iron-bearing minerals and, consequently, sediment magnetic properties. As reactive organic carbon is decomposed, electron acceptors such as manganese oxides, iron oxides, and finally sulphate are consumed, producing reduced porewater species (Mn^{2+} , Fe^{2+} , H_2S) that trigger dissolution of ferrimagnetic minerals like magnetite (Fe_3O_4) or promote the authigenic formation of iron sulphides including greigite (Fe_3S_4) and pyrite (FeS_2). In organic-rich environments, this process can lead to near-complete loss of magnetic minerals and a marked reduction in susceptibility, as magnetite dissolution proceeds rapidly once sulphidic conditions are established (Roberts, 2015). Overall, the study underscores that MS provides a sensitive proxy for tracking the intensity, depth, and spatial variability of organic-matter decomposition in terrestrial and marginal soils/sediments. Roberts et al. (1995) also provided an important complementary framework by demonstrating how mixtures of magnetic minerals with differing coercivities and grain sizes produce characteristic wasp-waisted hysteresis loops. Although not a study of decomposition per se, this behavior is directly relevant to terrestrial soils undergoing organic-matter degradation because redox-driven dissolution of magnetite and concurrent formation of iron sulphides create the mineral mixtures that generate such hysteresis signatures. Indeed, as reactive iron oxides are progressively removed and replaced by sulphidic phases, the

magnetic assemblage transitions from low-coercivity to mixed-coercivity populations, producing both hysteresis loop constriction and measurable changes in susceptibility (Roberts et al., 1995). Thus, hysteresis analysis enhances the interpretive power of MS by distinguishing between simple reductions in magnetic mineral concentration and more complex mineralogical transformations linked to organic-matter decay. Taken together, these studies suggested that magnetic susceptibility could represent a robust, non-destructive indicator of the presence of decomposing organic matter in terrestrial settings. High organic reactivity typically results in reduced MS due to magnetite dissolution, while localized sulphidisation may generate susceptibility anomalies indicative of concentrated organic decay. Integrating MS measurements with hysteresis-based mineralogical diagnostics therefore enables sensitive characterization of organic matter degradation in soil, supporting applications ranging from environmental reconstruction to forensic geoscience.

Within the present PhD project, MS and hysteresis analyses were performed exclusively on the GC group and revealed substantial differences between soil samples collected from the bottom of the graves at PBI 0 (*control soil*) and those collected from the corresponding HCDIs. With respect to MS, the control samples showed higher absolute values and a broader distribution along the oblique axis (Figure 3.30), a pattern indicative of greater variability. In contrast, the HCDI samples displayed slightly lower magnetic susceptibility values, which were more homogeneous and clustered toward the center of the graph. These differences suggested a higher concentration of ferromagnetic and paramagnetic minerals in the control samples, accompanied by greater grain-size variability.

The hysteresis curves analyzed both in their raw form (Figures 3.31a–c) and after manipulation (Figures 3.32a–c), revealed further differences: the control samples exhibited broader loops and greater dispersion in remanent magnetization (M_r) and saturation magnetization (M_s) values, whereas the HCDI samples showed thinner and more regular loops. This behavior is typical of systems dominated by multi-domain (MD) grains, as observed in the control samples, and by single-domain (SD) or pseudo-single-domain (PSD) grains in the HCDIs (Roberts et al., 2019).

The box plots of saturation magnetization (M_s) values for both raw and manipulated datasets (Figures 3.33 and 3.34) showed that mean values are comparable between the two groups, although the HCDI samples exhibited a smaller standard deviation. Such a pattern indicates a more consistent mineralogy and a more homogeneous texture within the HCDI (Reichel et al., 2016).

The Direct Current Demagnetization curves (Figure 3.35) also supported this interpretation: the control samples exhibited a broader spread across the plot, whereas the HCDI samples displayed more regular and symmetrical trajectories. The slope and shape of the curves suggested that the HCDI soils were composed primarily of single-domain (SD) or pseudo-single-domain (PSD) grains. The maximum backfield remanence values further confirmed the magnetic homogeneity that appears to characterize the HCDIs.

Finally, the Day diagram (Figure 3.36), constructed using the M_r/M_s and H_{cr}/H_c ratios, showed that most samples fall within the multi-domain (MD) sector or in the SD–MD transitional zone (Day et al., 1977). Here again, the control samples appeared more widely dispersed, whereas the HCDI samples cluster within a more restricted area, suggesting a more stable and coherent magnetic behavior.

Overall, all magnetic analyses conducted converged on the observation that the control samples exhibited greater mineralogical and grain-size variability, with a less homogeneous but stronger magnetic response, whereas the HCDI samples were more uniform and constrained, displaying a weaker yet more regular magnetic behavior.

Furthermore, the combined datasets highlighted a clear magnetic behavioral divergence: the control samples tended to show a predominant paramagnetic response, lacking well-defined remanence and coercivity, while the HCDI samples exhibited a stable ferromagnetic component. However, in the Day diagram, both groups fell within the MD field or the SD-MD transitional zone, suggesting a degree of grain-size continuity and the possible coexistence of magnetic phases with similar domain-size or domain-structure properties. This may be explained by the fact that all analyzed samples exhibited characteristics consistent with those of magnetite (Fe_3O_4), particularly with respect to susceptibility values, hysteresis loop shapes, and their position within the Day diagram (Dunlop, 2002). This interpretation is further supported by the values of saturation magnetization (M_s), coercivity (H_c), remanence (M_r), remanent coercivity (H_{cr}), and their corresponding ratios (Table 3.4), which are comparable to those reported for pure magnetite by Roberts et al. (1995).

Consequently, the results obtained here contrast with findings from studies focused on the surface-level use of magnetic responses to detect buried materials (Fenning & Donnelly, 2004; Pringle et al., 2015; Molina et al., 2016), which suggest that MS should increase in the presence of such materials. Instead, they align with the interpretation proposed by Roberts (2015), namely that in environments (or microenvironments, in this case) rich in organic matter, the

degradative activity of microorganisms induces chemical alterations capable of modifying the magnetic properties of iron-bearing minerals. This resulted in a localized but intense dissolution of ferrimagnetic minerals (in this case, magnetite), which can dramatically reduce magnetic susceptibility. It follows that the investigation of magnetic susceptibility, and more generally, the electromagnetic response of ferromagnetic minerals within the matrix in which a body or object is buried, constitutes a promising tool for the detection of clandestine burials. Hence, it must be considered that the anthropogenic and microbiological mechanisms responsible for such anomalies differ between field and laboratory conditions and therefore could produce opposite results.

However, the possibility cannot be excluded that the mechanical actions associated with digging and backfilling the graves may themselves have contributed, at least in part, to the results obtained in this experiment. Indeed, the study outcomes showed a clear difference between the control soil and the HCDI in terms of magnetic response and textural homogeneity, which may also be influenced by physical-mechanical processes related to soil removal, deposition, and subsequent reorganization of magnetic grains. The heterogeneity and broad variability observed in the undisturbed control samples suggested a complex sedimentary environment, likely shaped by episodic depositional events and limited grain-size selectivity. In contrast, the HCDI samples exhibited greater grain-size coherence and more uniform magnetic parameters, indicating a more stable depositional origin and a lower degree of post-depositional disturbance (Roberts, 2015; Dunlop & Özdemir, 2001). Particle transport dynamics may directly influence grain-size distribution and, consequently, magnetic susceptibility: the act of constructing the burials and depositing the cadavers may have produced a form of grain-size sorting, favoring the accumulation of single-domain (SD) or pseudo-single-domain (PSD) magnetic minerals, which exhibit a more stable magnetic response (Dunlop & Özdemir, 2001; Shirzaditabar & Heck, 2021). Finer-grained samples tended to fall within SD or PSD domains, showing consistent M_{rs}/M_s and H_{cr}/H_c ratios (Dunlop & Özdemir, 2001). Conversely, the control samples characterized by a more heterogeneous texture, likely contained a mixture of fine and coarse grains, which would respond differently to applied magnetic fields, generating the irregular hysteresis loops observed.

Another factor that may hold some relevance concerns textural reorganization processes. Indeed, the limited internal variability observed in the HCDI samples may suggest that, following burial, the environment underwent a period of relative physical stability. During this interval, magnetic grains may have gradually reorganized, promoting the formation of coherent

aggregates and low-coercivity structures. Such a process is consistent with the description provided by Roberts (2015), who notes that stable conditions can promote a form of magnetic diagenesis conducive to the preservation of coherent magnetic signals.

The examination of magnetic domains may also provide additional hypothetical insights into the physical processes involved. The HCDI samples, characterized by hysteresis loop distributions consistent with SD or PSD magnetite, exhibited magnetic parameters indicative of a reduced abundance of multi-domain (MD) grains, which were more prevalent in the control samples. This difference may reflect the physical conditions of the depositional environment and its subsequent textural evolution: SD and PSD grains are generally smaller and magnetically stable, responding coherently to applied magnetic fields, whereas MD grains are larger and contain multiple magnetic domains, producing more complex and less uniform signals (Dunlop & Özdemir, 2001; Roberts, 2015). According to Roberts et al. (2019), this difference may arise from physical conditions that limit grain growth or the formation of disordered magnetic aggregates. In particular, phenomena such as repeated oscillations in soil moisture content in surface horizons can prevent the stabilization of magnetic particles, promoting fragmentation and the presence of more disordered multi-domain (MD) grains with a less coherent magnetic response. This is consistent with what is well established regarding the influence of soil drainage and moisture conditions, as well as potential temporal fluctuations in water availability or seasonal contrasts, also influenced by temperature variations, which determine the formation of different mineralogical phases of iron oxides/hydroxides and their grain size under varying redox conditions (Schwertmann & Taylor, 1989; Huang & Wang, 1997; Lepre, 2023).

Despite the anomalies identified, the findings of the present study did not reveal any consistent trend either with increasing PBI or with the decomposition state in which the GC pig cadavers were found at the time of exhumation. Nevertheless, MS analysis remains a potentially useful tool in situations where a body has been buried or deposited and subsequently moved, as it may assist in reconstructing the sequence of events. Taken together, these results suggest that while magnetic susceptibility and related parameters may not reliably capture the temporal progression of decomposition within the HCDI, they can still offer meaningful insight into burial dynamics and post-depositional disturbance. Accordingly, magnetic analyses should be viewed as a complementary forensic approach, most effective when integrated with chemical, biological, and stratigraphic evidence, for reconstructing past depositional events and evaluating the integrity of a burial context.

5.2. Concealment Methods as Taphonomic Agents

The observation of a cadaver's decomposition state and the taphonomic alterations affecting it has traditionally constituted the starting point for PMI estimation performed by forensic anthropologists and pathologists (Micozzi, 1991; Megyesi et al., 2005). The importance of accurate PMI estimation lies in its potential to narrow the wide pool of missing individuals, thereby facilitating positive identification through fingerprints, DNA, biological profiling, or dental records (Cockle & Bell, 2015). Moreover, in the early stages of a homicide investigation, PMI estimation can help establish relevant time windows for examining forensic evidence, such as cell phone records or CCTV footage, and, in some cases, may support or challenge alibis provided by suspects (Cockle & Bell, 2015). However, in cases of accidental discoveries of cadavers, forensic experts are increasingly required to consider the specific depositional environment, in order to correctly interpret the evidence. Indeed, although certain processes observable during the normal progression of decomposition typically characterizes its successive stages (i.e., *Fresh*, *Bloat*, *Decay*, and *Dry*; Galloway et al., 1989), it is becoming increasingly clear that the environment of deposition can alter, and in some respects even drive, the course of these processes. The results obtained in this experiment generally supported these observations (Table 4.2): progression in the decomposition process was noted with increasing PMI and PBI, although the extent of such changes appeared to be influenced by the concealment methods employed.

At PBI1, the decomposition state of the pig cadavers was broadly homogeneous, with all individuals in the *Bloat* phase. It is worth noting, however, that subject GQ1 showed skin desiccation caused by the action of quicklime (Schotsmans et al., 2012), a condition that could potentially lead to an overestimation of the time since death.

Similarly, the observations made at PBI2 were relatively homogeneous, with the first signs of skeletal exposure, particularly in the cranial and limb regions, leading to the classification of this stage as *Decay*. Nevertheless, subject GQ2 again displayed a more advanced external appearance due to the pronounced desiccation of the skin, which could also have suggested a more advanced decomposition stage. Despite this, during disarticulation for subsequent laboratory maceration, the internal tissues were found to be remarkably well preserved, indicating the protective effect of the lime, which was progressively forming a hardening shell around the remains (Figures 4.13 and 4.14), an effect also reported in other similar studies (Schotsmans et al., 2014b).

With the onset of PBI3, the first signs of heterogeneity in the observed preservation states became evident, although all subjects were classified as being in the *Decay* or *Decay/Dry* phase, as in the case of GW3. The latter appeared to be in the most advanced stage of decomposition, with extensive bone exposure and moist decomposed tissue and fluids still present, whereas GN3 showed less skeletal exposure and a greater amount of putrefactive soft tissue remaining. In contrast, GQ3 displayed no evidence of skeletal exposure; instead, the skin was extremely dry, fused with the lime shell, and detached with minimal manipulation. Beneath it, the subcutaneous layer had taken on the appearance of a thin yet flexible and resistant membrane. Consequently, in this instance, quicklime treatment would have led to an underestimation of the PMI, contrary to what had been observed up to this point. Finally, GC3 also showed no signs of skeletal exposure, having undergone a conservative decomposition process resulting in wet mummification. This condition was likely the result of a combination of factors: the occurrence of intense rainfall events between PBI 2 and 3 (with approximately 200 mm of cumulative precipitation) and the restricted tissue transpiration caused by the tight adherence of the clothing to the body. Together, these factors would have created a humid, anoxic microenvironment conducive to the onset of this preservative state.

The external examination performed on the subjects at PBI4 revealed a regained homogeneity in their preservation state, all still within the *Decay phase*. All individuals showed a high amount of preserved fat tissue (adipocere), body fluids still present, and extensive bone exposure. Once again, however, GQ4 represented an exception: only partial skeletal exposure of the skull was observed, with preserved desiccated or mummified tissue, while the postcranial region was desiccated but well preserved, with the hooves still intact and articulated with the limbs. Thus, once again, quicklime treatment would lead to an underestimation of the PMI, especially considering that the substance had replaced the internal structures of the abdominal and limb regions, thereby maintaining the integrity of certain anatomical components.

Finally, at PBI5 all subjects had reached the *Dry* phase of decomposition, although with some variation. GN5 and GC5 were both completely skeletonized, with very limited traces of mummified tissue, almost absent in the case of GC5. In contrast, in GW5, bones were largely dry, but some decomposed tissue was still present and adipocere was abundant, while GQ5 displayed dry bones covered by mummified tissue, with the hooves still present.

Overall, there is a progressive, time-dependent evolution in the decomposition state of the subjects; however, its progression is far from linear due to the depositional environment and the concealment methods applied. For example, the long-standing assumptions regarding the caustic action of lime on decomposing bodies (D'Errico et al., 2011; Schotsmans et al., 2012, 2014a, 2014b), were disproved again. The present study suggested that quicklime does not accelerate tissue breakdown; rather, in the early stages of decomposition, it appears to induce pronounced dehydration of the outer skin layers, a condition that may lead to an overestimation of the PMI.

In addition, the observations indicated that the progressive formation of an external lime shell may protect the internal organs from exogenous microbial activity, resulting in their relatively good preservation for at least 101 days postmortem (PBI3). Such preservation could represent a potential advantage in medico-legal and pathological investigations, as it may enable the identification of lesions that would otherwise be difficult or impossible to recognize in decomposing soft tissues.

In the later stages, by contrast, lime appeared to exert a more preservative than degradative effect. Indeed, once its hygroscopic demands had been met and the quicklime had fully hydrated, the mummified skin remained preserved and was more abundant than in the other groups. A human burial exhibiting similar characteristics could therefore retain mummified skin, which in turn may preserve evidence of lesions or trauma-related features associated with the criminal act (Ventura et al., 2013). Moreover, the presence of mummified skin on the fingers and fingertips holds significant forensic potential: once rehydrated, such tissue may allow for the exceptional recovery of the victim's fingerprints, thereby contributing to their identification (Marais & van den Dool, 2021; Prabhakaran et al., 2024).

The presence of cotton clothing also appeared to influence the progression of decomposition. In this study, the results suggested that under conditions of high humidity (PBI3), the T-shirt adhered closely to the body, creating a microenvironment that may have contributed to wet mummification. At the subsequent PBIs, however, once the T-shirts had largely disintegrated, the preservation state of GC4 and GC5 became more comparable to that of the other groups, with the usual exception of the GQ group. Nevertheless, the biogeochemical anomalies observed in the HCDI of GC2 remain to be explained, as they do not correspond to the preservation state of the subject. Although the concentration of the analyzed compounds was significantly higher than in the other groups, the decomposition stage was assessed as

comparable to that of GW2. The hypothesis of a shift or displacement of the T-shirt, indeed found to be only minimally impregnated with decomposition fluids at recovery, remains plausible, as this would have allowed the passage of nutrients into the surrounding matrix. This hypothesis should be verified through analyses performed directly on fragments of the clothing (HauTEO et al., 2013; Card et al., 2015; Collins et al., 2020).

5.2.1. TBS of Buried Bodies: a Comparison of Three Methods

The observations collected on the decomposition state of the bodies were subsequently translated into quantitative terms through the calculation of the TBS, applying the methods of Megyesi et al. (2005), Keough et al. (2017), and Indra et al. (2024).

In agreement with the findings from the external examinations, at PBI1 all subjects achieved a TBS of 13 using the Megyesi et al. method, which, having been validated on a large human sample, includes broader decomposition stages than those adapted for animal models. Indeed, when applying the methods of Keough et al. and Indra et al., GQ1 obtained a slightly higher score than the others, whereas the lowest scores were assigned to GN1 using Keough et al. and to GN1 and GW1 using Indra et al.

At PBI2, a comparable homogeneity of results was again observed: GW2 and GC2 were assigned a TBS of 20, while GQ2 received a slightly higher score of 21. As in previous cases, this result is attributed to the desiccating effect of lime, particularly affecting the head and limbs.

The heterogeneity in the decomposition state observed macroscopically at PBI3 was also reflected in the assignment of the TBS. Indeed, subjects GN3 and GW3 reached scores of 22 and 25, respectively, whereas the preservation state of GQ3 resulted in a much lower score of 18, which would have led to an underestimation of the PMI. For GC3, the method could not be applied due to the onset of wet mummification.

At PBI4, the TBS values once again became relatively homogeneous: GN4 and GW4 both scored 27, while GC4 was assigned 28 points. In contrast, GQ4 again received a lower score than the other groups, although the discrepancy was smaller than in previous PBIs. Specifically, this subject was assigned 22 points using the Megyesi et al. method and 21 points using both the Keough et al. and Indra et al. methods.

Finally, at PBI5, the calculation of the TBS once again revealed discrepancies in the preservation state of the subjects. The highest score was assigned to GC5, corresponding to 35 with the Megyesi et al. method and 36 with the Indra et al. method, whereas the lowest score was recorded for GQ5 (27 points). In this case as well, the TBS highlighted how the preservative effect of lime prevented the destruction of certain tissues, thereby lowering the score. Subject GW5 also obtained a relatively low TBS, corresponding to 31 with the Megyesi et al. method and 32 with the Indra et al. method. Here, the issue was linked to the extensive presence of adipocere in the trunk region. In fact, the formation of adipocere on buried bodies is relatively

common, due to the ease with which an anoxic and moisture-rich hypogeal environment can develop (Fiedler & Graw, 2003; Forbes et al., 2005a; 2005b; Vass, 2011). According to Fiedler and Graw (2003), adipocere makes the corpse almost entirely resistant to decomposition and therefore makes the calculation of the TBS extremely challenging in such cases.

Overall, a progressive increase in score variability was observed with increasing PBI across all three applied methods (Figure 5.2). At the first two PBIs, variability among the different groups was very limited, reaching zero at PBI1 when using the Megyesi et al. method. This may be due to the fact that the method, validated on humans, may not fully capture all the stages that characterize the decomposition process in other species, whereas the other two methods, developed specifically to better quantify changes in decomposing pig cadavers, appeared more capable of assigning scores that reflected the observed phenomena.

Regardless of the method used, starting from PBI3, a clear increase in variability became evident and remained more or less constant throughout the remainder of the experiment. This highlights the significant influence exerted by concealment methods, which appear capable of altering the natural evolutionary sequence of decomposition processes.

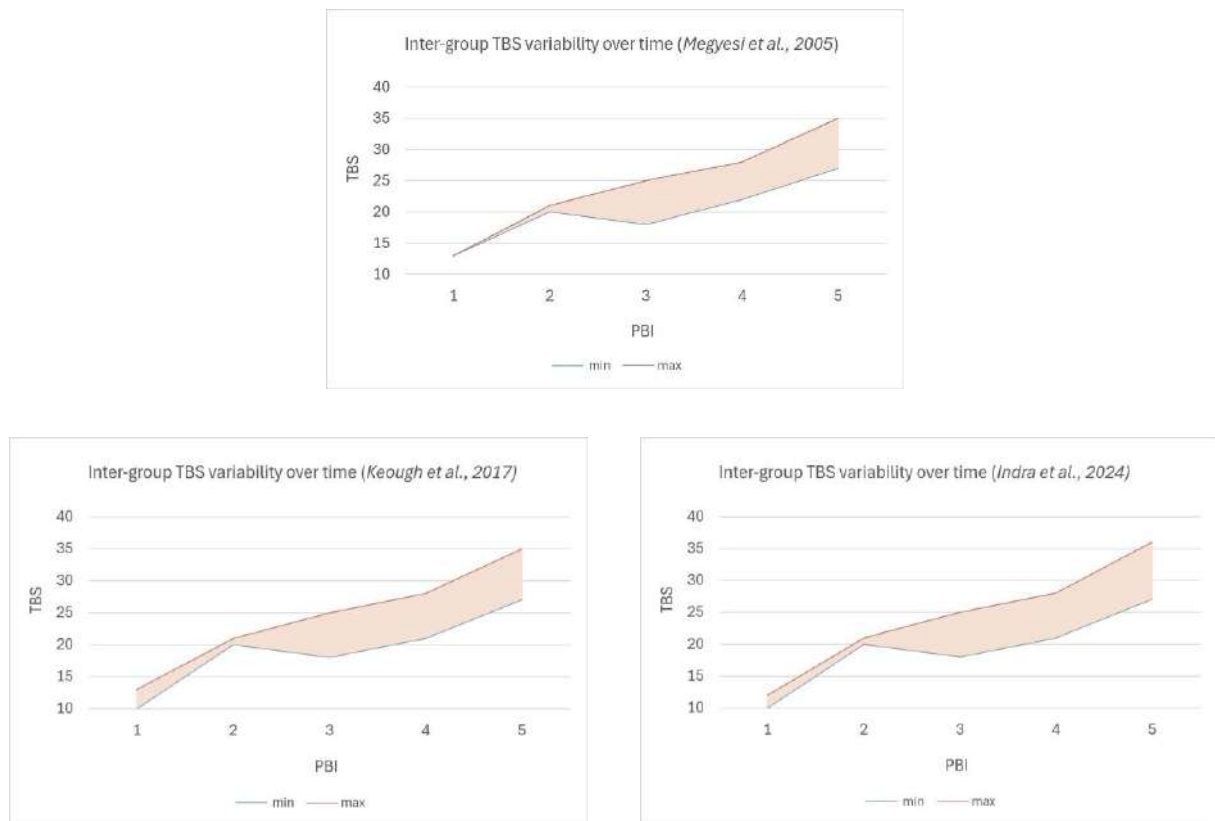


Figure 5.2: Comparison of the inter-group TBS variabilities over time according to the three methods considered.

5.2.2. Challenges in PMI Estimation: Insights from ADD and Vass' Universal Formula

The calculation of the TBS, in addition to providing a quantitative translation of the qualitative macroscopic observations of the subjects' decomposition state, also enabled the application of Accumulated Degree Days (ADD) to evaluate their effectiveness in the case of buried bodies subjected to multiple concealment methods or treatments. Overall, the calculation of ADD using the formula proposed by Megyesi et al. (2005) resulted in a marked underestimation of the PMI, regardless of the method used to compute the TBS. Moreover, the discrepancy between the actual and estimated postmortem interval increased with each PBI, at least up to the fourth exhumation (Figure 5.3).

In contrast, at PBI5, the ADD values calculated using the TBS derived from the Megyesi et al. (2005) method produced only a slight underestimation (with the exception of GQ5, already discussed), whereas applying the TBS obtained with the Indra et al. (2024) method resulted in a slight overestimation of the PMI for subject GC5. The reduction in error at PBI5 may be attributed to the near-complete skeletonization of the individuals, among whom GC5 retained the lowest amount of preserved mummified tissue.

Furthermore, even when including the ± 388 ADD error in the calculations performed, the method markedly underestimated the PMI up to PBI5 (397–467 days), at which point the underestimation decreased for subject GC5 (308–318 days), and then even resulted in an overestimation (443–461 days) when the TBS was calculated using the method by Indra et al. (2024). Similar observations regarding the inapplicability of the method in forensic casework have been made in other parts of the world, both on human and non-human samples (Marhoff et al., 2016; Suckling et al., 2016; Wescott et al., 2018). This would require a statistical recalculation of the method's estimated error, which should then be calibrated to the decomposition rate of buried bodies, so as to render it compliant with the *Daubert criteria*.

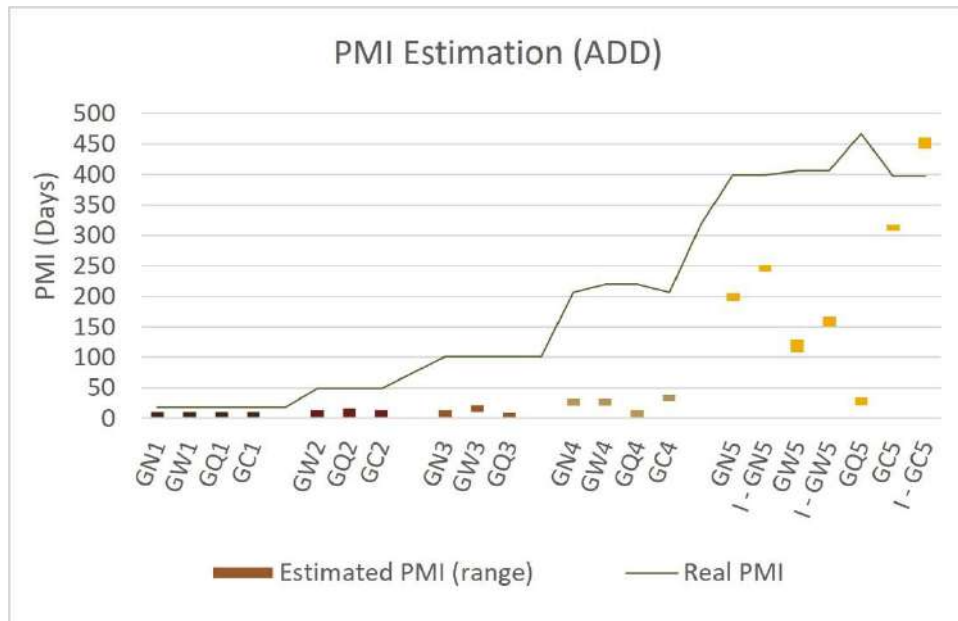


Figure 5.3: Graph showing the differences between the estimated PMI for each subject (calculated using the ADD formula (Megyesi et al., 2005) and the actual number of days elapsed between burial and recovery of the remains. The letter “I” indicates the subjects for whom the ADD calculation based on the TBS method proposed by Indra et al. (2024) yielded results that differed from those obtained using the Megyesi et al. method.

Overall, the calculation of ADDs for PMI estimation appears to be inefficient when applied to buried remains. This outcome is not entirely unexpected. The approach proposed by Megyesi et al. (2005) was originally developed under the assumption that atmospheric temperature is the predominant taphonomic factor (together with humidity, pH, and pO_2 ; Micozzi, 1991; Vass, 2011), particularly in the absence of insects, plants, or other matrices that might provide complementary information. For buried remains, however, atmospheric temperature influences the rate and sequence of decomposition only to a limited extent due to the insulating effect of the soil. This barrier also restricts the activity of necrophagous insects, whose access to the body becomes severely reduced (Simmons et al., 2010; Singh et al., 2016). As a result, not only did the method prove unsuitable due to the marked slowing of tissue degradation, but its limitations also became evident already at the stage of TBS assessment. Both methods developed on the animal model, in fact, included among their scoring parameters the presence of larval colonization and the subsequent larval migration during the early stages of decomposition (Keough et al., 2017; Indra et al., 2024).

These issues highlight the need for an *ad hoc* method capable of defining the decomposition stages of buried bodies and correlating them with time. The main challenge is that the hypogeal environment encompasses a wide range of factors that directly affect taphonomic processes, and these factors are themselves influenced by the broader environmental conditions in which they occur. This interplay risks making each case highly context-specific and, consequently, any potential method for taphonomic analysis difficult to standardize.

A further attempt to identify a PMI estimation method suitable for buried remains was proposed by Vass (2011) through the application of his *Elusive universal PMI formula*. Applying this method to the present experiment resulted in a substantial overestimation of the PMI (Table 4.5), particularly at PBIs 4 and 5 (Figure 5.4). Once again, the underlying issue may lie in the fact that the method was validated on a human model rather than an animal one, as in this project. Consequently, difficulties arise in assessing the decomposition status of the subjects expressed as a percentage. Although Vass provides a correspondence between the four main decomposition stages and specific percentage ranges, assigning a precise value is challenging, as different body districts often progress through different stages simultaneously. This issue is even more pronounced in the animal model, where the distribution of muscle and adipose tissue differs markedly from that of humans (Matuszewski et al., 2020; Dawson et al., 2022; Berezowski et al., 2024).

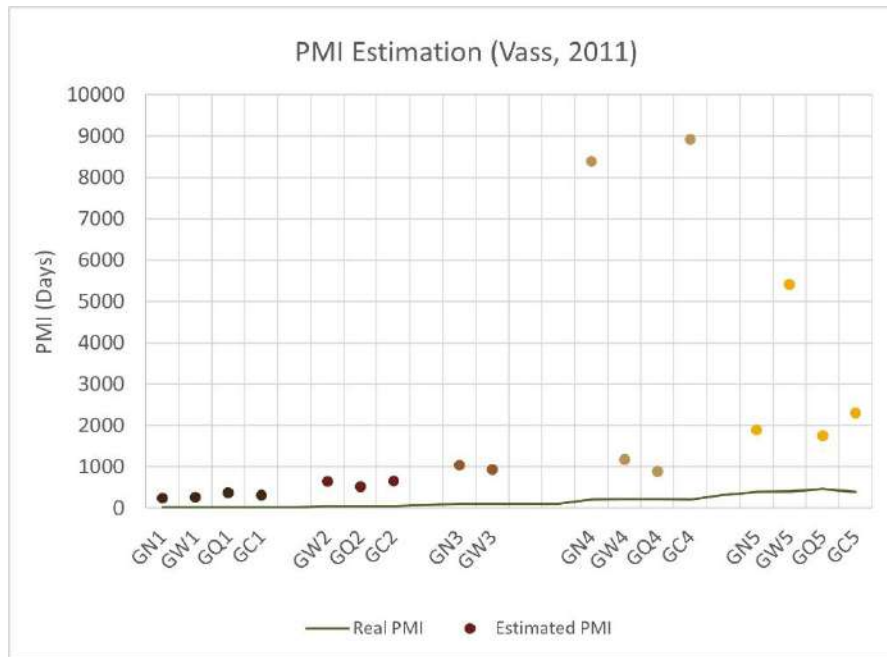


Figure 5.4: Graph showing the differences between the PMI estimated for each subject using Vass' (2011) formula for buried bodies and the actual number of days elapsed between burial and recovery of the remains. Subjects GQ3 and GN3 were excluded because their decomposition state could not be assessed.

Another issue affecting the universal applicability of the method may lie in the assessment of soil moisture collected from the burial context. In the experiment central to this PhD project, the soils were sandy in texture, and it is likely that their water retention capacity was relatively low. This would result in low moisture values which, being in the denominator of Vass' formula, would lead to an overestimation of the PMI. The phenomenon is particularly evident in the cases of subjects GN4 and GC4, both exhumed on the same day with a PBI of 206 days. In these cases, the moisture values of the soil samples were the lowest recorded in the entire experiment. Additionally, both subjects exhibited the presence of adipocere covering approximately 35–40% of the body. This contributed to further increases in the numerator values of the formula, resulting in a substantial overestimation of the PMI. Adipocere formation therefore represents an additional obstacle to applying the Vass formula to the animal model. The higher concentration of adipose tissue in the trunk region of piglets may explain the abundant formation of adipocere, which, in human remains, would presumably require a longer period to develop.

The results obtained are in agreement with what was stated by Cockle & Bell (2015). Indeed, they affirmed that it would be generally accepted that the estimation of a PMI based on the physical state of the body is inherently unreliable and constitutes just an educated starting point for the final evaluation, while environmental factors are those that should be taken more into consideration. Moreover, the authors criticized the use of the constant ratio of 4.6 in Vass' formula, which should "*represent a slowdown in the rate of decomposition of buried remains compared to exposed ones due to lack of oxygen*" (Vass, 2011). In their experiment, Cockle and Bell compared a set of 22 buried cases to 96 surface cases in Canada, finding that the rate of decomposition for buried compared to surface cases is extremely variable and dependent on the stage of decomposition reached. Indeed, for the first stages the progression was observed to be even faster for buried compared to surface cases, while for the stages of putrefaction the exposed bodies demonstrated a faster rate of decomposition. Moreover, the progression rate for the later stages of liquefaction was only slightly faster for buried cases. Such variability is sufficient to state that the use of a constant deceleration of the progression of the decomposition stages is not universally valid and, consequently, the formula for estimating the PMI is not applicable (Cockle & Bell, 2015). Overall, the findings of the study indicated that the factors influencing human decomposition may be more complex than assumed: region-specific humidity and temperature levels appear to exert a measurable effect, yet these variables are unlikely to be the sole determinants of decomposition dynamics. Intrinsic characteristics such as an individual's age, sex, cause and manner of death, and lifestyle, together with extrinsic elements including clothing, coverings, and the depositional environment, may also substantially influence both the rate and trajectory of human decomposition. Accordingly, the limited reliability of Vass' formula in estimating the PMI suggests that the development of a universal method remains an unresolved challenge. Given the current state of knowledge, it may be premature to infer broadly applicable patterns of decomposition. Nevertheless, with further retrospective analyses and additional experimental research, it may become possible to construct region-specific models by identifying the variables that characterize particular environmental and contextual settings.

In conclusion, also in this case, as in the calculation of ADD, the impossibility of validating the method and providing a known or potential rate of error demonstrates how difficult it is for PMI estimation to comply with all the Daubert criteria, especially due to the intrinsic and extrinsic variables that are difficult to express in quantitative terms.

5.2.3. Taphonomic and Chemical Analyses of Gunshot–Related Lesions on Buried Piglet Remains: a Complementary Approach

Taphonomic analysis, by itself, does not constitute a complete investigative method, as forensic investigations typically require the use of complementary techniques. This is particularly true when assessing the presence of wounds whose morphology and proportions have been altered by natural and depositional taphonomic agents. In the present PhD study, an interdisciplinary approach was therefore proposed, involving the complementary use of osteological and chemical analyses on gunshot wounds when the state of decomposition is so advanced that they become macroscopically unrecognizable (Gibelli et al., 2010).

The results of the external examinations conducted on the piglets from GW group made it possible to observe how, already from PBI2, the state of active decay had disrupted the appearance of a bullet exit wound, making it difficult to reach a diagnosis (Figure 5.5). In general, the progressive decomposition of the tissues was found to correlate closely with the PBI, reflecting the influence of taphonomic factors such as soil composition, humidity, and microbial activity. However, some fundamental ballistic features, including morphology, fracture patterns, and directionality, remained identifiable on bone tissue.



Figure 5.5: detail of the anterior exit wound of the gunshot on the skull of subject GW2.

The forensic pathological and osteological assessments conducted concurrently with the preparation of Dr. Ludovico Re's specialization thesis in Legal Medicine are briefly presented below. Particular attention is given to the discussion of the difficulties encountered in identifying gunshot-related lesions on the skull due to taphonomy, and distinguishing entrance from exit wounds.

Starting with the control piglet GW0 (which was never buried), on external autoptic examination, the good state of preservation of the cadaver resulted in the persistence of the animal's hair coat, which partially concealed the anterior entry wound, the posterior entry wound, and the posterior exit wound, while the only lesion exhibiting features indicative of a gunshot origin was the one in the right frontal region, corresponding to the anterior exit wound. After maceration and reconstruction of the skull, it was possible to observe and interpret the wounds:

- On the left side, at the maxillary region, a full-thickness defect was identified, exhibiting morphological features compatible with a gunshot lesion. The margins were sharp and smooth, with evident internal beveling at the zygomatic process, macroscopically consistent with the characteristics of an entry wound (Berryman & Symes, 1998). Based on the experimental setup, this lesion would be expected to correspond to the anterior entry wound of the projectile. However, its irregular morphology has also been described as a typical feature of exit wounds (Ortner, 1981; Smith et al., 1987), making definitive interpretation uncertain.
- On the right side of the skull, in the frontal region dorsal to the orbit, a full-thickness defect with a quadrangular morphology and external beveling was observed. The characteristics of the defect margins, together with the association of radial and concentric fractures, were interpreted as consistent with an exit wound (Smith et al., 1987; Symes et al., 2012), allowing a positive association with the anterior exit wound.

Once the anterior entry and exit wounds were characterized, the exploration of the intracranial trajectory using a probe allowed reconstruction of the bullet path, running dorsally-caudally-medially from the ventral region of the left maxillary bone to the lateral orbital and supraorbital region of the right frontal bone.

- With regard to the posterior gunshot, a full-thickness defect with a T-shaped morphology was noted in the occipital region, with comminuted fractures in the ventral and left lateral

areas, where the presence of an inward-displaced bone fragment was also observed. The interpretation of the nature of this lesion was not immediate: located in a median position on the occipital squama, it presented features typical of an exit wound (Smith et al., 1987; Symes et al., 2012), but the presence of internal beveling in the lateral area, the inward displacement of a bone fragment at the site of the comminuted fracture, and the retrieval of detached bone fragments constitute findings compatible with the characteristics of an entry wound (Smith et al., 1987; Berryman & Symes, 1998). Moreover, the associated fractures displayed a radial pattern: although present in both entry and exit wounds, their limited extent confirmed the features of an entry wound (Symes et al., 2012). An interpretative hypothesis that resolves these contradictions derives from the finding of a fracture of the lateral mass of the atlas: owing to its sharp margins, right angles, and granular surfaces, it was caused by high-energy trauma, well correlating with a gunshot injury (Symes et al., 2012). The concomitance between this lesion and the atlas fracture suggested a projectile trajectory caudal to the neurocranium, tangential to the external surface of the occipital squama. The above considerations, in light of this interpretative hypothesis, lead to confirming a positive association between the lesion and the posterior wounds.

Conversely, at the time of exhumation, specimen GW1 (PBI = 18 days) was in a moderate state of preservation, corresponding to the initial discoloration stage of putrefaction, with epidermolysis of the snout and collapse of the ocular globes. However, the body was extensively covered with soil and small stones, which made the identification of the gunshot wounds difficult. The lesions appeared as hyperchromic defects with poorly defined and non-assessable margins, with the exception of the posterior entry wound, which presented as a discontinuity at the base of the right auricular pavilion, elliptical in shape and characterized by regular margins. Regarding the interpretation of the wounds at bone level:

- On the left side, a defect with several morphological features consistent with firearm-related trauma was detected (Smith et al., 1987; Berryman & Symes, 1998). In particular, the morphology at the level of the maxillary and zygomatic bones was irregular, with external beveling, consistent with the characteristics of an exit wound (Smith et al., 1987; Berryman & Symes, 1998). Conversely, in the intermediate portion of the maxillary margin, external beveling is absent, and the margins are sharp, with right-angled surfaces, features typically associated with entrance wound. At the level of the body of the presphenoid and basisphenoid, the defect shows sharp, non-irregular margins, a

characteristic commonly observed in entrance wounds. However, the margin at the level of the basisphenoid wing shows loss of the external cortical layer and diploë with external beveling, as expected in exit wounds (Smith et al., 1987; Berryman & Symes, 1998).

Based on these observations, the isolated evaluation of this defect supports its interpretation as a firearm-related lesion. However, the coexistence of morphological features consistent with both entrance and exit wounds does not allow for a definitive diagnosis regarding the directionality of the projectile.

- The same considerations apply to the bone defect observed on the right hemicranium. The morphological features are compatible with firearm-related trauma; however, the morphological diagnosis remains inconclusive.

In this case, a comparative evaluation of the morphological features of the two lesions provided relevant interpretative information. Firstly, based on their coplanarity on the frontal plane, together with the absence of additional cranial bone defects and the presence of an intracranial tract observable upon inspection, it was concluded that the lesions represented a complete perforating injury, constituting the entrance and exit wounds of the projectile. Secondly, although some diagnostic uncertainty remained, the relatively greater abundance of radial fractures on the right side and the presence of unilateral diastasis of the right parietal bone, in association with bilateral temporal bone diastasis, suggest a difference in the intensity of forces transmitted to the bone tissue by the projectile, with greater force affecting the right side of the cranium. As is well established, such forces are typically greater at the exit wound (Berryman & Symes, 1998; Quatrehomme & İşcan, 1998; Symes et al., 2012). Based on these observations, a positive association was established between the left-sided defect and the anterior entrance wound, and between the right-sided defect and the anterior exit wound. Once characterized in this manner, analysis of the intracranial tract allowed reconstruction of the projectile trajectory, which followed a transverse left-to-right and cranio-caudal course. Ultimately, no skeletal evidence attributable to the posterior gunshot wound was identified.

Regarding specimen GW2 (PBI = 49 days), at the time of exhumation it was in the liquefactive stage of decomposition, with poor skin integrity and disarticulation of several skeletal elements. During examination, the skin was found to be entirely covered with agglomerates of soil and small stones which, once gently removed, revealed a whitish discoloration. A discontinuity with loss of cutaneous tissue was observed in the right cranial region, exposing cerebral material; this defect was interpreted as the anterior exit wound (Figure 5.5). The anterior entry wound,

as well as the posterior entry and exit wounds, could not be visualized. After maceration and reconstruction, defects and fractures were interpreted as follows:

- One lesion consisting of a full-thickness defect of the left maxillary bone and one involving the right maxillary bone, the right frontal bone, and the right lacrimal bone were found to have topographical correspondence on the frontal plane, rendering an intracranial trajectory between them discernible, indicating a complete bony-through lesion. Despite the morphology of the two lesions being not informative, comparative evaluation of the margins and fracture patterns was suitable for drawing conclusions. Firstly, the presence of internal beveling at the left zygomatic process and the presence of external beveling at the right zygomatic process suggested that the left-sided defect constituted the entry wound and the right-sided defect constituted the exit wound (Smith et al., 1987; Berryman & Symes, 1998; Symes et al., 2012). Secondly, a greater density of lesions was observed on the right facial massif, which was affected by more radial fractures and, on the nasal bone, by multiple fracture lines with comminuted fragments and a full-thickness loss of a bone fragment at the level of the right fronto-nasal suture. These findings indicated greater transmission of energy to the right side compared to the left: as already mentioned, more energy is typically transmitted to the bone tissue at the point of projectile exit from the skull (Berryman & Symes, 1998; Quatrehomme & Işcan, 1998; Symes et al., 2012). A positive association can therefore be made between the two defects, which constitute the anterior entry wound (left side) and the anterior exit wound (right side).
- On the same skull, a full-thickness defect of the left temporal bone with an elliptical morphology was also noted. Overall, this wound was the most indicative of a gunshot mechanism, as it presented a characteristic ellipsoidal morphology, with radial fractures extending across sutures, and margins displaying external beveling dorsally and internal beveling caudally (Smith et al., 1987; Berryman & Symes, 1998; Symes et al., 2012). The ellipsoidal morphology and the presence of internal beveling are features typical of an entry wound (Smith et al., 1987; Berryman & Symes, 1998). Hence, a positive association was made between this lesion and the posterior entry wound.
- Conversely, a full-thickness defect of the right parietal bone was observed. It exhibited a morphology that could be schematically represented as a triangle with a ventral–caudal apex. The caudal and ventral margins were sharp, with right-angled, granular-appearing surfaces; along the dorsal margin, a defect of the outer table and diploë was documented, with external beveling forming a semicircle with dorsal convexity. Its nature as a gunshot

- injury was suggested by the irregular morphology, the dorsal margin demonstrating regular semicircular external beveling with a regular free edge, and the presence of radial fractures extending to the sutures. Moreover, the dorsally flared margin, bevelled outward, supported a positive association with the posterior exit wound (Smith et al., 1987; Symes et al., 2012).
- In addition, a full-thickness defect was observed on the right side of the occipital squama, exhibiting an irregular morphology with sharp and regular margins and a bone splinter on the medial side with a dorsally directed apex. Six fractures were found to be in direct continuity with the defect. Overall, these features were considered consistent with a blunt-force trauma mechanism (Ortner, 1981; Galloway et al., 1999; Hart, 2005), although they did not provide differential indications regarding the producing weapon, as such characteristics may result either from the impact of a blunt object or from the blunt-force action of a firearm projectile.

Regarding subject GW4 (PBI = 220 days), exhumation revealed piglet remains in a state of partial skeletonization and advanced soft-tissue liquefaction. At preliminary inspection, only the dorsal surface of the cranial vault was visible; the morphology and spatial relationships of the residual soft tissues were not appreciable due to their advanced colliquative degradation. Consequently, neither entrance nor exit wounds could be identified during the external examination. Following the complete removal of the preserved soft tissues, the cranial lesions were evaluated as follows:

- A defect consistent with a firearm entrance wound was identified in the left maxillary bone at the level of the canine fossa: it displayed elliptical morphology, sharply delineated margins, loss of diploë and internal table (internal beveling), and a fracture in direct continuity, extending radially from the caudal margin. These peculiarities are ascribable to a firearm entry wound (Smith et al., 1987; Berryman & Symes, 1998).
- The right maxillary bone exhibited a defect involving the canine fossa, molar alveolar spaces, and palatine process, with characteristics typical of a firearm exit wound: irregular morphology, loss of outer table and diploë (external beveling) and fractures in direct continuity, extending radially from the defect's margins (Smith et al., 1987; Symes et al., 2012). The concomitant presence of entrance-wound features on the left maxillary bone and exit-wound features on the right maxillary bone allowed a positive association with the anterior entrance and exit lesions, respectively. Intracranial trajectory assessment indicated a left-to-right projectile path with a slightly dorsoventral and craniocaudal orientation.

- In the left temporal region, caudal and medial to the zygomatic process, a full-thickness defect of the squamous temporal bone was documented, exhibiting morphological criteria of a firearm entrance wound: elliptical/oval morphology, sharply defined margins, internal beveling, and loss of diploë and internal table (Smith et al., 1987; Berryman & Symes, 1998). On the contralateral side, the squamous part of the right temporal bone showed a defect consistent with a firearm exit wound, demonstrating irregular morphology, loss of external table and diploë (external beveling), and a fracture in direct continuity, extending radially from the lesion margins (Smith et al., 1987; Symes et al., 2012). The left temporal defect was therefore attributable to the posterior entrance.

Ultimately, at exhumation specimen GW5 (PBI=406 days) was found in a state of near-complete skeletonization, with disarticulated skeletal elements, detached cutaneous remnants, and plant roots infiltrating the cranial and mandibular regions. After restoration, the lesions were documented:

- An extensive bilateral defect was observed on the lateral and ventral surfaces of the maxillary bone. A complete intracranial bony channel connecting the left and right defects was evident, consistent with a through-and-through firearm wound. Both defects demonstrated irregular morphology, sharply demarcated margins, and radial fractures. The left maxillary defect exhibited no specific entrance- or exit-related features; however, the right maxillary lesion showed loss of external table and diploë (external beveling) of the lacrimal bone and the frontal process of the zygomatic bone, characteristic of an exit wound (Smith et al., 1987; Symes et al., 2012). Thus, despite the non-specific appearance of the left maxillary lesion, its association with the anterior entrance wound, and the right defect with the anterior exit wound, was supported. Intracranial pathway analysis indicated a left-to-right trajectory oriented nearly perpendicular to the lateral surface of the left maxillary bone.
- In the left parieto-temporal region, a “keyhole” defect (a morphological hallmark of tangential gunshot injury; Smith et al., 1987; Berryman & Symes, 1998; Symes et al., 2012) was identified. In such lesions, the oval component corresponds to the entrance or exit site and the assessment of margins’ beveling assists in determining wound directionality. Internal beveling was observed at the cranial half of the dorsal margin, supporting the positive association between the lesion and the posterior entrance wound (Smith et al., 1987; Symes et al., 2012).

- Conversely, the right temporal bone exhibited an irregular defect with granular fracture surfaces, orthogonal edges, and four fractures in direct continuity, extending radially from the margins. Although non-specific *per se*, these features are compatible with an exit wound when considered together with the contralateral keyhole-shaped defect (Smith et al., 1987; Symes et al., 2012). Trajectory reconstruction suggested a left-to-right, craniocaudal and dorsoventral projectile path.

Based on the forensic pathological examination, the findings suggest that an integrated approach combining forensic pathology and forensic anthropology may offer important interpretative advantages. In particular, osteological analysis appeared to gain increasing evidentiary relevance as the post-burial interval progressed and soft-tissue evidence became progressively unavailable. In the present case, restoration of the skeletal elements contributed to the assessment of the wound mechanism in the absence of soft tissues and supported the tentative reconstruction of the projectile trajectory through evaluation of the intracranial tract.

However, with the exception of subject GW4, whose cranial defects exhibited morphological features clearly attributable to entrance and exit wounds, it was not possible in the other samples to determine the nature of the lesions beyond reasonable doubt based on isolated evaluation alone. Definitive interpretation was only achievable through comparative assessment of the relationship between the observed contralateral defects, a procedure that may not always be possible.

In such circumstances, complementary analyses become necessary, most notably the detection of IGSR through SEM–EDX examination (Berryman et al., 2010; Gibelli et al., 2010; Amadasi et al., 2012a, 2012b, 2015; Nguyen-Hong et al., 2024). In this context, the analyses performed on the piglet skulls allowed for the characterization of the inorganic-chemical profile of the particulate matter.

Since the analysis of the quantity and composition of IGSR particles on the lesions was conducted not only to evaluate their potential for identifying gunshot-related lesions and distinguishing between entrance and exit wounds, but also to assess their persistence over the burial interval, the results are presented below in separate sections.

IGSR Analysis for the Detection of Gunshot-Related Wounds

In cases involving the investigation of lesions on bodies in an advanced state of decomposition, complementary IGSR analysis may become necessary, as this technique can provide useful indications for identifying firearm-related trauma and differentiating between entrance and exit wounds.

In the case of subject GW0, which was analyzed after a period of freezing following gunshot exposure, the results are consistent with those reported in the literature (Montoriol et al., 2021; Moussa et al., 2024; Nguyen-Hong et al., 2024). Specifically, the entrance wound yielded a higher number of particles compared to the exit wound (24 versus 21) and was the only lesion in which characteristic IGSR particles, defined by the combined presence of Pb–Ba–Sb, were detected. In this case, therefore, the method proved effective in supporting both the identification of the lesions as firearm-related and the differentiation between entrance and exit wounds.

By contrast, no particles classified as *characteristic of IGSR* were detected on the lesions of the buried subjects (the possible causes of this condition are discussed in the following section). Therefore, it was necessary to reconsider the criteria used to distinguish between entrance and exit wounds. Accordingly, the remaining particle classes with higher evidentiary value (i.e., *consistent with* and *commonly associated with IGSR*) were taken into consideration, while acknowledging the inherent limitation that contamination cannot be fully excluded in scenarios where appropriate control analyses are not feasible.

Similarly, buried subject GW1 also yielded a markedly higher number of IGSR particles on the entrance wound compared to the exit wound (22 versus 11). Particles classified as *consistent with* and *commonly associated with IGSR* were detected exclusively on the entrance wound. In contrast, only particles classified as *other IGSR*, according to the classification criteria adopted in this thesis, were identified on the exit wound.

With regard to piglet GW2, only a small number of particles were detected on the lesions; nevertheless, the entrance wound yielded a slightly higher number than the exit wound (3 versus 2). The distribution of particles between the two wounds was also atypical. Specifically, the entrance wound contained particles classified as both *commonly associated with IGSR* and *other IGSR*, whereas the exit wound yielded only particles classified as *commonly associated with IGSR*. From a taphonomic perspective, it should be noted that GW2 exhibited the poorest preservation of cranial soft tissues, which were in an advanced liquefactive stage of

decomposition. This condition made precise localization of the gunshot lesions more challenging and may have influenced the sampling process.

A similar trend was observed in subject GW4, in which a higher number of particles was detected on the entrance wound compared to the exit wound (29 versus 20). However, both lesions yielded a substantial number of particles classified as *commonly associated with IGSR* as well as *other IGSR*, indicating a less distinct differentiation between the two wounds based solely on particle distribution.

Finally, subject GW5 exhibited an inverse pattern, characterized by a markedly higher number of particles on the exit wound compared to the entrance wound (17 versus 8). Notably, particles classified as *commonly associated with IGSR* were detected on the exit wound but were absent on the entrance wound.

From the standpoint of cadaveric condition, it is notable that subjects GW4 and GW5 exhibited fully skeletonized skulls at the time of exhumation. This state may have facilitated access to particles that would ordinarily remain embedded within bone tissue following the projectile's passage (Berryman et al., 2010) and that, in the presence of soft tissues, it would have been more difficult to recover using swabbing techniques. Indeed, one of the swabs taken from the exit wound of GW5 inadvertently dislodged a small bone fragment bearing a groove (presumably produced by the bullet's trajectory) within which a metallic particle composed of Fe-Cr, and therefore identifiable as stainless steel, was detected (Figure 5.6). An alternative explanation is that gunshot-related lesions became more readily identifiable once the skulls were fully skeletonized, in contrast to earlier PBIs, when such injuries could only be approximated macroscopically. In either case, these findings highlight the value of SEM-EDX analysis for detecting IGSR in the recognition of gunshot trauma, particularly when such injuries are suspected but their macroscopic identification is impeded by advanced decomposition. Furthermore, advanced soft-tissue degradation and associated fluid release may have facilitated the post-depositional redistribution or loss of IGSR particles. The increased mobility of decomposition fluids within the burial matrix may promote the displacement, dilution, or removal of particles, thereby affecting their spatial distribution and potentially obscuring the original deposition pattern associated with firearm discharge.

The results of the IGSR analyses suggest that the application of this method to buried bodies in advanced stages of decomposition, in which the presence of firearm-related injuries is suspected, may allow the identification of traces of considerable investigative value. On the one

hand, the anthropological approach enabled the reconstruction of certain dynamics related to the projectile's passage through the tissues, particularly in cases where external examination made the recognition of the lesions impossible. On the other hand, however, the osteological analysis alone was not sufficient to identify and characterize all the features associated with gunshot trauma.

Conversely, IGSR analysis allowed the detection of metallic particles attributable to firearm discharge residues. Nonetheless, with increasing PBI, only particles belonging to classes less directly diagnostic of gunshot discharge were recovered (i.e., those classified as commonly associated with and other IGSR). On this basis, the possibility of expanding the range of particle characteristics considered suggestive of firearm discharge may be worth considering, at least in investigations involving bodies concealed underground and in advanced stages of decomposition.

Taken together, these findings underscore the importance of adopting a multidisciplinary analytical framework integrating forensic pathology, forensic anthropology, and chemical–environmental sciences. Such an approach may enhance the accuracy and robustness of interpretations while reducing the likelihood of analytical error.

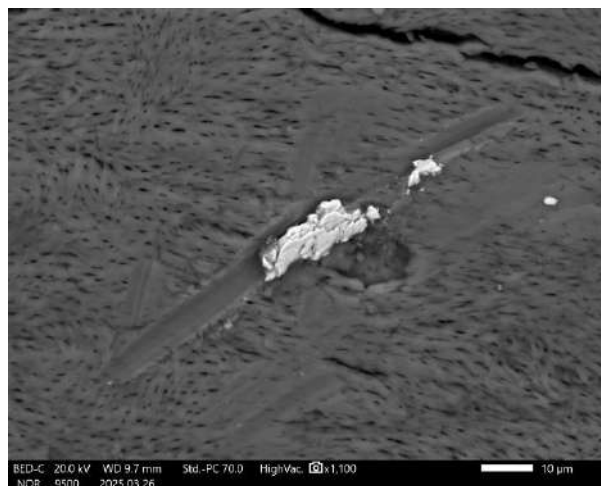


Figure 5.6: Bone fragment collected from the exit wound of GW5 during sampling. Metallic residues can be observed within the groove. Magnification 1,100x.

Persistence and Mobilization of IGSR in Hypogeal Environments: A Pre- and Post-Burial Comparison

SEM-EDX analyses conducted on the posterior lesions shortly after the shootings revealed a heterogeneous pattern that, in several respects, diverged from initial expectations. Specifically, a higher number of IGSR-related particles were not consistently detected on entrance defects compared to exit defects; this condition was observed only in subjects GW0 and GW4.

This outcome contrasts with the findings reported by Nguyen-Hong et al. (2024), who documented a greater abundance of IGSR-related particles in entrance holes than in exit holes in specimens that had been shot but not subsequently buried. Such divergence may be attributable to differences in ammunition type and shooting distance, as well as to variations in the animal models employed. In the aforementioned study, the shootings were performed using a rifle mounted on an easel at a distance of 36 cm and loaded with semi-jacketed bullets, whereas in the present experiment a markedly shorter shooting distance was used in conjunction with a semi-automatic pistol firing full-jacketed ammunition.

Moreover, inter-study variability may also stem from differences in the anatomical regions of the non-human animal models selected. Nguyen-Hong et al. utilized bovine feet, which contain a higher proportion of spongy tissue, while the present study involved porcine cranial vaults. In this latter context, the projectile traverses the interior of the skull, thereby passing through soft tissues and organs before exiting the body. These characteristics may influence projectile velocity and penetration dynamics, potentially accounting for the discrepancies observed in the distribution of IGSR-related particles. This pattern remains consistent when considering the total particle count, irrespective of classification.

However, when examining specific particle categories, particles classified as *characteristic of* or *consistent with IGSR* were more numerous on entrance holes than on exit holes. This is most likely related to the solidification and deposition behavior of residues within the firearm's expelled plume at the moment of discharge (Dalby et al., 2010), which primarily affects the surface of the target oriented toward the shooter (i.e., the entrance area).

Conversely, the distribution of particles *commonly associated with IGSR* were detected in an apparently random manner between entrance and exit holes. Nonetheless, it is noteworthy that such particles were consistently present, albeit with variable distribution and chemical composition, across all samples. Therefore, they may be regarded as indicative of a gunshot

event (assuming that contamination from alternative sources is excluded), although they are not suitable for differentiating between entrance and exit defects.

In contrast, particles classified as *other IGSR* were identified exclusively on exit holes. These particles predominantly consisted of Fe, Cu, and Zn (elements that may be associated with brass cartridge case or jacket fragmentation) as well as Fe-Cr-Ni, indicative of stainless steel (an iron–carbon alloy characterized by Cr and Ni as alloying elements). Such elemental compositions suggest potential contributions from the bullet core or jacket, which may generate these particles upon firing (Dalby et al., 2010; Charles et al., 2023). The exclusive presence of these particles on exit holes remains unexplained, although one possible interpretation involves instrumental limitations. Specifically, in SEM-EDX imaging, metallic particles appear brighter as the atomic weight of their constituent elements increases. It is therefore plausible that the comparatively high abundance of lighter-element particles belonging to other classes may have obscured the detection of these heavier particles. However, this explanation remains hypothetical and should be considered speculative pending further investigation.

The highlights obtained from the lesions prior to burial were subsequently compared with those collected from the same subjects (specifically from their anterior lesions) after exhumation at progressively increasing PBIs, or after a short interval in the refrigerated mortuary chamber in the case of GW0. The latter, although never buried, exhibited a reduced number of particles *characteristic of IGSR* on the entrance hole and none on the exit hole. This outcome may be related to the transport and freezing procedures carried out in the mortuary before the external examination. At the time of sampling, the skin surrounding the lesions appeared moist and blood-stained, conditions that may have facilitated the leaching or removal of IGSR particles.

In contrast, the results concerning the other particle categories were consistent with those obtained from the posterior lesion, showing no significant changes in their distribution or abundance. Before analyzing the samples collected from the buried subjects, particles present in the surrounding soil were examined to exclude the possibility of contamination or misinterpretation of soil-derived particles as IGSR. The soil from the experimental area fortunately produced particles whose composition and morphology were clearly distinct from those of firearm discharge residues, thereby allowing them to be reliably excluded during lesion analysis.

Overall, a higher number of particles was detected on entrance holes than on exit holes, with the exception of subject GW5, in whom the opposite pattern was observed. Notably, no particles

composed of Pb-Ba-Sb were detected in any subject. Particles classified as *consistent with IGSR* were identified only on the entrance hole of subject GW1 (buried for 18 days), amounting to a total of 11 particles composed of Pb and Ba, occasionally in association with Fe or Cu. Regarding particles classified as *commonly associated with IGSR*, their distribution between entrance and exit holes appeared to follow a time-dependent pattern. In subject GW1, these particles were found exclusively on the entrance hole and consisted of Ba or Pb associated with Ir.

In subjects GW2 and GW4, by contrast, these particles were more evenly distributed between entrance and exit holes and were composed of Ba, Sb, and Pb, with Pb frequently associated with Ir. Subject GW5 differed from the others in that such particles were present only on the exit hole, consisting of Ba, Pb, or Pb associated with Ir. This pattern is difficult to interpret and may be influenced by various environmental variables that are challenging to monitor, including subsurface humidity, soil pH modified by decomposition processes, and other post-burial conditions. From the standpoint of cadaveric condition, it is notable that subjects GW4 and GW5 exhibited fully skeletonized skulls at the time of exhumation. This state may have facilitated access to particles that would ordinarily remain embedded within bone tissue following the projectile's passage (Berryman et al., 2010) and that, in the presence of soft tissues, it would have been more difficult to recover using swabbing techniques. Indeed, one of the swabs taken from the exit wound of GW5 inadvertently dislodged a small bone fragment bearing a groove (presumably produced by the bullet's trajectory) within which a metallic particle composed of Fe-Cr, and therefore identifiable as stainless steel, was detected (Figure 5.6). An alternative explanation is that gunshot-related lesions became more readily identifiable once the skulls were fully skeletonized, in contrast to earlier PBIs, when such injuries could only be approximated macroscopically. In either case, these findings highlight the value of SEM-EDX analysis for detecting IGSR in the recognition of gunshot trauma, particularly when such injuries are suspected but their macroscopic identification is impeded by advanced decomposition.

These results show only partial agreement with those reported by Nguyen-Hong et al. (2024) for samples buried for 60 days, which are broadly comparable to subject GW2. In their study, the authors found a comparable number of particles on entrance and exit holes, with particles classified as *characteristic of IGSR* detected exclusively on the entrance hole. By contrast, in the present study such particles were absent, as were those classified as *consistent with IGSR*, which were instead well represented in Nguyen-Hong et al.'s samples. Furthermore, while

Nguyen-Hong et al. reported the presence of Pb-only particles on the exit hole, the present work also identified a Ba-containing particle in that location. Nonetheless, an overall greater persistence (though not an exclusive one) of Pb-based particles with increasing PBI was observed here, consistent with the trends described by Nguyen-Hong et al. (2024).

Apparent Loss of IGSR Over Time: A Possible Environmental Explanation

The progressive reduction and eventual disappearance of particles on the entrance holes may be attributable to leaching or weathering processes acting on metallic fragments. Because all subjects were buried on their right side, the entrance holes were more exposed both to atmospheric agents capable of infiltrating the burial environment and to the physicochemical properties of the surrounding soil. This specific dynamic has not yet been explicitly examined in the scientific literature. Nonetheless, several studies have investigated the environmental behavior of metals in shooting range soils, with particular attention to contamination levels and the chemical transformations undergone by metallic components.

For example, Black et al. (2021) reported that IGSR particles tend to accumulate within soil, where they interact with mineral components and organic matter. Over time, these particles may undergo oxidation, dissolution, and complexation processes. Such reactions particularly affect Pb and Sb: Pb may transform into carbonates (cerussite, PbCO_3), sulphates (anglesite, PbSO_4), or phosphates (pyromorphite, $\text{Pb}_5(\text{PO}_4)_3\text{OH}$), whereas Sb may shift from its metallic state to oxides (Sb_2O_3) or to soluble species such as $\text{Sb}(\text{OH})_6^-$ (Sanderson et al., 2018). Leaching processes may further contribute to particle removal by transporting Pb^{2+} and Sb^{3+} ions into deeper soil layers or into groundwater, especially in acidic or organic-rich soils. Overall, according to the authors, Sb is more mobile and more soluble than Pb, the latter tending instead to precipitate and remain bound to carbonate or phosphate phases within the soil matrix (Black et al., 2021). This behavior would account for the greater persistence of Pb-based particles at higher PBIs compared with Sb.

Finally, particles classified as *other IGSR* were also taken into consideration as they were found in considerable abundance on the lesions of the buried piglets, with the exception of subject GW2. From a taphonomic standpoint, GW2 represented the least well-preserved individual, being in an advanced liquefactive stage of decomposition, which made precise localization of the gunshot lesion difficult. Nevertheless, all subjects exhibited particles composed of Cu and Zn (previously associated with brass cartridge case or jacket fragmentation) as well as Fe-Cr-

Ni, indicative of stainless steel (Dalby et al., 2010; Charles et al., 2023), and Fe. All of these particles were present in quantities comparable to, or even exceeding, those observed on the same subjects' lesions prior to burial. According to Sanderson et al. (2018), Cu and Zn exhibit greater mobility in acidic environments, such as the soils of the experimental area.

However, decomposition involves a sequence of biochemical processes, including proteolysis, that result in the release of NH_4^+ ions and, consequently, an increase in soil pH (Carter et al., 2007; Benninger et al., 2008; Vass, 2017). This shift could favor the persistence of IGSR-related particles composed of these two elements. A similar interpretative framework may also account for the presence of particles composed of Fe and Fe-Cr-Ni. In alkaline soils, Fe typically precipitates into insoluble hydroxides or oxides and may further transform into stable mineral phases such as hematite (Fe_2O_3) and magnetite (Fe_3O_4), resulting in its immobilization (Zang et al., 2023). Likewise, under basic conditions Ni may form $\text{Ni}(\text{OH})_2$ or adhere to soil particles through adsorption processes, which similarly restrict its mobility (Rinklebe & Shaheen, 2017). By contrast, Cr generally displays increased mobility in acidic environments (Liang et al., 2021).

When particles composed of Fe-Cr or Fe-Cr-Ni are interpreted as fragments derived from stainless-steel components, it is also necessary to consider the alloy's internal microstructure. In these materials, Fe provides the metallic matrix forming the structural backbone of the alloy, whereas Cr and Ni are incorporated within its crystalline lattice as dissolved elements (Klaver et al., 2012). This structural arrangement may therefore help explain their comparatively reduced mobility within the burial environment. Although these hypotheses require further validation specifically within a forensic framework, the present study demonstrates that IGSR particles not derived from the primer can indeed persist in association with lesions for more than a year postmortem. This observation suggests that such particles may serve as indicative evidence of gunshot-related trauma even in remains exhibiting advanced decomposition. While the possibility cannot be excluded that brass or stainless-steel fragments originate from alternative sources or different weapon types (Gibelli et al., 2012), many of the particles detected in this study were identified by SEM-EDX as exhibiting a spherical morphology, including some composed exclusively of Fe, a feature strongly consistent with formation during a firearm discharge event.

5.3. Conclusions & Future Perspectives

The PhD project *Ticino 2023* presented in this thesis proposed an interdisciplinary examination of the taphonomic, biogeochemical, and environmental processes governing the interaction between a buried cadaver and the context here conceptualized as the Hypogeal Cadaver Decomposition Island (HCDI). By integrating macroscopic decomposition analysis with soil biogeochemistry, through the observation of decomposition-related anomalies such as Dissolved Organic Matter (DOM), volatile (VFA) and non-volatile organic acid profiles, pH variation, and the magnetic response of the matrix, this work sought to evaluate whether the hypogeal environment can serve as a reliable source of time-dependent information for postmortem and post-burial interval (PMI/PBI) estimation, and to explore how different concealment strategies may influence these relationships.

The outcomes of this research underscored both the complexity and the diagnostic potential of the hypogeal context, suggesting that burial is not a passive depositional environment but rather a dynamic biochemical system shaped by the combined effects of cadaveric decay, soil properties, and anthropogenic disturbance.

One of the main objectives of this project was to assess the capacity of the HCDI to record, retain, and reflect the biochemical signals produced by cadaveric decomposition. The results indicate that, despite the coarse texture, rapid drainage, and low natural retention capacity of the soil in the experimental area, decomposition-related anomalies were detectable across multiple analytical approaches. This is a non-trivial finding: sandy or gravel-rich soils are often assumed to rapidly leach decomposition products, thereby limiting their usefulness in forensic investigations.

In this study, however, the anomalies observed appeared to reflect both intrinsic soil processes and anthropogenic modifications, particularly the substantial soil mixing caused by excavation and backfilling. The mixing of horizons during burial preparation and closure markedly altered the vertical distribution of natural organic carbon, ultimately obscuring any predictable TOC pattern that might otherwise be attributable to decomposition. This outcome is consistent with previous research indicating that total organic carbon is an unreliable and often uninformative marker within CDIs, especially over short to medium timescales. The present experiment therefore further supports the view that TOC is too coarse a metric to function as a temporal indicator of burial-associated biogeochemical changes.

In contrast, DOM and its molecular signatures, detected through FT-IR spectrometry, proved to be considerably more responsive to decomposition and more sensitive to variations across PBIs, concealment treatments, and burial conditions. DOM concentration followed a broadly consistent temporal pattern in three of the four groups, peaking during intermediate PBIs (3 and 4) and declining as decomposition progressed toward skeletonization. Moreover, FT-IR spectrometry allowed the identification of specific molecular classes, including fatty acids, amides, protein degradation products, and adipocere-related compounds, whose presence and evolution paralleled the decomposition trajectories documented macroscopically. These findings support the hypothesis that DOM could potentially represent the most diagnostically informative fraction of soil organic matter within the HCDI and may constitute one of the most promising chemical tools for PMI/PBI estimation in burial contexts.

Equally significant were the pH trends observed within the HCDIs: an initial rise in pH, associated with ammonification and active decay, was followed by a gradual decrease as decomposition progressed and ammonium ions were depleted. This behavior mirrors classical CDI models and suggests that pH may serve as a rapid and informative first-screening indicator of burial activity. However, in graves treated with quicklime, the pH followed a markedly different alkaline trajectory, underscoring that concealment materials can mask, or even entirely override, biochemical signals that would otherwise be characteristic of decomposition.

The analysis of VFAs and selected non-volatile organic acids was designed to evaluate their suitability as biomarkers within a hypogeal microenvironment. The results indicate that VFAs do not exhibit clear time-dependent trends and therefore cannot be considered reliable standalone PMI estimators. Rather, their value appears to lie in confirming the presence of cadaveric fluids within the HCDI, even when the body is no longer actively releasing leachate, or may no longer be present at all. Propionic, butyric, and isovaleric acids showed sporadic yet meaningful peaks corresponding to periods of intense decomposition, whereas lactic and succinic acids appeared irregularly and showed no linear correspondence with macroscopic preservation states. Their occurrence may have been strongly influenced by external factors, including intense rainfall, soil retention capacity, late insect activity, and concealment strategies. Nevertheless, the detection of exceptionally high concentrations of VFAs in certain burials (notably GC2) suggests that, under specific conditions, organic acids can sharply capture pulses of cadaveric fluid release. Such pulses may hold contextual value, pointing to episodes of heightened microbial activity or to failures of materials intended to constrain decomposition fluids (e.g., clothing or lime). Taken together, these findings indicate that VFAs are

diagnostically useful for determining whether a decomposing body was present and active within a given soil volume, but they are poorly suited for temporal modeling unless integrated with additional markers.

Magnetic susceptibility (MS) and hysteresis analyses offered a complementary, non-destructive perspective on soil modification during burial, as observed on GC group. Contrary to observations from surface prospection studies, MS did not increase in the presence of the cadaver but instead slightly decreased relative to undisturbed control soils. This behavior aligns with models describing the dissolution of ferrimagnetic minerals, especially magnetite, under microenvironments rich in reactive organic matter and dominated by reductive microbial processes. The HCDI samples exhibited more homogeneous grain-size distributions, thinner and more coherent hysteresis loops, and magnetic responses consistent with single-domain or pseudo-single-domain magnetite, whereas control soils displayed greater heterogeneity and multi-domain behavior. These findings suggest that burial and the localized microbially mediated diagenesis triggered by decomposition can create a distinct magnetic response. Although MS resulted uncorrelated to PBI or decomposition stages, it proved sensitive to anthropogenic disturbance and organic matter decomposition, supporting its forensic value for detecting burial sites, reconstructing burial modifications, and identifying post-depositional disturbance. Its greatest utility would emerge when coupled with geochemical and stratigraphic indicators rather than as an isolated diagnostic tool.

From the perspective of the other substrate, namely the cadaver, one of the notable outcomes of this research was the observation that concealment methods can actively influence the taphonomic trajectory of buried remains. Quicklime, clothing, and the presence of wounds appeared to modify decomposition dynamics, at times accelerating, delaying, or otherwise altering tissue transformation in ways that may affect forensic interpretation.

In particular, the quicklime used in the GQ group produced marked early desiccation of the external tissues, which could give the misleading impression of a more advanced stage of decomposition. Internally, however, the tissues remained unexpectedly well preserved for more than 100 days, challenging the common assumption that lime accelerates decay. At later stages, lime appeared to contribute to longer-term preservation(especially of mummified skin) through the formation of a protective outer layer. This dual behavior (initial desiccation followed by sustained preservation) could indicate that quicklime has the potential to lead to both over- and under-estimation of PMI if its effects are not explicitly taken into account. Additionally, tissues

preserved under these conditions may offer valuable forensic information, such as supporting victim identification (e.g., when fingerprints remain intact) or enabling interpretation of lesions or pathological changes that would otherwise be lost.

Cotton garments, as observed in the GC group, appeared to exert variable effects on decomposition. In some cases, clothing seemed to restrict fluid dispersion enough to promote corification; in others, it absorbed substantial amounts of decomposition fluids, acting as a temporary reservoir that influenced the timing and extent of molecular diffusion into the surrounding soil. The associated FT-IR signatures (including cellulose-derived degradation products consistent with the breakdown of natural-fiber textiles) together with the observed increases in decomposition-related biomarkers, suggest that clothing can influence both DOM composition and the detectability of specific molecular signals.

Subjects from GW group, produced distinctive soil signatures, including early expression of amide III-associated FT-IR bands, which likely derived from initial blood infiltration into the HC DI. However, wounds did not appear to accelerate decomposition under burial conditions, reinforcing the idea that burial acts as an entomological barrier preventing the accelerated decay sometimes observed in surface contexts. Moreover, the taphonomic alterations resulting from burial are capable of disrupting and often obliterating lesions affecting the tissues that would otherwise retain diagnostic features, such as gunshot wounds. In such cases, an anthropological investigation of the appearance of the lesions on the bone tissue, correlated with a chemical analysis of gunshot residues, would represent the keystone for an accurate reconstruction of events.

Overall, across the experimental groups, concealment strategies appeared to exert a stronger influence on the trajectory of decomposition than the PBI itself, suggesting that PMI estimation should take these variables into account in order to reduce the risk of misinterpretation. The results of this PhD project also emphasize the potential value of adopting integrated analytical frameworks in forensic taphonomy, particularly when investigating clandestine burials, where concealment materials or environmental factors may obscure macroscopic evidence. In this context, the combined consideration of taphonomic, biogeochemical, and physical evidence may contribute to a more comprehensive understanding of burial decomposition processes than could be achieved by relying on a single analytical approach.

Several overarching conclusions arise from this multidimensional dataset, bearing direct relevance for forensic investigations involving buried remains:

- HCDI is a dynamic environment, strongly influenced by soil chemical, physical, and microbiological characteristics that should hence be considered during investigations.
- DOM and FT-IR characterization emerged as the most promising indicators of cadaver-derived inputs and a very reliable indicator of temporal biochemical evolution, independently of the concealment method considered. This technique could truly help in unraveling the role of organic carbon from HCDI. In this sense, soil chemical analysis should prioritize DOM, not TOC, to capture time-sensitive markers of decomposition.
- pH constitutes a rapid, inexpensive, and reliable first-line indicator of decomposition activity, except when concealment materials override natural trends. Therefore, pH measurement should be one of the first tests performed when assessing potential clandestine burials.
- VFAs and other organic compounds such as lactic and succinic acids are useful presence/absence biomarkers but lack temporal resolution unless integrated with other measures. However, their potential should be investigated further.
- Magnetic susceptibility can detect burial disturbance and microbially mediated mineralogical changes, though not decomposition stage. Therefore, magnetic methods can aid in identifying disturbed ground, especially when paired with stratigraphic or biogeochemical assessments.
- Concealment methods must be considered primary taphonomic agents, capable of fully altering expected decomposition trajectories and soil biogeochemical signals. In particular:
 - Quicklime does not accelerate decomposition and, on the contrary, may preserve tissues, meaning trauma, lesions, and soft-tissue features may remain identifiable long after expected.
 - Clothing may complicate PMI estimation, either by promoting conservative decomposition processes or altering soil biogeochemistry.
 - Burial can exacerbate the already high destructive potential of decompositional processes affecting tissues involved by lesions. The project demonstrated that the integration of anthropological and chemical analyses (via SEM-EDX) on gunshot

wounds was able to obtain a complete informational profile of the dynamics. A synergistic interdisciplinary approach should therefore always be envisaged in forensic practice.

With regard to these latter points, this research opens multiple avenues for refinement and advancement. Quicklime, in fact, should be considered not only as a taphonomic agent capable of disrupting the natural succession of decompositional processes, but also as a substrate that, due to its hygroscopic nature, can become informative. The FT-IR spectroscopic analyses conducted in this experiment were able to monitor the hydration processes that allowed quicklime (CaO) to transform into calcium hydroxide Ca(OH)_2 with increasing PBI. This could represent a starting point for understanding which and how many biomarkers present in putrefactive fluids are actually absorbed by the substance and how they interact with it.

Similarly, clothing should be considered a substrate capable of absorbing putrefactive fluids and, in doing so, potentially preserving information relevant to concealment dynamics. Although such lines of inquiry have been explored in some experimental forensic studies, research specifically examining the interaction between textile fibers, decomposition products, and the HCDI remains limited and would benefit from further systematic investigation. In particular, controlled studies comparing different textile types and burial coverings could help clarify how various materials influence decomposition processes and the preservation or alteration of associated biochemical markers.

As for the multidisciplinary investigation of lesions present on buried cadavers in an advanced state of decomposition, a similar approach will need to be applied to the other wounds inflicted on the piglets of the GW group (blunt-force and sharp-force trauma). In this way, it would be possible to expand both the catalogue of taphonomic alterations affecting lesions and the catalogue of metallic particles associated with specific weapons, assessing their characteristics and distribution.

The biogeochemical analyses conducted on the HCDI also suggest several directions for future research. In particular, the results indicate that the analysis of DOM deserves further targeted investigation, with the aim of validating the method and, in the long term, evaluating the relative abundance of this marker with respect to the natural background conditions of the investigation site over time. Such an approach could potentially provide an additional tool to support PMI estimation within forensic investigations, in conjunction with the other disciplines considered in the present project.

Furthermore, long-term monitoring of DOM and FT-IR signals may help clarify their behavior across multiple seasonal cycles and at more advanced stages of decomposition. This consideration likely applies to all the analytical approaches presented in this study.

To conclude, it seems reasonable to state that the task of any scientist working in taphonomy is to make use of all available tools to interpret the phenomena that can be observed. At the same time, it is essential to remember that this field requires monitoring the interaction between two inherently dynamic systems: a decomposing body and the natural environment in which it is concealed. Each system is governed by a complex network of bio(geo)chemical reactions driven by intrinsic and extrinsic processes, and these reactions inevitably intersect once the two substrates come into contact. As a result, they merge their respective dynamics, generating new patterns and signals that may be difficult to attribute exclusively to one source or the other. This challenge is exemplified by decomposing organic matter, where both plant- and animal-derived inputs can produce similar compounds. The most promising way to approach this puzzle is to deepen our understanding of each system independently and then focus on identifying the anomalous or emergent features that arise specifically from their interaction.

The instrument to achieve all this lies in interdisciplinarity and in collaboration among experts from different fields, with the purpose of ensuring justice.

– CHAPTER 6 –

SIDE PROJECT

THE CRYPT OF THE CA' GRANDA HOSPITAL

During the three years of doctoral research, part of the project was devoted to the investigation of the burial ground (*Sepolcreto*) located beneath the Crypt of the Church of the *Beata Vergine Annunciata* of the ancient *Ca' Granda* Hospital in Milan (Italy). Founded in 1456 by Francesco Sforza, the *Ospedale Maggiore* was conceived as both a medical and charitable institution that symbolized the city's humanistic ideals (Albini, 1982; Cosmacini, 1999). Within the church, the seventeenth-century crypt (built shortly after the plague) served as a burial site for patients who died in the hospital and was used for about seventy years before being abandoned due to the unhealthy accumulation of decomposing remains (Mattia, 2021). It was later reused as a patriotic burial ground in 1848 during the *Cinque Giornate di Milano*, only to fall back into neglect until its restoration in 2013 (Carlessi & Kluzer, 2015).

Architecturally, the *Sepolcreto* consists of 14 barrel-vaulted chambers (Figure 6.1; nine of which were found still containing human remains), connected to a system of drains intended for the discharge of decomposition fluids. Centuries of reuse created a complex stratigraphy of commingled and articulated bones, reflecting a long funerary tradition (Cattaneo et al., 2013) that, at the outset of the investigations, suggested the burial might have been of secondary nature. However, during more recent archaeological campaigns, stratigraphic excavation was applied for the first time, enabling the identification of clear depositional layers and several individuals preserved in anatomical connection, thereby supporting the interpretation of the *Sepolcreto* as a primary burial site. Following this development, it became possible to design a multidisciplinary research project aimed at retrieving information that could contribute to reconstructing this significant chapter in the history of the city of Milan and its inhabitants, through the study of the individuals interred in the burial ground (Mattia et al., 2022).

Documentation included 3D surveying, detailed taphonomic recording, and systematic cataloguing of remains in digital databases such as CORA, established by the Defense POW/MIA Accounting Agency (USA) for the management and curation of skeletal remains. Anthropological investigation involved over 2600 skeletal elements divided into two major research campaigns (Cattaneo et al., 2018; Mattia, 2021). Results revealed a population of adults of both sexes, many showing occupational stress markers, degenerative joint changes,

and evidence of chronic illness or trauma (Biehler-Gomez et al., 2021). Moreover, the examination of dentition highlighted heavy wear, caries, and abundant dental calculus, from which microresidues of plant starch and mineral particles were extracted, suggesting a modest diet (Mattia et al., 2022). Archaeobotanical studies also identified layers of soil rich in organic residues, together with pollen and phytoliths consistent with cereals, legumes, and herbs used for both food and healing (Caccia & Caccianiga, 2021).

Complementary analyses enriched the picture: toxicological testing detected the presence of mercury and lead, indicative of early modern medical treatments involving metallic compounds (Biehler-Gomez et al., 2022), while forensic toxicological analyses revealed the use of cannabis (Giordano et al., 2023) and coca plant (Giordano et al., 2024) already in the 1600s in Milan.

Moreover, histological and biomolecular approaches allowed the recovery of well-preserved soft tissue structures, while DNA analyses of teeth and dental calculus yielded partial mitochondrial profiles consistent with the genetic diversity of historical Milanese populations (Olivieri, 2021).

Through the integration of archaeology, anthropology, toxicology, natural sciences, and genetics, the study reconstructed four centuries of Milan's history from the perspective of its humblest inhabitants (Mattia, 2022). The *Sepolcreto* of the *Ca' Granda* thus becomes an extraordinary biocultural archive, preserving the physical traces of social inequality, medical experimentation, and urban life (Mattia, 2021). This interdisciplinary approach not only elucidates burial practices and health conditions of the early modern period but also proposes a methodological model for the scientific study of complex funerary contexts. In doing so, it restores to the forgotten dead of Milan a tangible identity and situates the *Ca' Granda* as a monument to both science and humanity (Mattia, 2022).

With regard to the study of the matrix, the hypothetical secondary nature of the burial could have justified the presence of allochthonous soil within the chambers, the provenance of which would have required investigation. However, the effective recognition of the depositional environment as primary made its characterization even more challenging. For this reason, a typically forensic approach to soil analysis was adopted for situations in which no comparison samples are available: compositional analysis by SEM-EDX, aimed at identifying sufficiently distinctive features to allow geographic attribution. Nonetheless, such investigation ultimately enabled the definition of a new soil category, the characteristics of which will be examined in greater detail in Section 6.1.

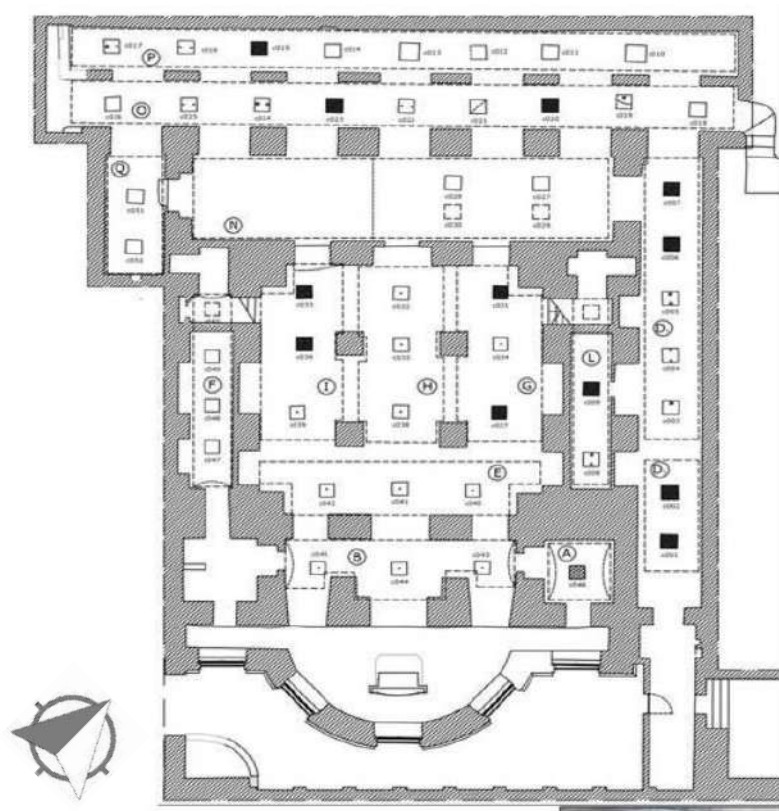


Figure 6.1: Plan of the Crypt of the Chiesa della Beata Vergine Annunziata (Image from Carlessi, Kluzer 2011).

6.1. *Thanatogenic Anthrosols: a Geoforensic Approach to the Exploration of the Sepolcreto of Ca' Granda (Milan) published in AAPP, Class of Physical, Mathematical and Natural Sciences Journal*

This manuscript, published in *AAPP, Class of Physical, Mathematical and Natural Sciences* 2023, 101(Suppl. 1), 1-21, is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Authors: Giulia Tagliabue (corresponding), Anna Masseroli, Mirko Mattia, Carlotta Sala, Elena Belgiovine, Daniele Capuzzo, Paolo Maria Galimberti, Fabrizio Slavazzi, Cristina Cattaneo and Luca Trombino.

DOI: <https://dx.doi.org/10.1478/AAPP.101S1A8>

Link: <https://www.mdpi.com/2076-3263/14/12/359>

The manuscript presents an innovative geoforensic investigation of burial soils within the seventeenth-century crypt of the *Ospedale Maggiore* in Milan. The study focuses on the mutual chemical interaction between human remains and the surrounding matrix, using Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM-EDX) to examine bone samples and matrix collected from chamber “O” of the burial ground. The results demonstrate a bidirectional exchange of elements, such as calcium, phosphorus, sulfur, nitrogen, and trace metals, between bone tissue and the burial substrate, reflecting long-term decomposition and anthropogenic influences. Moreover, the analyses allowed to identify an unprecedented material primarily composed of organic matter derived from human decomposition, intermixed with residual bone and soft tissues. To classify the substrate, we propose the new soil category of *Thanatogenic Anthrosols* defined as an anthropogenic soil formed through funerary activity in built, confined environments such as crypts and burial chambers. This concept extends existing soil taxonomies by acknowledging death-related processes as a formative factor in soil genesis. The study also reconstructs environmental and historical factors influencing the *Sepolcreto*, including the WWII bombing that altered its microstratigraphy, thus integrating archaeological, geochemical, and forensic perspectives to deepen understanding of the interplay between human remains and their burial environment.

**THANATOGENIC ANTHROSOLS:
A GEOFORENSIC APPROACH TO THE EXPLORATION
OF THE SEPOLCRETO OF THE CA'GRANDA (MILAN)**

GIULIA TAGLIABUE ^{ab*}, ANNA MASSEROLI ^a, MIRKO MATTIA ^b, CARLOTTA SALA ^{bc},
ELENA BELGIOVINE ^d, DANIELE CAPUZZO ^d, PAOLO MARIA GALIMBERTI ^e,
FABRIZIO SLAVAZZI ^f, CRISTINA CATTANEO ^b AND LUCA TROMBINO ^a

ABSTRACT. Soil is a dynamic matrix which can rapidly respond to disturbance events, such as the death and the deposition of an organism. Moreover, soil can be considered an archive of data due to its ability to record the traces of disturbance events. Accordingly, the biogeochemical analysis of the geopedological evidence could turn into a valuable tool for the study of decomposition processes. Hence, the aim of the present research is to detect the evidence of material exchange, linked to decomposition, between the bone tissue from two skeletal remains of the *Sepolcreto* (i.e., burial ground) in the crypt of the *Ca' Granda* (Milan, Italy), and the pedosedimentary matrix from the stratigraphic unit US3 in which they were immersed. Both biological and geopedological specimens were analysed using a Scanning Electron Microscope equipped with facility for energy-dispersive spectroscopy (SEM-EDS), which pointed out the presence of a mutual exchange of material between the two substrates, underlying the intensity of the interaction between the organisms (even whether dead) and the environment in which they are located. The results led to the detection of an inedited kind of material, mainly composed of organic matter resulted from the decomposition of human remains, and preserved bone and soft tissues, which could be considered for introducing a new type of Anthrosol.

1. Introduction

The death of an organism and its eventual deposition could be a disruptive influence on the surrounding environment, making the analytical techniques typical of geosciences a valid investigation tool when applied to the forensic field (Pye and Croft 2004; Ritz, Dawson, and Miller 2009; Fitzpatrick and Donnelly 2021; Tagliabue *et al.* 2023). Indeed, several geological and mineralogical analysis can contribute to solve forensic cases, such as X-ray diffraction analysis (Ruffell and Wiltshire 2004; Piga *et al.* 2009), infrared and Raman spectroscopy (Jehlka 2012; Ortiz-Herrero *et al.* 2021; Gatta, Mantovani, and Bromiley 2023), gas-chromatography (Brasseur *et al.* 2012; Mazzetto *et al.* 2019), chemical, microscopic and ultramicroscopic analysis (Graves 1979; Melo *et al.* 2008; Fitzpatrick and Raven 2012; Zangarini, Trombino, and Cattaneo 2016; Tagliabue *et al.* 2023). About the

latter, electron microscopy is frequently used in geosciences to examine the microscopic particles and, especially in the forensic field, the most common electron microscope is the SEM (Scanning Electron Microscope), often equipped with the facility for Energy-Dispersive Spectroscopy (EDS). Indeed, this analytical instrument can provide the chemical information needed for the identification of different microscopic objects, as well as their shapes and habits, which can be diagnostic (Dawson *et al.* 2008). As a matter of fact, the correct detection of the objects that can be found in soil, such as fossils (Tanaka and Local 1999), soil minerals (Petrosino, De Gennaro, and Mondillo 2019), pollen, spores (Jones and Bryant 2007), diatoms (Scott *et al.* 2014) and many more, can contribute to provide a reliable overview about the peculiar properties of a suspected material, which could be the object of a forensic investigation (Fitzpatrick 2008). Moreover, soil could also be detected as an archive of information, able to register the dynamic of the events (Young *et al.* 2014; Martín and Nanos 2016; Sangwan *et al.* 2020), as in the case of clandestine graves (Fiedler and Graw 2003; Tagliabue, Crespi, and Trombino 2021). Indeed, the decomposition of a corpse turns into a huge source of nutrients that are rapidly available for soil microorganisms (Vass *et al.* 1992), and therefore it alters the biochemical balance of the substrate. When the material from the cadaver enters gravesoil, it creates a concentrated area of fertility called Cadaver Decomposition Island (CDI) (Carter, Yellowlees, and Tibbett 2007), as a consequence of the decomposition of proteins, carbohydrates and lipids (Tortora and Grabowski 2000), which yield nitrogen, phosphorus, sulphur and carbon-based products that could be retained in soil (Benninger, Carter, and Forbes 2008). Specifically, proteins are reduced to compounds such as biogenic amines, namely putrescine and cadaverine, which can be accompanied by the emission of gases like methane and carbon dioxide (Ioan *et al.* 2017), as well as secondary products of the degradation of amino acids, containing sulphur atoms reduced to form ammonia (NH_3), hydrogen sulphide (H_2S) and sulphides (Forbes 2008). The latter are generally favoured by the anaerobic conditions that characterise the microenvironment of a burial soil (Fitzpatrick 2009), which is also acidic and, therefore, able to convert the ammonia in ammonium (NH_4^+) (Ioan *et al.* 2017). Conversely, the *post-mortem* decomposition of carbohydrates can lead to the conversion of glycogen to glucose monomers, that will be finally oxidised, forming carbon dioxide (CO_2), water (Dent, Forbes, and Stuart 2004; Forbes 2008) and several organic acids (Ioan *et al.* 2017), further contributing to the acidification of soil (Tibbett and Carter 2008). Finally, lipids can be decomposed in hydrocarbons, phosphorous, nitrogen and oxygenated compounds (Statheropoulos, Spiliopoulou, and Agapiou 2005), along with organic acids and volatile fatty acids (Forbes 2008). Finally, the decomposition process is ultimately mediated by the physicochemical (Junkins and Carter 2017) and textural (Tumer *et al.* 2013) characteristics of the gravesoil which selectively influences the decomposer community that has access to the remains (Finley *et al.* 2016). Furthermore, several other environmental parameters can affect the dynamics between the human remains and the burial environment: temperature (Von Lützwow and Kögel-Knabner 2009), moisture (Carter, Yellowlees, and Tibbett 2010), pH (Petřík *et al.* 2012) and anthropic disturbance (Capra *et al.* 2015) are just few of the numerous factors that influence the rate and evolution of the decomposition processes (Junkins and Carter 2017), making every burial site intrinsically unique.

For these reasons, the present research aimed to the cross-analysis of the human remains and the pedosedimentary matrix in which they were enclosed, from one of the 14

underground chambers of the *Sepolcreto* of the *Ca' Granda*, the ancient hospital of Milan, Italy (XVII century). Considering the uniqueness of the site, also due to the extremely prolonged contact between the two substrates, which lasted almost 400 years, this study consisted in the application of techniques typical of geoforensic sciences on the analysis of two representative bone fragments and as many samples of the matrix. The specimens were investigated by mean of a SEM-EDS, in order to identify, from an ultramicroscopic point of view, the mutual influence between the bone tissue and the surrounding matrix, with an emphasis on the noticeable alterations in the latter.

2. Materials and Methods

2.1. Study area. The *Sepolcreto* of the *Ca' Granda* (Figure 1) is a burial ground situated below the crypt of the church of *The Beata Vergine Annunciata*, the main place of worship of the ancient *Ospedale Maggiore* of Milano, whose building was commissioned by Francesco Sforza, at the time Duke of Milan. The hospital was operational approximately between 1637 and 1697 (Cosmacini 1999) and hosted a great amount of sick people from the most pauper social classes (Cattaneo and Slavazzi 2021). Therefore, the *Sepolcreto* constituted the only burial ground for the dead from the whole hospital, and it was estimated to contain around 150000 buried individuals (Cosmacini 1999; Sala 2020; Belgiovine and Capuzzo 2021; Cattaneo and Slavazzi 2021; Tagliabue 2022).

Until 2021, the previous archaeological and anthropological investigations provided some undefined results concerning the nature of the funerary depositions. Indeed, since the commingled state of the remains (Figure 2), interpreted as enclosed in a soil matrix hailing from a prior burial site, the *Sepolcreto* was considered an ossuary and, therefore, a secondary deposition (Osterholtz, Baustian, and Martin 2014; Sguazza 2014). Conversely, the most recent archaeological investigation of one of the underground chambers (namely the chamber “O”) was carried out through a preliminary area recognition by means of a photogrammetric image-based survey (Belgiovine and Capuzzo 2021) (Figure 3), followed by an actual stratigraphic survey. This approach led to the discovery of some anthropic conoid accumulations composed of bones and partially connected skeletons right below the square manholes located on the vault (Figure 4), allowing to identify it as a primary burial (Belgiovine and Capuzzo 2021; Tagliabue, Crespi, and Trombino 2021). Hence, the actual origin of the matrix detected inside the chamber “O”, which was renamed “pedosedimentary” due to its uncertain nature, needed to be thoroughly understood.

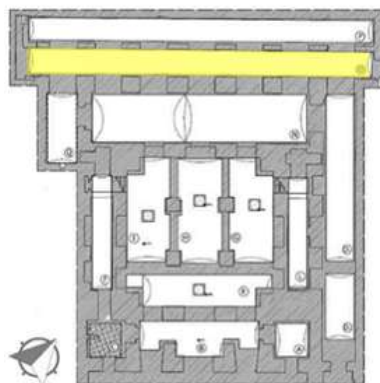


FIGURE 1. Representation of the 14 burial chambers of the *Sepolcreto* of the *Ca' Granda*. The chamber "O" is highlighted in yellow.



FIGURE 2. Commingled remains from a deposit of the chamber "O".



FIGURE 3. Photogrammetric image-based survey of a portion of the deposit of the chamber "O" (Courtesy of Belgiovine and Capuzzo 2021).



FIGURE 4. Anthropic conoid accumulations discovered right below the square manholes located on the vault of chamber “O” (Courtesy of Belgiovine and Capuzzo 2021).

2.2. Materials. The material considered in this study, consisting of samples of pedosedimentary matrix and bone fragments, came from the stratigraphic unit number 3 (US3) detected by the archaeologists (Archeosfera s.r.l. 2021). It was characterised by two different areas: the first one, named US3 SED1, was described as a thin blackish layer, whereas the second one, US3 SED2, was a reddish, sandy loam layer containing well preserved human bones (Archeosfera s.r.l. 2021). The osteologic material and the pedosedimentary matrix were collected undisturbed, as discovered inside the underground chamber, for the purpose of preserving the original contact between the two substrates for as long as possible (Figure 5). About the preparation of the specimens for the analysis, bone samples were reduced to cross-sections through a 2.5 mm thick diamond cut-off wheel, and subsequently included in epoxic resin (Araldite D, 10:1.5). Finally, the sections were reduced to 20-30 μm using a silicon carbide abrasive and sealed by a cover glass (Maat, Van Den Bos, and Aarents 2001). Moreover, a fragment of the surface of each bone sample was collected and analysed. Conversely, the pedosedimentary matrix samples were collected as bulk specimens and did not undergo any kind of preliminary treatment. In order to being analysed, the latter samples were collected on a specimen holder called “stub” by means of a carbon-based conductive glue (Petrosino, De Gennaro, and Mondillo 2019). The list and description of the samples is resumed in Table 1.



FIGURE 5. Example of a sampling from the US3, including bone and matrix samples, collected as discovered inside the chamber.

TABLE 1. Resume of the osteological and pedosedimentary samples collected in the chamber “O”.

Type of sample	Provenience	Sample ID	Description
Matrix	US3 SED1	Mat_SED1	Sandy loam blackish material
Matrix	US3 SED2	Mat_SED2	Sandy loam reddish material
Bone surface	US3 SED1	B_Surf_SED1	Surface fragment of a distal diaphysis of a right humerus (adult). Dark brown with darker stains.
Bone surface	US3 SED2	B_Surf_SED2	Surface fragment of a first left metatarsal bone (adult). Reddish brown.
Bone thin section	US3 SED1	B_Thin_SED1	Thin section from the distal diaphysis of the right humerus.
Bone thin section	US3 SED2	B_Thin_SED2	Thin section from the first metatarsal bone.

2.3. Methods. The analysis of both osteological and pedosedimentary specimens were carried out with a JSM-IT500 LV (Jeol) SEM instrument, imaging both secondary and back-scattered electrons to return a 3D compositional image of the objects detected on the surfaces of the samples (Petrosino, De Gennaro, and Mondillo 2019). Elemental analyses and 2D semiquantitative maps were produced with EDS instrument with an accelerating voltage of 30 kV, requiring a carbon-coated samples (Petrosino, De Gennaro, and Mondillo 2019). The analysed elements were standardized using several single-element standards, whereas elemental concentrations measured by EDS are reported as oxide weights normalized to 100%.

3. Results

3.1. Bone thin sections. The bone thin sections underwent ultramicroscopic analysis through SEM-EDS, which allowed the identification of a certain quantity of allochthonous objects nestled, in the osseous tissue, as well as the presence of biochemical traces on it. In particular, the analysis of the sample B_Thin_SED1 detected a chromatic anomaly between the periosteum and the cortical portion of the bone tissue which resulted as composed of sulphur ($S - 1.82 \pm 0.02$ Mass%), beside abundant phosphorus ($P - 29.71 \pm 0.08$ Mass%) and calcium ($Ca - 35.09 \pm 0.01$ Mass%), the main elements of hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_2$), that is the mineral constituent of bones and teeth (Crowder and Stout 2012) (Figure 6). Conversely, the ultramicroscopic analysis of the thin section B_Thin_SED2 showed an abundant presence of allochthonous objects nestled in the bone tissue and characterised by chemical elements ascribable to minerals, such as silicon ($Si - 38.69 \pm 0.09$ Mass%), calcium ($Ca - 9.81 \pm 0.04$ Mass%), iron ($Fe - 7.98 \pm 0.05$ Mass%), aluminium ($Al - 5.93 \pm 0.04$ Mass%), titanium ($Ti - 1.26 \pm 0.02$ Mass%), sodium ($Na - 0.47 \pm 0.01$ Mass%) and potassium ($K - 0.58 \pm 0.01$ Mass%) (Figure 7). Furthermore, the SEM-EDS

analysis of the same thin section detected a massive presence of lead (Pb – 66.99 ± 0.14 Mass%), often in association with iridium (Ir – 13.29 ± 0.07 Mass%), constituting some objects with a high atomic weight placed in a mineral matrix included in the bone tissue (Figures 8 and 9).

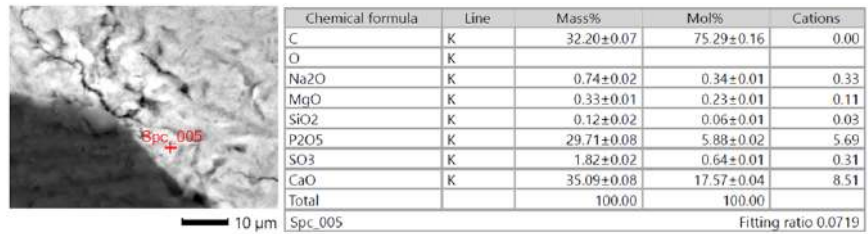


FIGURE 6. Point-like chemical analysis of the chromatic anomaly present on B_Thin_SED1 sample. The instrument highlighted the presence of sulphur (S), beside phosphorus (P) and calcium (Ca).

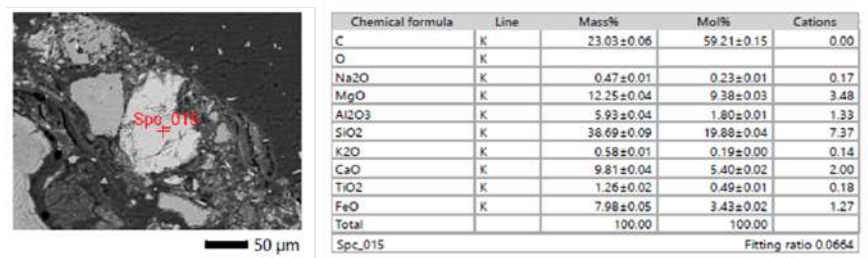


FIGURE 7. Point-like chemical analysis of one of the allochthonous objects nestled in the bone tissue in specimen B_Thin_SED2, mainly composed of elements ascribable to minerals, such as silicon (Si), magnesium (Mg), calcium (Ca), iron (Fe), alluminium (Al), titanium (Ti), sodium (Na) and potassium (K).

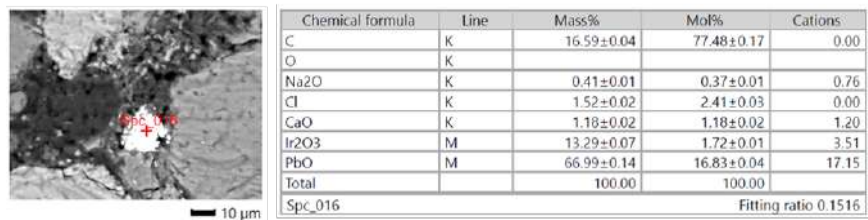


FIGURE 8. Point-like chemical analysis of the object constituted by lead (Pb) and iridium (Ir) present in the sample B_Thin_SED2.

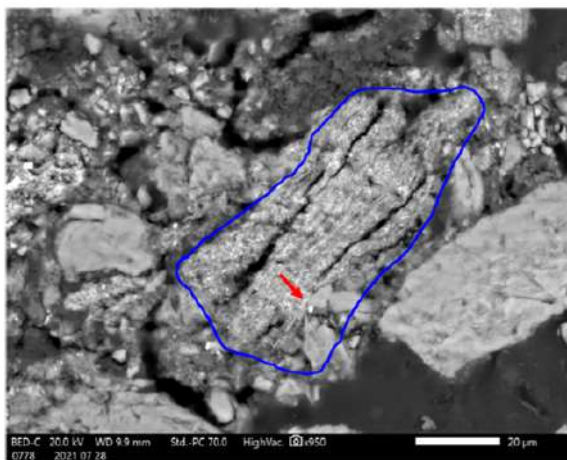


FIGURE 9. Compositional 3D image of the allochthonous object made of lead and iridium (red arrow), included in the mineral matrix (blue line) detected as nestled in the bone tissue of sample B_Thin_SED2.

3.2. Surface sample of bone tissue. The instrumental analysis of the sample B_Surf_SED1 allowed the production of a 2D elemental map of a point-like area (Figure 10). The SEM-EDS testified, beside the normal predominance of P and Ca, the main elements of hydroxylapatite, the presence of N and S, the latter to a lesser degree. The same method was applied for the specimen B_Surf_SED2 which, with the support of a 3D compositional image (Figure 11), detected a several yet localised coat of Pb, whereas the point-like chemical analysis of some high atomic weight allochthonous objects permitted to identify them as constituted of Pb (66.96 ± 0.12 Mass%) and Ir (18.65 ± 0.07 Mass%) (Figure 12).

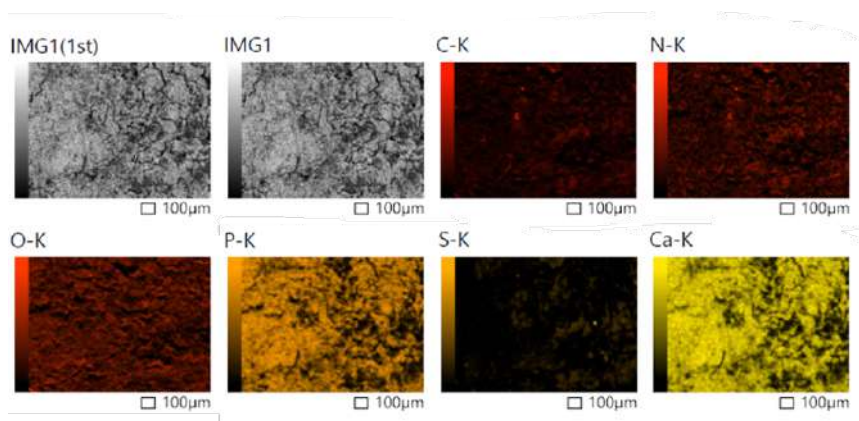


FIGURE 10. 2D elemental map of the surface sample B_Surf_SED1 that highlighted the diffusion of nitrogen (N) and sulphur (S).

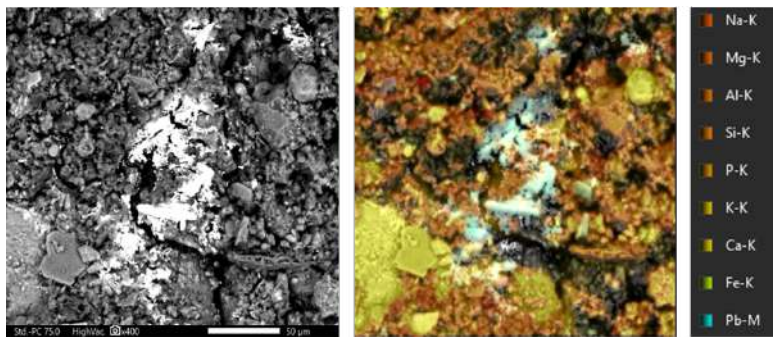


FIGURE 11. SEM-EDS analysis of the surface of the specimen B_Surf_SED2. On the left, a compositional 3-D image of an area of the sample presenting a lead (Pb) cover (400x magnifying). On the right, a superimposed image of the 2D elemental map showing the main chemical elements that characterise the investigated area, including lead (Pb) (400x magnifying).

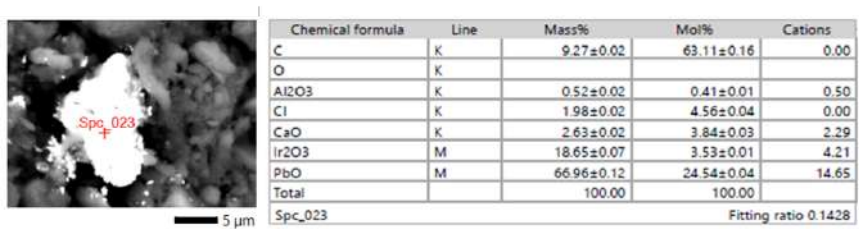


FIGURE 12. Point-like chemical analysis of the allochthonous object composed of lead (Pd) and iridium (Ir) detected on the surface of the sample B_Surf_SED2.

3.3. Pedosedimentary matrix. The elemental map carried out on the sample of pedosedimentary matrix named Mat_SED1 testified an unexpected scarcity of elements of mineral origin, namely Al, Si, K and Fe (Figure 13). Moreover, the same analysis showed an anomalous content of N associated to C and highlighted the presence of microscopic fragments of bones, marked out by the identification of Ca and P, the main elements of hydroxylapatite (Figure 13). The same specimen was analysed and represented, from an ultramicroscopic point of view, through the realisation of a 3D compositional image, whose observation allowed to recognise the presence of soft tissue, surrounded by micro-fragments of bone (Figure 14). Their identification was confirmed by means of an elemental qualitative and semi-quantitative point-like analysis, which underlined the predominance of S (15.11 ± 0.06 Mass%), Ca (9.20 ± 0.05 Mass%) and P (1.38 ± 0.02 Mass%) on the elements of mineral origin, namely Mg (0.40 ± 0.02 Mass%), Al (0.39 ± 0.02 Mass%) and Si (0.77 ± 0.02 Mass%) (Figure 15). Conversely, the 2D elemental map of the sample from the pedosedimentary matrix from US3 SED2 (Mat_SED2) (Figure 16) showed an abundance of bone fragments, identified through the association between Ca and P. Moreover, a noticeable diffusion of chemical elements typical of clay minerals was observed, namely Na, Al, Si,

K and Fe. Furthermore, some irregularly shaped objects with a high atomic weight were detected and recognised as mainly made of Pb (47.70 ± 0.19 Mass%) and Ir (10.02 ± 0.09 Mass%) (Figure 17).

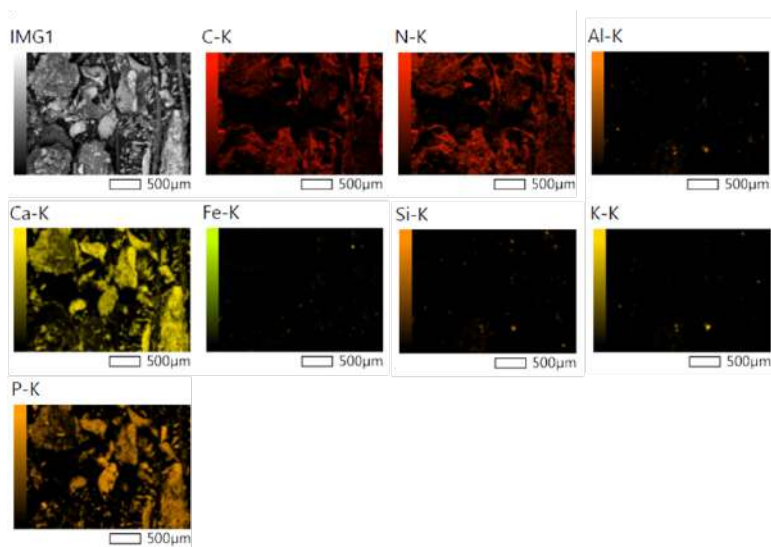


FIGURE 13. 2D elemental map of the pedosedimentary matrix sample Mat_SED1 from the chamber “O” (50x magnifying).

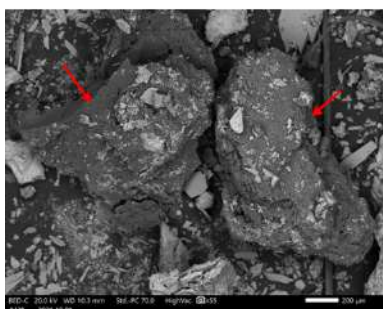


FIGURE 14. 3D compositional image of Mat_SED1. The red arrows point at two fragments of soft tissue surrounded by several microscopic bone fragments.

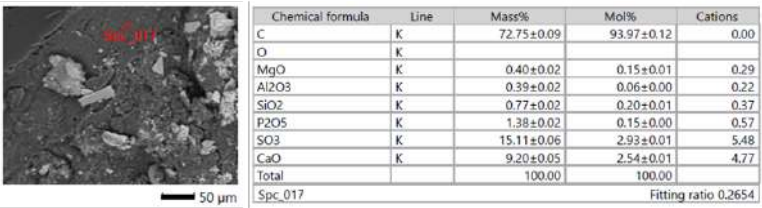


FIGURE 15. Point-like chemical analysis of a fragment of soft tissue in the sample Mat_SED1. The detection of calcium (Ca) and phosphorous (P) also confirm the presence of micro-fragments of bone tissue.

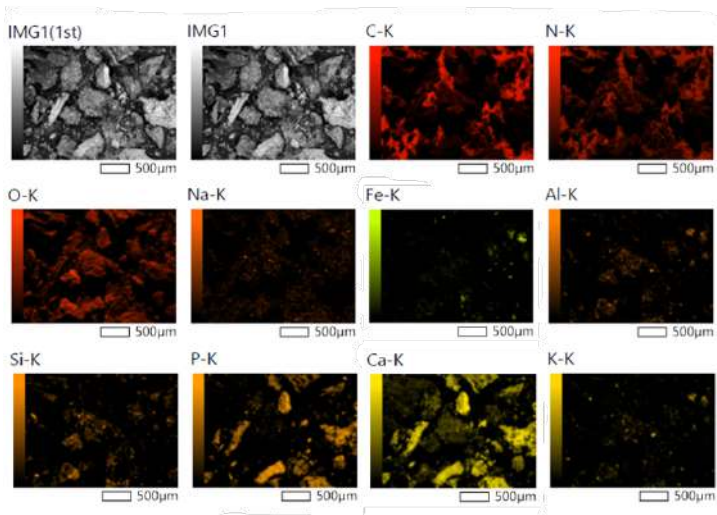


FIGURE 16. 2D elemental map of the pedosedimentary matrix sample Mat_SED2 from the chamber “O” (50x magnifying).

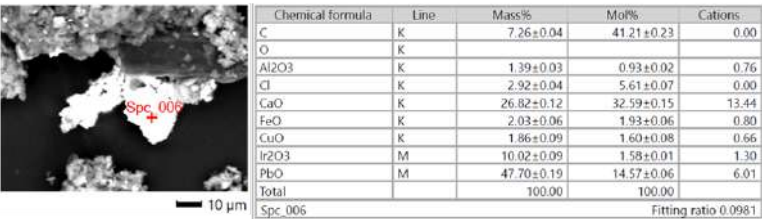


FIGURE 17. Point-like chemical analysis of the allochthonous object composed of lead (Pd), iridium (Ir) detected in the sample Mat_SED2.

4. Discussion

The ultramicroscopic analysis of the bone and pedosedimentary samples from the chamber “O” allowed to reveal the chemical reasons that stood behind the chromatic differences of the matrix between the two areas US3 SED1 and US3 SED2 (paragraph 4.1). Moreover, the study led to the detection of an inedited kind of material, which could be considered for introducing a new type of Anthrosol (paragraph 4.2).

4.1. The two areas. The area named US3 SED1 was characterised by sandy-loam blackish material and the specimens coming from this area (B_Thin_SED1, B_Surf_SED1 and Mat_SED1) testified some evidence of decomposition processes, stored in the two substrates. Indeed, the thin section of bone named B_Thin_SED1 showed a chromatic anomaly in its peripheral portion detected as made of S (Figure 6), which is ascribable to putrefactive processes: some amino acids that compose proteins can contain atoms of sulphur that, due to their decomposition, can be reduced by desulphydration (Forbes 2008) to yield ammonia, sulphides, and hydrogen sulphide gas (H_2S) (Hart *et al.* 1985). The latter could accumulate, especially in the abdominal cavity, during the emphysematous phase of decomposition (Clark, Worrell, and Pless 1997) and react with the hemoglobin, producing the sulphhemoglobin, the main responsible of the coloration of the cadaver when subjected to *livor mortis* (Clark, Worrell, and Pless 1997). The molecules of H_2S could also be absorbed in organic compounds, such as the bone tissue (Zangarini, Trombino, and Cattaneo 2016), through chemical fixation processes (Hart *et al.* 1985). Therefore, the anomaly observed in the thin section B_Thin_SED1 (Figure 6) was interpreted as trace of the fixation of H_2S in the bone tissue and, consequently, attributed to decomposition. Such a hypothesis was also supported by the observation of the 2D elemental map of the osteological specimen B_Surf_SED1 (Figure 10), where the S was still diffused, although the N was clearly more present. Such condition could be due to two biogenic amines, namely putrescine ($NH_2(CH_2)_4NH_2$) and cadaverine ($NH_2(CH_2)_5NH_2$). They are two nitric compounds resulting from microbial decarboxylation of amino acids (Hart *et al.* 1985), that is a biochemical process which takes place during the decomposition, in the putrefactive phase of proteins (Vass *et al.* 2002; Ioan *et al.* 2017). Putrescine and cadaverine are among the main cadaveric volatile organic compounds (VOCs) (as well as several acids, hydrocarbons, oxygenated compounds, and sulphides - Dekeirsschieter *et al.* 2009) beside being the main responsible of the typical odour ascribable to decomposition (Vass 2012) also identified during the recovery campaign. A noticeable diffusion of N was also observed in the 2D elemental map of the sample Mat_SED1 (Figure 13). Nevertheless, the temporal margin of permanence of these volatile compounds in such burial site, which has been sealed for centuries (Staurengghi 1916; Carlessi and Kluzer 2010), is still unclear. Indeed, it is possible to considerate an alternative justification of the N high concentration in this peculiar matrix: during the decomposition of a body, the amino acids release an amount of N in the form of ammonia (NH_3) (Hart *et al.* 1985). The persistent contact between a decomposing body and the surrounding matrix leads to an increased acidity level (Benninger, Carter, and Forbes 2008), which is able to transform ammonia in ammonium (NH_4^+) (Tibbett and Carter 2008) that could undergo denitrification (Dent, Forbes, and Stuart 2004). The latter normally involves anaerobic bacteria, which reduce nitrate in nitrite and, subsequently, gaseous N and nitrous oxide (Tibbett and Carter 2008; Ioan *et al.* 2017). In both cases, the actual research about the

origin of N in burial sites comparable to the *Sepolcreto* are lacking, whereas, conversely, the observation of this condition in forensic literature is more abundant. For instance, it is highly discussed how the ammonium ion, produced after the decomposition of the amino acids during the putrefaction processes, constitutes an important source of nourishment for the vegetation (Caccianiga, Bottacin, and Cattaneo 2012). As a matter of fact, an intense and continuous intake of N, as a consequence of the presence of a decomposing body, could imply a migration of some vegetal species, which can cover the grave and, therefore, become a fundamental detecting tool in the survey phase (Caccianiga, Bottacin, and Cattaneo 2012; Ioan *et al.* 2017). Finally, the 3D compositional image of Mat_SED1 (Figure 14) revealed its main structure being made of microscopic fragments of preserved decomposed soft and bone tissues, whereas the pedosedimentary matrix was absent.

Conversely, US3 SED2 was a reddish, sandy loam layer containing well preserved human bones whose ultramicroscopic analysis led to the identification of allochthonous microscopic objects included in the tissue. This situation not only testified the ability of skeletonised remains to entrap some evidence of the surrounding environment, but also allowed to reconstruct the circumstances that characterised the setting of a burial place, especially when subjected to such an intense anthropic influence. Indeed, some of the allochthonous objects found nestled in the bone tissue of the sample B_Thin_SED2 (Figure 7) proved to be composed of elements such as Si, Mg, Ca, Fe, Al, Ti, Na and K, which are ascribable to mineral constituents (Klein 2004). Especially, the predominant concentration of Si (38.69 ± 0.09 Mass%) followed by Ca (9.81 ± 0.04 Mass%), Fe (7.98 ± 0.05 Mass%) and Al (5.93 ± 0.04 Mass%) could be specifically attributed to the kneaded clay (Bergaya and Lagaly 2013), that is the constituent material of the bricks that composed (and still partially compose) the vault and the walls of the chamber “O” (Carlessi and Kluzer 2010). Moreover, the clay-bricks generally contain some inclusions which could be intentionally added during the production, in order to enhance the technical features of the matrix (Rice 1987), or they could be natural to the clay (Fernandes, Lourenço, and Castro 2010) or even be a newly-formed phase, due to the high firing temperatures during the cooking of the kneaded clay (Pérez-Monserrat *et al.* 2022). Indeed, the littler concentration of Ti (1.26 ± 0.02 Mass%), Na (0.47 ± 0.01 Mass%) and K (0.58 ± 0.01 Mass%) could suggest the presence of the mentioned inclusions, which are commonly found through the chemical-physical and mineralogical analysis of clary bricks from masonry of medieval age buildings, especially in Lombardy and North Italy (Negro Ponzi 2000; Pérez-Monserrat *et al.* 2022). However, their presence could be interpreted as some evidence, in the form of microscopic debris fragments, of the collapse of the vault after the bombing that almost destroyed the site during World War II (Carlessi and Kluzer 2010) (Figure 18). In support of this hypothesis, the SEM-EDS highlighted the presence of a cover of Pb on the sample B_Surf_SED2 (Figure 11), which could be ascribable to the fusion of the metal due to the high temperatures related to the explosion (Cashdollar and Zlochower 2007). Indeed, the same sample testified the occurrence of objects composed of Pb (66.96 ± 0.12 Mass%) and Ir (18.65 ± 0.07 Mass%) (Figure 12), whose co-existence constitutes a further clue of the presence of materials related to the bombing: the iridium is a component frequently used in the war industry and explosives production, since it is able to maintain its original properties even at temperatures over 1600°C (Hunt 1987). Moreover, besides being extremely resistant to corrosion and melting (Hunt 1987), the iridium is also the only chemical element able to resist to the

oxidative effects of the oxides produced by the melting of the lead (Richardson 1958), carried out for the production of weapons and explosives. Therefore, the two elements can be found together in the analysis of this kind of items and historical events (Richardson 1958; Hunt 1987; Cashdollar and Zlochower 2007). The analysis carried out on the sample of pedosedimentary matrix Mat_SED2 (Figure 16) presented an anomalous scarcity, in their concentration and distribution, of mineral chemical elements (namely Fe, Si, Mg, Al), which is a characteristic that is strongly incompatible with the classic definition of soil (Hartemink 2016). Moreover, the few mineral elements detected in the specimens (such as Na, Mg, Al, Fe, K, Ti and Si) tended to appear in the form of single objects, already attributed to the fragmentation of the clay-bricks as a result of the violent impact with the bomb that led to the collapse of the building (Carlessi and Kluzer 2010), along with some objects made of Pb (47.70 ± 0.19 Mass%) and Ir (10.02 ± 0.09 Mass%) (Figure 17), comparable to the ones detected in the sample B_Surf_SED2, where Pb and Ir resulted equally distributed (respectively 66.96 ± 0.12 Mass% and 18.65 ± 0.07 Mass%) (Figure 12). All the above-mentioned evidence could be considered the clear representation of the crucial role of anthropic activity over the whole evolution of the site, from the building of the *Sepolcreto* to its destruction and renovation.



FIGURE 18. A picture of the collapse that partially affected the Church of the *Beata Vergine Annunciata* during WWII (Archivio Fotografico di Milano).

4.2. Thanatogenic Anthrosols. On the basis of the ultramicroscopic analysis carried out on the pedosedimentary matrix of the chamber "O", it seemed legit to question its actual natural origin. Indeed, whether on one side the term "soil" could sound inadequate due to the scarcity of elements of mineral origin, on the other side its sedimentary character needs to be examined in depth. Firstly, the main factor affecting the evolution of the matrix of the site is the anthropic activity, which had been widely observed as an influencing factor of the

pedogenesis, from a geopedological and taxonomical point of view (Capra *et al.* 2015). The matrix of the *Sepolcreto* could potentially be considered an Anthrosol, which is described by the World Reference Base (WRB) as “soils that have been modified profoundly through human activities, such as addition of organic or mineral material, charcoal or household wastes, or irrigation and cultivation” (Food and Agriculture Organization of the United Nations 2014). Nevertheless, the here presented case displays a specific set of diagnostic characteristics and, therefore, would need a specially made definition able to represent both the anthropic influence and the funerary context that characterize the soil. About that, in 2003 the SSCRI (Slovakia Soil Classification System) proposed a new type of anthropogenic soil called “Necrosols” (Sobocká 2004), which would identify a soil formed after anthropic activity in cemeteries and sepulchral fields. Although Necrosols share some characteristics with the pedosedimentary matrix of the *Sepolcreto*, such as an increased concentration of P and the presence of preserved or decomposed human remains (Sobocká 2004), their origin is to be considered strongly different. Indeed, Necrosols are typical of outdoor areas affected by mechanical and biochemical disturbance, highlighted by an altered order of the horizons compared to the surrounding undisturbed area, as well as the alteration of the natural properties of the soil, like the texture, the organic matter (which is abundant even in the deepest horizons), and the oxides (Sobocká 2004). Clearly, this definition does not apply to the matrix detected and characterized in this study, since it does not present a developed profile that can be affected by the anthropic disturbance, neither it forms in a natural environment. Therefore, under this study, it seemed necessary to introduce a new term, able to adequately represent an Anthrosol specifically formed after the anthropic funerary activity in a built confined space. To achieve this, it was necessary to detect a diagnostic material, which the WRB defines as “materials that significantly influence pedogenetic processes or are indicative of them” (Food and Agriculture Organization of the United Nations 2014). Hence, considering the pedosedimentary matrix founded in the *Sepolcreto*, and taking into account the structure of the WRB classification system, it would be possible to define it as “Thanatogenic material”.

In order to make the definition fit as better as possible in the above-mentioned taxonomical system, it could be described as it follows:

General description

Thanatogenic material (from Greek θάνατος *Thánatos*, *i.e.*, death and -γενής *genés*, *i.e.*, born in a certain condition) is deeply linked to human funerary activities, such as mass burials in a confined built space (namely crypts, hypogea, burial grounds). They consist of organic matter resulted from the decomposition of human remains and preserved bone and soft tissues (e.g., through mummification and saponification), due to thanatological processes.

Diagnostic criteria

Thanatogenic material has:

1. abundant organic matter resulting from the decomposition of human remains and preserved fragments of bone and soft tissues in built confined spaces; and

2. a high content of nitrogen (N), calcium (Ca) associated to phosphorous (P), and sulfur (S) due to putrefaction processes and, of course, carbon (C).

It is a new and wide definition that, whether applied to the existing WRB classification, could be formalized as a suffix for Anthrosols, hence forming the term “Thanatogenic Anthrosols”.

5. Conclusion

The ultramicroscopic analysis of soil and bone tissue specimens collected from the chamber “O” of the *Sepolcreto* of the *Ca’ Granda* (Milan) allowed to detect the exchange of material between a corpse and the surrounding environment. This method of investigation proved to be particularly effective not only to outline the evolution of the decomposition processes and the different mechanisms and phases of deposition, but also to detect the evidence of anthropogenic events that have affected the history of the *Sepolcreto*, such as the World War II bombing. Moreover, it also permitted to recognise an inedited kind of material, which could be considered for introducing a new type of Anthrosols: “Thanatogenic material”. It could be described as organic matter resulted from the decomposition of human remains, due to thanatological processes and typically rich in nitrogen (N), calcium (Ca) associated to phosphorous (P), and sulfur (S) and carbon (C). Since it should be considered as deeply linked to human funerary activities, such as mass burials in a confined built space, it could be proposed as a suffix for Anthrosol, following the WRB classification system.

Acknowledgements

The authors would like to thank Crespi Stefania, Colzani Giovanni, Pasquale Poppa, and all the students and professionals who contributed to the investigation of the *Sepolcreto*.

Competing interests

We all declare that there is no Conflict of Interest, and this article is neither sent nor published in any other journal.

References

- Archeosfera s.r.l. (2021). *Report: Università degli Studi di Milano - Ca’ Granda*. Tech. rep.
- Belgiovine, E. and Capuzzo, D. (2021). “Analisi archeologica del Sepolcreto della Chiesa della Beata Vergine Annunciata in Ca’ Granda. Un approccio stratigrafico”. In: *Il Sepolcreto della Ca’ Granda, un Tesoro Storico e Scientifico di Milano. Volume conclusivo del progetto 2018, realizzato con il contributo di Regione Lombardia e diretto da Cristina Cattaneo e Fabrizio Slavazzi*. Ed. by M. Mattia. Ledizioni, pp. 71–80. URL: <http://digital.casalini.it/5215771>.
- Benninger, L. A., Carter, D. O., and Forbes, S. L. (2008). “The biochemical alteration of soil beneath a decomposing carcass”. *Forensic Science International* **180**(2-3), 70–75. DOI: [10.1016/j.forsciint.2008.07.001](https://doi.org/10.1016/j.forsciint.2008.07.001).
- Bergaya, F. and Lagaly, G. (2013). *Handbook of clay science*. Newnes. DOI: [10.1016/B978-0-08-098258-8.00028-6](https://doi.org/10.1016/B978-0-08-098258-8.00028-6).
- Brasseur, C., Dekeirsschieter, J., Schotsmans, E. M. J., Koning, S. de, Wilson, A. S., Haubruge, E., and Focant, J. F. (2012). “Comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry for the forensic study of cadaveric volatile organic compounds released in

- soil by buried decaying pig carcasses”. *Journal of Chromatography A* **1255**, 163–170. DOI: [10.1016/J.CHROMA.2012.03.048](https://doi.org/10.1016/J.CHROMA.2012.03.048).
- Caccianiga, M., Bottacin, S., and Cattaneo, C. (2012). “Vegetation Dynamics as a Tool for Detecting Clandestine Graves”. *Journal of Forensic Sciences* **57**(4), 983–988. DOI: [10.1111/j.1556-4029.2012.02071.x](https://doi.org/10.1111/j.1556-4029.2012.02071.x).
- Capra, G. F., Ganga, A., Grilli, E., Vacca, S., and Buondonno, A. (2015). “A review on anthropogenic soils from a worldwide perspective”. *Journal of Soils and Sediments* **15**(7), 1602–1618. DOI: [10.1007/s11368-015-1110-x](https://doi.org/10.1007/s11368-015-1110-x).
- Carlessi, M. and Kluzer, A. (2010). *Il cuore dell'antico Ospedale Maggiore di Milano. I luoghi dell'archivio e la chiesa della Beata Vergine Annunciata*. Silvana Editoriale. URL: <https://www.silvanaeditoriale.it/libro/9788836622283>.
- Carter, D. O., Yellowlees, D., and Tibbett, M. (2007). “Cadaver decomposition in terrestrial ecosystems”. *Naturwissenschaften* **94**(1), 12–24. DOI: [10.1007/s00114-006-0159-1](https://doi.org/10.1007/s00114-006-0159-1).
- Carter, D. O., Yellowlees, D., and Tibbett, M. (2010). “Moisture can be the dominant environmental parameter governing cadaver decomposition in soil”. *Forensic Science International* **200**(1-3), 60–66. DOI: [10.1016/j.forsciint.2010.03.031](https://doi.org/10.1016/j.forsciint.2010.03.031).
- Cashdollar, K. L. and Zlochower, I. A. (2007). “Explosion temperatures and pressures of metals and other elemental dust clouds”. *Journal of Loss Prevention in the Process Industries* **20**(4-6), 337–348. DOI: [10.1016/j.jlp.2007.04.018](https://doi.org/10.1016/j.jlp.2007.04.018).
- Cattaneo, C. and Slavazzi, F. (2021). *Il Sepolcreto della Ca' Granda, un Tesoro Storico e Scientifico di Milano*. Ed. by M. Mattia. Ledizioni. URL: www.ledizioni.it.
- Clark, M. A., Worrell, M. B., and Pless, J. E. (1997). “Postmortem Changes in Soft Tissue”. In: *Forensic Taphonomy: The Postmortem Fate of Human Remains*. Ed. by W. D. Haglund and M. H. Sorg. CRC Press LLC. URL: https://edisciplinas.usp.br/pluginfile.php/4551346/mod_resource/content/2/cap%209.pdf.
- Cosmacini, G. (1999). *La Ca' Granda dei milanesi. Storia dell'Ospedale Maggiore, Storia della sanità e della medicina*. Laterza. URL: <https://www.laterza.it/scheda-libro/?isbn=9788842057741>.
- Crowder, C. and Stout, S. (2012). *Bone Histology: an anthropological Perspective*. CRC Press. DOI: [10.1201/b11393](https://doi.org/10.1201/b11393).
- Dawson, L. A., Campbell, C. D., Hillier, S., and Brewer, M. J. (2008). “Methods of Characterizing and Fingerprinting Soils for Forensic Application”. In: *Soil analysis in forensic taphonomy: chemical and biological effects of buried human remains*. Ed. by M. Tibbett and D. Carter. CRC Press. DOI: [10.1201/9781420069921.ch11](https://doi.org/10.1201/9781420069921.ch11).
- Dekeirsschiet, J., Verheggen, F. J., Gohy, M., Hubrecht, F., Bourguignon, L., Lognay, G., and Haubruge, E. (2009). “Cadaveric volatile organic compounds released by decaying pig carcasses (*Sus domesticus* L.) in different biotopes”. *Forensic Science International* **189**(1-3), 46–53. DOI: [10.1016/j.forsciint.2009.03.034](https://doi.org/10.1016/j.forsciint.2009.03.034).
- Dent, B. B., Forbes, S. L., and Stuart, B. H. (2004). “Review of human decomposition processes in soil”. *Environmental Geology* **45**(4), 576–585. DOI: [10.1007/s00254-003-0913-z](https://doi.org/10.1007/s00254-003-0913-z).
- Fernandes, F. M., Lourenço, P. B., and Castro, F. (2010). “Ancient clay bricks: manufacture and properties”. In: *Materials, technologies and practice in historic heritage structures*, pp. 29–48. DOI: [10.1007/978-90-481-2684-2_3](https://doi.org/10.1007/978-90-481-2684-2_3).
- Fiedler, S. and Graw, M. (2003). “Decomposition of buried corpses, with special reference to the formation of adipocere”. *Naturwissenschaften* **90**(7), 291–300. DOI: [10.1007/s00114-003-0437-0](https://doi.org/10.1007/s00114-003-0437-0).
- Finley, S. J., Pechal, J. L., Benbow, M. E., Robertson, B. K., and Javan, G. T. (2016). “Microbial Signatures of Cadaver Gravesoil During Decomposition”. *Microbial Ecology* **71**(3), 524–529. DOI: [10.1007/s00248-015-0725-1](https://doi.org/10.1007/s00248-015-0725-1).

- Fitzpatrick, R. W. (2008). "Nature, Distribution, and Origin of Soil Materials in the Forensic Comparison of Soils". In: *Soil analysis in forensic taphonomy: chemical and biological effects of buried human remains*. Ed. by M. Tibbett and D. Carter. CRC Press. DOI: [10.1201/9781420069921.ch1](https://doi.org/10.1201/9781420069921.ch1).
- Fitzpatrick, R. W. (2009). "Soil: Forensic Analysis". In: *Wiley Encyclopedia of Forensic Science*. John Wiley & Sons, Ltd. DOI: [10.1002/9780470061589.fsa096](https://doi.org/10.1002/9780470061589.fsa096).
- Fitzpatrick, R. W. and Donnelly, L. J. (2021). "An introduction to forensic soil science and forensic geology: A synthesis". *Geological Society Special Publication* **492**(1), 1–32. DOI: [10.1144/SP492-2021-81](https://doi.org/10.1144/SP492-2021-81).
- Fitzpatrick, R. W. and Raven, M. D. (2012). "How Pedology and Mineralogy Helped Solve a Double Murder Case: Using Forensics to Inspire Future Generations of Soil Scientists". *Soil Horizons* **53**(5), 14. DOI: [10.2136/SH12-05-0016](https://doi.org/10.2136/SH12-05-0016).
- Food and Agriculture Organization of the United Nations (2014). *World Reference Base for Soil Resources 2014. International soil classification system for naming soils and creating legends for soil maps*. FAO.
- Forbes, S. L. (2008). "Decomposition Chemistry in a Burial Environment". In: *Soil Analysis in Forensic Taphonomy*. Ed. by M. Tibbett and D. Carter. CRC Press, Taylor and Francis Group, p. 203. DOI: [10.1201/9781420069921.ch8](https://doi.org/10.1201/9781420069921.ch8).
- Gatta, G. D., Mantovani, L., and Bromiley, G. D. (2023). "Raman Spectroscopy and Forensic Mineralogy". In: *Mineralogical Analysis Applied to Forensics. Soil Forensics*. Ed. by M. Mercurio, A. Langella, and R. M. C. P. di Maggio. Springer, Cham, pp. 141–169. DOI: [10.1007/978-3-031-08834-6_5](https://doi.org/10.1007/978-3-031-08834-6_5).
- Graves, W. J. (1979). "A Mineralogical Soil Classification Technique for the Forensic Scientist". *Journal of Forensic Sciences* **24**(2), 323–338. DOI: [10.1520/JFS10839J](https://doi.org/10.1520/JFS10839J).
- Hart, H., Hadad, C. M., Craine, L. E., and Hart, D. J. (1985). *Chimica organica*. Zanichelli. URL: www.zanichelli.it.
- Hartemink, A. E. (2016). "The definition of soil since the early 1800s". In: *Advances in Agronomy*. Vol. 137. Academic Press Inc, pp. 73–126. DOI: [10.1016/bs.agron.2015.12.001](https://doi.org/10.1016/bs.agron.2015.12.001).
- Hunt, L. B. (1987). "A History of Iridium: Overcoming the Difficulties of Melting and Fabrication". *Platinum Metals Rev* **31**(1), 32–41. URL: <https://technology.matthey.com/article/31/1/32-41/>.
- Ioan, B. G., Manea, C., Hanganu, B., Statescu, L., Solovastru, L. G., and Manoilescu, I. (2017). "The chemistry decomposition in human corpses". *Revista de Chimie* **68**(6), 1450–1454. DOI: [10.37358/rc.17.6.5672](https://doi.org/10.37358/rc.17.6.5672).
- Jehlika, J. (2012). "Infrared and Raman Spectroscopy: Forensic Applications in Mineralogy". In: *Infrared and Raman Spectroscopy in Forensic Science*. John Wiley & Sons, Ltd, pp. 419–445. DOI: [10.1002/9781119962328.ch8](https://doi.org/10.1002/9781119962328.ch8).
- Jones, G. D. and Bryant, V. M. (2007). "A comparison of pollen counts: Light versus scanning electron microscopy". *Grana* **46**(1), 20–33. DOI: [10.1080/00173130601173897](https://doi.org/10.1080/00173130601173897).
- Junkins, E. N. and Carter, D. O. (2017). "Relationships between Human Remains, Graves and the Depositional Environment". In: *Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment*. Ed. by E. M. J. Schotsmans, N. Márquez-Grant, and S. L. Forbes. First Edition. John Wiley & Sons Ltd. DOI: [10.1002/9781118953358.ch11](https://doi.org/10.1002/9781118953358.ch11).
- Klein, C. (2004). *Mineralogia*. Zanichelli.
- Maat, G. J., Van Den Bos, R. P., and Aarents, M. J. (2001). "Manual preparation of ground sections for the microscopy of natural bone tissue: update and modification of Frost's "rapid manual method"". *International Journal of Osteoarchaeology* **11**(5), 366–374. DOI: [10.1002/oa.578](https://doi.org/10.1002/oa.578).
- Martin, J. A. R. and Nanos, N. (2016). "Soil as an archive of coal-fired power plant mercury deposition". *Journal of Hazardous Materials* **308**, 131–138. DOI: [10.1016/j.jhazmat.2016.01.026](https://doi.org/10.1016/j.jhazmat.2016.01.026).
- Mazzetto, J. M. L., Melo, V. F., Bonfleur, E. J., Vidal-Torrado, P., and Dieckow, J. (2019). "Potential of soil organic matter molecular chemistry determined by pyrolysis-gas chromatography/mass

- spectrometry for forensic investigations”. *Science and Justice* **59**(6), 635–642. DOI: [10.1016/J.SCIJUS.2019.07.003](https://doi.org/10.1016/J.SCIJUS.2019.07.003).
- Melo, V. F., Barbar, L. C., Zamora, P. G. P., Schaefer, C. E., and Cordeiro, G. A. (2008). “Chemical, physical and mineralogical characterization of soils from the Curitiba Metropolitan Region for forensic purpose”. *Forensic Science International* **179**(2–3), 123–134. DOI: [10.1016/J.FORSCIINT.2008.04.028](https://doi.org/10.1016/J.FORSCIINT.2008.04.028).
- Negro Ponzi, M. M. (2000). “La produzione e l’uso dei laterizi nei siti rurali d’Italia settentrionale tra tardo antico e medioevo. I laterizi di Trino (VC)”. In: *I laterizi nell’alto medioevo italiano*. Società di Studi ravennati, pp. 53–74. URL: <https://hdl.handle.net/2318/49808>.
- Ortiz-Herrero, L., Uribe, B., Armas, L. H., Alonso, M. L., Sarmiento, A., Irurita, J., Alonso, R. M., Maguregui, M. I., Etxeberria, F., and Bartolomé, L. (2021). “Estimation of the post-mortem interval of human skeletal remains using Raman spectroscopy and chemometrics”. *Forensic Science International* **329**. DOI: [10.1016/J.FORSCIINT.2021.111087](https://doi.org/10.1016/J.FORSCIINT.2021.111087).
- Osterholtz, A. J., Baustian, K. M., and Martin, D. L. (2014). *Commingle and Disarticulated Human Remains: Working Toward Improved Theory, Method, and Data*. Springer. DOI: [10.1007/978-1-4614-7560-6](https://doi.org/10.1007/978-1-4614-7560-6).
- Pérez-Monserrat, E. M., Causarano, M. A., Maritan, L., Chavarria, A., Brogiolo, G. P., and Cultrone, G. (2022). “Roman brick production technologies in Padua (Northern Italy) along the Late Antiquity and Medieval Times: Durable bricks on high humid environs”. *Journal of Cultural Heritage* **54**, 12–20. DOI: [10.1016/j.culher.2022.01.007](https://doi.org/10.1016/j.culher.2022.01.007).
- Petrík, J., Prokes, L., Hofmanová, Z., Prokeš, L., Humpola, D., Fajkošová, Z., Kuča, M., Šabatová, K., and Kazdová, E. (2012). “Pedogeochemical Investigation of Bell Beaker Culture Graves from Hodonice and Těšetice- Kyjovice, Moravia, Czech Republic”. In: *Theoretical and Methodological Considerations in Central European Neolithic Archaeology*, pp. 45–64. URL: <https://www.researchgate.net/publication/221676476>.
- Petrosino, P., De Gennaro, R., and Mondillo, N. (2019). “Microscopia elettronica a scansione”. In: *Analisi mineralogiche in ambito forense*. Ed. by M. Mercurio, A. Langella, R. M. Di Maggio, and P. Cappelletti. First. Aracne Editrice, pp. 169–217. DOI: [10.1007/978-3-031-08834-6_10](https://doi.org/10.1007/978-3-031-08834-6_10).
- Piga, G., Thompson, T. J. U., Malgosa, A., and Enzo, S. (2009). “The potential of X-ray diffraction in the analysis of burned remains from forensic contexts”. *Journal of Forensic Sciences* **54**(3), 534–539. DOI: [10.1111/J.1556-4029.2009.01037.X](https://doi.org/10.1111/J.1556-4029.2009.01037.X).
- Pye, K. and Croft, D. J. (2004). *Forensic Geoscience: Principles, Techniques And Applications*. Geological Society, Special Publications. Geological Society. URL: www.geolsoc.org.uk.
- Rice, P. (1987). *Pottery Analysis: A Sourcebook*. Chicago, USA: University of Chicago Press. DOI: [10.2307/2804120](https://doi.org/10.2307/2804120).
- Richardson, F. D. (1958). “Iridium as a High Temperature Material”. *Platinum Metals Rev.* **2**(3), 83–85. URL: <https://www.ingentaconnect.com/content/matthey/pmr/1958/00000002/00000003/art00003#>.
- Ritz, K., Dawson, L. A., and Miller, D. (2009). *Criminal and Environmental Soil Forensics*. Springer. DOI: [10.1016/b978-0-12-404696-2.00012-6](https://doi.org/10.1016/b978-0-12-404696-2.00012-6).
- Ruffell, A. and Wiltshire, P. (2004). “Conjunctive use of quantitative and qualitative X-ray diffraction analysis of soils and rocks for forensic analysis”. *Forensic Science International* **145**(1), 13–23. DOI: [10.1016/J.FORSCIINT.2004.03.017](https://doi.org/10.1016/J.FORSCIINT.2004.03.017).
- Sala, C. (2020). “Il Sepolcreto della Ca’ Granda: studio di patologie infettive alle vie aeree superiori su un campione di resti osteologici”. Master Degree. Università degli Studi di Milano.
- Sangwan, P., Nain, T., Singal, K., Hooda, N., and Sharma, N. (2020). “Soil as a tool of revelation in forensic science: a review”. *Analytical Methods* **12**(43), 5150–5159. DOI: [10.1039/d0ay01634a](https://doi.org/10.1039/d0ay01634a).

- Scott, K. R., Morgan, R. M., Jones, V. J., and Cameron, N. G. (2014). "The transferability of diatoms to clothing and the methods appropriate for their collection and analysis in forensic geoscience". *Forensic Science International* **241**, 127–137. DOI: [10.1016/J.FORSIINT.2014.05.011](https://doi.org/10.1016/J.FORSIINT.2014.05.011).
- Sguazza, E. (2014). "Il Sepolcreto dell'Ospedale Maggiore (Ca' Granda) di Milano: indagini antropologiche di un singolare contesto di resti commisti". PhD Thesis. Università degli Studi dell'Insubria.
- Sobocká, J. (2004). "Necrosol as a New Anthropogenic Soil Type". In: *Soil Anthropization VIII*. Ed. by S. Science and C. R. I. of Bratislava, pp. 107–113. URL: https://www.vupop.sk/dokumenty/ine_soil_anthropization8.pdf#page=109.
- Statheropoulos, M., Spiliopoulou, C., and Agapiou, A. (2005). "A study of volatile organic compounds evolved from the decaying human body". *Forensic Science International* **153**(2-3), 147–155. DOI: [10.1016/j.forsciint.2004.08.015](https://doi.org/10.1016/j.forsciint.2004.08.015).
- Staurengi, C. (1916). *L'Ospedale di Milano e i Suoi Antichi Sepolcri: particolarmente il Foppone ora detto la Rotonda*. Ulrico Hoepli. URL: www.hoeplieditore.it.
- Tagliabue, G. (2022). "Geopedologia forense: indagine microscopica e ultramicroscopica di materiali provenienti da deposizioni sperimentali e sepolture archeologiche". Master Degree. MA thesis. Università degli Studi di Milano.
- Tagliabue, G., Crespi, S., and Trombino, L. (2021). "Il suolo come contenitore di evidenze: le Geoscienze applicate allo studio delle sepolture della cripta della Ca' Granda". In: *Il Sepolcreto della Ca' Granda, un Tesoro Storico e Scientifico di Milano*. Ed. by M. Mattia. Ledizioni LediPublishing, pp. 167–174. URL: www.ledizioni.it.
- Tagliabue, G., Masseroli, A., Ern, S. I. E., Comolli, R., Tambone, F., Cattaneo, C., and Trombino, L. (2023). "The Fate of Phosphorus in Experimental Burials: Chemical and Ultramicroscopic Characterization and Environmental Control of Its Persistency". *Geosciences* **13**(2), 14. DOI: [10.3390/geosciences13020024](https://doi.org/10.3390/geosciences13020024).
- Tanaka, H. and Local, J. (1999). "A microstructural investigation of Osaka Bay clay: the impact of microfossils on its mechanical behaviour". *Canadian Geotechnical Journal* **36**(3), 493–508. DOI: [10.1139/T99-009](https://doi.org/10.1139/T99-009).
- Tibbett, M. and Carter, D. O. (2008). *Soil analysis in forensic taphonomy: chemical and biological effects of buried human remains*. CRC Press. DOI: [10.1201/9781420069921](https://doi.org/10.1201/9781420069921).
- Tortora, G. J. and Grabowski, S. R. (2000). *Principles of Anatomy and Physiology*. 9th ed. John Wiley & Sons Inc. DOI: [10.1046/j.1469-7580.2000.197305138.x](https://doi.org/10.1046/j.1469-7580.2000.197305138.x).
- Tumer, A. R., Karacaoglu, E., Namli, A., Ketten, A., Farasat, S., Akcan, R., Sert, O., and Odabaşı, A. B. (2013). "Effects of different types of soil on decomposition: An experimental study". *Legal Medicine* **15**(3), 149–156. DOI: [10.1016/j.legalmed.2012.11.003](https://doi.org/10.1016/j.legalmed.2012.11.003).
- Vass, A. A. (2012). "Odor mortis". *Forensic Science International* **222**(1–3), 234–241. DOI: [10.1016/j.forsciint.2012.06.006](https://doi.org/10.1016/j.forsciint.2012.06.006).
- Vass, A. A., Barshick, S., Sega, G., Caton, J., Skeen, J. T., and Love, J. C. (2002). "Decomposition chemistry of human remains: a new methodology for determining the postmortem interval". *Journal of Forensic Science*, 542–553. DOI: [10.1520/jfs15294j](https://doi.org/10.1520/jfs15294j).
- Vass, A. A., Bass, W. M., Wolt, J. D., Foss, J. E., and Ammons, J. T. (1992). "Time since death determinations of human cadavers using soil solution". *Journal of Forensic Science* **37**(5), 1236–1253. DOI: [10.1520/jfs13311j](https://doi.org/10.1520/jfs13311j).
- Von Lützow, M. and Kögel-Knabner, I. (2009). "Temperature sensitivity of soil organic matter decomposition-what do we know?" *Biol. Fertil. Soils* **46**, 1–15. DOI: [10.1007/s00374-009-0413-8](https://doi.org/10.1007/s00374-009-0413-8).
- Young, J. M., Rawlence, N. J., Weyrich, L. S., and Cooper, A. (2014). "Limitations and recommendations for successful DNA extraction from forensic soil samples: A review". *Science & Justice* **54**(3), 238–244. DOI: [10.1016/j.scijus.2014.02.006](https://doi.org/10.1016/j.scijus.2014.02.006).
- Zangarini, S., Trombino, L., and Cattaneo, C. (2016). "Micromorphological and ultramicroscopic aspects of buried remains: Time-dependent markers of decomposition and permanence in soil in

experimental burial”. *Forensic Science International* **263**, 74–82. DOI: [10.1016/j.forsciint.2016.03.052](https://doi.org/10.1016/j.forsciint.2016.03.052).

-
- ^a Università degli Studi di Milano,
Dipartimento di Scienze della Terra “Ardito Desio”,
Via Mangiagalli 34, 20133 Milano, Italy
- ^b Università degli Studi di Milano, Dipartimento di Scienze Biomediche per la Salute,
LABANOF (Laboratorio di Antropologia e Odontologia Forense) Sezione di Medicina Legale,
Via Luigi Mangiagalli 37, 20133 Milano, Italy
- ^c Università Cattolica del Sacro Cuore, Beni Archeologici,
Scuola di Specializzazione (facoltà di Lettere e Filosofia),
Largo Gemelli 1, 20123 Milano, Italy
- ^d Società archeologica Archeosfera S.r.l.s.,
Via Volontari del Sangue 202, 20099 Sesto San Giovanni (MI), Italy.
- ^e Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico,
Via Sforza 28, 20122 Milano, Italy
- ^f Dipartimento di Beni Culturali e Ambientali,
Università degli Studi di Milano,
Via Noto 6, 20141 Milano, Italy
- * To whom correspondence should be addressed | email: giulia.tagliabue@unimi.it

Paper contributed to the workshop on “Advances and applications in geoforensics: Unraveling crimes with geology”,
held in Messina, Italy (26 September 2022) under the patronage of the *Accademia Peloritana dei Pericolanti*

Manuscript received 14 February 2023; published online 12 September 2023



© 2023 by the author(s); licensee *Accademia Peloritana dei Pericolanti* (Messina, Italy). This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/) (<https://creativecommons.org/licenses/by/4.0/>).

References

- Adams, B. J., Rainwater, C. W., Yim, A. D., & Alesbury, H. S. (2019). A retrospective study of intentional body dismemberment in New York City: 1996–2017. *Journal of forensic sciences*, 64(4), 1012-1016.
- Aitkenhead-Peterson, J. A., Owings, C. G., Alexander, M. B., Larison, N., & Bytheway, J. A. (2012). Mapping the lateral extent of human cadaver decomposition with soil chemistry. *Forensic science international*, 216(1-3), 127-134.
- Albini, G. (1982). *Assistenza e sanità nel ducato sforzesco*. Milano: Franco Angeli.
- Amadasi, A., Brandone, A., Rizzi, A., Mazzarelli, D., & Cattaneo, C. (2012a). The survival of metallic residues from gunshot wounds in cremated bone: A SEM–EDX study. *International Journal of Legal Medicine*, 126(4), 525–531.
- Amadasi, A., Borgonovo, S., Brandone, A., Di Giancamillo, M., & Cattaneo, C. (2012b). The survival of metallic residues from gunshot wounds in cremated bone: A radiological study. *International Journal of Legal Medicine*, 126(3), 363–369.
- Amadasi, A., Gibelli, D., Mazzarelli, D., Porta, D., Gaudio, D., Salsarola, D., Brandone, A., Rizzi, A., & Cattaneo, C. (2015). Assets and pitfalls of chemical and microscopic analyses on gunshot residues in skeletonized bodies: A report of five cases. *International Journal of Legal Medicine*, 129(4), 819–824.
- Anders, S., Kunz, M., Gehl, A., Sehner, S., Raupach, T., & Beck-Bornholdt, H. P. (2013). Estimation of the time since death—reconsidering the re-establishment of rigor mortis. *International journal of legal medicine*, 127(1), 127-130.
- Anderson, B., Meyer, J., & Carter, D. O. (2013). Dynamics of ninhydrin-reactive nitrogen and pH in gravesoil during the extended postmortem interval. *Journal of Forensic Sciences*, 58(5), 1348-1352.
- ASTM International. (2020). ASTM E1588-20. Standard practice for gunshot residue analysis by scanning electron microscopy/energy dispersive X-ray spectrometry.

- Baccino, E., De Saint Martin, L., Schuliar, Y., Guilloteau, P., Le Rhun, M., Morin, J. F., Leglise, D. & Amice, J. (1996). Outer ear temperature and time of death. *Forensic science international*, 83(2), 133-146.
- Balouet, J. C., Smith, K. T., Vroblesky, D., & Oudijk, G. (2009). Use of dendrochronology and dendrochemistry in environmental forensics: does it meet the Daubert criteria?. *Environmental Forensics*, 10(4), 268-276.
- Bansode, S., Morajkar, A., Ragade, V., More, V., & Kharat, K. (2025). Challenges and considerations in forensic entomology: A comprehensive review. *Journal of Forensic and Legal Medicine*, 102831.
- Barry, P. C. (1997). Admissibility of Scientific Evidence in the Remand of *Daubert v. Merrell Dow Pharmaceuticals, Inc.*: Questioning the Answers. In Widener L. Symp. J. (Vol. 2, p. 299).
- Barton, P. S., Strong, C., Evans, M. J., Higgins, A., & Quaggiotto, M. M. (2019). Nutrient and moisture transfer to insect consumers and soil during vertebrate decomposition. *Food Webs*, 18, e00110.
- Barton, P. S., Reboldi, A., Dawson, B. M., Ueland, M., Strong, C., & Wallman, J. F. (2020). Soil chemical markers distinguishing human and pig decomposition islands: a preliminary study. *Forensic Science, Medicine and Pathology*, 16(4), 605-612.
- Basavaiah, N., & Khadkikar, A. S. (2004). Environmental magnetism and its application towards palaeomonsoon reconstruction. *J. Ind. Geophys. Union*, 8(1), 1-14.
- Behrensmeyer, A. K. (1978). Taphonomic and ecologic information from bone weathering. *Paleobiology*, 4(2), 150-162.
- Behrensmeyer, A.K. & Kidwell, S.M. (1985). Taphonomy's contributions to paleobiology. *Paleobiology*, 11: 105-119.
- Benito, S., Abrego, Z., Sánchez, A., Unceta, N., Goicolea, M. A., & Barrio, R. J. (2015). Characterization of organic gunshot residues in lead-free ammunition using a new sample collection device for liquid chromatography–quadrupole time-of-flight mass spectrometry. *Forensic Science International*, 246, 79-85.

- Benninger, L. A., Carter, D. O., & Forbes, S. L. (2008). The biochemical alteration of soil beneath a decomposing carcass. *Forensic science international*, 180(2-3), 70-75.
- Berezowski, V., Moffat, I., Seckiner, D., Crebert, I., Ellis, J., & Mallett, X. (2024). The suitability of using domestic pigs (*Sus spp.*) as human proxies in the geophysical detection of clandestine graves. *Journal of Forensic Sciences*, 69(1), 316-328.
- Berk, R. E. (2009). Automated SEM/EDS analysis of airbag residue. II: Airbag residue as a source of percussion primer residue particles. *Journal of Forensic Sciences*, 54(1), 69-76.
- Berryman, H. E., & Symes, S. A. (1998). Recognizing gunshot and blunt cranial trauma through fracture interpretation. *Forensic osteology: advances in the identification of human remains*, 2, 333-352.
- Berryman, H. E., Kutyla, A. K., & Davis, J. R. (2010). Detection of gunshot primer residue on bone in an experimental setting—An unexpected finding. *Journal of Forensic Sciences*, 55(2), 488–492.
- Berryman, H. E. (2019). A systematic approach to the interpretation of gunshot wound trauma to the cranium. *Forensic science international*, 301, 306-317.
- Biehler-Gomez, L., Moro, C., Mondellini, M., Petrosino, D., & Morandini, P. (2021). Lo stato di salute della popolazione della Cripta della Ca'Granda. In: *Sepolcreto della Ca'Granda, un tesoro storico e scientifico di Milano*, 103-113.
- Biehler-Gomez, L., Mattia, M., Sala, C., Giordano, G., Di Candia, D., Messina, C., Sconfienza, L.M., Franchini, A.F., Porro, A., Galimberti, P.M., Slavazzi, F. & Cattaneo, C. (2022). Mercury poisoning in two patients with tertiary syphilis from the Ca'Granda hospital (17th-century Milan). *Archaeometry*, 64(2), 500-510.
- Black, O., Smith, S. C., & Roper, C. (2021). Advances and limitations in the determination and assessment of gunshot residue in the environment. *Ecotoxicology and environmental safety*, 208, 111689.
- Blau, S. (2009). *Handbook of forensic anthropology and archaeology* (Vol. 534). D. H. Ubelaker (Ed.). Walnut Creek: Left Coast Press.

- Blesa, M. J., Miranda, J. L., & Moliner, R. (2003). Micro-FTIR study of the blend of humates with calcium hydroxide used to prepare smokeless fuel briquettes. *Vibrational Spectroscopy*, 33(1-2), 31-35.
- Bona, D., Bertoldi, D., Borgonovo, G., Mazzini, S., Ravasi, S., Silvestri, S., Zaccone, C., Giannetta, B. & Tambone, F. (2023). Evaluating the potential of hydrochar as a soil amendment. *Waste Management*, 159, 75-83.
- Borrini, M. (2015). Forensic archaeology in Italy: the difficult birth of a discipline. *Forensic archaeology: A global perspective*, 91-97.
- Borsani, A. (2016). Relazione Geologica generale. Allegato A5.01 al PGT del Comune di Cassolnovo.
- Brooks, J.W., Sutton, L. (2018). Postmortem Changes and Estimating the Postmortem Interval. In: Brooks, J. (eds) *Veterinary Forensic Pathology*, Volume 1. Springer, Cham.
- Brožek-Mucha, Z. (2014). Scanning electron microscopy and X-ray microanalysis for chemical and morphological characterisation of the inorganic component of gunshot residue: selected problems. *BioMed Research International*, 2014(1), 428038.
- Byard, R. W. (2024). Concealed homicides—a postmortem study and review. *The American Journal of Forensic Medicine and Pathology*, 45(1), 20-25.
- Byard, R. W. (2025). Mummification—a forensic case series. *Australian Journal of Forensic Sciences*, 1-7.
- Caballero-Moreno, L., Luna, A., & Legaz, I. (2024). Lipidomes in cadaveric decomposition and determination of the postmortem interval: a systematic review. *International Journal of Molecular Sciences*, 25(2), 984.
- Caccia, G. (2022). *Interazione Corpo Ambiente: Il Fil Rouge Tra Analisi Tafonomiche e Tracce in Contesto Forense*. PhD Thesis. University of Milan.
- Caccia, G., & Caccianiga, M. (2021). Anticipazioni naturalistiche e merceologiche della Cripta della Ca'Granda. In: *Sepolcreto della Ca'Granda, un tesoro storico e scientifico di Milano*, 151-157.

- Caccianiga, M., Bottacin, S., & Cattaneo, C. (2012). Vegetation dynamics as a tool for detecting clandestine graves. *Journal of Forensic Sciences*, 57(4), 983-988.
- Caccianiga, M., Caccia, G., Mazzarelli, D., Salsarola, D., Poppa, P., Gaudio, D., Cappella, A., Franceschetti, L., Tambuzzi, S., Maggioni, L. & Cattaneo, C. (2021). Common and much less common scenarios in which botany is crucial for forensic pathologist and anthropologists: a series of eight case studies. *International Journal of Legal Medicine*, 135(3), 1067-1077.
- Cadée, G.C. (1991). The history of taphonomy. In (Donovan, S.K., ed.) *The Processes of Fossilization*. New York: Columbia University Press, pp. 3-21.
- Campobasso, C. P., Di Vella, G., & Introna, F. (2001). Factors affecting decomposition and Diptera colonization. *Forensic science international*, 120(1-2), 18-27.
- Canale A., Valente M.E., Ciotti A. (1984). Determination of volatile carboxylic acids and lactic acid in aqueous acid extract of silege by HPLC. *J. Sci.Food Agric.*, 35, 1178-1182.
- Card, A., Cross, P., Moffatt, C., & Simmons, T. (2015). The effect of clothing on the rate of decomposition and Diptera colonization on *Sus scrofa* carcasses. *Journal of forensic sciences*, 60(4), 979-982.
- Cardinetti, B., Ciampini, C., D'Onofrio, C., Orlando, G., Gravina, L., Ferrari, F., ... & Torresi, L. (2004). X-ray mapping technique: a preliminary study in discriminating gunshot residue particles from aggregates of environmental occupational origin. *Forensic science international*, 143(1), 1-19.
- Carlessi, M., & Kluzer, A. (2011). *Il cuore dell'antico Ospedale Maggiore di Milano. I luoghi dell'archivio e la chiesa della Beata Vergine Annunciata*. Silvana Editori,
- Carlessi, M., Kluzer, A. (2015). Past, Present and Future of the Forgotten Places in the Ancient "Ospedale Maggiore" (Ca' Granda) in Milan: Studies, Surveys, Analysis, Prospects and Projects. In: Toniolo, L., Boriani, M., Guidi, G. (eds) *Built Heritage: Monitoring Conservation Management*. Research for Development. Springer, Cham.
- Carter, D. O., Yellowlees, D., & Tibbett, M. (2007). Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften*, 94(1), 12-24.

- Carter, D. O., & Tibbett, M. (2008). Cadaver decomposition and soil: processes. In *Soil analysis in forensic taphonomy* (pp. 41-64). CRC Press.
- Casper, J. L. (1862). *A Handbook of the Practice of Forensic Medicine* (Vol. 1). New Sydenham Society.
- Castiglioni, G. B., Ajassa, R., Baroni, C., Biancotti, A., Bondesan, M., Brancucci, G., Castaldini, D., Castellaccio, E., Cavallin, A., Cortemiglia, F., Cortemiglia, G. C., Cremaschi, M., Da, O., Elmi, C., Favero Ferri, V., Gandini, F., Gasperi, G., & Zorzin, R. (1997). *Carta Geomorfologica della Pianura Padana*. 3 Fogli alla scala 1:250.000.
- Cattaneo, C., & Salsarola, D. (2009). Human remains and identity. *Wiley Encyclopedia of Forensic Science*.
- Cattaneo, C., Di Giancamillo, A., Campari, O., Orthmann, N., Martrille, L., Domeneghini, C., Jouineau, C. & Baccino, E. (2009). Infrared tympanic thermography as a substitute for a probe in the evaluation of ear temperature for post-mortem interval determination: a pilot study. *Journal of Forensic and Legal medicine*, 16(4), 215-217.
- Cattaneo, C., Poppa, P., Gibelli, D., Sassi, F., Porta, D. (2013). “Sit tibi terra levis”. Prime risultanze dall’analisi antropologica e paleopatologica del Sepolcreto dell’Ospedale Maggiore di Milano. In: Vaglianti, F., & Cattaneo, C. (edited by), *La popolazione di Milano dal Rinascimento*, pp. 61–68.
- Cattaneo, C., Maderna, E., Rendinelli, A., & Gibelli, D. (2015). Animal experimentation in forensic sciences: How far have we come?. *Forensic science international*, 254, e29-e35.
- Cattaneo, C., Mazzarelli, D., Cappella, A., Castoldi, E., Mattia, M., Poppa, P., De Angelis, D., Vitello, A., Biehler-Gomez, L. (2018). A modern documented Italian identified skeletal collection of 2127 skeletons: the CAL Milano Cemetery Skeletal Collection. *Forensic science international*, 287, 219-e1.
- Cecchetto, G., Amagliani, A., Giraudo, C., Fais, P., Cavarzeran, F., Montisci, M., Feltrin, G., Viel, G. & Ferrara, S. D. (2012). MicroCT detection of gunshot residue in fresh and decomposed firearm wounds. *International journal of legal medicine*, 126(3), 377-383.

- Charles, S., Geusens, N., & Nys, B. (2023). Interpol review of gunshot residue 2019 to 2021. *Forensic Science International: Synergy*, 6, 100302.
- Cheetham, P. N., & Hanson, I. (2016). Excavation and recovery in forensic archaeological investigations. In *Handbook of forensic anthropology and archaeology* (pp. 181-194). Routledge.
- Chen, Q., Song, Y., An, Y., Lu, Y., & Zhong, G. (2024). Soil microorganisms: Their role in enhancing crop nutrition and health. *Diversity*, 16(12), 734.
- Cholewa, M., Bonar, M., & Kadej, M. (2022). Can plants indicate where a corpse is buried? Effects of buried animal tissues on plant chemistry: Preliminary study. *Forensic Science International*, 333, 111208.
- Cockle, D. L., & Bell, L. S. (2015). Human decomposition and the reliability of a 'Universal' model for post mortem interval estimations. *Forensic science international*, 253, 136-e1.
- Collini, F., Andreola, S. A., Gentile, G., Marchesi, M., Muccino, E., & Zoja, R. (2014). Preservation of histological structure of cells in human skin presenting mummification and corification processes by Sandison's rehydrating solution. *Forensic Science International*, 244, 207-212.
- Collins, S., Stuart, B., & Ueland, M. (2020). Monitoring human decomposition products collected in clothing: an infrared spectroscopy study. *Australian Journal of Forensic Sciences*, 52(4), 428-438.
- Cosmacini, G. (1999). *Storia della medicina e della sanità a Milano*. Milano: Rizzoli.
- Cross, P., & Simmons, T. (2010). The influence of penetrative trauma on the rate of decomposition. *Journal of forensic sciences*, 55(2), 295-301.
- Crostack, C., Sehner, S., Raupach, T., & Anders, S. (2017). Re-establishment of rigor mortis: evidence for a considerably longer post-mortem time span. *International journal of legal medicine*, 131(4), 1039-1042.
- D'Errico, S., Turillazzi, E., Pomara, C., Fiore, C., Monciotti, F., & Fineschi, V. (2011). A novel macabre ritual of the Italian mafia ('Ndrangheta): Covering hands with gloves and burying the

corpse with burnt lime after execution. *The American Journal of Forensic Medicine and Pathology*, 32(1), 44-46.

Dalby, O., Butler, D., & Birkett, J. W. (2010). Analysis of gunshot residue and associated materials – a review. *Journal of forensic sciences*, 55(4), 924-943.

Day, R., Fuller, M., & Schmidt, V. A. (1977). Hysteresis properties of titanomagnetites: grain-size and compositional dependence. *Physics of the Earth and planetary interiors*, 13(4), 260-267.

Dawson, B. M., Barton, P. S., & Wallman, J. F. (2020). Contrasting insect activity and decomposition of pigs and humans in an Australian environment: a preliminary study. *Forensic science international*, 316, 110515.

Dawson, B. M., Wallman, J. F., & Barton, P. S. (2022). How does mass loss compare with total body score when assessing decomposition of human and pig cadavers?. *Forensic Science, Medicine and Pathology*, 18(3), 343-351.

Dearing, J. A. (1994). Environmental magnetic susceptibility: using the Bartington MS2 system. *Chi Pub*.

De Matteis, M., Giorgetti, A., Viel, G., Giraudo, C., Terranova, C., Lupi, A., ... & Montisci, M. (2021). Homicide and concealment of the corpse. Autopsy case series and review of the literature. *International journal of legal medicine*, 135(1), 193-205.

DeBruyn, J. M., Hoeland, K. M., Taylor, L. S., Stevens, J. D., Moats, M. A., Bandopadhyay, S., Dearth, S.P., Castro, H.F., Hewitt, K.K., Campagna, S.R., Dautartas, A.M., Vidoli G.M., Mundorff, A.Z., & Steadman, D. W. (2021). Comparative decomposition of humans and pigs: soil biogeochemistry, microbial activity and metabolomic profiles. *Frontiers in microbiology*, 11, 608856.

DeBruyn, J. M., Keenan, S. W., & Taylor, L. S. (2025). From carrion to soil: microbial recycling of animal carcasses. *Trends in Microbiology*, 33(2), 194-207.

Dekeirsschieter, J., Verheggen, F. J., Gohy, M., Hubrecht, F., Bourguignon, L., Lognay, G., & Haubruge, E. (2009). Cadaveric volatile organic compounds released by decaying pig carcasses (*Sus domesticus* L.) in different biotopes. *Forensic science international*, 189(1-3), 46-53.

- Delicato, F. (2025). Homicide with post-mortem dismemberment: The relationship between psychiatric disease of the killer, crime scene organization, and corpse concealment. (De) constructing Criminology: International Perspectives, 1(1), 70-86.
- Deng, M., Wu, H., Zhu, Z., Xie, S., Wang, G., Liang, X., Chen, R., Chen, Z., Cao, F., Wu, S., Deng, Z., Hu, G., Sun, Q. & Wang, Z. (2025). New perspective: An in vitro study on inferring the post-mortem interval (PMI) of human skeletal muscle based on ATR-FTIR spectroscopy combined with machine learning. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 126284.
- Dent, B. B., Forbes, A. S. L., & Stuart, A. B. H. (2004). Review of human decomposition processes in soil. *Environmental Geology*, 576–585.
- Dick, H. C., & Pringle, J. K. (2018). Inorganic elemental analysis of decomposition fluids of an in situ animal burial. *Forensic science international*, 289, 130-139.
- Dogan, K. H., Demirci, S., Deniz, I., & Erkol, Z. (2010). Decapitation and dismemberment of the corpse: a matricide case. *Journal of forensic sciences*, 55(2), 542-545.
- Donghi, M., Orsenigo, S., Manna, L., Profumo, A., Mattino, A., & Merli, D. (2024). Pervasiveness of inorganic gunshot residue (IGSR) in handguns after cleaning and conditioning procedures. *Journal of forensic sciences*, 69(3), 1035-1044.
- Dunlop, D. J. (2002). Theory and application of the Day plot. *Journal of geophysical research*, 107(B3), EPM5-1.
- Efremov, I.A. (1940). Taphonomy: a new branch of paleontology. *Pan American Geologist*, 74: 81-93.
- ERSAL, Ente Regionale di Sviluppo Agricolo della Lombardia (1996). I suoli del Parco del Ticino settore meridionale. Progetto “Carta Pedologica.”
- European Soil Bureau Network, E. C. (2005). *Soil Atlas of Europe*. Office for Official Publications of the European SBN.
- European Union. (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union* L276/33.

- Evis, L. H., Hanson, I., & Cheetham, P. N. (2016). An experimental study of two grave excavation methods: Arbitrary Level Excavation and Stratigraphic Excavation. *STAR: Science & Technology of Archaeological Research*, 2(2), 177–191.
- Fais, P., Giraudo, C., Boscolo-Berto, R., Amagliani, A., Miotto, D., Feltrin, G., Viel, G., Ferrara, S.D. & Cecchetto, G. (2013). Micro-CT features of intermediate gunshot wounds severely damaged by fire. *International journal of legal medicine*, 127(2), 419-425.
- Fancher, J. P., Aitkenhead-Peterson, J. A., Farris, T., Mix, K., Schwab, A. P., Wescott, D. J., & Hamilton, M. D. (2017). An evaluation of soil chemistry in human cadaver decomposition islands: Potential for estimating postmortem interval (PMI). *Forensic science international*, 279, 130-139.
- FAO, Food and Agriculture Organization of the United Nations (2006). *Guidelines for Soil Description*, 4th Ed., Rome.
- Feddema J.J. (2005). A Revised Thornthwaite-Type Global Climate Classification. *Physical Geography*, 26(6), 442–466.
- Feeney, W., Vander Pyl, C., Bell, S., & Trejos, T. (2020). Trends in composition, collection, persistence, and analysis of IGSR and OGSR: A review. *Forensic Chemistry*, 19, 100250.
- Feeney, W., Menking-Hoggatt, K., Vander Pyl, C., Ott, C. E., Bell, S., Arroyo, L., & Trejos, T. (2021). Detection of organic and inorganic gunshot residues from hands using complexing agents and LC-MS/MS. *Analytical Methods*, 13(27), 3024-3039.
- Felber, R., Stoeckli, S., & Calanca, P. (2018). Generic calibration of a simple model of diurnal temperature variations for spatial analysis of accumulated degree-days. *International journal of biometeorology*, 62(4), 621-630.
- Fenning, P. J., & Donnelly, L. J. (2004). *Geophysical techniques for forensic investigation*. Geological Society, London, Special Publications, 232(1), 11-20.
- Fiedler, S., & Graw, M. (2003). Decomposition of buried corpses, with special reference to the formation of adipocere. *Naturwissenschaften*, 90(7), 291-300.
- Fiedler, S., Schneckenberger, K., & Graw, M. (2004). Characterization of soils containing adipocere. *Archives of environmental contamination and toxicology*, 47(4), 561-568.

- Fitzpatrick, R. W. (2008). Nature, distribution, and origin of soil materials in the forensic comparison of soils. In *Soil analysis in forensic taphonomy* (pp. 13-40). CRC Press.
- Fitzpatrick, R. W., & Donnelly, L. J. (2021). An introduction to forensic soil science and forensic geology: A synthesis. Geological Society, London, Special Publications, 492(1), 1-32.
- Florencio-Silva, R., Sasso, G. R. D. S., Sasso-Cerri, E., Simões, M. J., & Cerri, P. S. (2015). Biology of bone tissue: structure, function, and factors that influence bone cells. *BioMed research international*, 2015(1), 421746.
- Forbes, S. L., Stuart, B. H., Dadour, I. R., & Dent, B. B. (2004). A preliminary investigation of the stages of adipocere formation. *Journal of Forensic and Sciences*, 49(3), JFS2002230-9.
- Forbes, S. L., Stuart, B. H., & Dent, B. B. (2005a). The effect of the burial environment on adipocere formation. *Forensic science international*, 154(1), 24-34.
- Forbes, S. L., Stuart, B. H., & Dent, B. B. (2005b). The effect of the method of burial on adipocere formation. *Forensic science international*, 154(1), 44-52.
- Forbes, S. L. (2008). Potential determinants of postmortem and postburial interval of buried remains. In *Soil analysis in forensic taphonomy* (pp. 237-258). CRC Press.
- Forbes, S. L., Wilson, M. E., & Stuart, B. H. (2011). Examination of adipocere formation in a cold water environment. *International journal of legal medicine*, 125(5), 643-650.
- Forbes, S. (2017). Body farms. *Forensic Science, Medicine and Pathology*, 13(4), 477-479.
- Forbes, M. N., Finaughty, D. A., Miles, K. L., & Gibbon, V. E. (2019). Inaccuracy of accumulated degree day models for estimating terrestrial post-mortem intervals in Cape Town, South Africa. *Forensic science international*, 296, 67-73.
- Franco, A., Mendes, S. D. S. C., Picoli, F. F., Rodrigues, L. G., & Silva, R. F. (2018). Forensic thanatology and the pink tooth phenomenon: From the lack of relation with the cause of death to a potential evidence of cadaveric decomposition in dental autopsies—Case series. *Forensic science international*, 291, e8-e12.

Frederickx, C., Dekeirsschieter, J., Brostaux, Y., Wathelet, J. P., Verheggen, F. J., & Haubruge, E. (2012). Volatile organic compounds released by blowfly larvae and pupae: New perspectives in forensic entomology. *Forensic Science International*, 219(1-3), 215-220.

Gale S.J. & Hoare P.G. (1991). *Quaternary Sediments*. Belhaven Press, London.

Gallidabino, M. D., & Weyermann, C. (2020). Time since last discharge of firearms and spent ammunition elements: state of the art and perspectives. *Forensic science international*, 311, 110290.

Galloway, A., Birkby, W. H., Jones, A. M., Henry, T. E., & Parks, B. O. (1989). Decay rates of human remains in an arid environment. *Journal of forensic sciences*, 34(3), 607-616.

Galloway, A., Symes, S. A., Haglund, W. D., & France, D. L. (1999). The role of forensic anthropology in trauma analysis. *Broken bones: anthropological analysis of blunt force trauma*. Springfield, IL: Charles C. Thomas. p, 5-31.

Geissenberger, J., Amendt, J., Klampfer, J., Thuemmel, L., Jakob, L., Monticelli, F. C., Steinbacher, P. & Pittner, S. (2024). Morphological changes and protein degradation during the decomposition process of pig cadavers placed outdoors or in tents—a pilot study. *Forensic Science, Medicine and Pathology*, 20(2), 508-517.

Gelderman, T., Stigter, E., Krap, T., Amendt, J., & Duijst, W. (2021). The time of death in Dutch court; using the Daubert criteria to evaluate methods to estimate the PMI used in court. *Legal medicine*, 53, 101970.

Gibelli, D., Brandone, A., Andreola, S., Porta, D., Giudici, E., Grandi, M. A., & Cattaneo, C. (2010). Macroscopic, microscopic, and chemical assessment of gunshot lesions on decomposed pig skin. *Journal of forensic sciences*, 55(4), 1092-1097.

Gibelli, D., Mazzarelli, D., Porta, D., Rizzi, A., & Cattaneo, C. (2012). Detection of metal residues on bone using SEM-EDS—part II: sharp force injury. *Forensic science international*, 223(1-3), 91-96.

Gigliotti, G., Giusquiani, P. L., Businelli, D., & Macchioni, A. (1997). Composition changes of dissolved organic matter in a soil amended with municipal waste compost. *Soil Science*, 162(12), 919-926.

- Giordano, G., Mattia, M., Boracchi, M., Biehler-Gomez, L., Cummaudo, M., Porro, A., Caccianiga, M., Sardanelli F., Slavazzi F., Galimberti P.M., Candia, D. & Cattaneo, C. (2023). Forensic toxicological analyses reveal the use of cannabis in Milano (Italy) in the 1600's. *Journal of Archaeological Science*, 160, 105873.
- Giordano, G., Mattia, M., Biehler-Gomez, L., Boracchi, M., Porro, A., Sardanelli, F., Slavazzi F., Galimberti P.M., Candia, D. & Cattaneo, C. (2024). Forensic toxicology backdates the use of coca plant (*Erythroxylum* spp.) in Europe to the early 1600s. *Journal of Archaeological Science*, 170, 106040.
- Giraud, C., Fais, P., Pelletti, G., Viero, A., Miotto, D., Boscolo-Berto, R., Viel, G., Montisci, M., Cecchetto, G. & Ferrara, S. D. (2016). Micro-CT features of intermediate gunshot wounds covered by textiles. *International journal of legal medicine*, 130(5), 1257-1264.
- Goldstein, J. I., Newbury, D. E., Michael, J. R., Ritchie, N. W., Scott, J. H. J., & Joy, D. C. (2017). *Scanning electron microscopy and X-ray microanalysis*. Springer.
- Guedes, A., Murray, R. C., Ribeiro, H., Sant'Ovaia, H., Valentim, B., Rodrigues, A., Leal, S. & Noronha, F. (2013). The potential application of magnetic susceptibility as a technique for soil forensic examinations. In: *Environmental and Criminal Geoforensics*, Geological Society, London, Special Publications.
- Haglund, W. D., & Sorg, M. H. (1997). *Forensic taphonomy: the postmortem fate of human remains*. CRC press.
- Harris, E. C. (2014). *Principles of archaeological stratigraphy*. Elsevier.
- Hart, G. O. (2005). Fracture pattern interpretation in the skull: differentiating blunt force from ballistics trauma using concentric fractures. *Journal of Forensic Sciences*, 50(6), JFS2004219-6.
- HauTEO, C., Hamzah, S. P. A., Osman, K., Ghani, A. A. A., & Hamzah, N. H. (2013). Post mortem changes in relation to different types of clothing. *The Malaysian journal of pathology*, 35(1), 77.

- Hayatsu, M., Tago, K., & Saito, M. (2008). Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. *Soil Science and Plant Nutrition*, 54(1), 33-45.
- Henssge, C. (1988). Death time estimation in case work. I. The rectal temperature time of death nomogram. *Forensic science international*, 38(3-4), 209-236.
- Henssge, C. (1992). Rectal temperature time of death nomogram: dependence of corrective factors on the body weight under stronger thermic insulation conditions. *Forensic science international*, 54(1), 51-66.
- Henssge, C., Althaus, L., Bolt, J., Freisleder, A., Haffner, H. T., Henssge, C. A., Hoppe, B. & Schneider, V. (2000). Experiences with a compound method for estimating the time since death. I. Rectal temperature nomogram for time since death. *International journal of legal medicine*, 113(6), 303-319.
- Henssge, C., & Madea, B. (2004). Estimation of the time since death in the early post-mortem period. *Forensic science international*, 144(2-3), 167-175.
- Higgins, O. A., Vazzana, A., Scalise, L. M., Riso, F. M., Buti, L., Conti, S., Bortoloni, E., Oxilia, G. & Benazzi, S. (2020). Comparing traditional and virtual approaches in the micro-excavation and analysis of cremated remains. *Journal of Archaeological Science: Reports*, 32, 102396.
- Hill, M. A., & Pokines, J. T. (2022). Comparative analysis of fetal pig decomposition processes in burials of variable depths and wrapping. *Journal of Forensic Sciences*, 67(6), 2192-2202.
- Hopkins, J., Brenner, L., & Tumosa, C. S. (1991). Variation of the amide I and amide II peak absorbance ratio in human hair as measured by Fourier transform infrared spectroscopy. *Forensic science international*, 50(1), 61-65.
- Huang, P. M., & Wang, M. K. (1996). Formation chemistry and selected surface properties of iron oxides.
- Hubig, M., Muggenthaler, H., & Mall, G. (2011). Influence of measurement errors on temperature-based death time determination. *International journal of legal medicine*, 125(4), 503-517.

- Huntley, B. J. (2023). Soil, water and nutrients. In *Ecology of Angola: Terrestrial biomes and ecoregions* (pp. 127-147). Cham: Springer International Publishing.
- Ilani, T., Schulz, E., & Chefetz, B. (2005). Interactions of organic compounds with wastewater dissolved organic matter: role of hydrophobic fractions. *Journal of Environmental Quality*, 34(2), 552-562.
- Indra, L., Giles, S., Alfsdotter, C., Errickson, D., & Lösch, S. (2024). Evaluation of porcine decomposition and total body score (TBS) in a central European temperate forest. *Journal of Forensic Sciences*, 69(3), 784-797.
- ISTAT Italian National Institute of Statistics data warehouse (2024). Report Vittime di Omicidio relativi all'anno 2023. StreetLib, November 2024.
- Institute of Laboratory Animal Resources (US). Committee on Care & Use of Laboratory Animals. (1986). Guide for the care and use of laboratory animals (No. 86). US Department of Health and Human Services, Public Health Service, National Institutes of Health.
- Ioan, B. G., Manea, C., Hanganu, B., Statescu, L., Solovastu, L. G., & Manoilescu, I. (2017). The Chemistry Decomposition in Human Corpses. *Rev. Chim*, 68(6), 1450–1454.
- Ismail, S. S., Pushpanathan, T., Alias, N. D. F., Ku Yusof, K. M. K., & Redzuan, N. S. (2025). Preliminary Observation of Physicochemical Changes associated with the Decomposition of a Partially Submerged Buried Cadaver in a Mangrove Soil System. *Malaysian Journal of Soil Science*, 29, 48-60.
- Jadhav, E. B., Parihar, K., Sankhla, M. S., & Sonone, S. (2022). Collection of Botanical Evidence. In *Manual of Crime Scene Investigation* (pp. 119-131). CRC Press.
- Janaway, R. C. (1996). The decay of buried remains and their associated materials, in *Studies in Crime: An Introduction to Forensic Archaeology* (J. Hunter, C. Roberts, and A. Martin, Eds.). London: Routledge, 58 – 85.
- Jeong, Y., Jantz, L. M., & Smith, J. (2016). Investigation into seasonal scavenging patterns of raccoons on human decomposition. *Journal of Forensic Sciences*, 61(2), 467-471.
- Jobbágy, E. G., & Jackson, R. B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological applications*, 10(2), 423-436.

- Jones, H., & Allsop, C. (2023). Missing, Murdered and Hidden. In *The Routledge International Handbook of Homicide Investigation* (pp. 278-295). Routledge.
- Kaatsch, H. J., Stadler, M., & Nietert, M. (1993). Photometric measurement of color changes in livor mortis as a function of pressure and time: Development of a computer-aided system for measuring pressure-induced blanching of livor mortis to estimate time of death. *International journal of legal medicine*, 106(2), 91-97.
- Kaatsch, H. J., Schmidtke, E., & Nietsch, W. (1994). Photometric measurement of pressure-induced blanching of livor mortis as an aid to estimating time of death: Application of a new system for quantifying pressure-induced blanching in lividity. *International journal of legal medicine*, 106(4), 209-214.
- Kaliszan, M., Hauser, R., Kaliszan, R., Wiczling, P., Buczyński, J., & Penkowski, M. (2005). Verification of the exponential model of body temperature decrease after death in pigs. *Experimental Physiology*, 90(5), 727-738.
- Kaliszan, M. (2011). Does a draft really influence postmortem body cooling?. *Journal of Forensic Sciences*, 56(5), 1310-1314.
- Kelly, J. A., Van Der Linde, T. C., & Anderson, G. S. (2009). The influence of clothing and wrapping on carcass decomposition and arthropod succession during the warmer seasons in central South Africa. *Journal of forensic sciences*, 54(5), 1105-1112.
- Keough N, L'Abbe EN, Steyn M, Pretorius S. Assessment of skeletal changes after post-mortem exposure to fire as an indicator of decomposition stage. *Forensic Sci Int* 2015; 246: 17–24.
- Keough, N., Myburgh, J., & Steyn, M. (2017). Scoring of decomposition: a proposed amendment to the method when using a pig model for human studies. *Journal of forensic sciences*, 62(4), 986-993.
- Klaver, T. P. C., Hepburn, D. J., & Ackland, G. J. (2012). Defect and solute properties in dilute Fe-Cr-Ni austenitic alloys from first principles. *Physical Review B—Condensed Matter and Materials Physics*, 85(17), 174111.

- Knight, B. (1992). Forensic science and animal rights. *Forensic science international*, 57(1), 1-3.
- Knight, B. (ed) (2002) *The Estimation of the Time since Death in the Early Postmortem Period*. Edward Arnold, London.
- Kocarek, P. (2003). Decomposition and Coleoptera succession on exposed carrion of small mammal in Opava, Czech Republic. *Eur. J. Soil Biol.* 39, 31–45.
- Kottek, M., Grieser, J., Beck, C., Rudolf, B., & Rubel, F. (2006). World Map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift*, 15, 259–263.
- Kotzé, Z., Aimar, S., Amendt, J., Anderson, G. S., Bourguignon, L., Hall, M. J., & Tomberlin, J. K. (2021). The forensic entomology case report—A global perspective. *Insects*, 12(4), 283.
- Krinsley, D. (1998). Models of rock varnish formation constrained by high resolution transmission electron microscopy. *Sedimentology*, 45(4), 711-725.
- Krompecher, T., Gilles, A., Brandt-Casadevall, C., & Mangin, P. (2008). Experimental evaluation of rigor mortis: IX. The influence of the breaking (mechanical solution) on the development of rigor mortis. *Forensic science international*, 176(2-3), 157-162.
- Kumar, A., Sharma, B. R., & Harish, D. (2013). Comparative evaluation of liver and rectal temperature in early postmortem interval estimation. *Journal of Forensic and Legal Medicine*, 20, 1025–1031.
- Kuriyavar, S. I., Vetrivel, R., Hegde, S. G., Ramaswamy, A. V., Chakrabarty, D., & Mahapatra, S. (2000). Insights into the formation of hydroxyl ions in calcium carbonate: temperature dependent FTIR and molecular modelling studies. *Journal of Materials Chemistry*, 10(8), 1835-1840.
- Laplace, K., Baccino, E., & Peyron, P. A. (2021). Estimation of the time since death based on body cooling: a comparative study of four temperature-based methods. *International Journal of Legal Medicine*, 135(6), 2479-2487.
- Laplace, K., Baccino, E., & Peyron, P. A. (2023). Influence of the ear temperature range on the measurement bias between an infrared thermometer and a reference probe for post-mortem interval determination. *Legal Medicine*, 64, 102271.

- Larson, D. O., Vass, A. A., & Wise, M. (2011). Advanced scientific methods and procedures in the forensic investigation of clandestine graves. *Journal of Contemporary Criminal Justice*, 27(2), 149-182.
- Ledergerber, T. D., Staymates, M., Dalzell, K. A., Arroyo, L. E., Jefferys, R., & Trejos, T. (2025). Uncovering gunshot residue flow and deposition mechanisms using novel visualization methods, real-time atmospheric particle sampling, and spectrochemical techniques. *Analytical Methods*, 17(17), 3415-3435.
- Leighton, M. (2015). Excavation methodologies and labour as epistemic concerns in the practice of archaeology. Comparing examples from British and Andean archaeology. *Archaeological Dialogues*, 22(1), 65-88.
- Lepre, C. (2023). Identifying temperature and moisture controls on Fe oxide origins. *Geophysical Research Letters*, 50(17).
- Lerchi, A., Krap, T., Eppenberger, P., & Pedernana, A. (2022). Implementation of adipocere fingerprinting in archaeology by applying a forensic approach. *TrAC Trends in Analytical Chemistry*, 157, 116801.
- Liang, J., Huang, X., Yan, J., Li, Y., Zhao, Z., Liu, Y., Ye, J. & Wei, Y. (2021). A review of the formation of Cr (VI) via Cr (III) oxidation in soils and groundwater. *Science of The Total Environment*, 774, 145762.
- Locard, E. (1930) Analyses of dust traces parts I, II and III. *Am J Police Sci* 276–298, 401–418, 496–514.
- Luong, S., Forbes, S. L., Wallman, J. F., & Roberts, R. G. (2018). Monitoring the extent of vertical and lateral movement of human decomposition products through sediment using cholesterol as a biomarker. *Forensic Science International*, 285, 93-104.
- Lyman, R. L. (2010). What taphonomy is, what it isn't, and why taphonomists should care about the difference. *Journal of Taphonomy*, 8(1), 1-16.
- Madea, B. (Ed.). (2014). *Handbook of forensic medicine*. John Wiley & Sons.
- Madea, B. (Ed.). (2023). *Estimation of the time since death*. CRC press.

- Madea, B. (2013) Estimation of the Time Since Death, Editor(s): Jay A. Siegel, Pekka J. Saukko, Max M. Houck, Encyclopedia of Forensic Sciences (Second Edition), Academic Press, 2013, Pages 229-238, ISBN 9780123821669.
- Madea, B., Kernbach-Wighton, G. (2013) Early and Late Postmortem Changes, Editor(s): Jay A. Siegel, Pekka J. Saukko, Max M. Houck, Encyclopedia of Forensic Sciences (Second Edition), Academic Press, 2013, Pages 217-228, ISBN 9780123821669.
- Madea, B., Henssge, C., Reibe, S., Tsokos, M., & Kernbach-Wighton, G. (2014). Postmortem changes and time since death. Handbook of forensic medicine, 75-133.
- Magni, P. A., Lawn, J., & Guareschi, E. E. (2021). A practical review of adipocere: Key findings, case studies and operational considerations from crime scene to autopsy. Journal of Forensic and Legal Medicine, 78, 102109.
- Maher, B. A. (1988). Magnetic properties of some synthetic sub-micron magnetites. Geophysical Journal International, 94(1), 83-96.
- Maile, A. E., Inoue, C. G., Barksdale, L. E., & Carter, D. O. (2017). Toward a universal equation to estimate postmortem interval. Forensic science international, 272, 150-153.
- Maisonhaute, J. É., & Forbes, S. L. (2023). Decomposition and insect succession on human cadavers in a humid, continental (Dfb) climate (Quebec, Canada). International Journal of Legal Medicine, 137(2), 493-509.
- Maitre, M., Kirkbride, K. P., Horder, M., Roux, C., & Beavis, A. (2017). Current perspectives in the interpretation of gunshot residues in forensic science: a review. Forensic science international, 270, 1-11.
- Maitre, M., Kirkbride, K. P., Horder, M., Roux, C., & Beavis, A. (2018). Thinking beyond the lab: organic gunshot residues in an investigative perspective. Australian Journal of Forensic Sciences, 50(6), 659-665.
- Manheim, M. H. (1997). Decomposition rates of deliberate burials: a case study of preservation. In Forensic taphonomy: the postmortem fate of human remains (pp. 469-481). CRC Press.

- Mann RW, Bass WM, Meadows L (1990) Time Since Death and Decomposition of the Human Body: Variables and Observations in Case and Experimental Field Studies. *J Forensic Sci* 35 (1): 103–111.
- Mansegosa, D. A., Giannotti, P. S., Marchiori, J. I., Jofré, F. N., Aballay, F. H., & Aisa, C. F. (2021). The story of a homicide: The location, exhumation, and multidisciplinary analysis of a clandestine burial. *Forensic Science International: Reports*, 3, 100165.
- Marhoff-Beard, S. J., Forbes, S. L., & Green, H. (2018). The validation of ‘universal’ PMI methods for the estimation of time since death in temperate Australian climates. *Forensic science international*, 291, 158-166.
- Marais, A. A., & van den Dool, A. H. (2021). Rehydration and restoration of fingerprint ridge detail in mummified post-mortem tissue: Literature review and investigation of a simplified formulation. *Journal of Forensic Sciences*, 66(6), 2252-2260.
- Marais-Werner, A., Myburgh, J., Becker, P. J., & Steyn, M. (2018). A comparison between decomposition rates of buried and surface remains in a temperate region of South Africa. *International Journal of Legal Medicine*, 132(1), 301-309.
- Marhoff, S. J., Fahey, P., Forbes, S. L., & Green, H. (2016). Estimating post-mortem interval using accumulated degree-days and a degree of decomposition index in Australia: a validation study. *Australian Journal of Forensic Sciences*, 48(1), 24-36.
- Martin, C., Vanderplanck, M., Boullis, A., Francis, F., Haubruge, E., & Verheggen, F. (2019). Impact of necrophagous insects on the emission of volatile organic compounds released during the decaying process. *Entomologia Generalis*, 39(1).
- Martin, C., Maesen, P., Minchilli, D., Francis, F., & Verheggen, F. (2021). Forensic taphonomy: characterization of the gravesoil chemistry using a multivariate approach combining chemical and volatile analyses. *Forensic Science International*, 318, 110569.
- Mason, A. R., McKee-Zech, H. S., Hoeland, K. M., Davis, M. C., Campagna, S. R., Steadman, D. W., & DeBruyn, J. M. (2022). Body mass index (BMI) impacts soil chemical and microbial response to human decomposition. *Msphere*, 7(5), e00325-22.

- Masseroli, A., Ern S., Rellini I. & Trombino L. (2018), Guida alla Descrizione del Suolo in Campagna.
- Mattia, M. (2021). Il Sepolcreto della Ca' Granda, un tesoro storico e scientifico di Milano. Ledizioni, Milan.
- Mattia, M. (2022). Quattrocento anni di storia di milano letti sui resti dei suoi abitanti. Recupero e studio del patrimonio archeologico e umano del sepolcreto della Ca' Granda. PhD Thesis. University of Milan.
- Mattia, M., Biehler-Gomez, L., Sguazza, E., Galimberti, P. M., Vaglianti, F., Gibelli, D., Poppa, P., Caccia, G., Caccianiga, M., Vanin, S., Manthey, L., Jantz, R.L., Di Candia, D., Maderna, M., Albin, G., Pawaskar, S., Damann, F., Fedeli, A.M., Belgiovine, E., Capuzzo, D., Slavazzi F. & Cattaneo, C. (2022). Ca'Granda, an avant-garde hospital between the Renaissance and Modern age: A unique scenario in European history. *Medical History*, 66(1), 24-33.
- Matuszewski, S., Hall, M. J., Moreau, G., Schoenly, K. G., Tarone, A. M., & Villet, M. H. (2020). Pigs vs people: the use of pigs as analogues for humans in forensic entomology and taphonomy research. *International journal of legal medicine*, 134(2), 793-810.
- Mazzoldi P., Nigro M., Voci C. (2010), Fisica (Legge di Stokes) - Volume I (seconda edizione), EdiSES, Napoli, ISBN 88-7959-137-1.
- Megyesi, M. S., Nawrocki, S. P., & Haskell, N. H. (2005). Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *Journal of forensic sciences*, 50(3), JFS2004017.
- Meyer, J., Anderson, B., & Carter, D. O. (2013). Seasonal variation of carcass decomposition and gravesoil chemistry in a cold (Dfa) climate. *Journal of forensic sciences*, 58(5), 1175-1182.
- Merritt, R. W., Snider, R., De Jong, J. L., Benbow, M. E., Kimbirauskas, R. K., & Kolar, R. E. (2007). Collembola of the grave: a cold case history involving arthropods 28 years after death. *Journal of Forensic Sciences*, 52(6), 1359-1361.
- Micozzi, M.S. (1991). Postmortem change in human and animal remains: A systematic approach. Springfield, Illinois: CC Thomas Publishers. xii + 124 pp.

- Miles, K. L., Finaughty, D. A., & Gibbon, V. E. (2020). A review of experimental design in forensic taphonomy: moving towards forensic realism. *Forensic sciences research*, 5(4), 249-259.
- Miller, J.J., Thurley, D.S.K. (2013) *Recovery of Human Remains*, Editor(s): Siegel, J.A., Saukko, P.J., Houck, M.M. *Encyclopedia of Forensic Sciences* (Second Edition), Academic Press, 2013, Pages 372-377, ISBN 9780123821669.
- Ministero dell'Interno - Ministry of the Interior (2025). Dipartimento della Pubblica Sicurezza. Direzione Centrale della Polizia Criminale, Servizio Analisi Criminale, and Ministero dell'Interno, "Omicidi Volontari consumati in Italia".
- Ministero per le Politiche Agricole - Ministry of Agricultural Policy (1999). Approvazione dei "Metodi ufficiali di analisi chimica del suolo", Decreto Ministeriale del 13/09/1999, Gazz. Uff. Suppl. Ordin. n° 248 del 21/10/1999.
- Minzière, V. R., Gassner, A. L., Gallidabino, M., Roux, C., & Weyermann, C. (2023a). The relevance of gunshot residues in forensic science. *Wiley Interdisciplinary Reviews: Forensic Science*, 5(1), e1472.
- Minzière, V. R., Robyr, O., & Weyermann, C. (2023b). Should inorganic or organic gunshot residues be analysed first?. *Forensic science international*, 348, 111600.
- Minzière, V. R., & Weyermann, C. (2024). Organic and inorganic gunshot residues on the hands, forearms, face, and nostrils of shooters 30 min after a discharge. *Science & Justice*, 64(5), 557-571.
- Moffatt, C., Simmons, T., & Lynch-Aird, J. (2016). An improved equation for TBS and ADD: establishing a reliable postmortem interval framework for casework and experimental studies. *Journal of Forensic Sciences*, 61, S201-S207.
- Mole, C. G., & Heyns, M. (2019). Animal models in forensic science research: justified use or ethical exploitation?. *Science and Engineering Ethics*, 25(4), 1095-1110.
- Molina, C. M., Pringle, J. K., Saumett, M., & Evans, G. T. (2016). Geophysical monitoring of simulated graves with resistivity, magnetic susceptibility, conductivity and GPR in Colombia, South America. *Forensic science international*, 261, 106-115.

- Montoriol, R., Guilbeau-Frugier, C., Aries, S., Onfroy, L., Lombardi, V., Payré, B., Cassard, H., Feki, N., Telmon, N. & Savall, F. (2021). Gunshot residue detection in stagnant water: SEM-EDX or ICP-MS? A preliminary study. *Journal of forensic sciences*, 66(4), 1267-1275.
- Moussa, C. E. K., Payré, B., Aries, S., Grill, S., Galland, S., Pestourie, L., Dedouit, F., Telmon, N., Savall, F. & Guilbeau-Frugier, C. (2024). Effects of carbonization on gunshot residue detection in an animal model. *Forensic Imaging*, 39, 200612.
- Movasaghi, Z., Rehman, S., & ur Rehman, Dr. I. (2008). Fourier Transform Infrared (FTIR) Spectroscopy of Biological Tissues. *Applied Spectroscopy Reviews*, 43(2), 134–179.
- Munsell Color (1994), Munsell Soil Color Charts, Revised edition, Macbeth Division of Kollmorgen Instruments Corporation, New Windsor, NY.
- Myburgh, J., L'Abbé, E. N., Steyn, M., & Becker, P. J. (2013). Estimating the postmortem interval (PMI) using accumulated degree-days (ADD) in a temperate region of South Africa. *Forensic science international*, 229(1-3), 165-e1.
- Nguyen-Hong, M., Payré, B., Aries, S., Dedouit, F., Telmon, N., Savall, F., & Guilbeau-Frugier, C. (2024). Comparison of SEM-EDX and ICP-MS for the analysis of gunshot residues after burial: An experimental study. *Forensic Imaging*, 39, 200604.
- Notter, S. J., Stuart, B. H., Rowe, R., & Langlois, N. (2009). The initial changes of fat deposits during the decomposition of human and pig remains. *Journal of Forensic Sciences*, 54(1), 195-201.
- O'Reilly, R. A., Ruffell, A., & Reimer, P. J. (2023). Suspect graveyard burial (South Armagh, N. Ireland): combined search, forensic anthropology and radiocarbon dating. *Science & Justice*, 63(1), 1-8.
- Olivieri, A. (2021). Dalle ossa ai genomi: analisi del DNA antico per ricostruire la nostra storia evolutiva. *Sepolcreto della Ca'Granda, un tesoro storico e scientifico di Milano*, 143-149.
- Orphanou, C. M. (2015). The detection and discrimination of human body fluids using ATR FT-IR spectroscopy. *Forensic science international*, 252, e10-e16.
- Ortner, D. (1981). Identification of pathological conditions in human skeletal remains. *Smithson. Contrib. Anthropol*, 28, 479.

- Ottone, C., & Rossetti, R. (1980). Condizioni termo-pluviometriche della Lombardia. *Atti Dell'Istituto Geologico Dell'Università Di Pavia*, 29, 27–48.
- Özcelik, S., Kuley, E., & Özogul, F. (2016). Formation of lactic, acetic, succinic, propionic, formic and butyric acid by lactic acid bacteria. *LWT*, 73, 536-542.
- Pavao, A., Graham, M., Arrieta-Ortiz, M. L., Immanuel, S. R. C., Baliga, N. S., & Bry, L. (2022). Reconsidering the in vivo functions of Clostridial Stickland amino acid fermentations. *Anaerobe*, 76, 102600.
- Paczkowski, S., & Schütz, S. (2011). Post-mortem volatiles of vertebrate tissue. *Applied microbiology and biotechnology*, 91(4), 917-935.
- Pecsi, E. L., Forbes, S., & Guillemette, F. (2024). Organic matter composition as a driver of soil bacterial responses to pig carcass decomposition in a Canadian continental climate. *Journal of Geophysical Research: Biogeosciences*, 129, e2024JG008355 (2024).
- Pellegrini, M., & Castaldi, D. (2008). Sorgenti di pianura: risorgive e fontanili. In *Atlante Tematico delle Acque d'Italia* (pp. 87–90). Brigati.
- Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., ... & Würbel, H. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *Journal of Cerebral Blood Flow & Metabolism*, 40(9), 1769-1777.
- Perdekamp, M. G., & Pollak, S. (2025). Autopsy findings in victims of gunshot injuries: wound ballistic considerations based on test shots to simulants and composite models. *International Journal of Legal Medicine*, 139(6), 2721.
- Perrault, K. A., Stefanuto, P. H., Stuart, B. H., Rai, T., Focant, J. F., & Forbes, S. L. (2015). Detection of decomposition volatile organic compounds in soil following removal of remains from a surface deposition site. *Forensic science, medicine, and pathology*, 11(3), 376-387.
- Perrault, K. A., & Forbes, S. L. (2016). Elemental analysis of soil and vegetation surrounding decomposing human analogues. *Canadian Society of Forensic Science Journal*, 49(3), 138-151.
- Pohjoismäki, J. L., Karhunen, P. J., Goebeler, S., Saukko, P., & Sääksjärvi, I. E. (2010). Indoors forensic entomology: colonization of human remains in closed environments by specific species of sarcosaprophagous flies. *Forensic Science International*, 199(1-3), 38-42.

- Pokines, J., & Symes, S. (2013). *Manual of Forensic Taphonomy*. CRC Press
- Polson CJ, Gee DJ, Knight B (1985) *The essentials of forensic medicine*, 4th ed. Pergamon Press, Oxford.
- Prabhakaran, P., Bakkannavar, S. M., Nayak, V. C., & Bhat, V. R. (2024). A review on rehydrating solutions for fingertips in dried decomposed bodies. *Forensic Science International: Reports*, 100399.
- Pringle, J. K., Cassella, J. P., & Jervis, J. R. (2010). Preliminary soilwater conductivity analysis to date clandestine burials of homicide victims. *Forensic Science International*, 198(1-3), 126-133.
- Pringle, J. K., & Jervis, J. R. (2010). Electrical resistivity survey to search for a recent clandestine burial of a homicide victim, UK. *Forensic science international*, 202(1-3), e1-e7.
- Pringle, J.K., Cassella, J.P., Jervis, J.R., Williams, A., Cross, P. and Cassidy, N.J. (2015a), Soilwater Conductivity Analysis to Date and Locate Clandestine Graves of Homicide Victims. *J Forensic Sci*, 60: 1052-1060. <https://doi.org/10.1111/1556-4029.12802>
- Pringle, J. K., Giubertoni, M., Cassidy, N. J., Wisniewski, K. D., Hansen, J. D., Linford, N. T., & Daniels, R. M. (2015b). The use of magnetic susceptibility as a forensic search tool. *Forensic science international*, 246, 31-42.
- Pringle, J. K., Stimpson, I. G., Wisniewski, K. D., Heaton, V., Davenward, B., Mirosch, N., ... & Jervis, J. R. (2020). Geophysical monitoring of simulated homicide burials for forensic investigations. *Scientific reports*, 10(1), 7544.
- Puccini, C. (2003) *Istituzioni di medicina legale*, CEA, Milano
- Quatrehomme, G., & İşcan, M. Y. (1998). Gunshot wounds to the skull: comparison of entries and exits. *Forensic science international*, 94(1-2), 141-146.
- Queiros, S. S., von der Lühse, B., Silva-Bessa, A., Brito-da-Costa, A. M., Caldas, I. M., Dawson, L., & Madureira-Carvalho, A. (2023). Lipidic compounds found in soils surrounding human decomposing bodies and its use in forensic investigations—a narrative review. *Science & Justice*, 63(3), 303-312.

- Rahim, M.K., Shariff, N., & Matsaat, G. (2018). Mechanical profiles of murder and murderers: An extensive review. *Malays. j. pathol*, 40(10).
- Rai, J. K., Pickles, B. J., & Perotti, M. A. (2022). The impact of the decomposition process of shallow graves on soil mite abundance. *Journal of forensic sciences*, 67(2), 605-618.
- Reichel, V., Kovács, A., Kumari, M., Bereczk-Tompa, É., Schneck, E., Diehle, P., Pósai, M., Hirt, A.M., Duchamp, M., Dunin-Borkowski R.E. & Faivre, D. (2017). Single crystalline superstructured stable single domain magnetite nanoparticles. *Scientific reports*, 7(1), 45484.
- Renfrew, C. & Bahn, P. (1991). *Archaeology: Theories, Methods, and Practice*. London: Thames and Hudson.
- Reuter, H., Gensel, J., Elvert, M., & Zak, D. (2020). Evidence for preferential protein depolymerization in wetland soils in response to external nitrogen availability provided by a novel FTIR routine. *Biogeosciences*, 17(2), 499-514.
- Rinklebe, J., & Shaheen, S. M. (2017). Redox chemistry of nickel in soils and sediments: A review. *Chemosphere*, 179, 265-278.
- Roberts, A. P., Cui, Y., & Verosub, K. L. (1995). Wasp-waisted hysteresis loops: Mineral magnetic characteristics and discrimination of components in mixed magnetic systems. *Journal of Geophysical Research: Solid Earth*, 100(B9), 17909-17924.
- Roberts, A. P. (2015). Magnetic mineral diagenesis. *Earth-Science Reviews*, 151, 1-47.
- Roberts, A. P., Hu, P., Harrison, R. J., Heslop, D., Muxworthy, A. R., Oda, H., Sato, T., Tauxe, L. & Zhao, X. (2019). Domain state diagnosis in rock magnetism: evaluation of potential alternatives to the Day diagram. *Journal of Geophysical Research: Solid Earth*, 124(6), 5286-5314.
- Romanelli, M. C., Marrone, M., Veneziani, A., Gianciotta, R., Leonardi, S., Beltempo, P., & Vinci, F. (2015). Hypostasis and time since death: State of the art in Italy and a novel approach for an operative instrumental protocol. *The American journal of forensic medicine and pathology*, 36(2), 99-103.
- Rillig, M. C., Caldwell, B. A., Wösten, H. A., & Sollins, P. (2007). Role of proteins in soil carbon and nitrogen storage: controls on persistence. *Biogeochemistry*, 85(1), 25-44.

- Sanderson, P., Qi, F., Seshadri, B., Wijayawardena, A., & Naidu, R. (2018). Contamination, fate and management of metals in shooting range soils—a review. *Current Pollution Reports*, 4(2), 175-187.
- Schlesinger, W.H., Bernhardt, E.S., (2020). *Biogeochemical Cycling on Land*, Editor(s): William H. Schlesinger, Emily S. Bernhardt, *Biogeochemistry* (Fourth Edition), Academic Press, Pages 183-248, ISBN 9780128146088.
- Schotsmans, E. M., Denton, J., Dekeirsschieter, J., Ivaneanu, T., Leentjes, S., Janaway, R. C., & Wilson, A. S. (2012). Effects of hydrated lime and quicklime on the decay of buried human remains using pig cadavers as human body analogues. *Forensic Science International*, 217(1-3), 50-59.
- Schotsmans, E. M., Denton, J., Fletcher, J. N., Janaway, R. C., & Wilson, A. S. (2014a). Short-term effects of hydrated lime and quicklime on the decay of human remains using pig cadavers as human body analogues: Laboratory experiments. *Forensic Science International*, 238, 142-e1.
- Schotsmans, E. M., Fletcher, J. N., Denton, J., Janaway, R. C., & Wilson, A. S. (2014b). Long-term effects of hydrated lime and quicklime on the decay of human remains using pig cadavers as human body analogues: Field experiments. *Forensic science international*, 238, 141-e1.
- Schotsmans, E.M.J. and de Voorde, W.V. (2017). Concealing the Crime: the Effects of Chemicals on Human Tissues. In *Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment* (eds E.M.J. Schotsmans, N. Márquez-Grant and S.L. Forbes).
- Schotsmans, E. M., Márquez-Grant, N., & Forbes, S. L. (Eds.). (2017). *Taphonomy of human remains: forensic analysis of the dead and the depositional environment*. John Wiley & Sons.
- Schultz, J. J. (2008). Sequential monitoring of burials containing small pig cadavers using ground penetrating radar. *Journal of Forensic Sciences*, 53(2), 279-287.
- Schwertmann, U. T. R. M., & Taylor, R. M. (1989). Iron oxides. *Minerals in soil environments*, 1, 379-438.

- Shirzaditabar, F., & Heck, R. J. (2021). Characterization of soil magnetic susceptibility: a review of fundamental concepts, instrumentation, and applications. *Canadian Journal of Soil Science*, 102(2), 231-251.
- Silva-Bessa, A., Madureira-Carvalho, Á., Azevedo, R. M., Dawson, L., Dinis-Oliveira, R. J., Forbes, S. L., & Ferreira, M. T. (2025). A soil-based approach on human taphonomy from five Portuguese public cemeteries. *Australian Journal of Forensic Sciences*, 57(1), 102-121.
- Simmons, T., Cross, P.A., Adlam, R.E. and Moffatt, C. (2010), The Influence of Insects on Decomposition Rate in Buried and Surface Remains. *Journal of Forensic Sciences*, 55: 889-892.
- Singh, R., Sharma, S., & Sharma, A. (2016). Determination of post-burial interval using entomology: a review. *Journal of Forensic and Legal Medicine*, 42, 37-40.
- Skinner, M., Alempijevic, D., & Djuric-Srejic, M. (2003). Guidelines for international forensic bio-archaeology monitors of mass grave exhumations. *Forensic science international*, 134(2-3), 81-92.
- Smith, O. B., Berryman, H. E., & Lahren, C. H. (1987). Cranial fracture patterns and estimate of direction from low velocity gunshot wounds. *Journal of Forensic sciences*, 32(5), 1416-1421.
- Smith, D. G., & Schenk, M. P. (2012). *A dissection guide & atlas to the fetal pig*. Morton Publishing Company.
- Smreczak, B., & Ukalska-Jaruga, A. (2021). Dissolved organic matter in agricultural soils. *Soil Science Annual*, 72(1).
- Soil Survey Staff. 2022. *Keys to Soil Taxonomy*, 13th edition. USDA Natural Resources Conservation Service.
- Soong, J. L., Calderón, F. J., Betzen, J., & Cotrufo, M. F. (2014). Quantification and FTIR characterization of dissolved organic carbon and total dissolved nitrogen leached from litter: a comparison of methods across litter types. *Plant and soil*, 385(1), 125-137.
- Smith, O.C., Berryman, H.E., and Lahern, C.H. (1987). Cranial fracture patterns and estimate of direction of low velocity gunshot wounds. *Journal of Forensic Sciences* 32: 1416– 1421.

- Spies, M. J., Finaughty, D. A., Friedling, L. J., & Gibbon, V. E. (2020). The effect of clothing on decomposition and vertebrate scavengers in cooler months of the temperate southwestern Cape, South Africa. *Forensic Science International*, 309, 110197.
- Statheropoulos, M., Spiliopoulou, C., & Agapiou, A. (2005). A study of volatile organic compounds evolved from the decaying human body. *Forensic science international*, 153(2-3), 147-155.
- Stavert, J. R., Drayton, B. A., Beggs, J. R., & Gaskett, A. C. (2014). The volatile organic compounds of introduced and native dung and carrion and their role in dung beetle foraging behaviour. *Ecological Entomology*, 39(5), 556-565.
- Steadman, D. W., Dautartas, A., Kenyhercz, M. W., Jantz, L. M., Mundorff, A., & Vidoli, G. M. (2018). Differential scavenging among pig, rabbit, and human subjects. *Journal of forensic sciences*, 63(6), 1684-1691.
- Stefanopoulos, P. K., Breglia, G. A., Bissias, C., Nikita, A. S., Papageorgiou, C., Tsiatis, N. E., Serafetinides, E., Gyftokostas, D.A., Aloizos, S. & Mikros, G. (2025). Firearm Injuries: A Review of Wound Ballistics and Related Emergency Management Considerations. *Emergency Care and Medicine*, 2(4), 52.
- Stuart, B. H., Forbes, S., Dent, B. B., & Hodgson, G. (2000). Studies of adipocere using diffuse reflectance infrared spectroscopy. *Vibrational Spectroscopy*, 24(2), 233-242.
- Suckling, J. K., Spradley, M. K., & Godde, K. (2016). A longitudinal study on human outdoor decomposition in Central Texas. *Journal of forensic sciences*, 61(1), 19-25.
- Sutherland, A., Myburgh, J., Steyn, M., & Becker, P. J. (2013). The effect of body size on the rate of decomposition in a temperate region of South Africa. *Forensic science international*, 231(1-3), 257-262.
- Swann, L., Chidlow, G. E., Forbes, S., & Lewis, S. W. (2010). Preliminary studies into the characterization of chemical markers of decomposition for geoforensics. *Journal of Forensic Sciences*, 55(2), 308-314.

- Symes, S. A., L'Abbé, E. N., Chapman, E. N., Wolff, I., & Dirkmaat, D. C. (2012). Interpreting traumatic injury to bone in medicolegal investigations. A companion to forensic anthropology, 340-389.
- Szelecz, I., Koenig, I., Seppey, C. V., Le Bayon, R. C., & Mitchell, E. A. (2018a). Soil chemistry changes beneath decomposing cadavers over a one-year period. *Forensic Science International*, 286, 155-165.
- Szelecz, I., Lösch, S., Seppey, C. V., Lara, E., Singer, D., Sorge, F., Tschui, J., Perotti, M.A. & Mitchell, E. A. (2018b). Comparative analysis of bones, mites, soil chemistry, nematodes and soil micro-eukaryotes from a suspected homicide to estimate the post-mortem interval. *Scientific reports*, 8(1), 25.
- Taborelli, A., Gibelli, D., Rizzi, A., Andreola, S., Brandone, A., & Cattaneo, C. (2012). Gunshot residues on dry bone after decomposition—A pilot study. *Journal of Forensic Sciences*, 57(5), 1281–1284.
- Tagliabue, G., Masseroli, A., Ern, S. I. E., Comolli, R., Tambone, F., Cattaneo, C., & Trombino, L. (2023). The Fate of Phosphorus in Experimental Burials: Chemical and Ultramicroscopic Characterization and Environmental Control of Its Persistency. *Geosciences*, 13(2), 24.
- Tagliabue, G., Masseroli, A., Caccia, G., Poppa, P., Cattaneo, C., & Trombino, L. (2024). Overview of Geosciences Applied to Forensic Taphonomy: The Interdisciplinary Approach of the Italian Non-Human Research Facility (Ticino-LEAFs). *Geosciences*, 14(12), 359.
- Tagliabue, G., Masseroli, A., Golinelli, A., Tambone, F., Cattaneo, C., & Trombino, L. (2026). Dissolved Organic Matter: Unraveling the Role of Organic Carbon from the Hypogeal Cadaver Decomposition Island. *Forensic Science International*, 383, 112887.
- Taleb, M. (2018). Assessment of Entomological remains from soil samples collected from a Pig (*Sus scrofa Domestica*) carcass decomposition site after 13 years. *Turkish Journal of Parasitology*.
- Taylor, L. S., Gonzalez, A., Essington, M. E., Lenaghan, S. C., Stewart, C. N., Mundorff, A. Z., Steadman D.W., & DeBruyn, J. M. (2023). Soil elemental changes during human decomposition. *PLoS One*, 18(6), e0287094.

- Thierry, A., Maillard, M. B., & Yvon, M. (2002). Conversion of L-leucine to isovaleric acid by *Propionibacterium freudenreichii* TL 34 and ITGP23. *Applied and Environmental Microbiology*, 68(2), 608-615.
- Thurn, B., Schotsmans, E. M., & Ueland, M. (2024). Lime and odour: A preliminary investigation into the effect of hydrated lime on the volatiles emitted from human remains. *Forensic Science International*, 358, 111745.
- Tibbett, M., & Carter, D. O. (Eds.). (2008). *Soil analysis in forensic taphonomy: chemical and biological effects of buried human remains*. CRC Press.
- Toms, C., Rogers, C. B., & Sathyavagiswaran, L. (2008). Investigation of homicides interred in concrete—the Los Angeles experience. *Journal of forensic sciences*, 53(1), 203-207.
- Trimpe, M. (2003). Analysis of fireworks for particles of the type Found in Primer Residue (GSR). *International Association for Microanalysis*, 4(1), 1-19.
- Tümer, A. R., Akçan, R., Karacaoğlu, E., Balseven-Odabaşı, A., Keten, A., Kanburoğlu, Ç., Ünal, M. & Dinç, A. H. (2012). Postmortem burning of the corpses following homicide. *Journal of forensic and legal medicine*, 19(4), 223-228.
- Tümer, A. R., Karacaoğlu, E., Namli, A., Keten, A., Farasat, S., Akcan, R., Sert, O. & Odabaşı, A. B. (2013). Effects of different types of soil on decomposition: An experimental study. *Legal medicine*, 15(3), 149-156.
- Turner, B., & Wiltshire, P. (1999). Experimental validation of forensic evidence: a study of the decomposition of buried pigs in a heavy clay soil. *Forensic Science International*, 101(2), 113-122.
- Ubelaker, D. H., & Zarenko, K. M. (2011). Adipocere: what is known after over two centuries of research. *Forensic science international*, 208(1-3), 167-172.
- Ueland, M., Nizio, K. D., Forbes, S. L., & Stuart, B. H. (2015). The interactive effect of the degradation of cotton clothing and decomposition fluid production associated with decaying remains. *Forensic Science International*, 255, 56-63.

- UK Animals Act (1986). Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. Presented to Parliament by the State for the Home Department pursuant to Elizabeth II 1986 C. 14 Section 21 (Animals Scientific Procedures) Act 1986.
- Usumoto, Y., Hikiji, W., Sameshima, N., Kudo, K., Tsuji, A., & Ikeda, N. (2010). Estimation of postmortem interval based on the spectrophotometric analysis of postmortem lividity. *Legal medicine*, 12(1), 19-22.
- Van Belle, L. E., Carter, D. O., & Forbes, S. L. (2009). Measurement of ninhydrin reactive nitrogen influx into gravesoil during aboveground and belowground carcass (*Sus domesticus*) decomposition. *Forensic Science International*, 193(1-3), 37-41.
- van Daalen, M. A., de Kat, D. S., Oude Grotebevelsberg, B. F., De Leeuwe, R., Warnaar, J., Oostra, R. J., & M Duijst-Heesters, W. L. (2017). An aquatic decomposition scoring method to potentially predict the postmortem submersion interval of bodies recovered from the North Sea. *Journal of forensic sciences*, 62(2), 369-373.
- Vanin, S., & Huchet, J. B. (2017). Forensic entomology and funerary archaeoentomology. *Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment: Forensic Analysis of the Dead and the Depositional Environment*, 167-186.
- Vass, A. A., Bass, W. M., Wolt, J. D., Foss, J. E., & Ammons, J. T. (1992). Time since death determinations of human cadavers using soil solution. *Journal of forensic sciences*, 37(5), 1236-1253.
- Vass, A. A. (2001). Beyond the grave understanding human decomposition. *Microbiology Today*, 28, 190–193.
- Vass, A. A., Barshick, S., Sega, G., Caton, J., Skeen, J. T., & Love, J. C. (2002). Decomposition chemistry of human remains: a new methodology for determining the postmortem interval. *Journal of Forensic Science*, 542–553.
- Vass, A. A., Smith, R. R., Thompson, C. V., Burnett, M. N., Wolf, D. A., Synstelien, J. A., Dulgerian, N. & Eckenrode, B. A. (2004). Decompositional odor analysis database. *Journal of Forensic and Sciences*, 49(4), 1-10.

- Vass, A. A., Smith, R. R., Thompson, C. V., Burnett, M. N., Dulgerian, N., & Eckenrode, B. A. (2008). Odor analysis of decomposing buried human remains. *Journal of Forensic Sciences*, 53(2), 384-391.
- Vass, A. A. (2012). Odor mortis. *Forensic Science International*, 222(1-3), 234-241.
- Vass, A. A. (2017). The Use of Volatile Fatty Acid Biomarkers to Estimate the Post-Mortem Interval. In: *Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment: Forensic Analysis of the Dead and the Depositional Environment*, 385-393.
- Vass, A. A. (2019). Death is in the air: Confirmation of decomposition without a corpse. *Forensic science international*, 301, 149-159.
- Vanezis, P., & Trujillo, O. (1996). Evaluation of hypostasis using a colorimeter measuring system and its application to assessment of the post-mortem interval (time of death). *Forensic science international*, 78(1), 19-28.
- Ventura, F., Portunato, F., Pizzorno, E., Mazzone, S., Verde, A., & Rocca, G. (2013). The need for an interdisciplinary approach in forensic sciences: perspectives from a peculiar case of mummification. *Journal of forensic sciences*, 58(3), 831-836.
- von der L  he, B., Fiedler, S., Mayes, R. W., & Dawson, L. (2017). Temporal fatty acid profiles of human decomposition fluid in soil. *Organic Geochemistry*, 111, 26-33.
- Voss, S. C., Forbes, S. L., & Dadour, I. R. (2019). The Soil Environment and Forensic Entomology. In *Forensic Entomology* (pp. 269-286). CRC Press.
- VV.AA. (2002) *Atlante della biodiversit   nel Parco Ticino - Edizione 2002. Elenchi Sistematici (Monografie). Consorzio Parco Lombardo della Valle del Ticino*. Updated 2024
- Walkley A., Black I. A (1933), An examination or the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method.
- Walsh-Haney, H. A., Galloway, A., & Byrd, J. H. (2019). Recovering buried bodies and surface scatter: the associated anthropological, botanical, and entomological evidence. In *Forensic Entomology* (pp. 187-212). CRC Press.

- Wescott, D. J., Steadman, D. W., Miller, N., Sauerwein, K., Clemmons, C. M., Gleiber, D. S., McDaneldb, C., Lauren Meckel, L. & Bytheway, J. A. (2018). Validation of the total body score/accumulated degree-day model at three human decomposition facilities. *Forensic Anthropology*, 1(3), 143-149.
- White, L., & Booth, T. J. (2014). The origin of bacteria responsible for bioerosion to the internal bone microstructure: results from experimentally-deposited pig carcasses. *Forensic science international*, 239, 92-102.
- Wilson, A. S., Janaway, R. C., Holland, A. D., Dodson, H. I., Baran, E., Pollard, A. M., & Tobin, D. J. (2007). Modelling the buried human body environment in upland climes using three contrasting field sites. *Forensic science international*, 169(1), 6-18.
- Wubshet, S. G., Måge, I., Böcker, U., Lindberg, D., Knutsen, S. H., Rieder, A., ... & Afseth, N. K. (2017). FTIR as a rapid tool for monitoring molecular weight distribution during enzymatic protein hydrolysis of food processing by-products. *Analytical Methods*, 9(29), 4247-4254.
- Yu, K., Zhang, H., Liu, Y., Wu, H., Cai, W., Wei, X., Liu, R., Wang, G., Sun, Q. & Wang, Z. (2021). Adipose tissue estimates the postmortem interval based on ATR-FTIR spectroscopy. *Microchemical Journal*, 164, 105977.
- Zang, Y., Huang, Y., Chang, X., Chen, J., Jiang, T., Wu, Z., Lu, L. & Tian, S. (2023). High soil pH and plastic-shed lead to iron deficiency and chlorosis of citrus in coastal saline–alkali lands: A field study in Xiangshan county. *Horticulturae*, 9(4), 437.
- Zangarini, S., Trombino, L., & Cattaneo, C. (2016). Micromorphological and ultramicroscopic aspects of buried remains: Time-dependent markers of decomposition and permanence in soil in experimental burial. *Forensic science international*, 263, 74-82.

Acknowledgment

I would like to thank those who have provided valuable input, resources, and collaboration throughout the progress of my PhD project.

My first acknowledgment goes to my PhD supervisors, Professor Luca Trombino and Professor Cristina Cattaneo, for giving me the opportunity to pursue my deep passion for forensic taphonomy and the biogeochemical principles that govern it.

I would also like to thank the current PhD Coordinator, Professor Giovanni Muttoni, and his predecessor, Professor Maria Iole Spalla, for giving me the opportunity to develop this project.

My thanks also go to the *Ticino Park*, represented by Dr. Monica Di Francesco and Mr. Stefano Sala, for enabling our Ticino-LEAFs facility to be established and to grow so fruitfully.

A special acknowledgment goes to the *Polizia Scientifica* of Milan, in particular to Dr. Anna Maria di Giulio and Dr. Dario Redaelli, for welcoming this project with great enthusiasm.

My sincere thanks also go to the Director of the *Civic Morgue of Milan* Daniele Aromataris, to his predecessor, Mauro Marrapodi, and to all the staff for their assistance and collaboration.

I would also like to thank the *Uboldo Shooting Range ASD* and its entire staff, without whom part of this project would not have been possible.

My gratitude goes to all the staff at *LABANOF*, especially Dr. Lucie Biehler-Gomez, who kindly offered her guidance despite having no obligation to do so. She has my utmost esteem and heartfelt appreciation. I am also grateful to Doctors Giulia Caccia, Debora Mazzarelli, Daniela Pezzucchi, Francesca Magli, Pasquale Poppa, Mirko Mattia, and Davide Porta for their collaboration and support.

I also extend my thanks to Doctors Stefano Tambuzzi, Ludovico Re, and all the other forensic pathologists and postgraduate trainees who contributed their expertise to this project.

Special thanks go to the *DISAA* of the University of Milan, especially Professor Fulvia Tambone, for her availability and interest, and to *LASA*, specifically Professor Giovanni Muttoni and Dr. Serena Perini, for supporting such an innovative and promising collaboration.

My heartfelt thanks go to my colleagues and friends at *LABANOF*: Doctors Carlotta Sala, Gaia Giordano, Claudia Moro, Marta Mondellini, Alice Tomaino, Daniele Petrosino, Lucrezia Rodella, and Andrea Palamenghi; to my fellow naturalists Doctors Anna Masseroli and Sara

Pescio and geologists Doctors Mara Fant, Giulia Amaglio, and Andrea Chiari. I do not believe I can adequately express how grateful I am to have shared this journey with each of them.

I would also like to thank all the students and thesis candidates I met throughout this project, whose contributions helped shape it into what it is today.

Finally, my deepest gratitude goes to my parents, to Daniele, and to my friends and family, without whom I would not have been able to reach this long-desired milestone.

And finally, to Piuma, for guiding me home.

Appendix

In the present chapter, the raw data collected from the analyses conducted as part of the PhD project are presented. In particular, the Appendix includes:

Tables	A.1 Comparison among the three methods used for the calculation of TBS (Megyesi et al., 2005; Keough et al., 2017; Indra et al., 2024).	Pag. 335
	A.2 Results of the TOC analysis (Walkley & Black method) on samples from group GN1–2.	Pag. 342
	A.3 Results of the TOC analysis (Walkley & Black method) on samples from group GW1–2.	Pag. 343
	A.4 Results of the TOC analysis (Walkley & Black method) on samples from group GQ1–2.	Pag. 344
	A.5 Results of the TOC analysis (Walkley & Black method) on samples from group GC1–2.	Pag. 345
	A.6 Results of the analyses of VFA, lactic acid, and succinic acid concentrations performed by HPLC.	Pag. 385
Spectra	Spectra obtained through FT-IR spectrophotometry performed on the DOM samples.	Pagg. 346-384
	Spectra obtained through HPLC analysis of VFAs, lactic acid, and succinic acid.	Pagg. 393-446
	Spectra and 3D compositional images of IGSR on lesions obtained through SEM-EDX analysis.	Pagg. 452-506
Figures	A.1 Form created for assessing the decomposition stage of the bodies and assigning the Total Body Score (TBS).	Pag. 447
	A.2 Example of an Excel spreadsheet for estimating the PMI based on the calculation of ADDs.	Pag. 448
	A.3 Example of an Excel spreadsheet for estimating the PMI based on the Vass formula for buried bodies.	Pag. 449
	A.4 Lateral-lateral radiographs of the piglets before inhumation and after the exhumations.	Pag. 450-451

Head & Neck				
Decomposition stage	Pt(s)	Megyesi et al. (2005) - humans	Keough et al. (2017) - pigs	Indra et al. (2024) - pigs
Fresh	1	Fresh, no discoloration	Fresh, no discoloration—slight lividity (pink/red)	Fresh, no discoloration—slight lividity (pink/red)
Early decomposition	2	Pink, white appearance, skin slippage, and some hair loss	Insect activity; pronounced lividity (dark pink/red)	Insect activity; pronounced lividity (dark pink/red)
	3	Gray-green discoloration, some flesh still relatively fresh	Dark-red discoloration with some flesh still relatively fresh; edema of ears; maggot colonization (mouth); initial bloating of neck and skin slippage	Possible gray-green discoloration, some flesh still relatively fresh, maggot colonization (mouth)
	4	Discoloration and/or brown shades particularly at edges, drying of nose, ears, and lips	Discoloration and/or brownish shades particularly at edges, drying of nose, ears, and lips; prominent bloating of neck; maggot colonization (mouth and eyes); purging of decompositional fluids (mouth)	Drying of nose, slight bloating of neck, hair loss and skin slippage, maggot colonization (mouth and eyes), ears might still be fresh

	5	Purging of decomposition fluids out of eyes, ears, nose, and mouth. Some bloating of neck and face	Purging of decompositional fluids (mouth, eyes, nose); brown discoloration; hair loss and skin slippage; drying of lips, nose and ears	Dark discoloration, extensive maggot colonization
	6	Brown-Black discoloration of flesh	Black discoloration of flesh; extensive maggot colonization and migration	Dark green-gray or purple-black discoloration of flesh, extensive maggot colonization and migration
Advanced decomposition	7	Caving in the flesh and tissues of eye and throat	Caving in of the flesh and tissues of eyes and throat	Caving in of the flesh and tissues of eyes and throat, ears might still be fresh
	8	Moist decomposition with bone exposure less than one half that of the area being scored	n/a	n/a
	9	Mummification with bone exposure less than one half that of the area being scored	n/a	n/a
Skeletonization	10	Bone exposure of more than half of the area being scored with greasy substances and decomposition tissue	n/a	n/a

11	Bone exposure of more than half of the area being scored with desiccated or mummified tissue	n/a	n/a
12	Bones largely dry, but retaining some grease	n/a	n/a
13	Dry bone	n/a	n/a

Trunk				
Decomposition stage	Pt(s)	Megyesi et al. (2005) - humans	Keough et al. (2017) - pigs	Indra et al. (2024) - pigs
Fresh	1	Fresh, no discoloration	Fresh, no discoloration—slight lividity (pink)	Fresh, no discoloration—slight lividity (pink)
Early decomposition	2	Pink, white appearance, skin slippage, and marbling present	Skin appears shiny/glossy with early bloating and may show purple-black discoloration over abdominal area	Skin might appear shiny/glossy, early bloating, localized gray-green discoloration over abdominal area
	3	Gray-green discoloration, some flesh still relatively fresh	Gray-purple to green discoloration: some flesh still relatively fresh; marbling of abdomen with maximum bloat	Gray-green discoloration, some flesh still relatively fresh, marbling of abdomen

	4	Bloating with green discoloration & purging of decompositional fluids	Purple-black discoloration and purging of decompositional fluids; skin slippage with maggot-filled blisters present; hair loss	Dark-green to purple-black discoloration, possible maggot colonization of anus
	5	Post-bloating following release of abdominal gases with discoloration changing from green to black	Post-bloating following release of the abdominal gases, with extensive skin slippage and drying out of blisters	Post-bloating following release of the abdominal gases, skin blisters, blisters possibly filled with maggots
Advanced decomposition	6	Decomposition of tissue producing sagging of flesh; caving in the abdominal cavity	n/a	n/a
	7	Moist decomposition with bone exposure less than one half that of the area being scored	n/a	n/a
	8	Mummification with bone exposure of less than one half that of the area being scored	n/a	n/a
Skeletonization	9	Bones with decomposed tissue, sometimes with body fluids and grease still present	n/a	n/a

10	Bones with desiccated or mummified tissue covering less than one half of the area being scored	n/a	n/a
11	Bones largely dry, but retaining some grease	n/a	n/a
12	Dry bone	n/a	n/a

Limbs				
Decomposition stage	Pt(s)	Megyesi et al. (2005) - humans	Keough et al. (2017) - pigs	Indra et al. (2024) - pigs
Fresh	1	Fresh, no discoloration	Fresh, no discoloration—slight lividity (pink) with rigor present	Fresh, no discoloration—slight lividity (pink) with rigor present
Early decomposition	2	Pink, white appearance, skin slippage of hands and feet	Pink-white appearance with bloating of proximal parts of limbs	Pink-white appearance with bloating of proximal parts of limbs, marbling
	3	Gray-green discoloration; marbling, some flesh still relatively fresh	Gray to green discoloration: marbling and shiny appearance of skin; some flesh still relatively fresh; skin slippage and hair loss	Gray to green discoloration, bloating of the entire limb

	4	Discoloration and/or brown shades particularly at edges, drying of fingers and toes and other projecting extremities	Discoloration and/or brownish shades particularly at edges, drying of skin (starting distal to proximal)	Discoloration and/or brownish shades particularly at edges, drying of skin (starting distal to proximal)
	5	Brown-black discoloration, skin having leathery appearance	Brown to black discoloration, skin having a leathery appearance	Dark-green to black discoloration, skin slippage, loss of hooves
Advanced decomposition	6	Moist decomposition with bone exposure less than one half that of the area being scored	n/a	n/a
	7	Mummification with bone exposure of less than one half that of the area being scored	n/a	n/a
Skeletonization	8	Bone exposure over one half the area being scored, some decomposed tissue and body fluids remaining	n/a	n/a
	9	Bones largely dry, but retaining some grease	n/a	Bones with desiccated or mummified tissue covering less than one half of the area being scored

10	Dry bone	n/a	Bones largely dry, but retaining some grease
11	/	/	Dry bone

Table A.1: Resume table of the categories and stages of decomposition for each of the three considered anatomical regions for the three methods. Note: n/a stands for no modifications with respect to previous methods.

<i>Sample ID</i>	<i>Weight (g)</i>	<i>Titration (mL)</i>	<i>Blank (mL)</i>	<i>TOC (g/kg)</i>	<i>OM (g/kg)</i>
IN GN1 0-10	0.2192	9.7	20.0	91.54	157.81
IN GN1 10-20	0.5030	16.5	20.0	13.56	23.37
IN GN1 20-30	1.0203	18.5	20.0	2.86	4.94
IN GN1 30-40	1.5820	16.6	20.0	4.19	7.22
IN GN1 40-50+	1.5726	16.3	20.0	4.58	7.90
EX GN1 0-10	0.5663	5.9	20.0	48.50	83.62
EX GN1 10-20	0.5255	10.4	20.0	35.59	61.35
EX GN1 20-30	0.6063	15.4	20.0	14.78	25.48
EX GN1 30-40	0.6033	11.8	20.0	26.48	45.65
EX GN1 40-50	0.6734	16.5	20.0	10.13	17.46
EX GN1 Pig	0.4562	16.5	20.0	14.95	25.77
EX GN1 HCDI	0.4312	16.4	20.0	16.26	28.04
IN GN2 0-10	0.2184	15.0	20.0	44.60	76.89
IN GN2 10-20	0.5516	17.0	20.0	10.59	18.27
IN GN2 20-30	1.1005	18.2	20.0	3.19	5.49
IN GN2 30-40	1.3600	16.6	20.0	4.87	8.40
IN GN2 40-50+	1.5584	17.7	20.0	2.88	4.96
EX GN2 0-10	0.6413	13.7	20.0	19.91	34.32
EX GN2 10-20	0.6165	8.0	20.0	30.85	53.19
EX GN2 20-30	0.7577	12.4	20.4	17.40	30.00
EX GN2 30-40	0.8781	13.0	20.4	14.34	24.72
EX GN2 40-50	0.9855	14.0	20.4	11.58	19.97
EX GN2 50-60	1.0552	16.8	20.4	16.80	28.96
EX GN2 60-80+	1.5332	19.0	20.4	1.74	3.01

Table A.2: Laboratory data for the TOC analysis (Walkley & Black method) on samples from group GN1-2. “IN” detects the samples collected before the inhumation from the burial wall; “EX” indicates the samples collected from the filling during the exhumations; “Pig” detects the soil that covered the bodies, directly in contact with them; “HCDI” indicates the soil collected below the bodies. For EX GN2 the sapling depth continued to 80 cm, due to the absence of the cadaver.

<i>Sample ID</i>	<i>Weight (g)</i>	<i>Titration (mL)</i>	<i>Blank (mL)</i>	<i>TOC (g/kg)</i>	<i>OM (g/kg)</i>
IN GW1 0-10	0.2203	12.2	20.2	70.04	120.75
IN GW1 10-20	0.2775	19.1	20.2	7.65	13.18
IN GW1 20-30	0.8536	18.5	20.2	3.84	6.62
IN GW1 30-40	1.0494	19.7	20.2	0.92	1.58
IN GW1 40-50	1.0985	19.4	20.2	1.40	2.42
IN GW1 50-60+	1.1954	18.7	20.2	2.42	4.17
EX GW1 0-10	0.2059	18.5	20.2	15.92	27.45
EX GW1 10-20	0.4786	16.7	20.2	14.11	24.32
EX GW1 20-30	0.2439	18.8	20.2	11.07	19.09
EX GW1 30-40	0.2622	18.7	20.2	11.03	19.02
EX GW1 40-50	0.3653	19.4	20.2	4.22	7.28
EX GW1 Pig	0.4776	18.4	20.3	7.64	13.16
EX GW1 HCDI	0.2674	18.4	20.2	12.98	22.38
IN GW2 0-10	0.2476	12.2	20.0	61.37	105.80
IN GW2 10-20	1.1274	10.6	20.0	16.24	28.00
IN GW2 20-30	1.0195	18.9	20.0	2.10	3.62
IN GW2 30-40	1.0041	19.6	20.0	0.78	1.34
IN GW2 40-50	1.1034	19.0	20.0	1.77	3.04
IN GW2 50-60+	1.5741	16.7	20.0	4.08	7.04
EX GW2 0-10	0.2517	14.8	20.3	41.94	72.30
EX GW2 10-20	0.2252	17.0	20.3	28.12	48.49
EX GW2 20-30	0.4054	14.2	20.3	28.88	49.79
EX GW2 30-40	0.6387	15.7	20.0	13.12	22.61
EX GW2 Pig	0.4560	14.0	20.3	26.52	45.71
EX GW2 HCDI	0.3110	18.2	20.3	12.96	22.34

Table A.3: Laboratory data for the TOC analysis (Walkley & Black method) on samples from group GW1-2. “IN” detects the samples collected before the inhumation from the burial wall; “EX” indicates the samples collected from the filling during the exhumations; “Pig” detects the soil that covered the bodies, directly in contact with them; “HCDI” indicates the soil collected below the bodies.

<i>Sample ID</i>	<i>Weight (g)</i>	<i>Titration (mL)</i>	<i>Blank (mL)</i>	<i>TOC (g/kg)</i>	<i>OM (g/kg)</i>
IN GQ1 0-10	0.2423	11.0	20.4	74.09	127.74
IN GQ1 10-20	0.5091	12.9	20.4	28.14	48.51
IN GQ1 20-30	1.1557	19.6	20.4	1.32	2.28
IN GQ1 30-40	1.3734	18.9	20.4	2.09	3.60
IN GQ1 40-50	1.5647	15.5	20.2	5.79	9.99
IN GQ1 50-60+	1.5738	18.6	20.2	1.96	3.38
EX GQ1 0-10	0.5828	16.1	20.2	13.57	23.39
EX GQ1 10-20	0.5165	14.5	20.2	21.29	36.70
EX GQ1 20-30	0.6766	10.2	20.2	28.51	49.15
EX GQ1 30-40	0.6675	7.7	20.2	36.12	62.27
EX GQ1 40-50	0.6648	4.2	20.2	46.42	80.03
EX GQ1 Pig	0.4149	14.6	20.2	26.03	44.88
EX GQ1 HCDI	0.4095	16.4	20.2	17.90	30.86
IN GQ2 0-10	0.2668	14.4	20.4	42.88	73.92
IN GQ2 10-20	0.5132	14.5	20.4	21.96	37.85
IN GQ2 20-30	1.0377	13.7	20.4	12.33	21.26
IN GQ2 30-40	1.3396	17.2	20.4	4.56	7.87
IN GQ2 40-50	1.5563	17.9	20.4	3.07	5.29
IN GQ2 50-60+	1.5225	15.7	20.4	5.90	10.16
EX GQ2 0-10	0.5034	15.0	20.4	20.49	35.32
EX GQ2 10-20	0.5504	13.0	20.4	25.68	44.27
EX GQ2 20-30	0.6236	13.1	20.4	22.36	38.54
EX GQ2 30-40	0.6324	13.0	20.4	22.35	38.53
EX GQ2 Pig	0.5237	17.7	20.4	9.85	16.98
EX GQ2 HCDI	0.4019	17.5	20.4	13.78	23.76

Table A.4: Laboratory data for the TOC analysis (Walkley & Black method) on samples from group GQ1-2. “IN” detects the samples collected before the inhumation from the burial wall; “EX” indicates the samples collected from the filling during the exhumations; “Pig” detects the soil that covered the bodies, directly in contact with them; “HCDI” indicates the soil collected below the bodies.

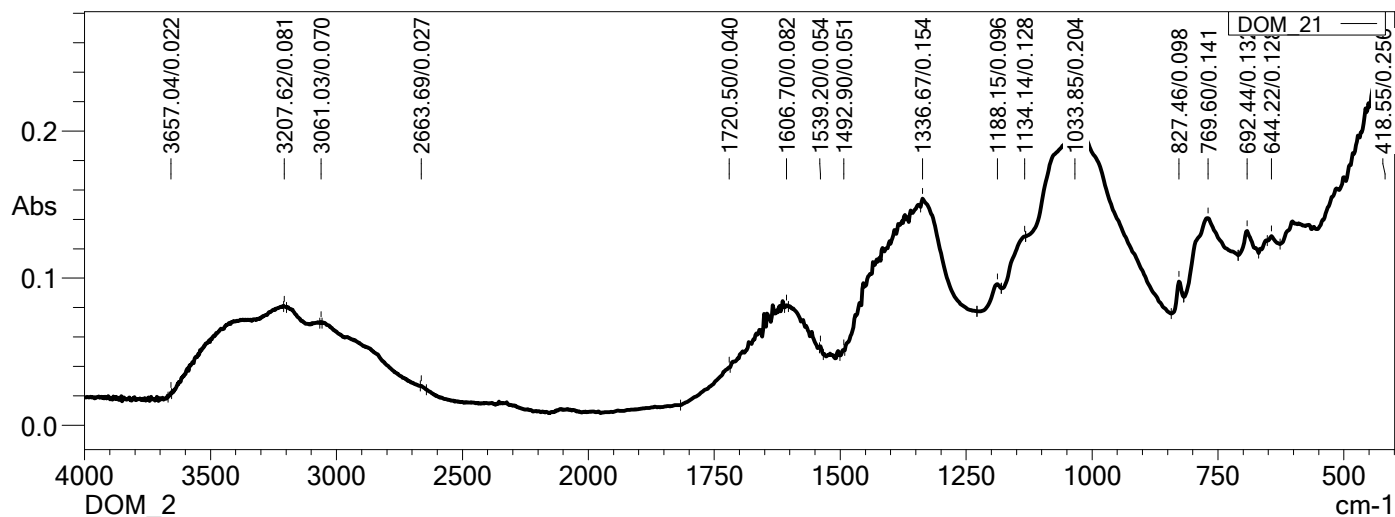
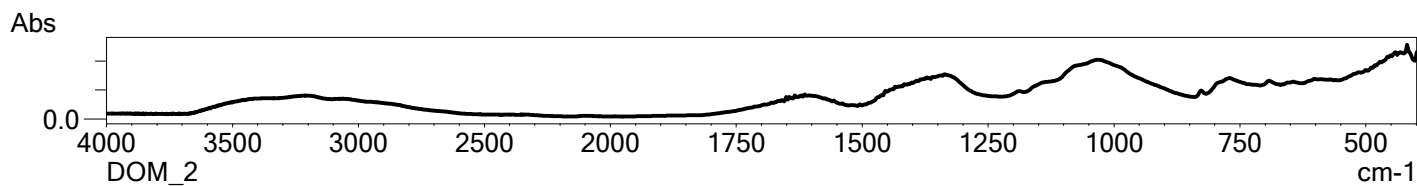
<i>Sample ID</i>	<i>Weight (g)</i>	<i>Titration (mL)</i>	<i>Blank (mL)</i>	<i>TOC (g/kg)</i>	<i>OM (g/kg)</i>
IN GC1 0-10	0.2188	9.8	20.2	91.68	158.05
IN GC1 10-20	0.5421	7.6	20.2	44.83	77.29
IN GC1 20-30	1.0791	12.9	20.2	13.05	22.49
IN GC1 30-40	1.3307	14.7	20.2	7.97	13.74
IN GC1 40-50	1.5487	15.6	20.2	5.73	9.88
IN GC1 50-60+	1.5374	13.0	20.2	9.03	15.57
EX GC1 0-10	0.5802	12.5	20.2	25.60	44.13
EX GC1 10-20	0.5191	13.0	20.2	26.75	46.12
EX GC1 20-30	0.6102	11.2	20.2	28.45	49.04
EX GC1 30-40	0.6102	13.0	20.2	22.76	39.24
EX GC1 40-50	0.6534	13.2	20.2	20.66	35.62
EX GC1 Pig	0.4115	15.6	20.2	21.56	37.17
EX GC1 HCDI	0.4421	16.2	20.2	17.45	30.09
IN GC2 0-10	0.2388	12.7	20.2	60.58	104.43
IN GC2 10-20	0.5070	18.0	20.2	8.37	14.43
IN GC2 20-30	1.1829	18.2	20.2	3.26	5.62
IN GC2 30-40	1.3140	18.5	20.2	2.50	4.30
IN GC2 40-50	1.5313	17.5	20.2	3.40	5.86
IN GC2 50-60+	1.5736	17.4	20.2	3.43	5.92
EX GC2 0-10	0.5159	18.0	20.2	8.22	14.18
EX GC2 10-20	0.5032	15.7	20.2	17.25	29.74
EX GC2 20-30	0.6128	15.3	20.2	15.42	26.59
EX GC2 30-40	0.6187	15.5	20.2	14.65	25.26
EX GC2 Pig	0.4610	18.0	20.2	9.20	15.87
EX GC2 HCDI	0.4490	16.7	20.2	15.03	25.92

Table A.5: Laboratory data for the TOC analysis (Walkley & Black method) on samples from group GC1-2. “IN” detects the samples collected before the inhumation from the burial wall; “EX” indicates the samples collected from the filling during the exhumations; “Pig” detects the soil that covered the bodies, directly in contact with them; “HCDI” indicates the soil collected below the bodies.

This section presents the individual FT-IR spectrometry spectra of the DOM samples extracted from the matrix at the bottom of the graves before inhumation (control soil) and from the HCDI after each exhumation. Correlated to each spectrum, a table is provided listing the specifications for each peak (from right to left). These include the wavenumber (cm^{-1}), absorbance intensity, corrected absorbance intensity, and the geometric characteristics of each peak (height (H), width (L), area, and corrected area. The section is organized as follows:

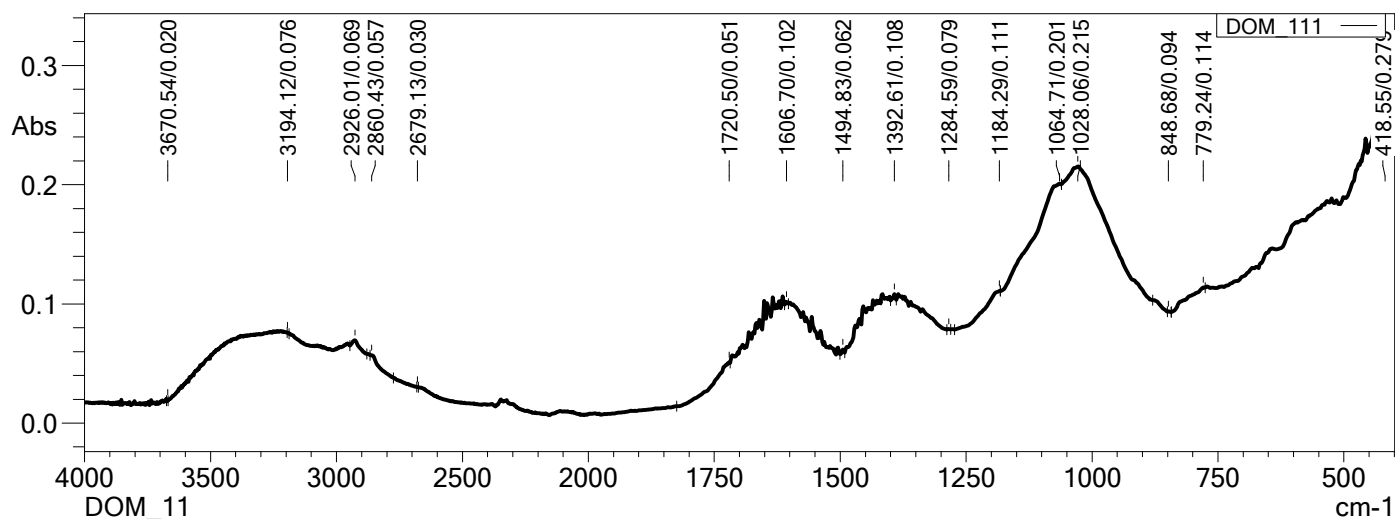
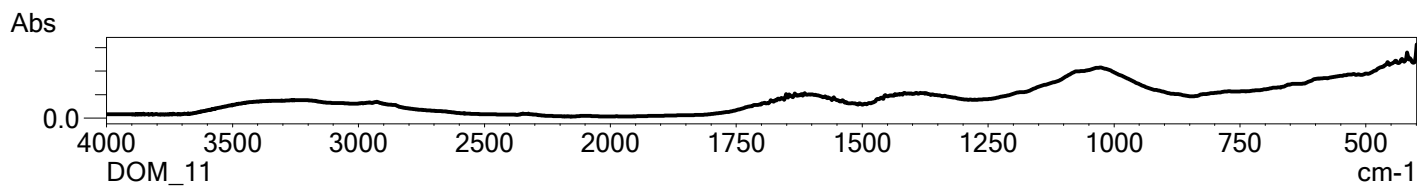
Page 347: GN1 Control soil	Page 367: GN1 HCDI
Page 348: GN3 Control soil	Page 368: GN3 HCDI
Page 349: GN4 Control soil	Page 369: GN4 HCDI
Page 350: GN5 Control soil	Page 370: GN5 HCDI
Page 351: GW1 Control soil	Page 371: GW1 HCDI
Page 352: GW2 Control soil	Page 372: GW2 HCDI
Page 353: GW3 Control soil	Page 373: GW3 HCDI
Page 354: GW4 Control soil	Page 374: GW4 HCDI
Page 355: GW5 Control soil	Page 375: GW5 HCDI
Page 356: GQ1 Control soil	Page 376: GQ1 HCDI
Page 357: GQ2 Control soil	Page 377: GQ2 HCDI
Page 359: GQ3 Control soil	Page 379: GQ3 HCDI
Page 360: GQ4 Control soil	Page 380: GQ4 HCDI
Page 361: GQ5 Control soil	Page 381: GQ5 HCDI
Page 362: GC1 Control soil	Page 382: GC1 HCDI
Page 363: GC2 Control soil	Page 383: GC2 HCDI
Page 364: GC3 Control soil	Page 384: GC3 HCDI
Page 365: GC4 Control soil	Page 385: GC4 HCDI
Page 366: GC5 Control soil	Page 386: GC5 HCDI

DOM_suolo_HC_t0



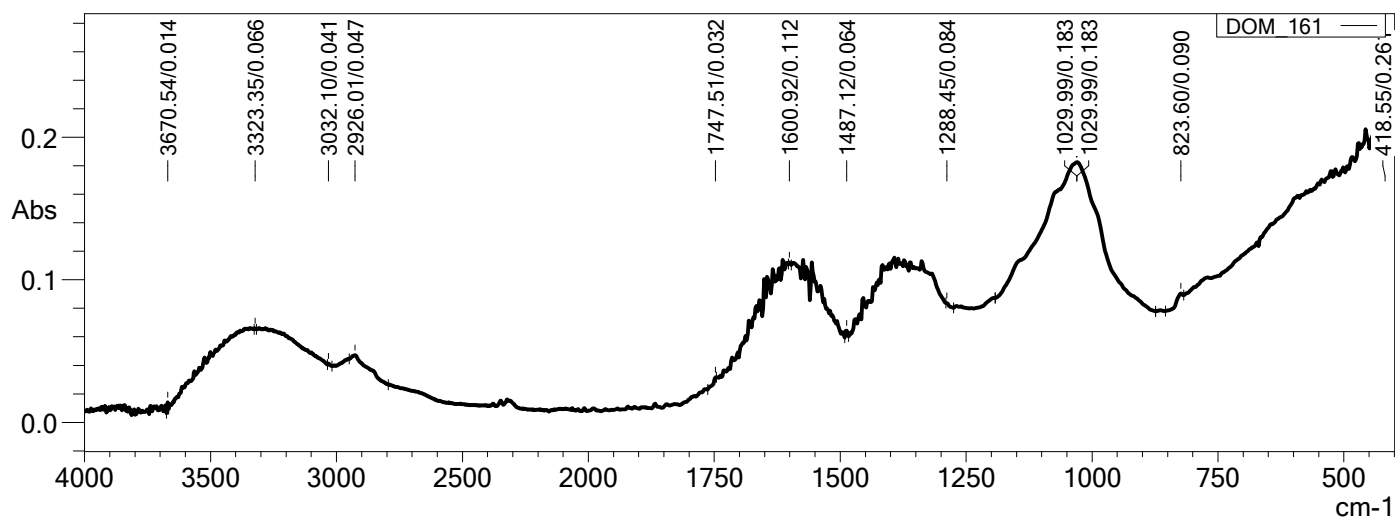
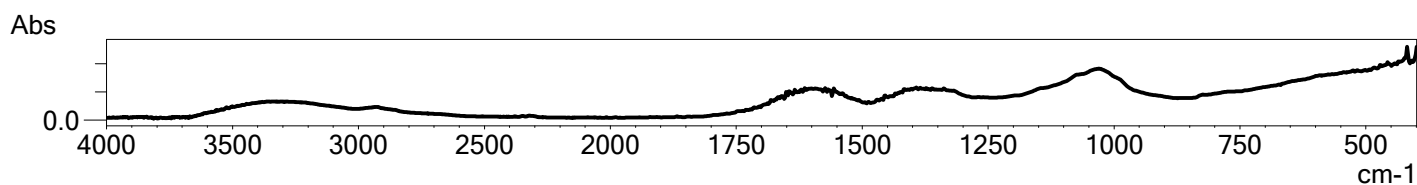
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	418.55	0.256	0.037	424.34	403.12	4.786	0.265	
2	644.22	0.128	0.004	651.94	626.87	3.151	0.033	
3	692.44	0.132	0.016	709.80	669.30	4.985	0.274	
4	769.60	0.141	0.041	817.82	709.80	13.186	2.237	
5	827.46	0.098	0.015	842.89	817.82	2.170	0.124	
6	1033.85	0.204	0.001	1132.21	1031.92	16.953	0.312	
7	1134.14	0.128	0.001	1180.44	1132.21	5.413	0.074	
8	1188.15	0.096	0.005	1228.66	1180.44	4.104	-0.007	
9	1336.67	0.154	0.008	1340.53	1228.66	12.000	-0.624	
10	1492.90	0.051	0.001	1500.62	1490.97	0.480	0.006	
11	1539.20	0.054	0.002	1541.12	1533.41	0.396	0.008	
12	1606.70	0.082	0.001	1610.56	1602.85	0.625	0.004	
13	1720.50	0.040	0.001	1816.94	1718.58	2.473	-0.141	
14	2663.69	0.027	0.000	2667.55	2642.48	0.647	0.007	
15	3061.03	0.070	0.001	3066.82	3057.17	0.675	0.005	
16	3207.62	0.081	0.000	3209.55	3197.98	0.933	0.002	
17	3657.04	0.022	0.001	3668.61	3655.11	0.280	0.004	

DOM_suolo_HC_t0



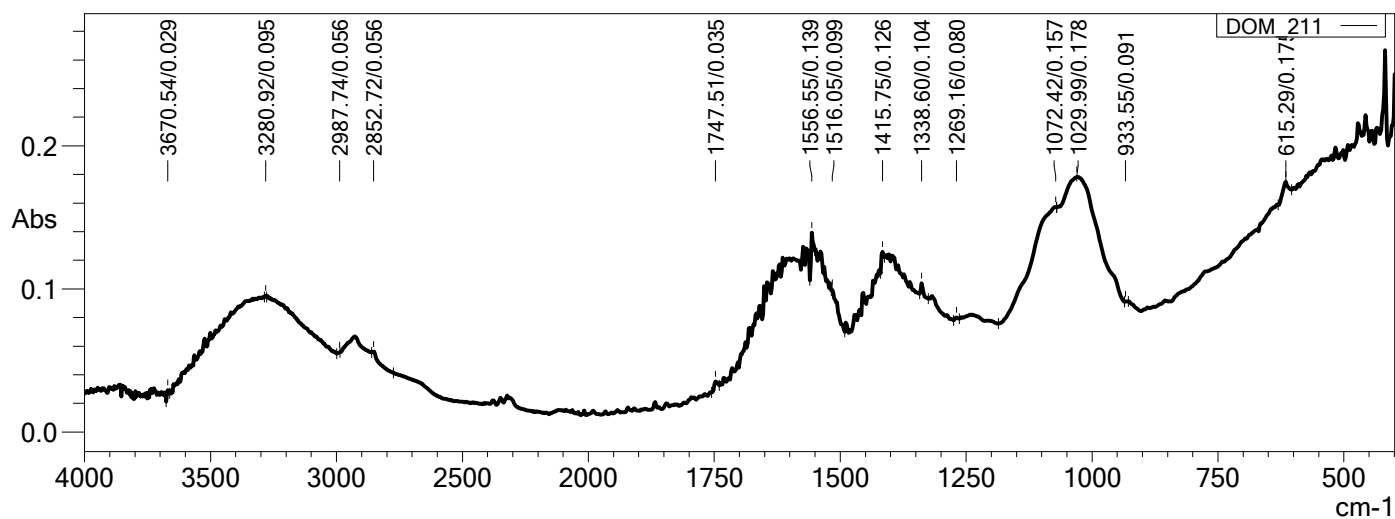
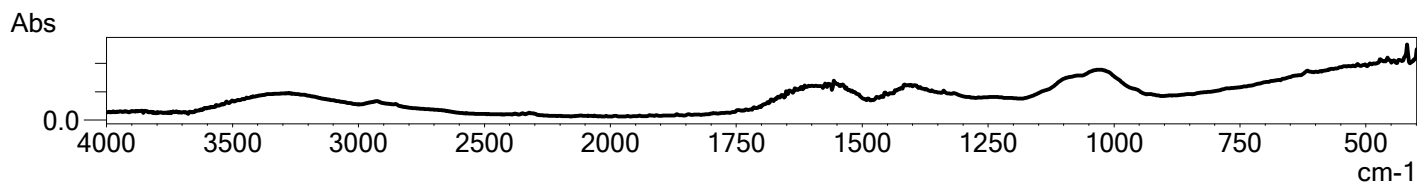
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	418.55	0.279	0.034	424.34	414.70	2.478	0.116	
2	779.24	0.114	0.001	842.89	775.38	7.071	0.093	
3	848.68	0.094	0.000	850.61	842.89	0.721	0.001	
4	1028.06	0.215	0.033	1060.85	879.54	29.600	2.083	
5	1064.71	0.201	0.003	1182.36	1060.85	18.885	-0.019	
6	1184.29	0.111	0.001	1273.02	1182.36	8.307	-0.271	
7	1284.59	0.079	0.000	1288.45	1280.73	0.608	0.002	
8	1392.61	0.108	0.005	1400.32	1388.75	1.217	0.023	
9	1494.83	0.062	0.003	1500.62	1490.97	0.575	0.012	
10	1606.70	0.102	0.002	1610.56	1602.85	0.777	0.007	
11	1720.50	0.051	0.001	1824.66	1718.58	2.995	-0.439	
12	2679.13	0.030	0.000	2681.05	2675.27	0.175	0.001	
13	2860.43	0.057	0.001	2866.22	2773.64	4.281	-0.110	
14	2926.01	0.069	0.007	2947.23	2879.72	4.330	0.179	
15	3194.12	0.076	0.000	3196.05	3188.33	0.584	0.001	
16	3670.54	0.020	0.001	3674.39	3668.61	0.110	0.002	

DOM_suolo_HC_t0



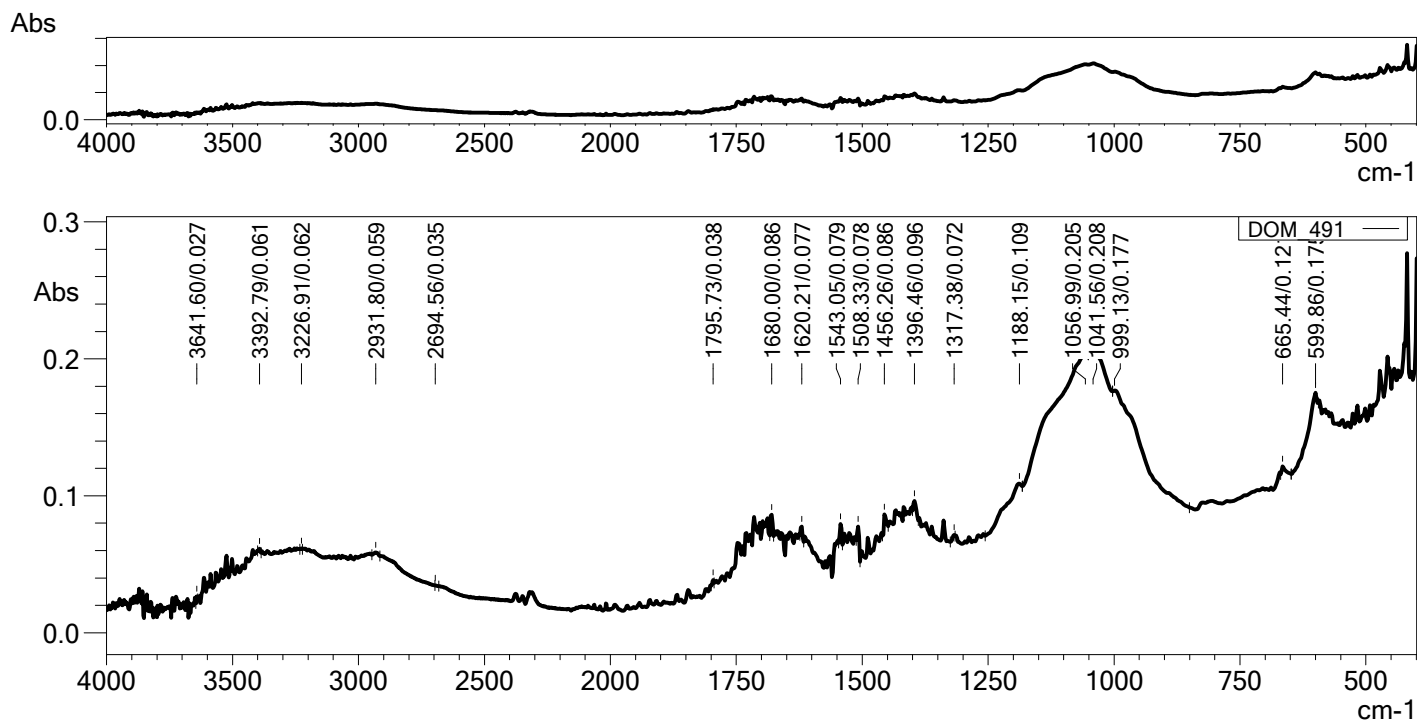
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	418.55	0.261	0.048	422.41	412.77	2.202	0.173	
2	823.60	0.090	0.003	854.47	817.82	3.055	-0.015	
3	1029.99	0.183	0.100	1192.01	873.75	39.551	13.292	
4	1029.99	0.183	0.100	1192.01	873.75	39.551	13.292	
5	1288.45	0.084	0.000	1290.38	1274.95	1.263	-0.003	
6	1487.12	0.064	0.004	1490.97	1483.26	0.483	0.021	
7	1600.92	0.112	0.001	1602.85	1597.06	0.645	0.005	
8	1747.51	0.032	0.001	1762.94	1745.58	0.468	-0.005	
9	2926.01	0.047	0.005	2949.16	2794.85	5.764	0.286	
10	3032.10	0.041	0.001	3035.96	3018.60	0.701	0.007	
11	3323.35	0.066	0.001	3327.21	3317.56	0.632	0.003	
12	3670.54	0.014	0.006	3674.39	3666.68	0.082	0.021	

DOM_suolo_HC_t0

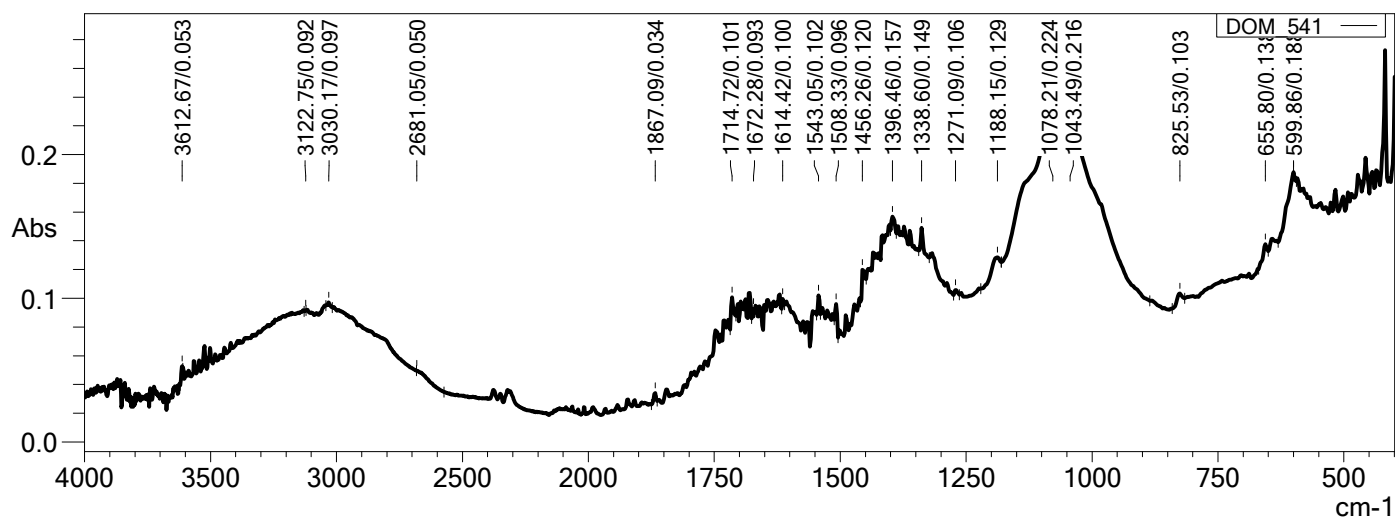
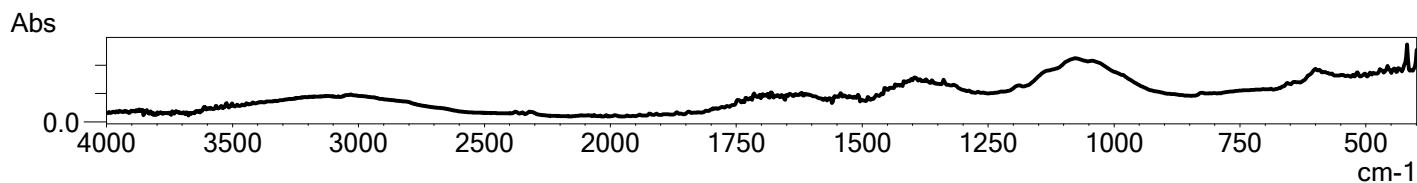


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	615.29	0.175	0.010	630.72	603.72	4.545	0.115	
2	933.55	0.091	0.000	935.48	927.76	0.703	0.001	
3	1029.99	0.178	0.041	1070.49	935.48	19.544	2.804	
4	1072.42	0.157	0.002	1186.22	1070.49	13.372	-0.101	
5	1269.16	0.080	0.001	1274.95	1263.37	0.918	0.009	
6	1338.60	0.104	0.008	1342.46	1325.10	1.683	0.035	
7	1415.75	0.126	0.009	1419.61	1411.89	0.942	0.043	
8	1516.05	0.099	0.003	1517.98	1490.97	2.291	0.014	
9	1556.55	0.139	0.025	1560.41	1550.77	1.230	0.108	
10	1747.51	0.035	0.005	1755.22	1739.79	0.500	0.035	
11	2852.72	0.056	0.002	2860.43	2773.64	4.096	-0.107	
12	2987.74	0.056	0.000	2999.31	2985.81	0.747	0.001	
13	3280.92	0.095	0.001	3286.70	3277.06	0.914	0.004	
14	3670.54	0.029	0.006	3676.32	3662.82	0.367	0.041	

DOM_suolo_HC_t0

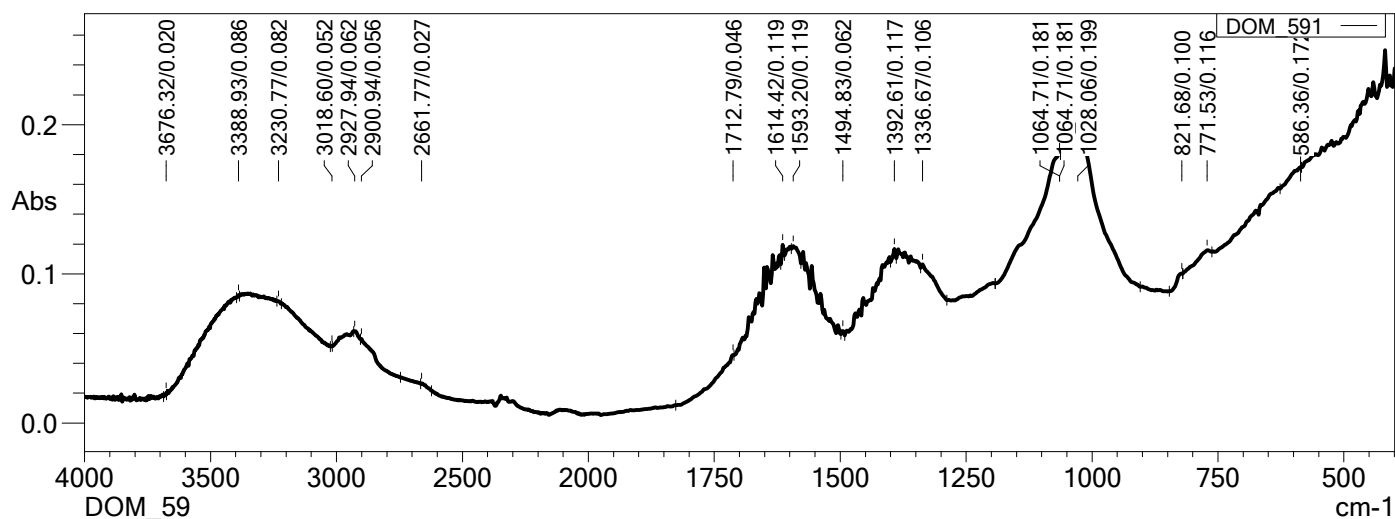
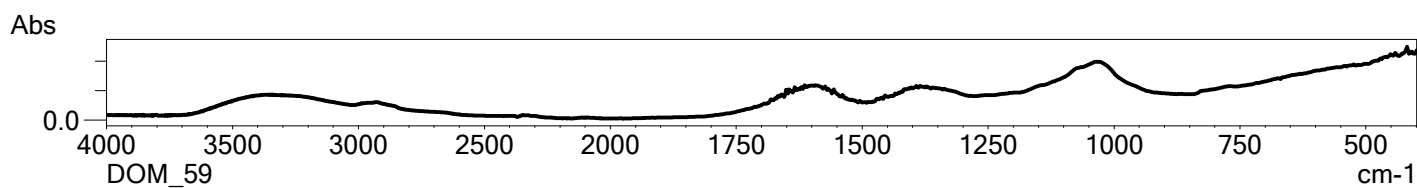


DOM_suolo_HC_t0



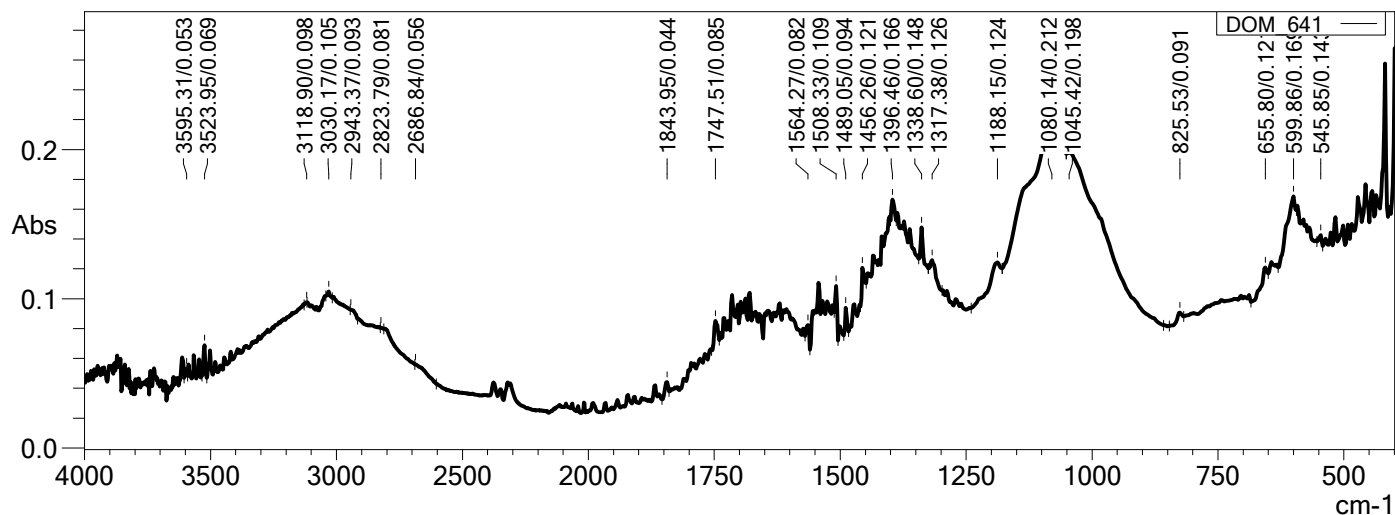
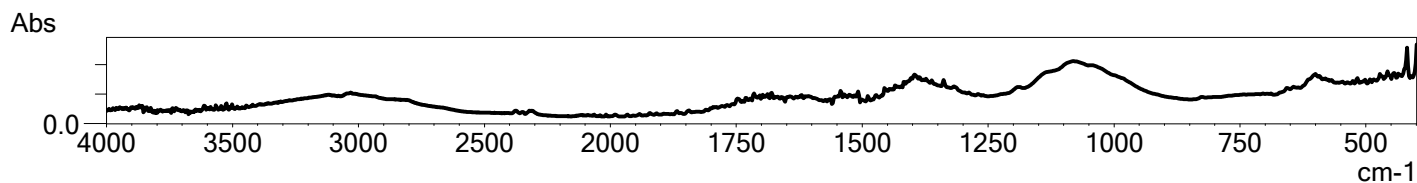
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	599.86	0.188	0.013	630.72	594.08	6.040	0.170	
2	655.80	0.138	0.009	669.30	650.01	2.510	0.070	
3	825.53	0.103	0.006	840.96	815.89	2.480	0.063	
4	1043.49	0.216	0.008	1051.20	885.33	25.183	-0.678	
5	1078.21	0.224	0.029	1180.44	1051.20	24.033	2.135	
6	1188.15	0.129	0.007	1220.94	1180.44	4.772	0.077	
7	1271.09	0.106	0.003	1274.95	1263.37	1.206	0.021	
8	1338.60	0.149	0.017	1344.38	1323.17	2.858	0.084	
9	1396.46	0.157	0.010	1400.32	1388.75	1.766	0.074	
10	1456.26	0.120	0.017	1460.11	1448.54	1.299	0.078	
11	1508.33	0.096	0.017	1512.19	1504.48	0.685	0.077	
12	1543.05	0.102	0.013	1546.91	1539.20	0.736	0.052	
13	1614.42	0.100	0.006	1616.35	1610.56	0.562	0.017	
14	1672.28	0.093	0.004	1676.14	1670.35	0.521	0.012	
15	1714.72	0.101	0.019	1718.58	1707.00	1.063	0.103	
16	1867.09	0.034	0.006	1874.81	1863.24	0.348	0.033	
17	2681.05	0.050	0.000	2682.98	2573.04	4.633	0.002	
18	3030.17	0.097	0.003	3041.74	3016.67	2.389	0.029	
19	3122.75	0.092	0.000	3128.54	3120.82	0.704	0.001	
20	3612.67	0.053	0.004	3620.39	3610.74	0.465	0.029	

DOM_suolo_HC_t0



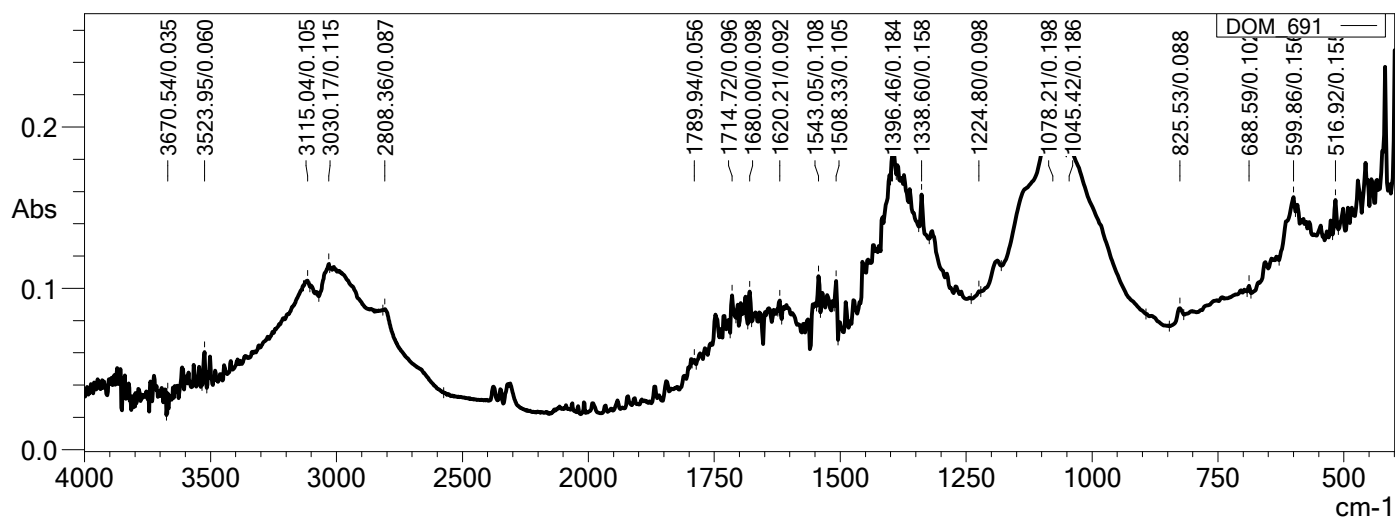
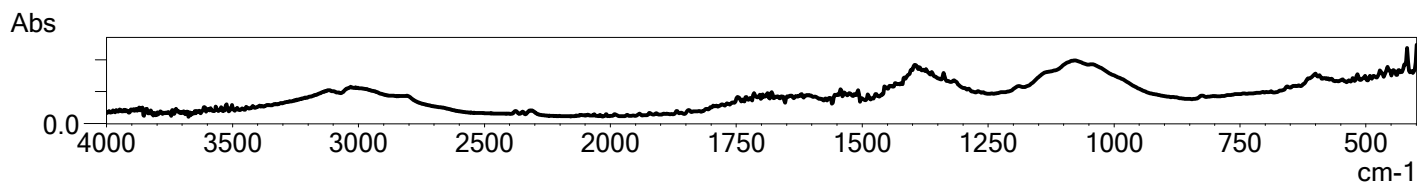
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	586.36	0.172	0.001	626.87	584.43	7.005	0.038	
2	771.53	0.116	0.004	819.75	761.88	6.313	0.094	
3	821.68	0.100	0.001	846.75	819.75	2.542	-0.002	
4	1028.06	0.199	0.004	1031.92	904.61	16.877	-1.522	
5	1064.71	0.181	0.002	1192.01	1062.78	16.976	-0.705	
6	1064.71	0.181	0.002	1192.01	1062.78	16.976	-0.705	
7	1336.67	0.106	0.004	1340.53	1288.45	4.955	0.097	
8	1392.61	0.117	0.007	1400.32	1388.75	1.297	0.035	
9	1494.83	0.062	0.003	1498.69	1490.97	0.468	0.011	
10	1593.20	0.119	0.004	1597.06	1577.77	2.232	0.073	
11	1614.42	0.119	0.010	1618.28	1610.56	0.877	0.033	
12	1712.79	0.046	0.001	1826.59	1710.86	2.845	-0.477	
13	2661.77	0.027	0.001	2665.62	2623.19	1.039	0.023	
14	2900.94	0.056	0.001	2904.80	2746.63	6.476	-0.391	
15	2927.94	0.062	0.001	2931.80	2904.80	1.606	0.019	
16	3018.60	0.052	0.000	3024.38	3016.67	0.397	0.001	
17	3230.77	0.082	0.001	3236.55	3219.19	1.408	0.006	
18	3388.93	0.086	0.001	3396.64	3385.07	0.983	0.005	
19	3676.32	0.020	0.001	3685.97	3674.39	0.219	0.007	

DOM_suolo_HC_t0



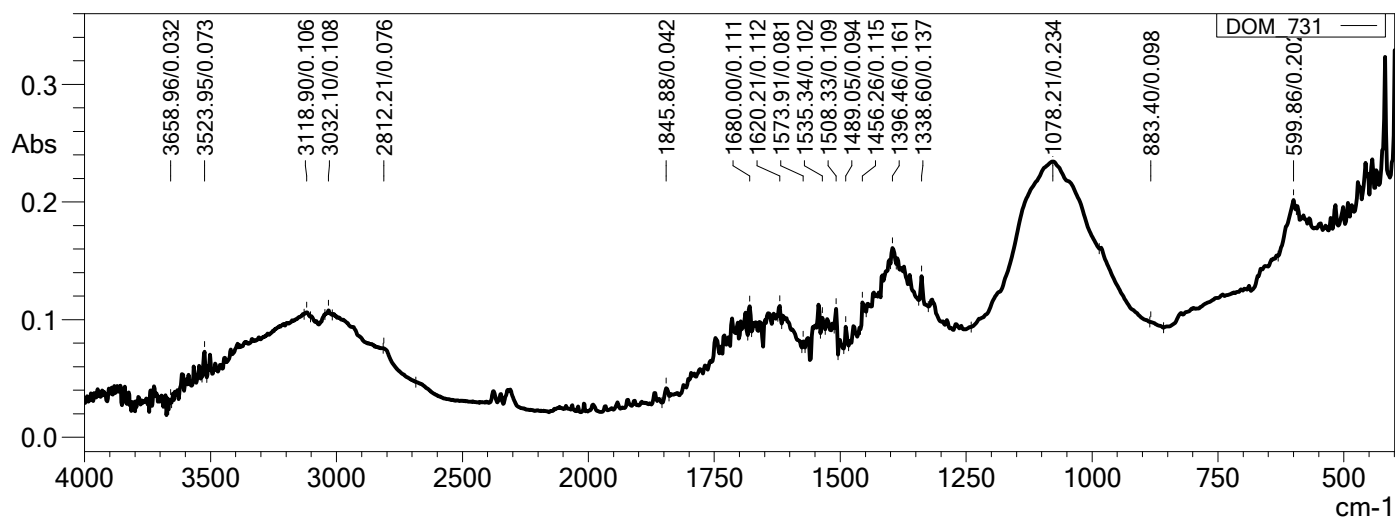
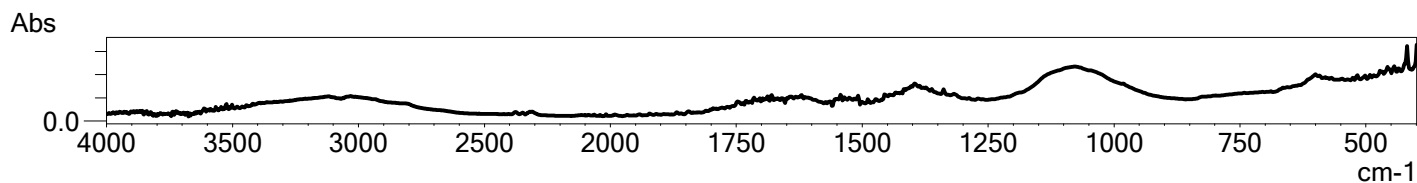
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	545.85	0.143	0.006	553.57	542.00	1.618	0.035	
2	599.86	0.169	0.015	630.72	594.08	5.401	0.266	
3	655.80	0.121	0.007	684.73	650.01	3.746	0.021	
4	825.53	0.091	0.004	846.75	817.82	2.482	0.025	
5	1045.42	0.198	0.004	1051.20	858.32	25.485	-1.451	
6	1080.14	0.212	0.032	1178.51	1051.20	22.746	2.534	
7	1188.15	0.124	0.008	1240.23	1178.51	6.666	0.060	
8	1317.38	0.126	0.010	1325.10	1298.09	3.143	0.093	
9	1338.60	0.148	0.023	1344.38	1325.10	2.492	0.108	
10	1396.46	0.166	0.014	1400.32	1388.75	1.854	0.092	
11	1456.26	0.121	0.021	1460.11	1448.54	1.282	0.094	
12	1489.05	0.094	0.017	1492.90	1483.26	0.809	0.070	
13	1508.33	0.109	0.027	1512.19	1504.48	0.744	0.116	
14	1564.27	0.082	0.013	1570.06	1560.41	0.763	0.083	
15	1747.51	0.085	0.016	1755.22	1739.79	1.182	0.120	
16	1843.95	0.044	0.008	1853.59	1840.09	0.528	0.052	
17	2686.84	0.056	0.000	2688.77	2603.90	4.272	0.075	
18	2823.79	0.081	0.000	2825.72	2812.21	1.083	0.000	
19	2943.37	0.093	0.000	2945.30	2916.37	2.628	0.036	
20	3030.17	0.105	0.004	3041.74	3016.67	2.570	0.033	
21	3118.90	0.098	0.000	3128.54	3116.97	1.124	0.005	
22	3523.95	0.069	0.021	3533.59	3514.30	1.087	0.170	
23	3595.31	0.053	0.005	3604.96	3591.46	0.686	0.044	

DOM_suolo_HC_t0



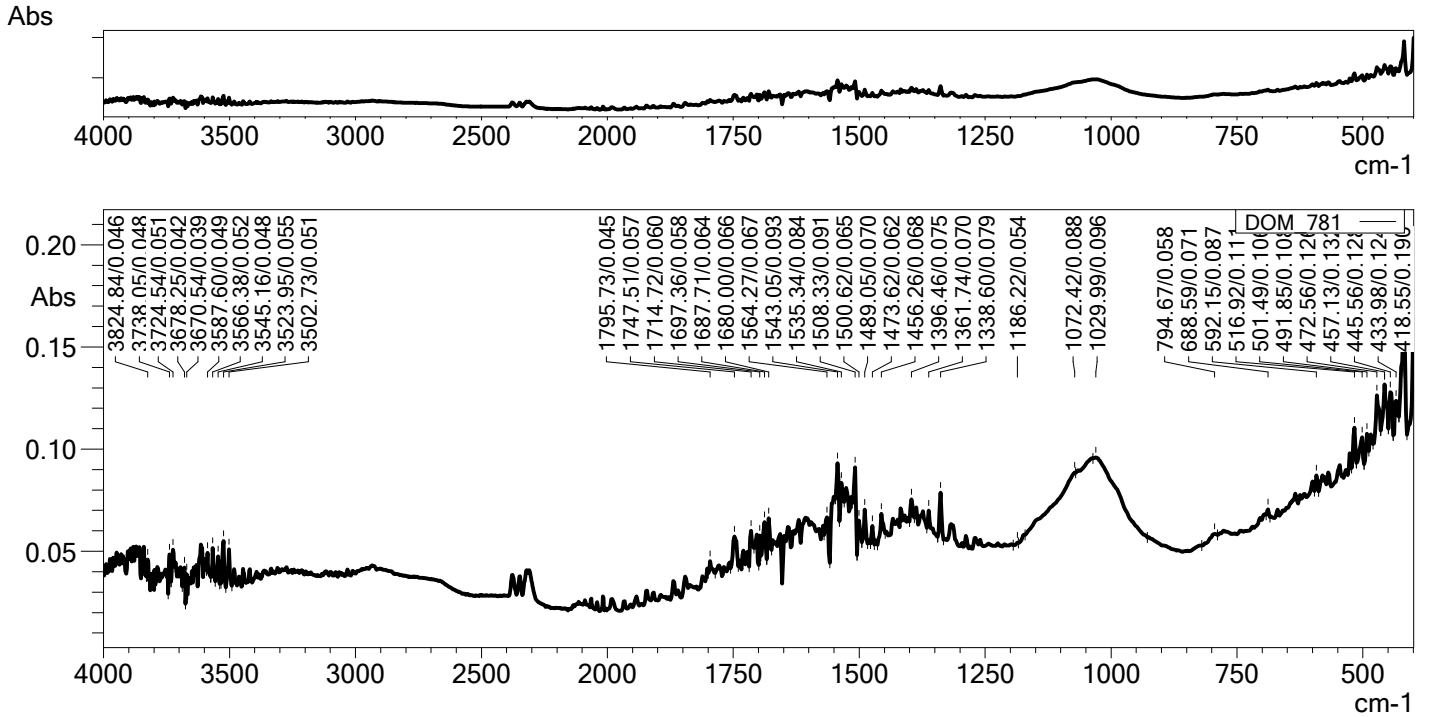
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	516.92	0.155	0.020	522.71	511.14	1.658	0.096	
2	599.86	0.156	0.012	628.79	596.00	4.579	0.235	
3	688.59	0.102	0.004	692.44	684.73	0.767	0.015	
4	825.53	0.088	0.006	846.75	817.82	2.363	0.046	
5	1045.42	0.186	0.005	1051.20	893.04	20.667	-0.578	
6	1078.21	0.198	0.028	1180.44	1051.20	21.409	2.113	
7	1224.80	0.098	0.001	1240.23	1220.94	1.847	0.001	
8	1338.60	0.158	0.022	1344.38	1323.17	2.948	0.095	
9	1396.46	0.184	0.016	1400.32	1388.75	2.067	0.112	
10	1508.33	0.105	0.026	1512.19	1504.48	0.716	0.110	
11	1543.05	0.108	0.020	1546.91	1539.20	0.757	0.085	
12	1620.21	0.092	0.009	1624.06	1616.35	0.676	0.033	
13	1680.00	0.098	0.019	1683.86	1676.14	0.698	0.092	
14	1714.72	0.096	0.020	1718.58	1707.00	0.997	0.104	
15	1789.94	0.056	0.002	1793.80	1786.08	0.418	0.007	
16	2808.36	0.087	0.002	2816.07	2574.97	13.441	-1.241	
17	3030.17	0.115	0.004	3070.68	3026.31	4.717	0.106	
18	3115.04	0.105	0.004	3130.47	3105.39	2.592	0.052	
19	3523.95	0.060	0.021	3533.59	3514.30	0.930	0.177	
20	3670.54	0.035	0.011	3674.39	3666.68	0.214	0.033	

DOM_suolo_HC_t0



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	599.86	0.202	0.014	630.72	594.08	6.588	0.217	
2	883.40	0.098	0.000	885.33	858.32	2.590	0.009	
3	1078.21	0.234	0.098	1240.23	985.62	44.805	12.484	
4	1338.60	0.137	0.022	1344.38	1325.10	2.293	0.098	
5	1396.46	0.161	0.013	1400.32	1388.75	1.797	0.090	
6	1456.26	0.115	0.018	1460.11	1448.54	1.234	0.086	
7	1489.05	0.094	0.018	1492.90	1483.26	0.810	0.070	
8	1508.33	0.109	0.028	1512.19	1504.48	0.744	0.114	
9	1535.34	0.102	0.012	1539.20	1529.55	0.922	0.052	
10	1573.91	0.081	0.005	1575.84	1570.06	0.461	0.020	
11	1620.21	0.112	0.012	1624.06	1616.35	0.815	0.044	
12	1680.00	0.111	0.023	1683.86	1676.14	0.795	0.113	
13	1845.88	0.042	0.009	1853.59	1840.09	0.491	0.056	
14	2812.21	0.076	0.000	2814.14	2684.91	7.536	-0.381	
15	3032.10	0.108	0.004	3045.60	3016.67	3.053	0.040	
16	3118.90	0.106	0.003	3128.54	3103.46	2.629	0.035	
17	3523.95	0.073	0.022	3533.59	3514.30	1.163	0.178	
18	3658.96	0.032	0.004	3666.68	3655.11	0.345	0.038	

DOM_suolo_HC_t0

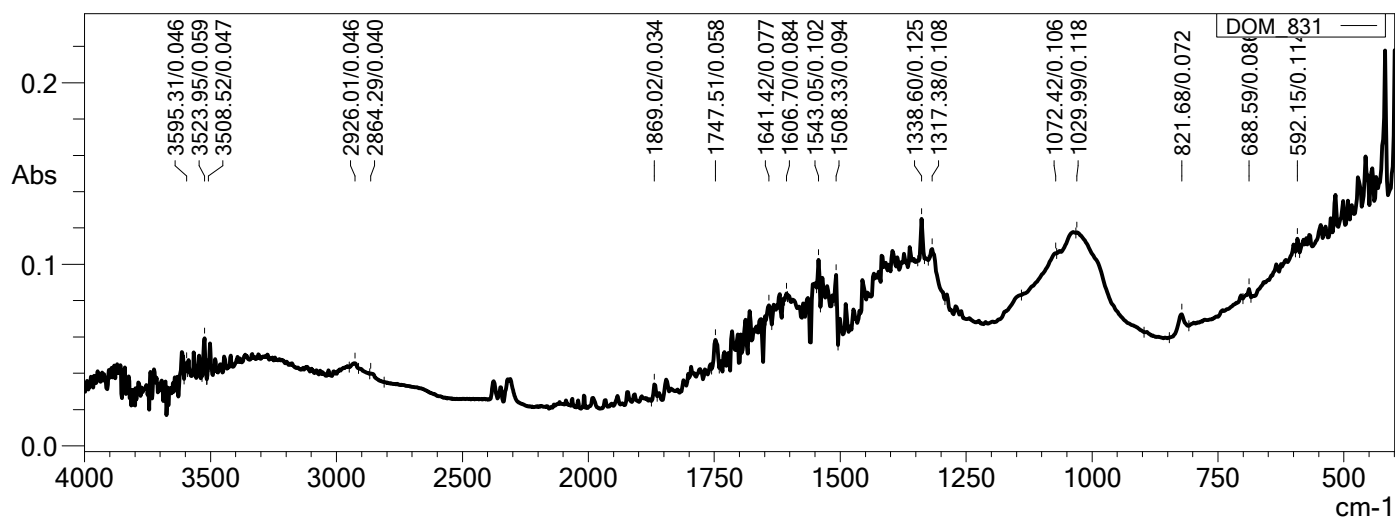
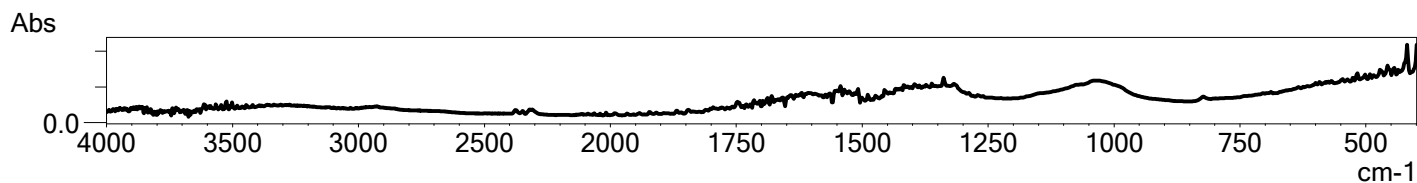


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	418.55	0.190	0.080	428.20	412.77	2.185	0.465	
2	433.98	0.124	0.012	439.77	428.20	1.348	0.060	
3	445.56	0.128	0.019	449.41	439.77	1.167	0.122	
4	457.13	0.132	0.022	464.84	449.41	1.845	0.154	
5	472.56	0.126	0.020	480.28	464.84	1.767	0.134	
6	491.85	0.108	0.011	497.63	487.99	0.967	0.043	
7	501.49	0.106	0.013	513.07	497.63	1.521	0.086	
8	516.92	0.111	0.018	522.71	513.07	0.975	0.088	
9	592.15	0.087	0.008	596.00	588.29	0.642	0.029	
10	688.59	0.071	0.003	692.44	684.73	0.530	0.011	
11	794.67	0.058	0.001	819.75	788.89	1.726	0.017	
12	1029.99	0.096	0.002	1035.77	927.76	8.246	0.023	
13	1072.42	0.088	0.001	1170.79	1070.49	7.239	-0.104	
14	1186.22	0.054	0.000	1193.94	1184.29	0.517	0.000	
15	1338.60	0.079	0.022	1344.38	1332.81	0.758	0.106	
16	1361.74	0.070	0.009	1365.60	1344.38	1.303	0.046	
17	1396.46	0.075	0.010	1400.32	1390.68	0.680	0.049	
18	1456.26	0.068	0.012	1463.97	1448.54	0.938	0.074	
19	1473.62	0.062	0.009	1477.47	1469.76	0.445	0.032	
20	1489.05	0.070	0.016	1494.83	1483.26	0.695	0.069	
21	1500.62	0.065	0.014	1504.48	1494.83	0.574	0.079	
22	1508.33	0.091	0.033	1514.12	1504.48	0.732	0.145	
23	1535.34	0.084	0.015	1539.20	1529.55	0.736	0.066	
24	1543.05	0.093	0.023	1546.91	1539.20	0.640	0.098	
25	1564.27	0.067	0.015	1570.06	1558.48	0.693	0.100	
26	1680.00	0.066	0.018	1683.86	1674.21	0.551	0.084	
27	1687.71	0.064	0.018	1695.43	1683.86	0.633	0.091	
28	1697.36	0.058	0.012	1699.29	1695.43	0.199	0.024	
29	1714.72	0.060	0.014	1718.58	1708.93	0.521	0.068	
30	1747.51	0.057	0.015	1755.22	1739.79	0.780	0.129	
31	1795.73	0.045	0.006	1799.59	1786.08	0.556	0.025	
32	3502.73	0.051	0.014	3506.59	3491.16	0.624	0.074	
33	3523.95	0.055	0.022	3533.59	3514.30	0.819	0.176	
34	3545.16	0.048	0.012	3550.95	3539.38	0.471	0.054	
35	3566.38	0.052	0.015	3574.10	3556.74	0.726	0.096	
36	3587.60	0.049	0.009	3591.46	3574.10	0.715	0.038	
37	3670.54	0.039	0.012	3674.39	3666.68	0.240	0.034	
38	3678.25	0.042	0.015	3687.90	3674.39	0.505	0.119	
39	3724.54	0.051	0.012	3732.26	3716.83	0.685	0.087	
40	3738.05	0.048	0.016	3743.83	3732.26	0.492	0.120	
41	3824.84	0.046	0.009	3830.63	3820.98	0.417	0.062	

DOM_suolo_HC_t0

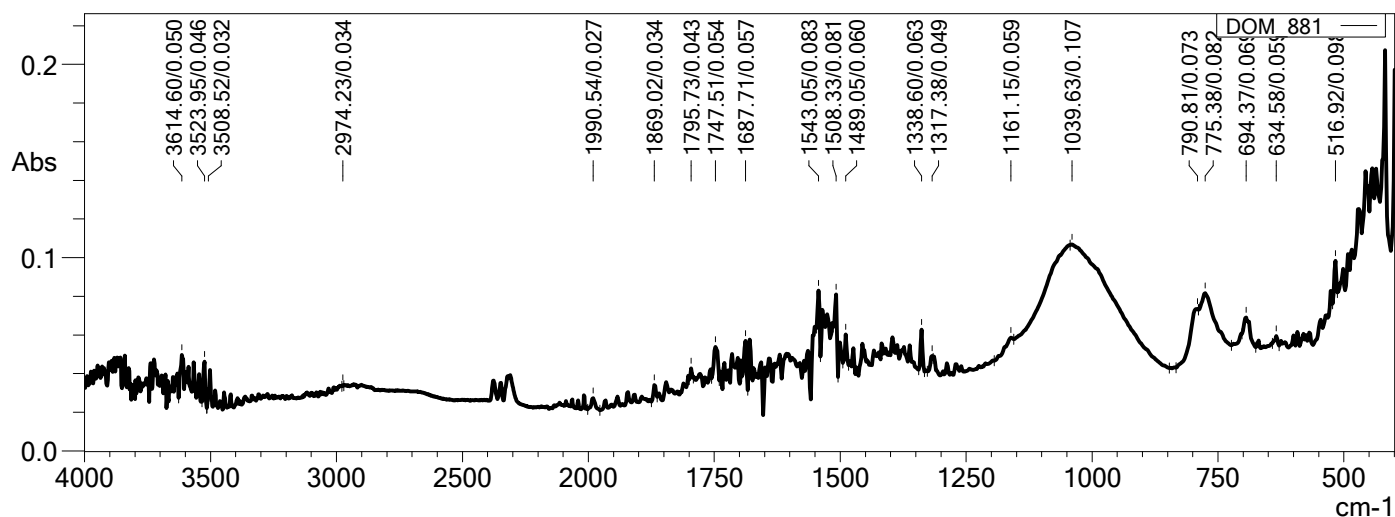
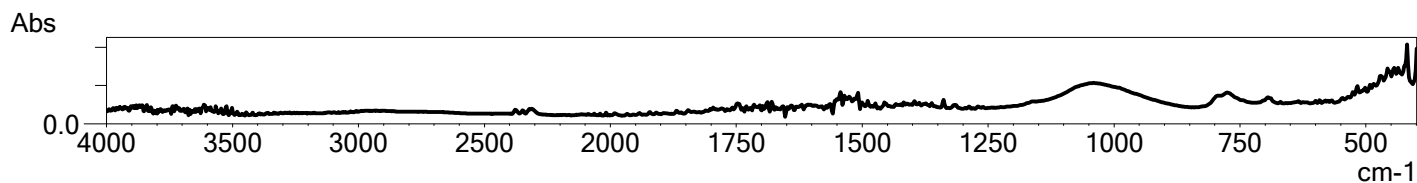
31	1795.73	0.045	0.006	1799.59	1786.08	0.556	0.025	
32	3502.73	0.051	0.014	3506.59	3491.16	0.624	0.074	
33	3523.95	0.055	0.022	3533.59	3514.30	0.819	0.176	
34	3545.16	0.048	0.012	3550.95	3539.38	0.471	0.054	
35	3566.38	0.052	0.015	3574.10	3556.74	0.726	0.096	
36	3587.60	0.049	0.009	3591.46	3574.10	0.715	0.038	
37	3670.54	0.039	0.012	3674.39	3666.68	0.240	0.034	
38	3678.25	0.042	0.015	3687.90	3674.39	0.505	0.119	
39	3724.54	0.051	0.012	3732.26	3716.83	0.685	0.087	
40	3738.05	0.048	0.016	3743.83	3732.26	0.492	0.120	
41	3824.84	0.046	0.009	3830.63	3820.98	0.417	0.062	

DOM_suolo_HC_t0



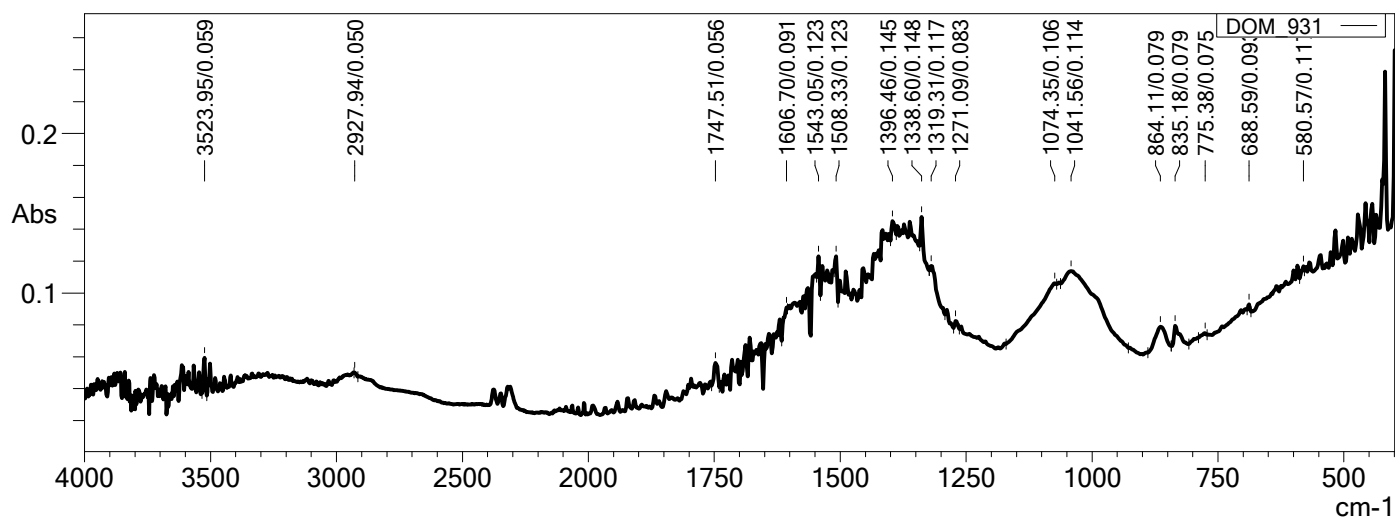
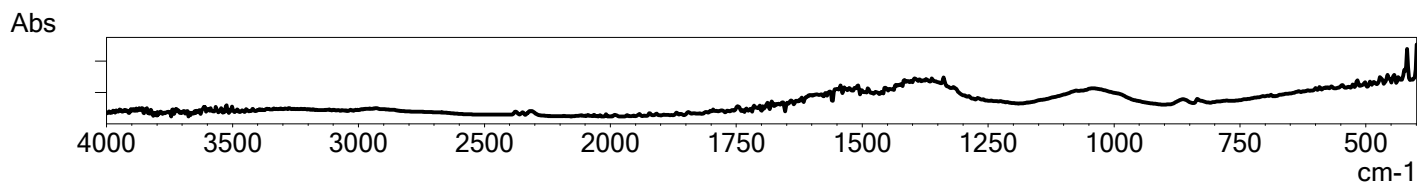
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	592.15	0.114	0.007	596.00	588.29	0.851	0.028	
2	688.59	0.086	0.005	700.16	684.73	1.287	0.031	
3	821.68	0.072	0.009	846.75	808.17	2.532	0.109	
4	1029.99	0.118	0.001	1031.92	896.90	11.663	-0.484	
5	1072.42	0.106	0.001	1139.93	1070.49	6.473	-0.088	
6	1317.38	0.108	0.011	1325.10	1292.31	3.131	0.134	
7	1338.60	0.125	0.022	1346.31	1332.81	1.485	0.100	
8	1508.33	0.094	0.026	1512.19	1504.48	0.629	0.107	
9	1543.05	0.102	0.021	1546.91	1539.20	0.714	0.083	
10	1606.70	0.084	0.002	1610.56	1604.77	0.477	0.007	
11	1641.42	0.077	0.009	1647.21	1635.64	0.853	0.065	
12	1747.51	0.058	0.016	1755.22	1739.79	0.789	0.132	
13	1869.02	0.034	0.008	1874.81	1863.24	0.341	0.040	
14	2864.29	0.040	0.000	2868.15	2810.28	2.159	-0.006	
15	2926.01	0.046	0.003	2949.16	2912.51	1.629	0.051	
16	3508.52	0.047	0.005	3514.30	3506.59	0.330	0.021	
17	3523.95	0.059	0.022	3533.59	3514.30	0.901	0.179	
18	3595.31	0.046	0.006	3604.96	3591.46	0.580	0.055	

DOM_suolo_HC_t0



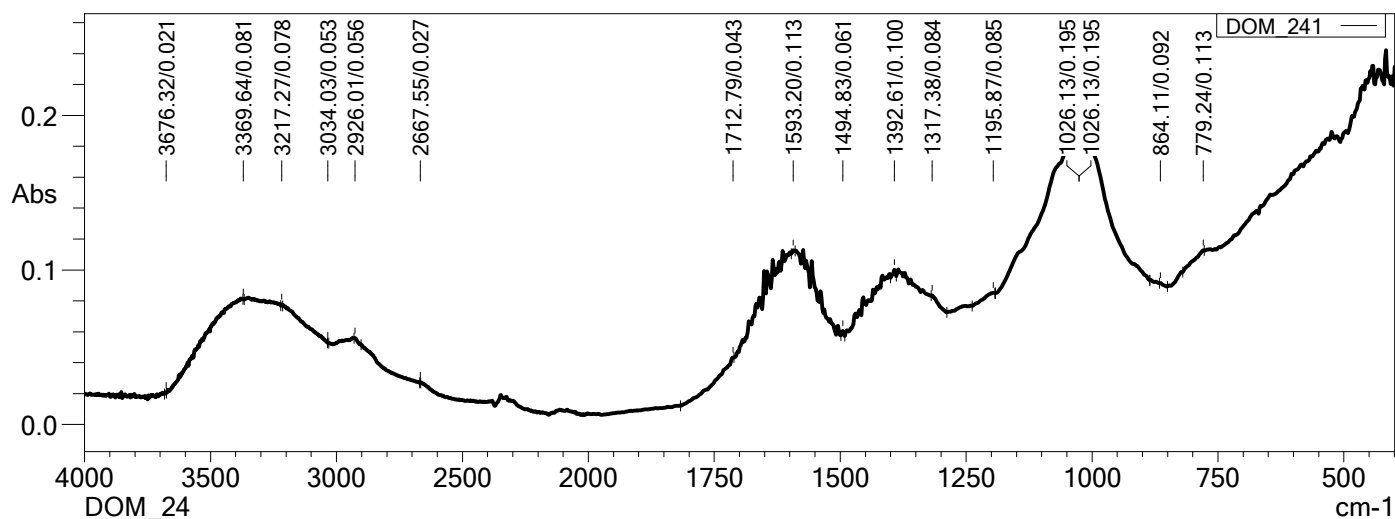
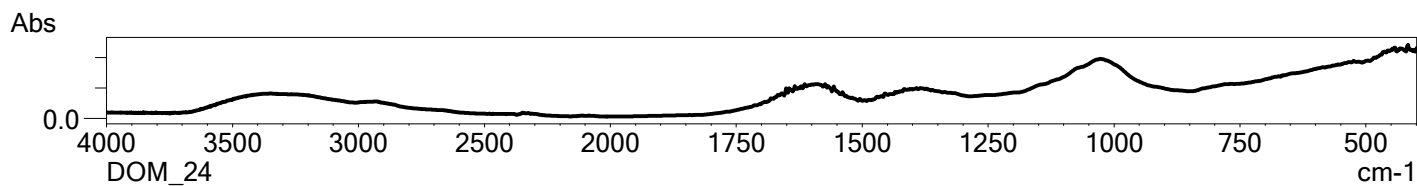
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	516.92	0.098	0.019	522.71	513.07	0.850	0.087	
2	634.58	0.059	0.004	642.30	628.79	0.770	0.027	
3	694.37	0.069	0.010	702.09	675.09	1.672	0.129	
4	775.38	0.082	0.012	788.89	723.31	4.436	0.249	
5	790.81	0.073	0.002	833.25	788.89	2.491	-0.085	
6	1039.63	0.107	0.002	1043.49	846.75	14.562	-0.126	
7	1161.15	0.059	0.002	1193.94	1155.36	2.039	0.026	
8	1317.38	0.049	0.002	1327.03	1315.45	0.521	0.006	
9	1338.60	0.063	0.021	1344.38	1332.81	0.577	0.097	
10	1489.05	0.060	0.015	1494.83	1483.26	0.583	0.063	
11	1508.33	0.081	0.032	1514.12	1504.48	0.635	0.142	
12	1543.05	0.083	0.027	1546.91	1539.20	0.553	0.120	
13	1687.71	0.057	0.023	1695.43	1683.86	0.527	0.122	
14	1747.51	0.054	0.017	1755.22	1739.79	0.723	0.152	
15	1795.73	0.043	0.005	1799.59	1791.87	0.304	0.016	
16	1869.02	0.034	0.007	1874.81	1863.24	0.347	0.037	
17	1990.54	0.027	0.006	2002.11	1977.04	0.588	0.056	
18	2974.23	0.034	0.000	2978.09	2970.38	0.261	0.002	
19	3508.52	0.032	0.005	3514.30	3506.59	0.218	0.024	
20	3523.95	0.046	0.022	3533.59	3514.30	0.647	0.187	
21	3614.60	0.050	0.008	3626.17	3610.74	0.648	0.076	

DOM_suolo_HC_t0



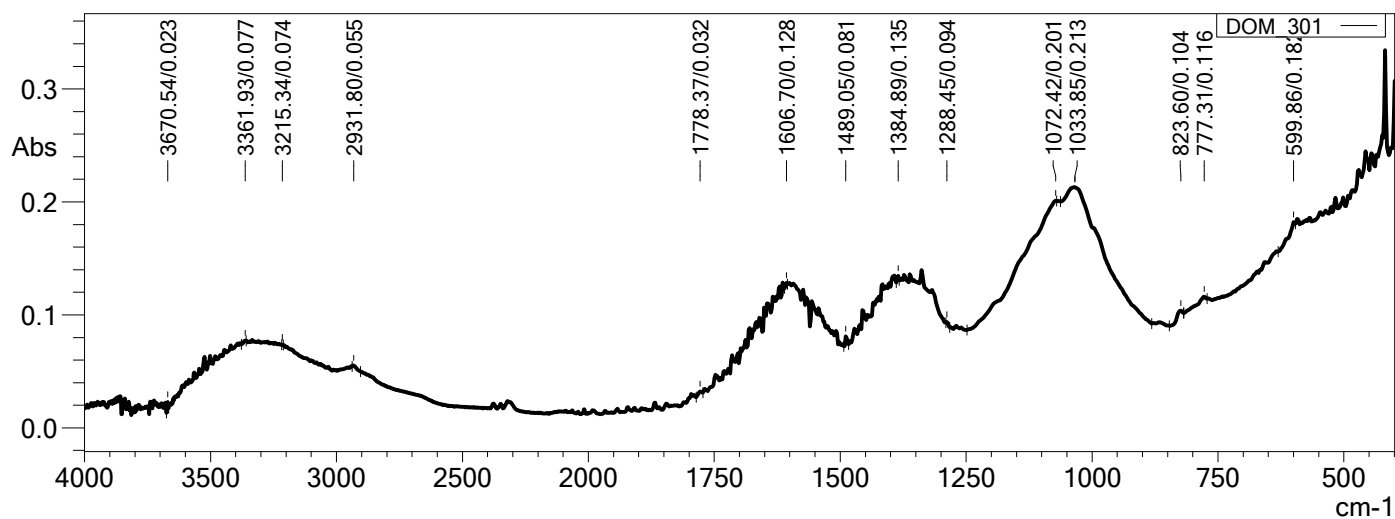
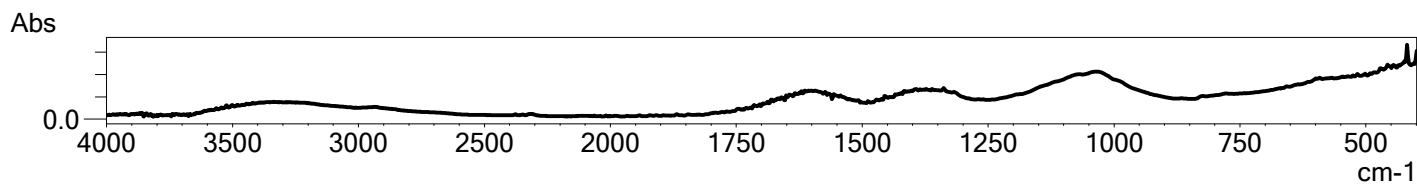
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	580.57	0.117	0.004	588.29	578.64	1.096	0.019	
2	688.59	0.093	0.004	692.44	684.73	0.700	0.014	
3	775.38	0.075	0.001	788.89	771.53	1.279	0.014	
4	835.18	0.079	0.012	842.89	808.17	2.493	0.155	
5	864.11	0.079	0.014	889.18	842.89	3.303	0.309	
6	1041.56	0.114	0.014	1062.78	927.76	12.629	1.048	
7	1074.35	0.106	0.002	1170.79	1070.49	8.625	-0.076	
8	1271.09	0.083	0.005	1274.95	1263.37	0.929	0.030	
9	1319.31	0.117	0.006	1323.17	1292.31	3.145	0.049	
10	1338.60	0.148	0.021	1342.46	1323.17	2.431	0.082	
11	1396.46	0.145	0.011	1400.32	1388.75	1.631	0.072	
12	1508.33	0.123	0.019	1512.19	1504.48	0.882	0.081	
13	1543.05	0.123	0.019	1546.91	1539.20	0.875	0.071	
14	1606.70	0.091	0.004	1616.35	1604.77	0.981	0.055	
15	1747.51	0.056	0.015	1755.22	1739.79	0.765	0.125	
16	2927.94	0.050	0.000	2929.87	2914.44	0.757	0.004	
17	3523.95	0.059	0.023	3533.59	3514.30	0.892	0.191	

DOM_suolo_HC_t0



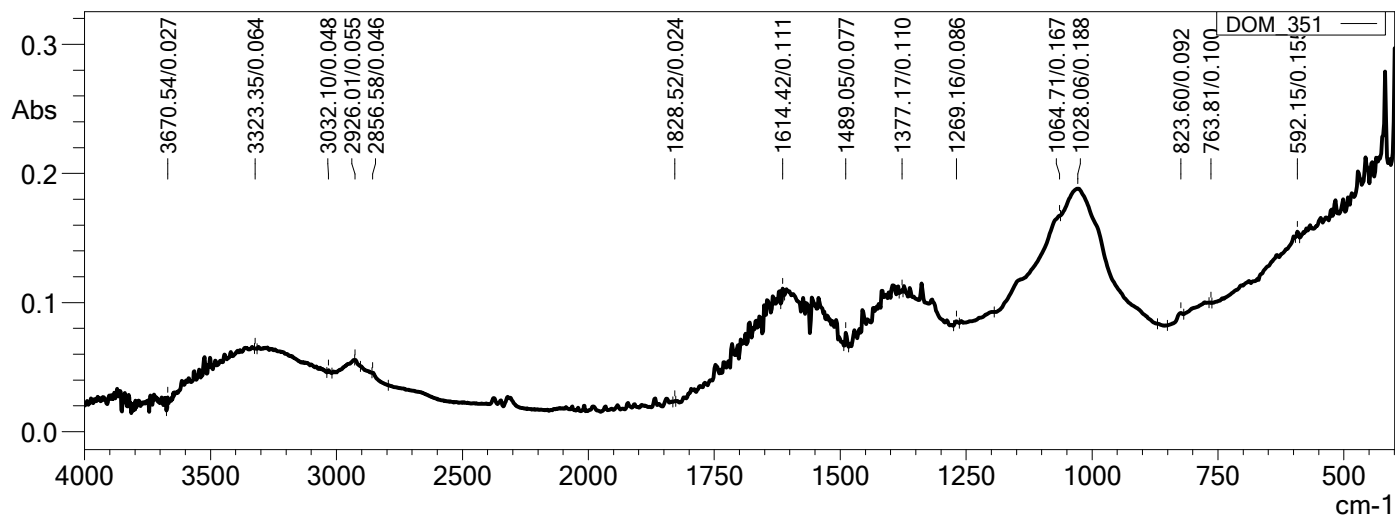
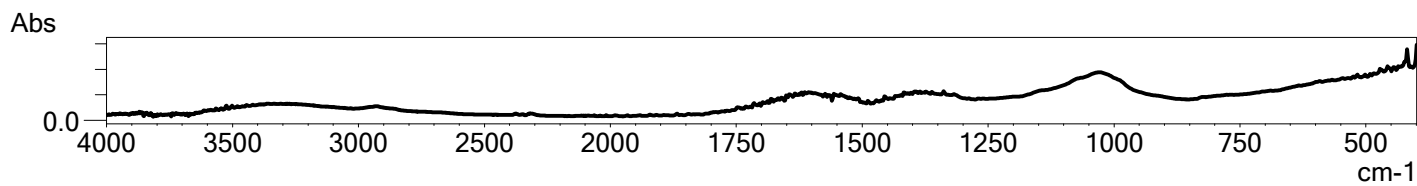
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	779.24	0.113	0.001	819.75	777.31	4.510	0.025	
2	864.11	0.092	0.000	866.04	850.61	1.394	-0.001	
3	1026.13	0.195	0.106	1192.01	885.33	41.736	14.401	
4	1026.13	0.195	0.106	1192.01	885.33	41.736	14.401	
5	1195.87	0.085	0.001	1238.30	1192.01	3.772	0.027	
6	1317.38	0.084	0.001	1319.31	1288.45	2.401	-0.004	
7	1392.61	0.100	0.004	1400.32	1388.75	1.128	0.023	
8	1494.83	0.061	0.003	1498.69	1490.97	0.457	0.014	
9	1593.20	0.113	0.001	1597.06	1589.34	0.864	0.005	
10	1712.79	0.043	0.001	1816.94	1710.86	2.637	-0.310	
11	2667.55	0.027	0.000	2669.48	2665.62	0.105	0.000	
12	2926.01	0.056	0.001	2929.87	2902.87	1.454	0.003	
13	3034.03	0.053	0.000	3035.96	3030.17	0.306	0.000	
14	3217.27	0.078	0.001	3221.12	3213.41	0.596	0.003	
15	3369.64	0.081	0.000	3371.57	3365.78	0.469	0.000	
16	3676.32	0.021	0.000	3682.11	3674.39	0.157	0.002	

DOM_suolo_HC_t0



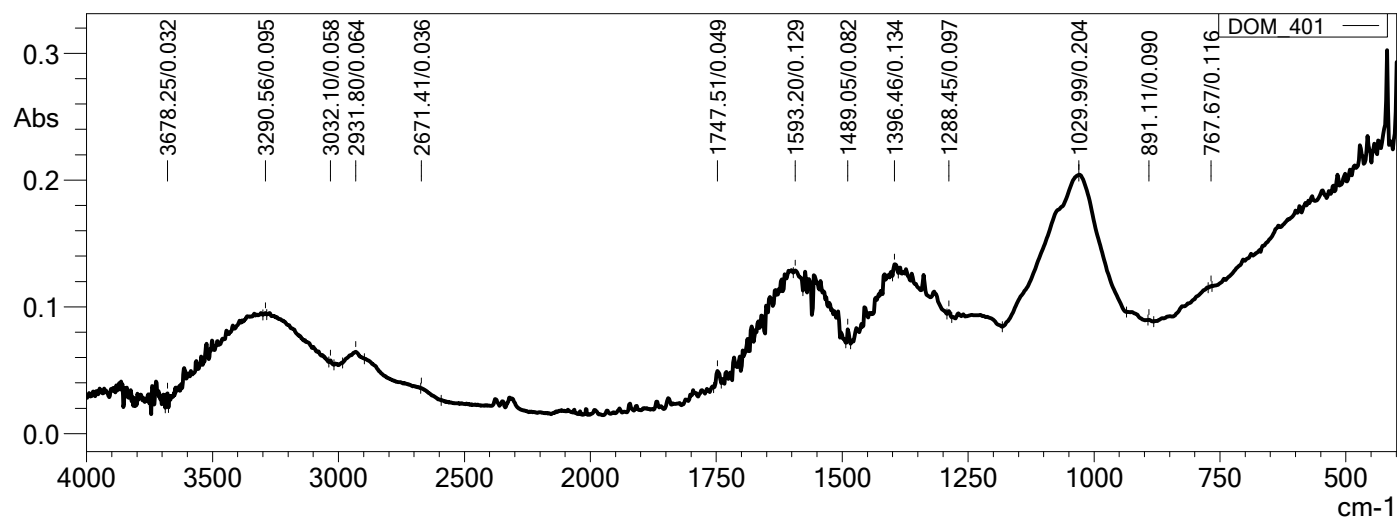
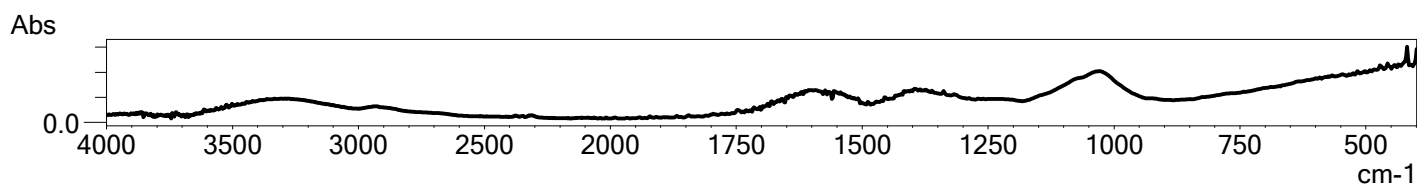
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	599.86	0.182	0.004	630.72	596.00	5.830	-0.017	
2	777.31	0.116	0.003	817.82	771.53	5.041	0.036	
3	823.60	0.104	0.004	846.75	817.82	2.807	0.027	
4	1033.85	0.213	0.030	1062.78	881.47	27.795	1.261	
5	1072.42	0.201	0.001	1247.94	1070.49	24.286	-1.233	
6	1288.45	0.094	0.002	1290.38	1282.66	0.709	0.006	
7	1384.89	0.135	0.005	1388.75	1382.96	0.766	0.016	
8	1489.05	0.081	0.008	1492.90	1483.26	0.737	0.031	
9	1606.70	0.128	0.002	1610.56	1604.77	0.732	0.006	
10	1778.37	0.032	0.003	1786.08	1772.58	0.413	0.021	
11	2931.80	0.055	0.002	2937.59	2904.80	1.731	0.022	
12	3215.34	0.074	0.000	3217.27	3211.48	0.425	0.000	
13	3361.93	0.077	0.001	3377.36	3358.07	1.466	0.009	
14	3670.54	0.023	0.008	3674.39	3666.68	0.144	0.026	

DOM_suolo_HC_t0



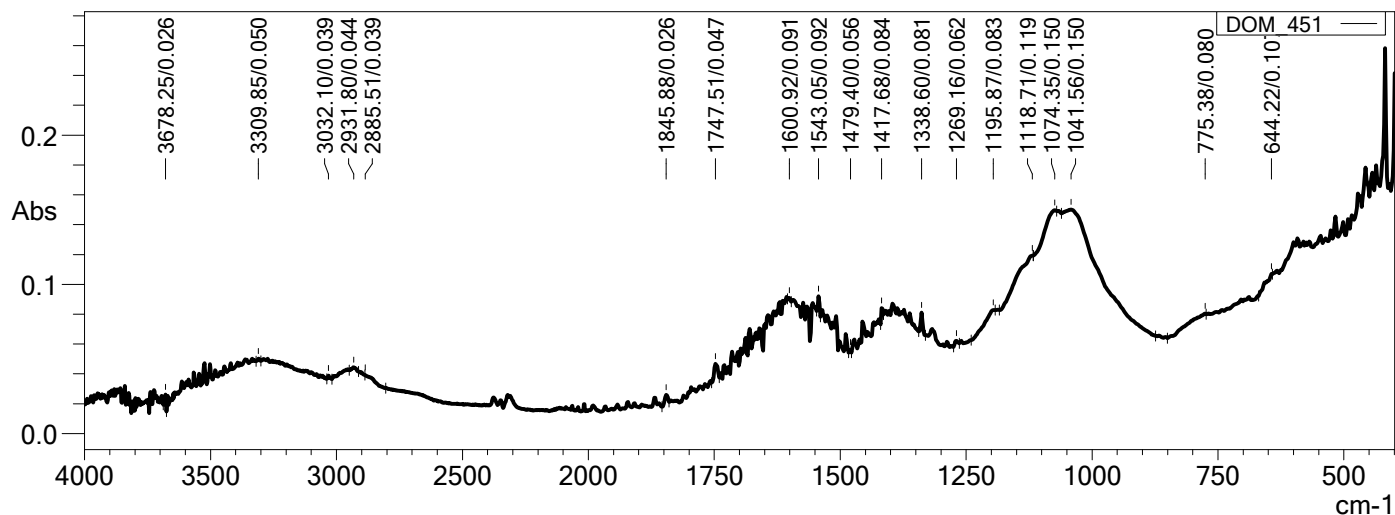
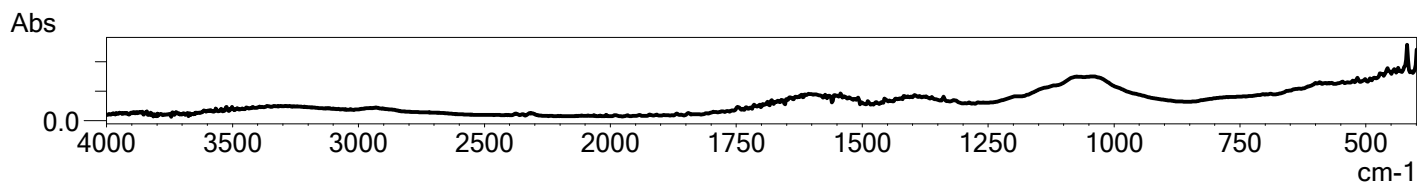
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	592.15	0.155	0.005	596.00	588.29	1.178	0.018	
2	763.81	0.100	0.000	767.67	761.88	0.578	0.001	
3	823.60	0.092	0.003	850.61	817.82	2.846	0.007	
4	1028.06	0.188	0.036	1062.78	869.90	25.783	1.583	
5	1064.71	0.167	0.001	1193.94	1062.78	16.444	-0.606	
6	1269.16	0.086	0.003	1274.95	1263.37	0.977	0.016	
7	1377.17	0.110	0.001	1382.96	1375.25	0.837	0.005	
8	1489.05	0.077	0.010	1492.90	1483.26	0.678	0.041	
9	1614.42	0.111	0.008	1618.28	1610.56	0.815	0.025	
10	1828.52	0.024	0.000	1832.38	1826.59	0.136	0.001	
11	2856.58	0.046	0.000	2858.51	2794.85	2.526	-0.068	
12	2926.01	0.055	0.000	2927.94	2904.80	1.229	0.002	
13	3032.10	0.048	0.002	3037.89	3018.60	0.895	0.014	
14	3323.35	0.064	0.000	3325.28	3315.63	0.620	0.000	
15	3670.54	0.027	0.007	3674.39	3666.68	0.174	0.026	

DOM_suolo_HC_t0



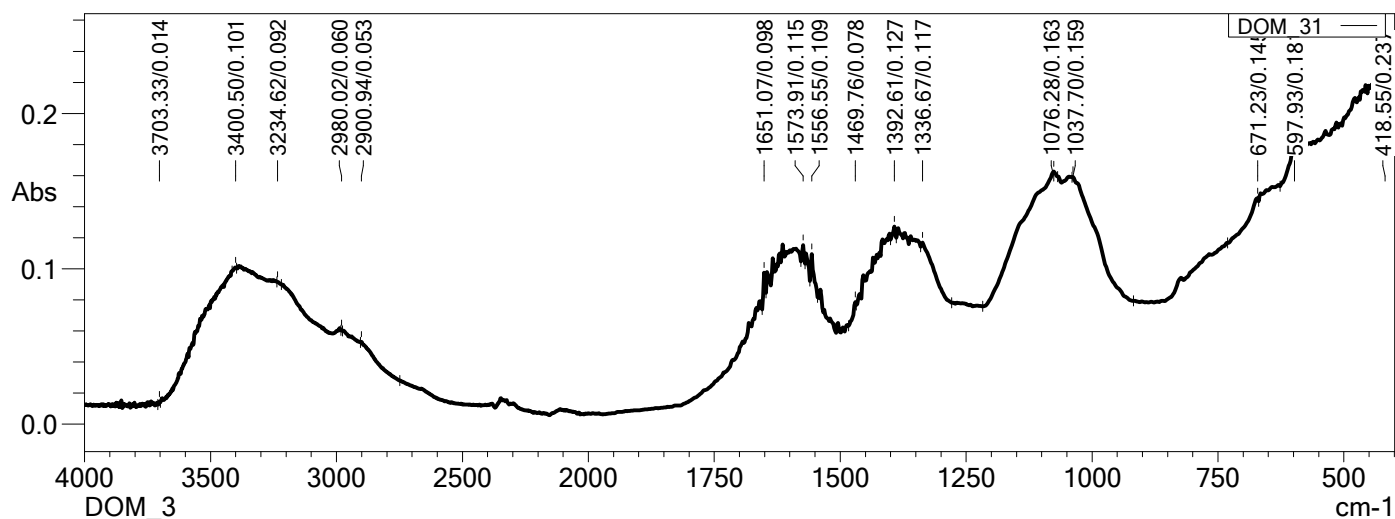
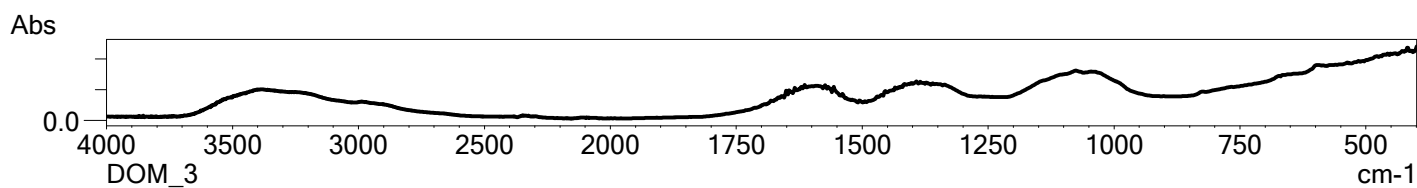
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	767.67	0.116	0.000	773.46	765.74	0.894	0.001	
2	891.11	0.090	0.000	893.04	881.47	1.031	0.001	
3	1029.99	0.204	0.113	1182.36	935.48	35.513	13.259	
4	1288.45	0.097	0.003	1292.31	1282.66	0.912	0.014	
5	1396.46	0.134	0.008	1400.32	1388.75	1.513	0.060	
6	1489.05	0.082	0.011	1492.90	1483.26	0.731	0.044	
7	1593.20	0.129	0.005	1597.06	1577.77	2.407	0.095	
8	1747.51	0.049	0.011	1755.22	1739.79	0.674	0.086	
9	2671.41	0.036	0.000	2673.34	2592.33	2.512	-0.003	
10	2931.80	0.064	0.007	2983.88	2897.08	5.296	0.309	
11	3032.10	0.058	0.002	3037.89	3018.60	1.080	0.012	
12	3290.56	0.095	0.001	3300.20	3284.77	1.456	0.010	
13	3678.25	0.032	0.011	3687.90	3674.39	0.373	0.097	

DOM_suolo_HC_t0



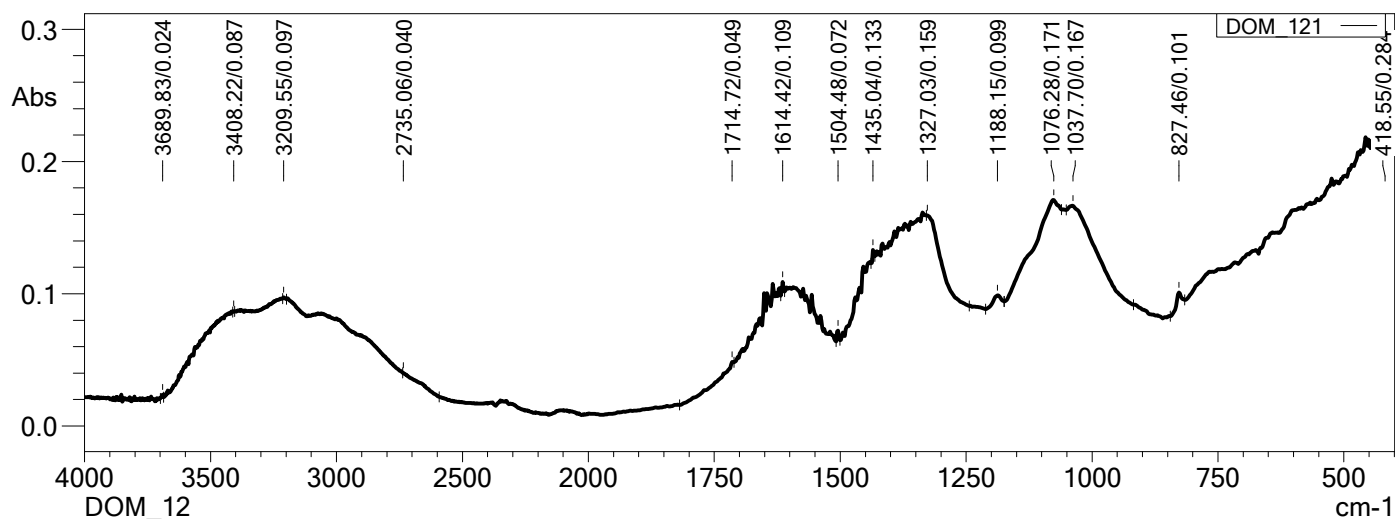
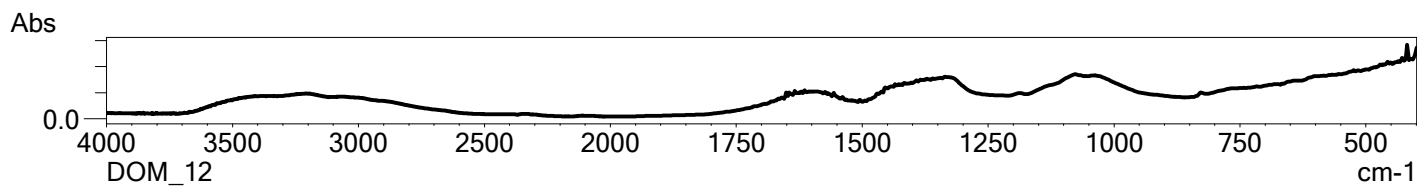
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	644.22	0.107	0.001	669.30	642.30	2.707	0.019	
2	775.38	0.080	0.001	850.61	773.46	5.603	0.051	
3	1041.56	0.150	0.011	1060.85	873.75	19.339	-0.592	
4	1074.35	0.150	0.003	1116.78	1070.49	6.274	0.066	
5	1118.71	0.119	0.001	1184.29	1116.78	6.916	0.100	
6	1195.87	0.083	0.002	1240.23	1192.01	3.514	0.012	
7	1269.16	0.062	0.003	1274.95	1263.37	0.706	0.019	
8	1338.60	0.081	0.014	1344.38	1330.88	0.967	0.060	
9	1417.68	0.084	0.009	1419.61	1413.82	0.471	0.028	
10	1479.40	0.056	0.002	1483.26	1477.47	0.320	0.006	
11	1543.05	0.092	0.012	1546.91	1539.20	0.663	0.044	
12	1600.92	0.091	0.002	1604.77	1597.06	0.694	0.005	
13	1747.51	0.047	0.011	1755.22	1739.79	0.637	0.085	
14	1845.88	0.026	0.006	1853.59	1840.09	0.303	0.038	
15	2885.51	0.039	0.000	2887.44	2804.50	2.877	-0.012	
16	2931.80	0.044	0.002	2949.16	2912.51	1.584	0.046	
17	3032.10	0.039	0.002	3037.89	3018.60	0.723	0.018	
18	3309.85	0.050	0.002	3319.49	3300.20	0.948	0.011	
19	3678.25	0.026	0.010	3687.90	3674.39	0.308	0.077	

DOM_suolo_HC_t0



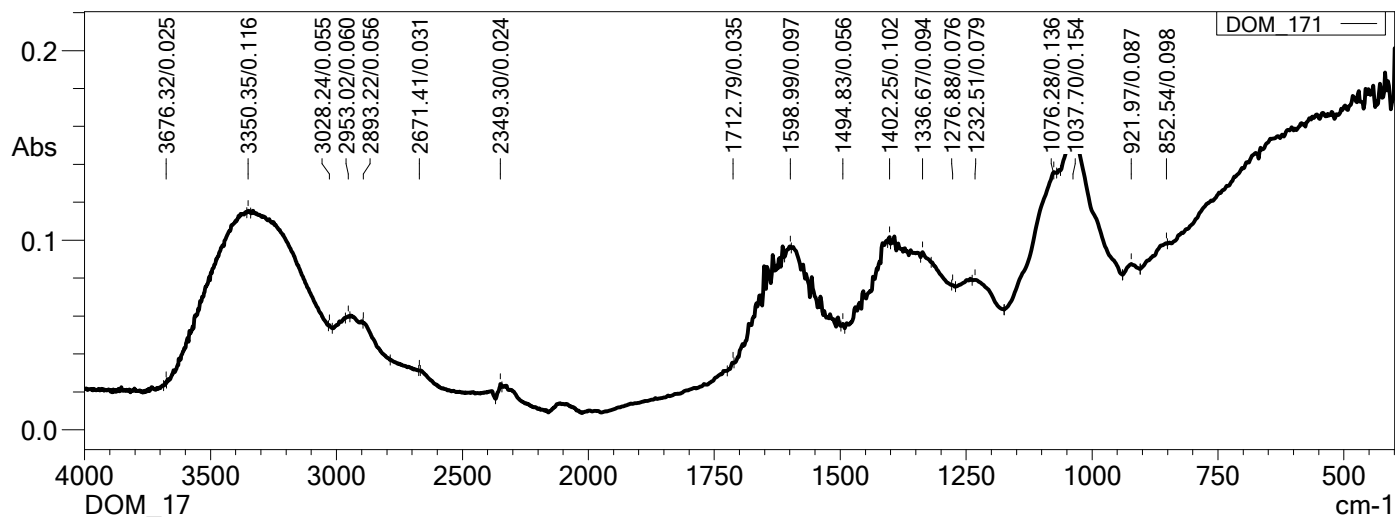
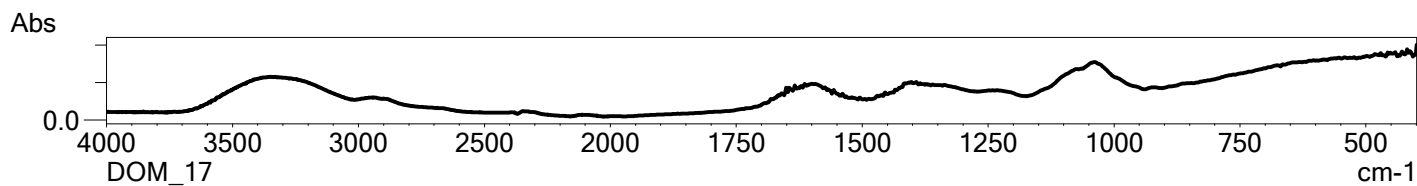
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	418.55	0.237	0.012	424.34	414.70	2.222	0.053	
2	597.93	0.181	0.002	626.87	596.00	5.107	-0.044	
3	671.23	0.145	0.003	731.02	669.30	7.921	-0.099	
4	1037.70	0.159	0.001	1039.63	918.12	13.773	-0.697	
5	1076.28	0.163	0.008	1217.08	1068.56	18.257	0.800	
6	1336.67	0.117	0.005	1340.53	1278.81	5.925	-0.007	
7	1392.61	0.127	0.008	1400.32	1388.75	1.420	0.038	
8	1469.76	0.078	0.003	1483.26	1467.83	1.082	0.002	
9	1556.55	0.109	0.020	1560.41	1544.98	1.471	0.130	
10	1573.91	0.115	0.011	1577.77	1570.06	0.841	0.039	
11	1651.07	0.098	0.018	1654.92	1647.21	0.670	0.060	
12	2900.94	0.053	0.001	2904.80	2748.56	6.081	-0.198	
13	2980.02	0.060	0.000	2981.95	2976.16	0.348	0.000	
14	3234.62	0.092	0.000	3236.55	3219.19	1.576	0.001	
15	3400.50	0.101	0.001	3414.00	3396.64	1.734	0.009	
16	3703.33	0.014	0.001	3709.11	3699.47	0.131	0.004	

DOM_suolo_HC_t0



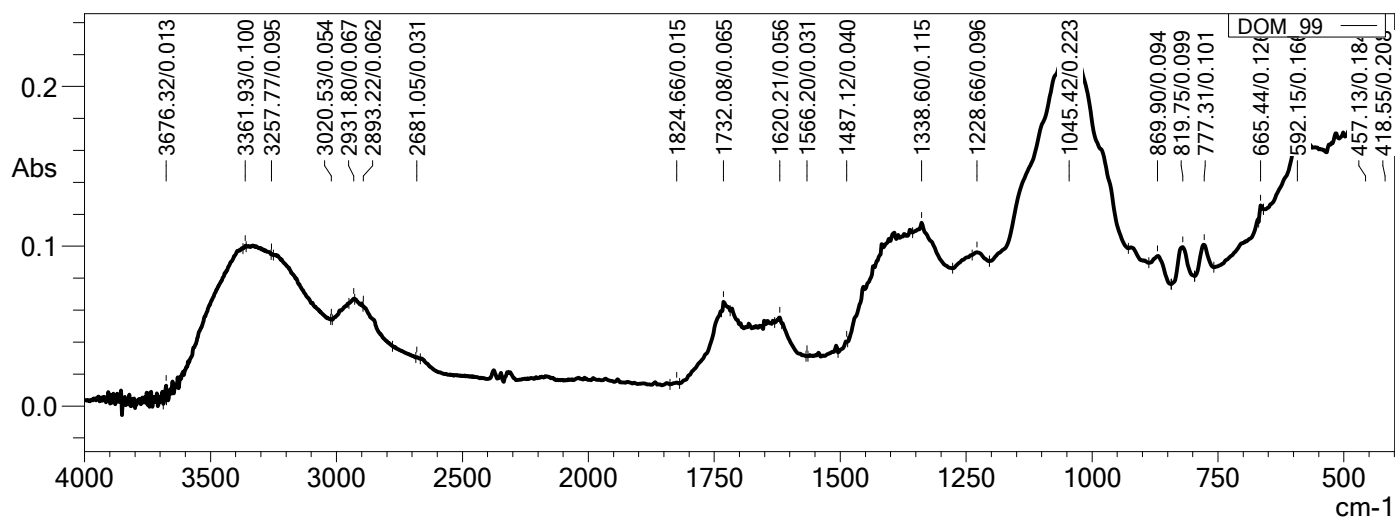
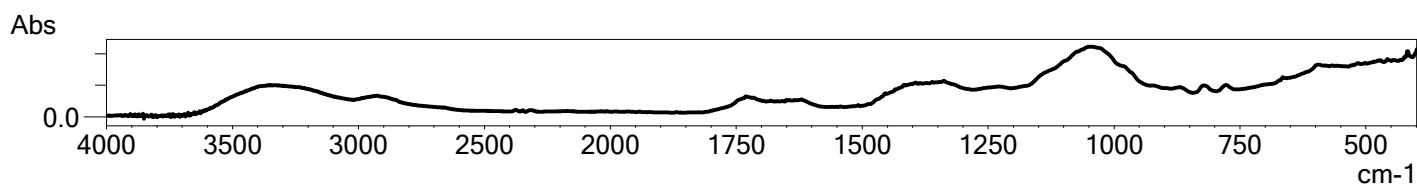
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	418.55	0.284	0.061	424.34	414.70	2.375	0.221	
2	827.46	0.101	0.011	844.82	815.89	2.681	0.099	
3	1037.70	0.167	0.010	1051.20	918.12	17.083	0.110	
4	1076.28	0.171	0.017	1174.65	1060.85	15.424	0.745	
5	1188.15	0.099	0.007	1211.30	1174.65	3.449	0.101	
6	1327.03	0.159	0.002	1328.95	1244.09	9.899	-0.708	
7	1435.04	0.133	0.008	1438.90	1431.18	0.990	0.020	
8	1504.48	0.072	0.008	1508.33	1500.62	0.531	0.034	
9	1614.42	0.109	0.009	1618.28	1610.56	0.802	0.030	
10	1714.72	0.049	0.001	1818.87	1710.86	3.140	-0.327	
11	2735.06	0.040	0.000	2736.99	2592.33	4.556	0.068	
12	3209.55	0.097	0.001	3213.41	3197.98	1.491	0.007	
13	3408.22	0.087	0.000	3412.08	3404.36	0.669	0.002	
14	3689.83	0.024	0.002	3699.47	3685.97	0.303	0.017	

DOM_suolo_HC_t0



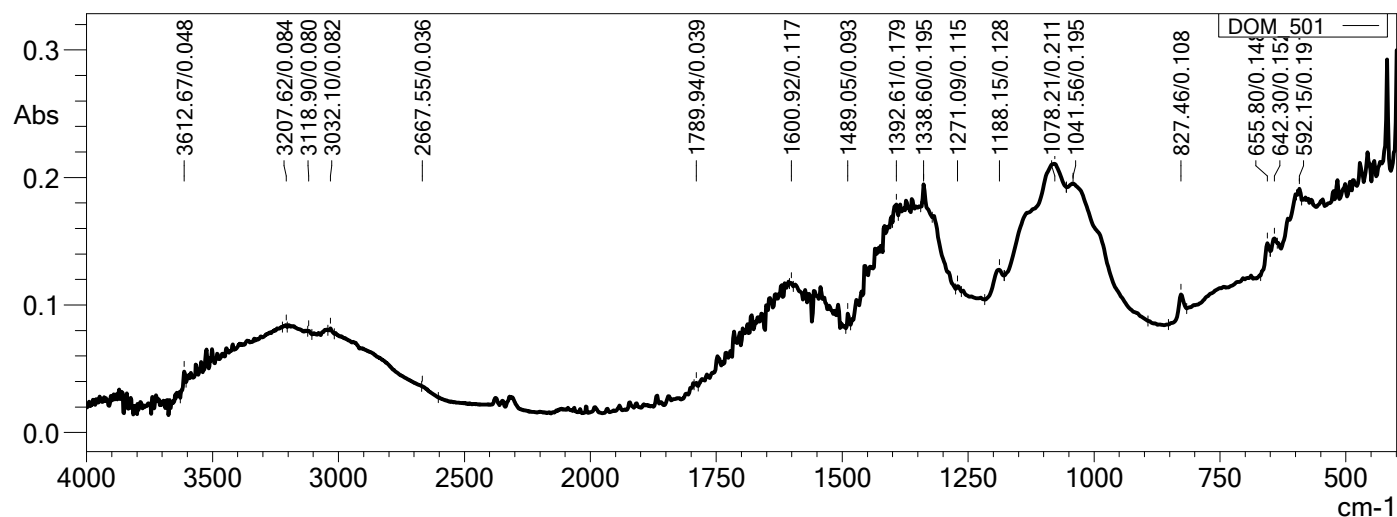
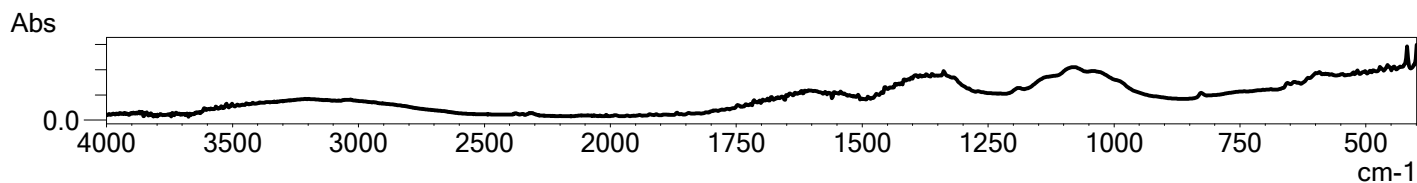
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	852.54	0.098	0.001	904.61	850.61	4.983	0.044	
2	921.97	0.087	0.004	939.33	904.61	2.968	0.077	
3	1037.70	0.154	0.029	1062.78	939.33	14.713	1.231	
4	1076.28	0.136	0.005	1174.65	1070.49	10.160	-0.189	
5	1232.51	0.079	0.002	1238.30	1174.65	4.611	0.087	
6	1276.88	0.076	0.000	1278.81	1271.09	0.586	0.001	
7	1336.67	0.094	0.003	1340.53	1319.31	1.932	0.029	
8	1402.25	0.102	0.003	1406.11	1400.32	0.581	0.011	
9	1494.83	0.056	0.002	1498.69	1490.97	0.425	0.007	
10	1598.99	0.097	0.002	1610.56	1597.06	1.282	0.019	
11	1712.79	0.035	0.001	1724.36	1710.86	0.450	0.003	
12	2349.30	0.024	0.003	2368.59	2343.51	0.519	0.030	
13	2671.41	0.031	0.000	2673.34	2667.55	0.181	0.000	
14	2893.22	0.056	0.000	2895.15	2787.14	4.942	-0.093	
15	2953.02	0.060	0.001	2964.59	2947.23	1.034	0.005	
16	3028.24	0.055	0.001	3032.10	3016.67	0.839	0.003	
17	3350.35	0.116	0.001	3356.14	3340.71	1.775	0.010	
18	3676.32	0.025	0.001	3685.97	3674.39	0.280	0.002	

DOM_suolo_HC_t0



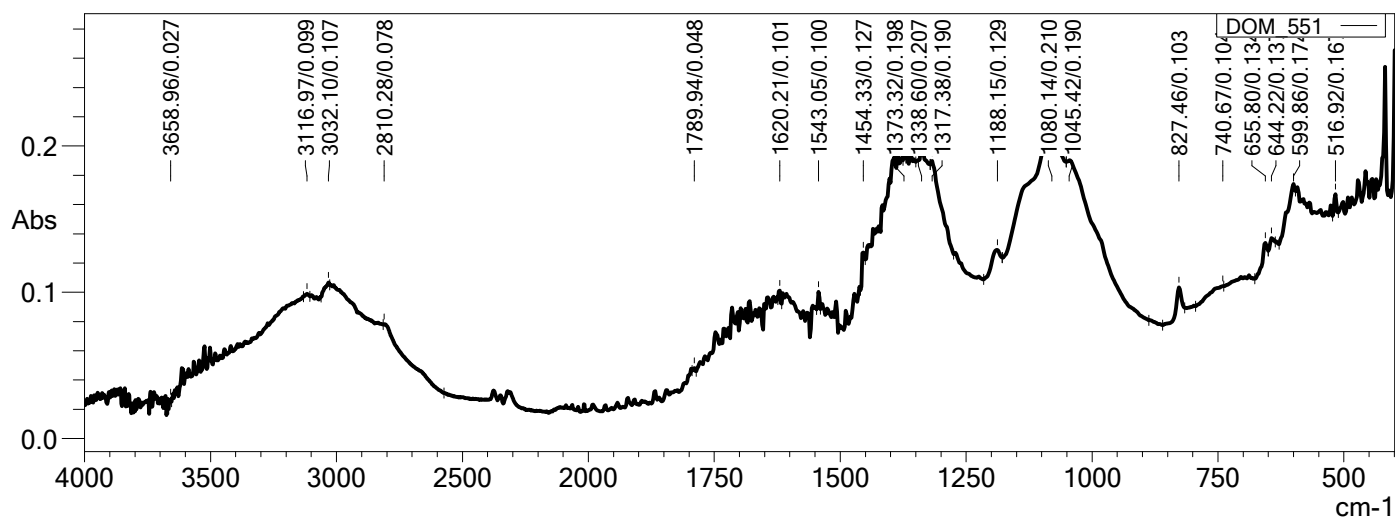
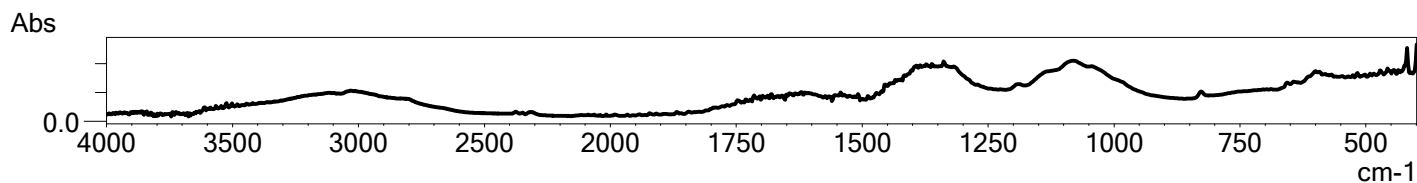
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	418.55	0.208	0.019	422.41	410.84	2.286	0.089	
2	457.13	0.184	0.009	464.84	449.41	2.754	0.059	
3	592.15	0.166	0.007	659.66	584.43	10.685	-0.057	
4	665.44	0.126	0.007	669.30	659.66	1.184	0.036	
5	777.31	0.101	0.017	796.60	758.02	3.537	0.295	
6	819.75	0.099	0.021	842.89	796.60	4.061	0.415	
7	869.90	0.094	0.009	887.26	842.89	3.872	0.195	
8	1045.42	0.223	0.127	1203.58	927.76	43.258	17.147	
9	1228.66	0.096	0.003	1238.30	1203.58	3.264	0.050	
10	1338.60	0.115	0.011	1355.96	1276.88	7.905	0.203	
11	1487.12	0.040	0.001	1504.48	1485.19	0.713	0.001	
12	1566.20	0.031	0.000	1568.13	1564.27	0.121	0.000	
13	1620.21	0.056	0.004	1629.85	1616.35	0.725	0.025	
14	1732.08	0.065	0.006	1735.93	1718.58	1.080	0.051	
15	1824.66	0.015	0.001	1838.16	1818.87	0.274	0.006	
16	2681.05	0.031	0.000	2684.91	2667.55	0.524	0.003	
17	2893.22	0.062	0.000	2895.15	2777.50	5.761	-0.106	
18	2931.80	0.067	0.001	2951.09	2927.94	1.523	0.008	
19	3020.53	0.054	0.000	3022.45	3016.67	0.313	0.001	
20	3257.77	0.095	0.000	3259.70	3250.05	0.915	0.002	
21	3361.93	0.100	0.001	3371.57	3358.07	1.342	0.004	
22	3676.32	0.013	0.009	3687.90	3674.39	0.089	0.054	

DOM_suolo_HC_t0



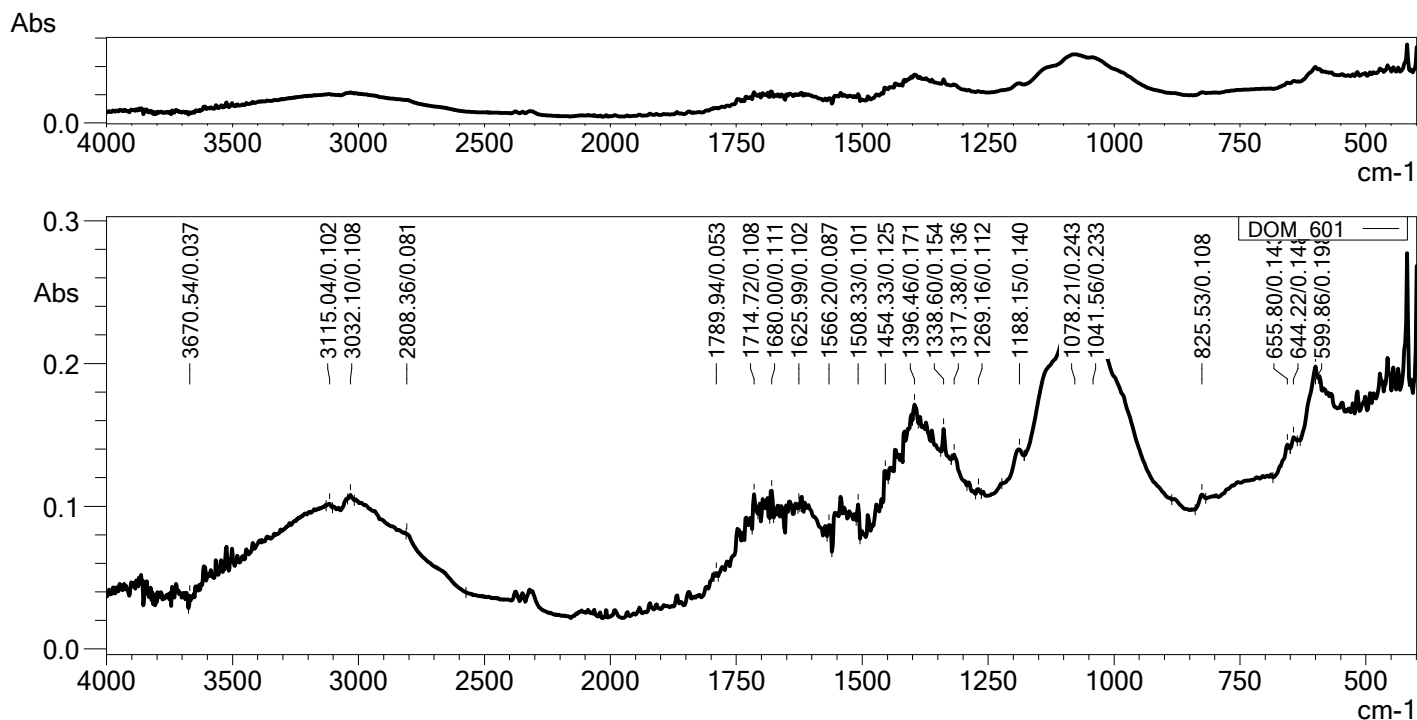
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	592.15	0.191	0.007	597.93	588.29	1.809	0.030	
2	642.30	0.152	0.006	650.01	636.51	2.006	0.045	
3	655.80	0.148	0.012	669.30	650.01	2.639	0.080	
4	827.46	0.108	0.015	852.54	815.89	3.461	0.125	
5	1041.56	0.195	0.012	1055.06	893.04	22.413	-0.250	
6	1078.21	0.211	0.031	1178.51	1055.06	21.713	2.249	
7	1188.15	0.128	0.009	1217.08	1178.51	4.523	0.139	
8	1271.09	0.115	0.003	1274.95	1263.37	1.311	0.019	
9	1338.60	0.195	0.020	1344.38	1321.24	4.130	0.126	
10	1392.61	0.179	0.010	1400.32	1388.75	2.021	0.082	
11	1489.05	0.093	0.011	1492.90	1483.26	0.843	0.041	
12	1600.92	0.117	0.002	1604.77	1597.06	0.898	0.005	
13	1789.94	0.039	0.002	1793.80	1786.08	0.292	0.006	
14	2667.55	0.036	0.000	2669.48	2603.90	2.100	0.005	
15	3032.10	0.082	0.003	3041.74	3016.67	2.000	0.025	
16	3118.90	0.080	0.001	3122.75	3105.39	1.366	0.008	
17	3207.62	0.084	0.002	3223.05	3203.76	1.610	0.017	
18	3612.67	0.048	0.012	3628.10	3604.96	0.899	0.127	

DOM_suolo_HC_t0

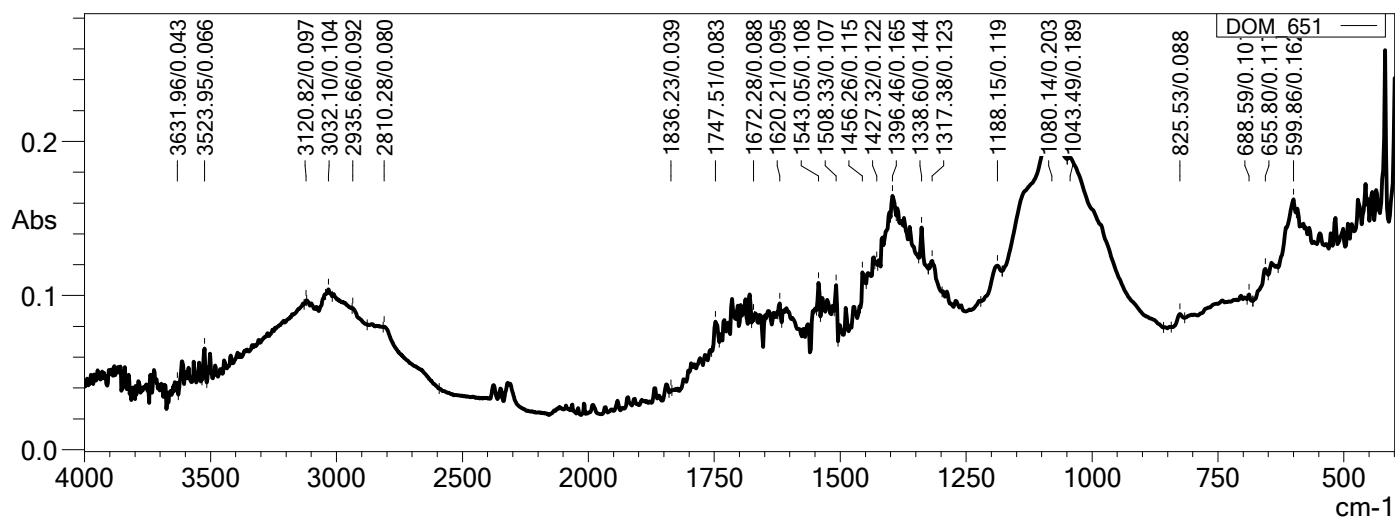
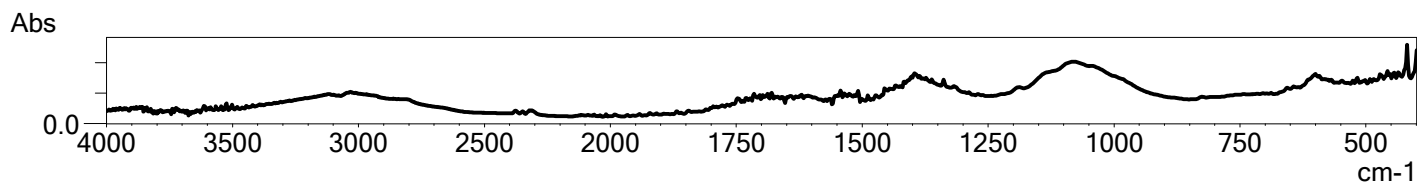


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	516.92	0.167	0.014	522.71	511.14	1.843	0.068	
2	599.86	0.174	0.009	628.79	596.00	5.078	0.127	
3	644.22	0.137	0.006	650.01	636.51	1.812	0.038	
4	655.80	0.134	0.010	677.01	650.01	3.259	0.052	
5	740.67	0.104	0.001	794.67	738.74	5.503	0.064	
6	827.46	0.103	0.017	860.25	815.89	3.827	0.127	
7	1045.42	0.190	0.005	1051.20	887.26	20.762	-1.380	
8	1080.14	0.210	0.035	1178.51	1051.20	22.407	2.493	
9	1188.15	0.129	0.009	1215.15	1178.51	4.405	0.146	
10	1317.38	0.190	0.007	1321.24	1274.95	7.314	0.058	
11	1338.60	0.207	0.019	1350.17	1321.24	5.567	0.120	
12	1373.32	0.198	0.007	1375.25	1369.46	1.128	0.023	
13	1454.33	0.127	0.013	1460.11	1450.47	1.145	0.066	
14	1543.05	0.100	0.012	1546.91	1539.20	0.725	0.044	
15	1620.21	0.101	0.008	1624.06	1616.35	0.748	0.027	
16	1789.94	0.048	0.002	1793.80	1786.08	0.362	0.006	
17	2810.28	0.078	0.001	2814.14	2573.04	12.344	-0.791	
18	3032.10	0.107	0.003	3061.03	3026.31	3.576	0.070	
19	3116.97	0.099	0.002	3130.47	3105.39	2.459	0.025	
20	3658.96	0.027	0.003	3666.68	3655.11	0.288	0.026	

DOM_suolo_HC_t0

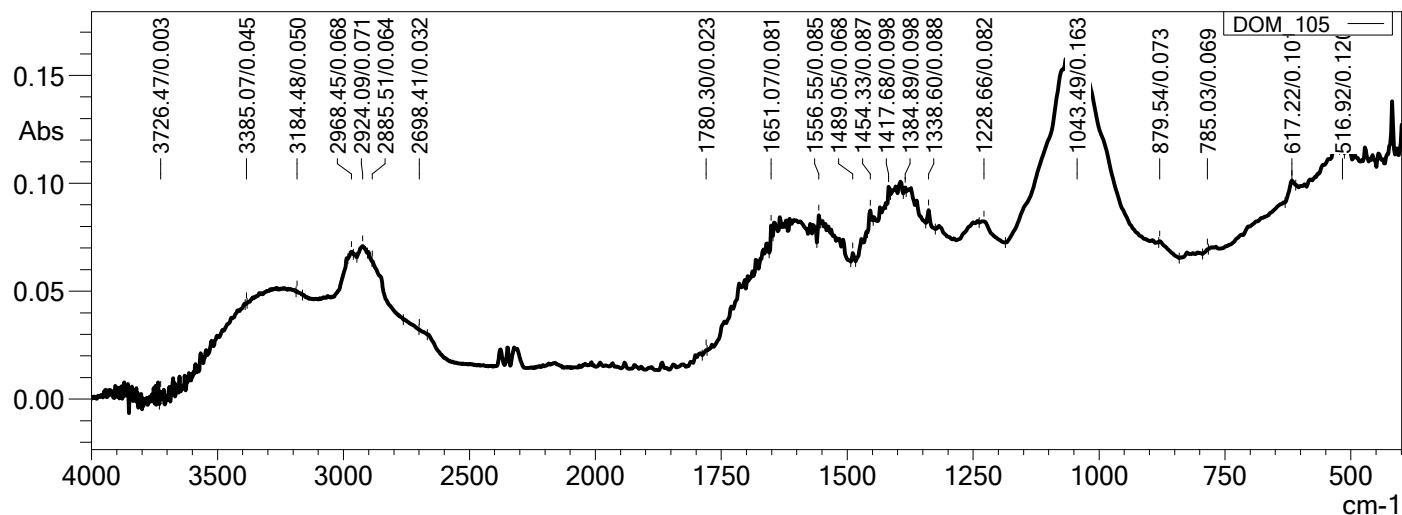
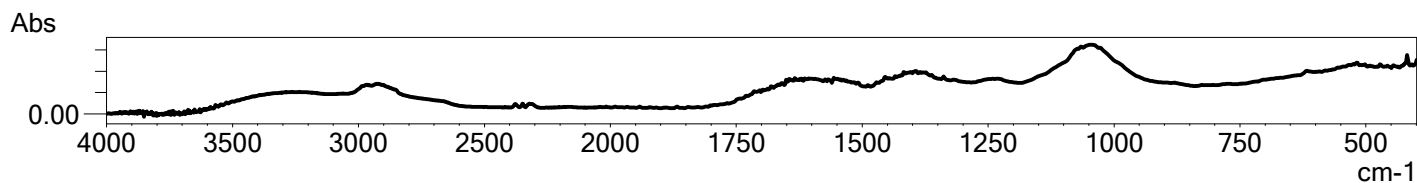


DOM_suolo_HC_t0



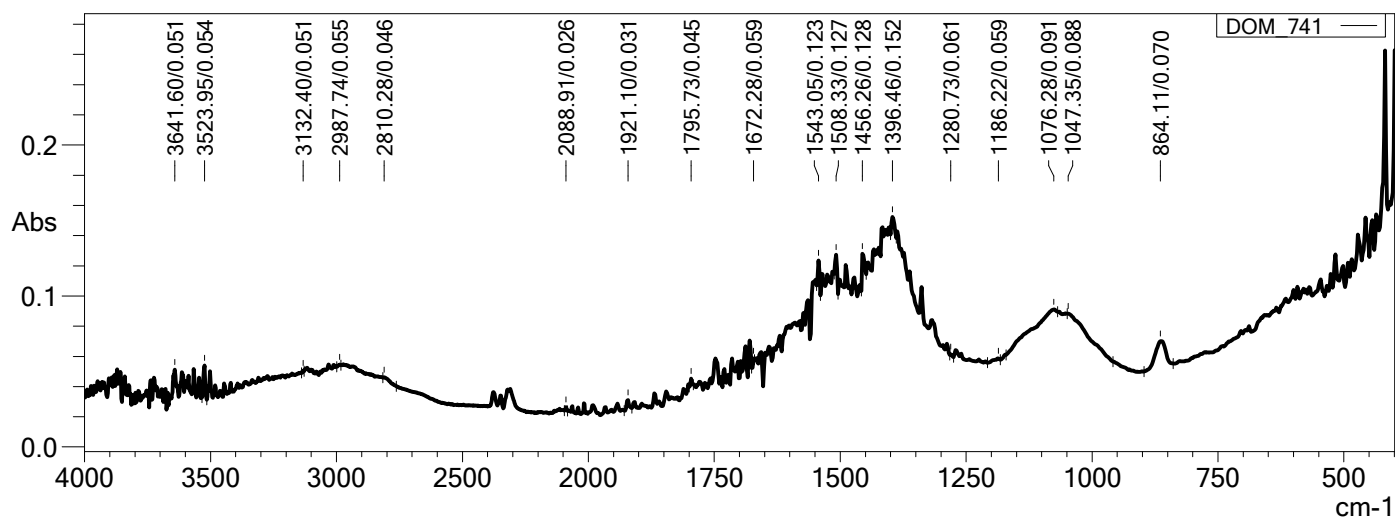
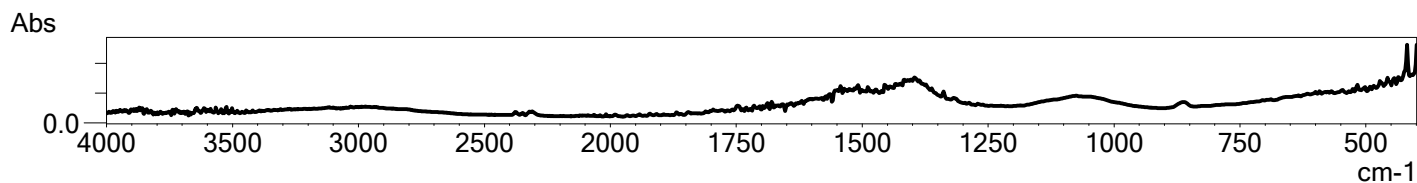
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	599.86	0.162	0.014	630.72	594.08	5.235	0.254	
2	655.80	0.117	0.007	680.87	650.01	3.287	0.049	
3	688.59	0.101	0.003	692.44	680.87	1.135	0.012	
4	825.53	0.088	0.005	842.89	815.89	2.266	0.039	
5	1043.49	0.189	0.004	1049.28	858.32	23.940	-1.627	
6	1080.14	0.203	0.032	1178.51	1049.28	22.231	2.566	
7	1188.15	0.119	0.008	1220.94	1178.51	4.599	0.101	
8	1317.38	0.123	0.010	1325.10	1298.09	3.059	0.092	
9	1338.60	0.144	0.022	1344.38	1325.10	2.431	0.100	
10	1396.46	0.165	0.014	1400.32	1388.75	1.840	0.090	
11	1427.32	0.122	0.002	1431.18	1425.40	0.700	0.005	
12	1456.26	0.115	0.018	1460.11	1448.54	1.240	0.083	
13	1508.33	0.107	0.026	1512.19	1504.48	0.730	0.111	
14	1543.05	0.108	0.020	1546.91	1539.20	0.763	0.081	
15	1620.21	0.095	0.009	1624.06	1616.35	0.694	0.035	
16	1672.28	0.088	0.003	1676.14	1670.35	0.493	0.005	
17	1747.51	0.083	0.016	1755.22	1739.79	1.154	0.123	
18	1836.23	0.039	0.001	1840.09	1834.30	0.220	0.001	
19	2810.28	0.080	0.001	2814.14	2592.33	12.715	-0.494	
20	2935.66	0.092	0.000	2937.59	2877.79	5.117	-0.041	
21	3032.10	0.104	0.004	3043.67	3016.67	2.745	0.042	
22	3120.82	0.097	0.001	3128.54	3118.90	0.922	0.002	
23	3523.95	0.066	0.021	3533.59	3514.30	1.030	0.172	
24	3631.96	0.043	0.005	3635.82	3628.10	0.313	0.018	

DOM_suolo_HC_t0



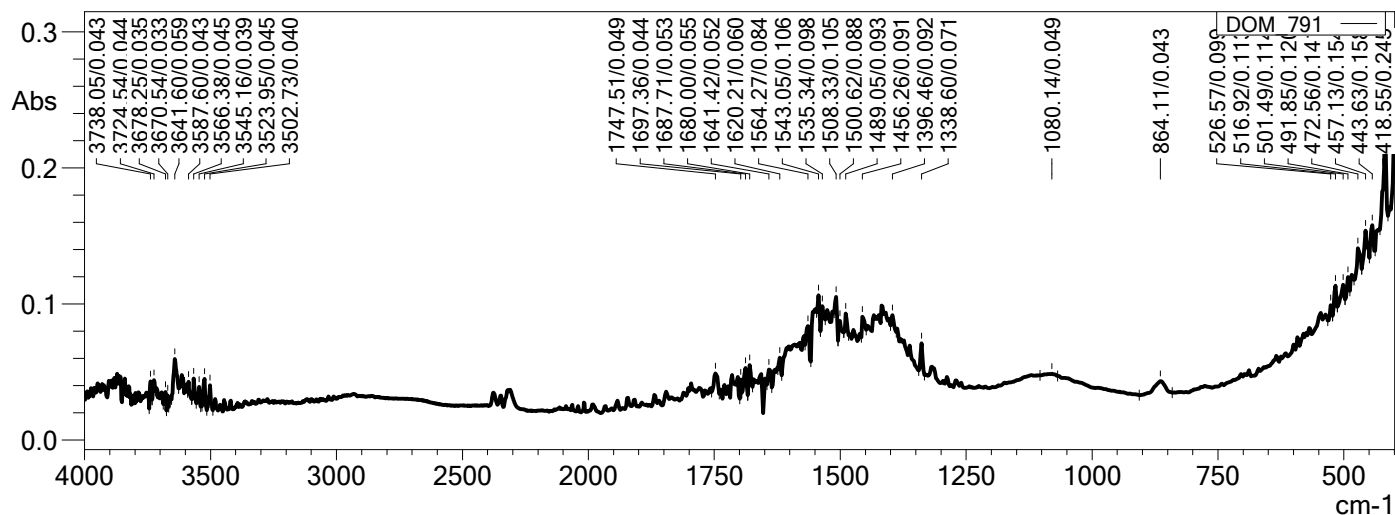
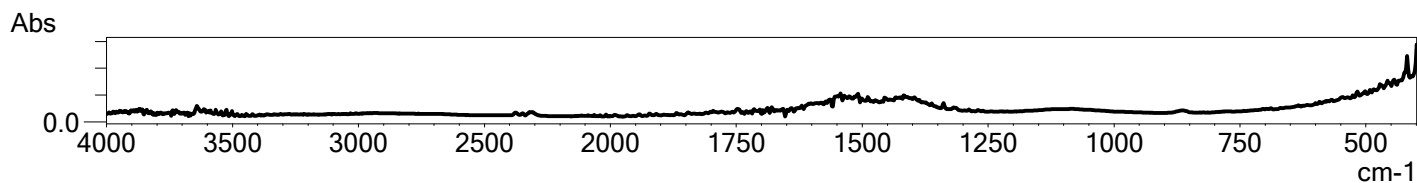
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	516.92	0.120	0.006	522.71	513.07	1.137	0.030	
2	617.22	0.101	0.005	630.72	609.51	2.068	0.051	
3	785.03	0.069	0.000	794.67	783.10	0.792	0.001	
4	879.54	0.073	0.001	881.47	840.96	2.799	0.005	
5	1043.49	0.163	0.008	1060.85	1029.99	4.939	0.158	
6	1228.66	0.082	0.002	1238.30	1186.22	4.027	0.008	
7	1338.60	0.088	0.007	1344.38	1325.10	1.580	0.033	
8	1384.89	0.098	0.002	1388.75	1382.96	0.562	0.008	
9	1417.68	0.098	0.006	1421.54	1413.82	0.731	0.017	
10	1454.33	0.087	0.007	1460.11	1448.54	0.963	0.037	
11	1489.05	0.068	0.004	1492.90	1483.26	0.631	0.017	
12	1556.55	0.085	0.008	1560.41	1552.70	0.623	0.028	
13	1651.07	0.081	0.009	1654.92	1647.21	0.576	0.023	
14	1780.30	0.023	0.001	1788.01	1778.37	0.210	0.002	
15	2698.41	0.032	0.000	2700.34	2667.55	1.020	0.003	
16	2885.51	0.064	0.000	2887.44	2762.06	6.013	-0.297	
17	2924.09	0.071	0.004	2947.23	2904.80	2.930	0.100	
18	2968.45	0.068	0.002	2981.95	2960.73	1.425	0.023	
19	3184.48	0.050	0.000	3188.33	3163.26	1.235	0.006	
20	3385.07	0.045	0.000	3390.86	3383.14	0.341	0.001	
21	3726.47	0.003	0.005	3730.33	3716.83	0.018	0.034	

DOM_suolo_HC_t0



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	864.11	0.070	0.017	896.90	839.03	3.436	0.396	
2	1047.35	0.088	0.001	1049.28	958.62	6.581	0.021	
3	1076.28	0.091	0.004	1170.79	1068.56	7.959	0.267	
4	1186.22	0.059	0.001	1207.44	1182.36	1.438	0.014	
5	1280.73	0.061	0.001	1282.66	1274.95	0.468	0.003	
6	1396.46	0.152	0.012	1400.32	1388.75	1.699	0.084	
7	1456.26	0.128	0.022	1458.18	1448.54	1.166	0.116	
8	1508.33	0.127	0.019	1512.19	1504.48	0.918	0.085	
9	1543.05	0.123	0.020	1546.91	1539.20	0.879	0.079	
10	1672.28	0.059	0.003	1676.14	1670.35	0.321	0.002	
11	1795.73	0.045	0.005	1799.59	1791.87	0.325	0.012	
12	1921.10	0.031	0.006	1928.82	1913.39	0.418	0.034	
13	2088.91	0.026	0.002	2094.69	2083.12	0.289	0.012	
14	2810.28	0.046	0.001	2816.07	2762.06	2.346	0.024	
15	2987.74	0.055	0.001	2999.31	2981.95	0.937	0.007	
16	3132.40	0.051	0.001	3138.18	3128.54	0.481	0.003	
17	3523.95	0.054	0.022	3533.59	3514.30	0.800	0.182	
18	3641.60	0.051	0.008	3645.46	3633.89	0.530	0.038	

DOM_suolo_HC_t0

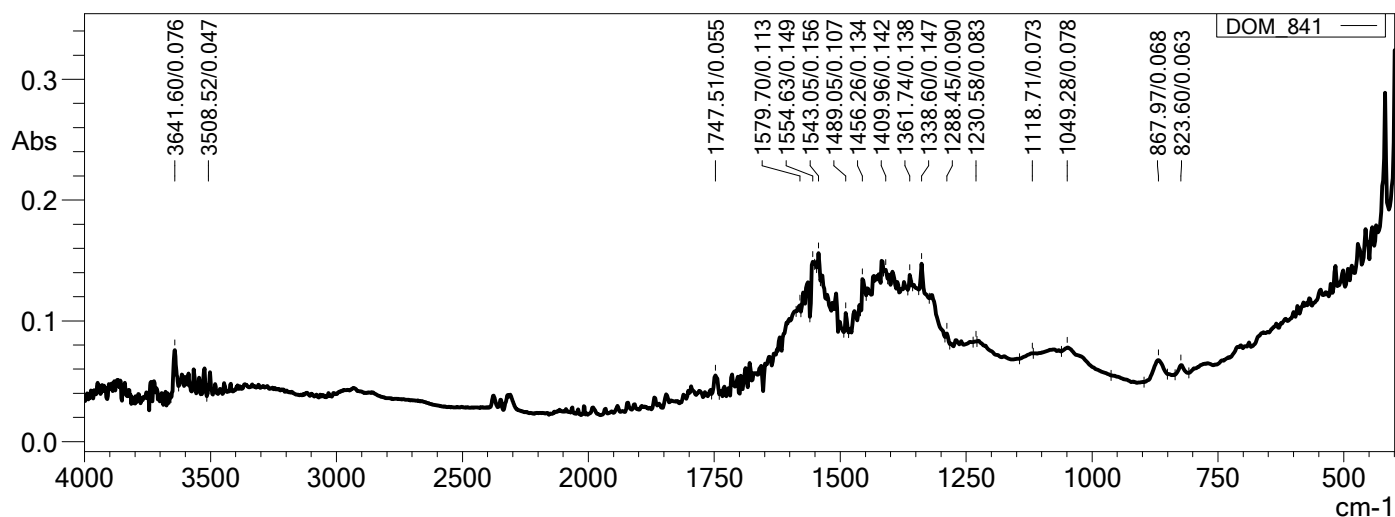
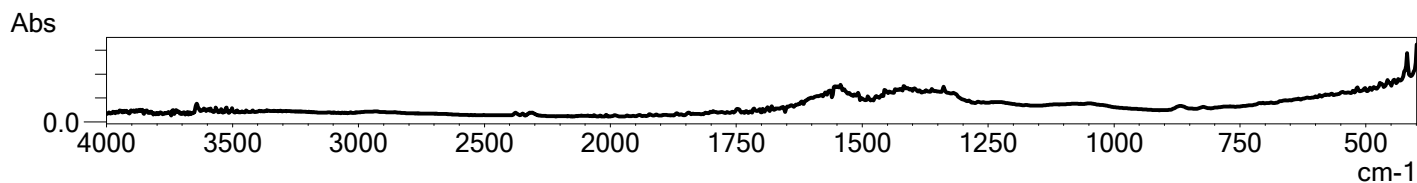


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	418.55	0.245	0.084	428.20	412.77	2.910	0.448	
2	443.63	0.158	0.021	449.41	437.84	1.709	0.129	
3	457.13	0.154	0.013	464.84	455.20	1.348	0.048	
4	472.56	0.141	0.019	480.28	464.84	2.007	0.129	
5	491.85	0.120	0.013	497.63	487.99	1.073	0.045	
6	501.49	0.114	0.012	513.07	497.63	1.627	0.074	
7	516.92	0.113	0.018	522.71	513.07	0.999	0.086	
8	526.57	0.099	0.009	532.35	522.71	0.898	0.034	
9	864.11	0.043	0.009	906.54	840.96	2.437	0.216	
10	1080.14	0.049	0.001	1103.28	1068.56	1.670	0.028	
11	1338.60	0.071	0.022	1344.38	1332.81	0.672	0.101	
12	1396.46	0.092	0.009	1400.32	1388.75	0.993	0.047	
13	1456.26	0.091	0.014	1458.18	1448.54	0.827	0.073	
14	1489.05	0.093	0.015	1492.90	1483.26	0.811	0.059	
15	1500.62	0.088	0.013	1504.48	1492.90	0.950	0.071	
16	1508.33	0.105	0.022	1512.19	1504.48	0.735	0.091	
17	1535.34	0.098	0.015	1539.20	1529.55	0.882	0.070	
18	1543.05	0.106	0.020	1546.91	1539.20	0.747	0.076	
19	1564.27	0.084	0.020	1570.06	1558.48	0.864	0.127	
20	1620.21	0.060	0.011	1624.06	1616.35	0.422	0.039	
21	1641.42	0.052	0.012	1645.28	1635.64	0.455	0.073	
22	1680.00	0.055	0.020	1683.86	1674.21	0.439	0.092	
23	1687.71	0.053	0.019	1695.43	1683.86	0.496	0.105	
24	1697.36	0.044	0.012	1699.29	1695.43	0.148	0.023	
25	1747.51	0.049	0.014	1755.22	1739.79	0.657	0.125	
26	3502.73	0.040	0.014	3506.59	3491.16	0.456	0.075	
27	3523.95	0.045	0.022	3533.59	3514.30	0.630	0.178	
28	3545.16	0.039	0.012	3550.95	3539.38	0.370	0.056	
29	3566.38	0.045	0.017	3574.10	3556.74	0.598	0.104	
30	3587.60	0.043	0.009	3591.46	3574.10	0.603	0.039	
31	3641.60	0.059	0.026	3655.11	3626.17	1.338	0.360	
32	3670.54	0.033	0.010	3674.39	3666.68	0.208	0.031	
33	3678.25	0.035	0.012	3687.90	3674.39	0.422	0.098	
34	3724.54	0.044	0.011	3732.26	3718.76	0.513	0.071	
35	3738.05	0.043	0.017	3743.83	3732.26	0.429	0.125	

DOM_suolo_HC_t0

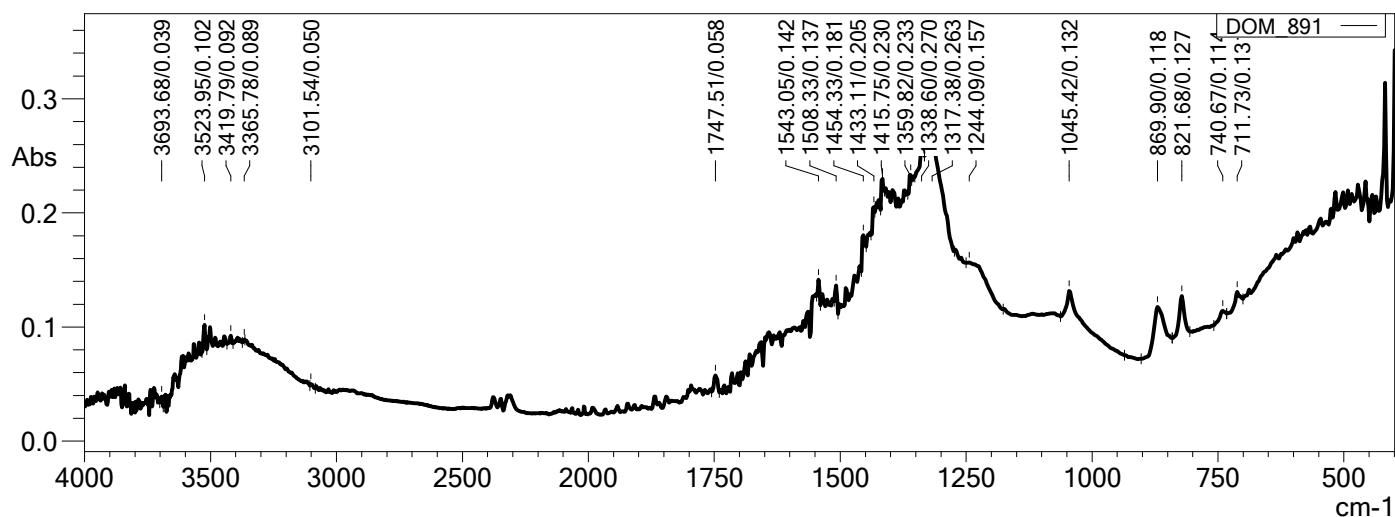
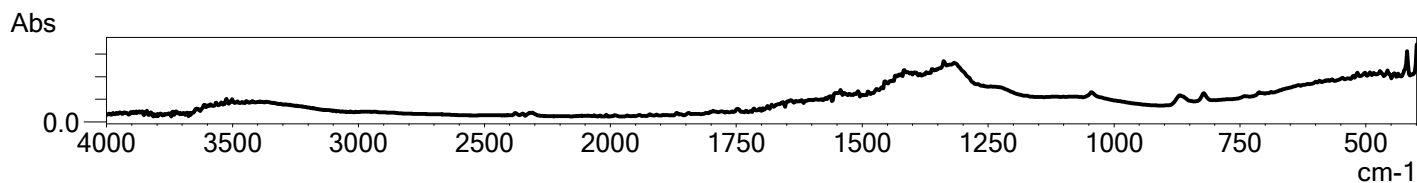
31	3641.60	0.059	0.026	3655.11	3626.17	1.338	0.360	
32	3670.54	0.033	0.010	3674.39	3666.68	0.208	0.031	
33	3678.25	0.035	0.012	3687.90	3674.39	0.422	0.098	
34	3724.54	0.044	0.011	3732.26	3718.76	0.513	0.071	
35	3738.05	0.043	0.017	3743.83	3732.26	0.429	0.125	

DOM_suolo_HC_t0



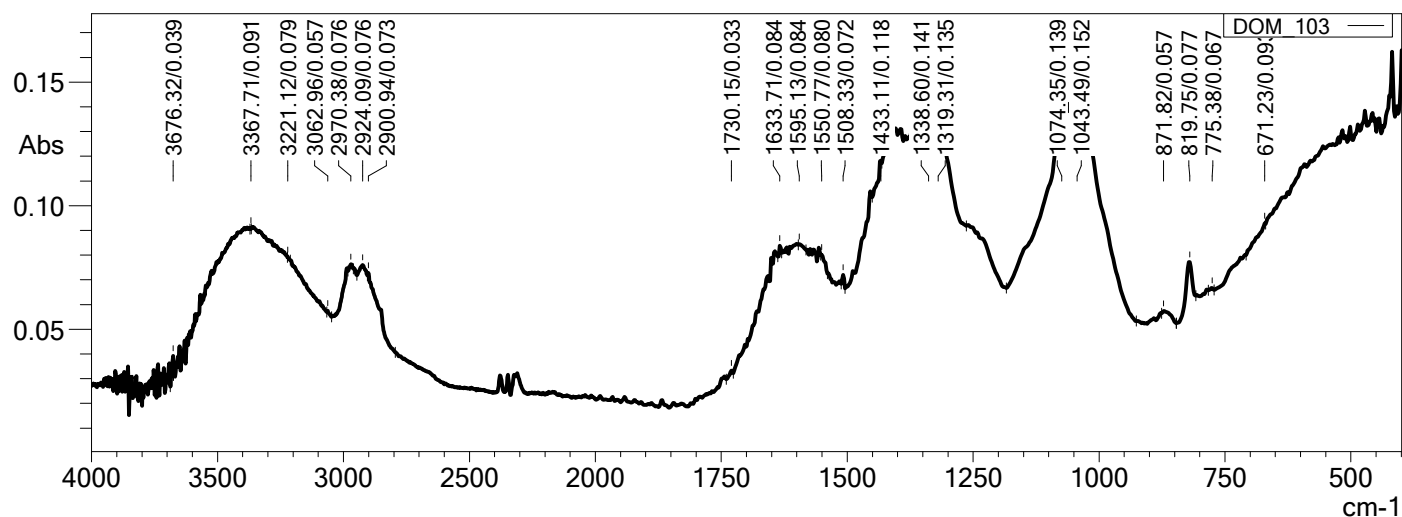
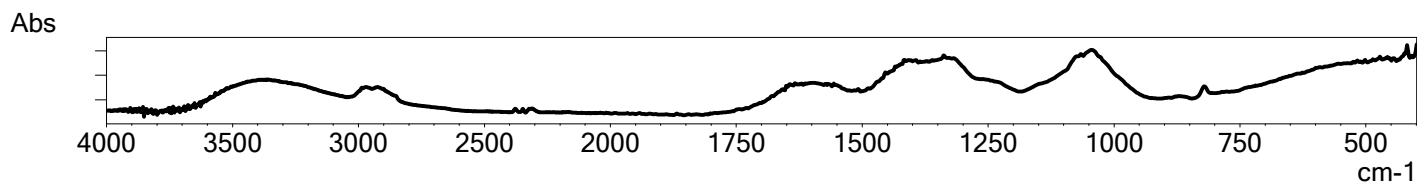
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	823.60	0.063	0.007	835.18	808.17	1.600	0.084	
2	867.97	0.068	0.014	896.90	850.61	2.713	0.278	
3	1049.28	0.078	0.006	1060.85	962.48	6.542	0.177	
4	1118.71	0.073	0.000	1143.79	1116.78	1.922	0.009	
5	1230.58	0.083	0.000	1236.37	1228.66	0.639	0.001	
6	1288.45	0.090	0.005	1292.31	1282.66	0.831	0.023	
7	1338.60	0.147	0.023	1344.38	1323.17	2.707	0.112	
8	1361.74	0.138	0.011	1365.60	1355.96	1.268	0.039	
9	1409.96	0.142	0.004	1413.82	1406.11	1.080	0.015	
10	1456.26	0.134	0.022	1460.11	1448.54	1.435	0.108	
11	1489.05	0.107	0.016	1492.90	1483.26	0.935	0.063	
12	1543.05	0.156	0.017	1546.91	1539.20	1.131	0.060	
13	1554.63	0.149	0.019	1560.41	1550.77	1.317	0.112	
14	1579.70	0.113	0.005	1587.42	1577.77	1.063	0.024	
15	1747.51	0.055	0.016	1755.22	1739.79	0.739	0.134	
16	3508.52	0.047	0.005	3514.30	3506.59	0.334	0.020	
17	3641.60	0.076	0.035	3657.04	3626.17	1.714	0.440	

DOM_suolo_HC_t0



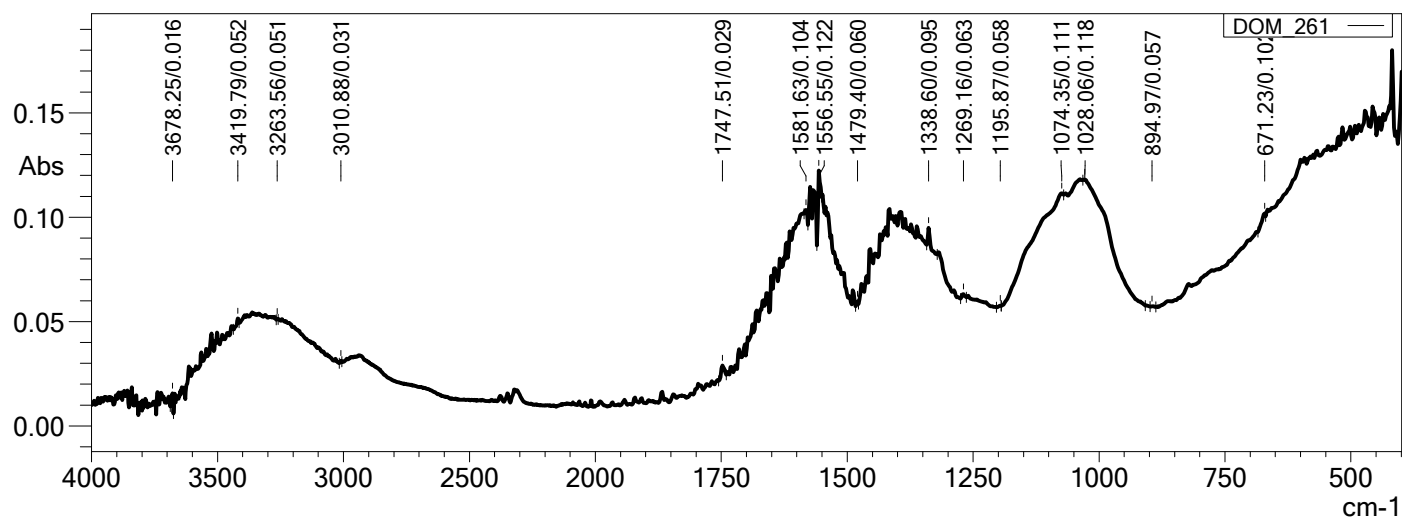
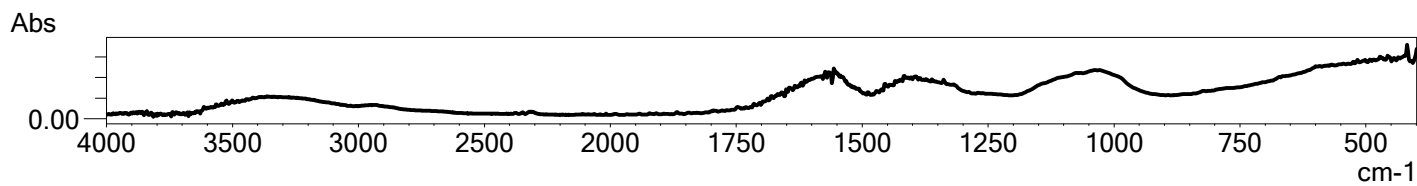
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	711.73	0.131	0.011	732.95	700.16	3.955	0.079	
2	740.67	0.114	0.006	758.02	732.95	2.733	0.060	
3	821.68	0.127	0.034	840.96	806.25	3.620	0.386	
4	869.90	0.118	0.036	902.69	840.96	5.719	0.706	
5	1045.42	0.132	0.027	1062.78	935.48	12.372	0.621	
6	1244.09	0.157	0.004	1249.87	1176.58	10.240	0.272	
7	1317.38	0.263	0.034	1332.81	1273.02	13.344	0.890	
8	1338.60	0.270	0.025	1352.10	1332.81	4.760	0.115	
9	1359.82	0.233	0.008	1365.60	1357.89	1.748	0.032	
10	1415.75	0.230	0.019	1419.61	1411.89	1.715	0.088	
11	1433.11	0.205	0.010	1438.90	1431.18	1.508	0.037	
12	1454.33	0.181	0.023	1458.18	1448.54	1.668	0.129	
13	1508.33	0.137	0.019	1512.19	1504.48	0.987	0.083	
14	1543.05	0.142	0.019	1546.91	1539.20	1.018	0.068	
15	1747.51	0.058	0.015	1755.22	1739.79	0.780	0.116	
16	3101.54	0.050	0.002	3105.39	3084.18	1.024	0.016	
17	3365.78	0.089	0.001	3373.50	3363.86	0.848	0.003	
18	3419.79	0.092	0.007	3435.22	3410.15	2.191	0.049	
19	3523.95	0.102	0.023	3533.59	3514.30	1.713	0.188	
20	3693.68	0.039	0.007	3701.40	3687.90	0.496	0.064	

DOM_suolo_HC_t0



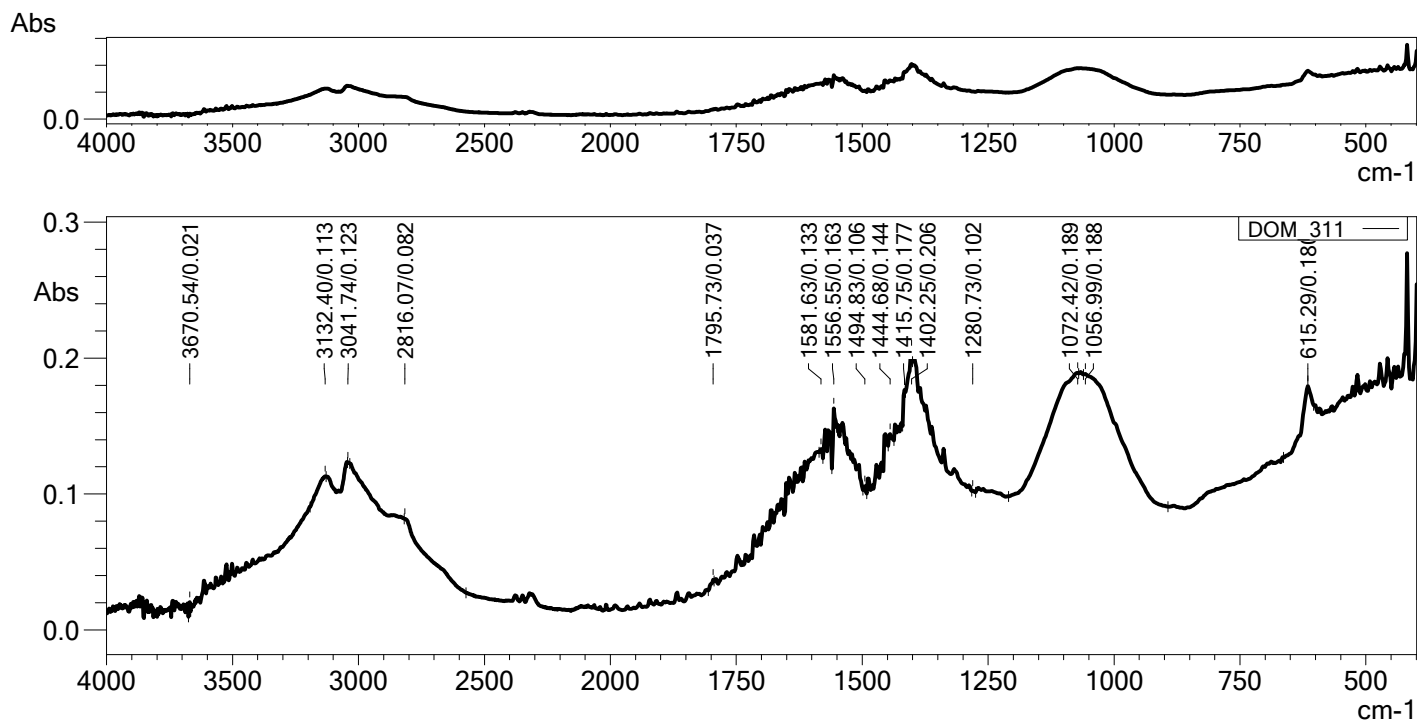
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	671.23	0.093	0.001	707.88	669.30	3.342	0.013	
2	775.38	0.067	0.001	783.10	771.53	0.766	0.004	
3	819.75	0.077	0.017	846.75	808.17	2.447	0.210	
4	871.82	0.057	0.002	875.68	846.75	1.611	0.036	
5	1043.49	0.152	0.023	1060.85	925.83	13.660	0.628	
6	1074.35	0.139	0.003	1184.29	1070.49	10.816	-0.873	
7	1319.31	0.135	0.002	1321.24	1263.37	6.389	-0.161	
8	1338.60	0.141	0.007	1357.89	1330.88	3.625	0.037	
9	1433.11	0.118	0.002	1450.47	1431.18	2.127	-0.002	
10	1508.33	0.072	0.005	1512.19	1504.48	0.540	0.020	
11	1550.77	0.080	0.001	1552.70	1539.20	1.045	0.015	
12	1595.13	0.084	0.000	1597.06	1581.63	1.289	0.006	
13	1633.71	0.084	0.004	1637.56	1629.85	0.630	0.011	
14	1730.15	0.033	0.002	1739.79	1726.29	0.428	0.009	
15	2900.94	0.073	0.002	2904.80	2794.85	6.028	-0.150	
16	2924.09	0.076	0.004	2947.23	2904.80	3.143	0.096	
17	2970.38	0.076	0.002	2981.95	2960.73	1.596	0.027	
18	3062.96	0.057	0.001	3066.82	3047.53	1.086	0.006	
19	3221.12	0.079	0.000	3223.05	3211.48	0.910	0.001	
20	3367.71	0.091	0.000	3371.57	3365.78	0.526	0.001	
21	3676.32	0.039	0.009	3687.90	3674.39	0.449	0.057	

DOM_suolo_HC_t0

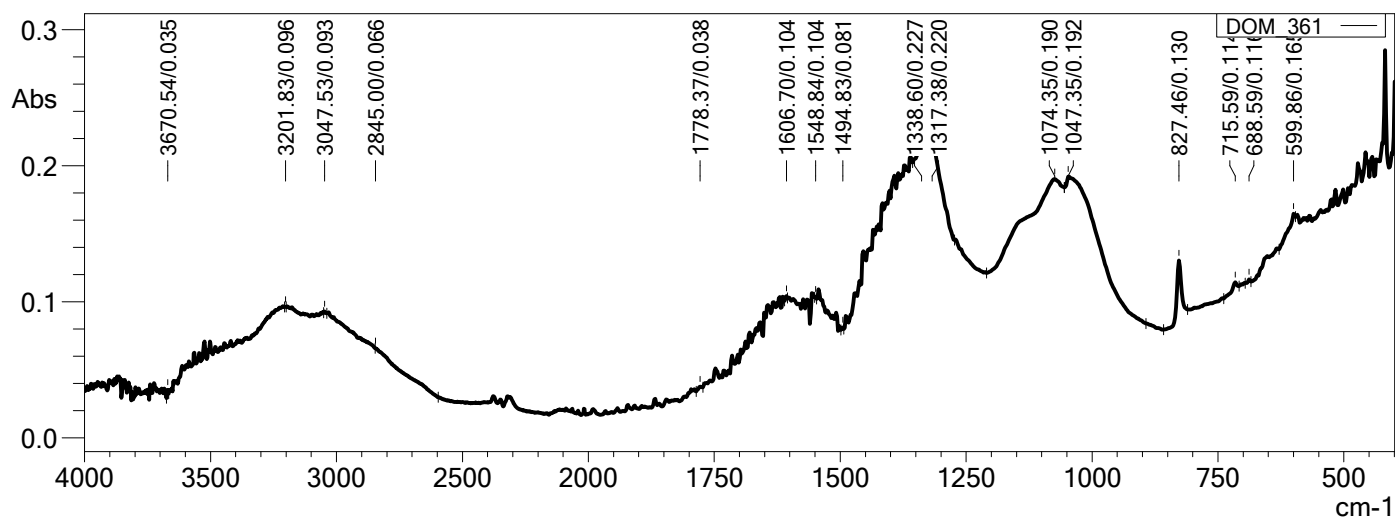
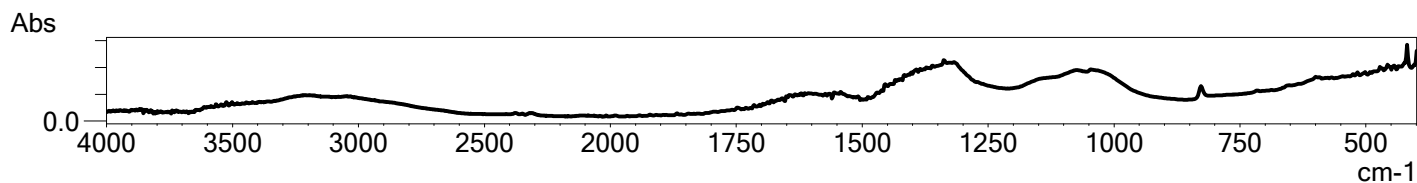


	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	671.23	0.102	0.002	684.73	669.30	1.511	0.020	
2	894.97	0.057	0.000	898.83	887.26	0.662	0.001	
3	1028.06	0.118	0.002	1031.92	908.47	10.581	-0.248	
4	1074.35	0.111	0.002	1193.94	1070.49	10.742	0.354	
5	1195.87	0.058	0.000	1203.58	1193.94	0.552	0.001	
6	1269.16	0.063	0.002	1274.95	1263.37	0.721	0.012	
7	1338.60	0.095	0.009	1342.46	1321.24	1.826	0.034	
8	1479.40	0.060	0.002	1483.26	1477.47	0.339	0.004	
9	1556.55	0.122	0.027	1560.41	1550.77	1.063	0.118	
10	1581.63	0.104	0.005	1585.49	1577.77	0.788	0.024	
11	1747.51	0.029	0.006	1755.22	1739.79	0.397	0.042	
12	3010.88	0.031	0.001	3016.67	3007.02	0.298	0.004	
13	3263.56	0.051	0.000	3267.41	3259.70	0.395	0.001	
14	3419.79	0.052	0.003	3437.15	3414.00	1.125	0.015	
15	3678.25	0.016	0.009	3687.90	3674.39	0.169	0.065	

DOM_suolo_HC_t0

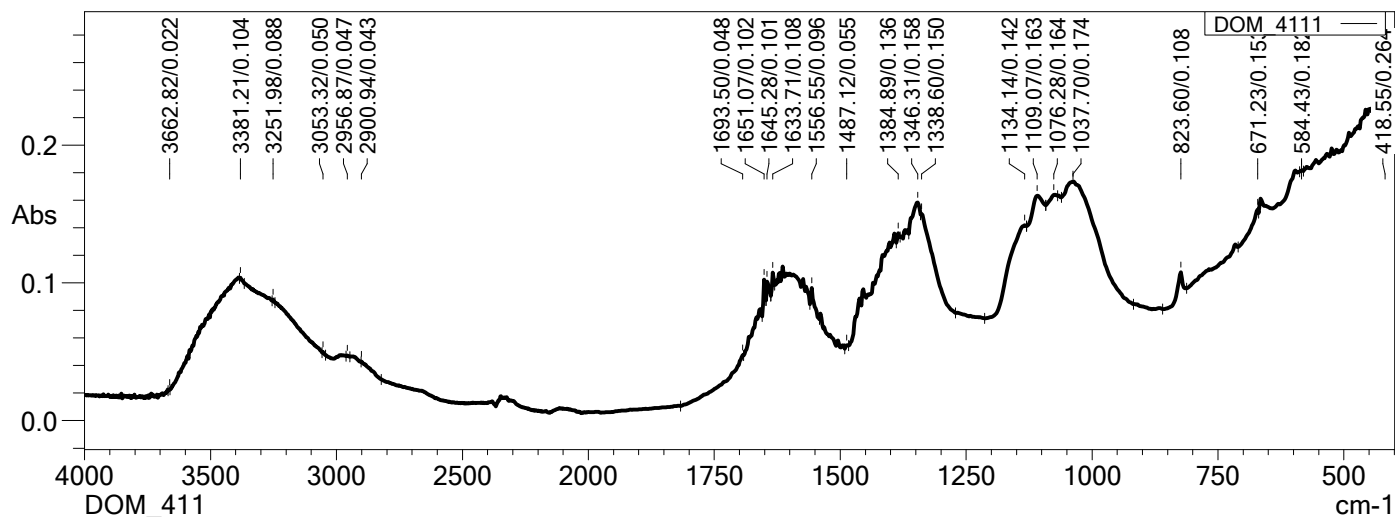
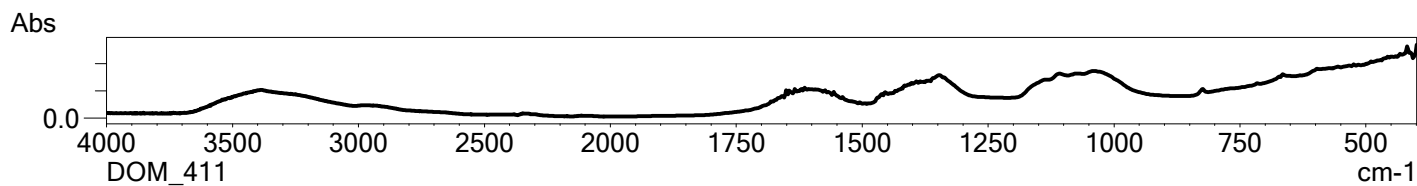


DOM_suolo_HC_t0



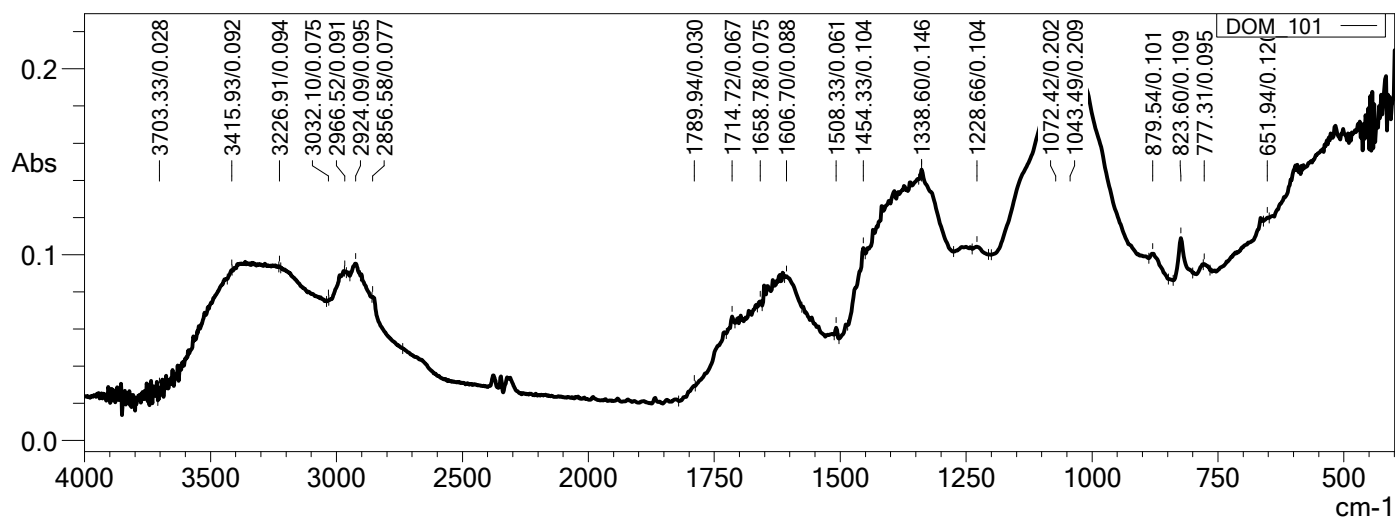
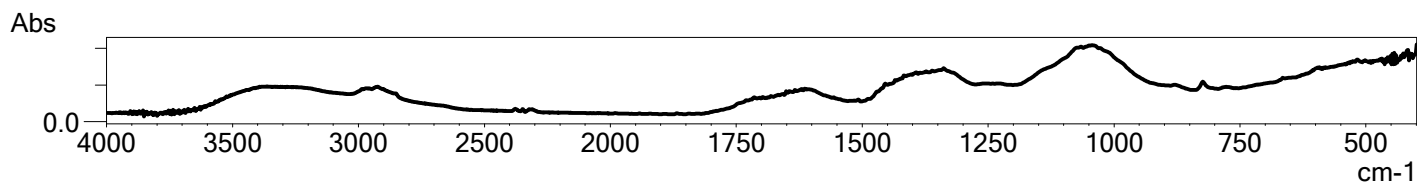
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	599.86	0.165	0.005	628.79	596.00	4.974	0.036	
2	688.59	0.116	0.002	696.30	684.73	1.330	0.007	
3	715.59	0.114	0.005	738.74	707.88	3.343	0.041	
4	827.46	0.130	0.041	858.32	810.10	4.549	0.358	
5	1047.35	0.192	0.013	1055.06	893.04	21.789	0.074	
6	1074.35	0.190	0.014	1209.37	1055.06	24.513	0.960	
7	1317.38	0.220	0.009	1321.24	1273.02	8.791	0.057	
8	1338.60	0.227	0.014	1355.96	1332.81	4.913	0.065	
9	1494.83	0.081	0.001	1498.69	1492.90	0.466	0.004	
10	1548.84	0.104	0.001	1550.77	1546.91	0.398	0.002	
11	1606.70	0.104	0.002	1610.56	1604.77	0.595	0.006	
12	1778.37	0.038	0.002	1786.08	1772.58	0.493	0.012	
13	2845.00	0.066	0.000	2846.93	2596.19	11.772	-0.225	
14	3047.53	0.093	0.001	3051.39	3037.89	1.245	0.005	
15	3201.83	0.096	0.000	3205.69	3199.91	0.557	0.000	
16	3670.54	0.035	0.004	3674.39	3666.68	0.254	0.017	

DOM_suolo_HC_t0



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	418.55	0.264	0.026	428.20	414.70	3.303	0.105	
2	584.43	0.182	0.001	588.29	580.57	1.400	0.003	
3	671.23	0.153	0.004	709.80	669.30	5.576	-0.029	
4	823.60	0.108	0.015	860.25	812.03	4.345	0.079	
5	1037.70	0.174	0.024	1060.85	918.12	18.766	1.177	
6	1076.28	0.164	0.003	1091.71	1068.56	3.734	0.034	
7	1109.07	0.163	0.014	1130.29	1091.71	5.984	0.247	
8	1134.14	0.142	0.004	1213.23	1130.29	8.694	-0.240	
9	1338.60	0.150	0.002	1340.53	1271.09	7.475	-0.427	
10	1346.31	0.158	0.012	1363.67	1340.53	3.506	0.206	
11	1384.89	0.136	0.005	1388.75	1381.03	1.028	0.016	
12	1487.12	0.055	0.001	1490.97	1483.26	0.418	0.007	
13	1556.55	0.096	0.014	1560.41	1541.12	1.605	0.083	
14	1633.71	0.108	0.013	1637.56	1629.85	0.773	0.044	
15	1645.28	0.101	0.013	1647.21	1637.56	0.923	0.066	
16	1651.07	0.102	0.021	1654.92	1647.21	0.696	0.067	
17	1693.50	0.048	0.001	1816.94	1691.57	2.987	-0.651	
18	2900.94	0.043	0.000	2902.87	2821.86	2.960	0.021	
19	2956.87	0.047	0.001	2962.66	2947.23	0.724	0.003	
20	3053.32	0.050	0.001	3057.17	3043.67	0.659	0.004	
21	3251.98	0.088	0.001	3255.84	3244.27	1.007	0.006	
22	3381.21	0.104	0.001	3385.07	3365.78	1.967	0.012	
23	3662.82	0.022	0.000	3668.61	3660.89	0.171	0.001	

DOM_suolo_HC_t0



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	651.94	0.120	0.001	659.66	648.08	1.380	0.004	
2	777.31	0.095	0.001	779.24	765.74	1.268	0.011	
3	823.60	0.109	0.021	839.03	800.46	3.684	0.283	
4	879.54	0.101	0.005	887.26	848.68	3.665	0.099	
5	1043.49	0.209	0.003	1047.35	1029.99	3.576	0.039	
6	1072.42	0.202	0.002	1199.72	1070.49	18.738	-0.727	
7	1228.66	0.104	0.002	1238.30	1205.51	3.361	0.030	
8	1338.60	0.146	0.008	1344.38	1274.95	8.572	0.164	
9	1454.33	0.104	0.007	1485.19	1450.47	2.883	0.063	
10	1508.33	0.061	0.005	1512.19	1502.55	0.559	0.020	
11	1606.70	0.088	0.002	1610.56	1575.84	2.850	0.080	
12	1658.78	0.075	0.003	1664.57	1654.92	0.712	0.015	
13	1714.72	0.067	0.005	1726.29	1708.93	1.080	0.031	
14	1789.94	0.030	0.001	1820.80	1788.01	0.820	-0.010	
15	2856.58	0.077	0.001	2858.51	2736.99	7.139	-0.554	
16	2924.09	0.095	0.007	2947.23	2904.80	3.910	0.150	
17	2966.52	0.091	0.000	2968.45	2960.73	0.701	0.000	
18	3032.10	0.075	0.000	3039.81	3030.17	0.725	0.000	
19	3226.91	0.094	0.000	3228.84	3221.12	0.721	0.002	
20	3415.93	0.092	0.001	3433.29	3414.00	1.729	0.007	
21	3703.33	0.028	0.005	3709.11	3699.47	0.243	0.020	

Sample ID	Ret. time (min)	Area	Acid	Concentration (mg soil kg ⁻¹ or ppm)	Dry soil (g)	Corr. Concentration (mg soil kg ⁻¹ or ppm)
Control soil 0-10	18.433	10588	acetic	18.83	2.846706803	66.15
	21.627	7260	propionic	20.44	2.846706803	71.80
Control soil 10-20	/	/	/	/	3.826988910	/
Control soil 20-30	/	/	/	/	5.249958419	/
Control soil 30-40	/	/	/	/	4.970623930	/
Control soil 40-50	/	/	/	/	5.181614003	/
GN1 HCDI	21.561	64410	propionic	86.42	4.308873720	200.55
	26.974	35925	butyric	59.11	4.308873720	137.18
	12.468	50164	lactic	40.90	4.579697987	89.31
GN3 HCDI	13.419	100108	lactic	80.76	4.579697987	176.35
	17.156	131539	formic	57.11	4.579697987	124.70
	21.474	208785	propionic	253.08	4.579697987	552.60
GN4 HCDI	11.166	8234	succinic	8.44	4.797137279	17.59

GN5 HCDI	11.193	6981	succinic	7.20	4.337046103	16.60
	12.197	9823	lactic	8.70	4.337046103	20.06
	20.580	17143	propionic	31.85	4.337046103	73.44
	28.407	18711	isovaleric	13.28	4.337046103	30.62
GW1 HCDI	/	/	/	/	4.368581315	/
GW2 HCDI	11.605	30362	succinic	30.28	4.402850000	68.76
	13.113	53262	lactic	43.37	4.402850000	98.50
	18.630	72351	acetic	68.41	4.402850000	155.38
	21.790	585334	propionic	687.75	4.402850000	1562.06
	27.445	107155	butyric	220.90	4.402850000	501.72
	12.444	28737	lactic	23.80	4.340282776	54.83
GW3 HCDI	17.193	14763	formic	7.11	4.340282776	16.38
	18.349	32522	acetic	36.44	4.340282776	83.95
	21.474	90805	propionic	116.88	4.340282776	269.29
	26.675	26128	butyric	36.86	4.340282776	84.93
	30.933	26729	isovaleric	38.25	4.340282776	88.12
	12.168	30344	lactic	25.08	4.710737538	53.24
GW4 HCDI	17.121	54967	formic	24.33	4.710737538	51.64
	18.435	112094	acetic	100.32	4.710737538	212.95

GW5 HCDI	21.675	262805	propionic	315.43	4.710737538	669.60
	27.201	155114	butyric	329.83	4.710737538	700.17
	31.658	111896	isovaleric	303.43	4.710737538	644.12
	11.196	8985	succinic	9.18	4.023561116	22.82
	20.631	20720	propionic	35.98	4.023561116	89.42
GQ1 HCDI	13.826	36174	lactic	29.73	4.648833552	63.95
	17.152	163058	formic	70.60	4.648833552	151.87
	18.422	105703	acetic	95.18	4.648833552	204.74
	21.494	201530	propionic	244.70	4.648833552	526.37
	26.926	31824	butyric	49.80	4.648833552	107.12
	31.071	164877	isovaleric	468.40	4.648833552	1007.56
GQ2 HCDI	13.046	13680	lactic	11.78	4.058808864	29.02
	17.090	25092	formic	11.53	4.058808864	28.41
	18.569	43749	acetic	45.45	4.058808864	111.98
	21.756	21386	propionic	36.75	4.058808864	90.54
	31.712	31410	isovaleric	52.82	4.058808864	130.14
GQ3 HCDI	17.150	98318	formic	42.89	4.232486631	101.33
	18.396	61205	acetic	59.46	4.232486631	140.48
	21.471	91441	propionic	117.62	4.232486631	277.90

GQ4 HCDI	26.754	37751	butyric	63.26	4.232486631	149.46
	30.854	191235	isovaleric	550.47	4.232486631	1300.58
	13.312	5001	lactic	4.85	5.006271473	9.69
	17.107	55560	formic	24.58	5.006271473	49.10
	18.428	44830	acetic	46.32	5.006271473	92.52
	31.363	41819	isovaleric	85.23	5.006271473	170.25
GQ5 HCDI	18.392	13005	acetic	20.77	4.420945263	46.98
	20.628	9578	propionic	23.12	4.420945263	52.30
	28.413	154897	isovaleric	437.32	4.420945263	989.20
GC1 HCDI	21.552	38870	propionic	56.93	4.518072289	126.01
	26.940	18660	butyric	19.90	4.518072289	44.04
	11.162	581116	succinic	573.75	4.117849899	1393.32
	12.494	546760	lactic	437.26	4.117849899	1061.86
	17.161	95409	formic	41.64	4.117849899	101.12
GC2 HCDI	18.351	132999	acetic	117.10	4.117849899	284.36
	21.470	1301782	propionic	1514.79	4.117849899	3678.59
	26.754	434822	butyric	965.14	4.117849899	2343.80
	30.929	102452	isovaleric	270.03	4.117849899	655.74
GC3 HCDI	11.174	10261	succinic	10.44	4.456979695	23.42

	12.303	35949	lactic	29.55	4.456979695	66.30
	17.185	30768	formic	13.96	4.456979695	31.32
	21.464	20203	propionic	35.38	4.456979695	79.38
GC4 HCDI	/	/	/	/	4.839040803	/
GC5 HCDI	20.614	33785	propionic	51.06	4.413237945	115.70
	28.395	20779	isovaleric	19.72	4.413237945	44.68

Table A.6: Laboratory data for the VFA and other organic acids analyses (HPLC) on soil samples collected from the control grave following a progressive depth before inhumation (control soil) and from the HCDI after each exhumation.

Below are the individual organic acid spectra obtained by HPLC analysis from samples collected at progressive depths in the control grave (control soil) and from the HCDI of each burial after exhumation. For each spectrum, a corresponding table is provided listing the characteristics of each peak, including retention time (minutes), area, and height.

The section is organized as follows:

Page 393: Control soil 0-10 cm

Page 395: Control soil 10-20 cm

Page 397: Control soil 20-30 cm

Page 399: Control soil 30-40 cm

Page 401: GN1 HCDI

Page 403: GN3 HCDI

Page 405: GN4 HCDI

Page 407: GN5 HCDI

Page 423: GQ1 HCDI

Page 425: GQ2 HCDI

Page 427: GQ3 HCDI

Page 429: GQ4 HCDI

Page 431: GQ5 HCDI

Page 409: GW1 HCDI

Page 411: GW2 HCDI

Page 413: GW3 HCDI

Page 415: GW4 HCDI

Page 417: GW5 HCDI

Page 433: GC1 HCDI

Page 435: GC2 HCDI

Page 437: GC3 HCDI

Page 439: GC4 HCDI

Page 441: GC5 HCDI



SHIMADZU
LabSolutions

Analysis Report

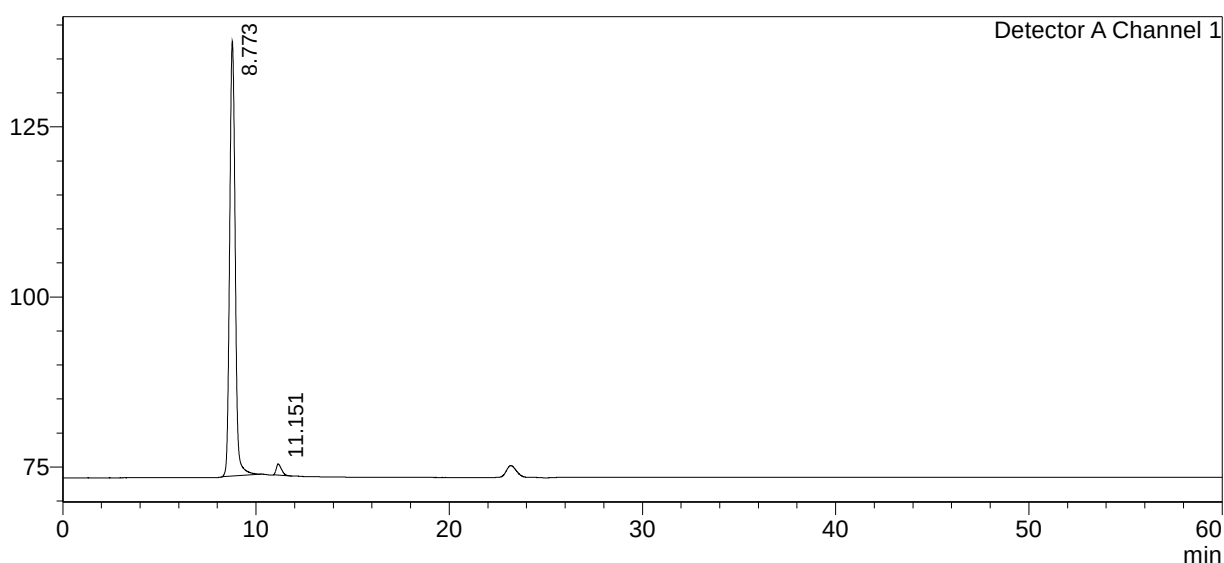
<Sample Information>

Sample Name : 88
Sample ID : A001
Data Filename : 98_011.lcd
Method Filename : AGV_digestate_24_Hi-plex.lcm
Batch Filename : AGV_prova_16-20.lcb
Vial # : 1-16
Injection Volume : 20 uL
Date Acquired : 11/04/2024 10:35:16
Date Processed : 11/04/2024 11:35:18

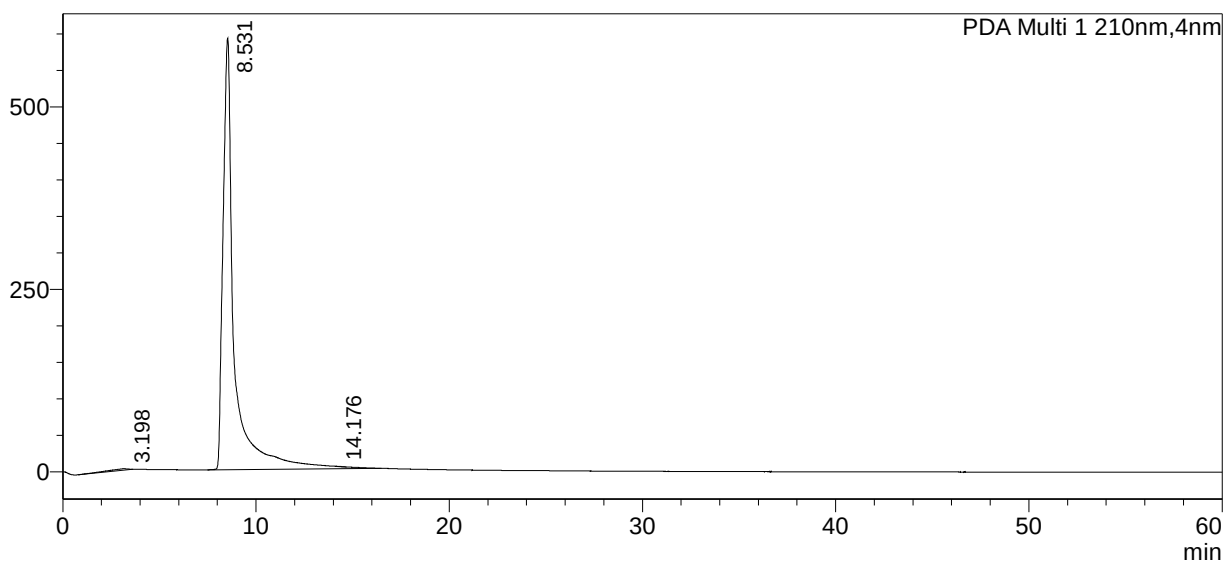
Sample Type : Unknown
Acquired by : Gruppo Ricicla
Processed by : Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.773	1283291	63940	0.000			
2	11.151	31494	1654	0.000			
Total		1314785	65594				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.198	162720	2022	0.000			
2	8.531	23791365	591066	0.000		S	
3	14.176	3091	152	0.000		T	
Total		23957176	593240				

**SHIMADZU**
LabSolutions

Analysis Report

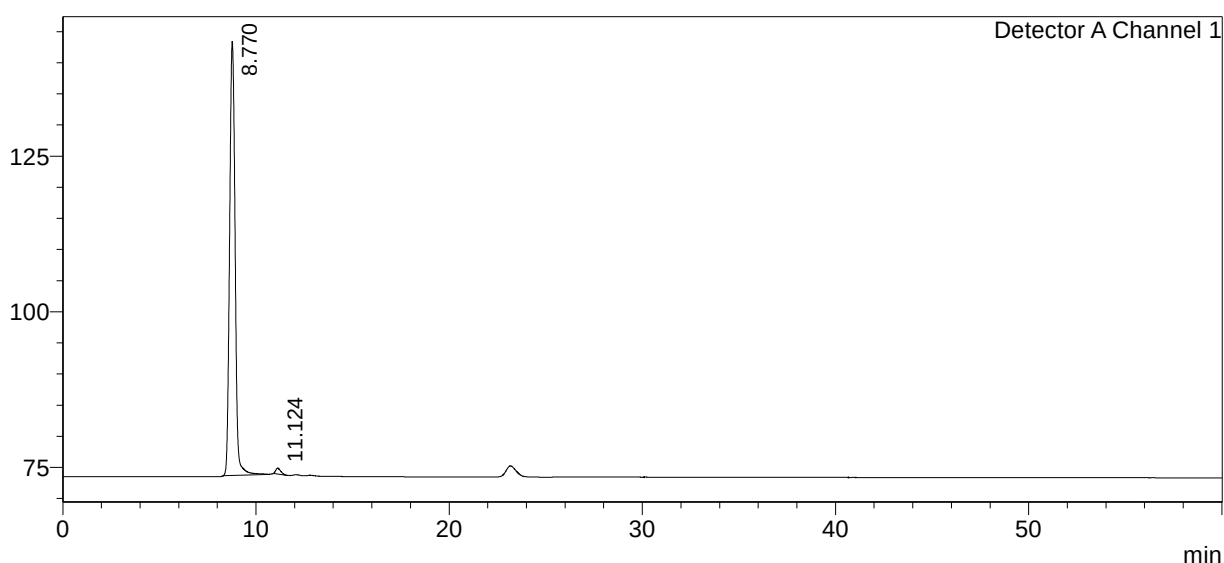
<Sample Information>

Sample Name : 89
Sample ID : A002
Data Filename : 99_012.lcd
Method Filename : AGV_digestate_24_Hi-plex.lcm
Batch Filename : AGV_prova_16-20.lcb
Vial # : 1-17
Injection Volume : 20 uL
Date Acquired : 11/04/2024 11:35:48
Date Processed : 11/04/2024 12:35:51

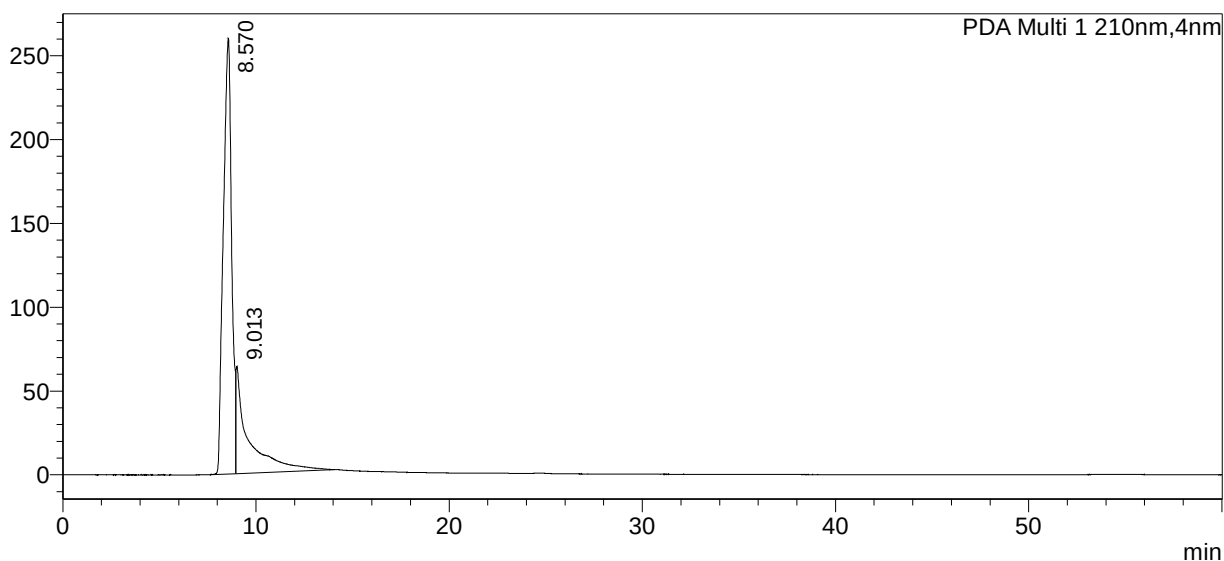
Sample Type : Unknown
Acquired by : Gruppo Ricicla
Processed by : Gruppo Ricicla

<Chromatogram>

mV



mAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.770	1389681	69770	0.000			
2	11.124	16517	948	0.000			
Total		1406197	70718				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.570	7767425	259970	0.000			
2	9.013	2943510	64074	0.000		V	
Total		10710935	324044				

**SHIMADZU**
LabSolutions

Analysis Report

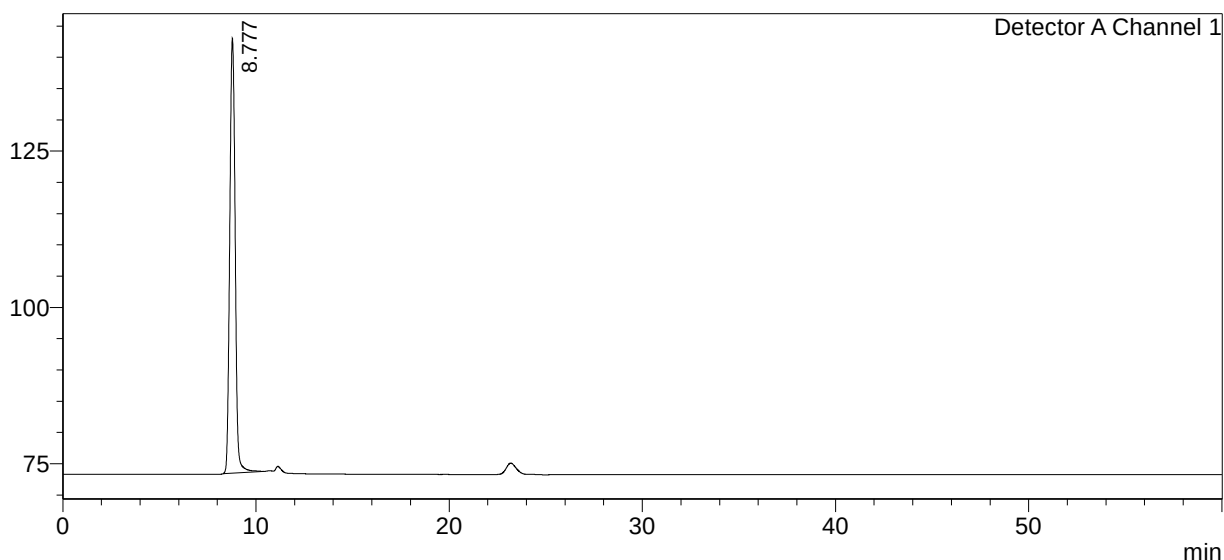
<Sample Information>

Sample Name : 90
Sample ID : A003
Data Filename : 100_013.lcd
Method Filename : AGV_digestate_24_Hi-plex.lcm
Batch Filename : AGV_prova_16-20.lcb
Vial # : 1-18
Injection Volume : 20 uL
Date Acquired : 11/04/2024 12:36:22
Date Processed : 11/04/2024 13:36:24

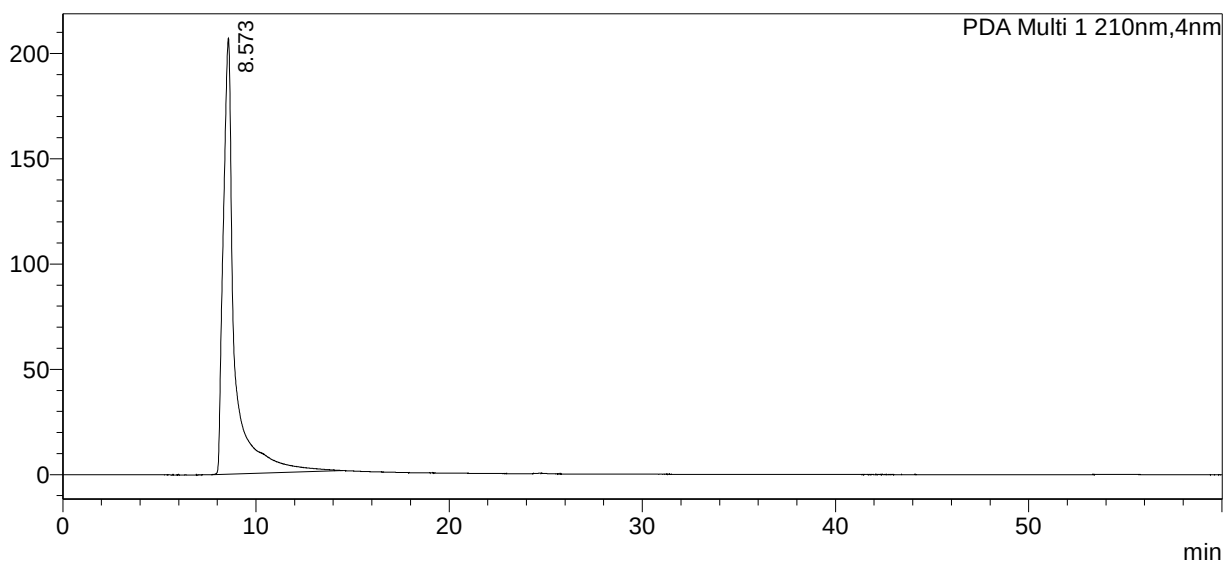
Sample Type : Unknown
Acquired by : Gruppo Ricicla
Processed by : Gruppo Ricicla

<Chromatogram>

mV



mAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.777	1371640	69570	0.000			
Total		1371640	69570				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.573	8399032	207010	0.000		S	
Total		8399032	207010				

**SHIMADZU**
LabSolutions

Analysis Report

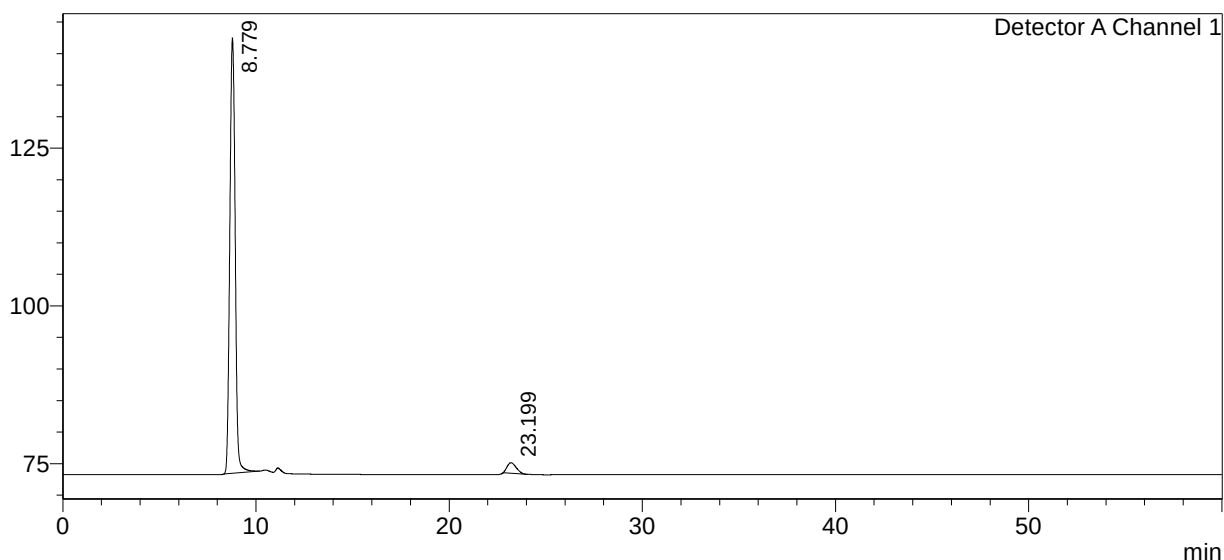
<Sample Information>

Sample Name : 91
Sample ID : A004
Data Filename : 101_014.lcd
Method Filename : AGV_digestate_24_Hi-plex.lcm
Batch Filename : AGV_prova_16-20.lcb
Vial # : 1-19
Injection Volume : 20 uL
Date Acquired : 11/04/2024 13:36:54
Date Processed : 11/04/2024 14:36:56

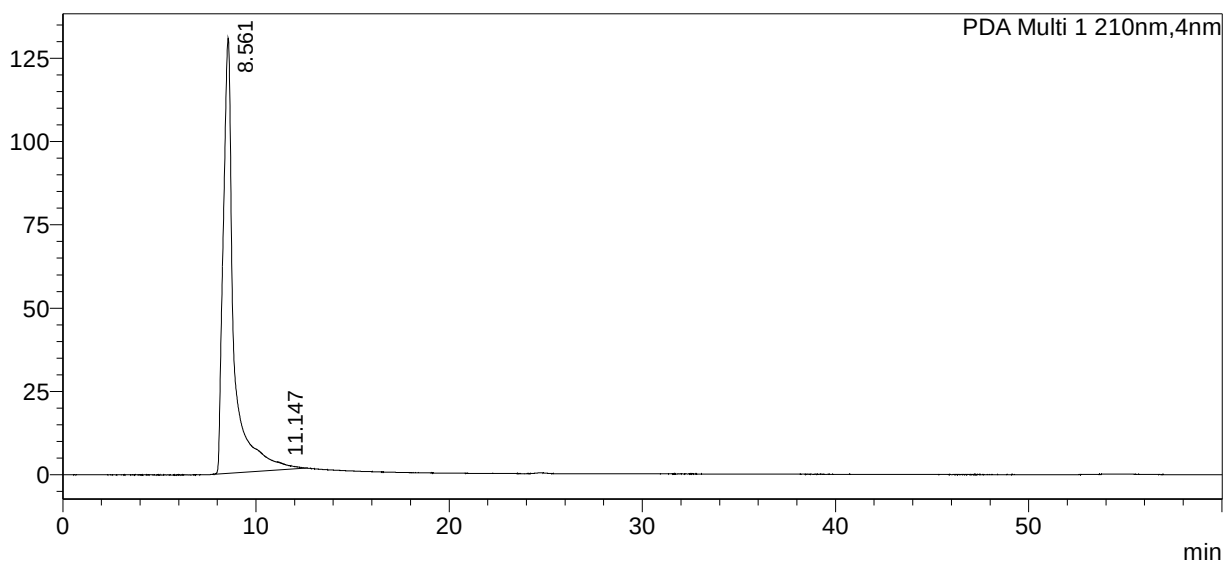
Sample Type : Unknown
Acquired by : Gruppo Ricicla
Processed by : Gruppo Ricicla

<Chromatogram>

mV



mAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.779	1345784	68994	0.000			
2	23.199	55192	1676	0.000			
Total		1400976	70670				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.561	4997312	130573	0.000		S	
2	11.147	1729	120	0.000		T	
Total		4999041	130693				



SHIMADZU
LabSolutions

Analysis Report

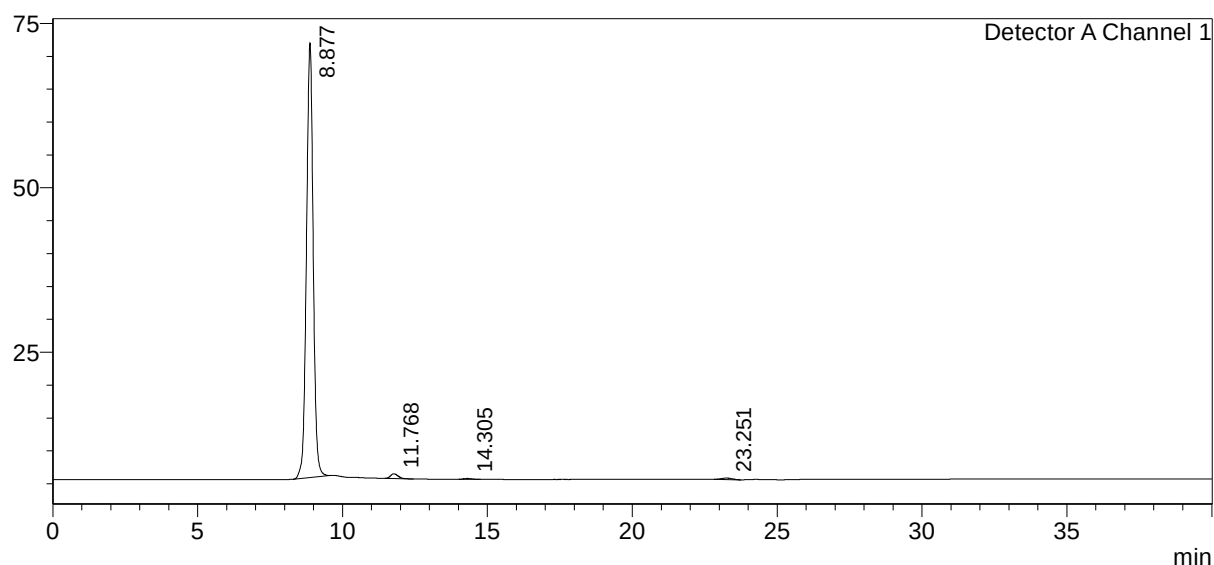
<Sample Information>

Sample Name : 19
 Sample ID : 19 a
 Data Filename : 19_002.lcd
 Method Filename : AGV_Andrea 60C-05.lcm
 Batch Filename : prova pig.lcb
 Vial # : 1-1
 Injection Volume : 20 uL
 Date Acquired : 12/09/2023 10:55:50
 Date Processed : 12/09/2023 11:35:52

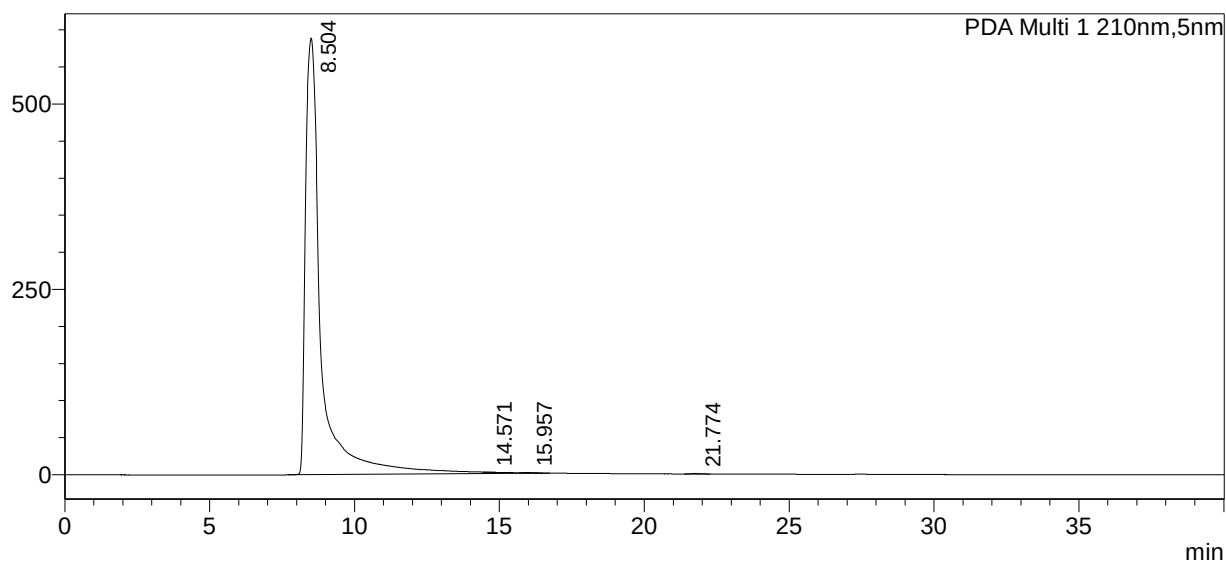
Sample Type : Unknown
 Acquired by : Gruppo Ricicla
 Processed by : Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.877	1070727	66066	0.000			
2	11.768	13513	741	0.000			
3	14.305	2522	128	0.000			
4	23.251	5833	223	0.000			
Total		1092595	67158				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.504	21993166	588665	0.000		S	
2	14.571	1424	101	0.000		T	
3	15.957	7999	371	0.000		T	
4	21.774	12811	523	0.000			
Total		22015400	589660				



SHIMADZU
LabSolutions

Analysis Report

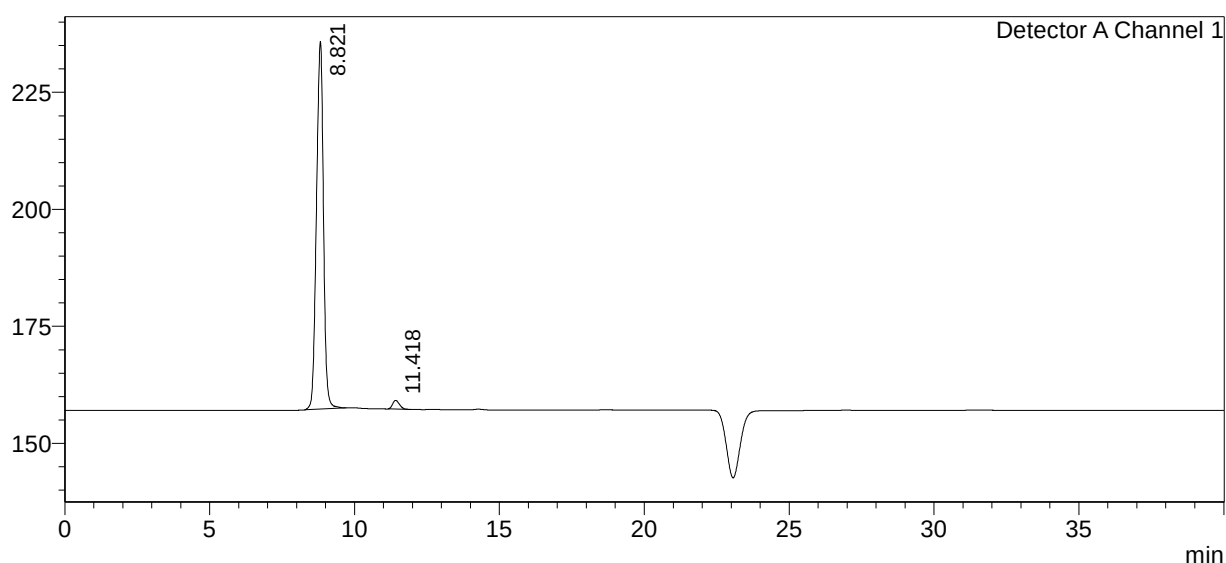
<Sample Information>

Sample Name : soil 53
 Sample ID : soil 53
 Data Filename : soil 53_002.lcd
 Method Filename : AGV_Andrea 60C-05.lcm
 Batch Filename : set soils_03.lcb
 Vial # : 1-2
 Injection Volume : 20 uL
 Date Acquired : 15/09/2023 14:20:47
 Date Processed : 15/09/2023 15:00:48

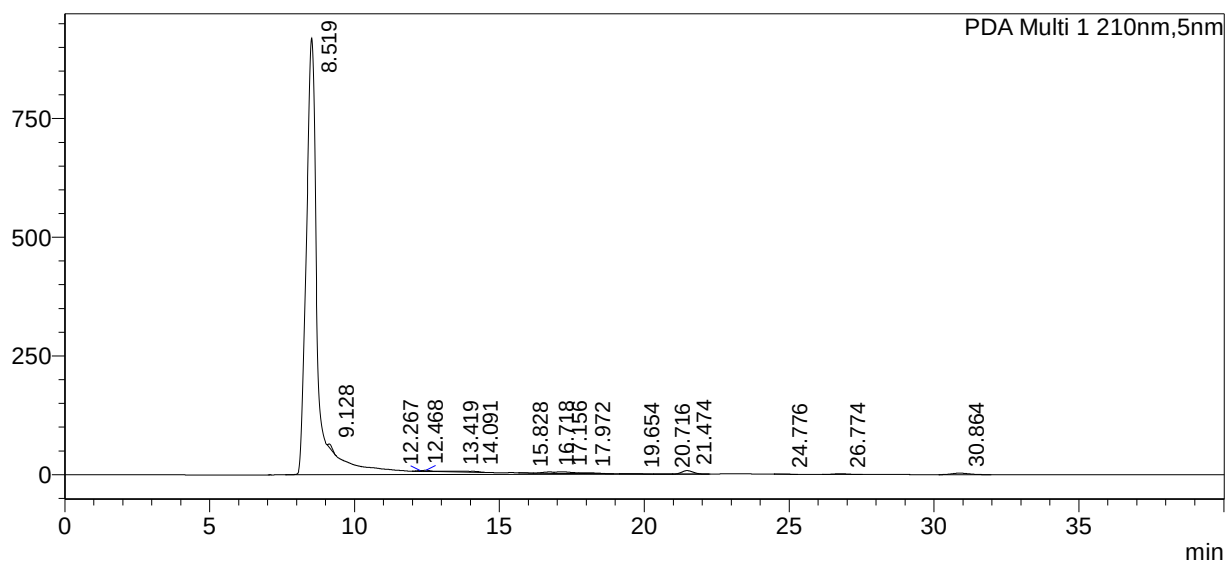
Sample Type : Unknown
 Acquired by : Gruppo Ricicla
 Processed by : Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.821	1325255	78632	0.000			
2	11.418	30372	1864	0.000			
Total		1355627	80496				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.519	26786251	919667	0.000		S	
2	9.128	67316	7361	0.000		T	
3	12.267	22420	1900	0.000		T	
4	12.468	50164	2525	0.000		TV	
5	13.419	100108	1751	0.000		TV	
6	14.091	35721	1834	0.000		TV	
7	15.828	5099	345	0.000		TV	
8	16.718	70010	2743	0.000		T	
9	17.156	131539	3331	0.000		TV	
10	17.972	79030	1899	0.000	mg/L	TV	Acetic acid
11	19.654	18441	605	0.000		T	
12	20.716	9185	400	0.000		T	
13	21.474	208785	7406	0.000		TV	
14	24.776	8835	347	0.000			
15	26.774	57114	1668	0.000			
16	30.864	133399	3150	0.000			
Total		27783416	956932				

**SHIMADZU**
LabSolutions

Analysis Report

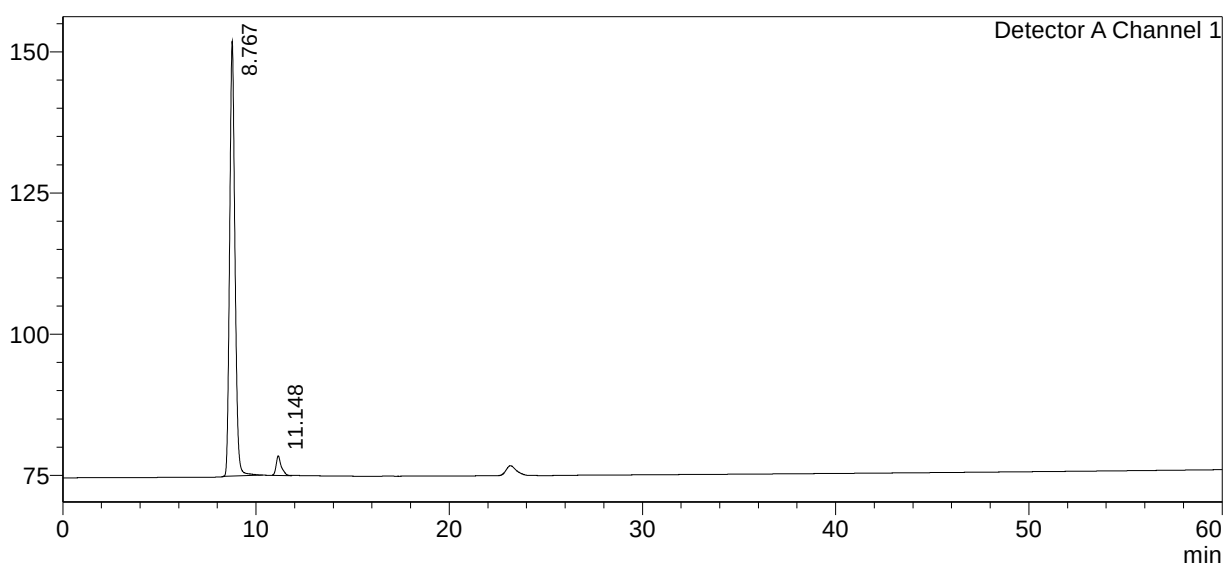
<Sample Information>

Sample Name : 86
Sample ID : A004
Data Filename : 86_004.lcd
Method Filename : AGV_digestate_24_Hi-plex.lcm
Batch Filename : AGV_prova_primi5.lcb
Vial # : 1-4
Injection Volume : 20 uL
Date Acquired : 09/04/2024 17:42:27
Date Processed : 09/04/2024 18:42:29

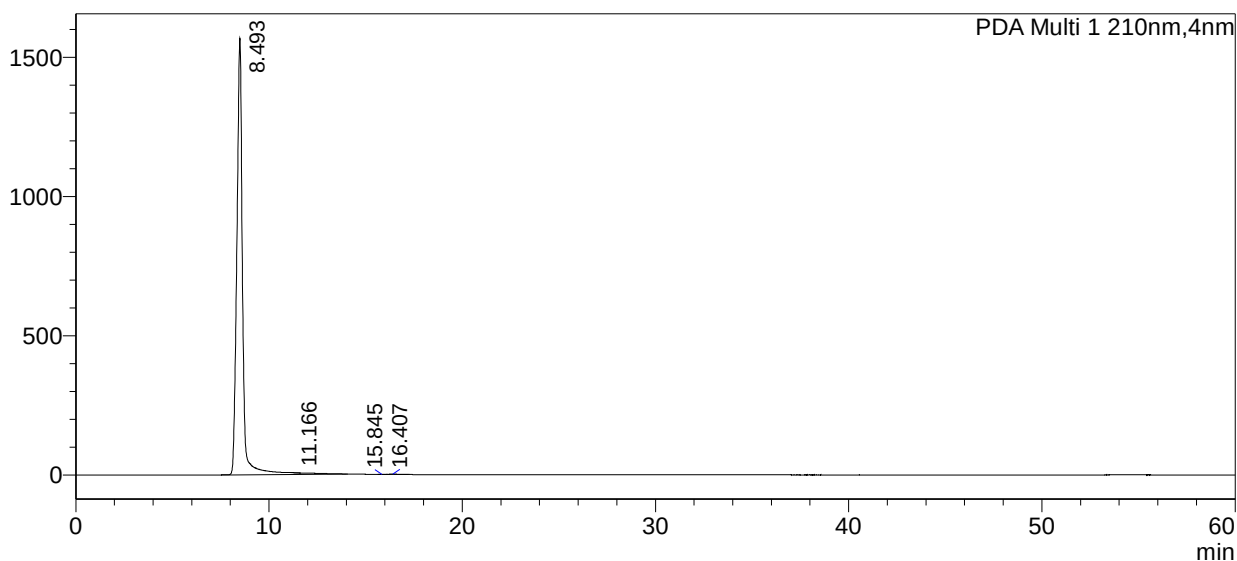
Sample Type : Unknown
Acquired by : Gruppo Ricicla
Processed by : Gruppo Ricicla

<Chromatogram>

mV



mAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.767	1478454	77020	0.000			
2	11.148	63287	3472	0.000			
Total		1541740	80492				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.493	32430536	1567453	0.000		S	
2	11.166	8234	692	0.000		T	
3	15.845	3105	228	0.000			
4	16.407	30548	1245	0.000			
Total		32472423	1569618				



SHIMADZU
LabSolutions

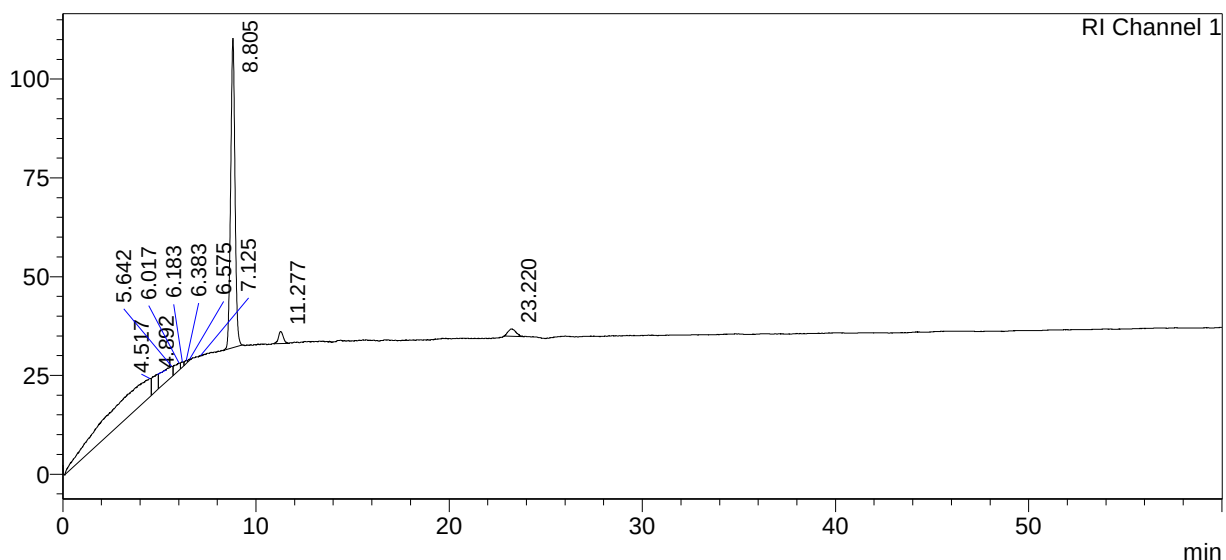
Analysis Report

<Sample Information>

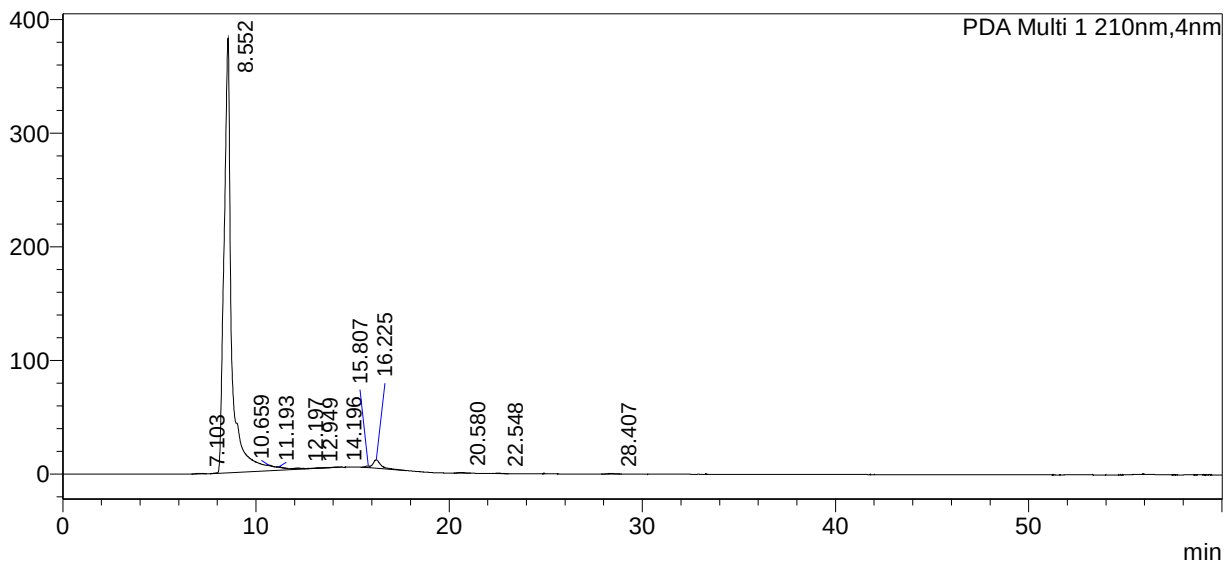
Sample Name	: 104		
Sample ID	: 104		
Data Filename	: 104_001.lcd		
Method Filename	: AGV_Matteo_Gabriele_feb_2025_Hi-plex.lcm		
Batch Filename	: 104.lcb		
Vial #	: 1-2	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 04/06/2025 11:16:40	Acquired by	: Gruppo Ricicla
Date Processed	: 04/06/2025 12:16:42	Processed by	: Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

RI Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	4.517	1153896	4572	0.000			
2	4.892	93124	3961	0.000		V	
3	5.642	143734	2605	0.000		V	
4	6.017	44402	1728	0.000		V	
5	6.183	14146	1318	0.000		V	
6	6.383	8813	747	0.000		V	
7	6.575	3450	277	0.000		V	
8	7.125	1034	143	0.000			
9	8.805	1232784	78375	0.000			
10	11.277	51734	3062	0.000			
11	23.220	54254	1875	0.000			
Total		2801369	98662				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	7.103	8776	398	0.000			
2	8.552	10377923	382309	0.000		S	
3	10.659	7298	598	0.000		T	
4	11.193	6981	554	0.000		T	
5	12.197	9823	477	0.000		T	
6	12.949	1450	84	0.000		T	
7	14.196	6765	246	0.000		T	
8	15.807	23097	1547	0.000			
9	16.225	217000	7528	0.000		V	
10	20.580	17143	715	0.000			
11	22.548	8292	335	0.016	g/L		Propionic acid
12	28.407	18711	547	0.000			
Total		10703259	395338				



SHIMADZU
LabSolutions

Analysis Report

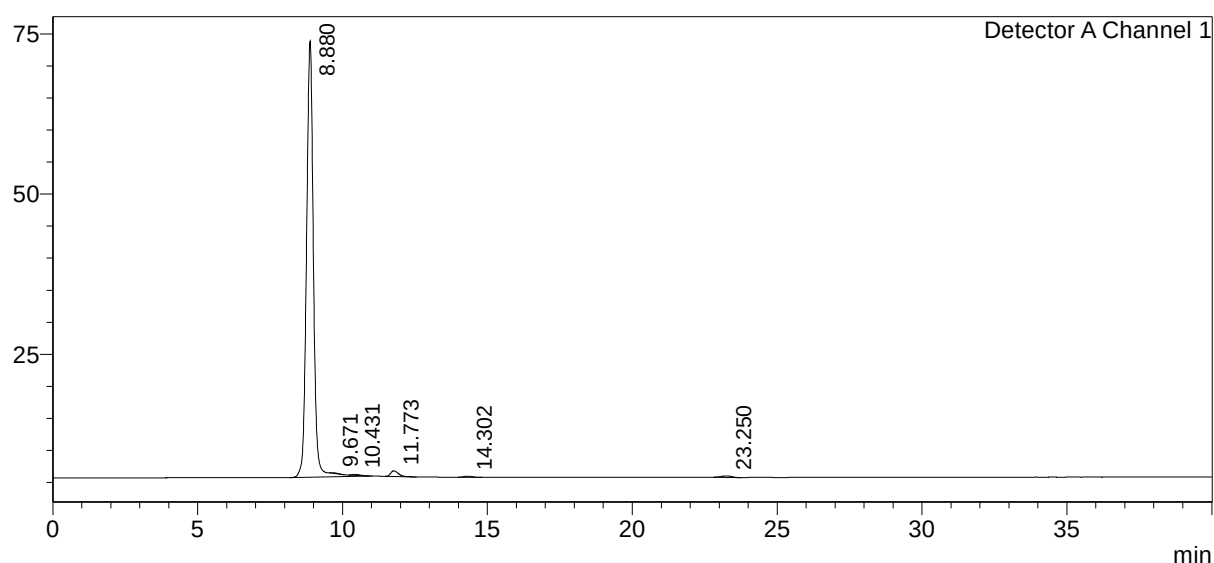
<Sample Information>

Sample Name : 21
Sample ID : 21 a
Data Filename : 21_003.lcd
Method Filename : AGV_Andrea 60C-05.lcm
Batch Filename : prova pig.lcb
Vial # : 1-2
Injection Volume : 20 uL
Date Acquired : 12/09/2023 11:36:22
Date Processed : 12/09/2023 12:16:26

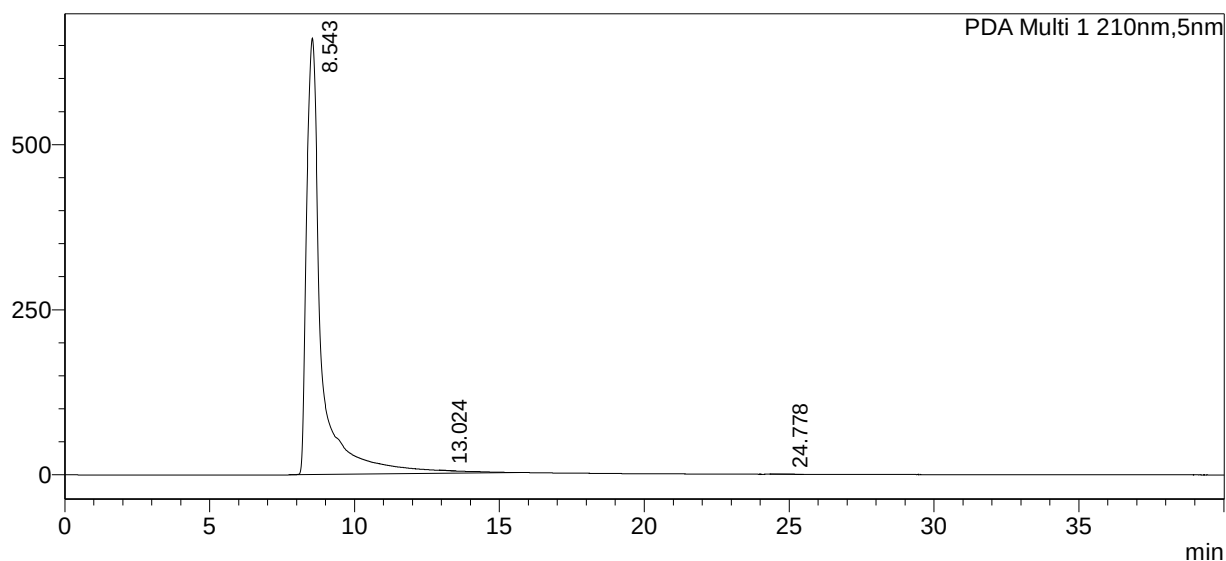
Sample Type : Unknown
Acquired by : Gruppo Ricicla
Processed by : Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.880	1134334	68037	0.000		S	
2	9.671	1542	90	0.000		T	
3	10.431	2526	147	0.000		T	
4	11.773	16749	905	0.000			
5	14.302	3400	165	0.000			
6	23.250	6063	230	0.000			
Total		1164614	69574				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.543	23522507	660803	0.000		S	
2	13.024	3824	200	0.000		T	
3	24.778	12035	358	0.000			
Total		23538366	661361				



SHIMADZU
LabSolutions

Analysis Report

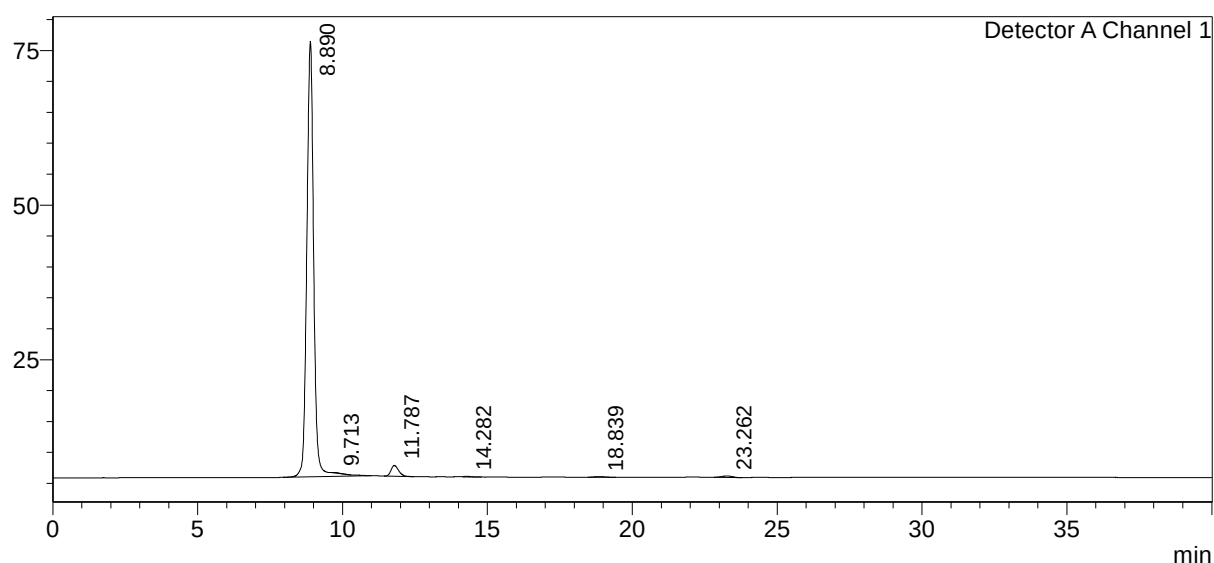
<Sample Information>

Sample Name : 41
 Sample ID : 41
 Data Filename : 41_002.lcd
 Method Filename : VFA_Analysis_60C-05.lcm
 Batch Filename : prova pig 2.lcb
 Vial # : 1-5
 Injection Volume : 20 uL
 Date Acquired : 12/09/2023 13:03:41
 Date Processed : 12/09/2023 14:56:59

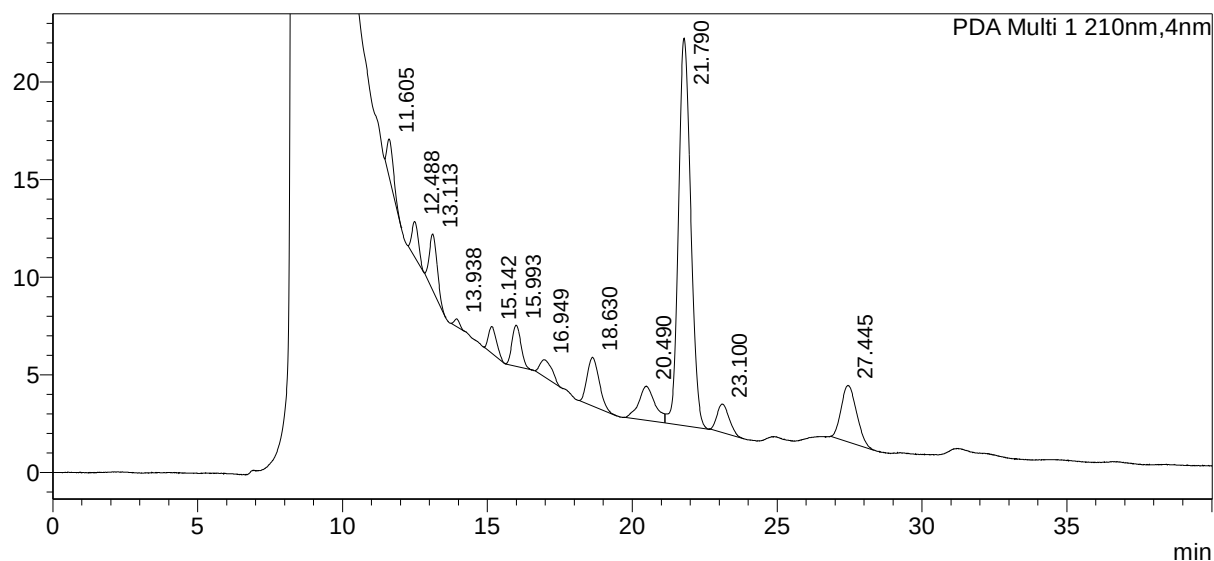
Sample Type : Unknown
 Acquired by : Gruppo Ricicla
 Processed by : Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.890	1170296	70461	0.000		S	
2	9.713	2144	108	0.000		T	
3	11.787	31823	1737	0.000			
4	14.282	1800	95	0.000		V	
5	18.839	3228	123	0.000			
6	23.262	6308	239	0.000			
Total		1215599	72763				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	11.605	30362	1944	0.000			
2	12.488	28295	1812	0.000			
3	13.113	53262	2890	0.000			
4	13.938	5226	402	0.000			
5	15.142	26393	1349	0.000			
6	15.993	45150	2109	0.000			
7	16.949	25677	859	0.000			
8	18.630	72351	2495	86.384	mg/L		RT:18.455
9	20.490	68909	1754	0.000			
10	21.790	585334	19843	0.000		V	
11	23.100	42478	1458	0.000			
12	27.445	107155	2886	0.000			
Total		1090592	39801				

Analysis Report

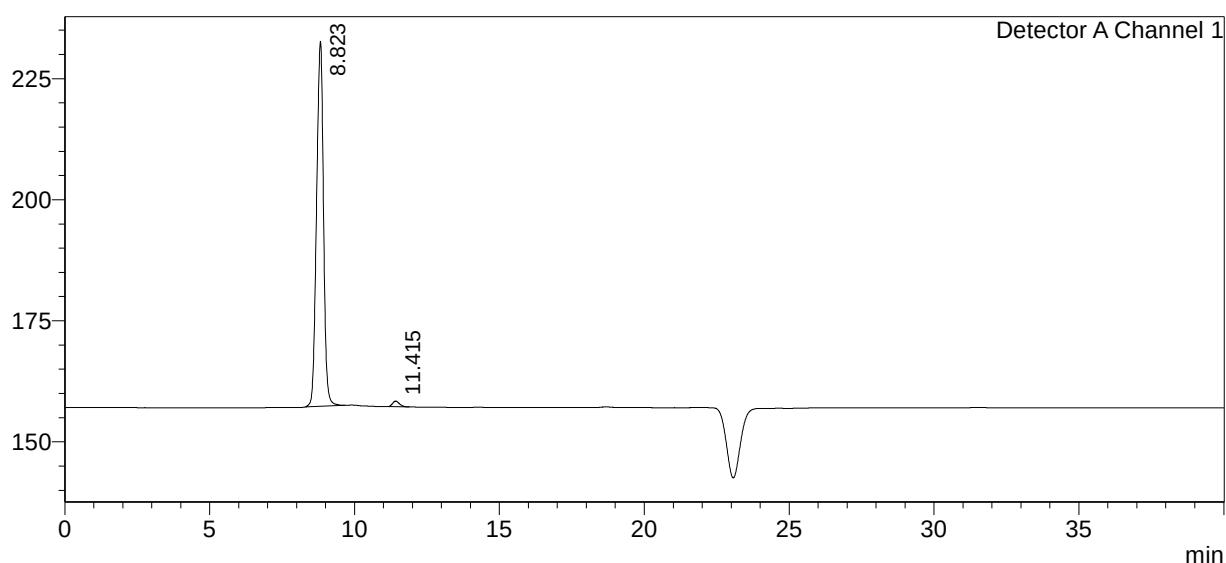
<Sample Information>

Sample Name : soil 62
 Sample ID : soil 62
 Data Filename : soil 62_003.lcd
 Method Filename : AGV_Andrea 60C-05.lcm
 Batch Filename : set soils_03.lcb
 Vial # : 1-3
 Injection Volume : 20 uL
 Date Acquired : 15/09/2023 15:01:19
 Date Processed : 15/09/2023 15:41:21

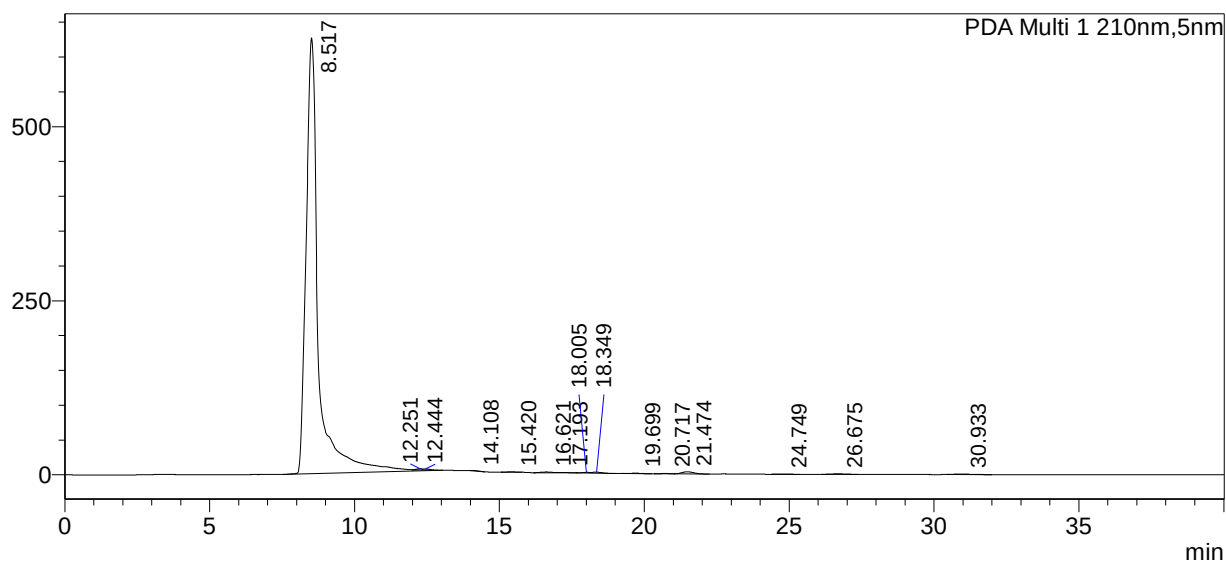
Sample Type : Unknown
 Acquired by : Gruppo Ricicla
 Processed by : Gruppo Ricicla

<Chromatogram>

mV



mAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.823	1259759	75422	0.000			
2	11.415	18199	1126	0.000			
Total		1277957	76548				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.517	18297571	625450	0.000		S	
2	12.251	22799	1771	0.000		T	
3	12.444	28737	1815	0.000		TV	
4	14.108	8907	512	0.000			
5	15.420	12564	648	0.000			
6	16.621	31672	1137	0.000			
7	17.193	14763	633	0.000		V	
8	18.005	23107	1068	0.000			
9	18.349	32522	1439	0.000	mg/L	V	Acetic acid
10	19.699	18055	650	0.000			
11	20.717	10561	424	0.000			
12	21.474	90805	3150	0.000		V	
13	24.749	11868	399	0.000			
14	26.675	26128	716	0.000			
15	30.933	26729	615	0.000			
Total		18656787	640428				



SHIMADZU
LabSolutions

Analysis Report

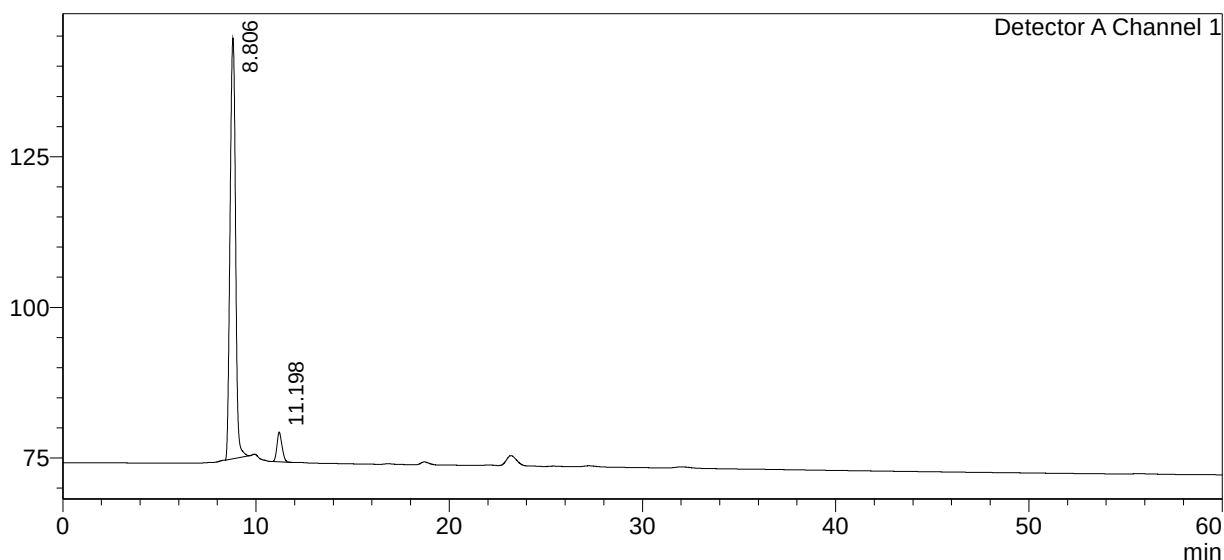
<Sample Information>

Sample Name : 93
Sample ID : A006
Data Filename : 93_006.lcd
Method Filename : AGV_digestate_24_Hi-plex.lcm
Batch Filename : AGV_Prova_6_15.lcb
Vial # : 1-11
Injection Volume : 20 uL
Date Acquired : 10/04/2024 22:20:42
Date Processed : 10/04/2024 23:20:44

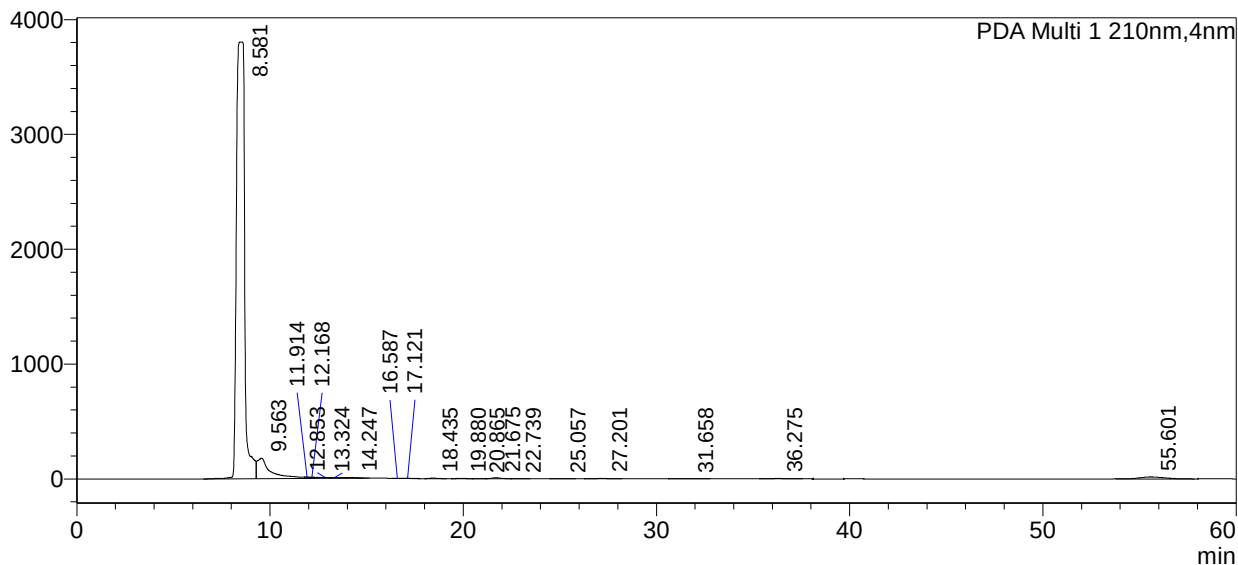
Sample Type : Unknown
Acquired by : Gruppo Ricicla
Processed by : Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.806	1380517	69790	0.000			
2	11.198	89630	4946	0.000			
Total		1470146	74737				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.581	111326345	3801142	0.000			
2	9.563	9496518	178204	0.000		SV	
3	11.914	13206	1178	0.000		T	
4	12.168	30344	1892	0.000		TV	
5	12.853	5546	306	0.000		T	
6	13.324	29453	856	0.000		TV	
7	14.247	76412	3431	0.000		TV	
8	16.587	24450	1037	0.000			
9	17.121	54967	2071	0.000		V	
10	18.435	112094	4138	0.179	g/L		Acetic acid
11	19.880	77475	2651	0.000			
12	20.865	28536	1135	0.000			
13	21.675	262805	7826	0.440	g/L	V	Propionic acid
14	22.739	19442	571	0.000		V	
15	25.057	21865	617	0.027	g/L		Isobutyric acid
16	27.201	155114	3314	0.272	g/L		Butyric acid
17	31.658	111896	1853	0.190	g/L		Isovaleric acid
18	36.275	170264	2950	0.340	g/L		Valeric acid
19	55.601	1512995	16861	0.000		V	
Total		123529726	4032033				



SHIMADZU
LabSolutions

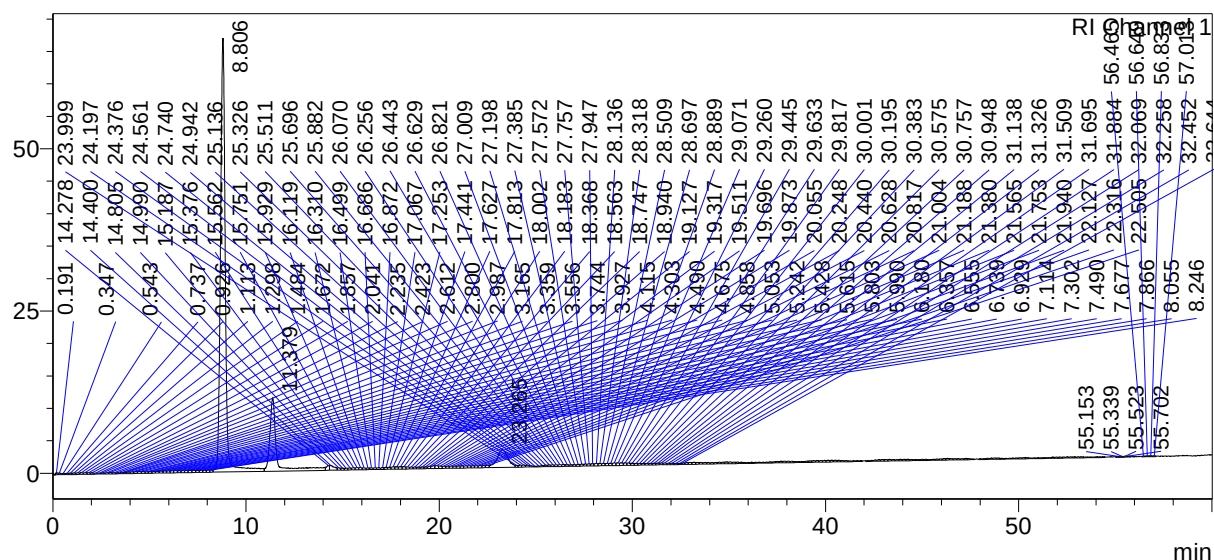
Analysis Report

<Sample Information>

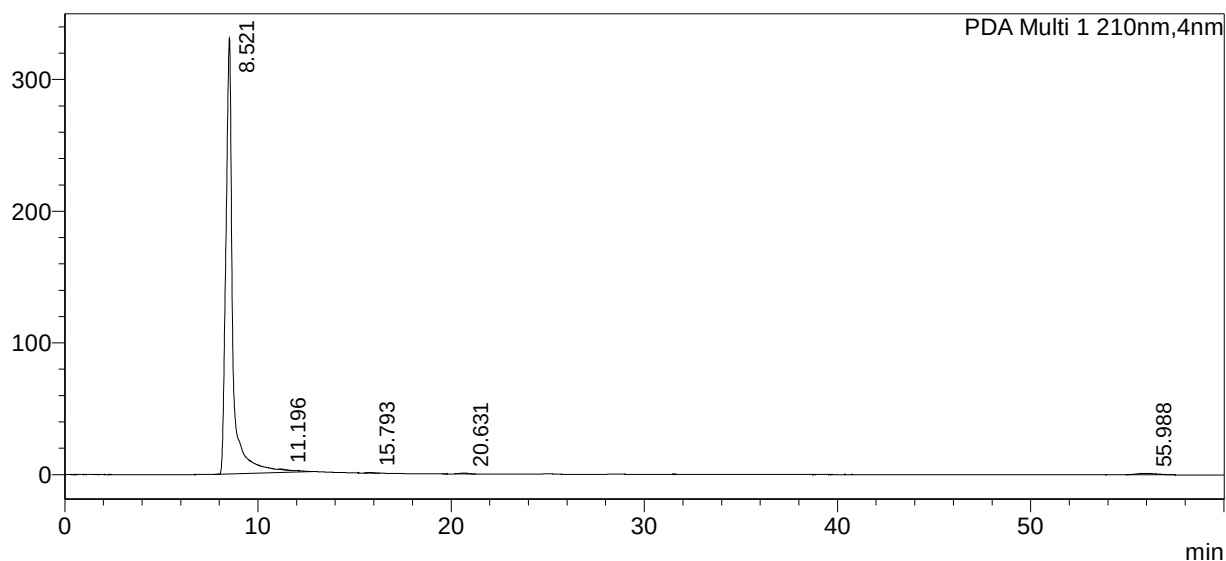
Sample Name	: 110		
Sample ID	: 110		
Data Filename	: 110_003.lcd		
Method Filename	: AGV_Matteo_Gabriele_feb_2025_Hi-plex.lcm		
Batch Filename	: 108_109_110.lcb		
Vial #	: 1-3	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 05/06/2025 12:07:22	Acquired by	: Gruppo Ricicla
Date Processed	: 05/06/2025 13:07:24	Processed by	: Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

RI Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.191	2556	306	0.000			
2	0.347	2446	256	0.000		V	
3	0.543	1763	196	0.000		V	
4	0.737	2116	213	0.000		V	
5	0.926	2417	239	0.000		V	
6	1.113	2673	262	0.000		V	
7	1.298	2658	266	0.000		V	
8	1.484	2804	271	0.000		V	
9	1.672	2660	268	0.000		V	
10	1.857	2641	257	0.000		V	
11	2.041	2187	222	0.000		V	
12	2.235	1758	186	0.000		V	
13	2.423	1683	178	0.000		V	
14	2.612	1757	187	0.000		V	
15	2.800	2034	205	0.000		V	
16	2.987	2303	233	0.000		V	
17	3.165	1991	202	0.000		V	
18	3.359	1433	161	0.000		V	
19	3.556	1588	175	0.000		V	
20	3.744	2201	225	0.000		V	
21	3.927	2705	268	0.000		V	
22	4.115	2735	275	0.000		V	
23	4.303	3071	296	0.000		V	
24	4.490	3282	320	0.000		V	
25	4.675	3243	301	0.000		V	
26	4.858	2437	266	0.000		V	
27	5.053	2748	269	0.000		V	
28	5.242	2842	289	0.000		V	
29	5.428	3212	310	0.000		V	
30	5.615	3358	320	0.000		V	
31	5.803	3324	330	0.000		V	
32	5.990	3440	341	0.000		V	
33	6.180	3785	360	0.000		V	
34	6.357	3735	354	0.000		V	
35	6.555	3599	342	0.000		V	
36	6.739	3489	343	0.000		V	
37	6.929	3575	354	0.000		V	
38	7.114	3823	360	0.000		V	
39	7.302	3790	360	0.000		V	
40	7.490	3540	347	0.000		V	
41	7.677	3563	353	0.000		V	
42	7.866	3671	350	0.000		V	
43	8.055	3561	352	0.000		V	
44	8.246	3451	373	0.000		V	
45	8.806	1165278	66866	0.000		SV	
46	11.379	264954	11301	0.000		SV	
47	14.278	8121	727	0.000		V	
48	14.400	12856	716	0.000		V	
49	14.805	3433	355	0.000		V	
50	14.990	3184	318	0.000		V	
51	15.187	3005	301	0.000		V	
52	15.376	3224	325	0.000		V	
53	15.562	3604	348	0.000		V	
54	15.751	3782	363	0.000		V	
55	15.929	3450	341	0.000		V	
56	16.119	3171	305	0.000		V	
57	16.310	2813	285	0.000		V	
58	16.499	2795	283	0.000		V	
59	16.686	2902	283	0.000		V	
60	16.872	2784	272	0.000		V	
61	17.067	2602	278	0.000		V	
62	17.253	3266	321	0.000		V	

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
63	17.441	3423	343	0.000		V	
64	17.627	4154	384	0.000		V	
65	17.813	3873	380	0.000		V	
66	18.002	4041	382	0.000		V	
67	18.183	3982	379	0.000		V	
68	18.368	3418	341	0.000		V	
69	18.563	3087	310	0.000		V	
70	18.747	3044	296	0.000		V	
71	18.940	2773	283	0.000		V	
72	19.127	2991	293	0.000		V	
73	19.317	2847	299	0.000		V	
74	19.511	3542	362	0.000		V	
75	19.696	4886	452	0.000		V	
76	19.873	5338	466	0.000		V	
77	20.055	3900	401	0.000		V	
78	20.248	3608	355	0.000		V	
79	20.440	3662	351	0.000		V	
80	20.628	3510	349	0.000		V	
81	20.817	3560	356	0.000		V	
82	21.004	3797	363	0.000		V	
83	21.188	3651	363	0.000		V	
84	21.380	3705	355	0.000		V	
85	21.565	3612	360	0.000		V	
86	21.753	3719	355	0.000		V	
87	21.940	3545	352	0.000		V	
88	22.127	3735	356	0.000		V	
89	22.316	3667	366	0.000		V	
90	22.505	3931	374	0.000		V	
91	23.265	106740	2738	0.000		V	
92	23.999	2492	299	0.000		V	
93	24.197	3212	330	0.000		V	
94	24.376	3614	335	0.000		V	
95	24.561	3321	297	0.000		V	
96	24.740	1708	215	0.000		V	
97	24.942	1093	133	0.000		V	
98	25.136	1388	162	0.000		V	
99	25.326	2003	212	0.000		V	
100	25.511	2488	261	0.000		V	
101	25.696	3004	285	0.000		V	
102	25.882	2825	287	0.000		V	
103	26.070	2838	288	0.000		V	
104	26.256	3022	295	0.000		V	
105	26.443	3040	301	0.000		V	
106	26.629	2741	269	0.000		V	
107	26.821	2442	251	0.000		V	
108	27.009	2546	262	0.000		V	
109	27.198	2941	291	0.000		V	
110	27.385	3095	314	0.000		V	
111	27.572	3510	341	0.000		V	
112	27.757	3482	346	0.000		V	
113	27.947	3539	351	0.000		V	
114	28.136	4202	390	0.000		V	
115	28.318	3839	379	0.000		V	
116	28.509	3720	368	0.000		V	
117	28.697	3949	376	0.000		V	
118	28.889	3973	394	0.000		V	
119	29.071	4419	417	0.000		V	
120	29.260	4493	425	0.000		V	
121	29.445	4250	418	0.000		V	
122	29.633	4293	404	0.000		V	
123	29.817	4036	385	0.000		V	
124	30.001	3300	328	0.000		V	

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
125	30.195	3056	296	0.000		V	
126	30.383	2583	271	0.000		V	
127	30.575	2886	286	0.000		V	
128	30.757	2768	271	0.000		V	
129	30.948	2497	257	0.000		V	
130	31.138	2634	272	0.000		V	
131	31.326	2971	294	0.000		V	
132	31.509	3076	299	0.000		V	
133	31.695	2875	283	0.000		V	
134	31.884	2380	243	0.000		V	
135	32.069	2288	231	0.000		V	
136	32.258	1785	190	0.000		V	
137	32.452	1665	187	0.000		V	
138	32.644	2349	246	0.000		V	
139	32.829	2829	293	0.000		V	
140	33.006	3206	305	0.000		V	
141	33.194	3232	311	0.000		V	
142	33.382	2945	294	0.000		V	
143	33.567	2881	278	0.000		V	
144	33.756	2469	248	0.000		V	
145	33.940	2418	238	0.000		V	
146	34.131	2158	220	0.000		V	
147	34.322	2383	237	0.000		V	
148	34.513	2465	255	0.000		V	
149	34.702	2740	271	0.000		V	
150	34.889	2908	286	0.000		V	
151	35.071	2776	271	0.000		V	
152	35.260	2402	246	0.000		V	
153	35.446	2340	235	0.000		V	
154	35.634	1860	195	0.000		V	
155	35.820	1549	166	0.000		V	
156	36.015	1396	154	0.000		V	
157	36.198	1460	157	0.000		V	
158	36.388	1409	150	0.000		V	
159	36.576	1493	163	0.000		V	
160	36.759	1208	137	0.000		V	
161	36.956	1453	163	0.000		V	
162	37.137	1664	177	0.000		V	
163	37.334	1645	179	0.000		V	
164	37.515	2107	224	0.000		V	
165	37.706	2459	248	0.000		V	
166	37.887	2632	261	0.000		V	
167	38.073	2452	243	0.000		V	
168	38.256	2172	217	0.000		V	
169	38.445	1652	176	0.000		V	
170	38.640	1507	164	0.000		V	
171	38.829	1610	174	0.000		V	
172	39.019	1963	204	0.000		V	
173	39.206	2204	231	0.000		V	
174	39.390	2527	252	0.000		V	
175	39.577	2659	262	0.000		V	
176	39.765	2580	263	0.000		V	
177	39.954	2645	269	0.000		V	
178	40.139	2854	278	0.000		V	
179	40.329	2826	277	0.000		V	
180	40.509	2741	269	0.000		V	
181	40.701	2373	243	0.000		V	
182	40.886	2111	211	0.000		V	
183	41.072	1672	178	0.000		V	
184	41.266	1386	152	0.000		V	
185	41.454	1379	148	0.000		V	
186	41.640	1420	153	0.000		V	

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
187	41.828	1152	131	0.000		V	
188	42.204	1107	130	0.000		V	
189	42.396	1380	154	0.000		V	
190	42.584	1818	199	0.000		V	
191	42.769	2283	232	0.000		V	
192	42.956	2534	253	0.000		V	
193	43.141	2603	256	0.000		V	
194	43.330	2546	252	0.000		V	
195	43.510	2104	223	0.000		V	
196	43.706	2181	220	0.000		V	
197	43.894	2234	234	0.000		V	
198	44.077	2337	231	0.000		V	
199	44.267	2199	220	0.000		V	
200	44.448	1923	194	0.000		V	
201	44.643	1535	171	0.000		V	
202	44.828	1700	175	0.000		V	
203	45.020	1833	190	0.000		V	
204	45.198	1654	173	0.000		V	
205	45.395	1205	136	0.000		V	
206	45.584	1466	158	0.000		V	
207	45.771	1703	186	0.000		V	
208	45.959	2055	210	0.000		V	
209	46.147	2242	236	0.000		V	
210	46.329	2543	250	0.000		V	
211	46.515	2417	237	0.000		V	
212	46.699	2120	218	0.000		V	
213	46.886	1899	190	0.000		V	
214	47.082	1773	190	0.000		V	
215	47.268	1998	204	0.000		V	
216	47.444	1648	171	0.000		V	
217	47.636	1021	115	0.000		V	
218	48.024	1042	124	0.000		V	
219	48.210	1414	151	0.000		V	
220	48.397	1726	179	0.000		V	
221	48.585	1840	194	0.000		V	
222	48.767	2145	216	0.000		V	
223	48.947	1878	194	0.000		V	
224	49.137	1255	137	0.000		V	
225	49.332	1047	119	0.000		V	
226	49.522	1170	131	0.000		V	
227	49.710	1280	142	0.000		V	
228	49.898	1406	154	0.000		V	
229	50.086	1734	180	0.000		V	
230	50.271	1962	201	0.000		V	
231	50.449	1796	180	0.000		V	
232	50.639	1159	130	0.000		V	
233	50.831	1151	131	0.000		V	
234	51.022	1235	134	0.000		V	
235	51.209	1385	149	0.000		V	
236	51.391	1255	139	0.000		V	
237	51.584	1113	128	0.000		V	
238	51.770	1759	185	0.000		V	
239	51.960	1698	179	0.000		V	
240	52.146	1817	185	0.000		V	
241	52.334	1824	188	0.000		V	
242	52.523	1826	194	0.000		V	
243	52.705	1837	187	0.000		V	
244	52.896	1679	178	0.000		V	
245	53.087	1753	179	0.000		V	
246	53.266	1652	173	0.000		V	
247	53.456	1258	136	0.000		V	
248	53.644	1188	135	0.000		V	

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
249	53.833	1095	123	0.000		V	
250	54.026	1369	149	0.000		V	
251	54.208	1406	149	0.000		V	
252	54.389	1124	121	0.000		V	
253	55.153	1117	126	0.000		V	
254	55.339	1336	141	0.000		V	
255	55.523	1501	159	0.000		V	
256	55.702	1346	144	0.000		V	
257	56.465	1183	135	0.000		V	
258	56.649	1414	157	0.000		V	
259	56.833	1506	153	0.000		V	
260	57.013	1289	139	0.000		V	
Total		2196641	146716				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.521	8206225	330948	0.000		S	
2	11.196	8985	574	0.000		T	
3	15.793	11943	557	0.000			
4	20.631	20720	736	0.000			
5	55.988	79780	1090	0.000			
Total		8327651	333905				



SHIMADZU
LabSolutions

Analysis Report

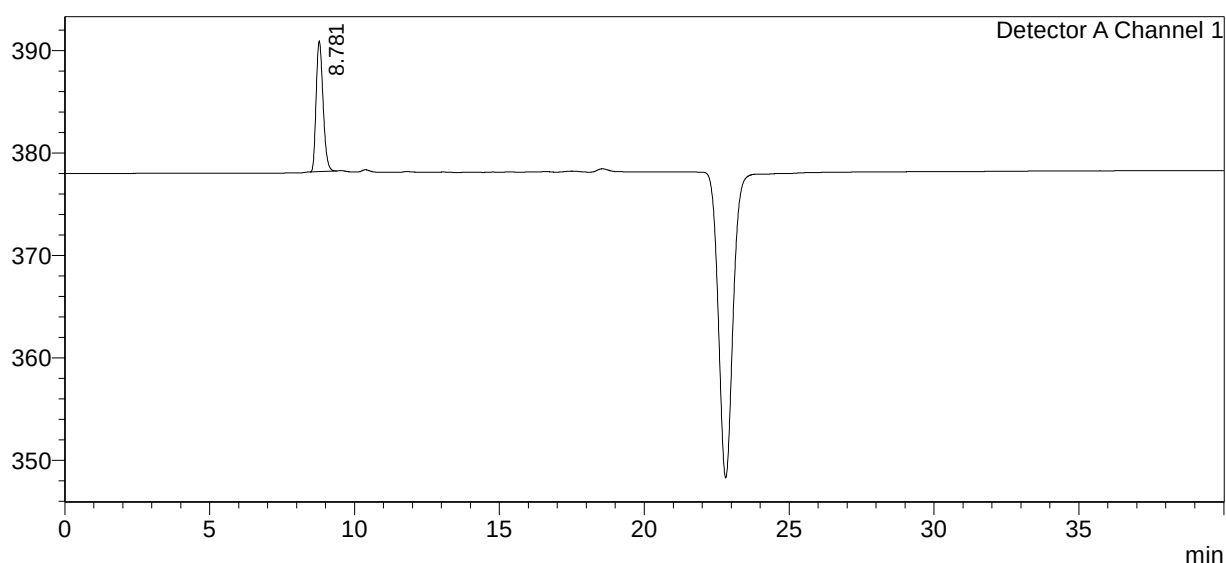
<Sample Information>

Sample Name : soil 24
Sample ID : soil 24
Data Filename : soil 24_004.lcd
Method Filename : AGV_Andrea 60C-05.lcm
Batch Filename : set soils_1.lcb
Vial # : 1-8
Injection Volume : 20 uL
Date Acquired : 13/09/2023 16:21:05
Date Processed : 13/09/2023 17:01:08

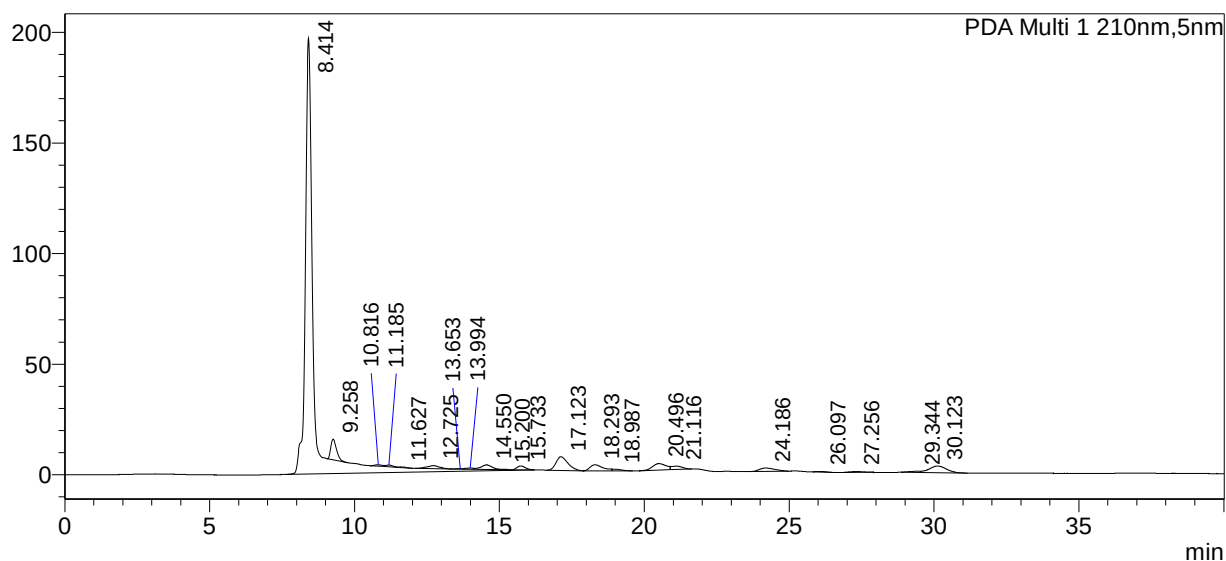
Sample Type : Unknown
Acquired by : Gruppo Ricicla
Processed by : Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.781	212291	12743	0.000			
Total		212291	12743				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.414	4053129	197013	0.000		S	
2	9.258	131128	9366	0.000		T	
3	10.816	11101	597	0.000		T	
4	11.185	14231	827	0.000		TV	
5	11.627	5827	308	0.000		TV	
6	12.725	42220	1396	0.000		T	
7	13.653	4849	289	0.000		T	
8	13.994	9881	565	0.000		TV	
9	14.550	56418	2090	0.000		TV	
10	15.200	3753	221	0.000		TV	
11	15.733	35732	1755	0.000		T	
12	17.123	178446	6168	0.000			
13	18.293	85394	2775	0.000	mg/L		Acetic acid
14	18.987	16495	767	0.000		V	
15	20.496	96216	2927	0.000			
16	21.116	40309	1520	0.000		V	
17	24.186	55744	1520	0.000			
18	26.097	6488	282	0.000			
19	27.256	13011	383	0.000			
20	29.344	13249	479	0.000			
21	30.123	123714	3034	0.000		V	
Total		4997335	234279				

SHIMADZU
LabSolutions

Analysis Report

<Sample Information>

Sample Name : 38
 Sample ID : 38
 Data Filename : 38_001.lcd
 Method Filename : VFA_Analysis_60C-05.lcm
 Batch Filename : prova pig 2.lcb
 Vial # : 1-4
 Injection Volume : 20 uL
 Date Acquired : 12/09/2023 12:23:10
 Date Processed : 13/09/2023 15:05:00

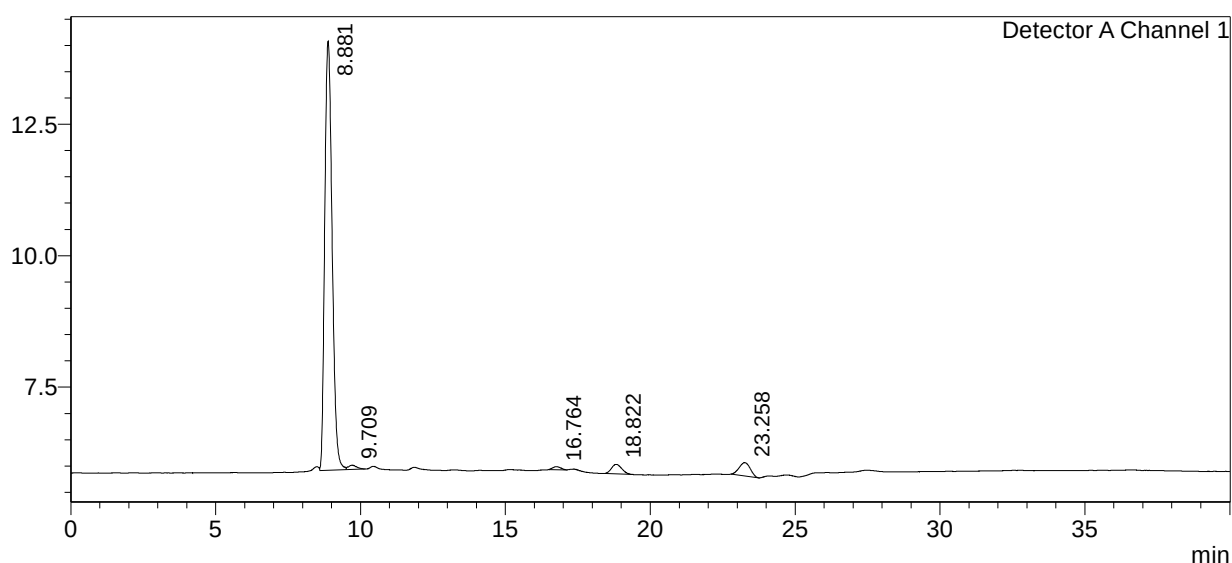
Sample Type : Unknown

Acquired by : Gruppo Ricicla

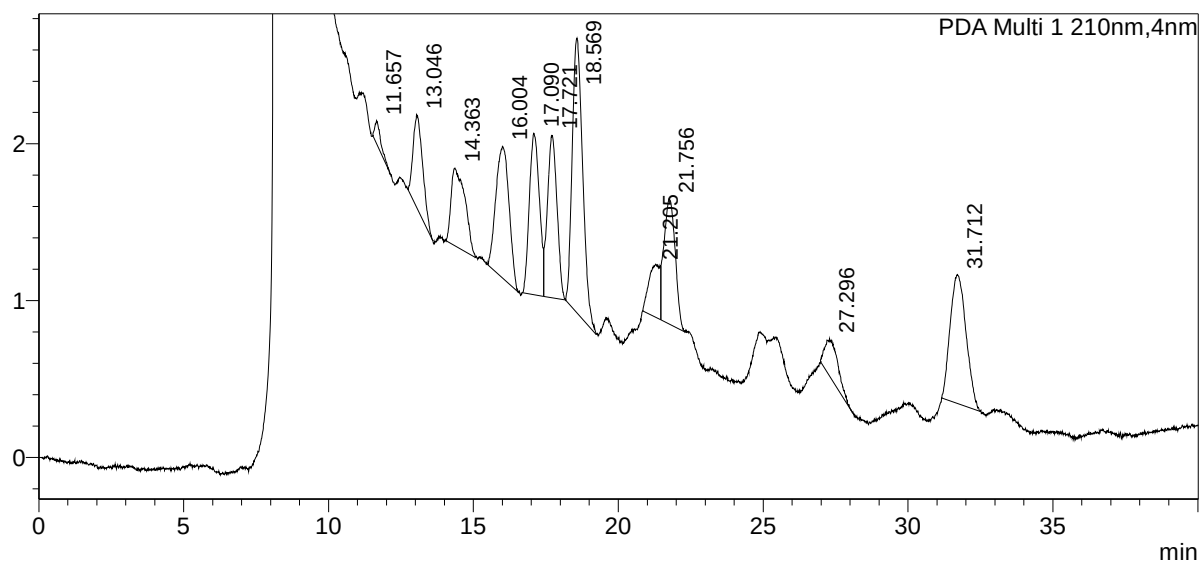
Processed by : Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.881	144819	8162	0.000		V	
2	9.709	1617	80	0.000		V	
3	16.764	1078	57	0.000			
4	18.822	4510	182	0.000			
5	23.258	6640	251	0.000			
Total		158664	8731				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	11.657	1937	144	0.000			
2	13.046	13680	594	0.000			
3	14.363	16661	494	0.000			
4	16.004	26424	839	0.000			
5	17.090	25092	1031	0.000			
6	17.721	23765	1036	0.000		V	
7	18.569	43749	1751	47.308	mg/L		RT:18.455
8	21.205	9092	314	0.000			
9	21.756	21386	800	0.000		V	
10	27.296	7802	242	0.000			
11	31.712	31410	823	0.000			
Total		220997	8068				



SHIMADZU
LabSolutions

Analysis Report

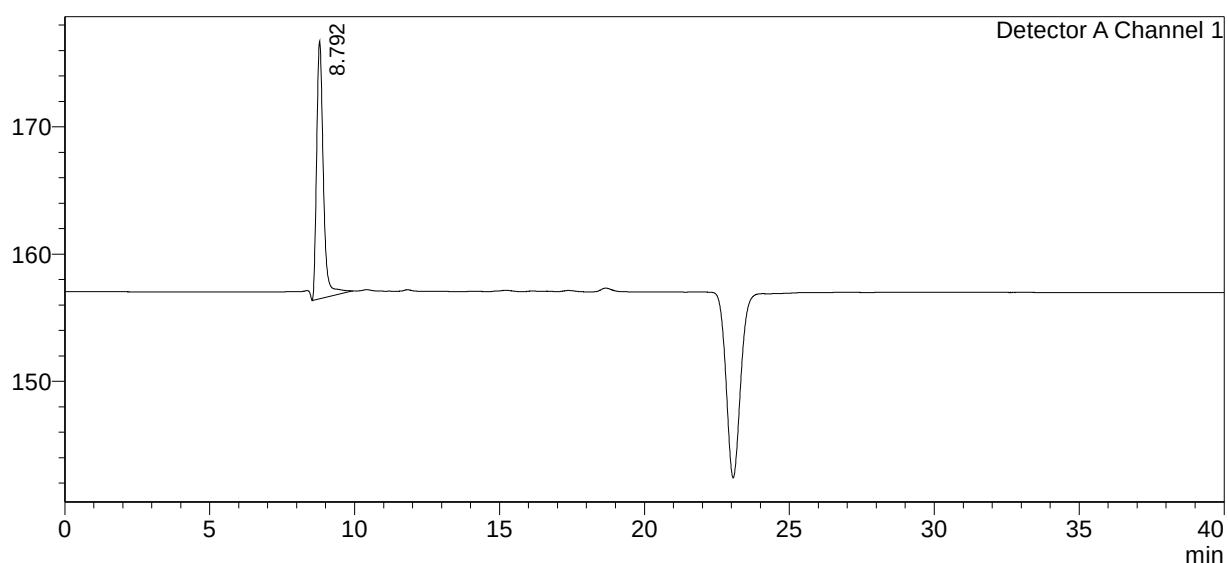
<Sample Information>

Sample Name : soil 71
Sample ID : soil 71
Data Filename : soil 71_005.lcd
Method Filename : AGV_Andrea 60C-05.lcm
Batch Filename : set soils_03.lcb
Vial # : 1-5
Injection Volume : 20 uL
Date Acquired : 15/09/2023 16:22:22
Date Processed : 15/09/2023 17:02:25

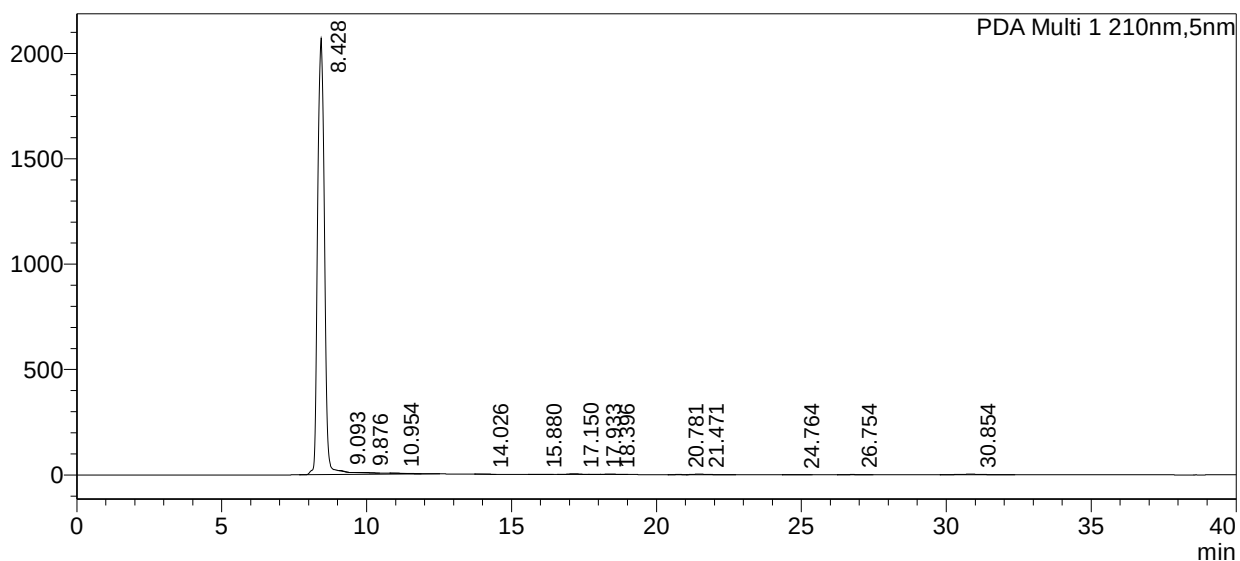
Sample Type : Unknown
Acquired by : Gruppo Ricicla
Processed by : Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.792	326562	20248	0.000			
Total		326562	20248				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.428	35048543	2071038	0.000		S	
2	9.093	18545	1788	0.000		T	
3	9.876	45164	1660	0.000		T	
4	10.954	14764	489	0.000		T	
5	14.026	13408	696	0.000			
6	15.880	17075	816	0.000			
7	17.150	98318	3806	0.000			
8	17.933	10581	568	0.000		V	
9	18.396	61205	2410	0.000	mg/L	V	Acetic acid
10	20.781	9843	391	0.000			
11	21.471	91441	2606	0.000		V	
12	24.764	12617	416	0.000			
13	26.754	37751	1143	0.000			
14	30.854	191235	3088	0.000			
Total		35670489	2090916				

**SHIMADZU**
LabSolutions

Analysis Report

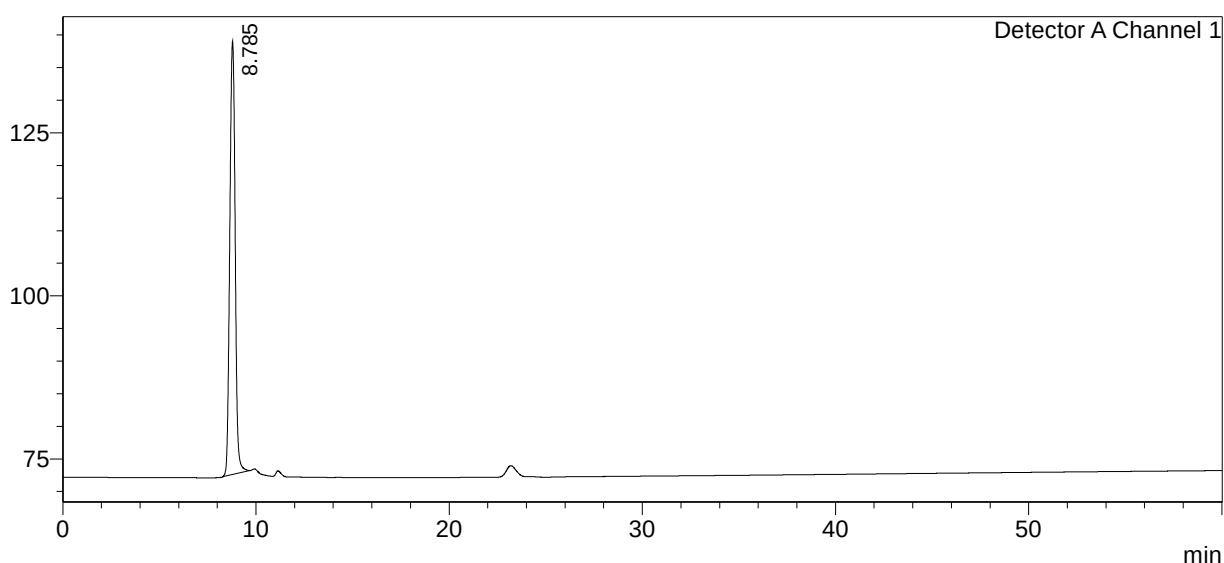
<Sample Information>

Sample Name : 94
Sample ID : A007
Data Filename : 94_007.lcd
Method Filename : AGV_digestate_24_Hi-plex.lcm
Batch Filename : AGV_Prova_6_15.lcb
Vial # : 1-12
Injection Volume : 20 uL
Date Acquired : 10/04/2024 23:21:15
Date Processed : 11/04/2024 00:21:18

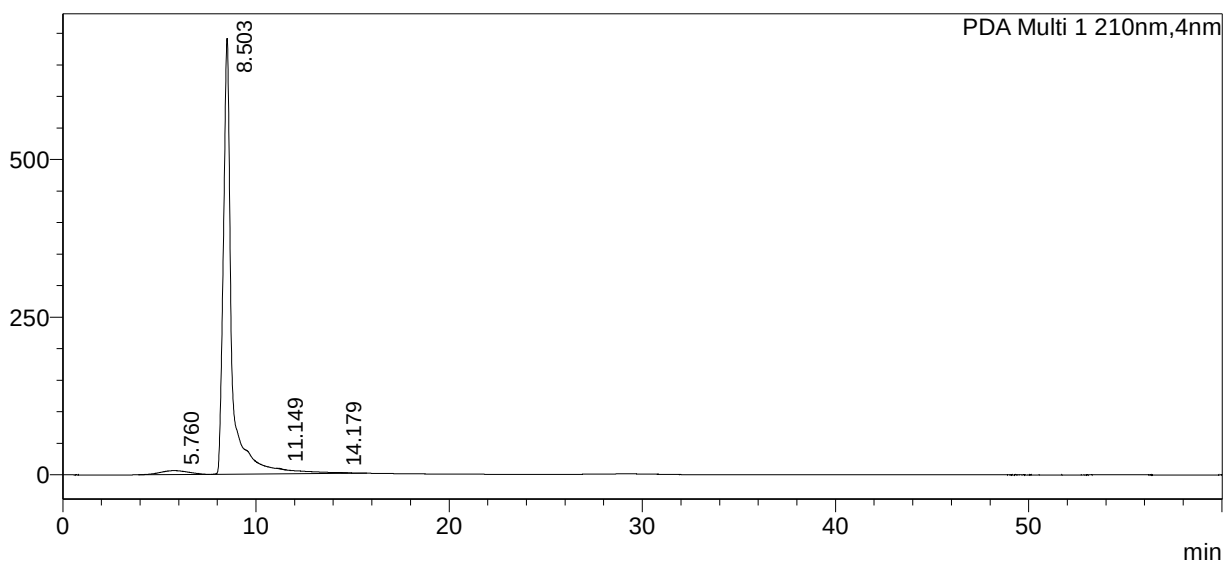
Sample Type : Unknown
Acquired by : Gruppo Ricicla
Processed by : Gruppo Ricicla

<Chromatogram>

mV



mAU



<Peak Table>

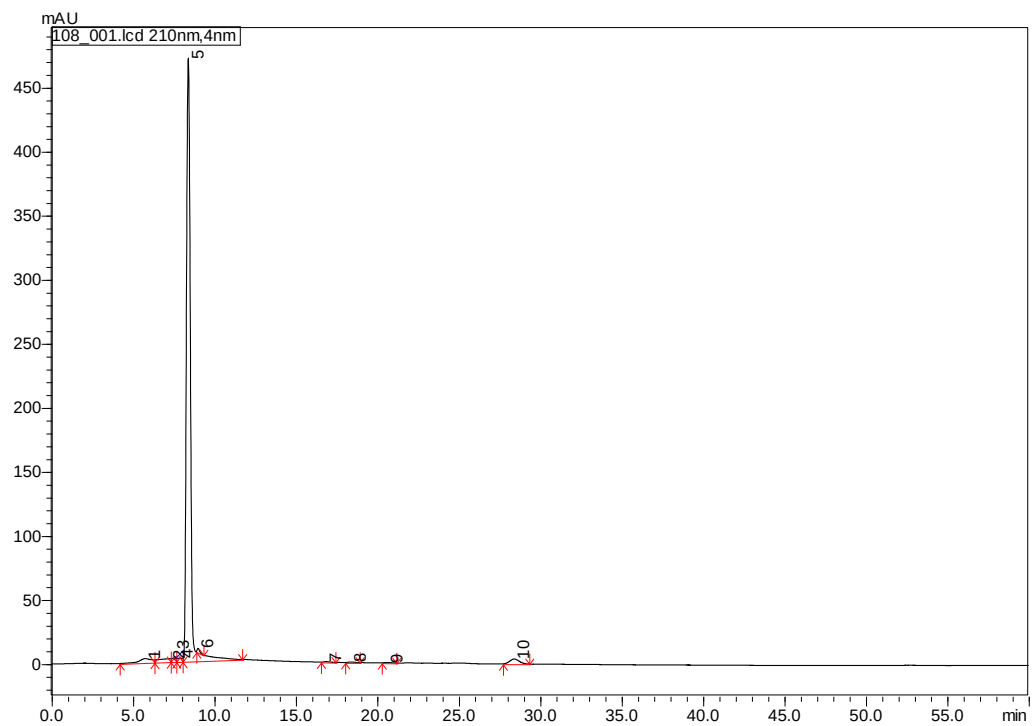
Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.785	1300494	66461	0.000			
Total		1300494	66461				

PDA Ch1 210nm

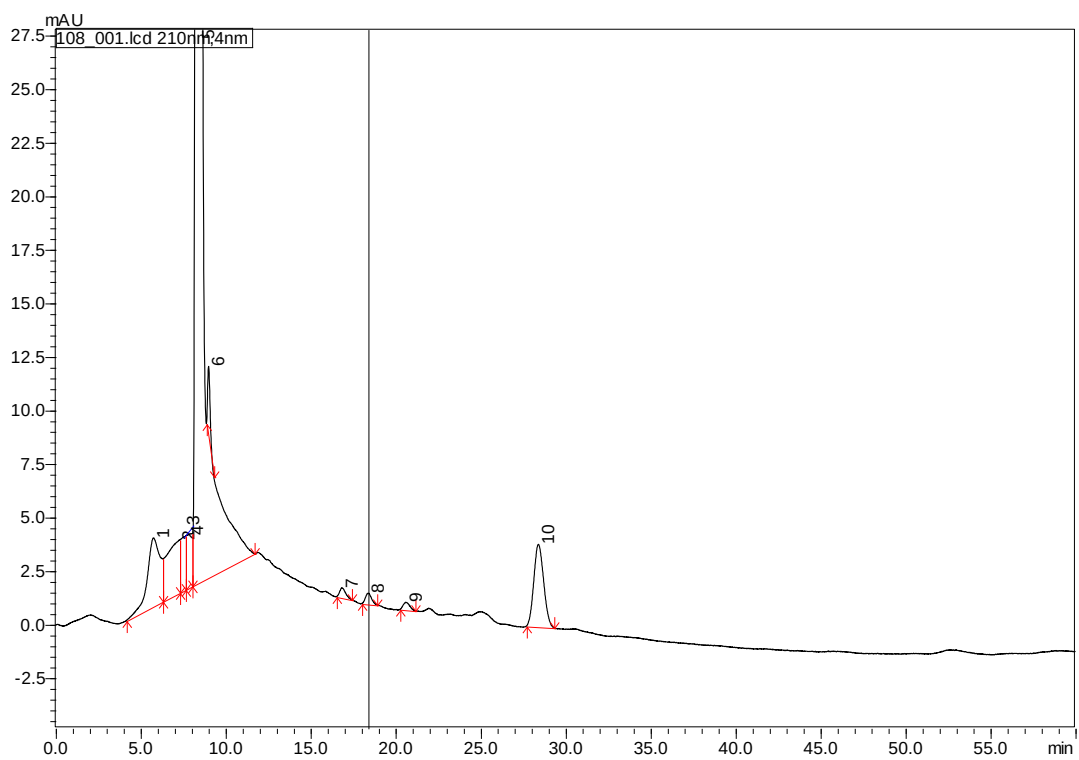
Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	5.760	646736	6309	0.000			
2	8.503	20982544	691559	0.000		S	
3	11.149	9036	571	0.000		T	
4	14.179	4176	187	0.000		T	
Total		21642493	698627				

Datafile Name:108_001.lcd
Sample Name:108
Sample ID:108



Datafile Name:108_001.lcd
Sample Name:108
Sample ID:108

in scala ridotta



Peak#	Ret. Time	Area
1	5.763	166276
2	7.264	135422
3	7.520	50932
4	7.765	55739
5	8.403	8110192
6	9.006	35259
7	16.863	11421
8	18.392	13005
9	20.628	9578
10	28.413	154897
Total		8742721



SHIMADZU
LabSolutions

Analysis Report

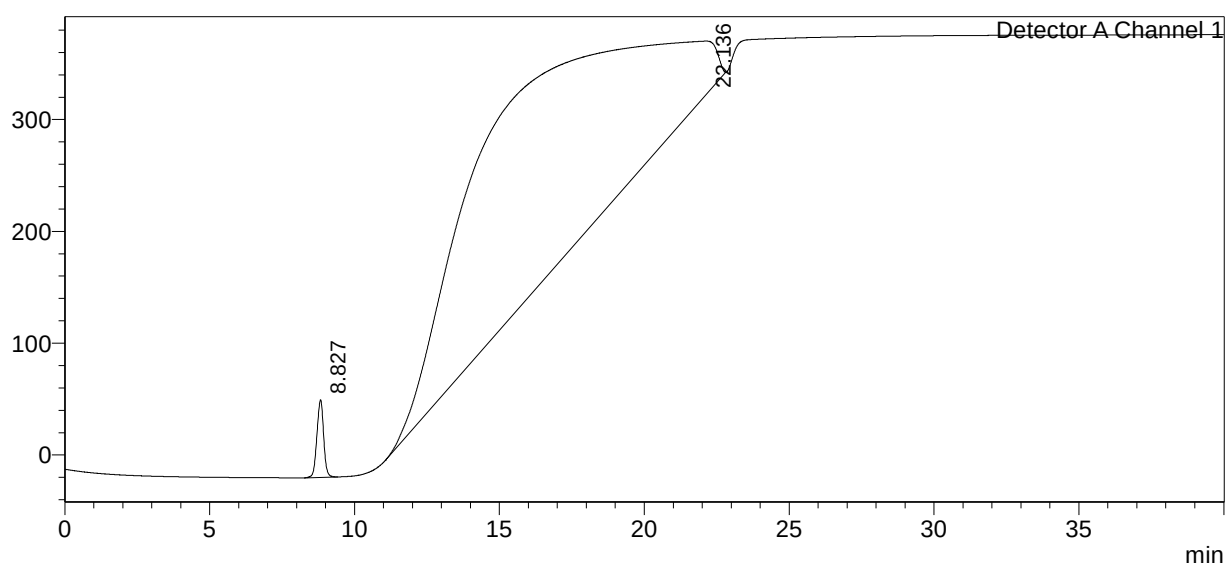
<Sample Information>

Sample Name : soil 2
 Sample ID : soil 2
 Data Filename : soil 2_001.lcd
 Method Filename : AGV_Andrea 60C-05.lcm
 Batch Filename : set soils_1.lcb
 Vial # : 1-5
 Injection Volume : 20 uL
 Date Acquired : 13/09/2023 14:19:28
 Date Processed : 13/09/2023 14:59:30

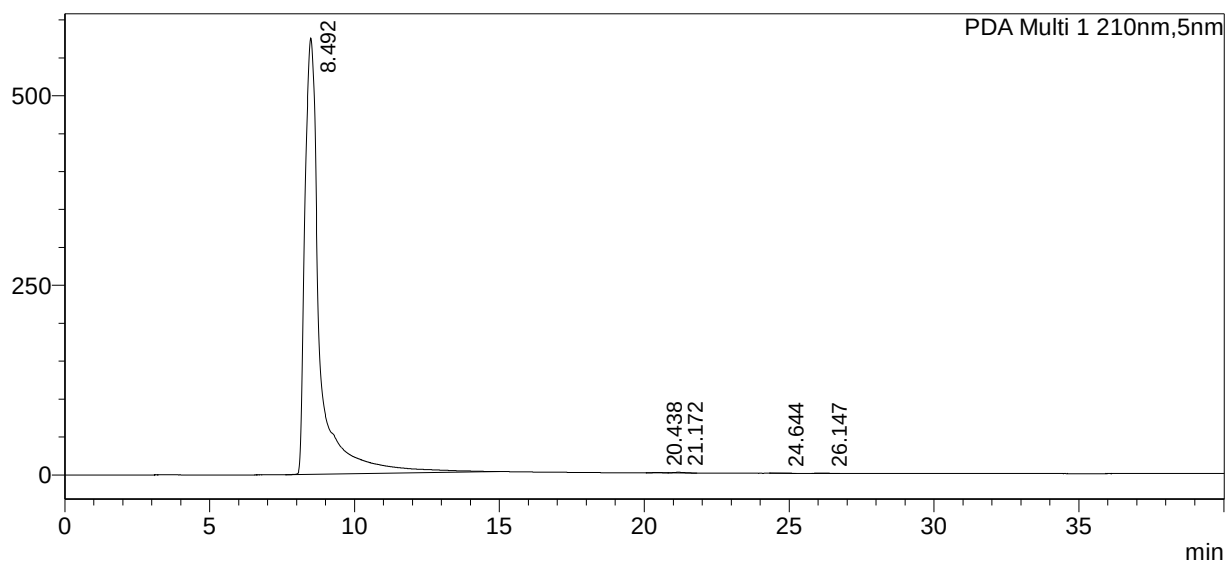
Sample Type : Unknown
 Acquired by : Gruppo Ricicla
 Processed by : Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.827	1125292	69378	0.000			
2	22.136	82454277	47804	0.000			
Total		83579569	117182				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.492	20705021	575147	0.000			
2	20.438	13656	550	0.000			
3	21.172	23821	898	0.000		V	
4	24.644	6716	232	0.000			
5	26.147	8368	286	0.000			
Total		20757582	577113				



SHIMADZU
LabSolutions

Analysis Report

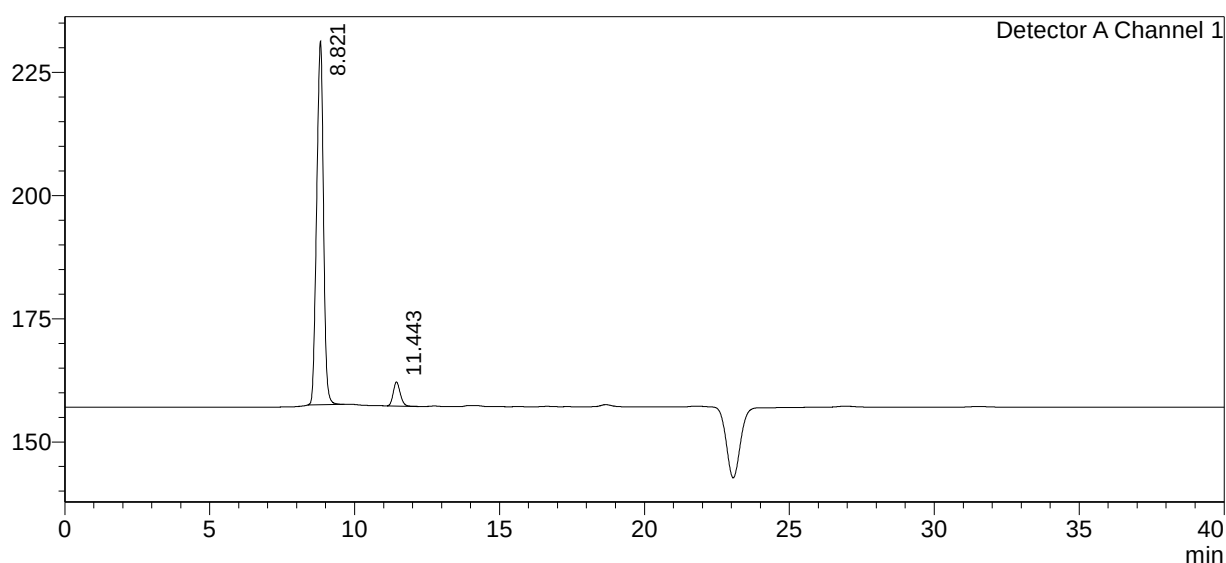
<Sample Information>

Sample Name : soil 51
Sample ID : soil 51
Data Filename : soil 51_001.lcd
Method Filename : AGV_Andrea 60C-05.lcm
Batch Filename : set soils_03.lcb
Vial # : 1-1
Injection Volume : 20 uL
Date Acquired : 15/09/2023 13:40:14
Date Processed : 15/09/2023 14:20:16

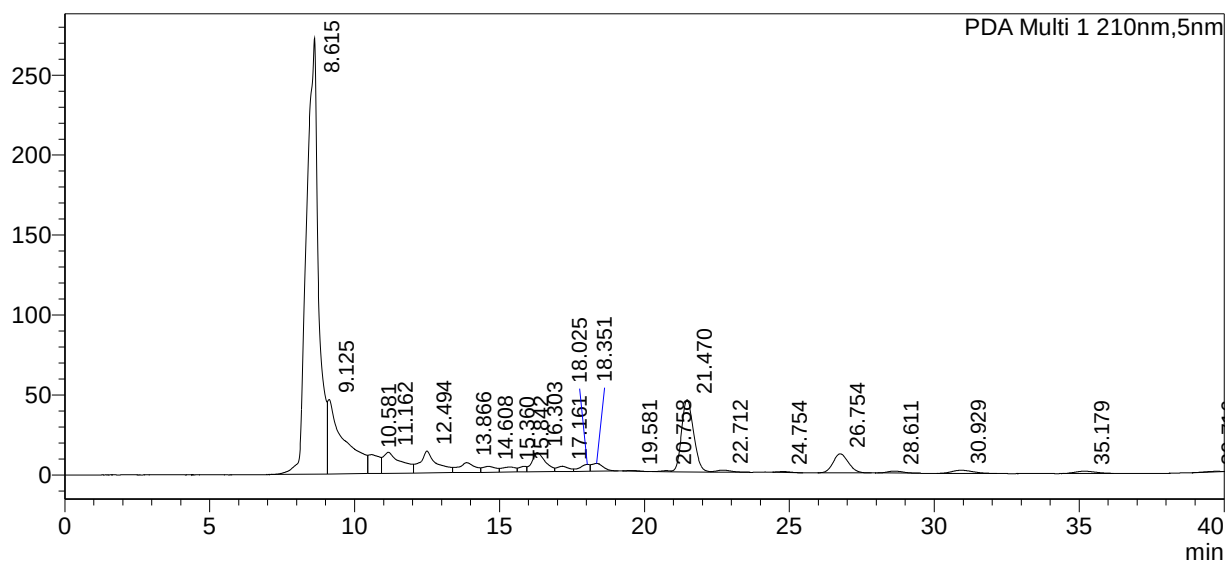
Sample Type : Unknown
Acquired by : Gruppo Ricicla
Processed by : Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.821	1207143	73814	0.000			
2	11.443	81697	4892	0.000			
Total		1288839	78706				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.615	7820149	272571	0.000			
2	9.125	1875902	46556	0.000		V	
3	10.581	312271	11774	0.000		V	
4	11.162	581116	13147	0.000		V	
5	12.494	546760	13698	0.000		V	
6	13.866	243118	6140	0.000		V	
7	14.608	118176	3647	0.000		V	
8	15.360	103751	3162	0.000		V	
9	15.842	63145	3458	0.000		V	
10	16.303	382293	11864	0.000		V	
11	17.161	95409	3285	0.000		V	
12	18.025	102201	4221	0.000		V	
13	18.351	132999	4839	0.000	mg/L	V	Acetic acid
14	19.581	11312	432	0.000			
15	20.758	14024	636	0.000			
16	21.470	1301782	45100	0.000		V	
17	22.712	43978	1269	0.000		V	
18	24.754	13237	463	0.000			
19	26.754	434822	11885	0.000			
20	28.611	46110	1244	0.000			
21	30.929	102452	1986	0.000			
22	35.179	69774	1416	0.000			
23	39.716	13378	366	0.000			
Total		14428157	463157				



SHIMADZU
LabSolutions

Analysis Report

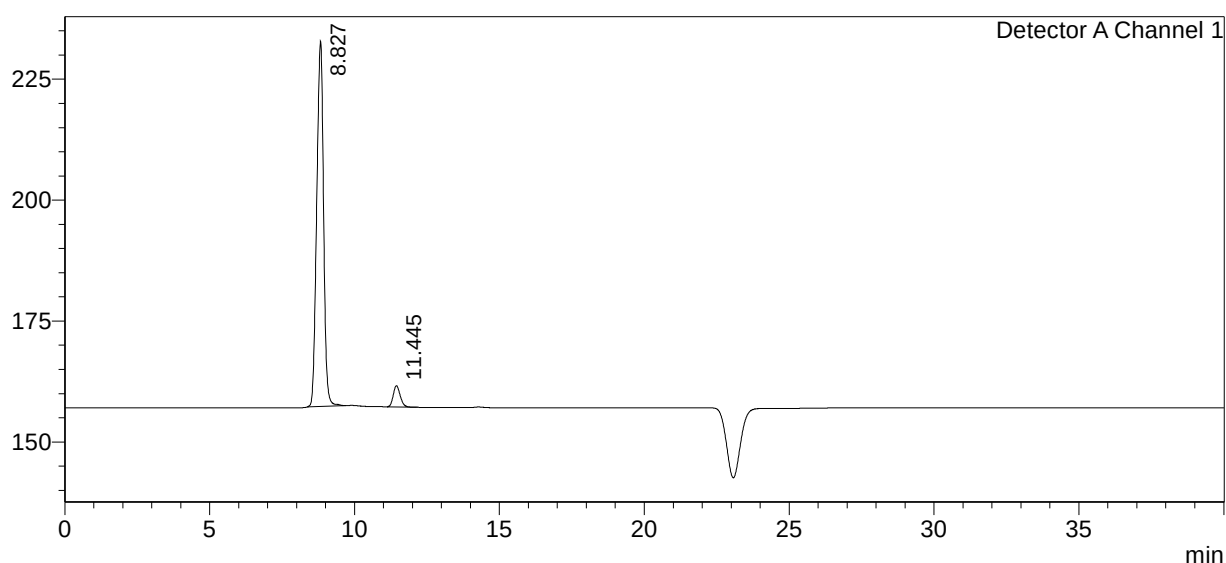
<Sample Information>

Sample Name : soil 66
Sample ID : soil 66
Data Filename : soil 66_004.lcd
Method Filename : AGV_Andrea 60C-05.lcm
Batch Filename : set soils_03.lcb
Vial # : 1-4
Injection Volume : 20 uL
Date Acquired : 15/09/2023 15:41:51
Date Processed : 15/09/2023 16:21:53

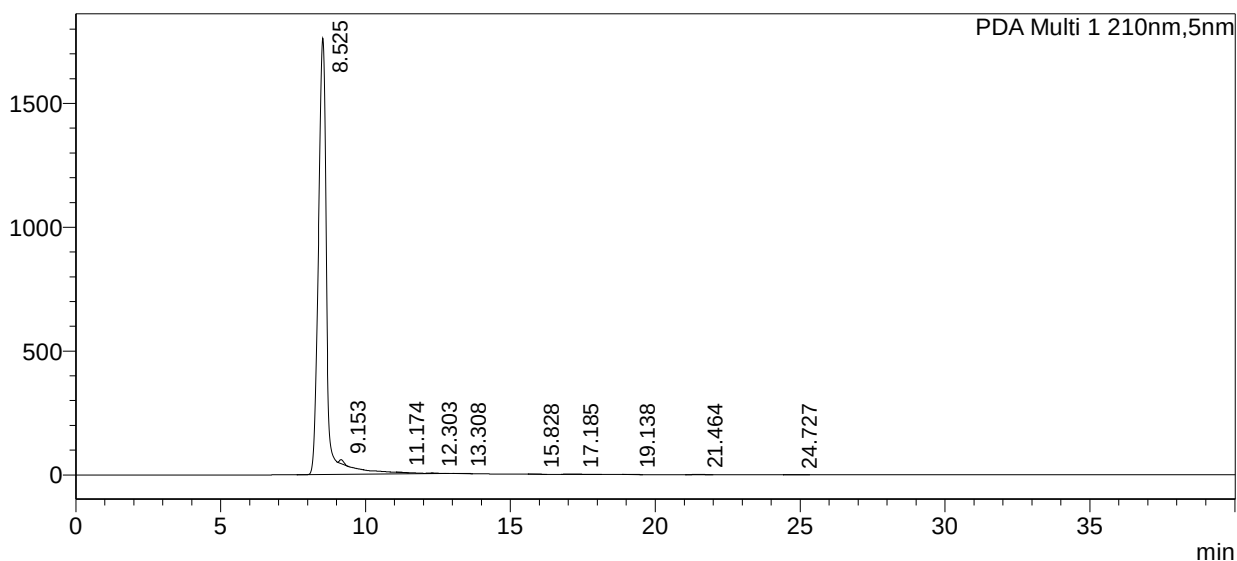
Sample Type : Unknown
Acquired by : Gruppo Ricicla
Processed by : Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.827	1244613	75537	0.000			
2	11.445	73048	4392	0.000			
Total		1317661	79929				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.525	35343069	1762095	0.000		S	
2	9.153	155571	15860	0.000		T	
3	11.174	10261	782	0.000		T	
4	12.303	35949	1921	0.000		T	
5	13.308	5184	239	0.000			
6	15.828	7041	410	0.000			
7	17.185	30768	972	0.000			
8	19.138	5761	275	0.000	mg/L		Acetic acid
9	21.464	20203	796	0.000			
10	24.727	6566	236	0.000			
Total		35620373	1783586				



SHIMADZU
LabSolutions

Analysis Report

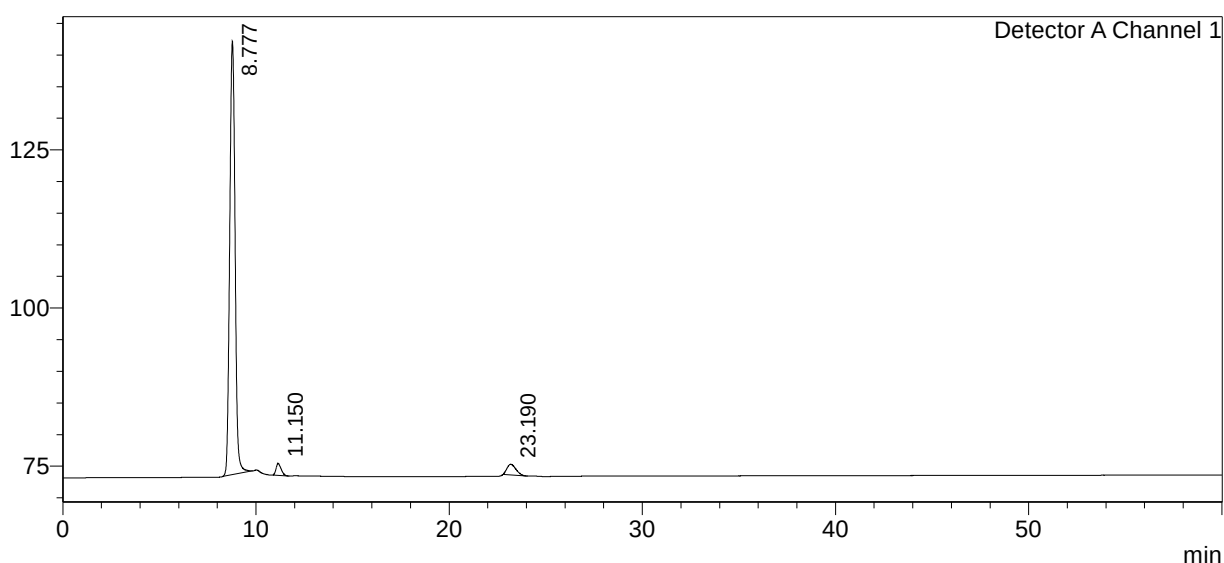
<Sample Information>

Sample Name : 90
Sample ID : A003
Data Filename : 90_003.lcd
Method Filename : AGV_digestate_24_Hi-plex.lcm
Batch Filename : AGV_Prova_6_15.lcb
Vial # : 1-8
Injection Volume : 20 uL
Date Acquired : 10/04/2024 19:19:02
Date Processed : 10/04/2024 20:19:05

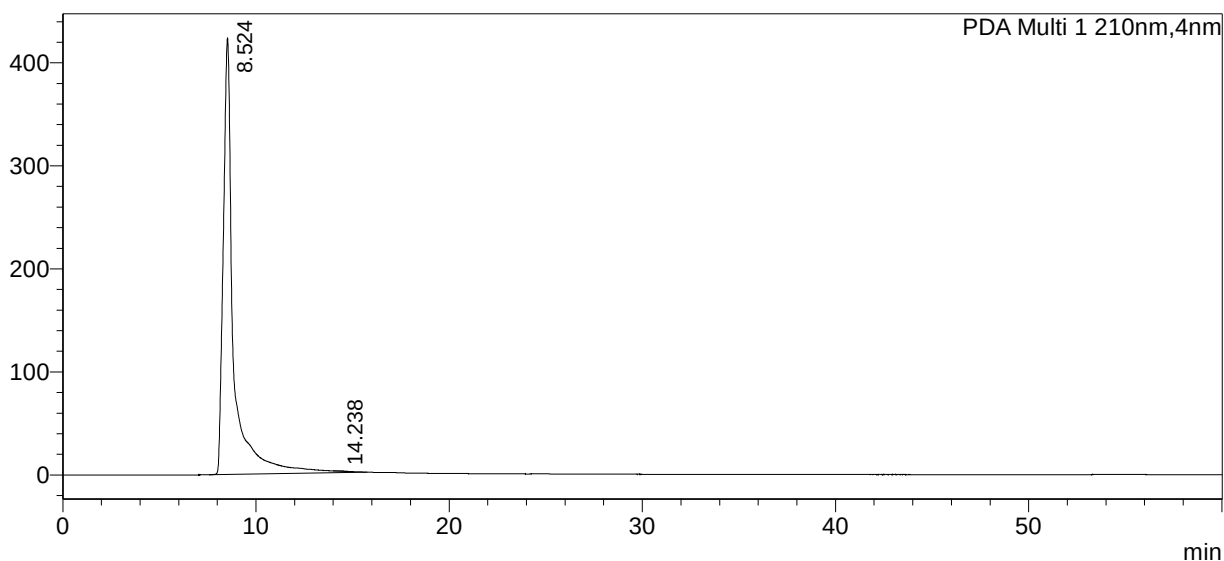
Sample Type : Unknown
Acquired by : Gruppo Ricicla
Processed by : Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

Detector A Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.777	1328486	68523	0.000			
2	11.150	33077	1882	0.000			
3	23.190	55201	1693	0.000			
Total		1416765	72098				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.524	15673386	423448	0.000		S	
2	14.238	9983	290	0.000		T	
Total		15683370	423738				



SHIMADZU
LabSolutions

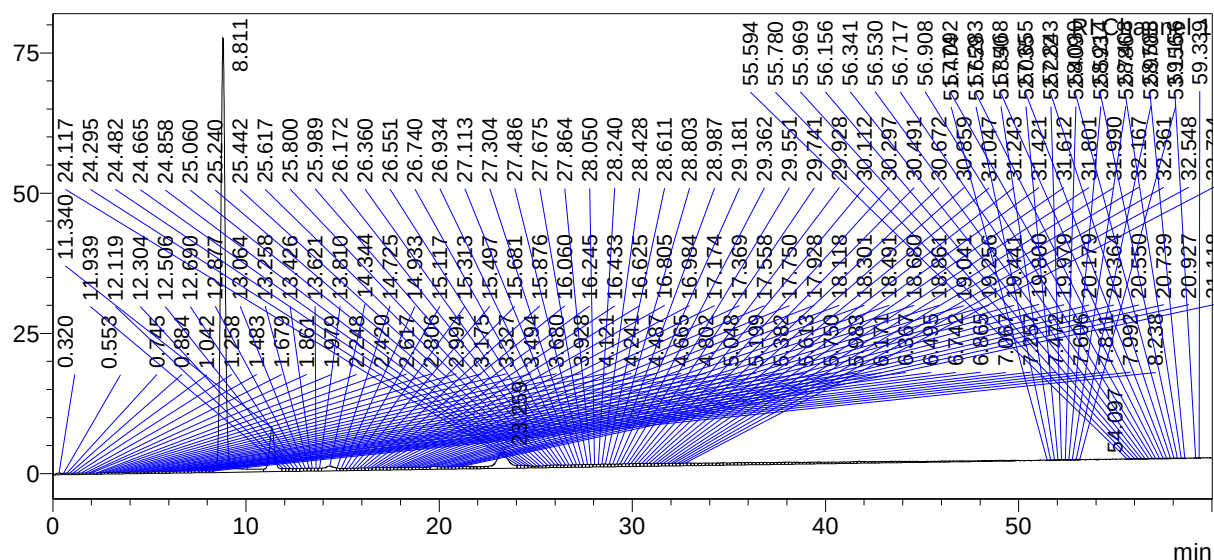
Analysis Report

<Sample Information>

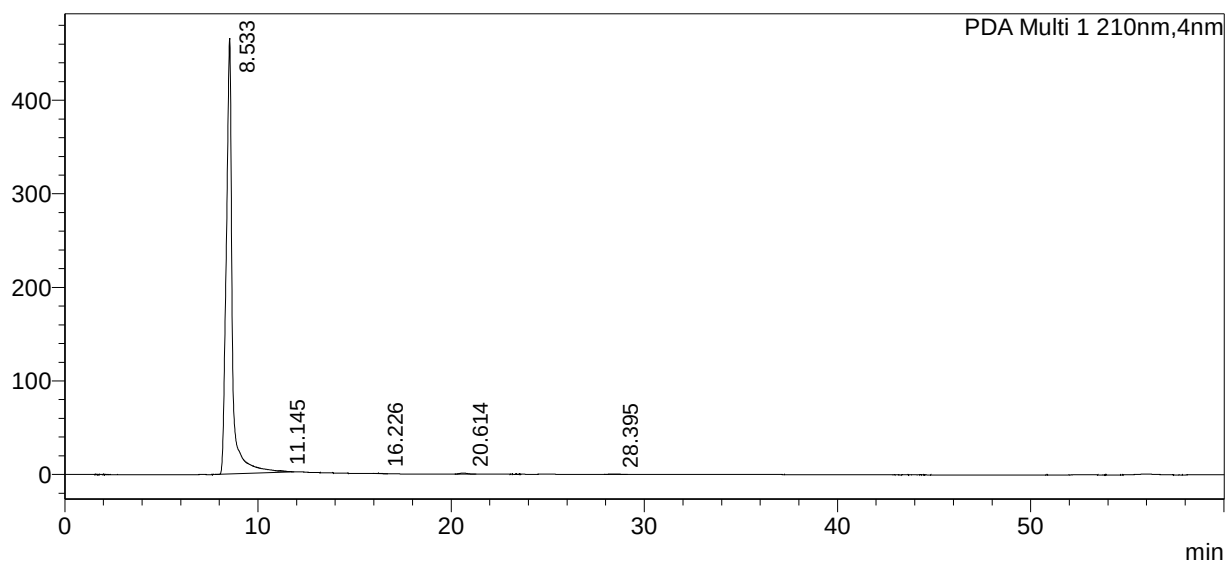
Sample Name	: 106		
Sample ID	: 106		
Data Filename	: 106_003.lcd		
Method Filename	: AGV_Matteo_Gabriele_feb_2025_Hi-plex.lcm		
Batch Filename	: 105_106_107.lcb		
Vial #	: 1-4	Sample Type	: Unknown
Injection Volume	: 20 uL		
Date Acquired	: 04/06/2025 15:29:36	Acquired by	: Gruppo Ricicla
Date Processed	: 04/06/2025 16:29:39	Processed by	: Gruppo Ricicla

<Chromatogram>

mV



MAU



<Peak Table>

RI Channel 1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.320	4867	296	0.000			
2	0.553	2898	268	0.000		V	
3	0.745	3037	273	0.000		V	
4	0.884	2992	271	0.000		V	
5	1.042	2819	246	0.000		V	
6	1.258	2601	254	0.000		V	
7	1.483	2854	258	0.000		V	
8	1.679	2586	252	0.000		V	
9	1.861	2918	262	0.000		V	
10	1.979	2832	255	0.000		V	
11	2.248	2367	234	0.000		V	
12	2.420	2696	242	0.000		V	
13	2.617	2613	244	0.000		V	
14	2.806	3108	283	0.000		V	
15	2.994	3067	284	0.000		V	
16	3.175	3148	281	0.000		V	
17	3.327	3016	285	0.000		V	
18	3.494	3025	274	0.000		V	
19	3.680	2649	260	0.000		V	
20	3.928	3069	279	0.000		V	
21	4.121	3424	313	0.000		V	
22	4.241	3320	312	0.000		V	
23	4.487	3402	303	0.000		V	
24	4.665	3144	291	0.000		V	
25	4.802	3201	290	0.000		V	
26	5.048	2982	276	0.000		V	
27	5.199	2995	280	0.000		V	
28	5.382	3342	291	0.000		V	
29	5.613	2835	276	0.000		V	
30	5.750	3098	281	0.000		V	
31	5.983	3308	285	0.000		V	
32	6.171	3061	297	0.000		V	
33	6.367	3777	349	0.000		V	
34	6.495	3806	358	0.000		V	
35	6.742	4190	360	0.000		V	
36	6.865	3899	371	0.000		V	
37	7.067	3699	343	0.000		V	
38	7.257	3710	363	0.000		V	
39	7.472	4614	384	0.000		V	
40	7.606	3636	361	0.000		V	
41	7.811	3984	346	0.000		V	
42	7.992	3352	320	0.000		V	
43	8.238	2853	289	0.000		V	
44	8.811	1299220	77413	0.000		SV	
45	11.340	151332	7830	0.000		V	
46	11.939	6156	552	0.000		V	
47	12.119	5430	529	0.000		V	
48	12.304	5739	516	0.000		V	
49	12.506	5423	506	0.000		V	
50	12.690	5519	515	0.000		V	
51	12.877	5927	532	0.000		V	
52	13.064	5733	532	0.000		V	
53	13.258	6525	564	0.000		V	
54	13.426	5797	544	0.000		V	
55	13.621	5553	517	0.000		V	
56	13.810	5548	518	0.000		V	
57	14.344	30889	864	0.000		V	
58	14.725	5102	511	0.000		V	
59	14.933	4075	418	0.000		V	
60	15.117	4427	416	0.000		V	
61	15.313	4930	428	0.000		V	
62	15.497	4584	429	0.000		V	

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
63	15.681	4636	436	0.000		V	
64	15.876	4960	448	0.000		V	
65	16.060	4817	454	0.000		V	
66	16.245	4982	448	0.000		V	
67	16.433	4827	452	0.000		V	
68	16.625	5388	483	0.000		V	
69	16.805	5689	517	0.000		V	
70	16.984	5025	477	0.000		V	
71	17.174	4702	446	0.000		V	
72	17.369	4848	438	0.000		V	
73	17.558	4656	440	0.000		V	
74	17.750	4745	448	0.000		V	
75	17.928	4929	445	0.000		V	
76	18.118	4920	447	0.000		V	
77	18.301	4660	441	0.000		V	
78	18.491	4560	430	0.000		V	
79	18.680	4738	428	0.000		V	
80	18.861	4805	423	0.000		V	
81	19.041	3693	371	0.000		V	
82	19.256	4009	383	0.000		V	
83	19.441	4367	415	0.000		V	
84	19.800	11079	523	0.000		V	
85	19.979	5461	488	0.000		V	
86	20.179	4300	427	0.000		V	
87	20.364	4413	422	0.000		V	
88	20.550	4579	414	0.000		V	
89	20.739	4553	431	0.000		V	
90	20.927	5000	442	0.000		V	
91	21.118	4245	420	0.000		V	
92	21.312	4858	443	0.000		V	
93	21.487	4644	441	0.000		V	
94	21.670	4597	425	0.000		V	
95	21.859	3938	374	0.000		V	
96	22.052	3887	369	0.000		V	
97	22.251	4133	395	0.000		V	
98	22.429	4421	420	0.000		V	
99	23.259	123100	2881	0.000		V	
100	24.117	5663	511	0.000		V	
101	24.295	5501	499	0.000		V	
102	24.482	5016	461	0.000		V	
103	24.665	4238	402	0.000		V	
104	24.858	3248	328	0.000		V	
105	25.060	3425	328	0.000		V	
106	25.240	3650	345	0.000		V	
107	25.442	4285	395	0.000		V	
108	25.617	4745	428	0.000		V	
109	25.800	5019	473	0.000		V	
110	25.989	5502	501	0.000		V	
111	26.172	5327	490	0.000		V	
112	26.360	4516	447	0.000		V	
113	26.551	4930	450	0.000		V	
114	26.740	4788	458	0.000		V	
115	26.934	5193	474	0.000		V	
116	27.113	5278	479	0.000		V	
117	27.304	4860	480	0.000		V	
118	27.486	5473	495	0.000		V	
119	27.675	5669	494	0.000		V	
120	27.864	4869	483	0.000		V	
121	28.050	5099	482	0.000		V	
122	28.240	5502	500	0.000		V	
123	28.428	5723	517	0.000		V	
124	28.611	5920	523	0.000		V	

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
125	28.803	4958	489	0.000		V	
126	28.987	5172	492	0.000		V	
127	29.181	5174	490	0.000		V	
128	29.362	5605	508	0.000		V	
129	29.551	5765	525	0.000		V	
130	29.741	5534	521	0.000		V	
131	29.928	5932	536	0.000		V	
132	30.112	5501	540	0.000		V	
133	30.297	6408	562	0.000		V	
134	30.491	6061	553	0.000		V	
135	30.672	5687	540	0.000		V	
136	30.859	5919	524	0.000		V	
137	31.047	4305	466	0.000		V	
138	31.243	5703	503	0.000		V	
139	31.421	5413	494	0.000		V	
140	31.612	4804	476	0.000		V	
141	31.801	5591	489	0.000		V	
142	31.990	5118	483	0.000		V	
143	32.167	5125	491	0.000		V	
144	32.361	5210	480	0.000		V	
145	32.548	4958	472	0.000		V	
146	32.734	5050	480	0.000		V	
147	32.922	5615	500	0.000		V	
148	33.108	4897	471	0.000		V	
149	33.295	4883	451	0.000		V	
150	33.483	4217	421	0.000		V	
151	33.676	4772	438	0.000		V	
152	33.856	4850	450	0.000		V	
153	34.043	4399	423	0.000		V	
154	34.236	4579	423	0.000		V	
155	34.420	4341	420	0.000		V	
156	34.614	4526	437	0.000		V	
157	34.795	5025	452	0.000		V	
158	34.979	4046	405	0.000		V	
159	35.170	4560	422	0.000		V	
160	35.362	4558	442	0.000		V	
161	35.545	4925	442	0.000		V	
162	35.737	4177	419	0.000		V	
163	35.919	4740	443	0.000		V	
164	36.105	4376	422	0.000		V	
165	36.293	4444	417	0.000		V	
166	36.486	4218	409	0.000		V	
167	36.668	4511	423	0.000		V	
168	36.856	4392	412	0.000		V	
169	37.040	3967	390	0.000		V	
170	37.233	3657	357	0.000		V	
171	37.419	3662	356	0.000		V	
172	37.607	4057	383	0.000		V	
173	37.796	3906	380	0.000		V	
174	37.982	4258	398	0.000		V	
175	38.167	4145	390	0.000		V	
176	38.354	3679	359	0.000		V	
177	38.543	3674	347	0.000		V	
178	38.728	3102	318	0.000		V	
179	38.916	3482	321	0.000		V	
180	39.110	3203	319	0.000		V	
181	39.293	3321	329	0.000		V	
182	39.479	3576	339	0.000		V	
183	39.665	3459	334	0.000		V	
184	39.854	3021	300	0.000		V	
185	40.046	3154	305	0.000		V	
186	40.229	2891	302	0.000		V	

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
187	40.415	3233	300	0.000		V	
188	40.603	2814	284	0.000		V	
189	40.794	2829	284	0.000		V	
190	40.980	2973	288	0.000		V	
191	41.163	2813	282	0.000		V	
192	41.358	3029	290	0.000		V	
193	41.552	3088	322	0.000		V	
194	41.730	3891	357	0.000		V	
195	41.913	3459	332	0.000		V	
196	42.102	2866	290	0.000		V	
197	42.291	2661	270	0.000		V	
198	42.476	2570	262	0.000		V	
199	42.666	2719	268	0.000		V	
200	42.854	2533	256	0.000		V	
201	43.040	2689	265	0.000		V	
202	43.231	2579	261	0.000		V	
203	43.416	2741	269	0.000		V	
204	43.602	2752	270	0.000		V	
205	43.789	2527	258	0.000		V	
206	43.974	2332	240	0.000		V	
207	44.165	2309	229	0.000		V	
208	44.352	2086	213	0.000		V	
209	44.536	2233	225	0.000		V	
210	44.722	2039	209	0.000		V	
211	44.911	1588	176	0.000		V	
212	45.106	1425	156	0.000		V	
213	45.288	1623	173	0.000		V	
214	45.477	1509	165	0.000		V	
215	45.664	1642	179	0.000		V	
216	45.850	1692	185	0.000		V	
217	46.041	1973	200	0.000		V	
218	46.222	1714	186	0.000		V	
219	46.412	1599	175	0.000		V	
220	46.601	1647	173	0.000		V	
221	46.787	1566	172	0.000		V	
222	46.972	1590	167	0.000		V	
223	47.164	1604	169	0.000		V	
224	47.344	1424	159	0.000		V	
225	47.536	1248	144	0.000		V	
226	47.726	1225	136	0.000		V	
227	47.913	1343	151	0.000		V	
228	48.103	1621	171	0.000		V	
229	48.285	1665	175	0.000		V	
230	48.472	1370	155	0.000		V	
231	48.664	1530	166	0.000		V	
232	48.847	1521	166	0.000		V	
233	49.035	1723	187	0.000		V	
234	49.218	1473	164	0.000		V	
235	49.414	1156	133	0.000		V	
236	49.598	1194	137	0.000		V	
237	49.782	1262	139	0.000		V	
238	51.474	1026	123	0.000		V	
239	51.659	1112	130	0.000		V	
240	51.850	1284	145	0.000		V	
241	52.035	1344	151	0.000		V	
242	52.222	1348	151	0.000		V	
243	52.409	1219	141	0.000		V	
244	52.593	1132	134	0.000		V	
245	52.786	1167	139	0.000		V	
246	52.970	1283	144	0.000		V	
247	53.156	1113	130	0.000		V	
248	54.097	1069	125	0.000		V	

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
249	55.594	1218	142	0.000		V	
250	55.780	1134	135	0.000		V	
251	55.969	1398	150	0.000		V	
252	56.156	1399	152	0.000		V	
253	56.341	1286	149	0.000		V	
254	56.530	1140	135	0.000		V	
255	56.717	1016	122	0.000		V	
256	56.908	1227	141	0.000		V	
257	57.092	1330	149	0.000		V	
258	57.283	1181	137	0.000		V	
259	57.468	1267	143	0.000		V	
260	57.655	1189	140	0.000		V	
261	57.843	1161	137	0.000		V	
262	58.030	1299	148	0.000		V	
263	58.214	1019	121	0.000		V	
264	58.408	1065	129	0.000		V	
265	58.588	1070	129	0.000		V	
266	59.156	1208	136	0.000		V	
267	59.339	1135	131	0.000		V	
Total		2554098	178808				

PDA Ch1 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8.533	10029657	465838	0.000		S	
2	11.145	4816	337	0.000		T	
3	16.226	7837	398	0.000			
4	20.614	33785	1231	0.000			
5	28.395	20779	595	0.000			
Total		10096874	468399				

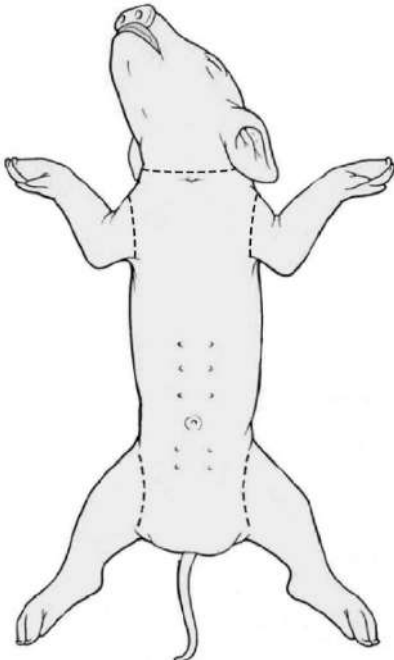
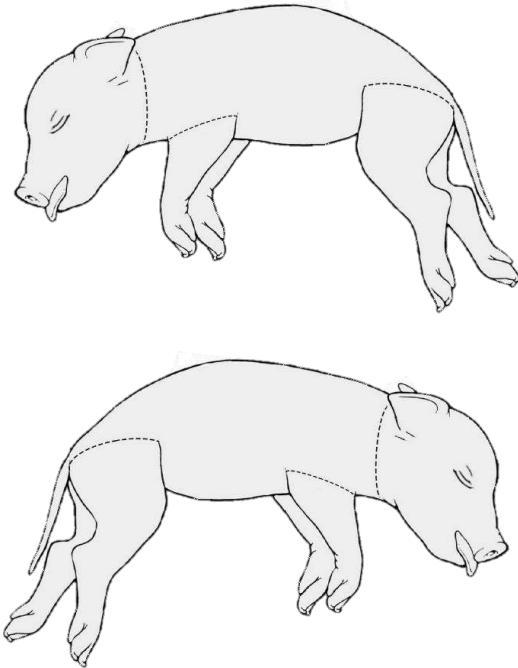
External Examination Date: __/__/__

Piglet ID:

Exhumation Date: __/__/__

PBI:

Samples:

TBS Assignment				
	Head & Neck (pt)	Trunk (pt)	Limbs (pt)	TBS
<i>Megyesi et al. (2005)</i>				
<i>Keough et al. (2017)</i>				
<i>Indra et al. (2024)</i>				

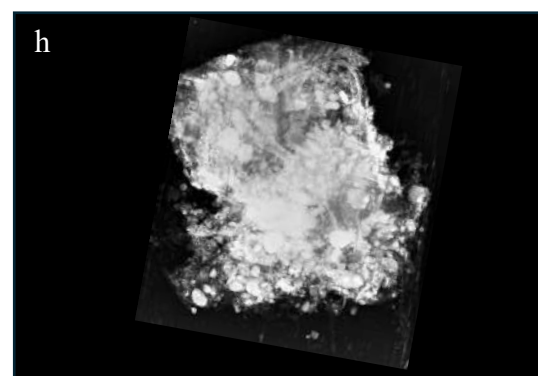
Figure A.1: Template of the form created for assessing the decomposition stage of the piglet bodies. The form allows recording observations on the remains both in the supine position and on both lateral sides (modified from Smith & Schenk, 2012). At the bottom, a table is included for assigning the Total Body Score (TBS), incorporating all three considered methods.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P		
1	PMI (Days)	Date	Temp °C	Temp °F	ADD accumul													
2	0	21/03/2023	11.8	53.24	53.24				Megyesi	2005	$ADD = 10^{(0.002 \times TBS^2 + 1.81)} \pm 388, 16$							
3	1	20/03/2023	10.9	51.62	104.86													
4	2	19/03/2023	10.6	51.08	155.94				TBS Megyesi et al. (2005)									
5	3	18/03/2023	9.7	49.46	205.40						TBS	ADD	ADD RANGE		PMI RANGE (days)	Real PMI (days)		
6	4	17/03/2023	9.1	48.38	253.78				PBI 1	GN	12	125.314117	-262.845883	513.474117	0-10	18		
7	5	16/03/2023	9.0	48.20	301.98					GW	13	140.604752	-247.555248	528.764752	0-10	18		
8	6	15/03/2023	11.2	52.16	354.14					GQ	13	140.604752	-247.555248	528.764752	0-10	18		
9	7	14/03/2023	11.3	52.34	406.48					GC	12	125.314117	-262.845883	513.474117	0-10	18		
10	8	13/03/2023	10.3	50.54	457.02													
11	9	12/03/2023	11.8	53.24	510.26													
12	10	11/03/2023	13.5	56.30	566.56				TBS Keough et al. (2017)									
13	11	10/03/2023	12.2	53.96	620.52						TBS	ADD	ADD RANGE		PMI RANGE (days)	Real PMI (days)		
14	12	09/03/2023	12.7	54.86	675.38				PBI 1	GN	9	93.7562007	-294.403799	481.916201	0-9	18		
15	13	08/03/2023	9.3	48.74	724.12					GW	10	102.329299	-285.830701	490.489299	0-9	18		
16	14	07/03/2023	8.2	46.76	770.88					GQ	11	112.719746	-275.440254	500.879746	0-9	18		
17	15	06/03/2023	8.5	47.30	818.18					GC	10	102.329299	-285.830701	490.489299	0-9	18		
18	16	05/03/2023	8.4	47.12	865.30													
19	17	04/03/2023	9.2	48.56	913.86													
20	18	03/03/2023	8.5	47.30	961.16	Real PMI			TBS Indra et al. (2024)									
21											TBS	ADD	ADD RANGE		PMI RANGE (days)	Real PMI (days)		
22									PBI 1	GN	9	93.7562007	-294.403799	481.916201	0-9	18		
23										GW	10	102.329299	-285.830701	490.489299	0-9	18		
24										GQ	12	125.314117	-262.845883	513.474117	0-10	18		
25										GC	10	102.329299	-285.830701	490.489299	0-9	18		
26																		

Figure A.2: Example of an Excel spreadsheet for estimating the PMI based on the calculation of ADDs. On the left are shown (in reverse order, from the exhumation date backward to the initial date) the daily mean temperatures provided by the Cerano (NO) weather station, converted to degrees Fahrenheit and summed to obtain the ADD. On the right, for each of the three methods considered, the TBS of the piglets exhumed at the first PBI is reported together with the corresponding ADD calculation (in absolute terms and as a range). Finally, a comparison between the estimated PMI and the actual PMI is provided.

	A	B	C	D	E	F	G	H
1	Exhumation Date	ID	Decomposition (%)	Adipocere (Multiplier)	Soil Temp (°C)	Soil Moist (%)	Estimated PMI (Days)	Real PMI (Days)
2	21/03/2023	GN1	5	1	11.8	14.68	166	18
3	21/03/2023	GW1	5	1	11.8	13.32	183	18
4	21/03/2023	GQ1	7	1	11.8	9.64	353	18
5	21/03/2023	GC1	12	1	11.8	11.22	520	18
6								
7		Adipocere			Decomposition			
8		% Adipocere formation	Multiplier		Stage	Warm conditions (%)	Cold conditions (%)	
9		<35	1		Fresh	1-10	1-20	
10		35	3		Bloat	11-35	21-45	
11		40	5		Decay	36-85	46-85	
12		45	5		Dry	86-100	86-100	
13		50	5					
14		55	5					
15		60	5					
16		65	5					
17		70	6					
18		75	7					
19		80	8					
20		85	11					
21		90	14					

Figure A.3: Example of an Excel spreadsheet for estimating the PMI based on the calculation of Vass formula (2011). In the upper table, the data used for the calculation are specified. References to the adipocere and decomposition scoring application are also reported. Finally, a comparison between the estimated PMI and the actual PMI is provided.



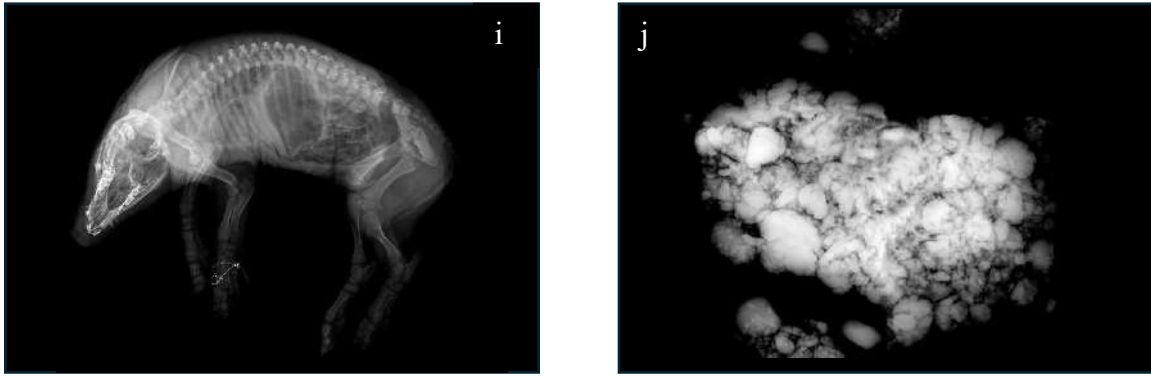


Figure A.4: Radiographies of the piglets of group GW, performed before the burials (left column) and after the exhumations (right column). a,b- GW0; c,d- GW1; e,f- GW2; g,h- GW4; i,j- GW5.

A substantial amount of data was yielded by SEM-EDX analyses conducted on IGSRs extracted from lesions on the subjects' skulls. Consequently, for each subject a 3D compositional image at 500x magnification of sample area detected as the most populated by IGSR will be reported, followed by the most significant particles detected. With regard to the control soil samples, only examples of particles that, in appearance or chemical composition, could be mistaken for IGSR are presented. The section is organized as follows:

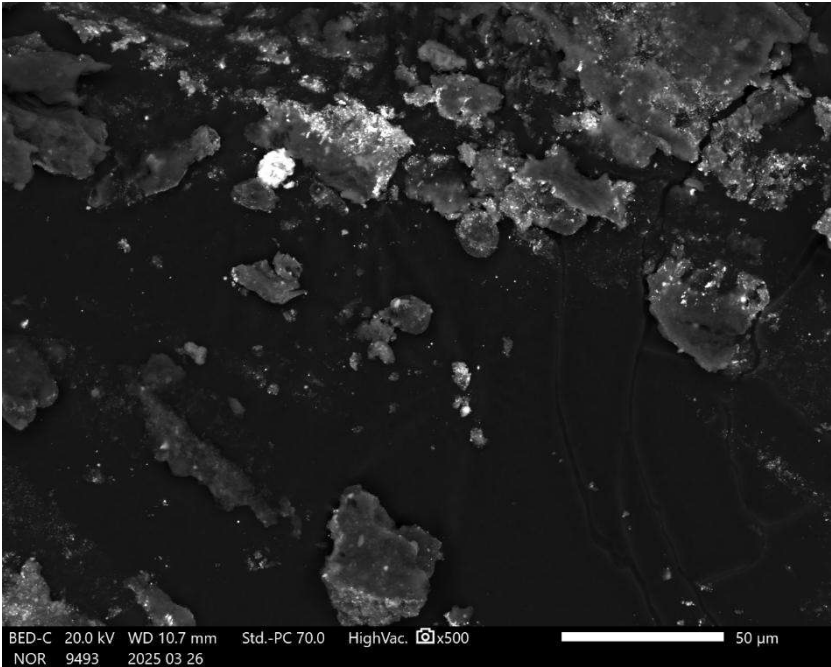
Pages 453-479: Posterior lesions (right after shooting/before burial)

Pages 480-504: Anterior lesion (control piglet/after burial)

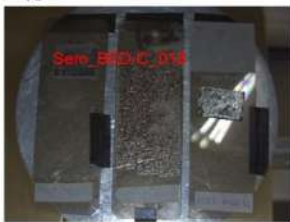
Pages 505-506: Control soil

Posterior lesions (right after shooting/before burial)

GW0 – Entry wound

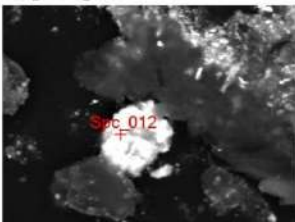


Smp_001



20 mm

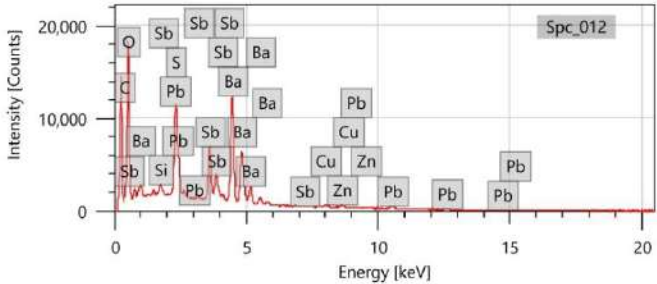
Sem_BED-C_018



5 µm

Signal BED-C
 Landing Voltage 20.0 kV
 WD 10.7 mm
 Magnification x2,700
 Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 2700
Process time	T1
Measurement detector	First
Live time	55.39 seconds
Real time	60.00 seconds
Dead time	8.00
Count rate	34666.00 CPS



Display name	Standard data	Quantification method	Result Type
Spc_012	Standardless	ZAF	Oxide (Number of oxygen 24)

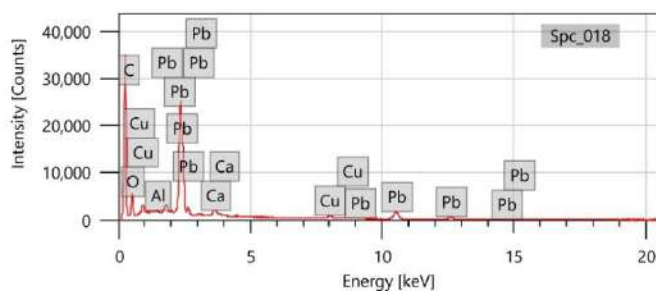
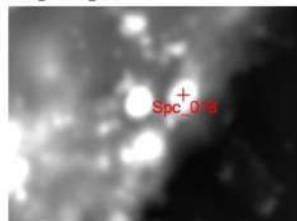
Chemical formula	Line	Mass%	Mol%	Cations
C	K	13.34±0.03	66.37±0.14	0.00
O	K			
SiO2	K	0.99±0.03	0.98±0.03	0.44
SO3	K	8.16±0.05	6.09±0.04	2.74
CuO	K	1.29±0.04	0.97±0.03	0.43
ZnO	K	1.48±0.04	1.09±0.03	0.49
Sb2O3	L	16.16±0.07	3.31±0.01	2.98
BaO	L	45.16±0.11	17.60±0.04	7.91
PbO	M	13.42±0.09	3.59±0.02	1.61
Total		100.00	100.00	

Spc_012 Fitting ratio 0.1612

Smp_001



Sem_BED-C_024



Signal BED-C
Landing Voltage 20.0 kV
WD 10.7 mm
Magnification x23,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 23000
Process time	T1
Measurement detector	First
Live time	55.19 seconds
Real time	60.00 seconds
Dead time	8.00
Count rate	31130.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

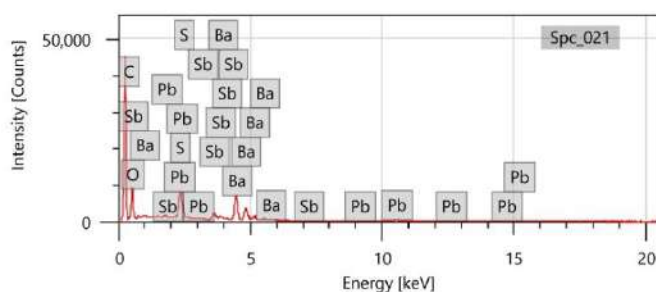
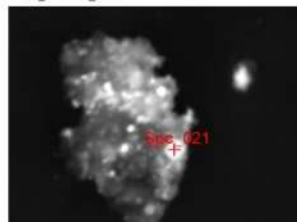
Chemical formula	Line	Mass%	Mol%	Cations
C	K	38.82±0.05	91.10±0.12	0.00
Al2O3	K	0.51±0.02	0.14±0.01	0.74
CaO	K	0.63±0.03	0.32±0.01	0.83
CuO	K	3.78±0.05	1.34±0.02	3.50
PbO	M	56.27±0.13	7.11±0.02	18.57
Total		100.00	100.00	

Spc_018 Fitting ratio 0.3823

Smp_001



Sem_BED-C_026



Signal BED-C
Landing Voltage 20.0 kV
WD 10.7 mm
Magnification x9,500
Vacuum Mode HighVacuum

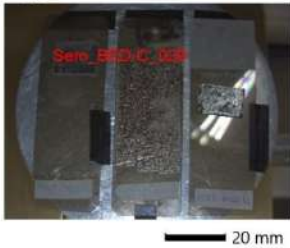
Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 9500
Process time	T1
Measurement detector	First
Live time	56.26 seconds
Real time	60.00 seconds
Dead time	6.00
Count rate	25806.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

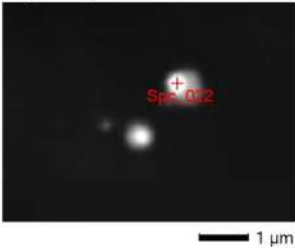
Chemical formula	Line	Mass%	Mol%	Cations
C	K	43.67±0.05	91.06±0.11	0.00
SO3	K	5.61±0.05	1.76±0.01	3.16
Sb2O3	L	5.24±0.05	0.45±0.00	1.62
BaO	L	31.96±0.10	5.22±0.02	9.38
PbO	M	13.52±0.09	1.52±0.01	2.73
Total		100.00	100.00	

Spc_021 Fitting ratio 0.4011

Smp_001

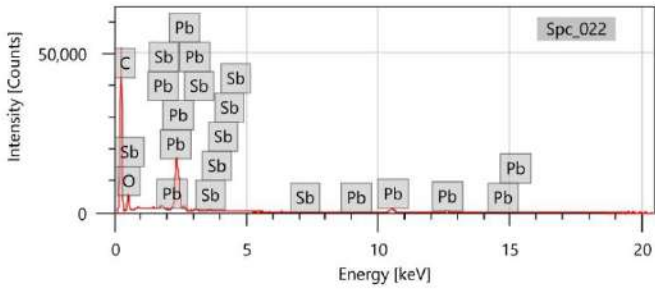


Sem_BED-C_029



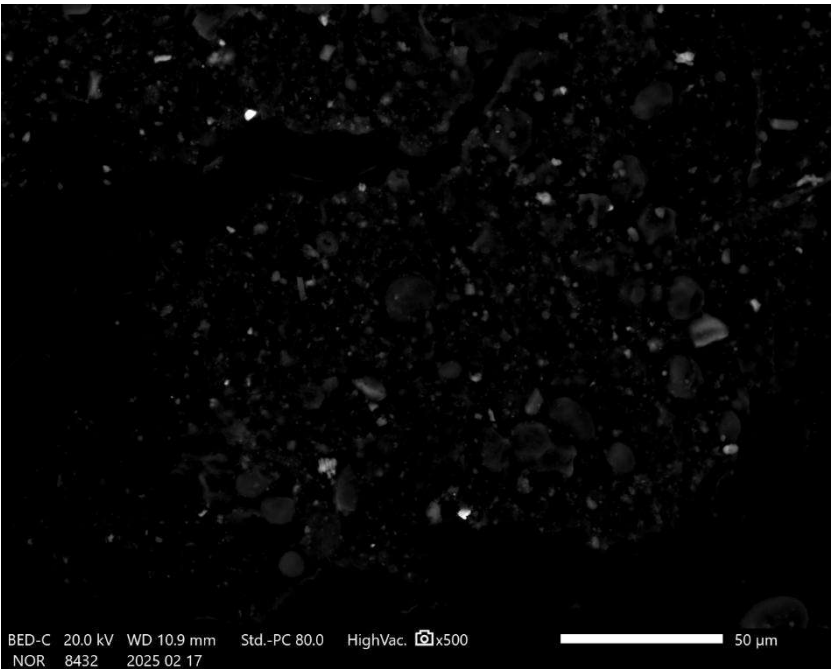
Signal BED-C
Landing Voltage 20.0 kV
WD 10.7 mm
Magnification x22,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 22000
Process time	T1
Measurement detector	First
Live time	55.77 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	26776.00 CPS



Display name	Standard data	Quantification method	Result Type	
Spc_022	Standardless	ZAF	Oxide (Number of oxygen 24)	
Chemical formula	Line	Mass%	Mol%	Cations
C	K	54.58±0.06	95.74±0.11	0.00
O	K			
Sb2O3	L	1.05±0.04	0.08±0.00	0.82
PbO	M	44.37±0.13	4.19±0.01	22.77
Total		100.00	100.00	
Spc_022			Fitting ratio 0.4940	

GW0 – Exit wound



Smp_002

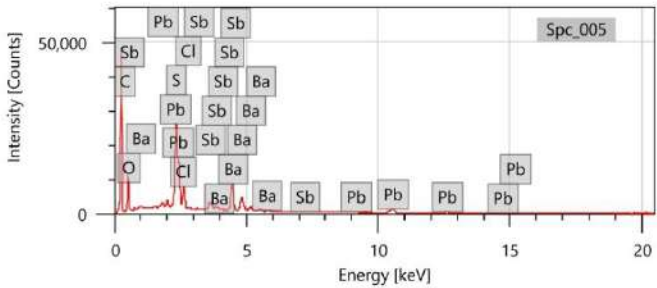


Sem_BED-C_005



Signal BED-C
Landing Voltage 20.0 kV
WD 11.0 mm
Magnification x6,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 6000
Process time	T4
Measurement detector	First
Live time	35.87 seconds
Real time	60.00 seconds
Dead time	40.00
Count rate	59315.00 CPS



Display name	Standard data	Quantification method	Result Type
Spc_005	Standardless	ZAF	Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	40.13±0.04	88.91±0.09	0.00
O	K			
SO3	K	5.36±0.03	1.78±0.01	3.28
Cl	K	2.91±0.01	2.16±0.01	0.00
Sb2O3	L	3.07±0.03	0.28±0.00	1.03
BaO	L	19.53±0.06	3.39±0.01	6.24
PbO	M	29.01±0.08	3.46±0.01	6.37
Total		100.00	100.00	

Spc_005 Fitting ratio 0.3019

Smp_002

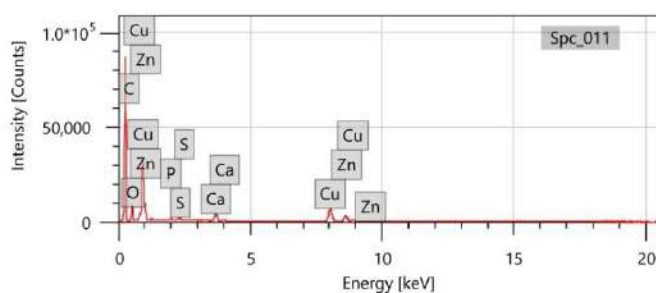


20 mm

Sem_BED-C_011



2 μm



Signal BED-C
Landing Voltage 20.0 kV
WD 10.9 mm
Magnification x9,000
Vacuum Mode HighVacuum

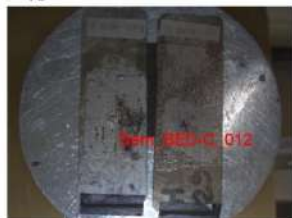
Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 9000
Process time	T4
Measurement detector	First
Live time	43.40 seconds
Real time	60.00 seconds
Dead time	28.00
Count rate	38458.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	61.49±0.05	91.25±0.07	0.00
O	K			
P2O5	K	0.72±0.01	0.09±0.00	0.45
SO3	K	1.27±0.01	0.28±0.00	0.70
CuO	K	2.65±0.02	0.84±0.00	2.09
CuO	K	22.33±0.07	5.00±0.02	12.41
ZnO	K	11.54±0.06	2.53±0.01	6.27
Total		100.00	100.00	

Spc_011 Fitting ratio 0.3664

Smp_002

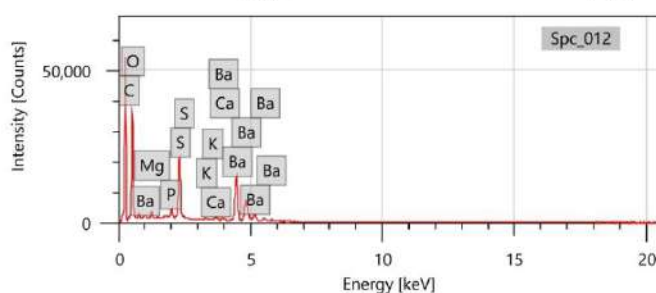


20 mm

Sem_BED-C_012



2 μm



Signal BED-C
Landing Voltage 20.0 kV
WD 10.9 mm
Magnification x9,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 9000
Process time	T4
Measurement detector	First
Live time	38.88 seconds
Real time	60.00 seconds
Dead time	35.00
Count rate	51214.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

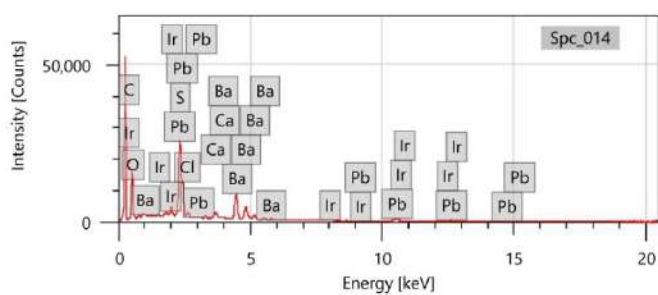
Chemical formula	Line	Mass%	Mol%	Cations
C	K	46.32±0.04	88.72±0.08	0.00
O	K			
MgO	K	1.48±0.02	0.85±0.01	0.89
P2O5	K	2.73±0.02	0.44±0.00	0.93
SO3	K	16.95±0.04	4.87±0.01	5.13
K2O	K	0.31±0.01	0.08±0.00	0.16
CuO	K	0.82±0.01	0.34±0.00	0.36
BaO	L	31.38±0.06	4.71±0.01	4.96
Total		100.00	100.00	

Spc_012 Fitting ratio 0.2413



Signal BED-C
Landing Voltage 20.0 kV
WD 11.0 mm
Magnification x5,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 5000
Process time	T4
Measurement detector	First
Live time	37.03 seconds
Real time	60.00 seconds
Dead time	38.00
Count rate	56226.00 CPS

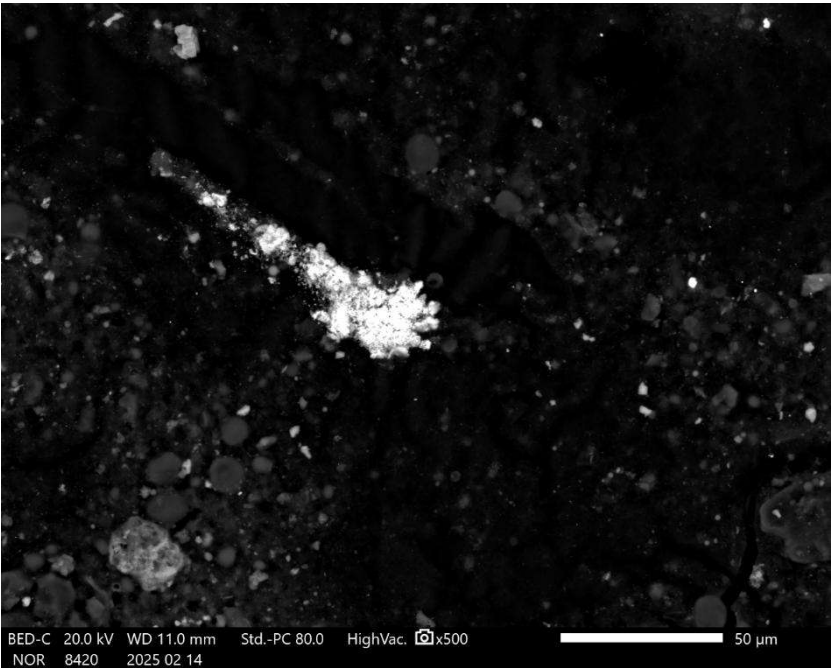


Display name	Standard data	Quantification method	Result Type
Spc_014	Standardless	ZAF	Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	42.40±0.04	89.95±0.09	0.00
O	K			
SO3	K	9.65±0.04	3.07±0.01	4.55
Cl	K	0.59±0.01	0.42±0.01	0.00
CaO	K	1.00±0.01	0.45±0.01	0.67
BaO	L	19.49±0.05	3.24±0.01	4.79
Ir2O3	M	3.81±0.03	0.22±0.00	0.67
PbO	M	23.05±0.07	2.63±0.01	3.90
Total		100.00	100.00	

Spc 014 Fitting ratio 0.3204

GW1 – Entry wound

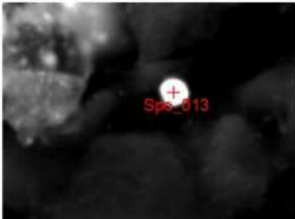


Smp_001



20 mm

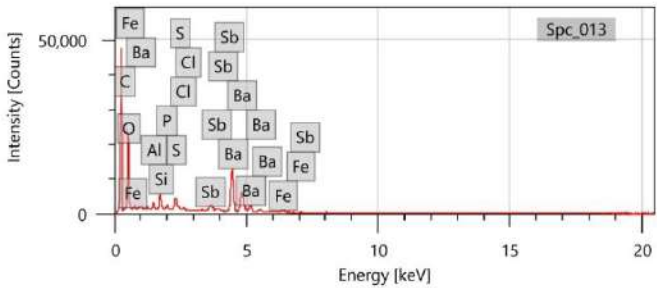
Sem_BED-C_018



2 µm

Signal BED-C
Landing Voltage 20.0 kV
WD 11.0 mm
Magnification x7,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 7500
Process time	T4
Measurement detector	First
Live time	43.73 seconds
Real time	60.00 seconds
Dead time	27.00
Count rate	36686.00 CPS



Display name	Standard data	Quantification method	Result Type
Spc_013	Standardless	ZAF	Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	45.46±0.05	89.48±0.09	0.00
Al2O3	K	2.06±0.03	0.48±0.01	1.40
SiO2	K	4.29±0.03	1.69±0.01	2.47
P2O5	K	1.85±0.02	0.31±0.00	0.90
SO3	K	3.55±0.03	1.05±0.01	1.54
Cl	K	0.52±0.01	0.34±0.01	0.00
FeO	K	1.39±0.02	0.46±0.01	0.67
Sb2O3	L	1.54±0.03	0.13±0.00	0.37
BaO	L	39.34±0.09	6.07±0.01	8.88
Total		100.00	100.00	

Spc_013 Fitting ratio 0.3017

Smp_001

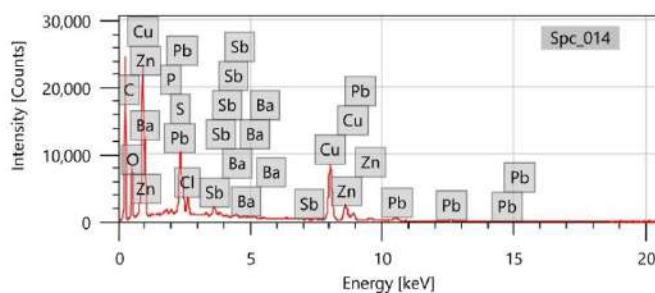


20 mm

Sem_BED-C_019



2 μm



Signal BED-C
Landing Voltage 20.0 kV
WD 10.9 mm
Magnification x9,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 9500
Process time	T4
Measurement detector	First
Live time	43.39 seconds
Real time	60.00 seconds
Dead time	27.00
Count rate	36765.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	32.00±0.05	78.81±0.11	0.00
O	K			
P2O5	K	0.81±0.02	0.17±0.00	0.36
SO3	K	1.98±0.03	0.73±0.01	0.77
Cl	K	1.28±0.01	1.07±0.01	0.00
CuO	K	33.73±0.10	12.55±0.04	13.25
ZnO	K	11.53±0.07	4.19±0.03	4.43
Sb2O3	L	2.31±0.03	0.23±0.00	0.49
BaO	L	1.32±0.02	0.25±0.00	0.27
PbO	M	15.04±0.06	1.99±0.01	2.11
Total		100.00	100.00	
Spc_014				Fitting ratio 0.2048

Smp_001

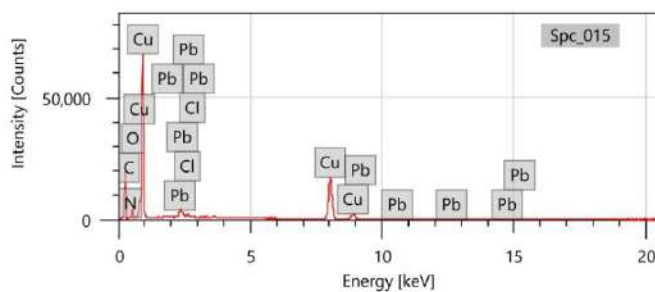


20 mm

Sem_BED-C_020



5 μm



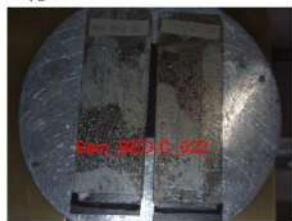
Signal BED-C
Landing Voltage 20.0 kV
WD 10.9 mm
Magnification x4,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 4500
Process time	T4
Measurement detector	First
Live time	41.59 seconds
Real time	60.00 seconds
Dead time	30.00
Count rate	41784.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

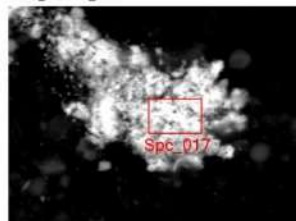
Chemical formula	Line	Mass%	Mol%	Cations
C	K	24.79±0.04	62.73±0.09	0.00
N	K	5.31±0.04	11.53±0.10	0.00
O	K			
Cl	K	0.60±0.01	0.51±0.01	0.00
CuO	K	64.22±0.13	24.54±0.05	23.34
PbO	M	5.08±0.04	0.69±0.01	0.66
Total		100.00	100.00	
Spc_015				Fitting ratio 0.0991

Smp_001

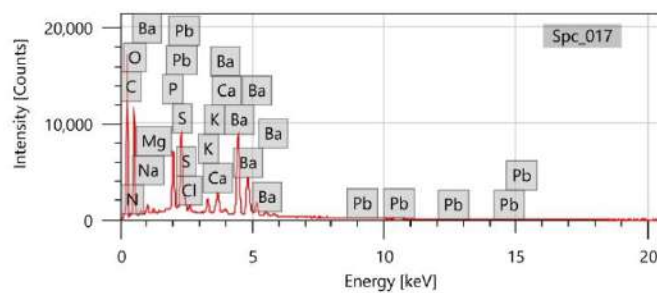


20 mm

Sem_BED-C_022



10 µm



Signal BED-C
Landing Voltage 20.0 kV
WD 11.1 mm
Magnification x2,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 2000
Process time	T4
Measurement detector	First
Live time	48.86 seconds
Real time	60.00 seconds
Dead time	18.00
Count rate	23083.00 CPS

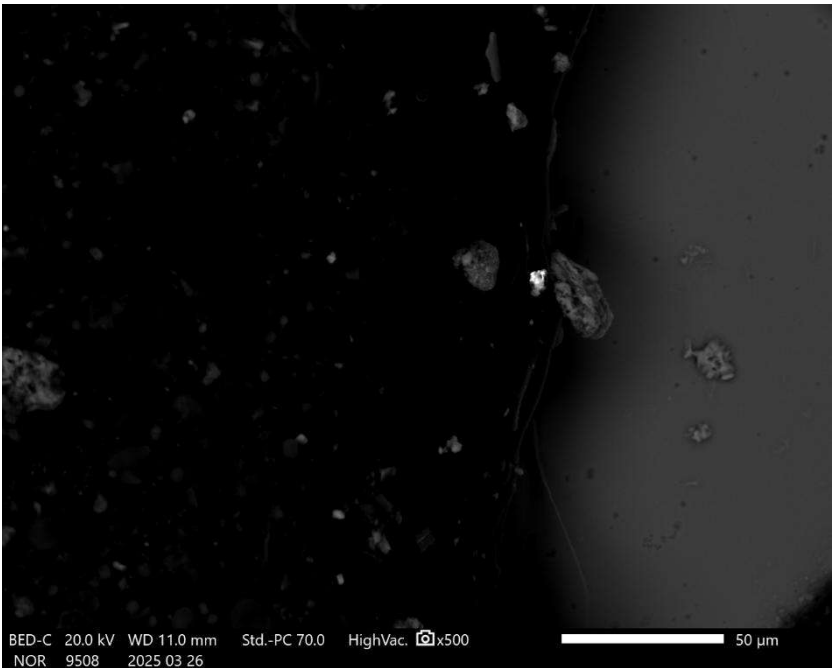
Display name: Standard data Quantification method: Result Type:
Spc_017 Standardless ZAF Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	28.73±0.05	69.81±0.12	0.00
Ni	K	6.84±0.07	14.24±0.16	0.00
O	K			
Na2O	K	1.09±0.02	0.51±0.01	0.78
MgO	K	0.51±0.02	0.37±0.01	0.28
P2O5	K	9.60±0.05	1.97±0.01	2.99
SO3	K	11.23±0.05	4.09±0.02	3.10
Cl	K	0.47±0.01	0.38±0.01	0.00
K2O	K	1.57±0.02	0.49±0.01	0.74
CaO	K	2.36±0.02	1.23±0.01	0.95
BaO	L	33.24±0.09	6.39±0.02	4.80
PbO	M	4.38±0.07	0.57±0.01	0.43
Total		100.00	100.00	

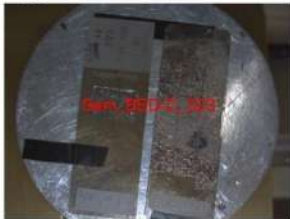
Spc_017

Fitting ratio 0.1853

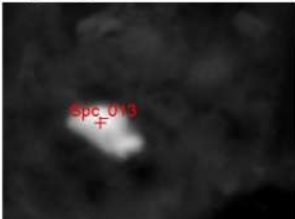
GW1 – Exit wound



Smp_002

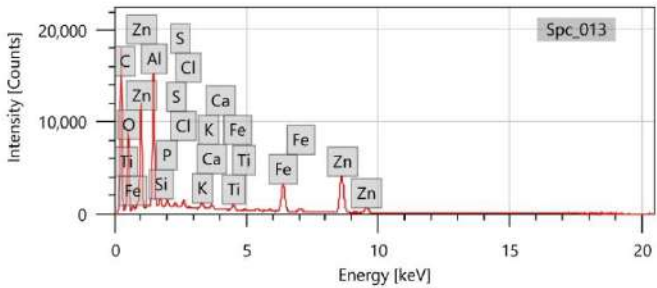


Sem_BED-C_023



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x12,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 12000
Process time	T1
Measurement detector	First
Live time	57.00 seconds
Real time	60.00 seconds
Dead time	5.00
Count rate	19071.00 CPS

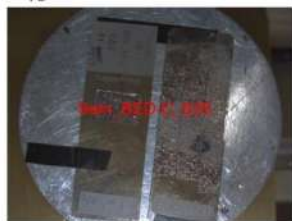


Display name	Standard data	Quantification method	Result Type
Spc_013	Standardless	ZAF	Oxide (Number of oxygen 24)

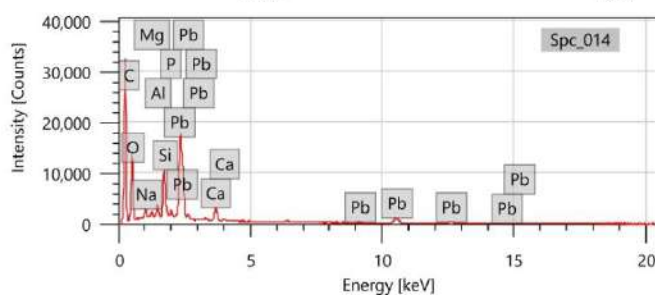
Chemical formula	Line	Mass%	Mol%	Cations
C	K	38.87±0.07	81.50±0.14	0.00
O	K			
Al2O3	K	16.23±0.06	4.01±0.02	6.78
SiO2	K	1.18±0.02	0.49±0.01	0.42
P2O5	K	1.20±0.02	0.21±0.00	0.36
SO3	K	0.68±0.02	0.21±0.01	0.18
Cl	K	0.47±0.01	0.34±0.01	0.00
K2O	K	0.54±0.01	0.19±0.00	0.25
CaO	K	0.55±0.01	0.29±0.01	0.21
TiO2	K	1.28±0.02	0.40±0.01	0.34
FeO	K	9.05±0.05	3.17±0.02	2.69
ZnO	K	29.94±0.13	9.27±0.04	7.84
Total		100.00	100.00	

Spc_013 Fitting ratio 0.2024

Smp_002



Sem_BED-C_025



Signal BED-C
Landing Voltage 20.0 kV
WD 10.9 mm
Magnification x15,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 15000
Process time	T1
Measurement detector	First
Live time	55.83 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	28037.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

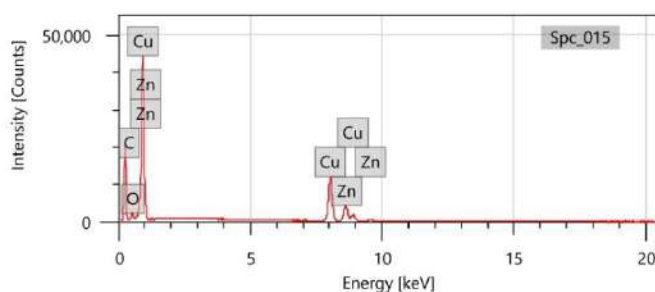
Chemical formula	Line	Mass%	Mol%	Cations
C	K	43.20±0.06	88.60±0.12	0.00
Na2O	K	1.62±0.02	0.65±0.01	1.78
MgO	K	0.84±0.02	0.51±0.01	0.71
Al2O3	K	1.98±0.03	0.48±0.01	1.32
SiO2	K	8.90±0.05	3.65±0.02	5.04
P2O5	K	2.01±0.03	0.35±0.00	0.96
CaO	K	3.61±0.03	1.58±0.01	2.18
PbO	M	37.84±0.11	4.18±0.01	5.76
Total		100.00	100.00	

Spc_014 Fitting ratio 0.3518

Smp_002



Sem_BED-C_027



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x5,500
Vacuum Mode HighVacuum

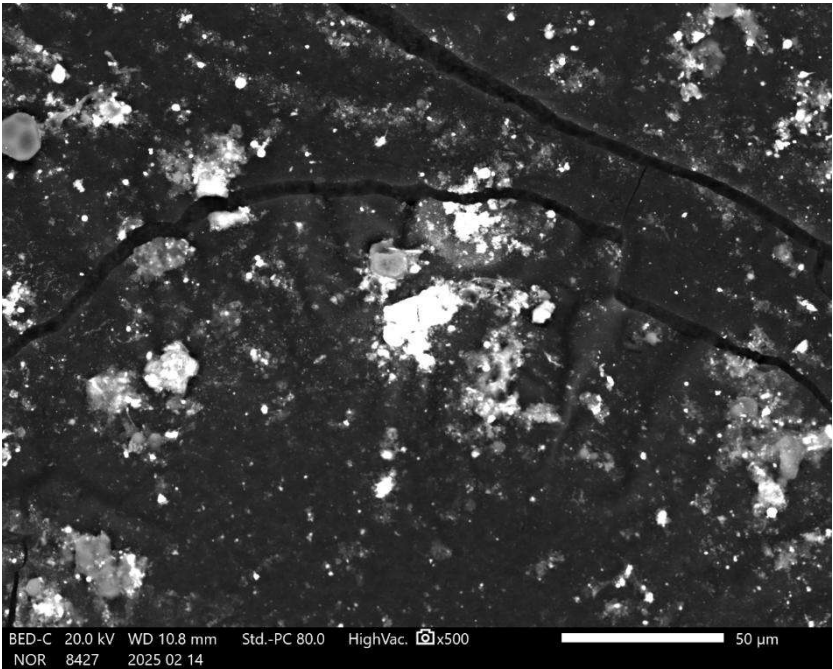
Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 5500
Process time	T1
Measurement detector	First
Live time	55.72 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	28242.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

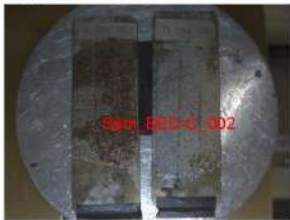
Chemical formula	Line	Mass%	Mol%	Cations
C	K	28.91±0.05	73.06±0.12	0.00
O	K	49.87±0.13	19.03±0.05	16.95
CuO	K	21.22±0.10	7.91±0.04	7.05
Total		100.00	100.00	

Spc_015 Fitting ratio 0.1541

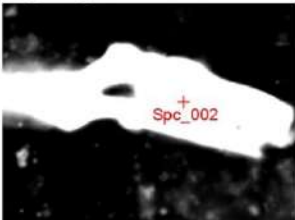
GW2 – Entry wound



Smp_003

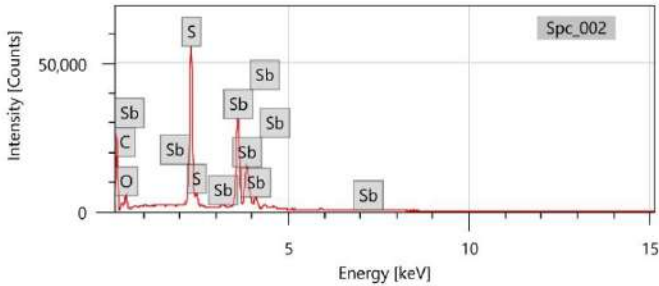


Sem_BED-C_002



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x5,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 5500
Process time	T4
Measurement detector	First
Live time	28.30 seconds
Real time	60.00 seconds
Dead time	52.00
Count rate	88255.00 CPS

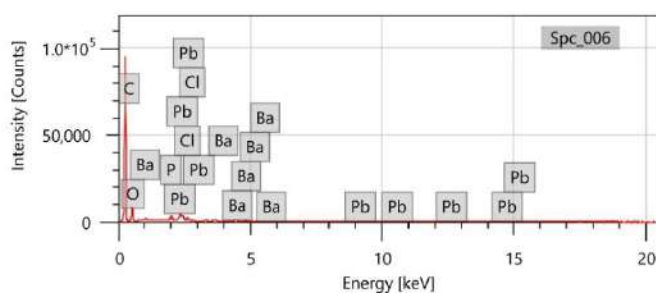


Display name	Standard data	Quantification method	Result Type	
Spc_002	Standardless	ZAF	Oxide (Number of oxygen 24)	
Chemical formula	Line	Mass%	Mol%	Cations
C	K	24.51±0.03	78.30±0.10	0.00
O	K			
SO3	K	33.85±0.05	16.22±0.02	5.98
Sb2O3	L	41.64±0.07	5.48±0.01	4.04
Total		100.00	100.00	
Spc_002			Fitting ratio 0.1098	

Smp_003



Sem_BED-C_007



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x13,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 13000
Process time	T4
Measurement detector	First
Live time	48.18 seconds
Real time	60.00 seconds
Dead time	20.00
Count rate	26016.00 CPS

Display name : Standard data Quantification method : ZAF Result Type : Oxide (Number of oxygen 24)

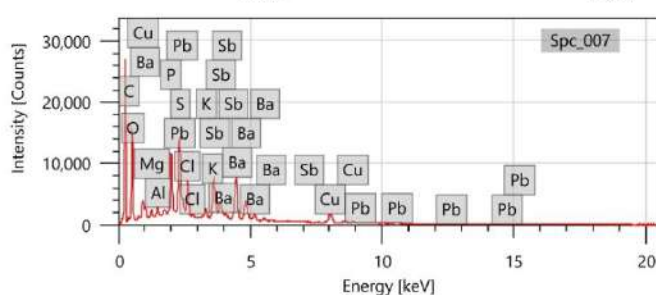
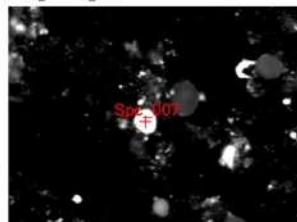
Chemical formula	Line	Mass%	Mol%	Cations
C	K	83.91±0.06	98.47±0.07	0.00
O	K			
P2O5	K	2.80±0.03	0.28±0.00	5.88
Cl	K	0.94±0.01	0.37±0.00	0.00
BaO	L	3.52±0.04	0.32±0.00	3.42
PbO	M	8.83±0.06	0.56±0.00	5.99
Total		100.00	100.00	

Spc_006 Fitting ratio 0.4976

Smp_003



Sem_BED-C_008



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x1,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 1500
Process time	T4
Measurement detector	First
Live time	40.89 seconds
Real time	60.00 seconds
Dead time	32.00
Count rate	44195.00 CPS

Display name : Standard data Quantification method : ZAF Result Type : Oxide (Number of oxygen 24)

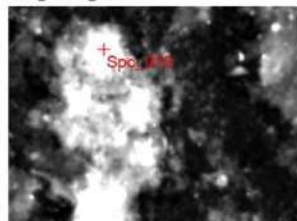
Chemical formula	Line	Mass%	Mol%	Cations
C	K	34.42±0.05	83.51±0.11	0.00
O	K			
MgO	K	0.82±0.02	0.39±0.01	0.43
Al2O3	K	1.09±0.02	0.31±0.00	0.46
P2O5	K	9.67±0.04	1.99±0.01	2.91
SO3	K	10.63±0.04	3.87±0.01	2.83
Cl	K	2.61±0.01	2.14±0.01	0.00
K2O	K	1.05±0.01	0.32±0.00	0.46
CuO	K	5.71±0.04	2.09±0.02	1.53
Sb2O3	L	10.68±0.05	1.07±0.00	1.56
BaO	L	17.85±0.06	3.39±0.01	2.48
PbO	M	5.49±0.06	0.72±0.01	0.52
Total		100.00	100.00	

Spc_007 Fitting ratio 0.2103

Smp_003

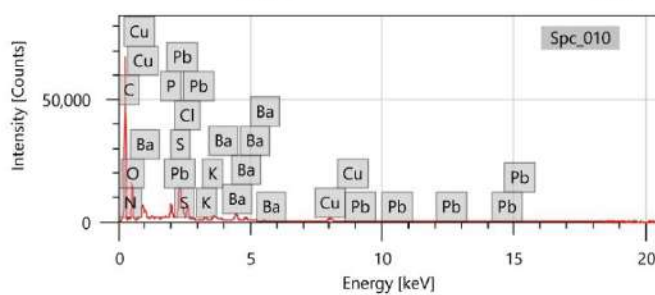


Sem_BED-C_012



Signal BED-C
Landing Voltage 20.0 kV
WD 10.9 mm
Magnification x4,500
Vacuum Mode HighVacuum

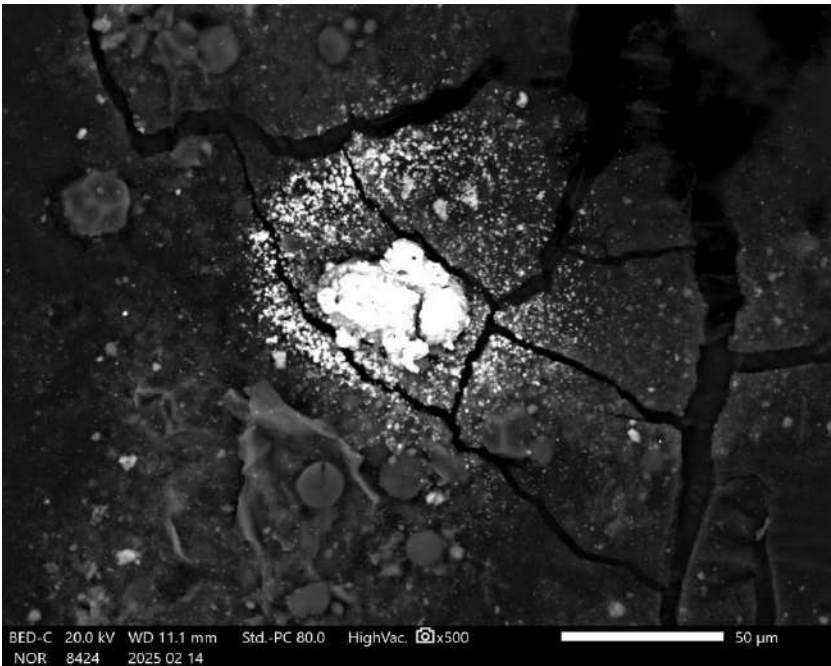
Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 4500
Process time	T4
Measurement detector	First
Live time	42.29 seconds
Real time	60.00 seconds
Dead time	29.00
Count rate	40954.00 CPS



Display name	Standard data	Quantification method	Result Type
Spc_010	Standardless	ZAF	Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	47.89±0.04	67.59±0.06	0.00
N	K	22.86±0.11	27.66±0.13	0.00
O	K			
P2O5	K	3.66±0.02	0.44±0.00	2.57
SO3	K	5.96±0.03	1.26±0.01	3.71
Cl	K	1.82±0.01	0.87±0.00	0.00
K2O	K	0.61±0.01	0.11±0.00	0.64
CuO	K	4.19±0.03	0.89±0.01	2.63
BaO	L	5.52±0.03	0.61±0.00	1.80
PbO	M	7.49±0.04	0.57±0.00	1.68
Total		100.00	100.00	
Spc_010				Fitting ratio 0.3712

GW2 – Exit wound



Smp_002

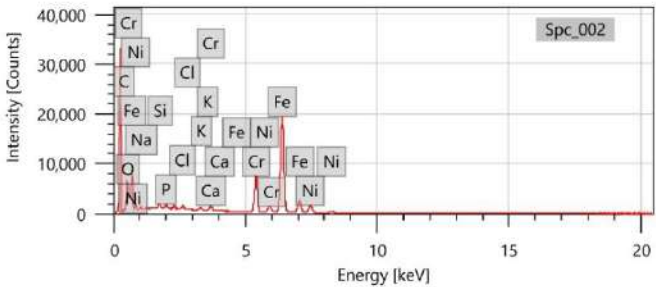


Sem_BED-C_004



Signal BED-C
Landing Voltage 20.0 kV
WD 11.0 mm
Magnification x7,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 7500
Process time	T4
Measurement detector	First
Live time	46.22 seconds
Real time	60.00 seconds
Dead time	23.00
Count rate	29457.00 CPS



Display name	Standard data	Quantification method	Result Type
Spc_002	Standardless	ZAF	Oxide (Number of oxygen 24)

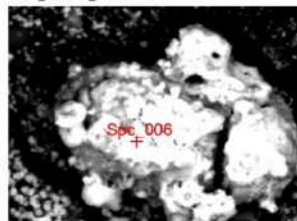
Chemical formula	Line	Mass%	Mol%	Cations
C	K	34.69±0.04	77.62±0.09	0.00
O	K			
Na2O	K	0.91±0.02	0.40±0.01	0.70
SiO2	K	0.74±0.02	0.33±0.01	0.29
P2O5	K	1.10±0.02	0.21±0.00	0.37
Cl	K	0.34±0.01	0.26±0.00	0.00
K2O	K	0.37±0.01	0.11±0.00	0.19
CaO	K	0.81±0.01	0.39±0.01	0.34
Cr2O3	K	11.79±0.04	2.09±0.01	3.68
FeO	K	44.23±0.06	16.59±0.03	14.60
NiO	K	5.01±0.04	1.81±0.01	1.59
Total		100.00	100.00	
Spc_002				Fitting ratio 0.1587

Smp_002

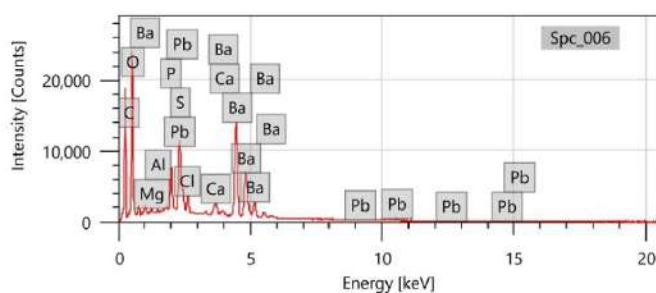


20 mm

Sem_BED-C_009



10 µm



Signal BED-C
Landing Voltage 20.0 kV
WD 11.0 mm
Magnification x2,300
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 2300
Process time	T4
Measurement detector	First
Live time	42.87 seconds
Real time	60.00 seconds
Dead time	28.00
Count rate	38806.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	25.33±0.04	78.28±0.13	0.00
O	K			
MgO	K	0.63±0.02	0.58±0.02	0.37
Al2O3	K	0.25±0.02	0.09±0.01	0.12
P2O5	K	8.47±0.04	2.21±0.01	2.80
SO3	K	9.44±0.04	4.38±0.02	2.77
Cl	K	1.50±0.01	1.57±0.01	0.00
CaO	K	1.10±0.02	0.73±0.01	0.46
BaO	L	43.57±0.09	10.55±0.02	6.67
PbO	M	9.72±0.07	1.62±0.01	1.02
Total		100.00	100.00	
Spc_006				Fitting ratio 0.1674

Smp_002

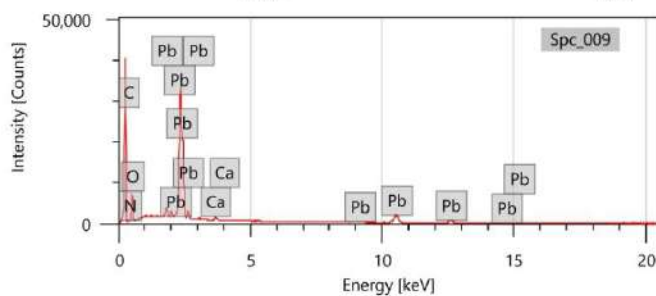


20 mm

Sem_BED-C_013



1 µm



Signal BED-C
Landing Voltage 20.0 kV
WD 11.0 mm
Magnification x11,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 11000
Process time	T4
Measurement detector	First
Live time	40.86 seconds
Real time	60.00 seconds
Dead time	31.00
Count rate	43147.00 CPS

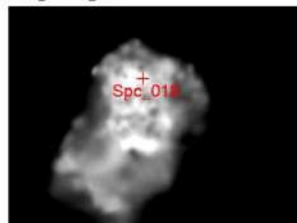
Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	34.13±0.04	72.13±0.08	0.00
N	K	11.83±0.09	21.43±0.16	0.00
O	K			
CaO	K	0.85±0.02	0.38±0.01	1.43
PbO	M	53.19±0.10	6.05±0.01	22.57
Total		100.00	100.00	
Spc_009				Fitting ratio 0.3171

Smp_002

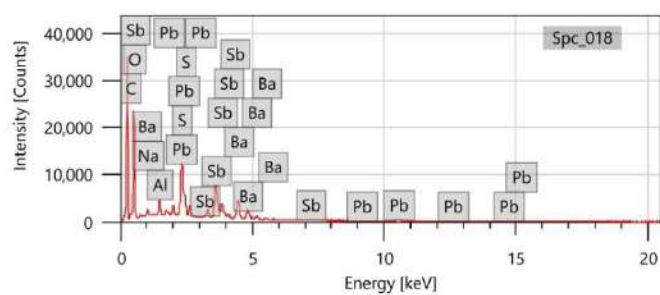


Sem_BED-C_027



Signal BED-C
Landing Voltage 20.0 kV
WD 11.0 mm
Magnification x8,500
Vacuum Mode HighVacuum

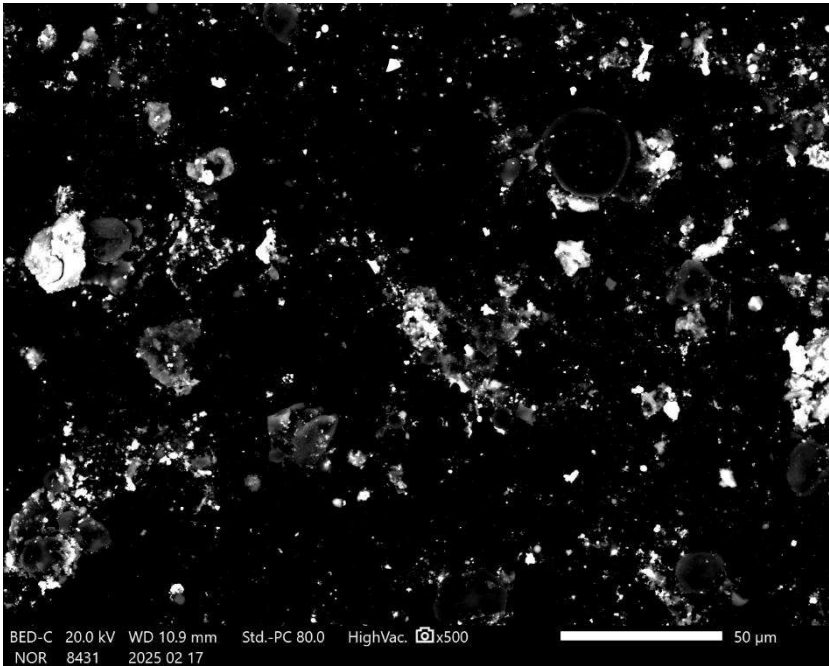
Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 8500
Process time	T4
Measurement detector	First
Live time	43.76 seconds
Real time	60.00 seconds
Dead time	27.00
Count rate	37088.00 CPS



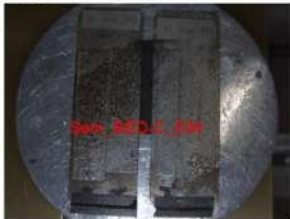
Display name: Standard data Quantification method: Result Type:
Spc_018 Standardless ZAF Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	28.64±0.05	89.27±0.10	0.00
O	K			
Na2O	K	1.43±0.02	0.64±0.01	1.44
Al2O3	K	3.26±0.03	0.89±0.01	1.98
SiO3	K	8.21±0.04	2.85±0.01	3.18
Sb2O3	L	17.09±0.07	1.63±0.01	3.64
BaO	L	14.70±0.06	2.66±0.01	2.98
PbO	M	16.66±0.08	2.07±0.01	2.32
Total		100.00	100.00	
Spc_018				Fitting ratio 0.3277

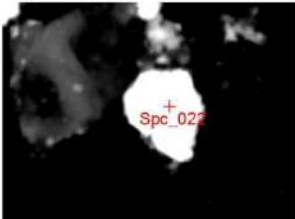
GW4 – Entry wound



Smp_001

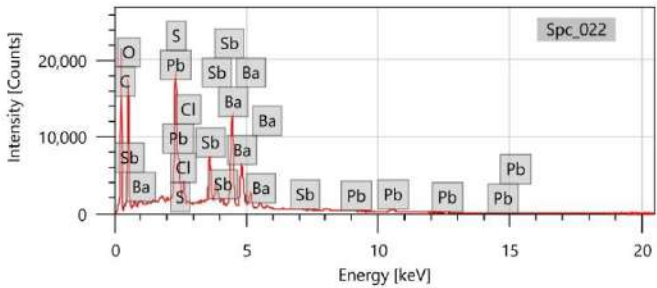


Sem_BED-C_034



Signal BED-C
Landing Voltage 20.0 kV
WD 10.9 mm
Magnification x7,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 7000
Process time	T4
Measurement detector	First
Live time	39.61 seconds
Real time	60.00 seconds
Dead time	34.00
Count rate	47907.00 CPS



Display name	Standard data	Quantification method	Result Type
Spc_022	Standardless	ZAF	Oxide (Number of oxygen 24)

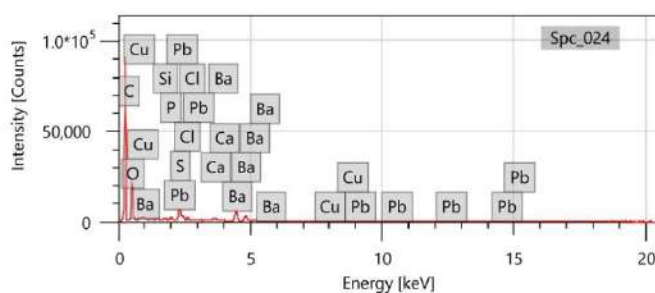
Chemical formula	Line	Mass%	Mol%	Cations
C	K	21.86±0.03	76.87±0.12	0.00
O	K			
SO3	K	13.25±0.05	6.99±0.03	4.25
Cl	K	1.46±0.01	1.74±0.01	0.00
Sb2O3	L	14.17±0.06	2.05±0.01	2.50
BaO	L	35.04±0.08	9.65±0.02	5.87
PbO	M	14.21±0.07	2.69±0.01	1.64
Total		100.00	100.00	

Spc_022 Fitting ratio 0.1821

Smp_001



Sem_BED-C_036



Signal BED-C
Landing Voltage 20.0 kV
WD 10.9 mm
Magnification x9,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 9000
Process time	T4
Measurement detector	First
Live time	44.34 seconds
Real time	60.00 seconds
Dead time	26.00
Count rate	35774.00 CPS

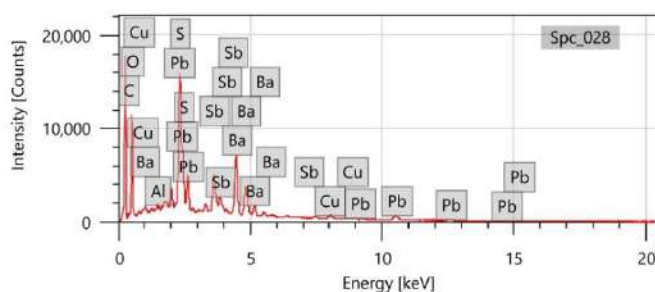
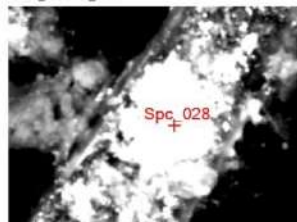
Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	68.69±0.05	95.79±0.07	0.00
O	K			
SiO2	K	0.57±0.02	0.16±0.00	0.60
P2O5	K	1.55±0.02	0.18±0.00	1.37
SO3	K	3.87±0.03	0.81±0.01	3.04
Cl	K	0.68±0.01	0.32±0.00	0.00
CuO	K	0.48±0.01	0.14±0.00	0.53
CuO	K	1.52±0.03	0.32±0.01	1.20
BaO	L	16.93±0.06	1.85±0.01	6.93
PbO	M	5.71±0.05	0.43±0.00	1.60
Total		100.00	100.00	
Spc_024				Fitting ratio 0.4304

Smp_001



Sem_BED-C_042



Signal BED-C
Landing Voltage 20.0 kV
WD 11.0 mm
Magnification x4,300
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 4300
Process time	T4
Measurement detector	First
Live time	44.20 seconds
Real time	60.00 seconds
Dead time	26.00
Count rate	34794.00 CPS

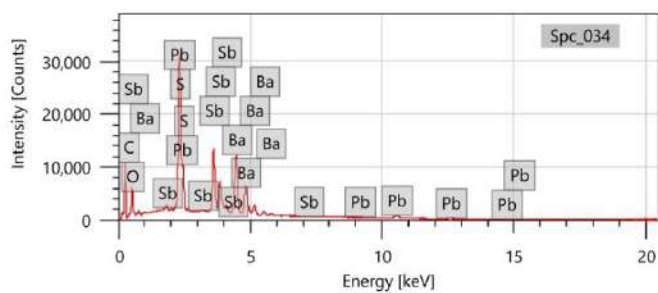
Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	22.90±0.04	79.24±0.13	0.00
O	K			
Al2O3	K	0.62±0.02	0.25±0.01	0.34
SO3	K	11.30±0.05	5.87±0.03	3.90
CuO	K	2.09±0.04	1.09±0.02	0.73
Sb2O3	L	10.75±0.07	1.53±0.01	2.04
BaO	L	26.71±0.08	7.24±0.02	4.82
PbO	M	25.63±0.10	4.77±0.02	3.18
Total		100.00	100.00	
Spc_028				Fitting ratio 0.2743

Smp_001



Sem_BED-C_051



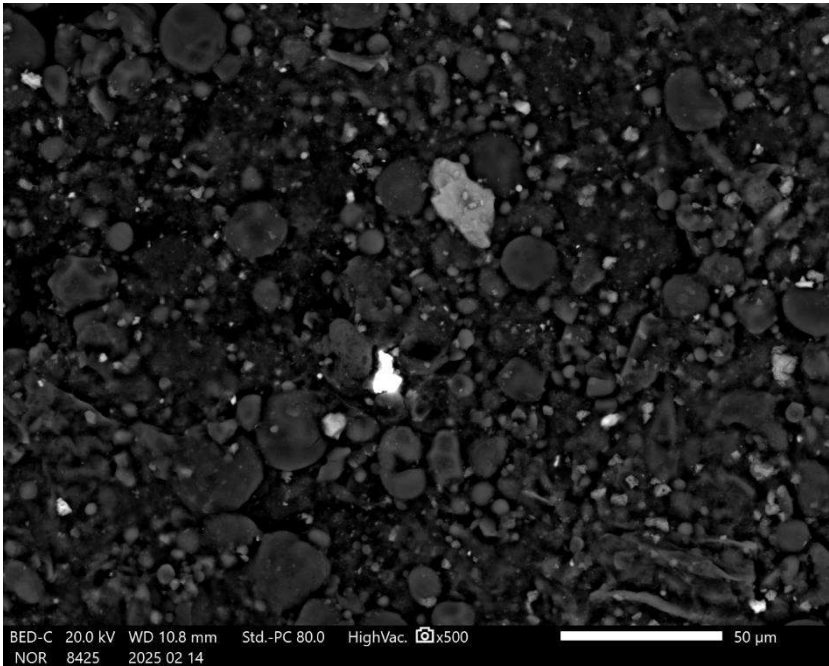
Signal BED-C
Landing Voltage 20.0 kV
WD 11.1 mm
Magnification x1,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 1000
Process time	T4
Measurement detector	First
Live time	35.03 seconds
Real time	60.00 seconds
Dead time	41.00
Count rate	61899.00 CPS

Display name: Standard data Quantification method: Result Type:
Spc_034 Standardless ZAF Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	10.29±0.02	58.63±0.12	0.00
O	K			
SO3	K	21.08±0.05	18.01±0.04	4.89
Sb2O3	L	23.34±0.06	5.48±0.01	2.98
BaO	L	28.67±0.07	12.79±0.03	3.48
PbO	M	16.62±0.07	5.09±0.02	1.38
Total		100.00	100.00	
Spc_034				Fitting ratio 0.0894

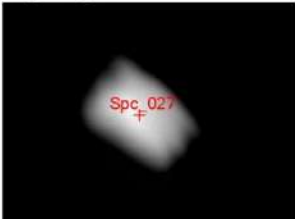
GW4 – Exit wound



Smp_002

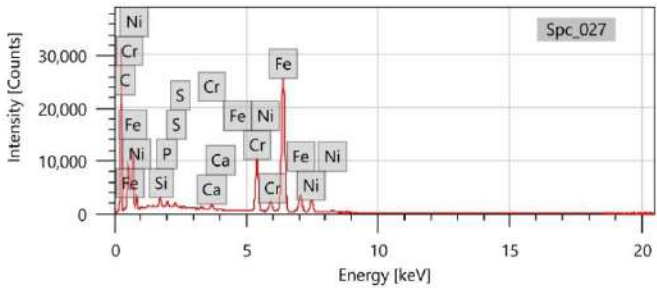


Sem_BED-C_040



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x27,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 27000
Process time	T4
Measurement detector	First
Live time	41.71 seconds
Real time	60.00 seconds
Dead time	30.00
Count rate	41371.00 CPS



Display name	Standard data	Quantification method	Result Type
Spc_027	Standardless	ZAF	Oxide (Number of oxygen 24)

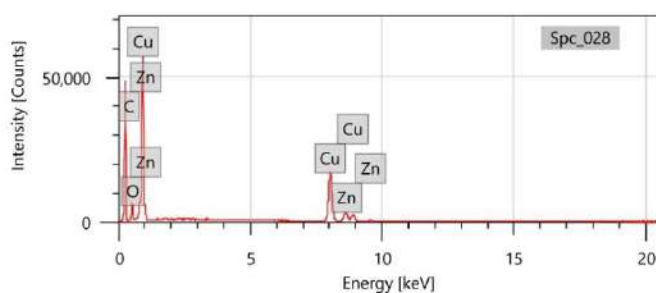
Chemical formula	Line	Mass%	Mol%	Cations
C	K	28.71±0.04	72.91±0.09	0.00
SiO2	K	1.18±0.02	0.60±0.01	0.42
P2O5	K	0.93±0.01	0.20±0.00	0.28
SO3	K	0.71±0.01	0.27±0.00	0.19
CaO	K	0.61±0.01	0.33±0.01	0.23
Cr2O3	K	13.45±0.04	2.70±0.01	3.76
FeO	K	48.11±0.08	20.42±0.03	14.24
NiO	K	6.30±0.04	2.57±0.02	1.79
Total		100.00	100.00	

Spc_027 Fitting ratio 0.1302

Smp_002



Sem_BED-C_041



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x8,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 8500
Process time	T4
Measurement detector	First
Live time	41.16 seconds
Real time	60.00 seconds
Dead time	31.00
Count rate	43202.00 CPS

Display name: Standard data Quantification method: Result Type:
Spc_028 Standardless ZAF Oxide (Number of oxygen 24)

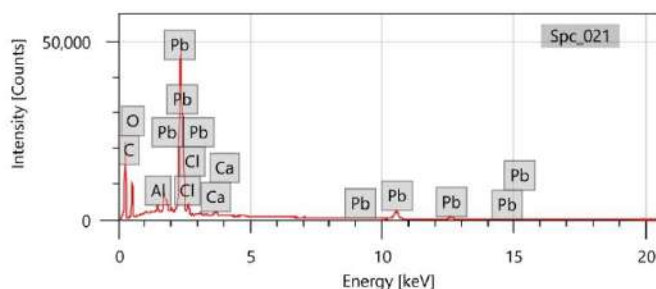
Chemical formula	Line	Mass%	Mol%	Cations
C	K	40.25±0.04	81.75±0.08	0.00
O	K			
CuO	K	48.60±0.10	14.91±0.03	19.60
ZnO	K	11.15±0.06	3.34±0.02	4.40
Total		100.00	100.00	

Spc_028 Fitting ratio 0.1955

Smp_002



Sem_BED-C_031



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x3,300
Vacuum Mode HighVacuum

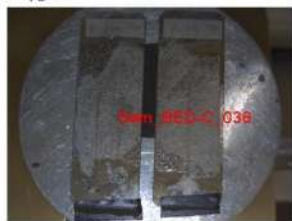
Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 3300
Process time	T4
Measurement detector	First
Live time	33.50 seconds
Real time	60.00 seconds
Dead time	44.00
Count rate	65958.00 CPS

Display name: Standard data Quantification method: Result Type:
Spc_021 Standardless ZAF Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	21.18±0.03	81.54±0.12	0.00
O	K			
Al2O3	K	1.27±0.02	0.58±0.01	1.51
Cl	K	1.02±0.02	1.33±0.02	0.00
CaO	K	1.13±0.02	0.93±0.02	1.22
PbO	M	75.40±0.12	15.62±0.02	20.51
Total		100.00	100.00	

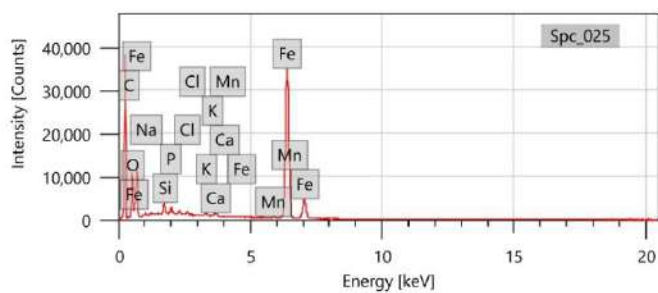
Spc_021 Fitting ratio 0.2139

Smp_002



20 mm

Sem_BED-C_036

1 μ m

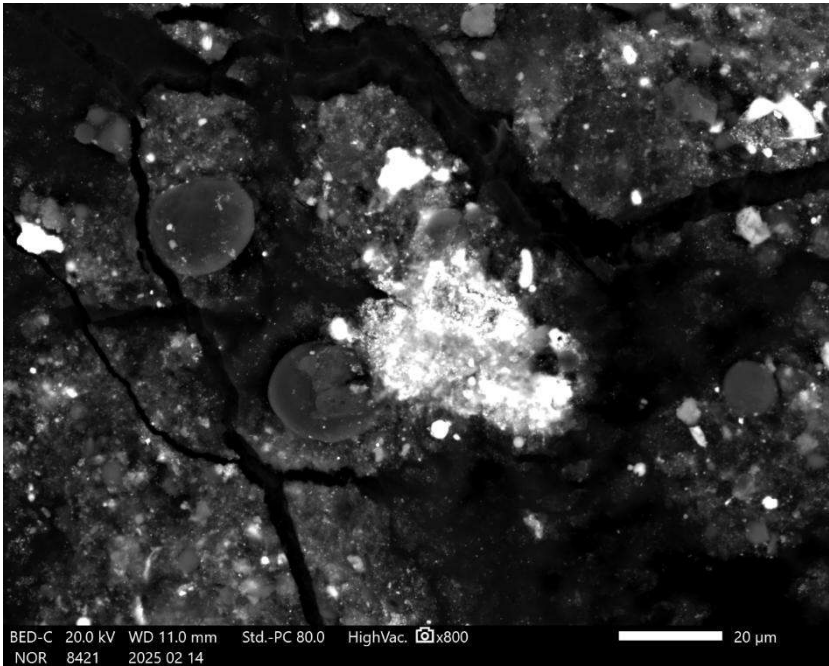
Signal BED-C
Landing Voltage 20.0 kV
WD 10.7 mm
Magnification x15,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 15000
Process time	T4
Measurement detector	First
Live time	41.94 seconds
Real time	60.00 seconds
Dead time	30.00
Count rate	40998.00 CPS

Display name: Standard data Quantification method: Result Type:
Spc_025 Standardless ZAF Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	32.77 $\pm$ 0.04	74.43 $\pm$ 0.08	0.00
O	K			
Na ₂ O	K	0.61 $\pm$ 0.02	0.27 $\pm$ 0.01	0.48
SiO ₂	K	1.68 $\pm$ 0.02	0.76 $\pm$ 0.01	0.67
P ₂ O ₅	K	1.18 $\pm$ 0.02	0.23 $\pm$ 0.00	0.40
Cl	K	0.21 $\pm$ 0.00	0.16 $\pm$ 0.00	0.00
K ₂ O	K	0.26 $\pm$ 0.01	0.07 $\pm$ 0.00	0.13
CaO	K	0.39 $\pm$ 0.01	0.19 $\pm$ 0.00	0.17
MnO	K	0.23 $\pm$ 0.01	0.09 $\pm$ 0.00	0.08
FeO	K	62.67 $\pm$ 0.09	23.80 $\pm$ 0.03	21.09
Total		100.00	100.00	
Spc_025				Fitting ratio 0.1338

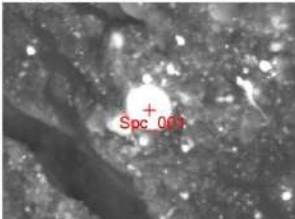
GW5 – Entry wound



Smp_001

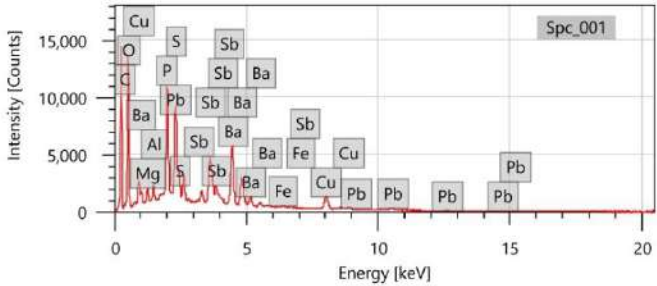


Sem_BED-C_001



Signal BED-C
Landing Voltage 20.0 kV
WD 11.0 mm
Magnification x3,300
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 3300
Process time	T4
Measurement detector	First
Live time	47.09 seconds
Real time	60.00 seconds
Dead time	21.00
Count rate	27563.00 CPS



Display name	Standard data	Quantification method	Result Type
Spc_001	Standardless	ZAF	Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	26.98±0.05	79.74±0.15	0.00
O	K			
MgO	K	1.44±0.02	1.28±0.02	0.65
Al2O3	K	1.53±0.02	0.58±0.01	0.55
P2O5	K	14.84±0.06	3.71±0.01	3.83
SO3	K	9.46±0.05	4.19±0.02	2.17
FeO	K	0.51±0.02	0.25±0.01	0.13
CuO	K	6.68±0.06	2.98±0.03	1.54
Sb2O3	L	7.79±0.06	0.95±0.01	0.98
BaO	L	20.48±0.07	4.74±0.02	2.45
PbO	M	10.29±0.08	1.64±0.01	0.85
Total		100.00	100.00	

Spc_001 Fitting ratio 0.2229

Smp_001

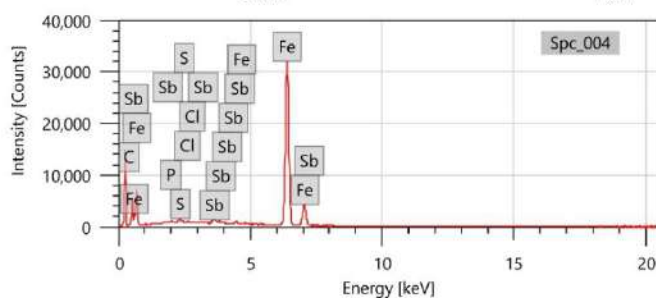


20 mm

Sem_BED-C_006



1 μm



Signal BED-C
Landing Voltage 20.0 kV
WD 11.0 mm
Magnification x11,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 11000
Process time	T4
Measurement detector	First
Live time	47.17 seconds
Real time	60.00 seconds
Dead time	21.00
Count rate	26412.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	19.01±0.03	58.69±0.11	0.00
O	K			
P2O5	K	0.46±0.01	0.12±0.00	0.14
SO3	K	0.68±0.01	0.31±0.01	0.18
Cl	K	0.18±0.01	0.19±0.01	0.00
FeO	K	78.54±0.12	40.54±0.06	22.89
Si2O3	L	1.14±0.03	0.15±0.00	0.16
Total		100.00	100.00	

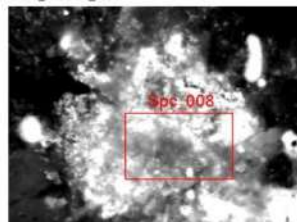
Spc_004 Fitting ratio 0.0921

Smp_001

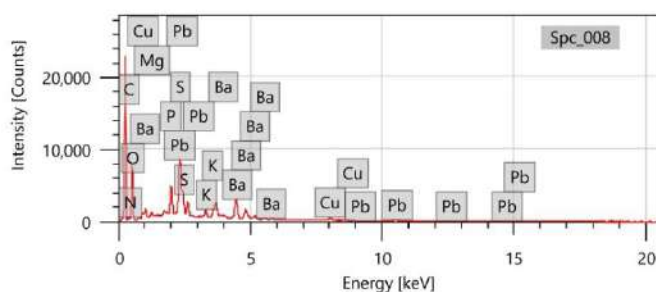


20 mm

Sem_BED-C_011



5 μm



Signal BED-C
Landing Voltage 20.0 kV
WD 11.0 mm
Magnification x2,700
Vacuum Mode HighVacuum

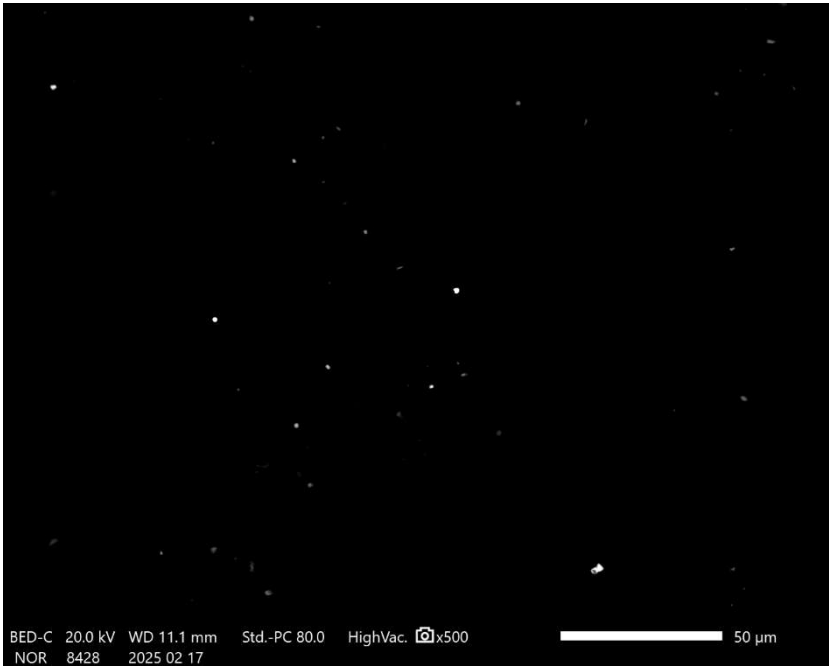
Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 2700
Process time	T4
Measurement detector	First
Live time	50.52 seconds
Real time	60.00 seconds
Dead time	16.00
Count rate	19715.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	39.43±0.06	71.56±0.10	0.00
N	K	13.29±0.12	20.68±0.19	0.00
O	K			
MgO	K	0.71±0.02	0.39±0.01	0.58
P2O5	K	6.78±0.04	1.04±0.01	3.14
SO3	K	7.28±0.05	1.98±0.01	2.99
K2O	K	1.31±0.02	0.30±0.00	0.91
CuO	K	2.46±0.04	0.67±0.01	1.02
BaO	L	12.76±0.07	1.81±0.01	2.74
PbO	M	15.98±0.09	1.56±0.01	2.36
Total		100.00	100.00	

Spc_008 Fitting ratio 0.3506

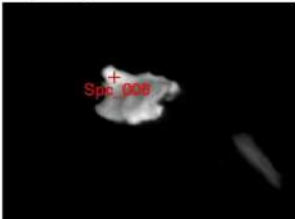
GW5 – Exit wound



Smp_001

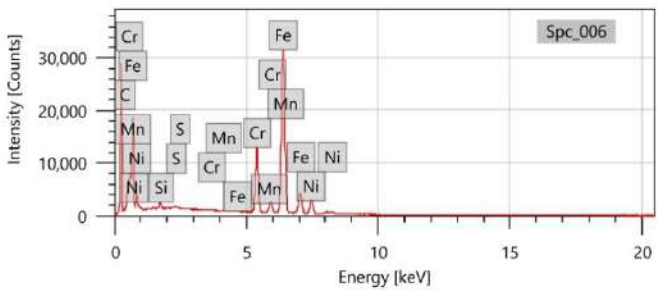


Sem_BED-C_010



Signal BED-C
Landing Voltage 20.0 kV
WD 10.9 mm
Magnification x4,300
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 4300
Process time	T4
Measurement detector	First
Live time	37.80 seconds
Real time	60.00 seconds
Dead time	37.00
Count rate	52819.00 CPS



Display name	Standard data	Quantification method	Result Type
Spc_006	Standardless	ZAF	Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	23.72±0.03	67.46±0.09	0.00
O	K			
SiO2	K	0.65±0.01	0.37±0.01	0.22
SO3	K	0.31±0.01	0.13±0.00	0.08
Cr2O3	K	14.56±0.04	3.27±0.01	3.95
MnO	K	1.05±0.02	0.51±0.01	0.31
FeO	K	53.15±0.08	25.27±0.04	15.27
NiO	K	6.55±0.04	3.00±0.02	1.81
Total		100.00	100.00	

Spc_006 Fitting ratio 0.0974

Smp_001

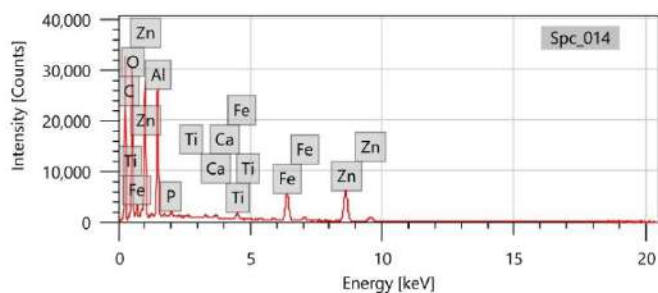


20 mm

Sem_BED-C_022



1 μm



Signal BED-C
Landing Voltage 20.0 kV
WD 11.0 mm
Magnification x10,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 10000
Process time	T4
Measurement detector	First
Live time	43.45 seconds
Real time	60.00 seconds
Dead time	27.00
Count rate	38029.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	41.77±0.05	83.55±0.10	0.00
Al ₂ O ₃	K	17.90±0.05	4.22±0.01	7.74
P ₂ O ₅	K	1.14±0.02	0.19±0.00	0.35
CaO	K	0.47±0.01	0.20±0.00	0.19
TiO ₂	K	1.60±0.02	0.48±0.00	0.44
FeO	K	10.10±0.04	3.38±0.01	3.10
ZnO	K	27.02±0.09	7.98±0.03	7.33
Total		100.00	100.00	

Spc_014 Fitting ratio 0.1872

Smp_001

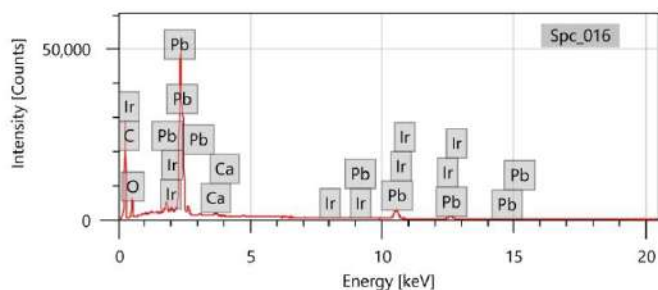


20 mm

Sem_BED-C_025



1 μm



Signal BED-C
Landing Voltage 20.0 kV
WD 11.0 mm
Magnification x11,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 11000
Process time	T4
Measurement detector	First
Live time	33.42 seconds
Real time	60.00 seconds
Dead time	44.00
Count rate	66718.00 CPS

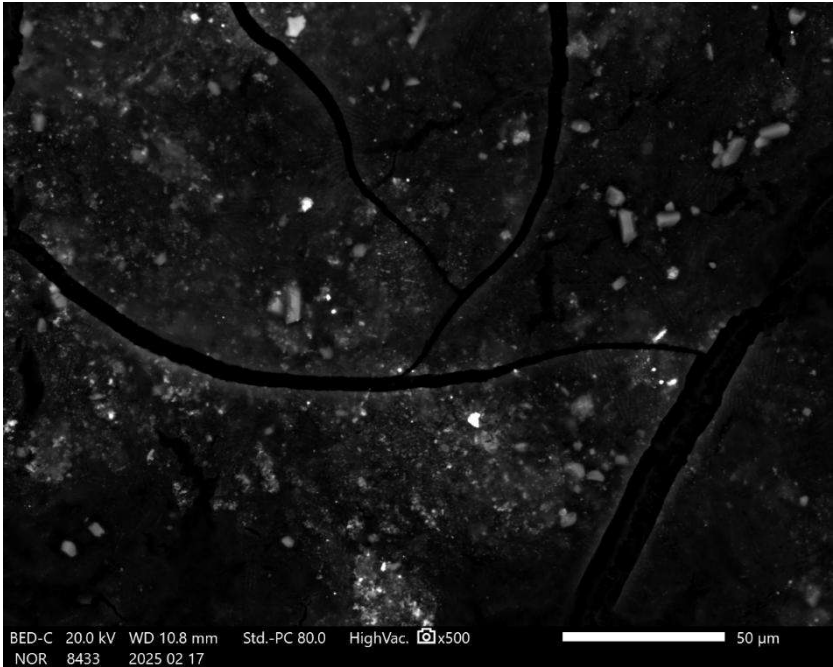
Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	23.59±0.03	84.92±0.11	0.00
O	K			
CaO	K	0.90±0.02	0.69±0.01	1.06
Ir ₂ O ₃	M	2.58±0.04	0.26±0.00	0.79
PbO	M	72.93±0.11	14.13±0.02	21.74
Total		100.00	100.00	

Spc_016 Fitting ratio 0.2355

Anterior lesions (control piglet/after burial)

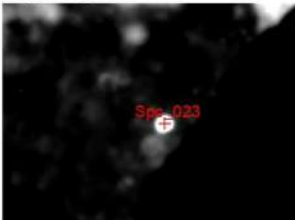
GW0 – Entry wound



Smp_002

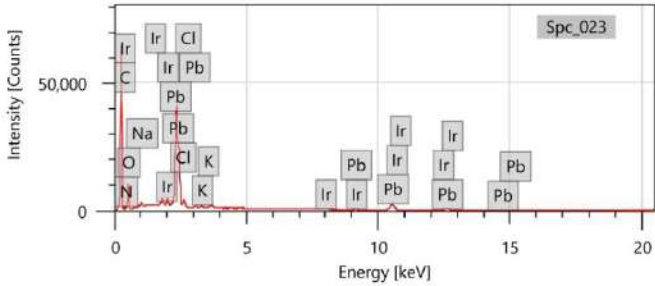


Sem_BED-C_036



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x9,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 9000
Process time	T4
Measurement detector	First
Live time	32.06 seconds
Real time	60.00 seconds
Dead time	46.00
Count rate	72333.00 CPS



Display name	Standard data	Quantification method	Result Type
Spc_023	Standardless	ZAF	Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	36.85±0.03	67.74±0.06	0.00
N	K	17.27±0.08	27.23±0.13	0.00
O	K			
Na2O	K	0.72±0.01	0.25±0.00	2.50
Cl	K	0.69±0.01	0.43±0.01	0.00
K2O	K	0.55±0.01	0.13±0.00	1.27
Ir2O3	M	2.86±0.03	0.15±0.00	1.43
PbO	M	41.06±0.07	4.06±0.01	19.96
Total		100.00	100.00	

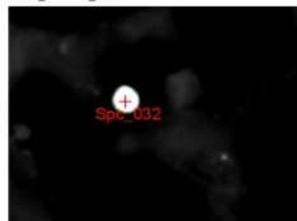
Spc_023 Fitting ratio 0.3486

Smp_002

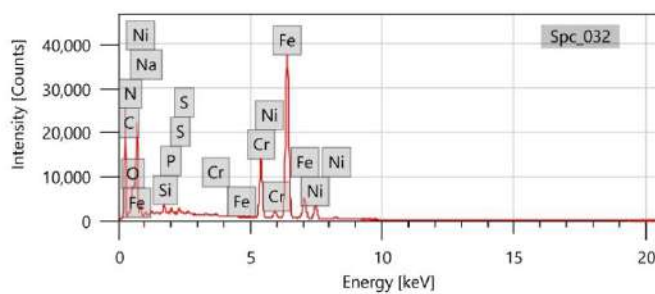


20 mm

Sem_BED-C_047



2 μm



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x7,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 7000
Process time	T4
Measurement detector	First
Live time	32.83 seconds
Real time	60.00 seconds
Dead time	45.00
Count rate	68754.00 CPS

Display name	Standard data	Quantification method	Result Type
Spc_032	Standardless	ZAF	Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	19.57±0.03	56.79±0.08	0.00
N	K	3.87±0.03	9.64±0.07	0.00
O	K			
Na2O	K	0.70±0.01	0.39±0.01	0.45
SiO2	K	0.98±0.01	0.57±0.01	0.33
P2O5	K	0.87±0.01	0.21±0.00	0.25
SO3	K	0.61±0.01	0.27±0.00	0.15
Cr2O3	K	13.10±0.03	3.01±0.01	3.47
FeO	K	53.54±0.08	25.97±0.04	15.01
NiO	K	6.76±0.03	3.15±0.02	1.82
Total		100.00	100.00	
Spc_032				Fitting ratio 0.0689

Smp_002

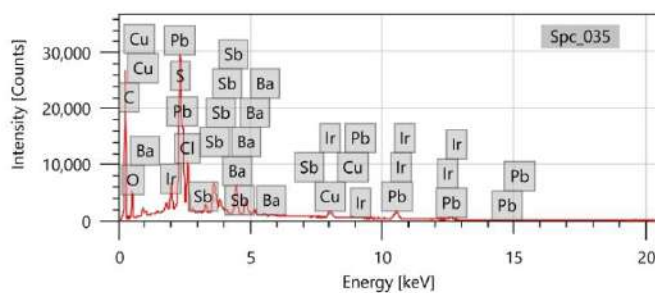


20 mm

Sem_BED-C_051



20 μm



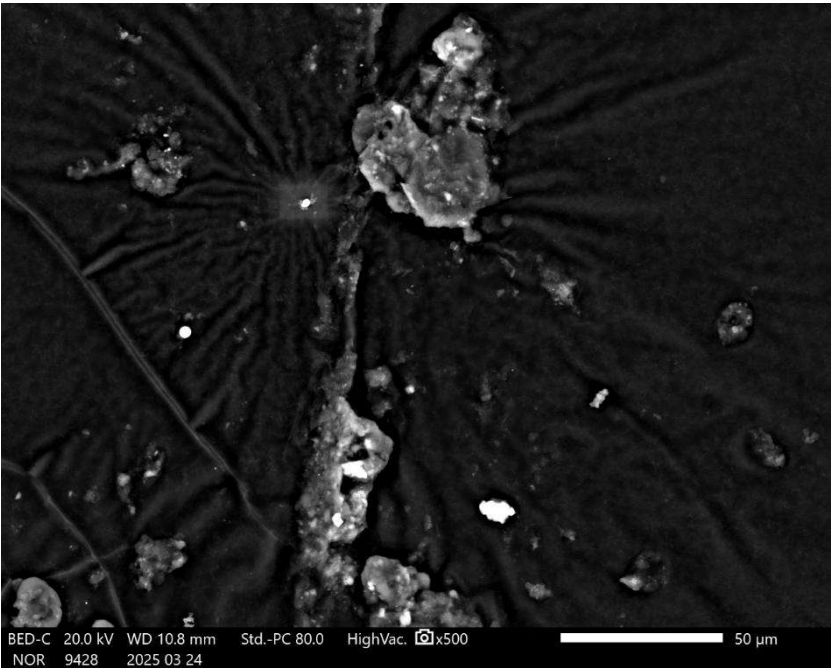
Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x950
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 950
Process time	T4
Measurement detector	First
Live time	33.55 seconds
Real time	60.00 seconds
Dead time	43.00
Count rate	65867.00 CPS

Display name	Standard data	Quantification method	Result Type
Spc_035	Standardless	ZAF	Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	26.72±0.04	81.21±0.11	0.00
O	K			
SO3	K	7.01±0.04	3.20±0.02	3.18
Cl	K	4.03±0.02	4.15±0.02	0.00
CuO	K	3.93±0.04	1.80±0.02	1.80
Sb2O3	L	7.99±0.05	1.00±0.01	1.99
BaO	L	12.26±0.05	2.92±0.01	2.91
Ir2O3	M	6.32±0.04	0.53±0.00	1.06
PbO	M	31.74±0.08	5.19±0.01	5.17
Total		100.00	100.00	
Spc_035				Fitting ratio 0.2444

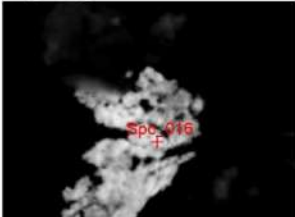
GW0 – Exit wound



Smp_001

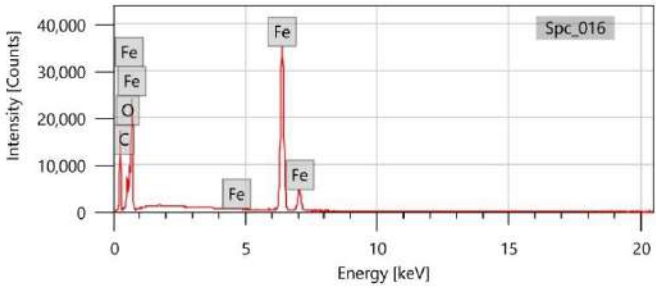


Sem_BED-C_031



Signal BED-C
Landing Voltage 20.0 kV
WD 10.7 mm
Magnification x3,300
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 3300
Process time	T4
Measurement detector	First
Live time	43.12 seconds
Real time	60.00 seconds
Dead time	28.00
Count rate	37295.00 CPS

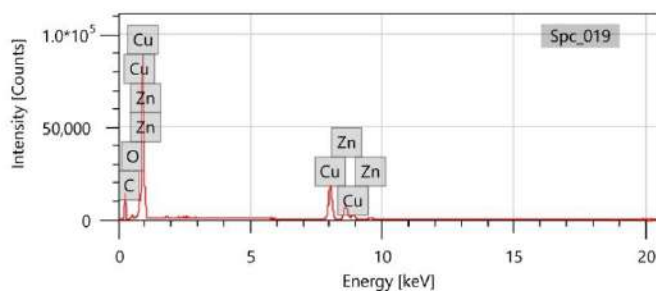


Display name	Standard data	Quantification method	Result Type	
Spc_016	Standardless	ZAF	Oxide (Number of oxygen 24)	
Chemical formula	Line	Mass%	Mol%	Cations
C	K	20.26±0.03	60.32±0.10	0.00
O	K			
FeO	K	79.74±0.11	39.68±0.06	24.00
Total		100.00	100.00	
Spc_016			Fitting ratio 0.0656	

Smp_001



Sem_BED-C_035



Signal BED-C
Landing Voltage 20.0 kV
WD 10.7 mm
Magnification x3,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 3000
Process time	T4
Measurement detector	First
Live time	33.49 seconds
Real time	60.00 seconds
Dead time	44.00
Count rate	66905.00 CPS

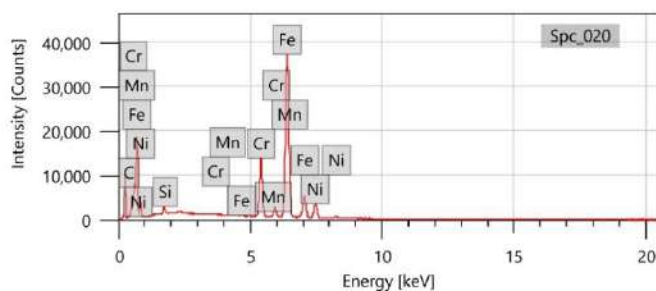
Display name: Standard data Quantification method: Result Type:
Spc_019 Standardless ZAF Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	17.94±0.03	59.32±0.11	0.00
O	K			
CuO	K	54.60±0.11	27.27±0.05	16.09
ZnO	K	27.47±0.09	13.41±0.04	7.91
Total		100.00	100.00	
Spc_019				Fitting ratio 0.0664

Smp_001



Sem_BED-C_037



Signal BED-C
Landing Voltage 20.0 kV
WD 10.7 mm
Magnification x9,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 9500
Process time	T4
Measurement detector	First
Live time	36.66 seconds
Real time	60.00 seconds
Dead time	39.00
Count rate	55506.00 CPS

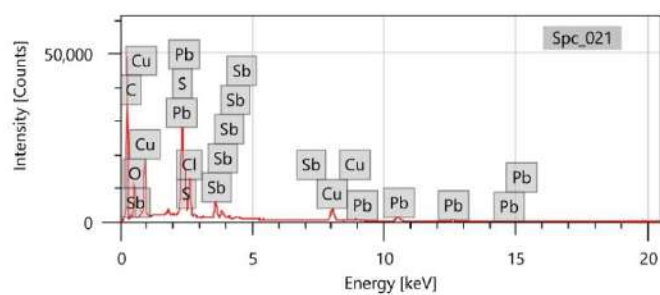
Display name: Standard data Quantification method: Result Type:
Spc_020 Standardless ZAF Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	9.79±0.02	41.75±0.09	0.00
O	K			
SiO2	K	1.00±0.02	0.86±0.01	0.29
Cr2O3	K	15.89±0.04	5.35±0.01	3.68
MnO	K	0.43±0.02	0.31±0.01	0.11
FeO	K	64.29±0.09	45.83±0.06	15.76
NiO	K	8.60±0.04	5.89±0.03	2.03
Total		100.00	100.00	
Spc_020				Fitting ratio 0.0290

Smp_001



Sem_BED-C_038



Signal BED-C
Landing Voltage 20.0 kV
WD 10.7 mm
Magnification x9,500
Vacuum Mode HighVacuum

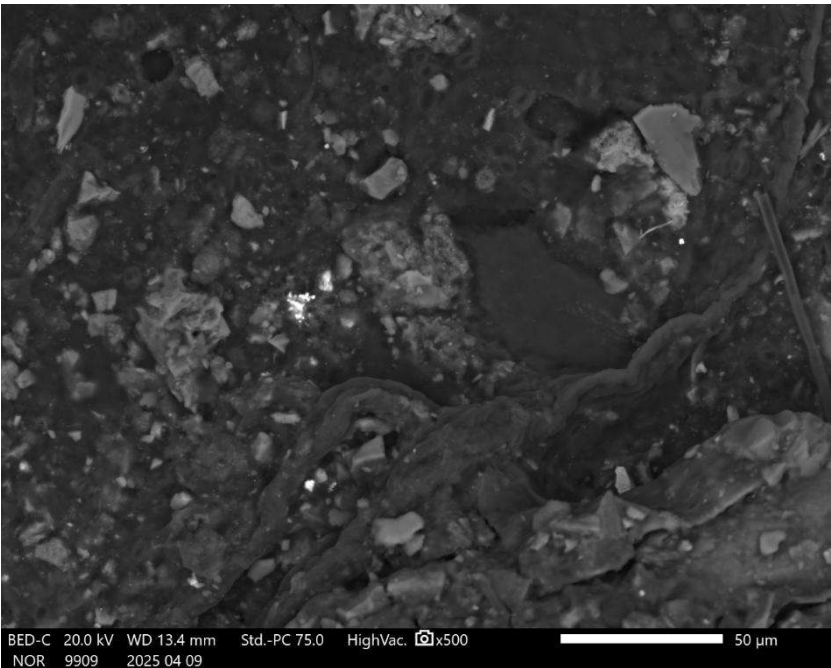
Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 9500
Process time	T4
Measurement detector	First
Live time	33.16 seconds
Real time	60.00 seconds
Dead time	44.00
Count rate	68986.00 CPS

Display name: Standard data Quantification method: Result Type:
Spc_021 Standardless ZAF Oxide (Number of oxygen 24)

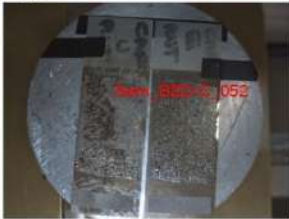
Chemical formula	Line	Mass%	Mol%	Cations
C	K	45.02±0.05	88.84±0.09	0.00
O	K			
SO3	K	5.24±0.03	1.55±0.01	2.95
Cl	K	4.53±0.02	3.03±0.01	0.00
CuO	K	10.35±0.05	3.08±0.02	5.87
Sb2O3	L	8.50±0.04	0.69±0.00	2.63
PbO	M	26.35±0.07	2.80±0.01	5.33
Total		100.00	100.00	

Spc_021 Fitting ratio 0.2762

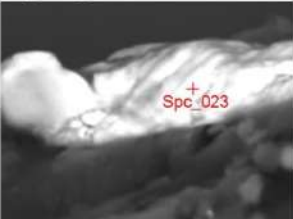
GW1 – Entry wound



Smp_001

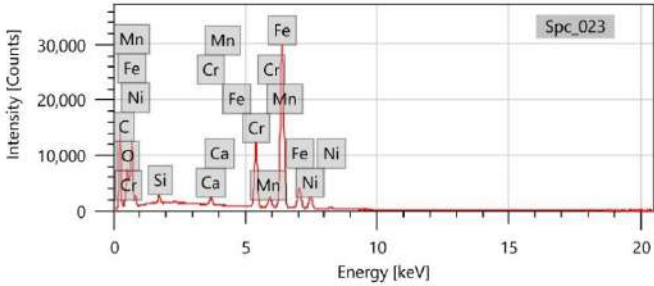


Sem_BED-C_052



Signal BED-C
Landing Voltage 20.0 kV
WD 13.4 mm
Magnification x5,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 5500
Process time	T1
Measurement detector	First
Live time	55.49 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	34914.00 CPS



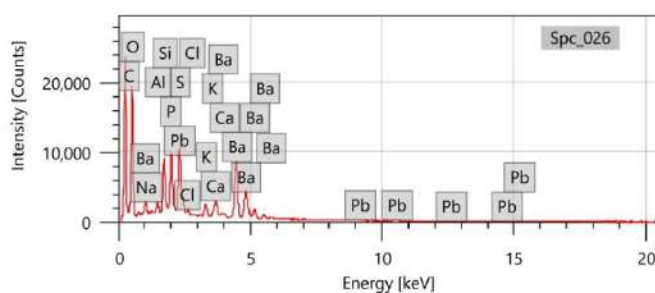
Display name	Standard data	Quantification method	Result Type
Spc_023	Standardless	ZAF	Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	14.47±0.03	52.82±0.10	0.00
O	K			
SiO2	K	0.92±0.02	0.67±0.01	0.28
CaO	K	0.92±0.01	0.72±0.01	0.30
Cr2O3	K	15.96±0.05	4.61±0.01	3.87
MnO	K	0.98±0.02	0.61±0.01	0.26
FeO	K	59.90±0.10	36.56±0.06	15.37
NiO	K	6.84±0.04	4.01±0.02	1.69
Total		100.00	100.00	
Spc_023				Fitting ratio 0.0585

Smp_001



Sem_BED-C_059



Signal BED-C
Landing Voltage 20.0 kV
WD 13.0 mm
Magnification x5,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 5000
Process time	T1
Measurement detector	First
Live time	56.17 seconds
Real time	60.00 seconds
Dead time	6.00
Count rate	29594.00 CPS

Display name: Standard data Quantification method: Result Type:
Spc_026 Standardless ZAF Oxide (Number of oxygen 24)

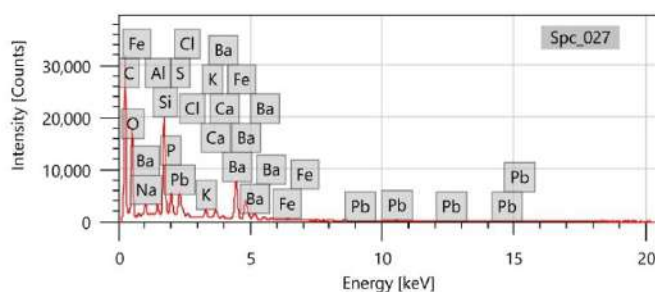
Chemical formula	Line	Mass%	Mol%	Cations
C	K	33.03±0.05	81.69±0.13	0.00
O	K			
Na2O	K	1.56±0.02	0.75±0.01	0.94
Al2O3	K	1.36±0.02	0.40±0.01	0.50
SiO2	K	7.13±0.04	3.52±0.02	2.22
P2O5	K	10.59±0.05	2.22±0.01	2.79
SO3	K	9.40±0.04	3.49±0.02	2.19
Cl	K	0.39±0.01	0.39±0.01	0.00
K2O	K	1.50±0.02	0.47±0.00	0.59
CaO	K	2.10±0.02	1.11±0.01	0.70
BaO	L	27.08±0.08	5.23±0.01	3.90
PbO	M	5.87±0.07	0.78±0.01	0.49
Total		100.00	100.00	

Spc_026 Fitting ratio 0.2197

Smp_001



Sem_BED-C_060



Signal BED-C
Landing Voltage 20.0 kV
WD 13.0 mm
Magnification x8,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 8500
Process time	T1
Measurement detector	First
Live time	56.27 seconds
Real time	60.00 seconds
Dead time	6.00
Count rate	29080.00 CPS

Display name: Standard data Quantification method: Result Type:
Spc_027 Standardless ZAF Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	39.34±0.06	83.64±0.12	0.00
O	K			
Na2O	K	1.90±0.02	0.78±0.01	1.23
Al2O3	K	1.65±0.02	0.41±0.01	0.65
SiO2	K	16.61±0.06	7.06±0.02	5.54
P2O5	K	5.67±0.04	1.02±0.01	1.60
SO3	K	3.96±0.03	1.26±0.01	0.99
Cl	K	0.36±0.01	0.26±0.01	0.00
K2O	K	1.22±0.01	0.33±0.00	0.52
CaO	K	1.49±0.02	0.68±0.01	0.53
FeO	K	0.57±0.02	0.20±0.01	0.16
BaO	L	23.78±0.07	3.96±0.01	3.11
PbO	M	3.45±0.05	0.39±0.01	0.31
Total		100.00	100.00	

Spc_027 Fitting ratio 0.3725

Smp_001

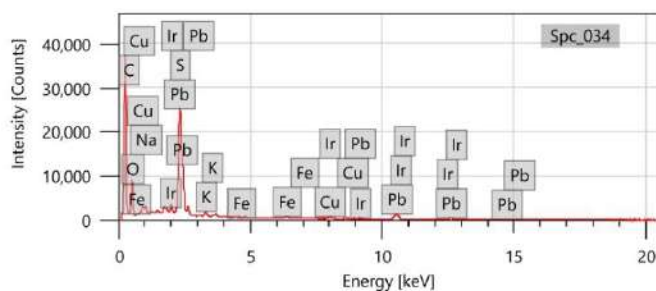


20 mm

Sem_BED-C_068



2 μm



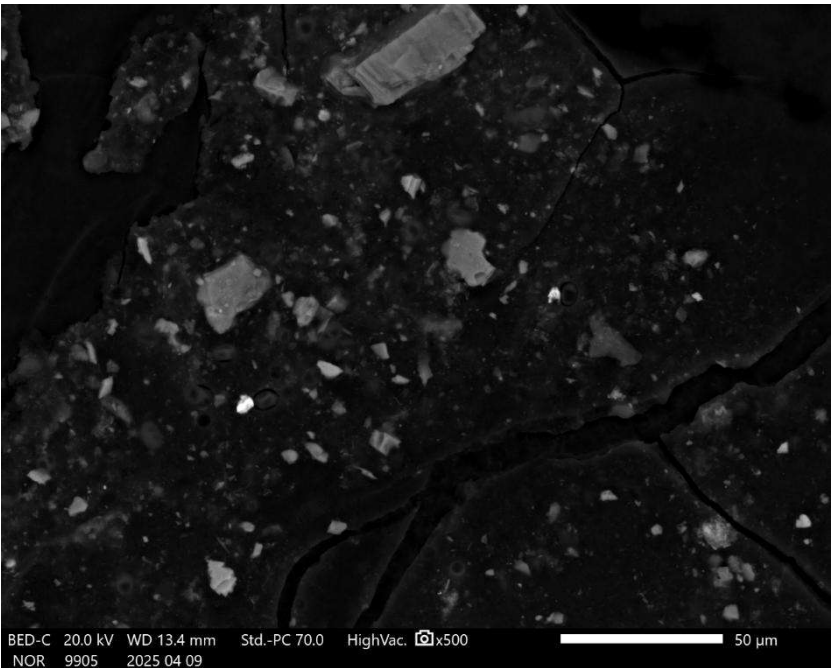
Signal BED-C
Landing Voltage 20.0 kV
WD 13.3 mm
Magnification x9,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 9500
Process time	T1
Measurement detector	First
Live time	55.64 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	30730.00 CPS

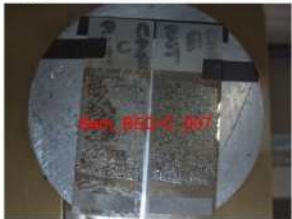
Display name: Standard data Quantification method: Result Type:
Spc_034 Standardless ZAF Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	45.59±0.06	90.58±0.12	0.00
O	K			
Na2O	K	1.09±0.02	0.42±0.01	1.09
SO3	K	14.34±0.06	4.27±0.02	5.57
K2O	K	1.00±0.02	0.25±0.00	0.66
FeO	K	1.06±0.02	0.35±0.01	0.46
CuO	K	1.99±0.04	0.60±0.01	0.78
Ir2O3	M	4.10±0.04	0.23±0.00	0.59
PbO	M	30.84±0.11	3.30±0.01	4.30
Total		100.00	100.00	
Spc_034				Fitting ratio 0.3931

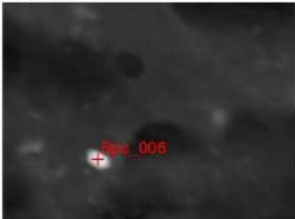
GW1 – Exit wound



Smp_001

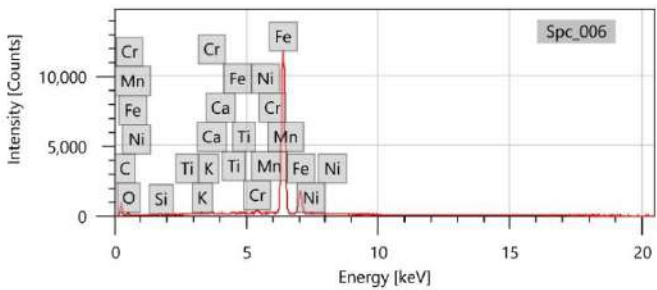


Sem_BED-C_007



Signal BED-C
Landing Voltage 20.0 kV
WD 13.5 mm
Magnification x3,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 3500
Process time	T1
Measurement detector	First
Live time	57.33 seconds
Real time	60.00 seconds
Dead time	4.00
Count rate	6601.00 CPS

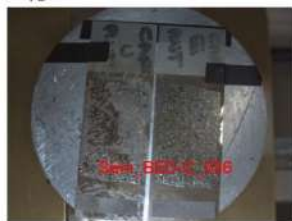


Display name	Standard data	Quantification method	Result Type
Spc_006	Standardless	ZAF	Oxide (Number of oxygen 24)

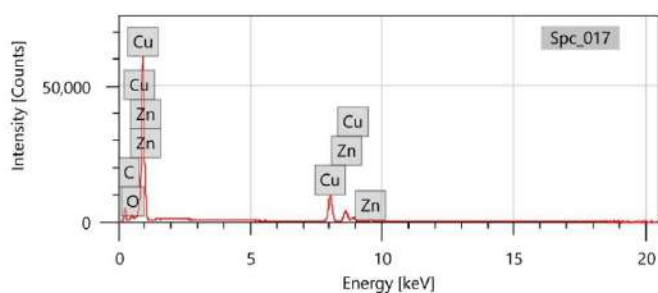
Chemical formula	Line	Mass%	Mol%	Cations
C	K	3.90±0.03	19.64±0.15	0.00
O	K			
SiO2	K	0.26±0.02	0.26±0.02	0.08
K2O	K	0.18±0.01	0.12±0.01	0.07
CaO	K	0.19±0.01	0.20±0.02	0.06
TiO2	K	0.24±0.02	0.18±0.01	0.05
Cr2O3	K	1.18±0.03	0.47±0.01	0.27
MnO	K	0.42±0.03	0.36±0.03	0.11
FeO	K	92.89±0.23	78.18±0.20	22.95
NiO	K	0.74±0.04	0.60±0.03	0.18
Total		100.00	100.00	

Spc_006 Fitting ratio 0.0194

Smp_001



Sem_BED-C_036



Signal BED-C
Landing Voltage 20.0 kV
WD 13.4 mm
Magnification x4,000
Vacuum Mode HighVacuum

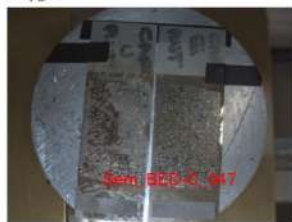
Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 4000
Process time	T1
Measurement detector	First
Live time	55.98 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	29913.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

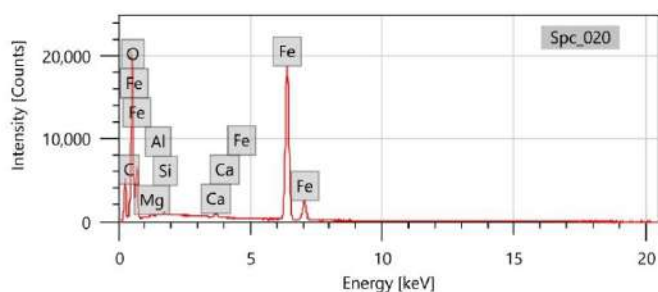
Chemical formula	Line	Mass%	Mol%	Cations
C	K	12.10±0.04	47.86±0.16	0.00
O	K			
CuO	K	58.94±0.17	35.22±0.10	16.21
ZnO	K	28.97±0.13	16.92±0.08	7.79
Total		100.00	100.00	

Spc_017 Fitting ratio 0.0758

Smp_001



Sem_BED-C_047



Signal BED-C
Landing Voltage 20.0 kV
WD 13.4 mm
Magnification x1,900
Vacuum Mode HighVacuum

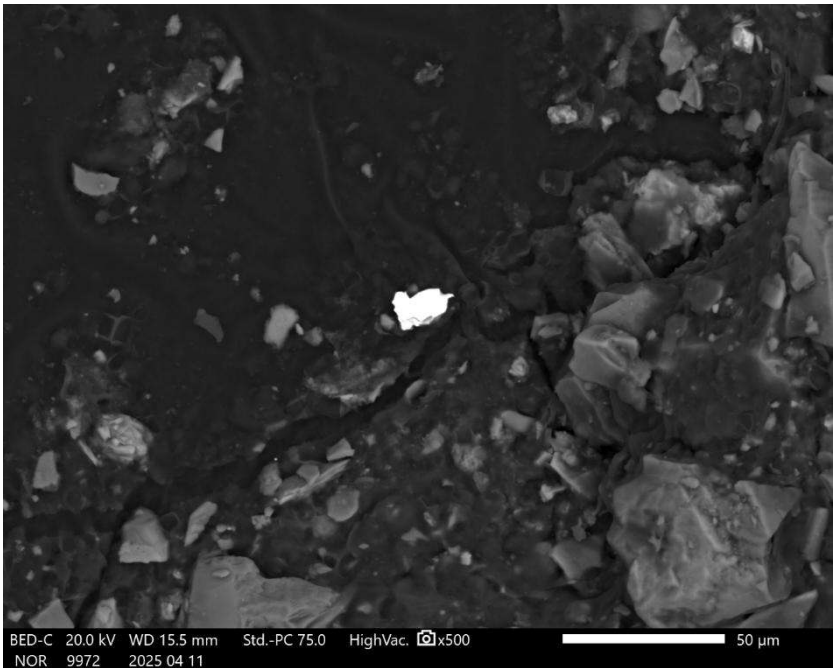
Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 1900
Process time	T1
Measurement detector	First
Live time	57.21 seconds
Real time	60.00 seconds
Dead time	5.00
Count rate	18431.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	12.79±0.04	46.56±0.15	0.00
O	K			
MgO	K	0.45±0.03	0.49±0.03	0.22
Al2O3	K	0.28±0.03	0.12±0.01	0.11
SiO2	K	0.46±0.03	0.34±0.02	0.15
CaO	K	0.72±0.02	0.56±0.01	0.25
FeO	K	85.30±0.17	51.93±0.10	23.08
Total		100.00	100.00	

Spc_020 Fitting ratio 0.0737

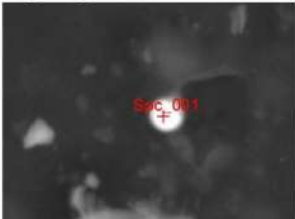
GW2 – Entry wound



Smp_001

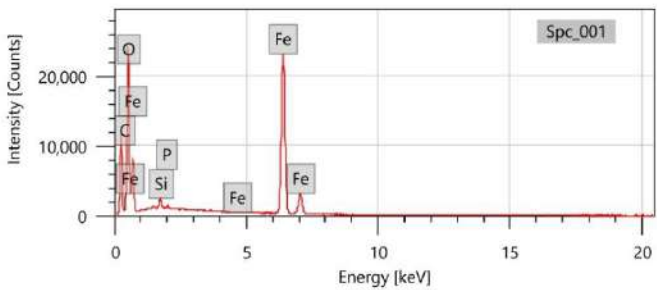


Sem_BED-C_002



Signal BED-C
Landing Voltage 20.0 kV
WD 15.6 mm
Magnification x5,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 5500
Process time	T1
Measurement detector	First
Live time	56.72 seconds
Real time	60.00 seconds
Dead time	5.00
Count rate	23718.00 CPS



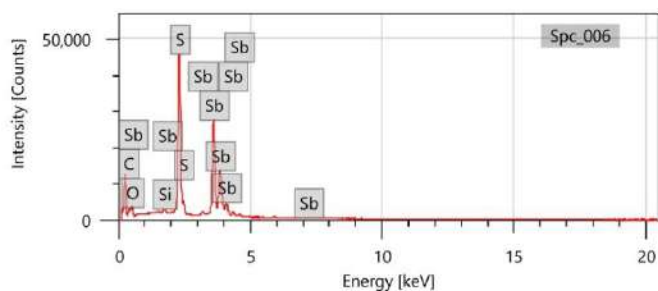
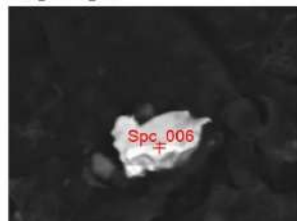
Display name	Standard data	Quantification method	Result Type
Spc_001	Standardless	ZAF	Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	20.27±0.04	60.34±0.13	0.00
O	K			
SiO2	K	1.67±0.03	1.00±0.02	0.58
P2O5	K	0.71±0.02	0.18±0.01	0.21
FeO	K	77.34±0.14	38.48±0.07	22.32
Total		100.00	100.00	
Spc_001				Fitting ratio 0.0957

Smp_001



Sem_BED-C_009



Signal BED-C
Landing Voltage 20.0 kV
WD 15.6 mm
Magnification x2,300
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 2300
Process time	T1
Measurement detector	First
Live time	54.97 seconds
Real time	60.00 seconds
Dead time	8.00
Count rate	41767.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

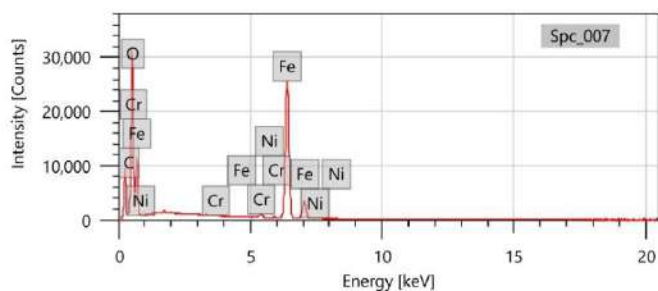
Chemical formula	Line	Mass%	Mol%	Cations
C	K	14.32±0.03	65.17±0.14	0.00
O	K			
SiO2	K	0.64±0.02	0.58±0.02	0.13
SiO3	K	36.95±0.06	25.23±0.04	5.83
Si2O3	L	48.09±0.09	9.02±0.02	4.17
Total		100.00	100.00	

Spc_006 Fitting ratio 0.0741

Smp_001



Sem_BED-C_011



Signal BED-C
Landing Voltage 20.0 kV
WD 15.6 mm
Magnification x1,700
Vacuum Mode HighVacuum

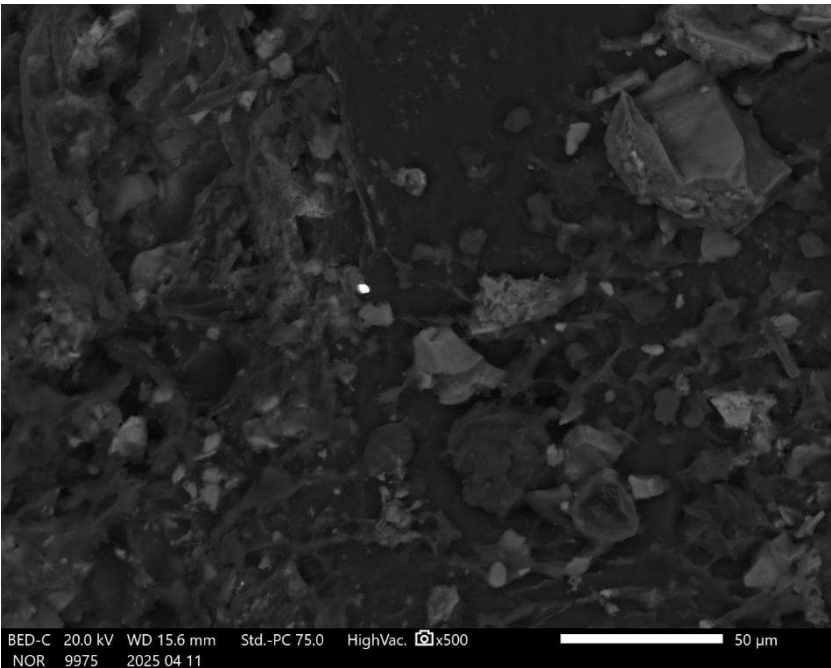
Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 1700
Process time	T1
Measurement detector	First
Live time	56.40 seconds
Real time	60.00 seconds
Dead time	6.00
Count rate	27633.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

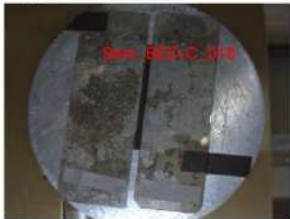
Chemical formula	Line	Mass%	Mol%	Cations
C	K	14.75±0.04	51.05±0.12	0.00
O	K			
Cr2O3	K	1.28±0.02	0.35±0.01	0.34
FeO	K	83.44±0.14	48.30±0.08	23.35
NiO	K	0.53±0.02	0.30±0.01	0.14
Total		100.00	100.00	

Spc_007 Fitting ratio 0.0786

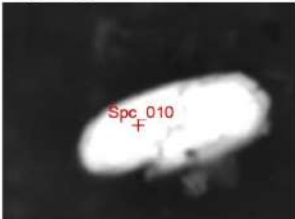
GW2 – Exit wound



Smp_001

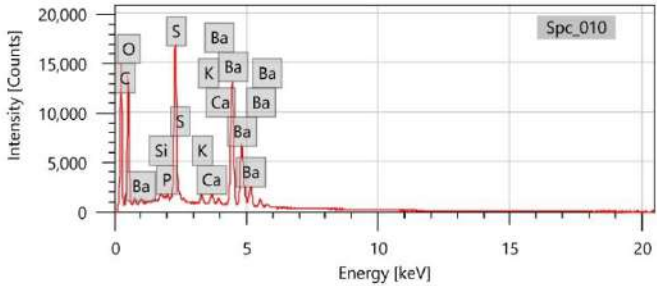


Sem_BED-C_018



Signal BED-C
Landing Voltage 20.0 kV
WD 15.5 mm
Magnification x8,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 8500
Process time	T1
Measurement detector	First
Live time	56.35 seconds
Real time	60.00 seconds
Dead time	6.00
Count rate	25724.00 CPS



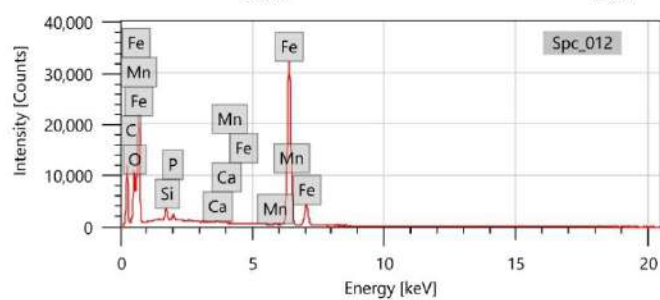
Display name	Standard data	Quantification method	Result Type
Spc_010	Standardless	ZAF	Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	23.57±0.05	74.81±0.14	0.00
O	K			
SiO2	K	0.24±0.02	0.15±0.02	0.07
P2O5	K	1.06±0.02	0.28±0.01	0.28
SO3	K	23.79±0.07	11.33±0.03	5.53
K2O	K	0.85±0.01	0.34±0.01	0.34
CaO	K	1.23±0.02	0.84±0.01	0.41
BaO	L	49.26±0.11	12.25±0.03	5.98
Total		100.00	100.00	
Spc_010				Fitting ratio 0.1405

Smp_001



Sem_BED-C_023



Signal BED-C
Landing Voltage 20.0 kV
WD 15.7 mm
Magnification x9,500
Vacuum Mode HighVacuum

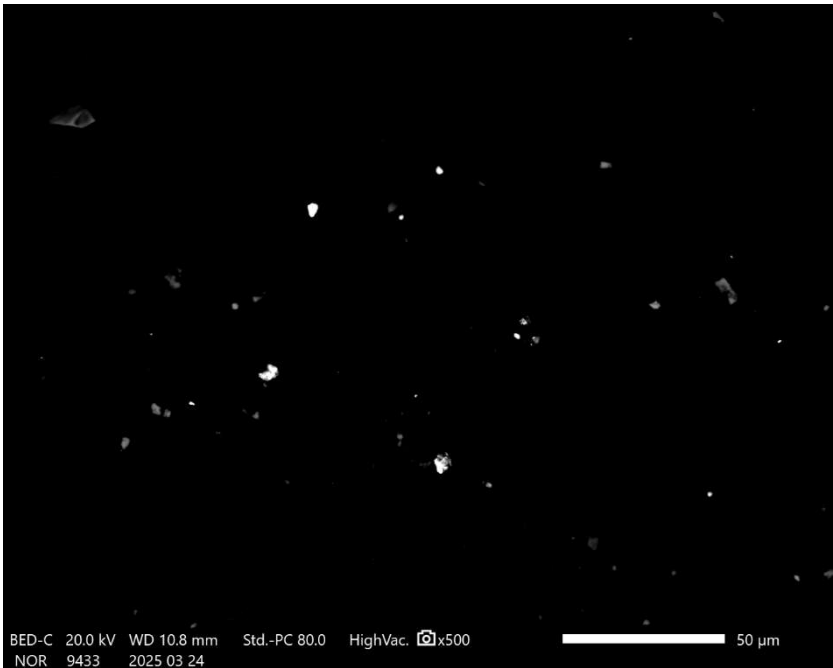
Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 9500
Process time	T1
Measurement detector	First
Live time	55.95 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	30522.00 CPS

Display name: Standard data Quantification method: Result Type:
Spc_012 Standardless ZAF Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	15.35 ± 0.03	52.05 ± 0.11	0.00
O	K			
SiO2	K	2.04 ± 0.03	1.39 ± 0.02	0.66
P2O5	K	1.20 ± 0.02	0.34 ± 0.01	0.33
CaO	K	0.30 ± 0.01	0.22 ± 0.01	0.10
MnO	K	0.39 ± 0.02	0.23 ± 0.01	0.11
FeO	K	80.72 ± 0.12	45.77 ± 0.07	21.66
Total		100.00	100.00	

Spc_012 Fitting ratio 0.0608

GW4 – Entry wound



Smp_001

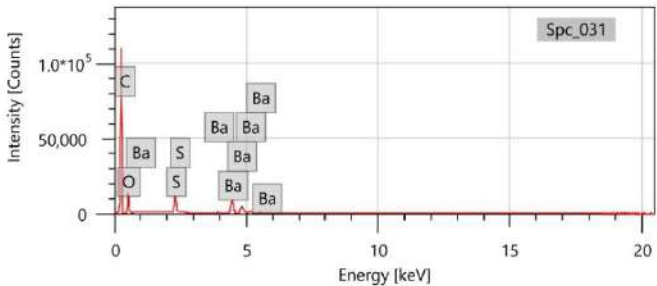


Sem_BED-C_057



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x14,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 14000
Process time	T4
Measurement detector	First
Live time	43.88 seconds
Real time	60.00 seconds
Dead time	27.00
Count rate	37008.00 CPS



Display name	Standard data	Quantification method	Result Type
Spc_031	Standardless	ZAF	Oxide (Number of oxygen 24)

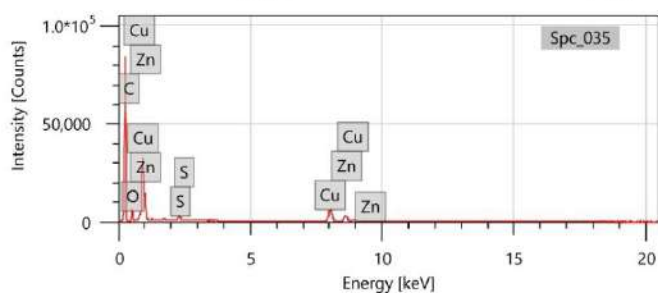
Chemical formula	Line	Mass%	Mol%	Cations
C	K	68.67±0.05	95.58±0.07	0.00
O	K			
SO3	K	10.06±0.03	2.10±0.01	5.85
BaO	L	21.27±0.06	2.32±0.01	6.46
Total		100.00	100.00	

Spc_031 Fitting ratio 0.4172

Smp_001



Sem_BED-C_063



Signal BED-C
Landing Voltage 20.0 kV
WD 10.7 mm
Magnification x8,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 8500
Process time	T4
Measurement detector	First
Live time	43.70 seconds
Real time	60.00 seconds
Dead time	27.00
Count rate	37426.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

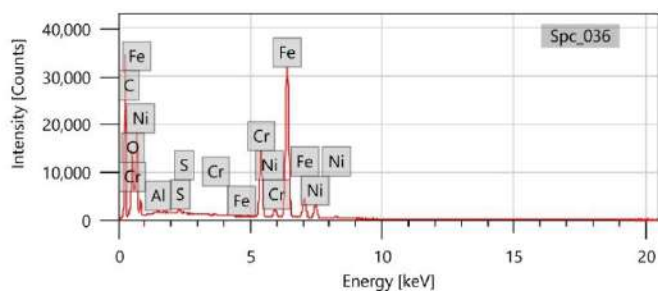
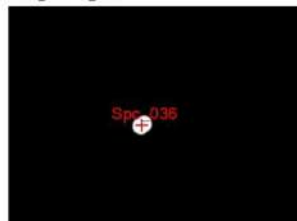
Chemical formula	Line	Mass%	Mol%	Cations
C	K	62.97±0.05	91.91±0.07	0.00
O	K			
SO3	K	1.80±0.02	0.39±0.00	1.06
CuO	K	21.88±0.07	4.82±0.02	13.03
ZnO	K	13.36±0.07	2.88±0.01	7.78
Total		100.00	100.00	

Spc_035 Fitting ratio 0.3819

Smp_001



Sem_BED-C_064



Signal BED-C
Landing Voltage 20.0 kV
WD 10.7 mm
Magnification x5,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 5500
Process time	T4
Measurement detector	First
Live time	36.28 seconds
Real time	60.00 seconds
Dead time	39.00
Count rate	56954.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	26.62±0.03	71.03±0.08	0.00
O	K			
Al2O3	K	0.38±0.01	0.12±0.00	0.16
SO3	K	0.51±0.01	0.20±0.00	0.14
Cr2O3	K	15.25±0.04	3.22±0.01	4.28
FeO	K	50.62±0.08	22.59±0.03	15.04
NiO	K	6.62±0.04	2.84±0.02	1.89
Total		100.00	100.00	

Spc_036 Fitting ratio 0.1031

Smp_001

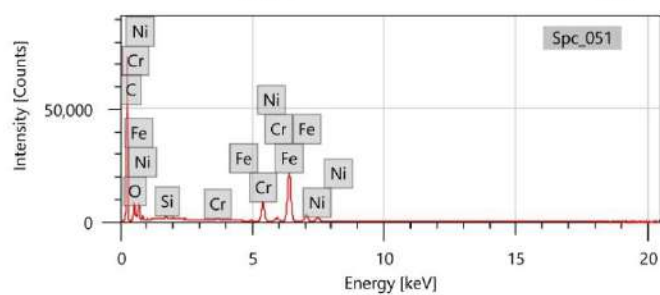


20 mm

Sem_BED-C_083



1 μm



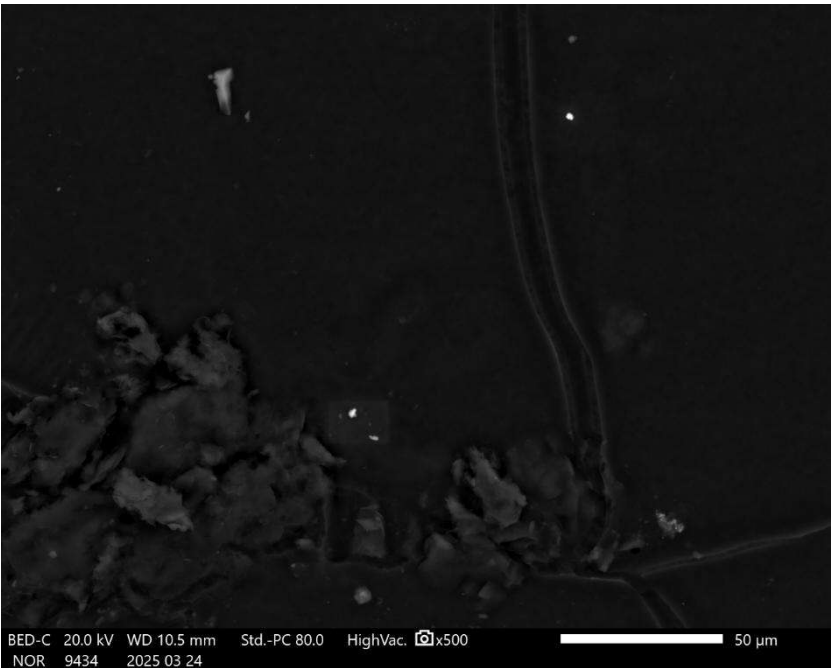
Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x14,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 14000
Process time	T4
Measurement detector	First
Live time	41.81 seconds
Real time	60.00 seconds
Dead time	30.00
Count rate	41652.00 CPS

Display name: Standard data Quantification method: Result Type:
Spc_051 Standardless ZAF Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	46.81±0.04	85.41±0.07	0.00
O	K			
SiO2	K	0.69±0.01	0.25±0.00	0.34
Cr2O3	K	10.07±0.03	1.45±0.00	3.93
FeO	K	37.82±0.07	11.53±0.02	15.60
NiO	K	4.61±0.03	1.35±0.01	1.83
Total		100.00	100.00	
Spc_051				Fitting ratio 0.2497

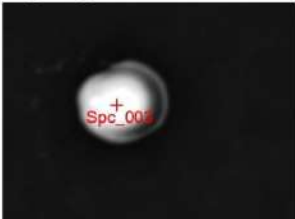
GW4 – Exit wound



Smp_002

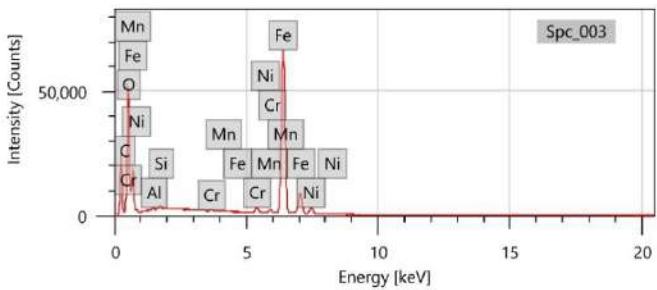


Sem_BED-C_007



Signal BED-C
Landing Voltage 20.0 kV
WD 10.5 mm
Magnification x5,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 5000
Process time	T1
Measurement detector	First
Live time	52.09 seconds
Real time	60.00 seconds
Dead time	13.00
Count rate	68542.00 CPS



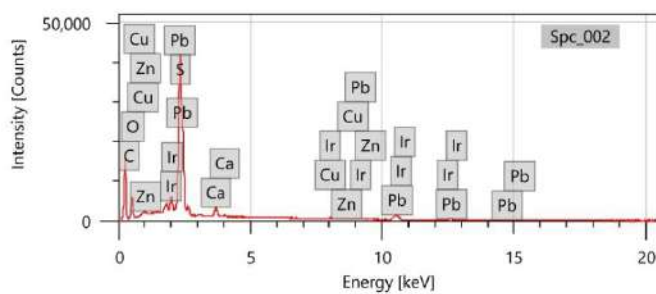
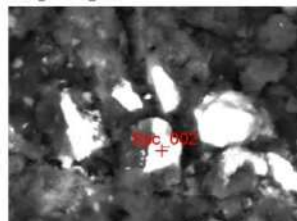
Display name	Standard data	Quantification method	Result Type
Spc_003	Standardless	ZAF	Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	13.69±0.02	49.00±0.08	0.00
O	K			
Al2O3	K	0.50±0.01	0.21±0.01	0.19
SiO2	K	0.56±0.01	0.40±0.01	0.18
Cr2O3	K	1.58±0.01	0.45±0.00	0.41
MnO	K	1.25±0.01	0.75±0.01	0.34
FeO	K	77.65±0.08	46.44±0.05	21.14
NiO	K	4.77±0.03	2.74±0.02	1.25
Total		100.00	100.00	
Spc_003				Fitting ratio 0.0700

Smp_001



Sem_BED-C_002



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x2,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 2000
Process time	T1
Measurement detector	First
Live time	55.05 seconds
Real time	60.00 seconds
Dead time	8.00
Count rate	37552.00 CPS

Display name	Standard data	Quantification method	Result Type
Spc_002	Standardless	ZAF	Oxide (Number of oxygen 24)

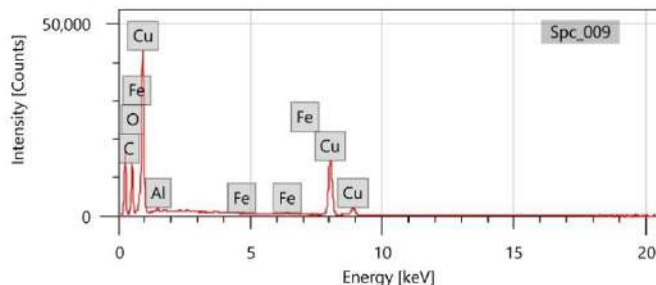
Chemical formula	Line	Mass%	Mol%	Cations
C	K	20.42±0.04	76.99±0.14	0.00
O	K			
SO3	K	14.86±0.06	8.40±0.03	4.87
CaO	K	2.55±0.02	2.06±0.02	1.19
CuO	K	0.85±0.03	0.48±0.02	0.28
ZnO	K	1.02±0.03	0.57±0.02	0.33
Ir2O3	M	7.46±0.05	0.78±0.01	0.91
PbO	M	52.84±0.12	10.72±0.02	6.22
Total		100.00	100.00	

Spc_002 Fitting ratio 0.2008

Smp_001



Sem_BED-C_011



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x5,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 5500
Process time	T1
Measurement detector	First
Live time	55.93 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	30299.00 CPS

Display name	Standard data	Quantification method	Result Type
Spc_009	Standardless	ZAF	Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	25.27±0.05	69.17±0.12	0.00
O	K			
Al2O3	K	1.04±0.03	0.34±0.01	0.51
FeO	K	0.68±0.02	0.31±0.01	0.24
CuO	K	73.00±0.17	30.18±0.07	22.99
Total		100.00	100.00	

Spc_009 Fitting ratio 0.1418

Smp_001

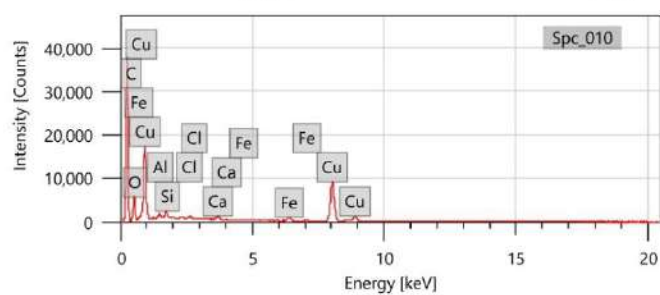


Sem_BED-C_012



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x6,500
Vacuum Mode HighVacuum

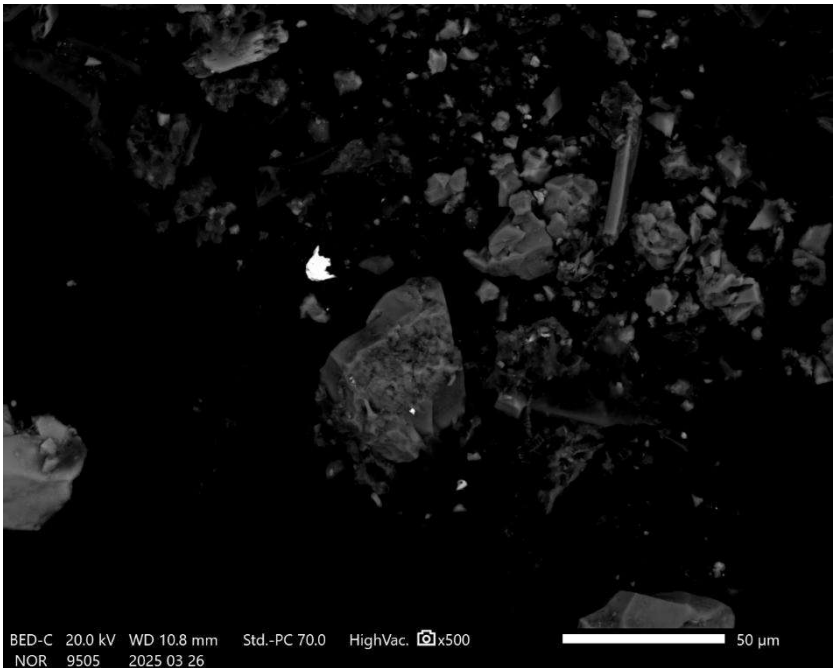
Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 6500
Process time	T1
Measurement detector	First
Live time	56.70 seconds
Real time	60.00 seconds
Dead time	5.00
Count rate	22061.00 CPS



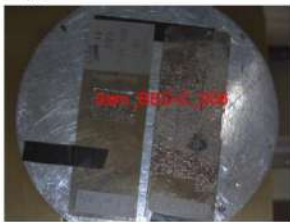
Display name: Standard data Quantification method: Result Type:
Spc_010 Standardless ZAF Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	45.32±0.06	84.30±0.11	0.00
O	K			
Al2O3	K	1.14±0.02	0.25±0.00	0.72
SiO2	K	1.65±0.03	0.61±0.01	0.89
Cl	K	0.31±0.01	0.20±0.00	0.00
CaO	K	0.76±0.01	0.30±0.01	0.44
FeO	K	2.09±0.02	0.65±0.01	0.94
CuO	K	48.73±0.14	13.69±0.04	19.77
Total		100.00	100.00	
Spc_010				Fitting ratio 0.3007

GW5 – Entry wound



Smp_002

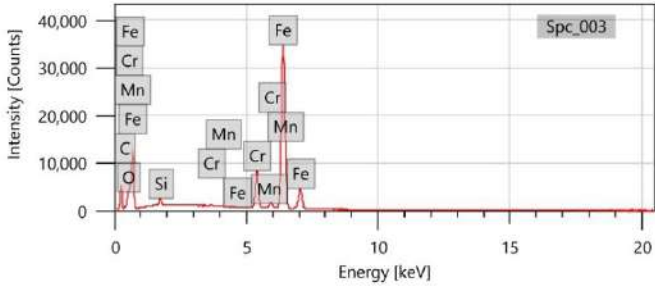


Sem_BED-C_006



Signal BED-C
Landing Voltage 20.0 kV
WD 10.7 mm
Magnification x5,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 5500
Process time	T1
Measurement detector	First
Live time	55.72 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	30178.00 CPS



Display name	Standard data	Quantification method	Result Type	
Spc_003	Standardless	ZAF	Oxide (Number of oxygen 24)	
Chemical formula	Line	Mass%	Mol%	Cations
C	K	6.63±0.02	31.20±0.10	0.00
O	K			
SiO2	K	1.09±0.02	1.03±0.02	0.31
Cr2O3	K	11.66±0.04	4.34±0.02	2.65
MnO	K	0.48±0.02	0.38±0.02	0.12
FeO	K	80.14±0.12	63.05±0.09	19.28
Total		100.00	100.00	
Spc_003			Fitting ratio 0.0267	

Smp_002

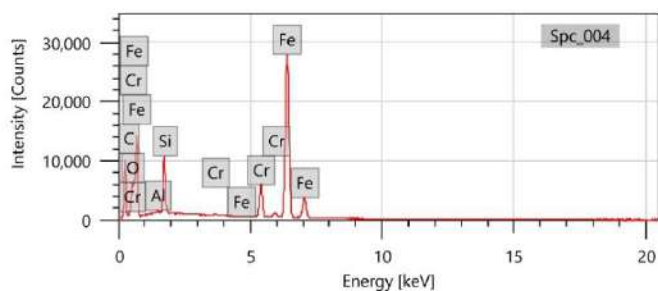


20 mm

Sem_BED-C_007



2 μm



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x8,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 8000
Process time	T1
Measurement detector	First
Live time	55.98 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	28455.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	13.55±0.03	49.33±0.11	0.00
Al2O3	K	0.43±0.02	0.18±0.01	0.14
SiO2	K	8.42±0.04	6.13±0.03	2.36
Cr2O3	K	8.95±0.04	2.58±0.01	1.98
FeO	K	68.65±0.11	41.79±0.07	16.09
Total		100.00	100.00	

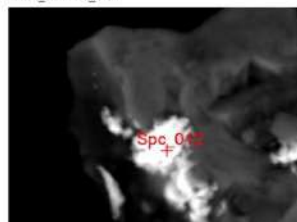
Spc_004 Fitting ratio 0.0478

Smp_002

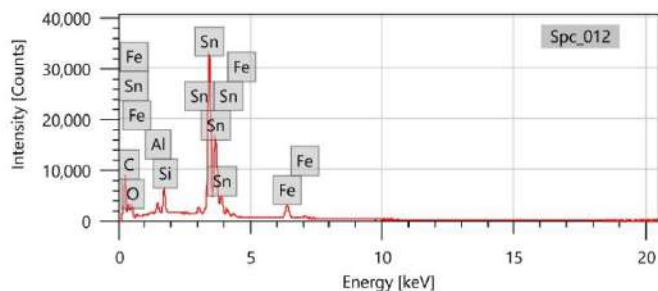


20 mm

Sem_BED-C_019



5 μm



Signal BED-C
Landing Voltage 20.0 kV
WD 10.9 mm
Magnification x5,000
Vacuum Mode HighVacuum

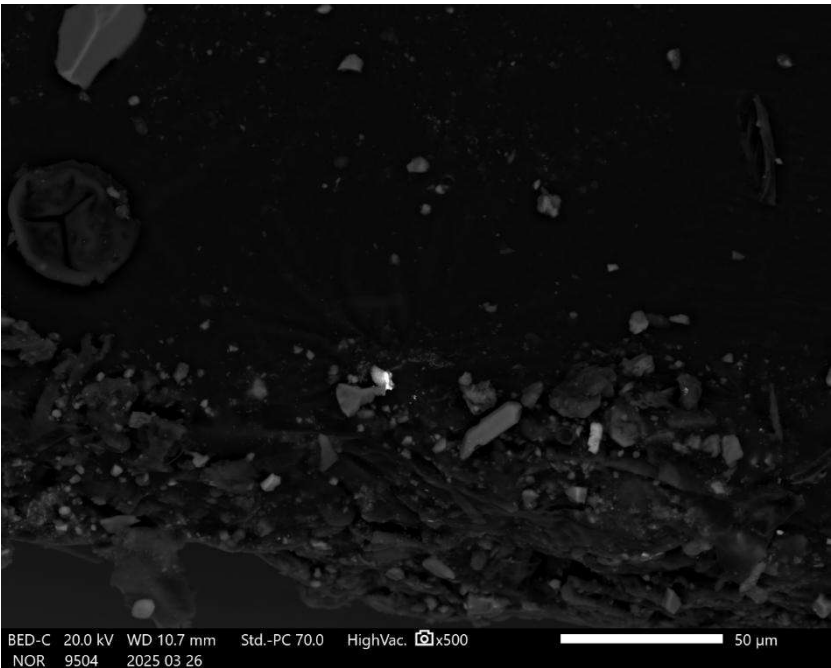
Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 5000
Process time	T1
Measurement detector	First
Live time	55.30 seconds
Real time	60.00 seconds
Dead time	8.00
Count rate	33426.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

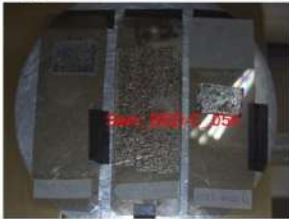
Chemical formula	Line	Mass%	Mol%	Cations
C	K	5.50±0.02	38.24±0.12	0.00
O	K			
Al2O3	K	2.03±0.03	1.66±0.02	0.69
SiO2	K	4.95±0.04	6.88±0.06	1.42
FeO	K	7.74±0.05	8.99±0.05	1.86
SnO2	L	79.79±0.13	44.23±0.07	9.14
Total		100.00	100.00	

Spc_012 Fitting ratio 0.0799

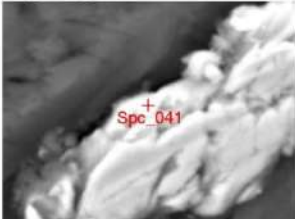
GW5 – Exit wound



Smp_001

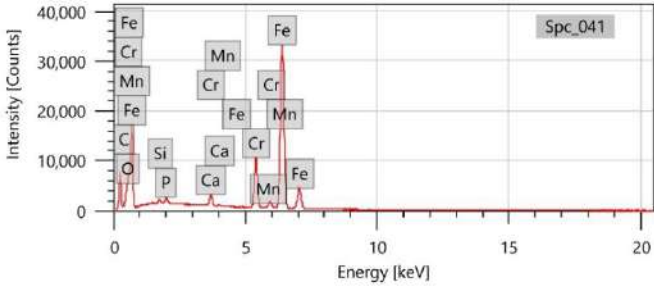


Sem_BED-C_054



Signal BED-C
 Landing Voltage 20.0 kV
 WD 9.7 mm
 Magnification x8,000
 Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 8000
Process time	T1
Measurement detector	First
Live time	55.53 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	34046.00 CPS



Display name	Standard data	Quantification method	Result Type
Spc_041	Standardless	ZAF	Oxide (Number of oxygen 24)

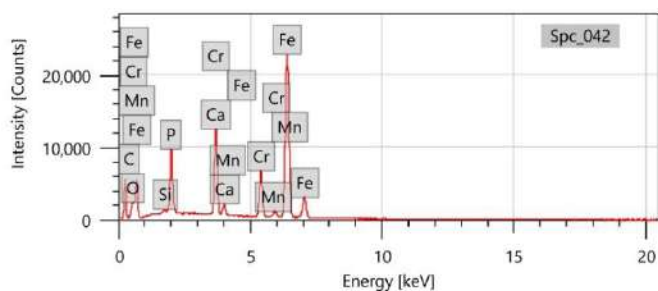
Chemical formula	Line	Mass%	Mol%	Cations
C	K	8.17±0.02	36.73±0.10	0.00
O	K			
SiO2	K	0.58±0.02	0.52±0.02	0.16
P2O5	K	1.16±0.02	0.44±0.01	0.28
CaO	K	1.73±0.02	1.66±0.02	0.53
Cr2O3	K	14.66±0.05	5.21±0.02	3.29
MnO	K	0.42±0.02	0.32±0.01	0.10
FeO	K	73.28±0.11	55.11±0.08	17.41
Total		100.00	100.00	

Spc_041 Fitting ratio 0.0313

Smp_001



Sem_BED-C_055



Signal BED-C
Landing Voltage 20.0 kV
WD 9.7 mm
Magnification x8,000
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 8000
Process time	T1
Measurement detector	First
Live time	56.32 seconds
Real time	60.00 seconds
Dead time	6.00
Count rate	25480.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

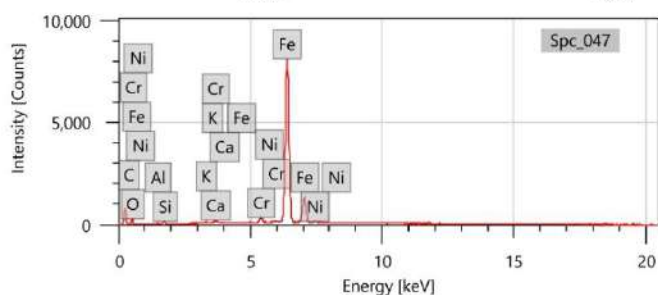
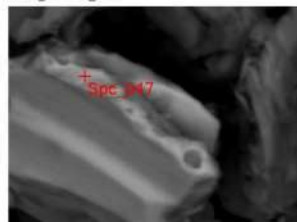
Chemical formula	Line	Mass%	Mol%	Cations
C	K	8.49±0.03	37.71±0.11	0.00
O	K			
SiO2	K	0.39±0.02	0.35±0.01	0.10
P2O5	K	9.87±0.04	3.71±0.02	2.09
CaO	K	10.97±0.04	10.44±0.03	2.93
Cr2O3	K	11.31±0.04	3.97±0.02	2.23
MnO	K	0.31±0.02	0.24±0.01	0.07
FeO	K	58.66±0.11	43.58±0.08	12.24
Total		100.00	100.00	

Spc_042 Fitting ratio 0.0319

Smp_001



Sem_BED-C_062



Signal BED-C
Landing Voltage 20.0 kV
WD 10.9 mm
Magnification x3,700
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 3700
Process time	T1
Measurement detector	First
Live time	56.43 seconds
Real time	60.00 seconds
Dead time	6.00
Count rate	5224.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	5.44±0.04	25.75±0.19	0.00
O	K			
Al2O3	K	0.22±0.02	0.12±0.01	0.08
SiO2	K	0.71±0.03	0.67±0.03	0.21
K2O	K	0.10±0.01	0.06±0.01	0.04
CaO	K	0.36±0.02	0.37±0.02	0.12
Cr2O3	K	1.61±0.04	0.60±0.01	0.38
FeO	K	90.47±0.27	71.60±0.22	22.50
NiO	K	1.08±0.06	0.82±0.04	0.26
Total		100.00	100.00	

Spc_047 Fitting ratio 0.0277

Smp_001

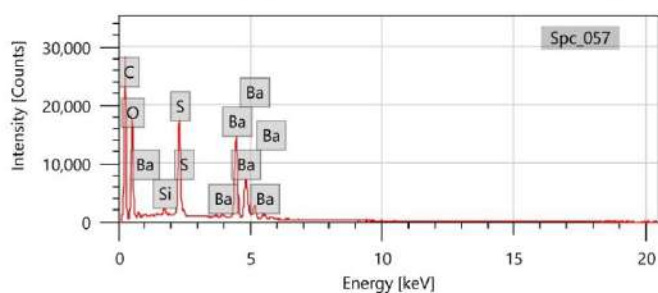


20 mm

Sem_BED-C_073



2 μm



Signal BED-C
Landing Voltage 20.0 kV
WD 10.8 mm
Magnification x7,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 7500
Process time	T1
Measurement detector	First
Live time	55.99 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	29339.00 CPS

Display name: Standard data Quantification method: Result Type:
Spc_057 Standardless ZAF Oxide (Number of oxygen 24)

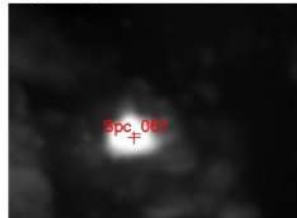
Chemical formula	Line	Mass%	Mol%	Cations
C	K	33.20±0.05	83.02±0.12	0.00
O	K			
SiO2	K	0.79±0.02	0.39±0.01	0.29
SO3	K	20.41±0.06	7.66±0.02	5.62
BaO	L	45.59±0.10	8.93±0.02	6.56
Total		100.00	100.00	
Spc_057				Fitting ratio 0.1998

Smp_001

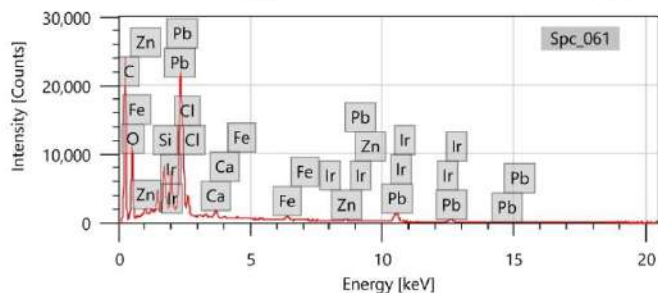


20 mm

Sem_BED-C_077



2 μm



Signal BED-C
Landing Voltage 20.0 kV
WD 10.6 mm
Magnification x8,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 8500
Process time	T1
Measurement detector	First
Live time	55.48 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	30182.00 CPS

Display name: Standard data Quantification method: Result Type:
Spc_061 Standardless ZAF Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	33.11±0.05	86.92±0.13	0.00
O	K			
SiO2	K	5.20±0.04	2.73±0.02	4.03
Cl	K	1.52±0.02	1.35±0.02	0.00
CaO	K	1.33±0.02	0.75±0.01	1.10
FeO	K	1.72±0.02	0.75±0.01	1.11
ZnO	K	1.07±0.04	0.41±0.01	0.61
Ir2O3	M	12.20±0.06	0.89±0.00	2.63
PbO	M	43.86±0.11	6.20±0.02	9.16
Total		100.00	100.00	
Spc_061				Fitting ratio 0.3111

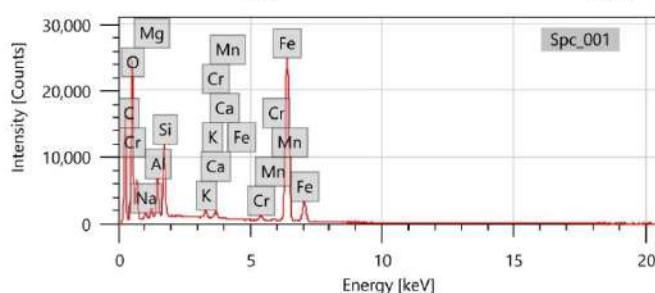
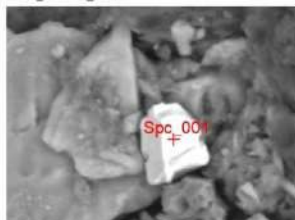
Control soil

Surface soil samples

Smp_002



Sem_BED-C_001



Signal BED-C
Landing Voltage 20.0 kV
WD 11.8 mm
Magnification x2,300
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 2300
Process time	T1
Measurement detector	First
Live time	56.02 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	30483.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

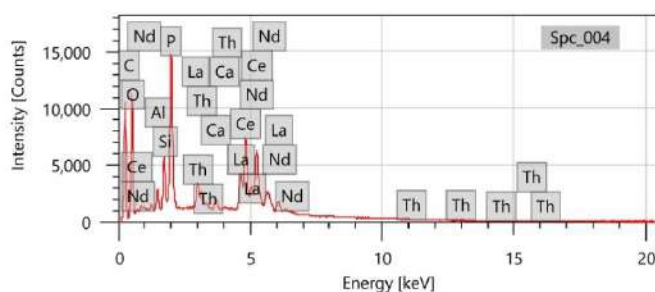
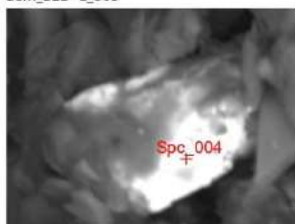
Chemical formula	Line	Mass%	Mol%	Cations
C	K	22.70±0.04	63.45±0.11	0.00
O	K			
Na2O	K	0.95±0.02	0.51±0.01	0.55
MgO	K	1.12±0.02	0.88±0.02	0.50
Al2O3	K	4.63±0.03	1.52±0.01	1.63
SiO2	K	8.88±0.04	4.96±0.02	2.65
K2O	K	0.75±0.01	0.27±0.00	0.29
CaO	K	0.78±0.01	0.47±0.01	0.25
Cr2O3	K	0.99±0.02	0.22±0.00	0.23
MnO	K	0.49±0.02	0.23±0.01	0.12
FeO	K	58.71±0.10	27.48±0.05	14.68
Total		100.00	100.00	

Spc_001 Fitting ratio 0.0991

Smp_002



Sem_BED-C_005



Signal BED-C
Landing Voltage 20.0 kV
WD 12.4 mm
Magnification x4,300
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 4300
Process time	T1
Measurement detector	First
Live time	55.51 seconds
Real time	60.00 seconds
Dead time	7.00
Count rate	30943.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	13.74±0.03	68.71±0.15	0.00
O	K			
Al2O3	K	2.18±0.03	1.28±0.02	0.67
SiO2	K	5.40±0.04	5.40±0.04	1.42
P2O5	K	18.99±0.07	8.04±0.03	4.23
CaO	K	0.99±0.02	1.06±0.02	0.28
La2O3	L	11.62±0.07	2.14±0.01	1.13
CeO2	L	26.63±0.10	9.30±0.03	2.45
Nd2O3	L	11.96±0.08	2.14±0.01	1.12
ThO2	M	8.49±0.07	1.93±0.02	0.51
Total		100.00	100.00	

Spc_004 Fitting ratio 0.1773

Deep soil samples

Smp_002

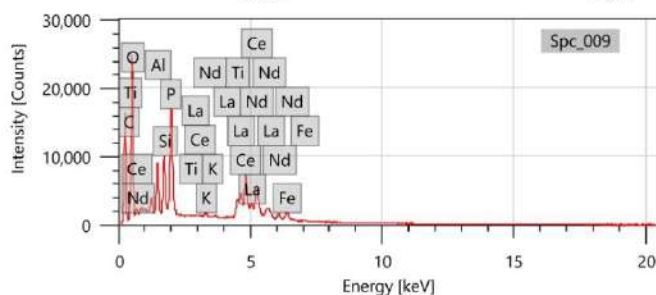


20 mm

Sem_BED-C_018



2 μm



Signal BED-C
Landing Voltage 20.0 kV
WD 12.1 mm
Magnification x8,500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 8500
Process time	T1
Measurement detector	First
Live time	55.30 seconds
Real time	60.00 seconds
Dead time	8.00
Count rate	37141.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	20.44±0.04	74.29±0.14	0.00
O	K			
Al2O3	K	6.42±0.04	2.75±0.02	1.84
SiO2	K	7.66±0.04	5.57±0.03	1.86
P2O5	K	18.19±0.06	5.58±0.02	3.74
K2O	K	0.40±0.01	0.19±0.00	0.13
TiO2	K	3.99±0.03	2.18±0.02	0.73
FeO	K	2.46±0.03	1.50±0.02	0.50
La2O3	L	10.39±0.07	1.39±0.01	0.93
CeO2	L	21.41±0.08	5.48±0.02	1.82
Nd2O3	L	8.62±0.07	1.12±0.01	0.75
Total		100.00	100.00	

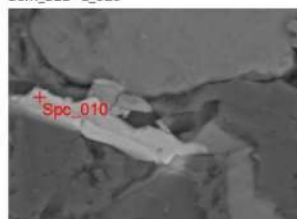
Spc_009 Fitting ratio 0.2102

Smp_002

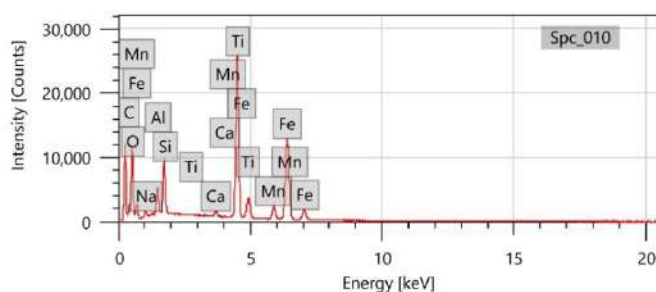


20 mm

Sem_BED-C_020



5 μm



Signal BED-C
Landing Voltage 20.0 kV
WD 12.2 mm
Magnification x2,700
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 2700
Process time	T1
Measurement detector	First
Live time	56.21 seconds
Real time	60.00 seconds
Dead time	6.00
Count rate	29219.00 CPS

Display name: Standard data Quantification method: ZAF Result Type: Oxide (Number of oxygen 24)

Chemical formula	Line	Mass%	Mol%	Cations
C	K	13.76±0.03	49.77±0.11	0.00
O	K			
Na2O	K	0.99±0.02	0.69±0.01	0.42
Al2O3	K	3.20±0.03	1.36±0.01	0.83
SiO2	K	6.56±0.04	4.75±0.03	1.44
CaO	K	0.69±0.01	0.53±0.01	0.16
TiO2	K	39.14±0.08	21.29±0.04	6.47
MnO	K	4.29±0.03	2.63±0.02	0.80
FeO	K	31.37±0.08	18.97±0.05	5.76
Total		100.00	100.00	

Spc_010 Fitting ratio 0.0653

Publications and Dissemination Activities

Scientific articles on international peer-reviewed journals:

Tagliabue, G., Masseroli, A., Golinelli, A., Tambone, F., Cattaneo, C., & Trombino, L. (2026). Dissolved Organic Matter: Unraveling the Role of Organic Carbon from the Hypogeal Cadaver Decomposition Island. *Forensic Science International*, 112887. <https://doi.org/10.1016/j.forsciint.2026.112887>

Tagliabue, G., Masseroli, A., Caccia, G., Poppa, P., Cattaneo, C., & Trombino, L. (2024). Overview of Geosciences Applied to Forensic Taphonomy: The Interdisciplinary Approach of the Italian Non-Human Research Facility (Ticino-LEAFs). *Geosciences*, 14(12), 359. <https://doi.org/10.3390/geosciences14120359>

Tagliabue, G., Masseroli, A., Mattia, M., Sala, C., Belgiovine, E., Capuzzo, D., Galimberti, P.M., Slavazzi, F., Cattaneo, C. & Trombino, L. (2023). Thanatogenic anthrosols: a geoforensic approach to the exploration of the sepolcreto of the Ca'Granda (Milan). *AAPP, Class of Physical, Mathematical and Natural Sciences*, 101 (Suppl. 1), 1-21. <https://doi.org/10.1478/AAPP.101S1A8>

Tagliabue, G., Masseroli, A., Ern, S. I. E., Comolli, R., Tambone, F., Cattaneo, C., & Trombino, L. (2023). The Fate of Phosphorus in Experimental Burials: Chemical and Ultramicroscopic Characterization and Environmental Control of Its Persistency. *Geosciences*, 13(2), 24. <https://doi.org/10.3390/geosciences13020024>

Book chapter:

Tagliabue, G., Crespi, S., & Trombino, L. (2021). Il suolo come contenitore di evidenze: le Geoscienze applicate allo studio delle sepolture della cripta della Ca' Granda. In: Mattia, M. (Ed) *Il Sepolcreto della Ca' Granda, un tesoro storico e scientifico di Milano*, 167-174. Ledizioni LediPublishing; 2022. ISBN: 9788855265973

Congresses:

Tagliabue, G., Caccia, G., Tambuzzi, S., Redaelli, D., Trombino, L., Cattaneo C. Soil as an Unconventional Substrate for IGSR Detection: Does Dirt Matter? EAFS25, The European Academy of Forensic Science, Dublin (Ireland). May 26-30, 2025 (oral presentation).

Tagliabue, G., Masseroli, A., Golinelli, A., Tambone, F., Cattaneo, C., Trombino, L. Evaluating the potential of Dissolved Organic Matter (DOM) analysis and characterization for the investigation of clandestine graves. EGU25, EGU General Assembly, Vienna (Austria). April 27-May 2, 2025 (oral presentation).

Tagliabue, G., Cattaneo, C., Trombino, L. The application of soil analysis in forensic taphonomy: using pigs as analogues for human corpses. EGU23, EGU General Assembly Vienna (Austria). April 23-28, 2023 (poster).

Tagliabue, G., Masseroli, A., Mattia, M., Sala, C., Belgiovine, E., Capuzzo, D., Giordano G., Galimberti, P.M., Slavazzi, F., Cattaneo, C., Trombino, L. The unique contextual situation of the Ca' Granda burial ground: from taphonomic observations towards a new type of anthropogenic soil. EGU23, EGU General Assembly Vienna (Austria). April 23-28, 2023 (poster).

Scientific Dissemination:

Curry, A. (2025). Thousands buried in 17th-century Italian crypt reveal lives of working poor. Science (News). Available at: <https://www.science.org/content/article/thousands-buried-17th-century-italian-crypt-reveal-lives-working-poor>