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THANATOGENIC ANTHROSOLS: A GEOFORENSIC APPROACH TO THE EXPLORATION OF THE SEPOLCRETO OF THE CA'GRANDA (MILAN)

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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 (Jehlika 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 Lützow 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.

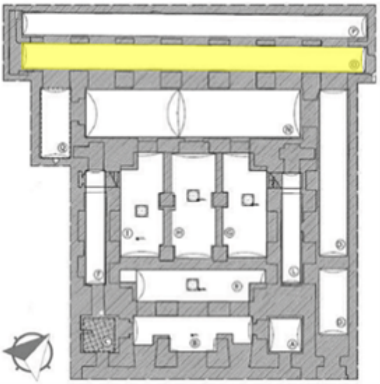


FIGURE 1. Representation of the 14 burial chambers of the *Sepolcreto* of the *Ca' Granda*. The chamber "O" is highlighted in yellow.

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.

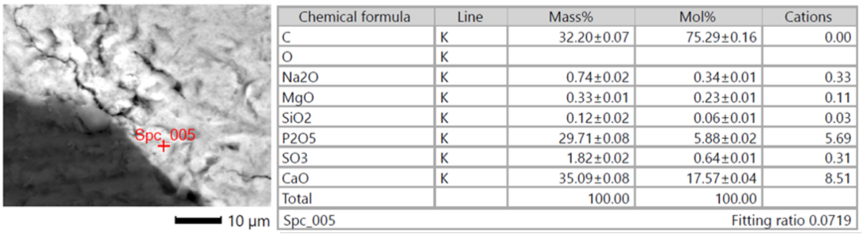
TABLE 1. Resume of the osteological and pedosedimentary samples collected in the chamber "O".

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 backscattered 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%.

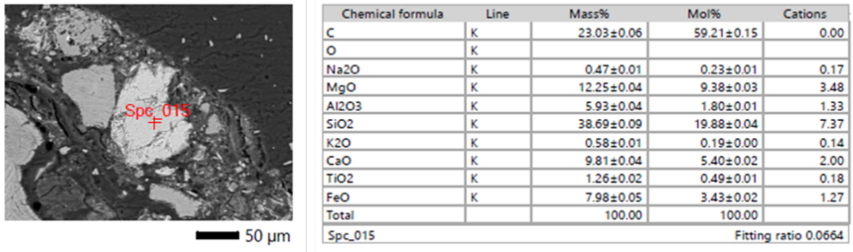
3. Results

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 ± 0.02 Mass%), beside abundant phosphorus (P – 29.71 ± 0.08 Mass%) and calcium (Ca – 35.09 ± 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 ± 0.09 Mass%), calcium (Ca – 9.81 ± 0.04 Mass%), iron (Fe – 7.98 ± 0.05 Mass%), aluminium (Al – 5.93 ± 0.04 Mass%), titanium (Ti – 1.26 ± 0.02 Mass%), sodium (Na – 0.47 ± 0.01 Mass%) and potassium (K – 0.58 ± 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).



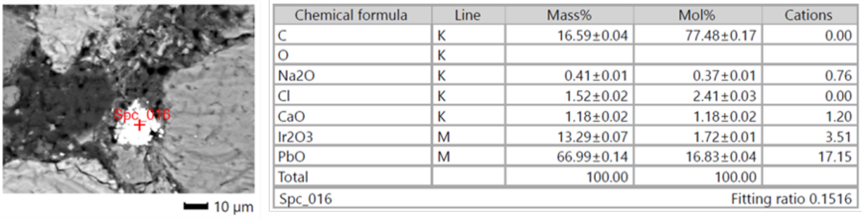
Chemical formula	Line	Mass%	Mol%	Cations
C	K	32.20±0.07	75.29±0.16	0.00
O	K			
Na2O	K	0.74±0.02	0.34±0.01	0.33
MgO	K	0.33±0.01	0.23±0.01	0.11
SiO2	K	0.12±0.02	0.06±0.01	0.03
P2O5	K	29.71±0.08	5.88±0.02	5.69
SO3	K	1.82±0.02	0.64±0.01	0.31
CaO	K	35.09±0.08	17.57±0.04	8.51
Total		100.00	100.00	
Spc_005				Fitting ratio 0.0719

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).



Chemical formula	Line	Mass%	Mol%	Cations
C	K	23.03±0.06	59.21±0.15	0.00
O	K			
Na2O	K	0.47±0.01	0.23±0.01	0.17
MgO	K	12.25±0.04	9.38±0.03	3.48
Al2O3	K	5.93±0.04	1.80±0.01	1.33
SiO2	K	38.69±0.09	19.88±0.04	7.37
K2O	K	0.58±0.01	0.19±0.00	0.14
CaO	K	9.81±0.04	5.40±0.02	2.00
TiO2	K	1.26±0.02	0.49±0.01	0.18
FeO	K	7.98±0.05	3.43±0.02	1.27
Total		100.00	100.00	
Spc_015				Fitting ratio 0.0664

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).



Chemical formula	Line	Mass%	Mol%	Cations
C	K	16.59±0.04	77.48±0.17	0.00
O	K			
Na2O	K	0.41±0.01	0.37±0.01	0.76
Cl	K	1.52±0.02	2.41±0.03	0.00
CaO	K	1.18±0.02	1.18±0.02	1.20
Ir2O3	M	13.29±0.07	1.72±0.01	3.51
PbO	M	66.99±0.14	16.83±0.04	17.15
Total		100.00	100.00	
Spc_016				Fitting ratio 0.1516

FIGURE 8. Point-like chemical analysis of the object constituted by lead (Pb) and iridium (Ir) present in the sample B_Thin_SED2.

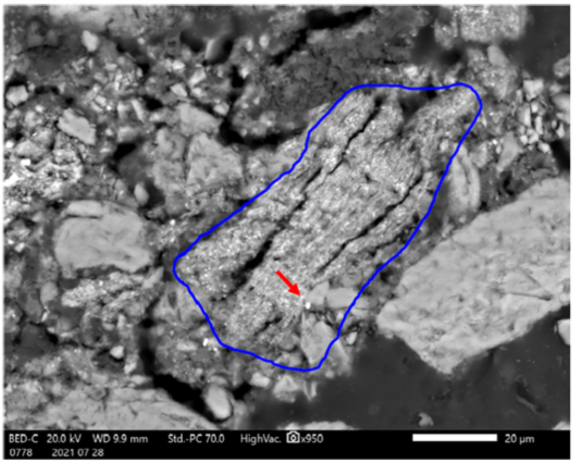


FIGURE 9. Compositional 3D image of the allochthonous object made of lead and iridium (red arrow), included in the mineral matrix (blue line) detected as nestled in the bone tissue of sample B_Thin_SED2.

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).

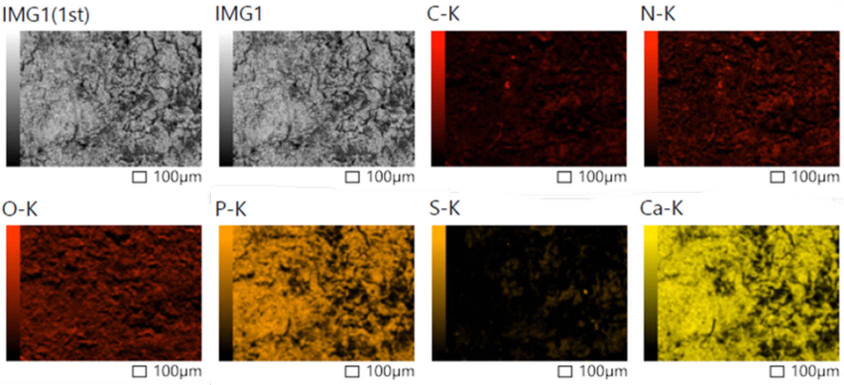


FIGURE 10. 2D elemental map of the surface sample B_Surf_SED1 that highlighted the diffusion of nitrogen (N) and sulphur (S).

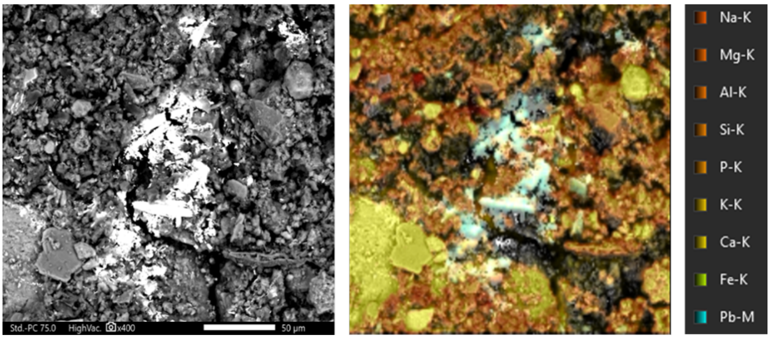
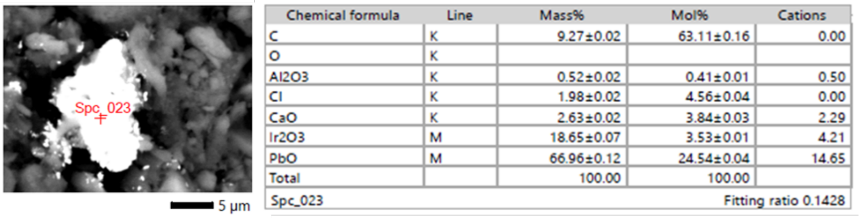


FIGURE 11. SEM-EDS analysis of the surface of the specimen B_Surf_SED2. On the left, a compositional 3-D image of an area of the sample presenting a lead (Pb) cover (400x magnifying). On the right, a superimposed image of the 2D elemental map showing the main chemical elements that characterise the investigated area, including lead (Pb) (400x magnifying).



Chemical formula	Line	Mass%	Mol%	Cations
C	K	9.27±0.02	63.11±0.16	0.00
O	K			
Al2O3	K	0.52±0.02	0.41±0.01	0.50
Cl	K	1.98±0.02	4.56±0.04	0.00
CaO	K	2.63±0.02	3.84±0.03	2.29
Ir2O3	M	18.65±0.07	3.53±0.01	4.21
PbO	M	66.96±0.12	24.54±0.04	14.65
Total		100.00	100.00	
Spc_023				Fitting ratio 0.1428

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).

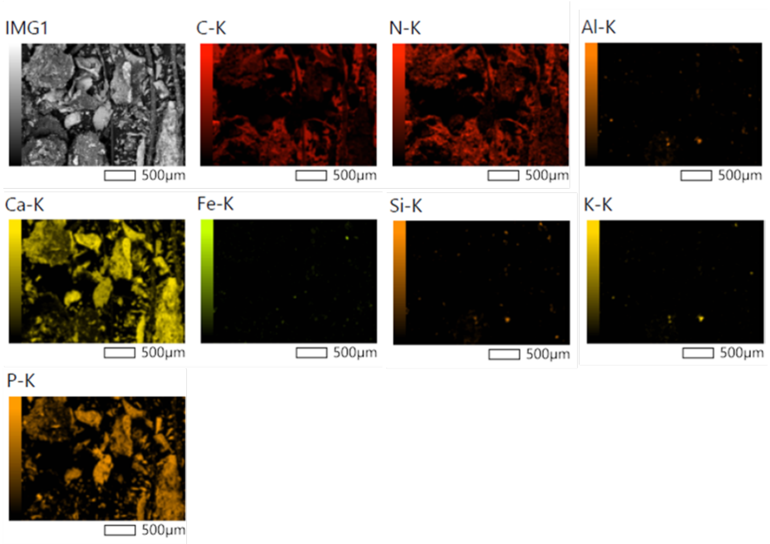


FIGURE 13. 2D elemental map of the pedosedimentary matrix sample Mat_-SED1 from the chamber “O” (50x magnifying).

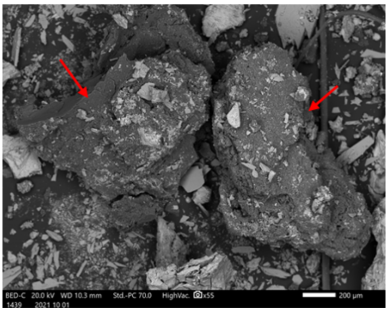
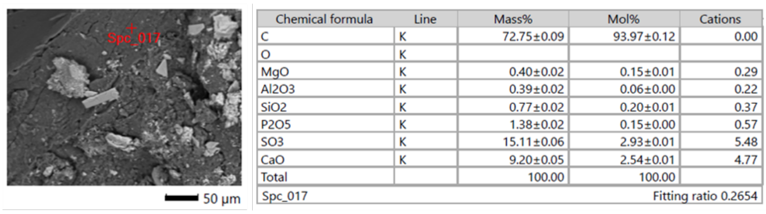


FIGURE 14. 3D compositional image of Mat_SED1. The red arrows point at two fragments of soft tissue surrounded by several microscopic bone fragments.



Chemical formula	Line	Mass%	Mol%	Cations
C	K	72.75±0.09	93.97±0.12	0.00
O	K			
MgO	K	0.40±0.02	0.15±0.01	0.29
Al2O3	K	0.39±0.02	0.06±0.00	0.22
SiO2	K	0.77±0.02	0.20±0.01	0.37
P2O5	K	1.38±0.02	0.15±0.00	0.57
SO3	K	15.11±0.06	2.93±0.01	5.48
CaO	K	9.20±0.05	2.54±0.01	4.77
Total		100.00	100.00	
Spc_017				Fitting ratio 0.2654

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.

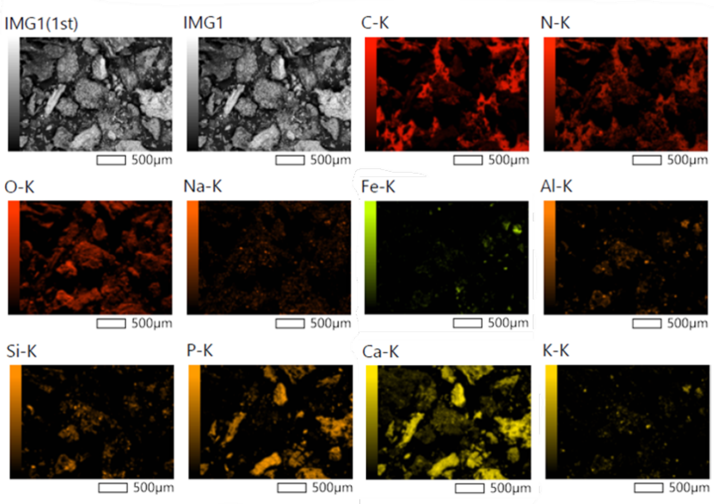
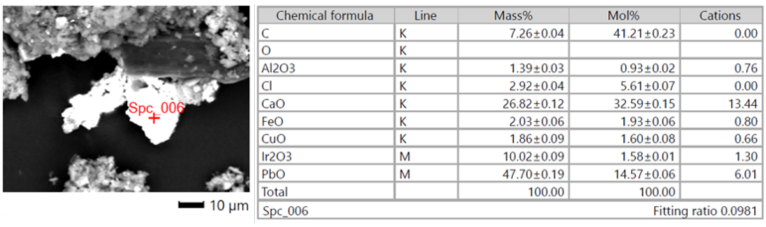


FIGURE 16. 2D elemental map of the pedosedimentary matrix sample Mat_SED2 from the chamber “O” (50x magnifying).



Chemical formula	Line	Mass%	Mol%	Cations
C	K	7.26±0.04	41.21±0.23	0.00
O	K			
Al2O3	K	1.39±0.03	0.93±0.02	0.76
Cl	K	2.92±0.04	5.61±0.07	0.00
CaO	K	26.82±0.12	32.59±0.15	13.44
FeO	K	2.03±0.06	1.93±0.06	0.80
CuO	K	1.86±0.09	1.60±0.08	0.66
Ir2O3	M	10.02±0.09	1.58±0.01	1.30
PbO	M	47.70±0.19	14.57±0.06	6.01
Total		100.00	100.00	
Spc_006				Fitting ratio 0.0981

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 desulphydralation (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 sulphemoglobin, 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 (Staurenghi 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_2^+) (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.

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Competing interests

We all declare that there is no Conflict of Interest, and this article is neither sent nor published in any other journal.

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