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Micromorphological and ultramicroscopic aspects of buried remains: Time-dependent markers of decomposition and permanence in soil in experimental burial

Sara Zangarini [a,b,*], Luca Trombino [a], Cristina Cattaneo [b]

[a] Dipartimento di Scienze della Terra, Università degli Studi di Milano, Via Mangiagalli 34, I-20133 Milano, Italy
[b] LABANOF Laboratorio di Antropologia e Odontologia Forense, DMU, Sezione di Medicina Legale, Università degli Studi di Milano, Via Mangiagalli 37, I-20133 Milano, Italy

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ABSTRACT

A buried body not only determines an environmental response at the deposition site but it is also affected by the soil. The experiment was performed using eleven swine carcasses buried in an open site (Northern Italy). Changes occurring in bone tissue at different post-burial intervals were evaluated observing thin sections of bones through micromorphological and ultramicroscopic (SEM-EDS) techniques. These methods allowed the identification of: (a) magnesium phosphate ($Mg_3(PO_4)_2$) crystallizations, probably linked to decomposition of bones and soft tissues; (b) significant sulphur levels which seem to be related to hydrogen sulphide (H_2S) fixation in bone tissue; (c) metal oxide concentrations in the form of unusual violet-blue colorations, which probably are evidence of the soil's action and penetration in bones, also testified by (d) the presence of mineral grains enclosed in the osseous tissue. The results underline the possibility of identifying both time-dependent markers of decomposition and indicators of permanence in soil in buried bones.

Every year in northern Italy several forensic cases concerning the burial of victims of organized crime, which occurred 10–30 years ago, are investigated. Thus, the application of different forensic disciplines in these investigations is becoming more and more important, in order to precisely analyze each evidence in clandestine graves [1].

In this framework, the importance of the role played by soil scientists in the modern forensic sciences is increasing, in particular when the above described buried human remains, strongly decomposed or skeletonized, are found in different environmental situations. However, among the different techniques normally used in geo-forensic approaches, soil micromorphology (including both optical microscopy and ultramicroscopy) has never been properly assessed for various reasons [2]. Forensic geopedology has an important role in linking corpses to the crime scenes: geopedological analysis not only investigate the soil residues found on the victims, but also other essentials aspects concerning the inhumation site features and changes caused by the long-term burial of corpses in soil [3–5].

This study is part of a wider project, in which an interdisciplinary team worked on several sets of buried swine carcasses in order to study their decomposition and the environmental responses to the burial [6–8] Several geopedological analyses (i.e. physical, chemical – including also HPLC on organic compounds – mineralogical, and micropedological) have been already carried out for each grave before the inhumations and after the exhumations, in order to determine soil characteristics variation over time [9], together with geological, entomological and medical analyses.

The present work is focused specifically on micropedological aspects of buried bones, i.e. the micromorphological (petrographic microscope) and ultramicroscopic (SEM-EDS) cross characterization of the osseous tissue, in order to describe bone alteration pathways due both to decomposition and permanence in soil.

Buried bone characterization for forensic purposes has been already taken in account in several articles. The study of experimental burials allowed the collection of a significant number of data about the decay of human (or swine) remains in different burial environments over time. The authors suggest that many factors may affect the decomposition of a buried corpse, as the different geoclimatic conditions [10,11], the moisture [12], the

* Corresponding author at: Dipartimento di Scienze della Terra, Università degli Studi di Milano, Via Mangiagalli 34, I-20133 Milano, Italy. Tel.: +39 3346292523.
E-mail address: sara.zangarini@gmail.com (S. Zangarini).

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microbial activity [13] or the soil properties [14,15]. However, some of these factors may also be recorded through bone tissue mineralogical changes, for instance the formation of vivianite ($Fe_2(PO_4)_2{\cdot}8H_2O$) [16,17]. Therefore, the micropedological approach was selected in order to analyze bone tissue changes from a new perspective in the study of the sequence of post-mortem events.

In particular, the authors set out to assess the presence in bone of possible markers of permanence in soil of remains.

1. Materials and methods

1.1. Experimental design

Eleven complete carcasses of domestic pigs (*Sus scrofa*), who died of natural causes, were used as experimental material, considering that most of swine anatomic, physiological and genetic features are comparable to human ones (weight, fat-to-muscle ratio and hair coverage) [18]. All specimens were weighted and photographed before being buried in each grave (subjects 4 and 5 were inhumed in the same grave).

The experiment lasted 924 days (2009 May – 2011 December). The specimens' exhumations were divided in five main time intervals (7 weeks, 29–35 weeks, 54–56 weeks, 102–103 weeks and 128–130 weeks) (Table 1).

Moreover, macroscopic analyses were performed for each specimen in order to record information concerning morphological characteristics at different decomposition stages [7] according to Megyesi et al. [19,20].

1.2. Experimentation area

The experimental field was placed inside the Ticino River Regional Park (Pavia, Italy) at 95 m above sea level (45°23′ N 8°50′ E). Coarse fluvial deposits composed of crystalline rocks and poorly developed soils characterize the area. The climate is suboceanic with highest rainfall in spring and autumn and the average rainfall is 1050 mm/year [21].

Table 1
Summary table of all the specimens characteristics.

Area	Specimen	Analyzed bone	PBI (postburial interval)	Weight (kg)
A	M1	Femur, lumbar vertebra (c)	47 days (7 weeks)	80
	M2	Femur, lumbar vertebra (c)	207 days (29 weeks)	80
	M3	Femur, cervical vertebra (c)	396 days (56 weeks)	80
		Radius, lumbar vertebra (u)		
	M4	Femur, lumbar vertebra (u)	923 days (130 weeks)	48
	M5	Femur, lumbar vertebra (u)	923 days (130 weeks)	52
	M6	Femur, lumbar vertebra (c)	711 days (102 weeks)	90
		Radius, cervical vertebra (u)		
B	M7	Femur, vertebra (c)	42 days (7 weeks)	80
	M8	Tibia, toracic vertebra (u)	383 days (54 weeks)	80
		Radius, lumbar vertebra (c)		
	M9	Femur, lumbar vertebra (u)	924 days (128 weeks)	90
	M10	Tibia, cervical vertebra (u)	726 days (103 weeks)	90
	M11	Femur, vertebra (c)	235 days (35 weeks)	80

(c), soft tissues covered bones; (u), uncovered bones.

Ten ditches were excavated by a digging machine in two areas covered by different vegetation. The A site was an open area occupied by dry grasslands, dominated by grasses and sedges (*Bromus sterilis*, *Aira caryophyllea* and *Koeleria* [5]); in this site, the graves were 90 cm deep. In the B site the vegetation cover was represented by acidophilic peduncolate oak (*Quercus robur*) woodlands, sometimes replaced by degraded communities of *Robinia pseudoacacia* and by highly invasive *Prunus serotina* [5]; in this site, the graves were 80 cm deep. The chosen depth represents an average value encountered in forensic scenarios (e.g., intermediate between "shallow" values of 50–60 cm and "deep" values of 100–110 cm [4]).

1.3. Soil descriptions

Soil profiles descriptions were recorded for each grave and soil samples were taken during the inhumation phase [8,22].

The site is characterized by poorly developed soils: the horizons have high permeability, rapid drainage and sandy texture in the first meter. These are non-calcareous soils, with a pH of about 4.5–5.5 at the surface and 5.6–6.6 at 50 cm depth. The base-cation saturation ratio is very low as the AWC (Available Water Capacity) [21]. These soils are currently classified as *Typic Udorthents sandy-skeletal, mixed mesic* [23] and here follows a detailed soil type-profile description for the study area:

O organic material lying under herbaceous vegetation

A1 dark brown (7.5 YR 3/2); loamy and sandy-loam, weak fine granular structure; very porous; clear wavy boundary to

A2 dark brown (7.5 YR 4/2); loamy and sandy-loam, weak fine granular structure; very porous; clear wavy boundary to

C1 dark brown (7.5 YR 4/2); sandy loamy, frequent centimetric clasts (2–5 cm), weak structure; very porous, frequent roots; clear linear boundary to

C2 brown (7.5 YR 5/2); sand, frequent centimetric clasts (5–10 cm), loose; clear linear boundary to

C3 brown (7.5 YR 5/2); sand, overlapping levels of decimetric and centimetric clasts, loose; clear linear boundary to

C4 brown (7.5 YR 5/2); sand, dominant decimetric and centimetric clasts, fining upward, loose; lower boundary not exposed.

1.4. Analytical procedures

Twenty-six cross-sections were extracted both from compact bone (e.g. femur, radius or tibia) and from spongy bone (e.g. vertebra) of all specimens (Table 1). Subsequently, bone samples were included in epoxic resin (Araldite D, 10:1 ratio) and the sections were reduced to 20–30 μm of thickness using silicon carbide abrasive.

Thin sections were observed in transmitted light, both plane polarized (therein PPL) and cross polarized (therein XPL), at the petrographic microscope *Leitz Laborlux 12 POL*. All the specific features (i.e. mineral grains, bone tissue alterations, etc.) observed in each section were successively studied with a Cambridge 360 scanning electron microscope (SEM), imaging both secondary and back-scattered electrons. Ultramicroscopic analyses were performed with an energy dispersive X-ray analysis (EDS Link Isis 300) requiring that thin section be covered with graphite. Moreover, bone tissue analyses were carried out following a specific pattern: the evaluation spots were homogeneously allocated all over the bone section surface (both of compact and of spongy bone).

The magnesium phosphate semi-quantitative description makes use of frequency classes determined according to the general-purpose classes according to Stoops [24]: very few (<5%),